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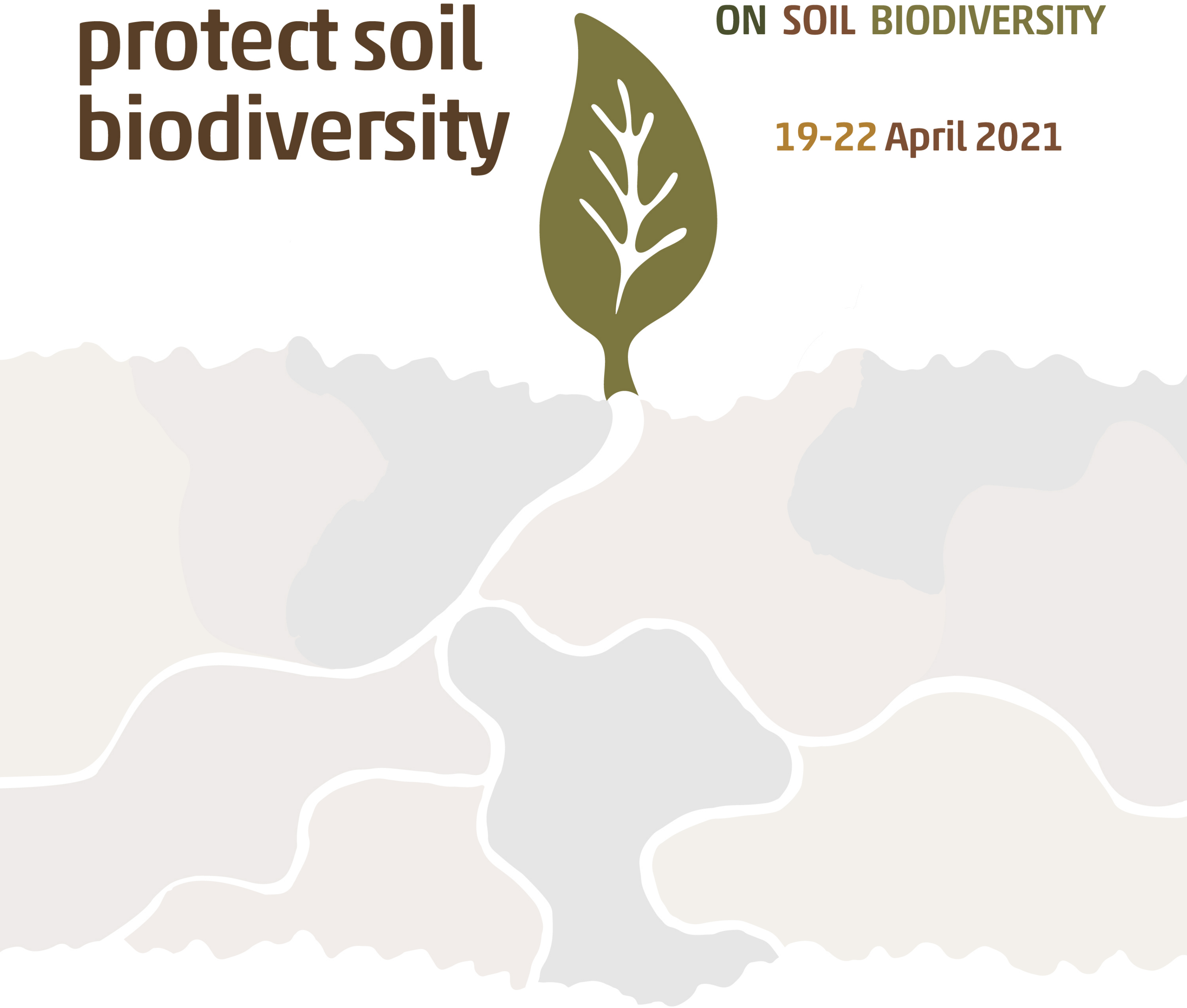

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Contents

Theme 1.

State of knowledge on soil biodiversity

Keep soil alive, protect soil biodiversity

Earthworm diversity and soil-related processes in disturbed Caspian forest

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Abstract summary

This study focuses the effects of forest disturbance (i.e. tree uprooting) on earthworm diversity and some of soil properties in Hyrcanian forest, northern Iran. For this, twenty seven single-tree gap sites in mixed beech forests were found, seventeen sites dominated by beech (*Fagus orientalis* Lipsky) and ten by hornbeam (*Carpinus betulus* L.) at 700-1300 m altitude range. Four microsites were distinguished including mound top (mound), the pit bottom (pit), the gap in the canopy (gap) and closed canopy (canopy) at each site. Soil samples were taken at 10cm depth from all microsites. The ecological groups of earthworms (i.e. epigeic, anecic and endogeic) were collected simultaneously with the soil sampling by hand sorting. Soil pH, water content, total carbon, total nitrogen and carbon to nitrogen ratio measured in the laboratory. The effect of uprooting disturbance on soil properties were found, significantly. Total earthworm number and biomass differed significantly among mentioned sites and microsites. Number and biomass of earthworms showed decreasing trend from undisturbed (closed canopy) to disturbed sites (gap, pit and mound). This trend is mainly caused by number and biomass of endogeic ecological group of earthworms. Thus, the windthrow generally reduced the activity and abundance of the earthworms.

Keywords: Ecological groups of earthworms, epigeic, anecic, endogeic, soil function.

Introduction

Soil formation processes are affected by different disturbances in forest ecosystems. One of the most important effects of windthrow is through the influence on the rate and quality of soil formation processes (Samonil *et al.*, 2008). Soil processes are controlled by a set of relatively independent state factors including climate, organisms, relief, parent material, time and by a group of interactive controls such as disturbance regime and human activities. Forest gaps are a key component of the disturbance regime and examples of natural interactive controls with direct impacts on state factors including climate and organisms. Forest gaps represent dramatic top - down trophic interactions between vegetation and the soil microbial mediated processes (Chapin *et al.*, 2002). Gaps may be responsible for the creation of nutrient hot spots or islands of fertility that increase forest productivity and overall pedodiversity (Scharenbroch and Bockheim, 2008). Earthworms are perhaps the most important soil organisms in terms of their influence on organic matter breakdown, soil structural development, and nutrient cycling, especially in productive ecosystems (Kooch *et al.*, 2008). Despite of the vast increase in scientific literature on earthworms in recent years, much remains to be known in their basic biology and ecology (Nachtergale *et al.,* 2002). However, determine of relation among biomass and diversity of earthworms with pit and mound disturbances and soil properties are essential for management of forest ecosystems. The goal of this study was to investigate of windthrow effects on soil properties, earthworm biomass and species diversity in hyrcanian forests of Iran that is the first survey in these forests.

Materials and Methods

This research performed in Sardabrood forests that are located in the lowland and midland of Mazandaran province in north of Iran. Twenty-seven single - tree gap sites in mixed beech forests were found, seventeen sites dominated by beech (Fagus orientalis Lipsky) and ten by hornbeam (Carpinus betulus L.) at 700 - 1300 m altitude range. In all of areas, the pit and mounds resulted from the fall of a single tree. Four microsites were distinguished including mound top (mound), the pit bottom (pit), the gap in the canopy (gap) and closed canopy (canopy) at each site. Soil samples were taken at 10cm depth from all microsites to laboratory analysis. The earthworms were collected simultaneously with the soil sampling by hand sorting, washed in water and weighed with milli gram precision. Species of earthworms were identified by external characteristics using the key of BOUCH (see Figure. 1). Biomass was defined as the weight of the worms after drying for 48 hours on filter paper at room temperature (60˚C). Analysis of whole data was done in SPSS Ver. 13.5 of statistical program. Factor analysis is statistic technique for achievement to complex relationships among variables. For this purpose, relationships between microsites and earthworms species were analysed by Principle Component Analysis (PCA).

Figure 1: Earthworm's representative of different ecological groups

Earthworm's representative of different ecological groups. (a) Epigeic species inhabit organic rich surface layers and feed mainly on surface organic matter. (b) Endogeic species, consume more mineral soil than epigeic species, and mix mineral and organic soil layers together. (c) Anecic species live in deep vertical burrows, feed mainly on surface litter, and incorporate litter into the soil as well as transporting mineral soil to the surface from deeper soil layers (Pfiffner, 2014).

Results

Analysis of variance is indicating soil characteristics have significant differences in investigated microsites of beech and hornbeam sites. The maximum and minimum of acidity were observed in mound and canopy microsites, respectively for both of sites. The most water content (moisture) devoted in pit microsite and the least was observed on mound. In beech site, maximum of carbon related to canopy and the least devoted on mound microsite, but in hornbeam site, the highest observed in gap microsite. Nitrogen had higher amounts in canopy and least value related to mound. Carbon to nitrogen ratio was less in canopy and the highest amounts observed in pit for beech site and on mound in hornbeam site (Table 1). Analysis of data showed that number and biomass of earthworm's ecological groups had significant differences among microsites and sites. Earthworm's number and biomass had more amounts in canopy and the least were observed on mound microsites. Hornbeam site involved more abundance of earthworms in compare to beech site (Tables 2 and 3). In beech site, PCA showed that percentage of eigenvalue for the first and second axis are about 53.91percent and 31.69 percent, respectively. In hornbeam site, the first second of the PCA accounted for 81.38 percent of the total variance; 48.13 percent by axis 1, 33.25 percent by axis 2 (Figure 2).

Table 1: Mean of soil characteristics in different microsites of sites

Site	Microsite	pH	Water content	Carbon	Nitrogen	Carbon to nitrogen ratio
Beech	Mound	6.82 (0.03)a	15.98 (0.84)d	2.59 (0.13)b	0.12 (0.006)c	21.05 (1.39)ab
	Pit	6.11 (0.09)b	55.72 (1.97)a	2.68 (0.18)b	0.12 (0.003)c	22.00 (1.69)a
	Gap	6.68 (0.04)a	29.38 (1.26)c	3.00 (0.16)ab	0.17 (0.008)b	17.67 (1.37)bc
	Canopy	6.03 (0.07)b	43.80(1.75)b	3.34 (0.15)a	0.24 (0.006)a	14.04 (0.68)c
Hornbeam	Mound	7.66 (0.05)a	14.84 (0.84)c	2.35 (0.04)b	0.11 (0.005)b	20.37 (0.90)a
	Pit	7.43 (0.01)b	53.74 (4.32)a	2.37 (0.29)b	0.13 (0.007)b	18.67 (2.75)a
	Gap	7.65 (0.04)a	37.61 (2.39)b	3.04 (0.17)a	0.25 (0.006)a	12.05 (0.74)b
	Canopy	7.34 (0.08)b	37.49 (2.34)b	2.84 (0.03)ab	0.26 (0.007)a	10.95 (0.38)b

Table 2: Kruskal - Wallis analysis for number and biomass of earthworms in different microsites

Site - Microsite / Statistical character		Earthworm Groups	Epigeic		Anecic		Endogeic	
			Number	Biomass	Number	Biomass	Number	Biomass
Beech	Microsite	Mound	0	0	0	0	0	0
		Pit	1	10.47	0	0	0	0
		Gap	0	0	1.47	10.76	0	0
		Canopy	0.52	2.76	0	0	1.23	7.41
	Statistical characters	Chi square	15.242	15.849	30.477	30.459	19.404	19.398
		DF	3	3	3	3	3	3
		Sig.	0.002**	0.001**	0.000**	0.000**	0.000**	0.000**
Hornbeam	Microsite	Mound	0	0	0	0	0	0
		Pit	2.10	31	0.30	1	0	0
		Gap	1	5.50	2.80	34.30	0	0
		Canopy	1.30	10.40	4.50	13.20	2.80	26.90
	Statistical characters	Chi square	12.105	12.444	17.775	18.338	28.800	28.783
		DF	3	3	3	3	3	3
		Sig.	0.007**	0.006**	0.000**	0.000**	0.000**	0.000**

Table 3: Mann - Whitney analysis for number and biomass of earthworms in sites

Earthworm / Statistical character	Epigeic		Anecic		Endogeic	
	Number	Biomass	Number	Biomass	Number	Biomass
Mann - Whitney U	945	950.50	957.50	951.50	1207	1201
Wilcoxon	3291	3296.50	3303.50	3297.50	3553	3547
Z	-3.31	-3.26	-3.41	-3.46	-1.66	-1.733
Sig.	0.001**	0.001**	0.001**	0.001**	0.095 ns	0.083 ns

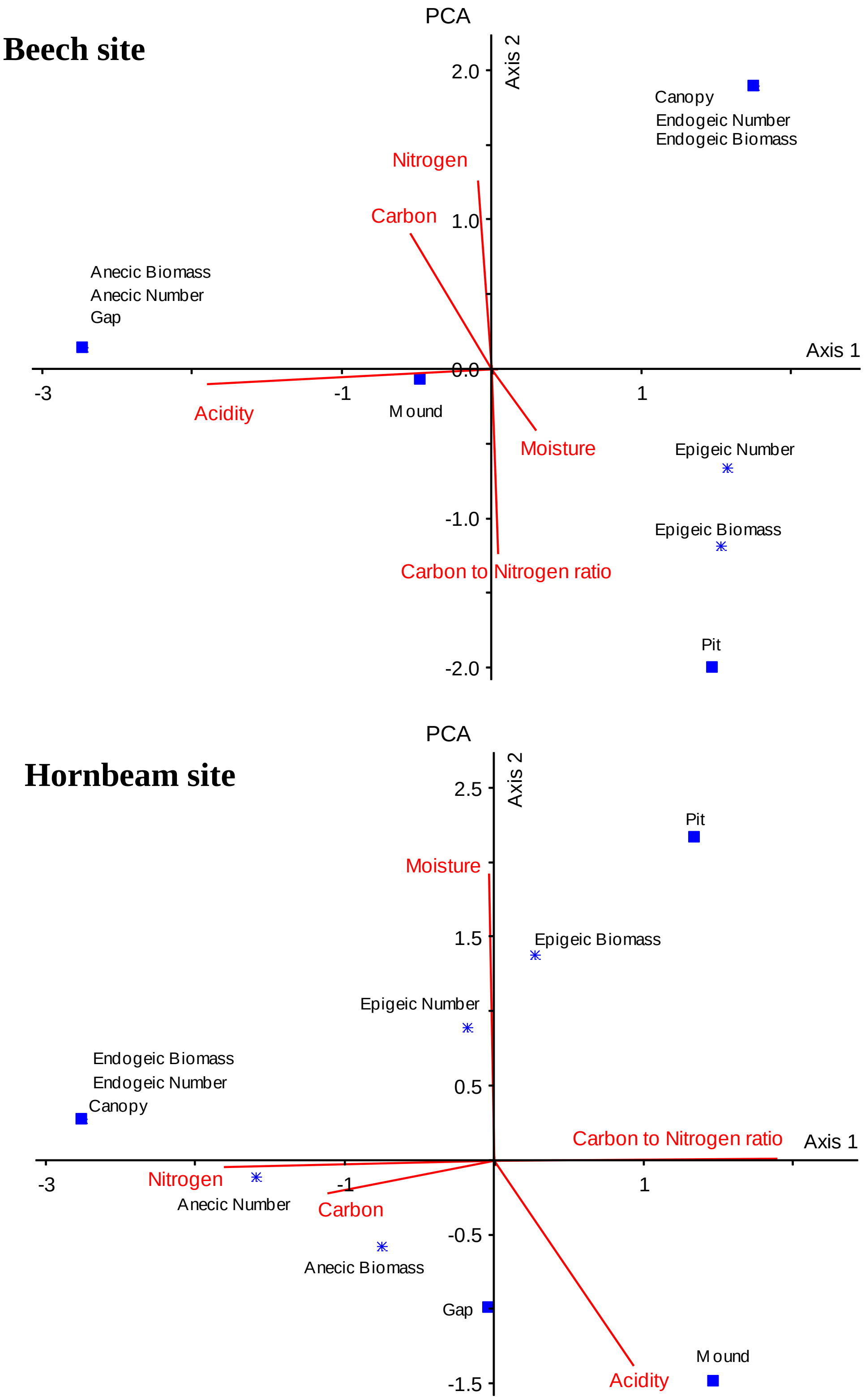

Figure 2: PCA bibplots of microsites, soil characteristics and earthworm species

Discussion

It is conceivable that gap creation increased soil acidity and low pH observed in canopy microsite. Faguetum litters have low pH, but within gaps because of disturbance creation and extensive changes, the decomposition velocity is higher and organic matter cycles are better in compared to canopy. Therefore, the soil pH includes higher amounts in gap microsite that is according to the results of Muscolo, Sidari and Mercurio (2007)'s research. In beech site, epigeic ecological groups (that were belonging to epi - anecic under category) found in pit and canopy microsites that were in relation to moisture high amounts within these microsites. Almost, 80 to 90 percent of earthworms fresh weight constituted of water, thus soil moisture are essential for theirs live and will kill by reason of soil drying (Saleh Rastin, 1978). With considering moisture of 55.72 percent in pit microsite, this moisture amount is due to more gathering of epigeic. The most earthworms, especially anecic species prefer the positions with rich nutrition. These species consuming litters with low C/N content. Endogeic and anecic species are more resistant to soil inappropriate textures and drought conditions. They are able to migration more beneath layers and avoid of soil drought, especially in summer season (Hale and Host, 2005). In hornbeam site, earthworm's different groups were found in studied microsites except of mound microsite. Mounds creates hilly surfaces on superficial soil and include more soil volume and temperature in compared to other surfaces (Londo and Mroz, 2001). On the other hand, soil temperature is effective on number and biomass of earthworms and distribution (Brady, 1990). Therefore, low moisture and high temperature created fatal conditions for earthworms on mound microsite (Nachtergale *et al.*, 2002). High assemblage of epigeics biomass within pit is related to earthworm's hygrophilous. Some of earthworms are semi aquatic and prefer positions with high moisture and deep water (Schwert, 1990).

Conclusion

Results of this study showed that earthworms can be as bioindicator for evaluation of forest stands changes after disturbance events. The windthrow generally reduced the activity and abundance of the earthworms. Our results suggest that windthrow should be considered as an effective factor on pedodiversity that are tied to forest ecology. This is significant for evaluating forest management policies and practices with respect to impacts on soil and also for the use of soils as indicators of forest ecosystems.

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Litter decomposition and organic matter turnover by soil fauna in a sustainably managed olive grove

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Abstract summary

In Mediterranean orchards, soil organic matter (SOM) plays a crucial role and its level is principally determined by the continuous physical and chemical action of soil fauna. The aim of this study was to characterize and compare C/N dynamics and other soil physico-chemical parameters, soil macrofauna abundance, bioturbation and litter/SOM decomposition indices in a Mediterranean olive (*Olea europaea* L.) orchard subjected to two different soil management systems (namely sustainable, S_{mng}, and conventional, C_{mng}) for 18 years. The adoption of the S_{mng} system significantly increased almost three times the abundance of earthworms and two times that of other macrofauna. Bioturbation due to soil fauna and roots was significantly higher in the S_{mng} system, and this caused a significantly faster SOM decomposition measured both in local litter bags and in tea bags. The results highlighted that a great part of the soil quality and fertility of the S_{mng} system could be due to the bioturbation activity of soil fauna, together with its interaction with decomposing microorganisms. It emerged that the role of soil fauna should be seriously taken into account in future land management strategies not exclusively focused on fruit yield and quality.

Keywords: bioturbation; litter decomposition; olive orchards; soil fauna; sustainable land use; tea bags.

Introduction, scope and main objectives

Soils and crops in Mediterranean agro-ecosystems are particularly vulnerable to climate change and environmental stresses, and they will be more and more in the next future (IPCC, 2019). Particularly, Mediterranean fruit orchards are endangered by an increasing water shortage often due to changes in rainfall frequency and distribution, and rise of soil aridity and desertification, with resulting critically low levels of soil organic matter (SOM) and contents of macro- and micronutrients, all of which essential for water storage and plant growth (Palese *et al.*, 2009; Pascazio *et al.*, 2018; Sofo *et al.*, 2019). In order to 'break' this vicious circle, nature-based solutions based on increased carbon inputs are required to facilitate sustainable use and conservation of soils.

The amount and types of SOM are principally determined by the continuous physical and chemical action of soil organisms, as soil fauna participates to SOM shredding, transformation and decomposition, in cooperation with microorganisms (Matson *et al.,* 1997; Six *et al.,* 2004). Moreover, Soil fauna-SOM interactions are of particular relevance in determining soil physical, chemical and microbiological fertility (Giller *et al.,* 1997; FAO, 2017; Totsche *et al.,* 2018).

In this research, olive (*Olea europaea* L.), a typical and widely-spread Mediterranean fruit crop, has been chosen for its multifunctional role. On this basis, the aim of this study was to characterize and compare C/N dynamics and other soil physico-chemical parameters, soil macrofauna abundance, bioturbation and litter/SOM decomposition indices in a mature Mediterranean olive orchard subjected to two different soil management systems (namely sustainable, S_{mng}, and conventional, C_{mng}) over a long term period of 18 years.

Methodology

The trial was carried out in a 2-ha olive orchard (*Olea europaea* L., cv. 'Maiatica'; 70-year.old plants with a distance of 8 x 8 m; NE orientation) located in Ferrandina (Southern Italy, Basilicata region; N 40° 29′; E 16° 28′). Half of the orchard (1 ha) has been managed using sustainable/conservation agricultural practices for 18 years (2000-2018) (sustainable management, S_{mng}), while the other half (1 ha) was kept as 'control' plot and was conducted with a locally conventional management (C_{mng}) (Table 1). In June 2018, soil sampling was performed in the inter-row area of both the systems (S_{mng} and C_{mng}). For both the soil management systems, five composite samples (n = 5) were prepared.

On soil composite samples (soil depths of 0-5, 5-10 and 15-20 cm), total organic carbon (TOC), total carbonates, total N (TN), pH and bulk density were determined according to Pansu and Gautheyrou (2006).

Soil macrofauna was hand-sorted and chemically expelled in the field using mustard oil solution. In order to evaluate soil bioturbation, mesh bags were prepared, installed for one year in the field and then recovered for evaluating biogenic structures due to macrofauna presence. An experiment using tea and and local litter was also carried out. Tea bags and local litter bags were buried for 90 days in the soil. After that, their decomposition indices were calculated according to Keuskamp *et al.* (2013) and Harmon, Nadelhoffer & Blair (1999).

Table 1: Agricultural practices adopted in the sustainable system (S_{mng}) and in the conventional system (C_{mng})

Practice	S_{mng}	C_{mng}
Soil tillage	No tillage. Spontaneous weeds and grasses mowed at least twice a year. Crop residues were cut and left on the ground as mulch.	Tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare.
Fertilization	Guided fertilization: fertigation based on a nutrient balance approach which takes into account nutrient input (by wastewater), output (by yield), and recycling/immobilisation in the grove system (by pruned material, senescent leaves, cover crops). The average values of organic C, N, P and K contained in the treated wastewater were 124, 54, 3 and 50 kg ha^{-1} $year^{-1}$. An integrative amount of 40 kg ha^{-1} $year^{-1}$ of N-NO_3^- was distributed in the early spring.	Mineral fertilization carried out empirically once per year in early spring by using granular product applied to the soil (NPK 20-10-10 fertilizer at doses ranging from 300 to 500 kg ha^{-1} $year^{-1}$).
Irrigation	Guided drip irrigation (6 self-compensating drippers per tree delivering 8 L h^{-1}) with treated municipal wastewater. The irrigation was based on crop evapotranspiration, calculated according to FAO equation: $ET_c = K_r \times K_c \times ET_o$ (K_r = reduction coefficient; K_c = crop coefficient; ET_o = potential evapotranspiration).	No irrigation (about 35 m^3 rainfall $plant^{-1}$ $year^{-1}$).
Pruning	Light winter pruning was performed each year in order to reach vegetative-reproductive balance of trees. Pruning material was cut and left on the ground as mulch.	Heavy pruning carried out every two years. Pruned residues burned out of the olive grove.

Results

The profiles of soil total organic carbon (SOC) and soil total nitrogen (STN) in the two management systems, and particularly in the topsoil (0-5 cm), were considerably different. Here, SOC levels were significantly higher ($p \leq 0.05$) in the S_{mng} system, compared to the C_{mng} one, while the differences in SOC levels were not significant in 5-10 cm layer, and reversed in the 10-20 cm one. The contribution of soil inorganic carbon (SIC) as fraction of soil total carbon (STC) was higher with increasing soil depths, but no significant differences were found between the two soil systems. The levels of STN were significantly higher in the S_{mng} system at 0-5 cm, while the differences in the remaining soil depths and soil systems were not statistically significant. The SOC/STN ratios in the both the soil systems were significantly different between the 0-5 cm soil layer and the other two depths, and were statistically higher in the S_{mng} system. In the litter of the S_{mng} system, the values of SOC and STN were 43.38 g kg^{-1} and 5.90 g kg^{-1}, while no litter was found in the C_{mng} system due to the soil management adopted. In the S_{mng} system, the values of soil pH and bulk density increased with rising soil depth and were significantly lower from those of the C_{mng} system.

Both the number (7 ± 1 specimens) and total weight (4.011 ± 0.702 g) of the collected earthworms were higher in the S_{mng} system, compared to the C_{mng} values (3 ± 1 specimens and 1.397 ± 0.334 g, respectively). Similar trends were found for other macrofauna specimens' number and total weight. The mean weight of earthworms was not statistically different between the two soil management systems, while that of other macrofauna was higher in the S_{mng} system.

The weight of the biogenic structures in the mesh bags with holes (access to macrofauna) were significantly higher in the S_{mng} system than in the C_{mng} one, with differences marked in the 0-5 cm soil depth (10.058 and 3.952 g in the S_{mng} and C_{mng} systems, respectively). The same trend was found in the mesh bags without holes (access to smaller fauna only) (3.710 and 1.392 g in the S_{mng} and C_{mng} systems, respectively, at 0-5 cm soil depth). At the deepest soil depths (5-10 and 10-20 cm) bioturbation was very low compared to the 0-5 cm soil layer.

The weight differences of the tea inside the two types of the tea bags (green and red), allowed to calculate the decomposition indices. Among these, the fraction of remaining green and red tea (Wr_t and Wr_t, respectively) were lower in the S_{mng} system. The stabilisation factor (S) resulted to be significantly higher in the C_{mng} system (0.670 vs 0.585), while the decomposition rate constant (k) showed a reverse trend (0.018 in the S_{mng} system and 0.010 in the C_{mng} one).

The fraction of remaining local litter (Xl_t) in the bags kept in the soil for one year was significantly higher in the C_{mng} system (0.847) than in the S_{mng} one (0.626). Regarding the litter decomposition constant (z), it resulted to be 0.515 in the S_{mng} system and 0.168 in the C_{mng} system, being significantly different at $p \leq 0.05$. Similar trends of local litter bags decomposition parameters, but with higher remaining litter and lower z values were found in the bags kept in the soil for 90 days.

Figure 1: (a) Earthworms, (b) mesh bags, (c) local litter bags, and (d) tea bags recovered from the soils studied in the experiment

Discussion

A great part of the soil quality and fertility in the S_{mng} system could be due to the bioturbation activity of soil macrofauna, together with its interaction with decomposing microorganisms and roots. From the general analysis of the data obtained, it appears that a S_{mng} system increased macrofauna abundance and bioturbation, with repercussions on SOC decomposition determined both in litter and tea bags. Generally, higher microbial and faunal biodiversity in agro-ecosystems leads to greater stability and multifunctionality (Giller *et al.*, 1997; Sofo *et al.*, 2019; Wu and Wang, 2019). From a productive point of view, in soil fauna-plant interactions both the animal and the plant profit from each other, and these interactions could play an important role in fruit growing, positively affecting plant status, water and nutrient uptake and improving product quality (Brussard *et al.*, 2007).

On this basis, the role of soil fauna should be seriously taken into account in future land management strategies focused not only to fruit yield and quality but also to multifunctionality of agro-ecosystems. From an ecological point of view, understanding the relationship between local changes (e.g., soil fauna/microorganisms, soil quality and fertility, soil carbon storage capacity, nutrient cycling and soil water retention) and global effects (e.g., soil quality/fertility, soil environmental importance, climate change mitigation and adaptation) can be particularly important in fruit orchards, whose products are a relevant source of income for many farmers operating in the Mediterranean area and have a potential role for climate change mitigation (Pergola *et al.*, 2013).

Conclusions

In view of circular economy principles and to capitalize on natural potential of soils, strategies have to be developed for sustainable land use practices that optimize nutrient and energy use. This will reduce SOM decline, soil erosion and soil degradation but also promote ecosystem services and foster biodiversity, with consequent benefits to the whole agro-ecosystem stability and its resilience against biotic and abiotic factors.

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Biodiversity in a soil sequence under different use in an area in the interior of the Valencian community, (Spain)

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Abstract summary

Methods based on the study of microfauna are useful for assessing soil quality and studying the effect of degradation of biological and chemical properties. The area used for the study is a rural area of the Valencian Community that presents an area with natural forest soils, and another with soils affected by agricultural uses. Soils with different degradation states (natural soils with potential vegetation, and cultivated soils without irrigation) were included on different parental materials.

Soil properties were characterized by classifying the different soils. The sampled soils are representative of the Mediterranean area of the Iberian Peninsula and correspond to the following soil subunits (WRB-FAO): Molic and rendzinic Leptosoils, and calcareous Regosoils. Moreover, the aspects involved in the biological activity of the soil were studied determining: the organic matter content of the soil, degree of humification, breathing rate and populations of microarthropods. The results showed significant variations in the organic matter content of the soil, with high values of the degree of humification. It is obtained that soil chemical characteristics and biological properties are related to microarthropod populations indicating that the effects of potential vegetation degradation, and inappropriate land use are a direct cause of decrease in organic matter, degree of humification, breathing rate, and decreased populations of microarthromerates in soils.

Keywords: soil sequence, biodiversity

Introduction

Different agronomic techniques can affect the physical, chemical and biological properties of the soil. Certain soil bioindicators are very useful for detecting changes in soil quality, among them is mainly its organic content and soil fauna, important data to monitor the health of the soil (Gardi and Jeffery, 2008; Gómez-Pamiesi, Godoy and Coronel, 2016).

The decrease in biodiversity is in many cases the result of the alteration of the ecosystem due to changes in land use and use (Brussaard, Ruiter and Brown, 2007). In Mediterranean ecosystems there are links between the organic C content and the soil breathing with different stages of degradation or the plant succession in which the ecosystem is located (Rutigliano *et al.,* 2009) with respect to soil fauna, specifically microarthropods, it has been found that there is greater diversity and abundance in natural or forest soils versus cultivated or degraded soils (Arroyo and Iturrondobeitia, 2006; Brown *et al.*, 2007). The objective of this work is to study the variations of different indexes indicators of soil quality depending on its use, in two areas with different lithology with two different types of uses, one corresponding to a climax soil and another to a soil dedicated to cereal cultivation.

Methodology

Soil sampling was carried out in a study area located in the north of the province of Valencia. Units were selected based on geological material; and in turn, each unit was subdivided according to vegetation, type and land use. The study area presents a dry-sub-humid Mediterranean climate, Systematic sampling was performed. Two subunits were sampled in each unit: Climax vegetation (C1, and T1), and cereal crops (C3) and almond and vine (T3). Each subunit was divided into two plots, within each plot the sample was composed of three or four subsamples taken at a depth between 0-15 cm and 50 cm away being the total ground mass of 5 kg. In the same way, unchanged samples were taken in cylinders 5 cm high and 5 cm in diameter, in each plot in order to determine the microarthropods present. Organic matter was determined by the Walkley and Black method (MAPA, 1994). In the fractionation of organic matter from soil the samples were treated with NaOH (0.1 M) in 1:10 ratios, in continuous agitation for 2 hours (Baddi *et al.,* 2004). The organic C content in each fraction was determined by the Walkley-Black method. The soil breathing rate was determined by measure of O2 consumption due to soil microorganisms, using OxiTop-C (Roca-Pérez, Arévalo and Boluda, 2005) breaths, the incubation time was a maximum of 15 days at 20 oC and in darkness. In order to identify microarthropods, extraction was performed with the Berlesse funnel; the counting and classification of the catches was made with a 4x binocular magnifying glass on a petri dish later (Liñan, 1998).

Results and discussion

The organic matter (MO) values obtained in our study in each of the subunits and their characteristics are presented in Table 1. The highest percentages of organic matter were obtained in soils under forest tree cover (climax vegetation), and the lowest percentages occurred in soils dedicated to cultivation, regardless of the geological material on which Developed. Therefore, depending on the use of land we can observe a clear variation in the percentage of organic matter. Microarthropod communities are important as bioindicators in the assessment of the different agronomic regimes. The evaluation has been carried out using the QBS indices (arthropods and collembola) (Lakshmi and Ammini, 2017; Menta *et al.*, 2018). The QBS results showed the variation due to expected use and were more affected by the agronomic regime than by soil characteristics, with the lowest values recorded for agricultural soils.

Table I: Total microarthropods and breathing rate ((mg O_2 g^{-1}ss,), of the soil for 15 days incubation

Sample	Land use	Lithology	Bioclimatic floor	Soil (Deckers and Nachtergaele, 1998)	Micro arthropods/m^2	Humification grade	Respiration (15 days incubation)
C1	Forest	Limestone Cretácic	Supra Mediterranean	*Leptosoil móllic*	21.435	13	4532±321
C3	Agricultural	Limestone Cretácic	Meso Mediterránean	*Leptosoil háplic*	2.340	3	321±14
T1	Forest	Conglomerates of the Tertiary	Meso Mediterránean	*Leptosoil móllic*	18.932	11	3721±266
T3	Agricultural	Conglomerates of the Tertiary	Meso Mediterránean	*Regosoil calcáric*	3.679	5	412±21

The various organic indexes in soils were quantified according to their use and source material. The values of the degree of humification defined as the relationship $(C_{AH}+C_{AF})/Ct$ ranged from 3 to 13, with notable differences between forest and agricultural soils regardless of bioclimatic floor and geological material, which could be related to the characteristics of organic matter and dense plant cover. The titration of the O_2 consumed show that, regardless of the lithology the less degraded the ecosystem, (soils under potential vegetation < soils dedicated to dry crops) and higher content of organic matter has soil (Table 1), the greater its biological activity.

The number and type of microarthropod populations varied depending on land use, being generally higher in natural Leptosoles with potential carrascal vegetation. The largest microarthropod populations were those of the Groups Colémbolos and Acarus, Orders Astigmata and Prostigmata. This fact suggests that geological material and bioclimate are not the determining factors.

The application of different relationships indicated by Karg (1963) between the types of individuals in the study samples show that the Astigmados/Prostigmados ratio favors the Astigmados for forest soils while for the soils under cultivation, favors the Prostigmados. This indicated that areas that are poorly vegetated or in disturbed media appear imbalances, instability and a decrease in soil fertility manifesting an imbalance of mesofauna groups (Havlicek, 2012). In our case, the relationship of Colermbola and other meso or microarthropods (Oribatida, Astigmata, Gamásides, Prostigmatas and Psocopteras) indicate that these relationships tend to increase in agricultural soils (T3).

Conclusions

In the Mediterranean Region, climatic and lithological conditions together with relief and anthropic activity increase soil degradation processes due to loss of organic matter, and it is essential to determine qualitatively and quantitatively parameters that are indicators of the effects on soil loss of quality and biodiversity. The study of the content of organic matter, humic substances, the breathing rate of the soil microbiota and the number of microarthropods in Mediterranean soil systems in an area of the province of Valencia, comparing them on different lithologies climax soils versus agricultural soils emphasized that the parameters used function as good indicators of soil degradation. Assuming the degradation of potential vegetation as a loss of biodiversity, the results showed that its loss influenced the biological properties of the soil rather than variations due to bioclimate or source material, with a gradient negative of the soils of the most preserved ecosystems towards those in deforested and cultivated areas

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Soil biodiversity and physico-hydraulic function: How earthworm and plant root interaction contribute to ecosystem services?

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Abstract summary

Many valuable ecosystem services are driven by soil organisms, and earthworms are the key representatives. Earthworm activity underpins several soil properties that directly influence soil hydrological functioning and food production. However, the influence of different earthworm ecotypes and plant-roots they interact with is largely unknown. Laboratory and field experiments were conducted to investigate these effects.

The data show that earthworms and plant roots interaction can significantly change soil physico-hydraulic properties. However, laboratory experiments indicated that there are species effects. The vertical-burrowing earthworm *Lumbricus terrestris* increased soil water-flow rates, but when their burrows are not connected to drainage systems, plant roots had a greater effect. The lateral-burrowing earthworm *Allolobophora chlorotica* had a greater impact on soil hydraulic properties than *L. terrestris*. The presence of *A. chlorotica* in soils resulted in the greatest and most rapid increase in soil water-flow as a result of their interaction with plant roots.

In a field experiment, the conversion of arable soil to ley caused significant changes to soil properties; earthworms' presence in the ley was responsible for a significant proportion of these changes. However, the magnitude of earthworm impacts is significantly controlled by seasonal climate variations. Furthermore, the presence of earthworms significantly increased plant biomass.

Keywords: Lumbricus terrestris, Allolobophora chlorotica, Soil water flow, Soil water storage, plant growth, hydraulic conductivity, soil water release curves, water stable aggregates.

Introduction, scope and main objectives

Relatively little is known about life in the soil, particularly the interactions between its components. Many of these interactions drive soil processes that give rise to ecosystem services (ES) supporting human wellbeing.

The earthworm is a significant ES driver in soil systems, dominating the biomass of soil invertebrates. Previous reviews (Bertrand *et al.*, 2015; Blouin *et al.*, 2013) have highlighted the main ES provided indirectly by earthworms via their activity in the soil. Earthworms can be classified into three ecological groups, epigeic (litter dwelling), endogeic (shallow burrowing) and anecic (deep burrowing) (Bouché, 1977), and these have differing effects on ES such as soil water regulation. Many factors interact in a complex fashion to impact soil water regulation such as earthworm species and biomass (Alegre, Pashanasi and Lavelle, 1996; Blouin, Lavelle and Laffray, 2007) bulk density (Blanchart *et al.*, 1997), burrowing and casting activities (Bastardie *et al.*, 2003; Le Couteulx *et al.*, 2015), soil texture, soil structure and initial water content (Fischer *et al.*, 2014; Pérès *et al.*, 1998). While there are studies related to each of those factors, relatively little is known about how the effects are different in the presence of plant roots which earthworms interact with.

The primary aim of this work was to examine the influence of different earthworm ecotypes and the plant roots which they interact with on soil properties that directly influence soil hydrological functioning and food production. To achieve this aim, a series of laboratory experiments and a field experiment involving different earthworm ecotypes common in the UK were conducted. The experiments were carried out with and without plants and in the presence and absence of earthworms in soils of differing texture (loam, silt loam and sandy loam soils; two of the fields from which these soils were collected were used in the field experiment).

Methodology

Two laboratory experiments were carried out to examine: 1) the effects of the vertical burrowing earthworm *L. terrestris* on hydraulic function of different soil textures and whether these effects are different in the presence of plant roots which earthworms interact with; 2) then determined whether the interaction effects on soil hydraulic function are different for the lateral burrowing earthworm *A. chlorotica* and how they change with time. A field experiment was then conducted to determine the effects of earthworms as a community, composed of different ecological groups of earthworms, on soil hydraulic function when exposed to natural climate and how those affects change through seasons. The research also explored the resulting effects on plant growth.

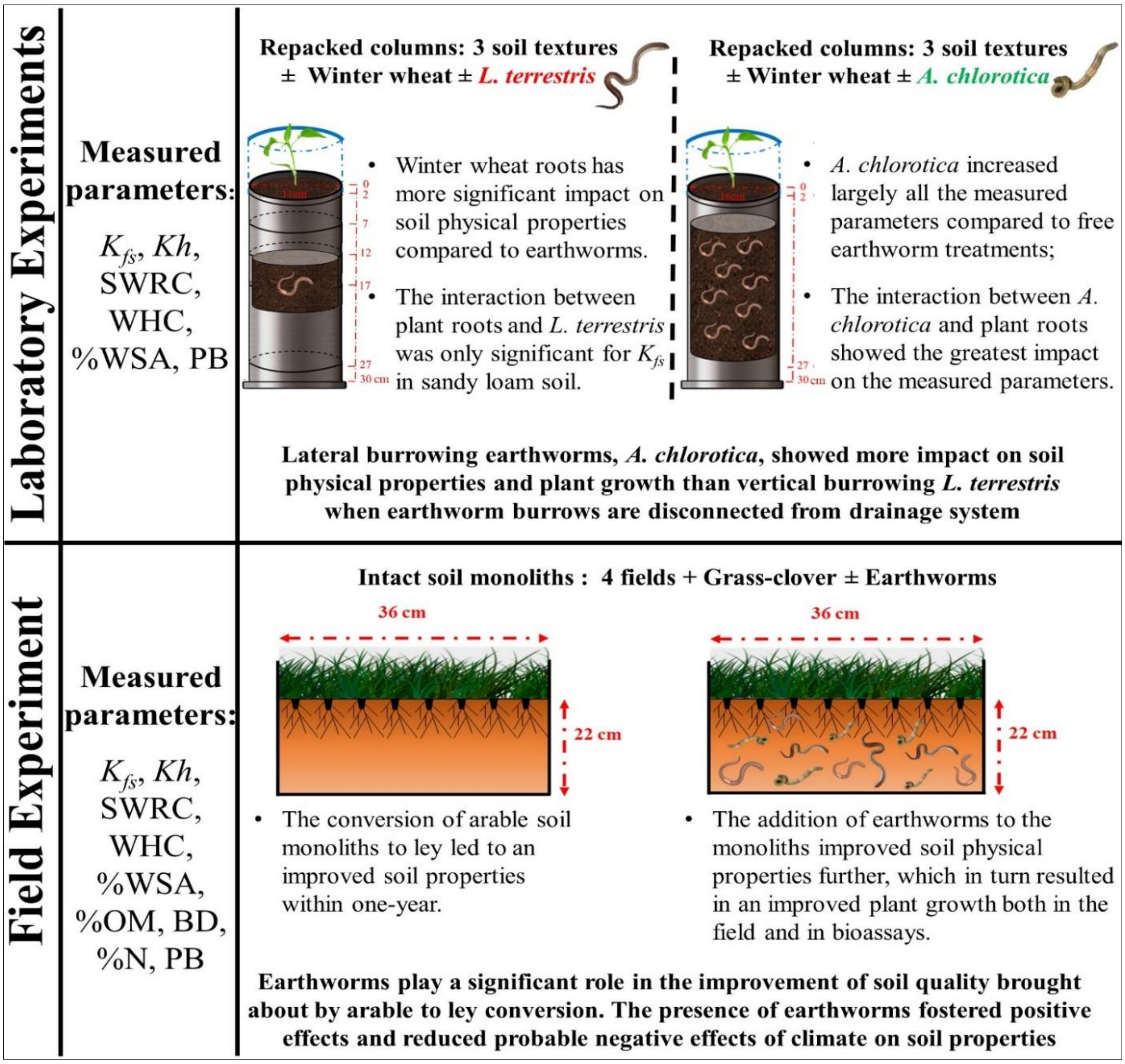
In the two laboratory experiments, either *L. terrestris* or *A. chlorotica* were added to repacked columns with three different soil textures, loam, silt loam and sandy loam collected from the top 20 cm from three fields at the University of Leeds commercial farm, (53° 51′ 44″ N 1° 20′ 35′′W). The soils were air-dried, sieved to < 2 mm, then analysed for pH, organic matter content, dry bulk density and soil particle size (data not shown). Soils were repacked into PVC columns to a dry bulk density of 1.3 g cm^{-3} and wetted gravimetrically with deionized water up to 25 – 30 percent to sustain earthworm activity (Butt and Lowe, 2011; Lowe and Butt, 2005). All columns had a 3 cm thick buffer soil layer at their base separated from the bulk soil by a fine nylon mesh. The columns were planted with pre-germinated winter wheat seedlings then earthworms were added to the earthworm-present treatments. The columns were placed in a controlled temperature room (15 ± 1°C and 60 ± 7 percent rh) under LED lights for 16 weeks. Four replicate columns with the presence and absence of both individual winter wheat plants and either *L. terrestris* or *A. chlorotica* earthworms were established. At the end of the experiment, the field saturated and unsaturated hydraulic conductivity, the partitioning between macropore flow and micropore flow, soil water release curves, soil water holding capacity, water stable aggregates and plant biomass were measured.

For the field experiment, undisturbed monoliths were extracted from four fields that had been under arable use for at least 8 years. From each of the four fields, seven monoliths were collected, six of which were defaunated by deep freezing at -20 °C for three weeks and one monolith was left unfrozen as a control. After defaunation, all monoliths were planted with a ryegrass-clover ley then placed back in an excavated hole in the fields from which the monoliths had been extracted. Within each field, earthworms at population density and diversity similar to that recorded in nearby pasture fields were added at three of the defaunated monoliths. Earthworm addition to the three defaunated monoliths was repeated after the high summer temperatures to ensure earthworm inoculation success. The produced treatments are: defaunated monoliths with earthworms' addition (n = 3), defaunated monoliths without earthworms' addition (n = 3) and a control (Control, n = 1). During the experiment period, hydraulic conductivity at potentials of -6, -3, -1 and -0.5 cm (K0.5) was measured once a season, percent water flow through different pores classes (<0.5, 0.5-1, 1-3 and >3 mm in diameter) was calculated, and grass-clover shoot biomass was collected halfway through the experiment and at the end of the experiment. All monoliths were extracted after 12 months of experimentation - samples from the monoliths were collected for measurement of soil water-release-curves, water-holding capacity, bulk density, percentage water-stable aggregates, organic matter, total nitrogen content (percentN) and grass-clover belowground biomass. Earthworms were recovered from the monoliths during the sampling process and by hand-sorting the remaining soil in the monoliths. Wheat bioassays were conducted on the soil from each monolith.

Figure 1 in the following section shows in part a schematic drawing of both the PVC columns used for the laboratory and the monoliths used for the field experiment.

Results and discussion

The main findings of the research are summarised in the Figure 1 below:



- Winter wheat roots has more significant impact on soil physical properties compared to earthworms.
- The interaction between plant roots and *L. terrestris* was only significant for K_{fs} in sandy loam soil.

- *A. chlorotica* increased largely all the measured parameters compared to free earthworm treatments;
- The interaction between *A. chlorotica* and plant roots showed the greatest impact on the measured parameters.

Lateral burrowing earthworms, *A. chlorotica*, showed more impact on soil physical properties and plant growth than vertical burrowing *L. terrestris* when earthworm burrows are disconnected from drainage system

- The conversion of arable soil monoliths to ley led to an improved soil properties within one-year.

- The addition of earthworms to the monoliths improved soil physical properties further, which in turn resulted in an improved plant growth both in the field and in bioassays.

Earthworms play a significant role in the improvement of soil quality brought about by arable to ley conversion. The presence of earthworms fostered positive effects and reduced probable negative effects of climate on soil properties

Figure 1: Diagram summarising the main results of the research project

Letters refer to the measured parameters; %WSA = percentage of water stable aggregates, WHC = water holding capacity, K_{fs} = field saturated hydraulic conductivity, Kh = unsaturated hydraulic conductivity, SWRC = Soil water release curves, %OM = percentage of organic matter content, BD = bulk density, %N = percentage of total nitrogen, and PB = plant dry biomass. Symbol ± refers to present or absent

The laboratory experiments showed that in conditions where burrows do not connect to drainage systems, the vertical burrowing earthworms *L. terrestris* increased water flow at saturation; however, the impact of plants was more significant. Under the same conditions, the lateral burrowing earthworm *A. chlorotica*, showed more significant impact on water flow than both plant roots and the vertical burrowing earthworm *L. terrestris*. The interaction between winter wheat roots and either *L. terrestris* or *A. chlorotica* showed the greatest effect on water flow. In the *A. chlorotica* experiment, the increase in saturated hydraulic conductivity occurred more quickly in the earthworm + winter wheat than the other treatments. In the *L. terrestris* experiment, the contribution of water flow in pores > 3 mm was greatest in the plant-present treatments. In contrast in the *A. chlorotica* experiment water flow in pores > 3mm was greatest in the *A. chlorotica*-present treatments. Similarly, for soil water release curves, the water content in the soil was greatest in the plant-present treatments in the *L. terrestris* experiment. In the *A. chlorotica* experiment water content in the soil was greatest in the *A. chlorotica*-present treatments. *A. chlorotica* burrowing and casting behaviour increased soil surface roughness (micro-dams) and built complex sinuous burrow systems of small diameter that helped to hold more water in soil than *L. terrestris*. These results show the important role of soil biota in modifying soil hydrological processes and suggest that the action of soil biota can mitigate surface water erosion, or perhaps even reduce downstream flood risks. The results show that endogeic earthworm species can have a significant effect on soil hydraulic properties and could have a greater role to play in mitigating the impacts of flooding and drought compared to vertical burrowing earthworms. Also, to reap the maximum benefit of earthworms for successful land management requires the use of the appropriate species.

The use of a mixed population of earthworms under field conditions confirmed the significant effect of earthworms on soil water flow as in the laboratory experiment. However, their impact was affected significantly by seasonal changes. Earthworm addition to ley increased hydraulic conductivity in summer and spring and decreased it in winter. Earthworm addition to ley also increased the soil water content at saturation and at field capacity, water holding capacity and available water to plants. This reflects the ability of earthworms to contribute to soil water regulation and support crop growth, two key services provided by soil, through water transfer and storage processes. The decreased soil bulk density and increased organic matter content in the ley monoliths suggest that arable to ley conversion improved soil properties, but the further increase in organic matter content in the presence of earthworms shows the important role of earthworms in fostering/enhancing these effects. The differences in hydraulic conductivity between seasons can affect modelling of hydraulic processes and importantly this suggests the need to use the appropriate hydraulic conductivity for the appropriate season.

The soil from the fields with higher organic matter content had higher water flow rates and water holding capacity in all the experiments compared to other soils with lower organic matter content. Overall, the increase in plant dry biomass in all experiments reflected the increase in soil water holding capacity or the availability of water to plants. Plant growth was the highest in the earthworm addition treatments which suggests improved soil properties for crops in the presence of earthworms.

Conclusions

Overall, earthworms that occupy different ecological niches have differing effects on soil physico-hydraulic properties. However, the interaction between plant roots and earthworms changed soil properties which resulted in enhanced percolation rates, together with more water being retained by the soil at different volumetric water contents, which in turn result in higher plant growth. The increased soil water flow and storage could help alleviate exposure of many human systems and ecosystem services that are derived from soils to negative effects of extreme events due to current climate change. Boosting earthworm populations is a worthwhile practice to ensure successful and sustainable land restoration and the improvement of soil qualities adversely impacted by intensive agriculture.

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Kinetic diversity indices for the characterization of topsoil formation in natural and augmented ecosystems

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Abstract summary

We introduce the chemical kinetics for the evaluation of soil ion diversity in order to characterize the relationship between vegetation growth and topsoil formation.

Using two measures that represent invariant features of chemical reaction rate with respect to the total ion concentration and ion balance, we analysed soil mineral databases from geographically continuous Brazilian savanna and highly biodiverse farming plots and surrounding natural environment in Japan. Combined with the model of self-organized vegetation growth, the developed measures showed distinctiveness and complementarity to interpret the topsoil formation processes through a wide range of spatio-temporal ecological transition. Such a framework of analysis is applicable to wider ecological interactions that can be formalized as a concentration-dependent reaction network.

Keywords: Soil ion diversity, chemical kinetics, Brazilian Savanna, Synecoculture, augmented ecosystems, ecological optimum, topsoil formation, diversity measure

Introduction, scope and main objectives

We applied two measures of diversity based on the chemical kinetics, to evaluate the topsoil formation with respect to the spatial transition and temporal succession of ecosystems with natural and artificially augmented vegetation. The main objective is to qualitatively characterize the development process of aboveground and underground ecosystems for 1) the total amount, and 2) balance of soil ions, both contribute to maximize chemical reaction rate in soil solution.

Methodology

Databases: Soil mineral databases of A: four different depths and four transitional vegetation difference from the Cerrado (Brazilian savanna) (Cruz Ruggiero *et al.*, 2002), and B: with two different depths, 34 and 104 samples respectively from natural and augmented ecosystems with edible plant species in Synecoculture project (Funabashi, 2019).

Measures: 1) **Dilution-Invariant Mean Entropy Gradient (DIMEG)** and 2) **Regularized Weighted Self-Information Product (RWSIP)** defined based on the invariant property of the entropy of chemical reaction (Funabashi, 2019).

Summary of derivation of the measures: Consider the chemical reaction rate $v = k\prod_{i=1}^{m}[X_i]^{r_i}$ with m reactants, each with concentration $[X_i]$, coefficients r_i, and rate constant k. We convert the unit $[X_i]$ to a probability form, such that ${[X_i]}/{c^n}$, with constant $c > 0$ and scale $n \geq 0$ being the dilution factor. Adding the concentration of inactive elements $q = 1 - \sum_{i=1}^{m}\frac{[X_i]}{c^n} > 0$, the set of $\{[X_i], q\}$ forms a probability distribution, on which we define the Mean Entropy $S = -\sum_{i=1}^{m} r_i \frac{[X_i]}{c^n}\log\frac{[X_i]}{c^n} - q\log q$ as a diversity measure. Since soil solution is largely influenced by external factors in actual open-field situations (e.g., precipitation), we are interested in the measure of chemical reaction that is not affected by a simple dilution. Using the asymptotic analysis, we derive the constant gradient of the mean entropy with respect to the change in dilution parameters represented in the form of standard function ${\log c^n}/{c^n}$:

$$\lim_{n\to\infty}\left(S/\frac{\log c^n}{c^n}\right) = \lim_{n\to\infty}\sum_{i=1}^{m}\left(-r_i\frac{[X_i]}{c^n}(\log[X_i] - n\log c) - q\log q\right)\left(\frac{c^n}{n\log c}\right) = \sum_{i=1}^{m} r_i[X_i],$$

from which we define DIMEG as the dilution-invariant measure of reaction rate,

$$DIMEG \coloneqq \sum_{i=1}^{m} r_i[X_i].$$

For a given c and n, larger DIMEG value generally represents greater reaction rate. See Figure 1A-1 and B-1 for more intuitive meanings.

Since DIMEG is based on the sum, it can take the same value for different balances of reactants. To distinguish the different reaction rates under the same DIMEG, we develop a complementary measure based on the product of term-wise entropy regularized by DIMEG:

$$\lim_{n\to\infty}\left(\frac{\prod_{i=1}^{m}\left(-\frac{[X_i]}{c^n}\log\frac{[X_i]}{c^n}\ /\ \frac{\log c^n}{c^n}\right)^{r_i}}{\left(S\ /\ \frac{\log c^n}{c^n}\right)^{\sum_{i=1}^{m} r_i}}\right) = \frac{\prod_{i=1}^{m}[X_i]^{r_i}}{DIMEG^{\sum_{i=1}^{m} r_i}},$$

the limit value of which we define as RWSIP,

$$RWSIP := \frac{\prod_{i=1}^{m}[X_i]^{r_i}}{DIMEG^{\sum_{i=1}^{m} r_i}}.$$

It corresponds to the gradient of the weighted self-information product $WSIP := \prod_{i=1}^{m}\left(-\frac{[X_i]}{c^n}\log\frac{[X_i]}{c^n}\right)^{r_i}$ with respect to the dilution parameters $\left({\log c^n}/{c^n}\right)^{\sum_{i=1}^{m} r_i}$. Note that the numerator of RWSIP coincides with the solubility product constant. See Figure 1A-2 and B-2 for the exact relationship between WSIP and RWSIP.

The database analyses in Figure 1 and 2 took after the general chemical equation of soil ion equilibrium:

$$Cl^- + NO_3^- + 2SO_4^{2-} + HCO_3^- + H_2PO_4^- = 2Ca^{2+} + 2Mg^{2+} + K^+ + Na^+$$

The conversion of unit from measured concentration to the probability form of these ions can be calculated from the molecular weight, and in case of choosing $c = 10$, it is situated at the range of $4 < n < 4.7$, which satisfies the convergence of the gradients of Mean Information and WSIP to DIMEG and RWSIP, respectively, in the actual databases as shown in Figure 1.

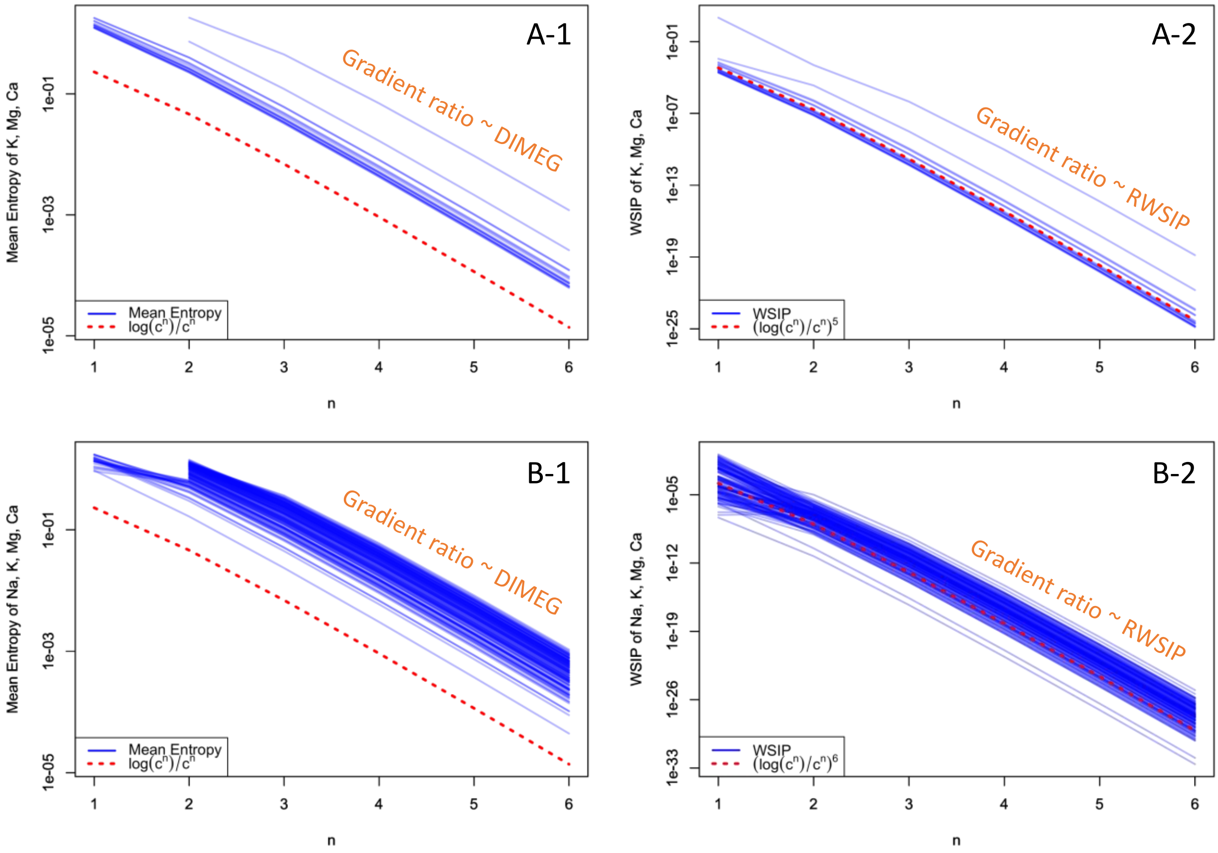

Figure 1: Asymptotic behavior of A: Mean Entropy and B: Weighted Self-Information Product (WSIP) in blue lines, applied to soil mineral databases of A-1 and B-1: Brazilian savanna (Cruz Ruggiero *et al.*, 2002), and A-2 and B-2: Synecoculture fields and surrounding natural ecosystems (Funabashi, 2019)

With respect to the dilution parameters $c = 10$ and $n \geq 1$, the gradient ratios between the blue lines and red dotted standard functions converge to constant values, which provide DIMEG and RWSIP scores for each sample. Actual soil solution remains within $4 < n < 4.7$.

Results

Figure 2 summarize the results of DIMEG and RWSIP scores. In the soil mineral database of Brazilian savanna, the DIMEG scores that represent the total concentration of measured minerals K, Mg, Ca show gradual increase along the vegetation transition from lower savannic formation to semideciduous forest, and the concentration becomes higher towards the soil surface.

The RWSIP scores, on the contrary, take the maximum values at higher savannic formation such as cerrado sensu stricto and cerradão (i.e.

ecotonic formation between grassland and forest), and remains lower at both ends of campo cerrado and semideciduous forest. The increase of RWSIP towards the soil surface is generally observed, except the low score at the soil surface of semideciduous forest (i.e. established ecosystem with high and dense vegetation).

Results from Synecoculture fields and surrounding ecosystems also showed higher concentration of soil minerals Na, K, Mg, Ca at the surface (0-10cm) level, with statistically significant increases of mean DIMEG scores compared to the 30-40cm depth layer (p < 1 percent). While the surface soil of natural ecosystems showed higher DIMEG values than Synecoculture fields (p < 5 percent), at the deeper level (30-40cm) the Synecoculture samples contained more concentration of total minerals (p < 5 percent). There was no significant difference between the overall samples of Synecoculture vs. natural environment.

The RWSIP values between Synecoculture vs. surrounding ecosystems showed higher mean value for Synecoculture fields (p < 1 percent), with significant difference at the surface 0-10cm layer (p < 1 percent). Only in Synecoculture fields the increase of RWSIP scores towards the surface was significant (p < 5 percent). The p-values are summarized in Tab.1.

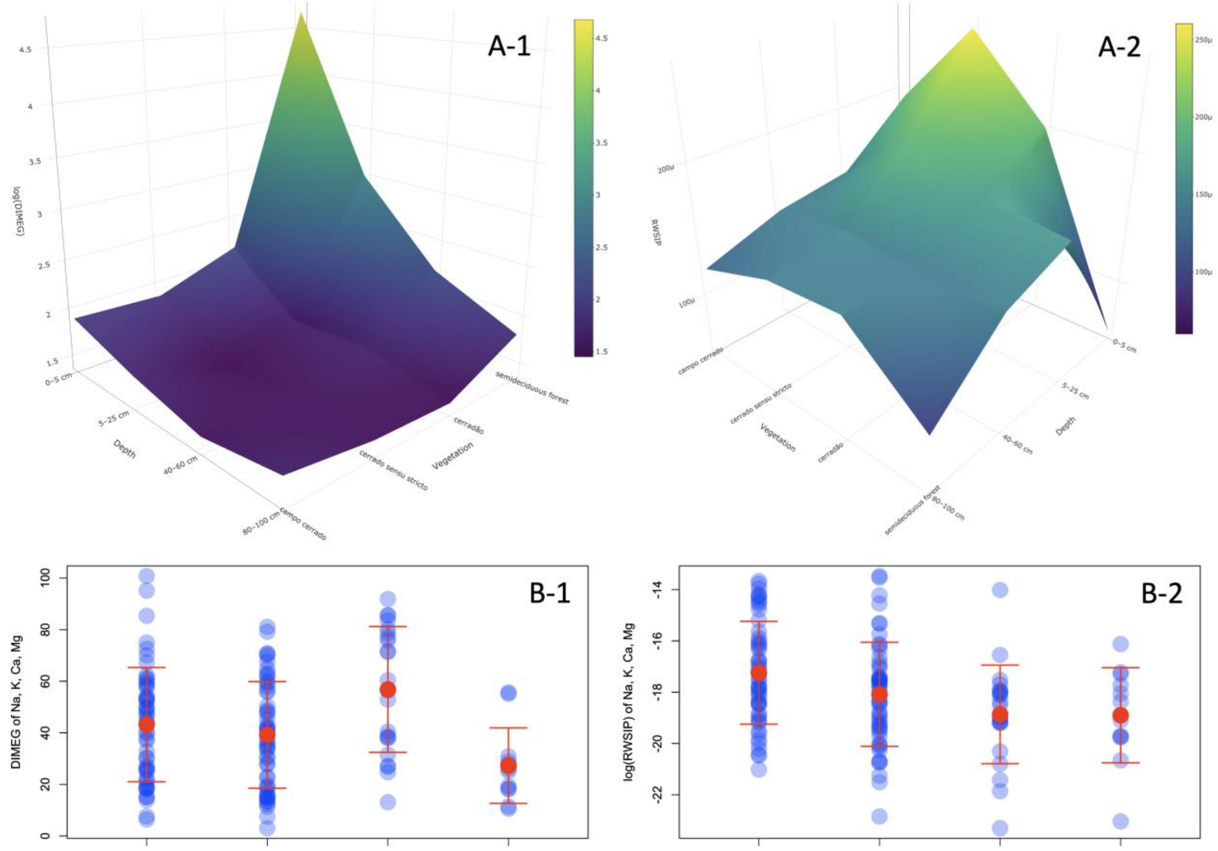

Figure 2: A: DIMEG and B: RWSIP scores of A-1 and B-1: Brazilian savanna [a] (mesh surface), and A-2 and B-2: Synecoculture fields and surrounding natural ecosystems (Funabashi, 2019) (blue circles with mean value ± standard deviation in red circles and lines)

Table 1: Results of t-test for the difference of mean values between two sample groups from Synecoculture fields ("Syneco") and secondary forests ("Natural") in the same area in Japan

Measure	Sample group 1	Magnitude relation	Sample group 2	p-value
DIMEG	Syneco 0-10cm	>	Syneco 30-40cm	0.0052
DIMEG	Syneco 0-10cm	<	Natural 0-10cm	0.031
DIMEG	Natural 0-10cm	>	Natural 30-40cm	0.00011
DIMEG	Syneco 30-40cm	>	Natural 30-40cm	0.029
DIMEG	Syneco All	<	Natural All	0.29
log(RWSIP)	Syneco 0-10cm	>	Syneco 30-40cm	0.038
log(RWSIP)	Syneco 0-10cm	>	Natural 0-10cm	0.0021
log(RWSIP)	Natural 0-10cm	>	Natural 30-40cm	0.96
log(RWSIP)	Syneco 30-40cm	>	Natural 30-40cm	0.20
log(RWSIP)	Syneco All	>	Natural All	0.0021

The notation "0-10cm" and "30-40cm" are the sampling depths. "Syneco All" gathers all samples of "Syneco 0-10cm" and "Syneco 30-40cm", and "Natural All" that of "Natural 0-10cm" and "Natural 30-40cm". With respect to the magnitude relationship of mean values, significant differences are highlighted with red (significance level 1%) and blue (5%). To satisfy the normality of distribution, RWSIP was tested in logarithmic scale.

Discussion

The complementarity between DIMEG and RWSIP shed new insights on the interpretation of savannic formation: while total ion concentration showed monotonal increase towards highly developed vegetation, the soil ion balance became most diverse at the transitional ecotonic formation between grassland and forest. Since natural plant communities that comprise multiple species follow the self-organization process known as ecological optimum (Funabashi, 2016), the qualitative dynamics of DIMEG and RWSIP can be mapped into the different phases of the sigmoidal growth curve (Figure 3): The DIMEG can be interpreted as a proxy of the growth curve of underground ion diversity, while RWSIP corresponds to the growth rate (i.e. differential of the growth curve) because maximizing the diversity in a given concentration leads to the maximization of chemical reaction rate. This model coherently explains the relative magnitude relationship between DIMEG and RWSIP with respect to the spatial transition of vegetation in Brazilian savanna (light blue in Figure 3).

Higher mineral concentration at the surface level is another evidence of ecological optimum, where vegetative organic matters mainly stack and decompose at the surface level, which forms nutrient cycle and exerts soil functions supported by chemical buffering capacity (e.g. adsorptive filtration of water through electro-chemical property of soil particles). In coherence with the theory of augmented ecosystems, the qualitative differences between Synecoculture (1-3 years of implementation with an enhanced level of biodiversity) and natural soils (lower species diversity but longer period of topsoil formation) was consistent with such a self-organization view: In both cases soil surface maintained higher concentration of ions, and despite the shorter period of vegetation succession, Synecoculture fields accumulated the same level of soil minerals as in natural ecosystems with longer succession. Major difference was observed at the balance of these ions, which corresponds to the different phases of the growth curve between the ecotonic transition of Synecoculture (i.e. the orange-shaded range in Figure 3, with higher RWSIP values represented as a magenta line) and more established and saturated stage of natural ecosystems (i.e. the green-shaded range in Figure 3, with lower RWSIP values represented as a cyan line).

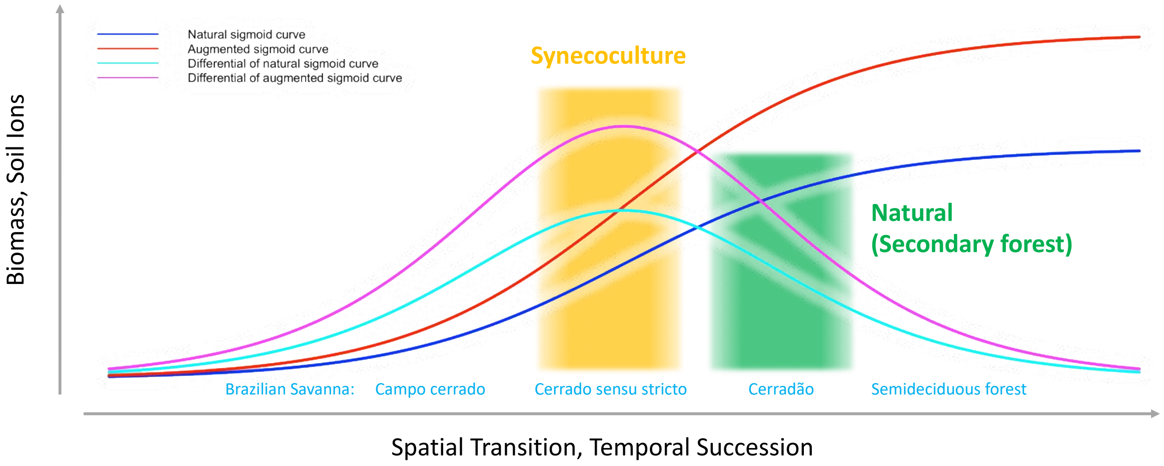

Figure 3: Qualitative model of vegetation development and soil formation based on the self-organization process of ecological optimum

The cases of Brazilian savanna and Synecoculture experiments are integrated with respect to the relative differences between DIMEG (total ion concentration) and RWSIP (ion balance) scores, which correspond to the sigmoidal growth curves (blue and red lines) and its gradient curves (cyan and magenta lines) , respectively, of the biodiversity measures such as aboveground biomass and underground ion diversity. The blue curve and its cyan gradient represent the spatial transition and temporal succession of natural ecosystems, while the red curve and its magenta gradient those of augmented ecosystems such as Synecoculture fields. The correspondence with analysed databases is shown as: light blue words indicating the savannic transition in Brazil; orange shade for the succession stage of Synecoculture fields; and green shade for its surrounding ecosystems' samples.

Conclusions

Two novel diversity indices (DIMEG and RWSIP) incorporating the mechanism of chemical reaction in soil have been constructed, and validated its effectiveness in characterizing natural and augmented ecosystems that contain variability in vegetation transition and succession stages. The developed indices are robust against the open-field fluctuation such as soil water content and provide consistent measures that can be applied to the reinterpretation of existent soil ion databases under variable measurement conditions, and combine with related information on biodiversity. Since the soil chemical property is the essential environment for soil biodiversity, the developed indices may provide important information for the estimation of soil microbial communities using massive data and artificial intelligence

(Funabashi, forthcoming). The complementarity between arithmetic and geometric means is one of the essential strategies for the characterization of complex systems, and further variants of the proposed diversity measures could be considered to unveil hierarchical structure in higher-dimensional databases (Funabashi, forthcoming). These indices being formally defined on chemical equation, there should be direct applications to dynamical equilibria of general ecological interaction networks beyond soil ion reactions, such as the food chain of soil microorganisms.

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Soils of Georgia highlands and their biodiversity

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Abstract summary

Georgia, according to WWF definition is included in the list of "hot spots" of biodiversity in the Caucasus and Eastern Europe. That's why to study its biodiversity is very interesting, especially in the highland regions. Ecological changes caused by global warming and human impact, study the High Mountain ecosystems and facilitating maximal development of soil is one of the most actual problems for scientists.

In High Mountain regions of Georgia livestock farming is the main agricultural branch. Hence, the considerable amount of meadows in High Mountain regions of Georgia are used as pastures and the others in adjacent areas are occupied with technical cultures.

Ecosystems of relatively simple structures are most convenient for biogeocenological research and at the same time are less studied. High Mountain regions are areas wherein forming rather extreme conditions for soils' ecosystems.

Investigations were conducted in different regions of Georgia at an altitude of 800 – 2395 m asl. There were determined the basic groups of High Mountain meadows' soil inhabitants – invertebrates-saprophagous.

Keywords: Soil inhabitants, Highlands, Biodiversity, Caucasus.

Introduction, scope and main objectives

Georgia is one of the main countries in the Caucasus, lying between Western Asia and Eastern Europe. It is bounded to the west by the Black Sea, to the north by Russia, to the south by Turkey, and to the southeast and east by Armenia and Azerbaijan (Kokhia and Golovatch, 2020). The area is mainly high montane, situated between latitudes 41° and 44°N, and longitudes 40° and 47°E.The peculiar location of the Caucasus, the extremely diverse climatic conditions and the heterogeneous nature of its vegetation determine the soil biodiversity.

The most of Georgia's territory is occupied by hilly meadow soddy primitive soils, which extend from 1100 to 2600 m asl. In most mountainous areas, the following types of soils are found:

1. Forest light brown soils;

2. Mountain-valley landscape with alluvial soils;

3. Mountain meadow soils;

4. Mountain glacial landscape with subnival and nival belts (Kokhia, *et al.*, 2021).

Georgia's highlands flora also is diverse and represented more than 4130 species of plants. Among the countries of moderate climate, Georgia is considered as one of the richest floristically; it is at the 5th place in Europe, after Italy (5663), Spain (4916), Greece (4900) and France (4500). High endemic level of Georgia's flora reflects its richness. Approximately 21percent of the flora in Georgia is endemic and consists of about 90 endemic species. Among them about 600 species are Caucasian and about 300 are Georgian endemic.

The Greater Caucasus Mountain Range plays an important role in moderating Georgia's climate and protects the nation from the penetration of colder air masses from the north. The Lesser Caucasus Mountains partially protect the region from the influence of dry and hot air masses from the south (Bondyrev, Davitashvili, Singh, 2015). According to Gulisashvili (1964), the high-altitude natural zonation varies quite clearly in different parts of Georgia (Figure. 1) in relation to climatic gradients.

Ecosystems of relatively simple structures are mostly convenient for biogeocenological researche and at the same time are less studied. High mountain regions are just those areas relatively rather extreme conditions for living organisms' ecosystems of the most simple structures are formed and developed. Nowadays influenced by the environmental changes caused by global warming and human impact, the study of high mountain ecosystems and facilitating maximal development of soil is one of the most actual problems for scientists.

That's why it is so interesting to study the biodiversity of the highland regions, especially of the soil and its inhabitants. In the presence of climate change, land degradation and biodiversity loss, soils have become one of the most vulnerable and actual problems for scientists of the world.

In high mountain regions of Georgia livestock farming is the main agricultural branch. Finding an optimal balance between livestock production and grazing impact on animal diversity is important for the development of sustainable grazing systems. Hence, the considerable amount of meadows in High Mountain regions of Eastern Georgia are used as pastures and the others in adjacent areas are occupied with technical cultures (potato, cereal crops). The rate of plant residue decomposition, their mineralization, soil structure and humus horizon formation, the rate of organic compound turnover is determined and significantly depends on the soil inhabitants' life-activity (millipedes, earthworms, nematodes, and other invertebrates).

The main objective of the research is to study soil inhabitants their species composition and distribution in high mountain ecosystems.

Soil invertebrates appear as bioindicators of soil state. We consider that the study of high mountain soils macrofauna structure, estimation of their role in soil layers mixing, increasing of water flowing and aeration, to improvement of soil physical and chemical characteristics and enrichment of organic matter with products of their vital activity should be the most effective method for ecological monitoring of such type of ecosystems. The representatives of macrofauna as a saprophagous-humificators take an active part in bringing organic compounds of plant litter into deep layers of soil, enriching soil mineral horizon which leads to deepening and formation of a soil profile. Invertebrate-saprophagues release energy and nutrient elements accumulated by green plants.

Methodology

To study specific composition of soil inhabitants of high mountain ecosystems, their distribution and trophicstructure standard methods applied in soil zoology were used. For gathering and definition of soil invertebrates' dominant species, traps were used. There were provided the complex research of high-mountainous ecosystems and soil inhabitants' vital activity were investigated in some High Mountain sites of Georgia. There were determined the basic groups of high mountain meadows' invertebrate-saprophagous (millipedes, earthworms, and Woodlice).

In the fields with Lucerne, the prevalence of saprophagues number was clearly expressed and approximately was 60 percent. The structure of invertebrates' population in these places was characterized by the variety of trophic groupings and poly-dominance. In the lower mountain forest zone canopy saprophagues predominate and compose 76 percent of the total number and are represented also with earthworms, woodlice and diplopods. In the mining soils of alpine meadow there was found just one species of diplopods - *Catamicrophyllum caucasicum* and their number comprised more than 200 ind./m^2 or 88 percent (Kokhia, 2011). It is necessary to note that the presence of rare and endemic forms is characteristic for the macrofauna of the alpine meadow of alpine zone.

Results

Long-term studies of the Georgian soil fauna have shown that the main soil inhabitants are saprophagous-invertebrates, including Insects, Myriapoda and Earthworms (Kokhia, 2011). At the same time, we studied the Nematodes: phitoparasites, free-living and entomopathogenic forms among them.

In Georgia, recorded about 2480 species of insects.

Table 1: Soil inhabitants' diversity and distribution in Georgia

	Myriapoda	Earthworms	Nematode
Western Georgia	71	80	361
Eastern Georgia	63	30	300
Total	134	110	661

Lumbricidae is characteristic of shallow soils, while they are almost not found on medium and humus-rich soils. The anthropogenic factor is the reason for this since shallow soils were mainly used as pastures and the powerful and humus-rich soils as agricultural land. Therefore, these soils are richer with the earthworms' species composition.

Studying the lumbricofauna of Georgian mountainous chernozems, it was established that earthworms occupy the leading positions among the soil macrofauna and play a decisive role in soil formation processes, so their number varies from 240 to 400 ind/m^2. It should be noted right away that in Eastern Georgia, namely in the Vashlovany Nature Reserve, the number of earthworms was only 8 to 14 ind/m^2. Nowadays, in mountain chernozems, were found earthworms species, which are typical for the forest brown soils, such as *Dendrobaenaveneta, D. byblica, D. penteri, Eisenia grandis perelae*, etc, after the destruction of forest vegetation, they adapted to the conditions of mountain chernozems (Kvavadze, 1985).

Discussion

The diplopod fauna of Georgia, Transcaucasia, is very rich given the country's relatively small territory because it presently comprises 103 species from 44 genera, 12 families, and 7 orders. Investigation has shown that a significant number of millipede species is noted in Western Georgia (52 percent) and about 19 percent were noted in Eastern Georgia (Kokhia, 2018).

Proceeding from the above-stated principles Kvavadze (1999) has classified earthworms of the Caucasus and singled out the following ecological groupings: inhabiting soil surface or epiedaphobionts, soil-litter or deviedaphobionts, forms living in humus horizon or hemiedaphobionts, euedaphobionts or forms living in deep layers of soil, amphibionts earthworms living in water and land and hydrobionts or secondary water forms of earthworms.

In this respect, in the Adjara region (Bukhnikashvili and Beltadze, 2012), for example, earthworms are grouped as follows:

Table 2: Ecological earthworm groups

Ecological Groups	Earthworms
Epiedaphobionts	7
Deviedaphobionts	5
Hemiedaphobionts	10
Euedaphobionts	4
Euedaphobionts	3

Hydrobionts	1

Conclusions

As it was expected, the meadow soil macrofauna significantly differed from the ones of forests' soil. It was mostly observed in dominant groups. As for main groups, they were unchangeable. In all height level sand in all associations earthworms had the priority condition, though in other groups among them insects and diplopods were in small quantities. It was due to earthworm density of soil invertebrates that was quite high, especially in the lower alpine zone (1250-1300 m asl). The zoological studies of south Georgian mountain chernozems' confirmed that earlier they were such as brown forest soils and later approached to the chernozems (Kvavadze, 2013).

In subalpine meadows the great bulk of macrofauna was concentrated in the topsoil horizon (0-30 cm) and on soil surfaces under shelters. The largest number of animals at the edge of stony taluses was higher than it is confirmed by rules of the ecotone effect. During the experiments the tendency of transition of soil animals to dwelling on a soil surface at the big heights was defined with the basic limiting factor of the deficiency of heat in soil increasing with height.

In accordance with the studies, it can be concluded that soil inhabitants play an important role in the soil formation process. Soil aeration, layer mixing depends on the life-activity of inhabitants, as a result of their nutrition (Kokhia, 2011, Wallis De Vries, *et al.*, 2007), a powerful humus layer is formed. Based on this, it is necessary should be continued studies to protect the biodiversity of soil inhabitants.

Studies based on climatic conditions and landscape diversity has shown that the soil inhabitants of Eastern Georgia are more numerous, while the species composition is more abundant in Western Georgia (Jgenti, 2008, Tskitishvili *et al.*, 2018).

It should be noted that the soil invertebrates can be used as sensitive indicators for soil diagnostics

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The biodiversity of soil microfungi in Colombia

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Abstract summary

Although Colombia is one of the first tropical countries where an effort was made to gather mycological flora, contributions to the taxonomy, diversity and ecology of soil microfungi are still scarce. In this study, the diversity of soil microfungi was studied collecting biodiversity data from literature according to the six Colombian natural regions: Andean, Amazonian, Caribbean, Orinoquía, Pacific and Insular. The analysis showed that most data comes from the Andean region. This may be due to the fact that the Andean region is the most populated and most accessible one to research. The other regions were much less studied, with the Insular one with no data at all. More than 300 species of soil microfungi were recorded for Colombia, belonging to 126 different genera and 6 phyla (*Ascomycota*, *Basidiomycota* *Mucoromycota*, *Glomeromycota*, *Mortierellomycota*, and *Olpidiomycota*). Among them, arbuscular mycorrhizae represent the most widely studied group. The most recorded genera in Colombian soils were *Acaulospora* and *Glomus*, with ca. 20 species each. The other genera were rarely isolated and consequently less studied. It is, therefore, evident the need to continue and complement the existing research on microfungi in Colombia to have a better understanding of soil fungal biodiversity and its properties.

Keywords: Fungi, Biological diversity, Soil, South America, Colombia

Introduction, scope and main objectives

Soil hosts an incredible diversity and abundance of microbial life, composed mainly by bacteria and fungi (Fierer, 2017). It is estimated that 1 g of soil contains 10^5 – 10^6 fungal cells (Pepper, 2019). Soil fungi are responsible for a wide array of important ecological functions, such as influencing carbon sequestration through plant life and nutrient mineralization (Bardgett & van der Putten, 2014). The mycological flora of the tropical zones of the world is less known than that of the temperate zones. One of the first tropical countries where an effort was made to study mycological flora was Colombia. Mycological studies in Colombia have focused mainly on macroscopic fungi, and contributions to the taxonomy, diversity and ecology of microfungi are still scarce, so this area of mycology is little known for the country. The attention on microfungi has increased only in the last decades and its research has been directed especially to certain metabolites and functional groups in ecosystems. The present analysis aims to provide an overview of the current state of knowledge on soil fungal biodiversity of Colombia, in order to establish a starting point for future investigations of the soil-plant-animal-man system, in relation to the pattern of geographical division of the Colombian territory. This study focuses on the variety of soil microfungal species, which is a significant source of potential wealth to develop strategies for the rational use and management of available soil resources, both biologically and socially, considering agroecosystems and the conservation of biological diversity.

Methodology

A literature search was performed on Google Scholar and Web of Knowledge using the key words "Colombia" AND "soil" AND "fungi" both in English and in Spanish in order to collect all the publications that represented the state of the art on native soil microfungi in Colombia. The research studies and the found fungal species were divided according to the pattern of geographical division of the Colombian territory that includes the six natural regions: Andean, Amazon, Caribbean, Insular, Orinoquía and Pacific.

Results

The previous studies on soil fungi of Colombia reported a total of 300 identified species belonging to 126 different genera. The different available papers and taxa are considered in relation to the Colombian natural regions. The most studied region was by far the Andean one, with more than 300 soil fungal taxa, followed by the Orinoquía (47 taxa), Caribbean (38 taxa), Amazon (37 taxa), and Pacific (7 taxa) (Figure 1). The phyla that were more frequently recorded were Glomeromycota, Ascomycota, and Mucoromycota (Figure 2).

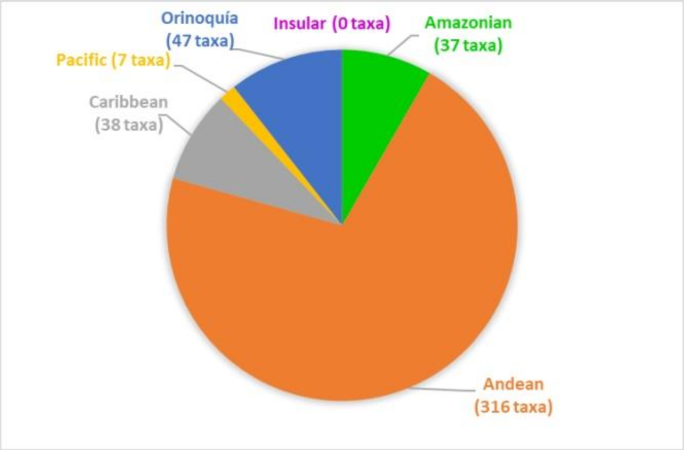

Figure 1: Number of soil microfungal taxa recorded from the natural regions of Colombia (Landínez-Torres *et al.* 2020)

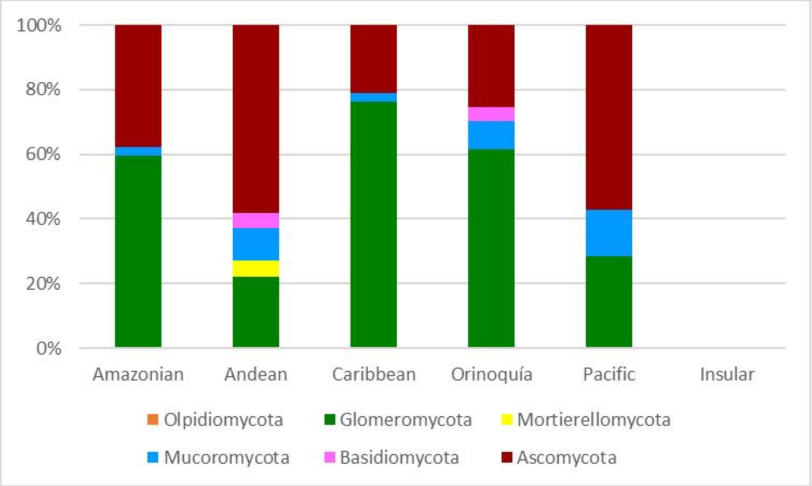

Figure 2: Relative abundance of soil microfungi at phylum level (expressed in %) recorded from the natural regions of Colombia (Landínez-Torres *et al.* 2020)

According to our analysis of the available literature, the most found genera in Colombian soils are *Penicillium*, *Glomus*, and *Acaulospora* with ca. 20 species each, *Mortierella* with 15 species, *Aspergillus*, *Fusarium*, *Mucor*, *Rhizophagus*, and *Trichoderma* with ca. 10 species each. The other genera were less abundant and with a lower diversity of species in the soil.

Discussion

The review of the studies on the microscopic fungi presents in the soil of Colombia showed that the Andean region was the most favored, since the highest number of species and genera were isolated from this region. The insular region was never considered in the studies analysed and data on soil fungi were not found for this region. The high diversity present in the Andean region may be due to its extension, eco-geographical and climatic diversity and to the fact that it is the most populated region, which could favor its study and therefore a deeper knowledge of its mycological diversity.

The Amazon, unlike the other regions, has soil fungi recordings for each of its administrative regions. This phenomenon is with no doubt related to the research efforts that universities, institutions and government made over time in this strategic area of life, both biologically and culturally.

This analysis showed that the methodologies for fungal taxonomic determination should be enriched, since the characterization based only on morphology through identification keys makes it difficult to classify the soil fungi at species level. Therefore, it is necessary to complement this type of analysis with molecular and metagenomic techniques, as it was done by Landínez-Torres *et al.* (2019). In fact, mycologists should focus on these important aspects which are essential to enrich knowledge in this promising area.

Referring to the soil fungi of the country, arbuscular mycorrhizae represent the most widely studied group, perhaps because of the importance of these fungi in the tropics, with their increase in plant nutrition, especially phosphorus absorption (Cardoso, 2017). The genera *Glomus* and *Acaulospora* were dominant in the mycorrhizal composition of Colombia. However, it is necessary to continue studies on all soil fungi to assess the actual diversity and distribution, especially in areas of the country where they have not yet been carried out.

Conclusions

Knowledge about the biological diversity of soil microfungi as well as the understanding of their ecology regarding soil microbial interactions is vital for a developing country like Colombia. Studies on this subject will contribute to solutions to problems such as optimization of agroecosystems, recovery of highly anthropized areas and conservation of natural ecosystems, especially considering the great functional potential of soil fungi such as arbuscular mycorrhizae, cellulolytic and lignolytic fungi, potential biological control agents, antagonists, phosphate and calcium solubilizers.

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A soil biodiversity survey coupled with the national soil quality monitoring network?

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Abstract summary

Despite its importance for human activities, soil biodiversity remains largely unknown and threatened. Aware of the need for large-scale monitoring, we studied the feasibility to add a soil biodiversity survey (RMBS-Biodiversity) to the already existing French soil quality monitoring network (RMQS). We worked with soil biodiversity experts to couple RMBS-Biodiversity with RMQS i.e. to choose soil taxons and functions, the methodologies and the sampling design. According to the experts, the sampling design of RMQS fits with a soil biodiversity survey. We propose sampling protocols for *i)* bacteria and fungi, *ii)* protists, *iii)* nematodes, *iv)* mesofauna (particularly springtails), *v)* below-ground macrofauna (earthworms), *vi)* surface macrofauna (beetles and spiders), and considering functions, *vii)* soil porosity, *viii)* enzymatic activity and *ix)* organic matter degradation. The RMBS-Biodiversity data would be used to assess the distribution and monitor the changes in soil biodiversity at the scale of the French territory. In addition, coupling data providing from RMBS with data from RMQS-Biodiversity, allows deciphering links between biodiversity, physical-chemical characteristics of the soil and human activities (practices, heavy metal loading, and pesticide residues for instance).

Keywords: Large-scale survey, soil taxons, soil functions, soil physical-chemical characteristics, practices, biodiversity distribution

Introduction, scope and main objectives

Soil biodiversity is essential for human activities and particularly for agriculture, by governing soil structure, organic matter degradation, nutrient and water cycles (Wagg *et al.,* 2014). Despite of its importance, soil biodiversity remains largely unknown and threatened by human activities (Decaëns, 2010; Orgiazzi *et al.,* 2016).

From the early 1990's, research programs are launched in order to know better soil biodiversity. In France, several surveys exist or have existed, varying according to sampling design, spatio-temporal breadth and studied taxon and functions: ENVASSO (Bispo *et al.,* 2009), EcoFINDERS (CORDIS. 2015), LUCAS (Toth, Jones & Montanarella, 2013), Bioindicator programme (Bispo, Grand et Galsomies, 2009) and Landmark (LANDMARK, 2015). It remains the need to a global surveillance network of soil biodiversity based on standardized methodologies, covering the whole French territory (metropolitan and ultra-marine areas), including almost the main soil biodiversity components as it is done elsewhere (Dragicevic, 2008; McKenzie, Van Leeuwen et Pinder, 2009; Rutgers, 2011; Schmidt *et al.,* 2011; CENBAM, 2012).

Building a large-scale survey with several methodologies needs a strong organization and substantial resources to cover large spatiotemporal scales. It could be advantageous to lean on an existing survey, already operational. Beyond the organization benefit, this coupling allows to study the links between different data with a high statistical power. Besides, linking different biodiversity surveys is one of the main goals of the French Biodiversity Office (OFB) (Touroult *et al.,* 2017). However, it is necessary to assess if the sampling design of the existing survey could fit with the new one. For example, the grid pixel size and the number of studied size can impact the results (Nielsen *et al.,* 2009; Soberón *et al.,* 2007).

Aware of the need for large-scale monitoring and of the opportunity of this coupling for research issues, we studied the feasibility to add a soil biodiversity survey to the already existing French soil quality monitoring network (RMQS). The objectives of the present work were: 1) Constitute an expert group involving in the feasibility evaluation of a long-term soil biodiversity survey, 2) Choose protocols and assess human, financial and technical needs and 3) Propose a sampling design accommodating all the biodiversity methodologies and the pedologic measurements.

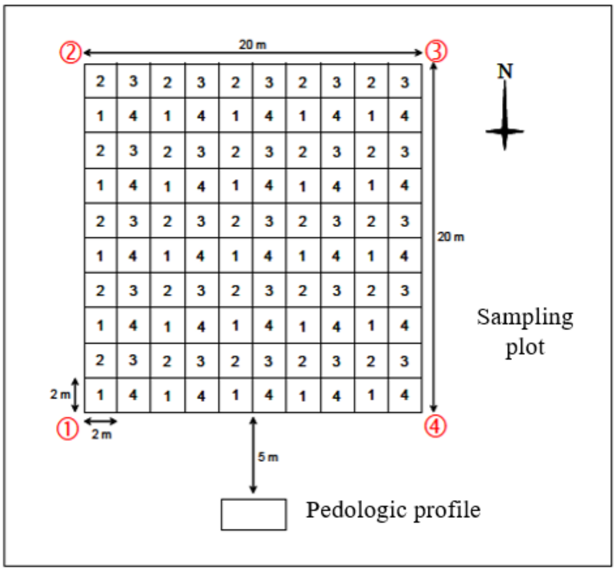
Methodology

In order to study the feasibility to add a soil biodiversity survey (RMBS) to the already existing RMQS, we first installed a group of experts and discussed the way we could adapt the existing sampling design to biodiversity measurements.

RMQS

The first campaign of the RMQS took place from 2000 to 2015 in continental France and overseas territories (French Guiana, West Indies, Reunion and Mayotte islands) and the second campaign began in 2016. The RMQS is based on the monitoring of 2,240 sites spread over a 16*16 km grid on French territory. These sites can be agricultural plots, meadows, urban gardens, wastelands, forests, vineyards and orchards and other types of natural environments.

For each site, a sampling area of 20m*20m, divided into 2m*2m plots, was selected. Monitoring is carried out on 25 of these plots (those with plot n°2 for the second RMQS campaign, Figure 1). A pedological profile is carried out near the sampling area.



2	3	2	3	2	3	2	3	2	3
1	4	1	4	1	4	1	4	1	4
2	3	2	3	2	3	2	3	2	3
1	4	1	4	1	4	1	4	1	4
2	3	2	3	2	3	2	3	2	3
1	4	1	4	1	4	1	4	1	4
2	3	2	3	2	3	2	3	2	3
1	4	1	4	1	4	1	4	1	4
2	3	2	3	2	3	2	3	2	3
1	4	1	4	1	4	1	4	1	4

Figure 1: Sampling design of the RMQS

The data collected correspond to the physico-chemical characteristics of the soil, including contaminants, organic carbon stocks and human activities (agricultural or soil management practices). Some biodiversity monitoring has already been done on RMQS plots in three projects. Flora and forestry characteristics are measured since 2000 in plots of the ICP forest network. In the ECOMIC-RMQS project, microorganism community was determined for all RMQS plots. Similarly, fungi analysis is in progress. In the RMQS-Biodiv, several taxa (microorganisms, mites, springtails, nematodes, earthworms) and functions (organic matter degradation and enzymatic activity) were sampled in 107 Brittany sites. More recently, the enzymatic activity is measured on RMQS soil samples collected since 2016 (5 enzymes in 2016 and 2017, 10 enzymes from 2018).

Setting up the soil biodiversity survey

The OFB expressed the need to build a survey of soil biodiversity, which will be part of the larger survey of terrestrial biodiversity. The first step is to demonstrate the feasibility of such surveillance, taking into account scientific and practical aspects. Thus, we contacted around 30 soil biodiversity experts to install a working group where we built a survey. The questionnaire included 59 questions divided into 4 main categories: sampling (16 questions), laboratory and data analysis (23 questions), costs (5 questions) and interpretation of the results (15 questions). The survey was used to gather the opinions and knowledge of the experts (e.g. usefulness of the taxon, budget, way of implementation, interpretation limits) and collect the details of both field and lab methods for each taxon and function. We circulated the survey within the group. We performed face-to-face interviews to discuss more practical aspects (such as field and analysis time, workload, coordination with other teams, analysis difficulties) and collect suggestions for the most efficient way of working. Finally, during the 4 plenary meetings we all together i) discuss the results of the questionnaire, ii) study the feasibility of coupling RMBS with RMQS, iii) choose soil taxa, functions and associated methods, iv) figure out the sampling design and v) list practical needs (labour supply, costs, material, time). Such meetings were also used to explain the aims of the project, present the monitoring network, the advancement of the work, make a synthesis of the different information and reach agreements.

Results

During the last two years, 21 completed answers and 2 uncompleted answers to the questionnaire were received, together with 14 interviews. We discussed the results and shared our views during 4 plenary meetings with all the experts.

The working groups composed by the contacted experts, is representative of almost all the soil biodiversity taxonomic groups, and some functions. According to experts, the sampling design of RMQS seems be appropriate to perform the soil biodiversity survey. However, some aspects of the RMQS protocols were discussed to be adapted to the RMBS-Biodiversity. We decided that the frequency (10-15 years) and season of sampling, and the sampling grid (16*16 Km) of RMQS fits to the objectives of RMBS-Biodiversity.

We collectively propose a survey of *i)* bacteria and fungi, *ii)* protists, *iii)* nematodes, *iv)* below-ground mesofauna (springtails mainly), *v)* below-ground macrofauna, *vi)* surface macrofauna (beetles and spiders) and mesofauna, and considering functions, *vii)* soil porosity, *viii)* enzymatic activity and *ix)* organic matter degradation (Tab. 1). Flora and sporocarp fungi should also be part of the RMBS but discussions about sampling protocols are still in progress. The expert group still need to be set up for French ultramarine areas.

The development of RMBS-Biodiversity has also allowed federating a multidisciplinary network around soil biodiversity, composed of researchers (pedologists, agronomists and ecologists), botanical conservatories, nature managers, naturalist associations, the administration and private research offices.

Table 1: Taxons, functions and methodologies selected

Taxons-Functions	Data collection	Lab analysis
Bacteria and fungi	Composite sample	Mass sequencing
Protists	Composite sample	Sequencing
Nematodes	Composite sample	Taxonomic identification
Mesofauna	Soil corers	Taxonomic identification
Belowground macrofauna	Spade test and mustard application	Taxonomic identification
Surface macrofauna and mesofauna	Pitfall traps	Taxonomic identification
Soil porosity	Soil column	Soil column scan
Enzymatic Activity	Composite sample	Absorbance measure
Organic matter degradation	Cotton strips	Tension test

Discussion

After 2 years of discussion, meetings and literature review, we considered RMQS sampling design adapted to monitor soil biodiversity at large scale. As there are no studies at different scales of soil biodiversity, so it is difficult to define the most suitable sampling design density and therefore the most appropriate grid cell. Coupling a soil biodiversity survey with a project such as the RMQS, will certainly provide some answers to this question. Indeed, a soil biodiversity survey (RMBS-Biodiversity) using the RMQS tool represents a real opportunity to conduct a study on a national scale. Even if the study grid is quite large in relation to existing work (e.g., plot scale), it will provide a global vision at a national scale of soil biodiversity and thus provide a better understanding of the biogeography of soil organisms. Due to the lack of information about the wide scale distribution of soil organisms, the adaptation of RMQS protocols to soil organism’s study should constitute a starting point to understand the main drivers influencing soil biodiversity at large scale.

The RMBS-Biodiversity will start with a testing phase in 2020. A soil expert group is engaged in this project and methodologies were chosen.

The next steps are to identify methodologies to survey Flora and fungi sporocarps and to define sampling designs in ultramarine territories.

Conclusions

Adding a soil biodiversity survey (RMBS-Biodiversity) to the RMQS seems feasible and will be tested. It is a promising project that will contribute to a better knowledge of soil biodiversity on the French territory and to understand links between different kind of soil biodiversity, physical-chemical characteristics of the soil and human activities.

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Use the metabolic fingerprint in microbial communities to evaluate the anthropogenic impact on soils

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Abstract summary

Soil microbes provide ecological services to determine health in soils. Microbial communities in soils can be monitored by checking their metabolic properties. EcoPlate (Biolog, Inc.) can be used to do that by inoculating a mixed culture of microorganisms on a system of 96-well microplates with 31 different carbon sources, where microbes grow in a 5 days test. The growing is measure by optical density. The objective of this work was to evaluate the metabolic fingerprint of microbes in three soil samples of the same area (The Will County at Illinois State USA): a natural reserve, an industrial park and a farmland with 15 years of herbicide use (Glyphosate). Results showed differences between samples and functional diversity on carbon sources (carbohydrates, polymers, carboxylic acids, amino acids, amines, and phenolic compounds) by each microbial community. Communities in the farmland by all carbon sources where lower than the Natural Reserve and the Industrial Park (30 to 90 percent). Natural Reserve has the highest values of optical densities of almost all the carbon sources. Polymers are the only carbon source higher of Industrial Park. These findings indicated that is possible to evaluate anthropogenic impact on soils by using the metabolic fingerprint of microbial communities of soils.

Keywords: Metabolic Fingerprint, Microbial Communities, Ecoplate, Carbon Sources, Anthropogenic Impact, Soils

Introduction, scope and main objectives

The microbial community is defined as an “assembly” of populations of microorganisms that interact with each other, as well as with the spatial and temporal environment. These interactions give it attributes by which it can be characterized (Atlas and Bartha, 2002). Soil microbial communities play an important role in agroecosystem functioning and are essential for plant nutrition and health (Gattinger, Palojärvi and Schloter, 2008). EcoPlate (Biolog, Inc.) is a technique to determine functional analysis diversity and structure of microbial communities, where different carbon sources are used to establish a metabolic profile of microorganisms and their behavior. It detects if bacteria can oxidize the different carbon sources where an electron transfer from the respiratory electronic transport chain to the tetrazolium salt of the medium, producing a color change that is measured in absorbance terms (Preston-Mafham, Boddy and Randerson, 2002). The aim of this study was to evaluate the anthropogenic impact on three soil samples of The Will County area at Illinois State USA: a natural reserve, an industrial park and a farmland with 15 years of herbicide use by determine the metabolic fingerprint of microbes on microplates EcoPlate (Biolog, Inc.).

Methodology

Soil samples were collected at Midewin National Tallgrass Prairie (41°20’53.4624”N 88°11’27.2862”W), North Island Industrial Park (41°18’34.041”N 88°0.705”W), and Mazon Farm (15 years of Glyphosate use) (41°10’15.905”N 88°27’16.851”W). From each site, soil samples were collected utilizing a soil recovery tube and sorted during collection into Whirl-Pak bags. Organic Carbon (OC), pH, Nitrate (NO_3^-) and Phosphate ($PO_4^=$) were measure for each soil. OC was measured by the Walkley-Black Method and the other ones using the Soil Analysis Hach Test Kit Cat. No. 24959-00. Table 1 shows the data of the soils. The temperature ranged from 74-87°F and the humidity ranged from 47-64 percent. The soils were prepared for use in the EcoPlate microplate by mixing 5 g of soil from the top 4 inches and 5 g of soil from the bottom 4 inches from each collection site with 95 mL of phosphate-buffered saline (PBS) in a waring commercial blender for one minute. The soil suspension was diluted 1:100, and 1:1000. Each well of the EcoPlate was inoculated with 120 µL of the 1:100 and 1:1000 dilutions. The EcoPlates were incubated at 25°C. The absorbance values (optical density) of the wells were determined using a Perkin Elmer Victor x3 multilabel plate reader and measured at 24, 48, 72, 96, and 120-hour time intervals. Triplicates were done and data were compared using Analysis of Variance (ANOVA), with a $p<0.05$ as significant. IBM SPSS 25 was used to perform the analyses.

Table 1: Physicochemical Characteristics of the Natural Reserve, Industrial Park and Farmland Soils

Physicochemical Characteristics	Natural Reserve	Industrial Park	Farmland
pH	6,92	7.75	6.49
Organic Carbon (%)	2.72	1.56	1.67
Nitrate (NO_3^-) $mg.L^{-1}$	16	6.67	8
Phosphate ($PO_4^=$) $mg.L^{-1}$	9.9	41.8	90.2

Results

Table 2 shows optical densities of microbial communities to Natural Reserve, Industrial Park and Farmland from day 1 (D1) to 5 (D5) of 1:100 and 1:1000 dilutions. Data indicates growing among days and differences between carbon sources of each soil indicating the influence of the anthropogenic activities having in mind that all soil are from the same zone (Will County). In addition, Figure 1 shows the comparative use of carbon sources (carbohydrates, polymers, carboxylic acids, amino acids, amines, and phenolic compounds) by microbial communities of each soil. As is observed Natural Reserve has the highest values of optical densities of almost all the carbon sources (1:100 and 1:1000 dilutions). Polymers are the only carbon source higher to Industrial Park 1:100 dilution, maybe due industrial additional source of this type and microbial adaptation. Communities in the farmland by all carbon sources where lower than the Natural Reserve and the Industrial Park (30 to 90 percent). Clearly these findings indicated that is possible to evaluate anthropogenic impact on soils by using the metabolic fingerprint of microbial communities of soils.

Table 2: Optical Densities to Natural Reserve, Industrial Park and Farmland Microbial Soil Communities from D1 to D5 of 1:100 and 1:1000 Dilutions

	Natural Reserve 1-100					Natural Reserve 1-1000				
Carbon Source	**D1**	**D2**	**D3**	**D4**	**D5**	**D1**	**D2**	**D3**	**D4**	**D5**
Carbohydrates	0,04410	1,08703	1,37357	1,66843	1,81513	0,01907	0,64230	0,95577	0,95470	1,09177
Polymers	0,05683	0,58817	1,38942	1,67333	1,89233	0,02017	0,24958	0,69350	1,05800	1,29525
Carboxylic Acids	0,00900	0,85119	1,36376	1,46610	1,66195	0,00452	0,20186	0,58090	0,67043	0,80490
Amino Acids	0,00678	0,58978	1,04467	1,15322	1,42283	0,00350	0,10622	0,48822	0,80833	1,01394
Amines	0,00233	0,72933	1,42200	1,41233	1,51633	0,00300	0,26317	0,56183	0,81550	1,13983
Phenolic Compounds	0,00067	0,26633	1,00700	0,97150	1,14767	0,00083	0,06083	0,41100	0,70583	0,72833

	Industrial Park 1-100					Industrial Park 1-1000				
Carbon Source	**D1**	**D2**	**D3**	**D4**	**D5**	**D1**	**D2**	**D3**	**D4**	**D5**
Carbohydrates	0,062767	1,097300	1,338167	1,605267	1,776967	0,0427	0,4318	0,6158	0,7229	0,9956
Polymers	0,062417	0,578083	1,340000	1,917750	2,103917	0,0193	0,1319	0,3753	0,4860	0,7447
Carboxylic Acids	0,029095	1,048952	1,489476	1,439143	1,636143	0,0085	0,3577	0,6795	0,7142	0,8001
Amino Acids	0,020889	0,803167	1,254778	1,377000	1,630667	0,0014	0,2382	0,5762	0,7339	1,0197
Amines	0,022000	1,135667	1,704000	1,577333	1,796333	0,0017	0,2602	0,9220	0,9345	1,1355
Phenolic Compounds	0,000000	0,384667	0,791000	0,937000	1,044500	0,0000	0,0010	0,0142	0,0283	0,0550

	Farmland 1-100					Farmland 1-1000				
Carbon Source	**D1**	**D2**	**D3**	**D4**	**D5**	**D1**	**D2**	**D3**	**D4**	**D5**
Carbohydrates	0,01083	0,11603	0,22187	0,52297	1,09177	0,00262	0,00217	0,00285	0,01630	0,05503
Polymers	0,01067	0,05600	0,18075	0,28192	1,29525	0,01199	0,01025	0,00875	0,01108	0,03158
Carboxylic Acids	0,01905	0,06138	0,28505	0,48210	0,80490	0,00285	0,02295	0,09467	0,09433	0,07419
Amino Acids	0,00100	0,06928	0,21556	0,47461	1,01394	0,00293	0,00583	0,03372	0,06556	0,11344
Amines	0,01233	0,01050	0,00983	0,03733	1,13983	0,07621	0,07867	0,07267	0,00100	0,00067
Phenolic Compounds	0,01633	0,00317	0,00583	0,03783	0,72833	0,00318	0,00067	0,00000	0,00100	0,01850

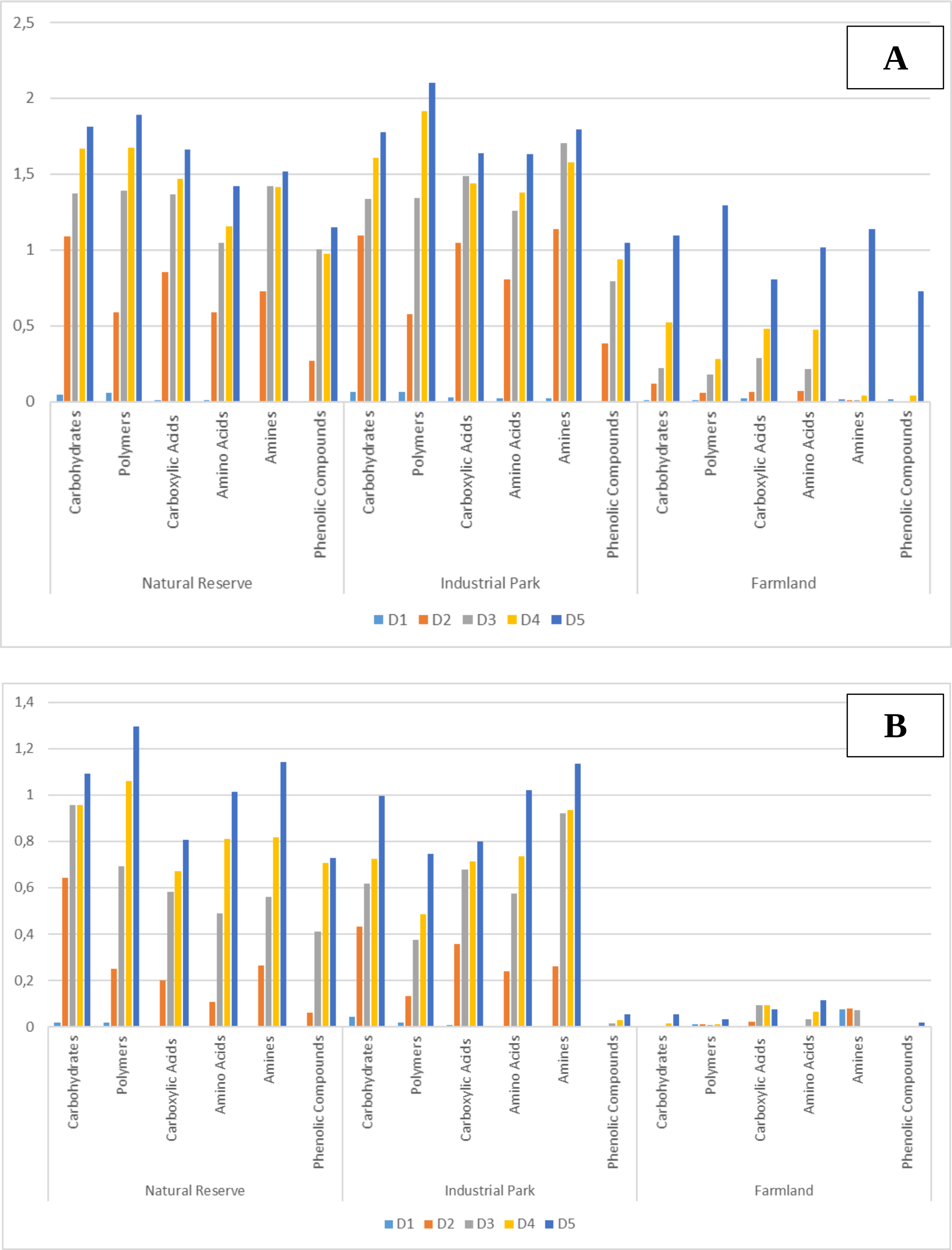
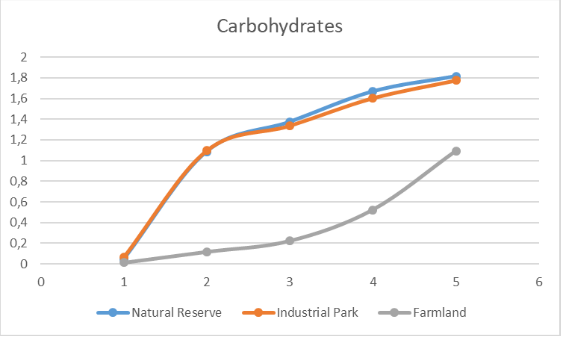
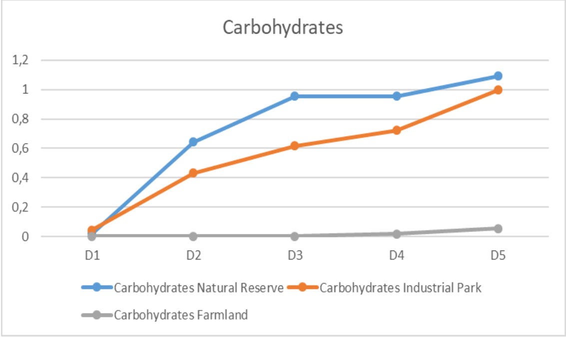
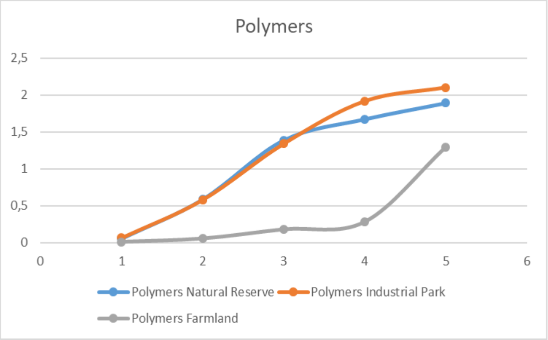
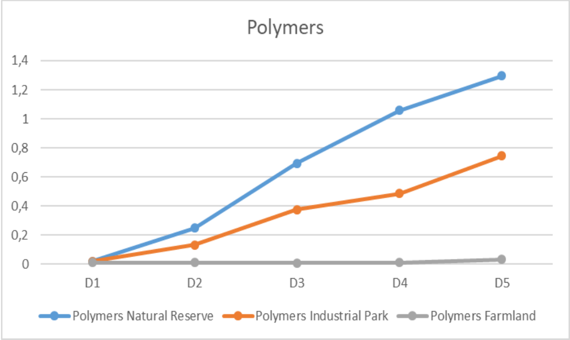
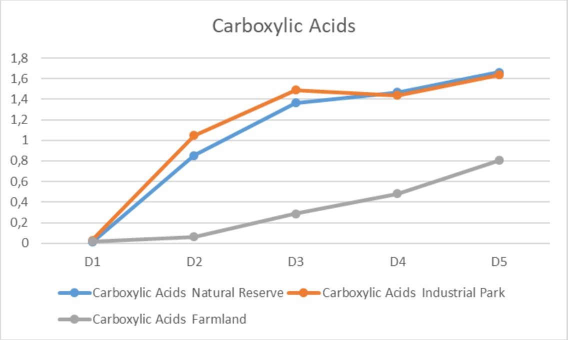
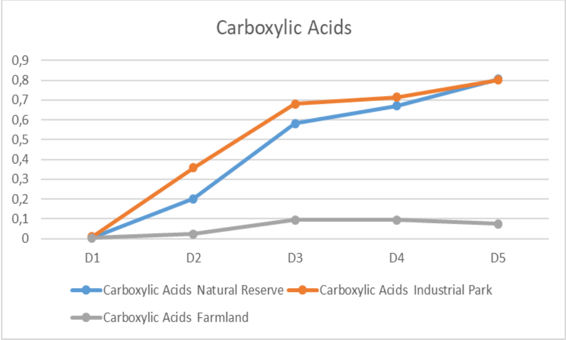
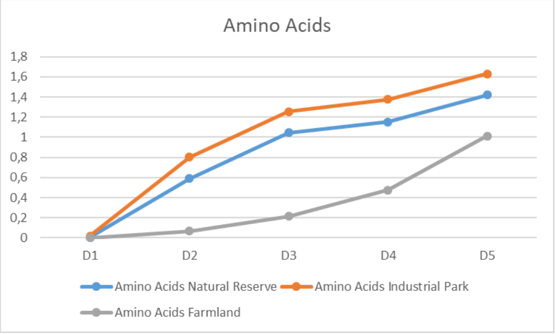
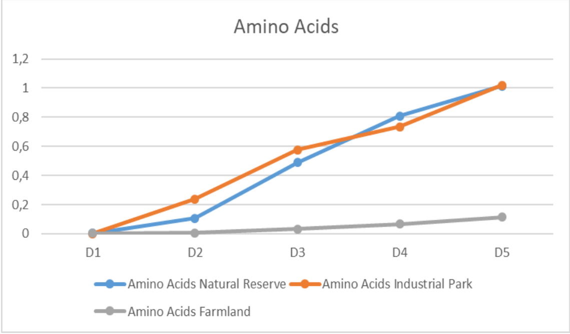


Figure 1: Optical Densities to Natural Reserve, Industrial Park and Farmland Microbial Soil Communities from D1 to D5 of 1:100 (A) and 1:1000 (B) Dilutions

Figure 2 shows optical densities by carbon sources: carbohydrates, polymers, carboxylic acids, amino acids, amines, and phenolic compounds of microbial soil communities by natural reserve, industrial park and farmland from D1 to D5 of 1:100 and 1:1000 dilutions. Data shows that carbohydrates and polymers are the carbon sources with higher optical densities rather than the other ones. Phenolic compounds are the carbon source less used by microbial communities of the soils studied. Natural Reserve and Industrial Park have a similar behaviour in most of the carbon sources. Farmland of all carbon sources has the lowest optical densities values compare with the other ones. In addition is observed the growing of microbial communities using different carbon sources day by day. This is especially high by the natural preserve and industrial park. The adaptation of farmland microbes is slow compare with the other two soils. Is interesting to observe how natural reserve microbes can be use all carbon sources rather than farmland. For it is difficult to use polymers, amines and phenolic compounds.

Dilution 1:100	**Dilution 1:1000**
	
	
	
	

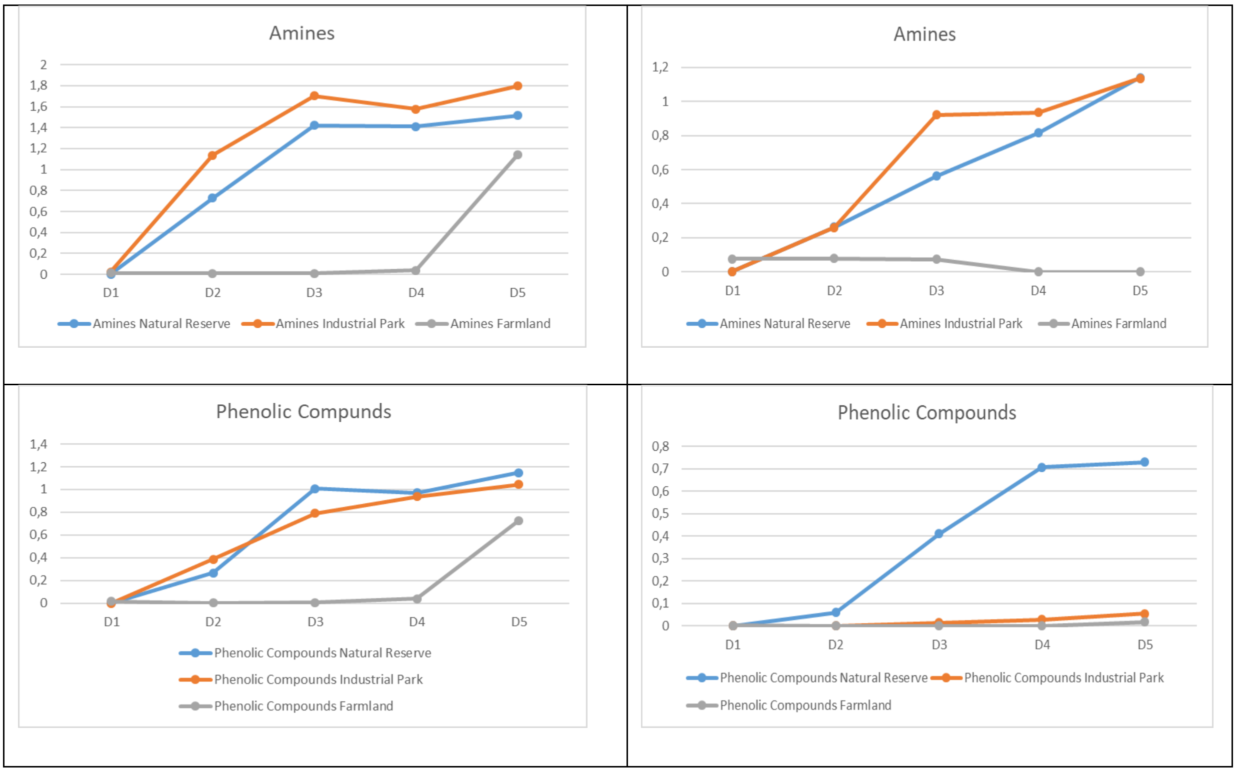


Figure 2: Optical densities by Carbon Sources: carbohydrates, polymers, carboxylic acids, amino acids, amines, and phenolic compounds of microbial soil communities by Natural Reserve, Industrial Park and Farmland from D1 to D5 of 1:100 and 1:1000 dilutions

Discussion

According to The European Soil Data Centre (ESDAC, 2020), soil biodiversity is the variation in soil life, from genes to communities, and the ecological complexes of which they are part, that is from soil micro-habitats to landscapes. In this context, microbial metabolic responses obtained in this study, provides important information about impact that anthropogenic sources made on soils. Through the collection of environmental samples, it was found that soil samples obtained from areas with high glyphosate exposure (Farmland) had lower optical densities than areas with no glyphosate exposure (Natural Reserve and Industrial Park). This aligns with the findings that suggests that long-term applications of glyphosate influence microbial diversity and community composition (Newman *et al*, 2016; Kuklinsky-Sobral *et al*, 2005; Lancaster *et al.*, 2010). In addition to that, data showed how the communities exhibit differences in adaptation through time when they must use to specific carbon sources. This agrees Arteaga, Gómez and Martínez (2016) where they determined the physiological profile of the microbial community using a set of substrates and carbon sources to establishing a characteristic response pattern without isolation of axenic crops.

Conclusion

Results indicate that EcoPlate (Biolog, Inc.) can be used to monitor changes in soil microbial communities over time, as well as, the metabolic use of carbon sources of these communities in soils. On the other hand, the study gave evidence of changes in soils where anthropogenic activities are on it. The extensive herbicide use is an example of that. This example indicated that the adaptation period of microbes is higher than the ones in natural environments to be capable to use different carbon sources. Future work is needed to determine how additional carbon of anthropogenic sources can be use as food by microbes and their effect over time.

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Lewis University, Ill USA

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Underestimated biodiversity of edaphic prokaryotic metacommunity in the western Amazon: risks may be higher

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Abstract summary

Transformations in forest landscape promoted in particular by the extensive cattle ranching is considered one of the factors with the greatest impact on biodiversity conservation in the Brazilian Amazon. Considering soil beyond pedosphere as an ecosystem becomes important to identify effects of these changes on soil microbial communities, as well as their ecological functions and services. We identified how the relationships between land-use and edaphic environments affects the structure of bacterial metacommunities in the mineral (0-10 cm), rhizosphere and litter layers of Brazilian Western Amazon. Relevant regions for conservation and high anthropogenic pressure were selected. Amplicon sequence variants (ASVs) were identified and processed using the DADA2 algorithm. Our results indicate that the inclusion of litter and rhizosphere as part of the soil environment reveals a microbial diversity so far underestimated at the regional scale, indicating that the risks associated with maintaining soil functionality in tropical forests under high anthropogenic pressure may be higher.

Keywords: Soil ecosystem; 16S rRNA gene; Wester Amazon; next-generation-sequencing; microbial biodiversity; land use change

Introduction, scope and main objectives

Climate change, deforestation, habitat fragmentation and land-use intensification can be considered as the main factors by which the biodiversity of tropical forests has been declining at an alarming rate (Nobre *et al.,* 2016).

In the last decade, important studies have focused on understanding the relationship between land-use intensification and impacts on the structure (Jesus *et al.,* 2009; Rodrigues *et al.,* 2013; de Carvalho *et al.,* 2016) and function (Mendes *et al.,* 2015) of soil microbial communities in different landscape contexts of the Brazilian Amazon. These studies agree with each other on the impacts of landscape simplification on soil microbial biodiversity, as well as which soil attributes shape their richness, equitability and dominance in "local" alpha diversity, especially for the Bacteria domain. Nevertheless, it is not yet clear whether intensification of converted systems may contribute to microbial homogenization at regional scales of diversity. Another shortcoming of microbial ecology study carried out until recently is a general conception of soil as being restricted to the surface layer of the pedosphere, which is the concept used for the studies cited above. The borderline range between the soil profile and its top organic layers harbours a complex biotic web associated with the soil matrix, and these has not been taken into account. Disregarding components of this compartment can lead us to underestimating important information for measuring the effects of land-use change on soil microbial biodiversity.

Our study is positioned in this context, bringing consistent results from a geographic gradient that encompasses distinct locations and soil types of the Brazilian Western Amazon. In addition, we direct efforts to understand how land-uses in regions with high anthropogenic pressure are affecting the prokaryotic metacommunity (PM) in different compartments of the edaphic environment. We hypothesized that the joint evaluation of these compartments increases heterogeneity of the PM at the regional scale of diversity.

Methodology

This study was carried out in the Brazilian Western Amazon, within a geographical range of ±900 km.

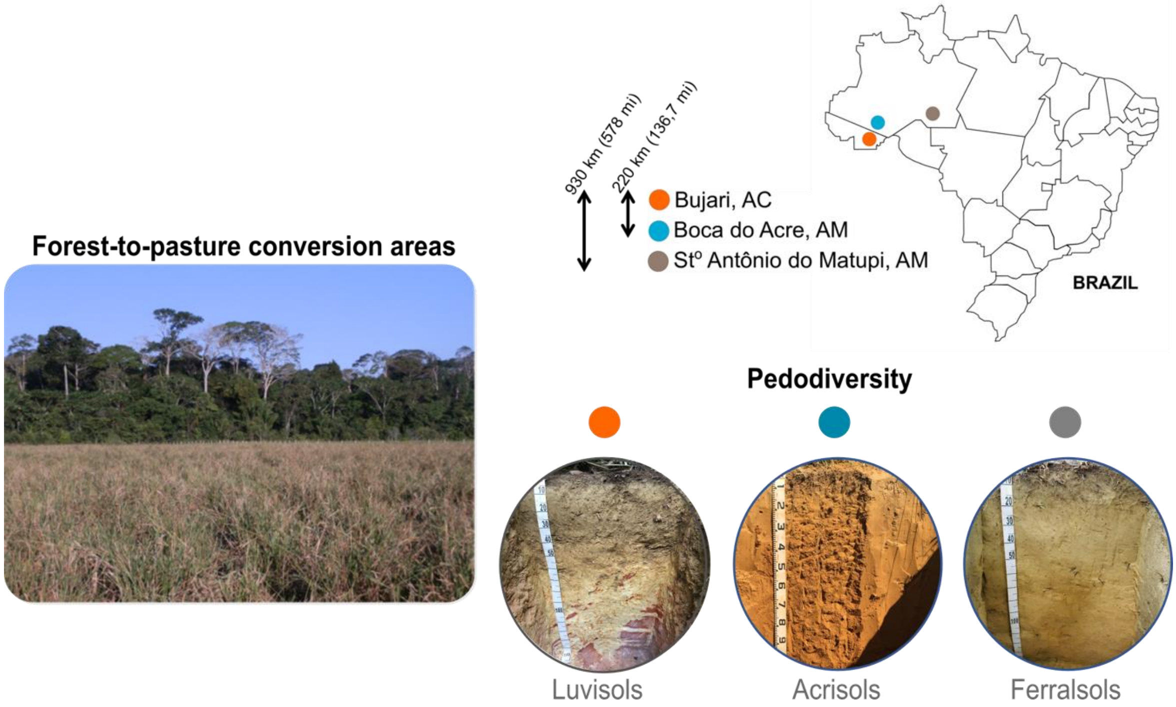

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Figure 1: Scheme of forest-to-pasture conversion areas in the Brazilian Western Amazon and predominant soil typs in each study region

Regions and land-uses were selected where the advance of livestock activity has been reported as one of the main drivers of deforestation (Fonseca *et al.*, 2018), as well as contact with environmental entities.

Sampling and experimental design

Sampling took place in August 2017 following an experimental design similar to the one used by the Sustainable Amazon Network (Gardner *et al.,* 2013), with a total of 65 composite sampling points between forest and pasture systems. The following compartments of the forest edaphic environment were collected: mineral (soil at a depth of 0-10 cm), litter (deposited leaves, branches, reproductive structures and other materials on the soil surface) and rhizosphere (soil particles adhered to the roots between the litter and the mineral compartments). All material for molecular analysis was immediately stored in sterile pouches, put on ice, and refrigerated at -80ºC after taken to the laboratory. In this study, only the mineral layer was used to characterize the pasture areas. Soil chemical and physical attributes were analysed at the National Soil Research Center, Rio de Janeiro, Brazil.

DNA extraction and 16S rRNA gene sequencing

DNA extraction from all layers was performed using the standard DNeasy PowerSoil kit protocol (MO BIO Laboratories Inc.). Amplification of the 16S rRNA gene was performed as described in Caporaso *et al.* (2012). PCR products were purified and subjected to library preparation and sequencing with Illumina MiSeq at the Argonne National Laboratory, USA.

Sequence analysis

Sequence separation was performed in a Phyton 2 environment, based on primer barcodes and processed using DADA2 (Callahan *et al.,* 2016) with quality filtering over 250 bp. The repeated sequences were removed and grouped into ASVs (Amplicon Sequence Variants) and assigned the taxonomy by 'Silva reference database v132' classifier. The package ‘dada2’ v.1.14.0 (Callahan *et al.,* 2016) was used in R 3.6.1 environment (R Core Team, 2018).

Community analysis

The ecological distance between sites and land-uses was measured by Bray-Curtis dissimilarity index. Permutational analysis of variance (PERMANOVA) and homogeneity (PERMDISP) were used to test the statistical significance of the observed differences between the PM. Environmental variables were selected by using generalized additive models, as non-linear measure to explain the importance of each selected variable on the prokaryotic metacommunity matrix. Analyses were carried out in R environment, mainly supported by ‘phyloseq’ v.1.30.0 (McMurdie and Holmes, 2013) and ‘vegan’ v.2.5-6 (Oksanen *et al.,* 2013) packages and dependencies.

Diversity partitioning

Tsallis entropy was turned into Hill numbers, which generate effective numbers of equally frequent species for each value of “*q*”, making possible the interpretation and comparison between the effective numbers (Marcon and Hérault, 2015). The order of diversity "*q*" attaches importance to rare species: "*q=0*" for richness, "*q=1*" for Shannon diversity and "*q=2*" for dominance. Diversity partitioning means that, in a given area, the γ-diversity of all individuals found can be divided internally (α-diversity) and between the local assemblies (β-diversity). Analyses were performed in R using the 'entropart' v.1.6-1 package (Marcon and Hérault., 2015).

Results

Prokaryote communities' structure in the evaluated regions is affected by the land-use system (PERMANOVA, F=8.56, p<0.001), which determined the taxonomic predominance of each system and by edaphic environment variables (F=9.93, p<0.001), especially related to soil acidity (r=0.74; p<0.001) modelling the community structure of this environment in different regions, especially between Bujarí and the others.

Diversity partitioning analysis showed that the ASV richness (*q=0*) of the belonging to the soil mineral compartment (0-10 cm) is significantly higher in the pastures than in forests, for all diversity scales and study regions (Welch F-test; F=16.41, p<0.05). Shannon's gamma diversity (*q=1*) was also significantly higher in the pastures (p=0.015, F=5.43). The general analysis of the soil mineral layer resulted that there are no significant differences between the contribution of forest and pasture PM to the "local" alpha diversity (F=3.23, p>0.05). However, beta and gamma diversities were sufficiently higher in pastures ((β) F=6.94, p<0.001; (γ) F=5.43, p=0.013). No difference was detected for dominance (*q=2*), meaning that both systems have similar numbers of dominant species acting at all scales of diversity.

Otherwise, when considering all layers of the edaphic environment together and re-analysing the diversity models, we observed that the difference between alpha diversity is no longer observed for any order of diversity (F=2.19, p=0.133). The richness of the forest edaphic environment (*q=0*) was significantly higher at the regional diversity scales ((β) F=24.61, p=0.011; (γ) F=26.76, p=0.015) as well as for Shannon beta diversity (*q=1*; F=14.04, p=0.025), which has less influence in the case of a high number of rare ASVs (i.e.: low number of sequences). Again, when considering all Hill numbers for PM at each measured scale, there is no difference in "local" alpha diversity between land-use systems except for BAC (F=6.11, p=0.015). A high difference between land-use systems was identified on the beta diversity scale (F=15.76, p<0.001) for all regions.

Discussion

Our analysis of the effects of forest-to-pasture conversion on the contribution of soil PM to diversity scales allowed us to observe how disregarding the soil as an ecosystem (Ponge, 2015), can be risky in terms of understanding biodiversity loss. As far we know, this is the first report that analysed this integrated view of the edaphic environment to measuring biodiversity in tropical ecosystems at risk by the advance of extensive livestock. Increases in alpha diversity in more disturbed systems, such as pasture and mechanized agriculture, is an important issue in microbial ecology research (Rodrigues *et al.*, 2013; de Carvalho *et al.*, 2016). However, our results suggest that the joint evaluation of different compartments of the soil ecosystem should be added to the diversity analyses, especially to studies that evaluate the effect of land-use change at regional diversity scales (beta and gamma).

Conclusions

The forest-to-pasture conversion shapes a critical impact in edaphic environment, leading to changes its prokaryotic metacommunity reducing the spatial turnover of species in the Brazilian Western Amazon soils.

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Knowledge of biodiversity and ecosystem services of South American mycorrhiza through research networking

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Abstract summary

The South American Mycorrhizal Research Network originated in Chile in 2017, as an horizontal scientific community directed towards the progress of mycorrhizal applications, research, and outreach in South America. By conducting a scientific literature review, and experimental settings, the state of knowledge of mycorrhizal fungi diversity, types, and ecosystem services is shown. The continent host 186 morphospecies of arbuscular mychorrizal fungi, representing the 60 percent of the global diversity. Patagonian Nothofagaceae forests present the highest species richness of ectomycorrhizal fungi compared to other biogeographic regions of the continent. In the temperate rainforests of the continent, arbuscular mycorrhizal fungi associate with 85 percent of vascular plants, while ectomycorrhizal fungi associate with less than 3 percent. The roles of mycorrhizal associations in the bedrock' biogenic weathering and in increasing the tolerance of cereals to high aluminum concentrations are shown as examples of the ecosystem services provided by this symbiosis. Research and geographic gaps in plants, soil, and mycorrhizal fungi of entire biomes/countries of South America are caused by monetary, linguistic, geographic, technical, and political barriers, and also by different research interests. These limitations can be overcome through collaborative and horizontal networking aimed at integrate the biodiversity and ecosystem services of mycorrhiza in South America.

Keywords: biodiversity-ecosystem functioning, community structure, knowledge gaps, mycorrhiza, networking, South America.

Introduction, scope and main objectives

Vast regions of South America remain unstudied in terms of their soil biodiversity and ecosystem services, despite the great ecosystem diversity of this continent (Guerra *et al.*, 2020). Although the emergence of new and more efficient molecular and macroecological approaches in the last decades has boosted global soil biodiversity studies, geographical data gaps are still large in South America because of monetary, linguistic, geographic, and political barriers (Amano and Sutherland, 2013). Thus, many regions, biomes, soil organisms, and soil functions have now been assessed in the continent (Guerra *et al.*, 2020).

Mycorrhizal fungi, a crucial symbiosis for 92 percent of terrestrial plants (Brundrett and Tedersoo, 2018), are involved in many soil ecosystem services as food production and nutrient cycling (Van der

Heijden *et al.*, 2015). The geographic and research gaps regarding the biodiversity and ecosystem services of mycorrhiza in South America have limited the ability of scientists to address many ecological and evolutionary questions. These limitations are caused by an historic shortage of connections between researchers in and out of the continent, and also by different research interests (Marín and Bueno, 2019).

In this context, research networks are a necessary tool to surpass local constraints (Richter *et al.,* 2018). The South American Mycorrhizal Research Network (SAMRN; https://southmycorrhizas.org/) was established in 2017, as a horizontal scientific community directed towards the progress of mycorrhizal research and knowledge, along with applications and public outreach in South America. In its short existence, the SAMRN has reinforced scientific interactions between researchers, stakeholders, and students from the continent, organizing two symposia: in Valdivia, Chile, in 2017 (Bueno *et al.*, 2017; Godoy *et al.*, 2017), and in Bariloche, Argentina, in 2019 (Mujica *et al.*, 2019). The cooperative effort of our members has also resulted in the first book on mycorrhizal fungi in South America (Pagano and Lugo, 2019).

This work aimed at highlighting the main findings of the SAMRN regarding the biodiversity and ecosystem functions of mycorrhizal fungi in South America.

Methodology

Three areas of research were explored regarding the biodiversity and ecosystem functions of mycorrhizal fungi in South America: i. Biodiversity of arbuscular mycorrhizal and ectomycorrhizal fungi in the continent; ii. Mycorrhizal types of plant species in a biodiversity hotspot of the continent -its southern temperate rainforests; and iii. Ecosystem services provided by mycorrhiza.

Biodiversity of mycorrhizal fungi

A detailed compilation of published studies was made in Google Scholar for arbuscular mycorrhizal fungi (Cofré *et al.,* 2019) and ectomycorrhizal fungi (Nouhra *et al.,* 2019).

Mycorrhizal types

In order to determine the mycorrhizal types of the plant species in southern temperate rainforests of South America, 17 plots (30 m × 30 m) in southern Chile were selected. The mycorrhizal type was determined by analysis of the mycorrhizal colonization of roots (i.e. fixation, root staining, and microscope quantification) (Godoy and Marín, 2019).

Mycorrhizal ecosystem services

One year *in situ* experiments with phyllosilicates (muscovite and biotite) contained in bags buried at a 15 cm soil depth, and analysed by confocal laser microscopy, were implemented to evaluate the bedrock' biogenic weathering in southern Chile (Marín, 2018). The effects of high aluminum concentrations on the community structure of the arbuscular mycorrhizal fungi associated with six cereal species (*Avena sativa*, *Hordeum vulgare*, *Triticum durum*, x. *Triticosecale Wittmack*, *Secale cereale*, and *Triticum aestivum*) were studied in southern Chile by morphological analyses of spores, roots' mycorrhizal colonization, and glomalin related soil protein quantification (Aguilera *et al.*, 2017).

Results

Biodiversity of mycorrhizal fungi

Cofré *et al.* 2019 compiled a total of 110 articles identifying a total of 186 morphospecies of arbuscular mycorrhizal fungi in South America, approximately the 60 percent of the global biodiversity of these fungi. Brazil (158) and the Atlantic Forest (120), were the country and biogeographic region, respectively, with most arbuscular mycorrhizal fungi species (Cofré *et al.*, 2019). Though, this may be explained by a geographic bias as much of South America has not been studied (Figure 1). Nouhra *et al.* (2019) compared morphological and molecular methods regarding ectomycorrhizal fungi richness, finding very similar results with both methods: the most abundant ectomycorrhizal lineages were *cortinarius*, *russula*-*lactarius*, *amanita*, and *inocybe*; and the Patagonian Nothofagaceae forests showed the higher diversity.

Mycorrhizal types

In the temperate rainforests of southern Chile, from a total of 245 vascular plant species, 208 species (85 percent) have mycorrhizal associations (Table 1) (Godoy and Marín, 2019). A total of 187 plant species associated with arbuscular mycorrhizal fungi, 10 with ericoid mycorrhizal fungi, seven with ectomycorrhizal fungi, four with orchid mycorrhizal fungi, and 37 plant species did not form any mycorrhizal association (Table 1) (Godoy and Marín, 2019).

Mycorrhizal ecosystem services

Ecosystem age was related to the degree of biogenic weathering in southern Chile, showing also a higher degree on forests dominated by ectomycorrhizal fungi (Nothofagaceae) (Marín, 2018). Furthermore, hyphae channels were seen on phyllosilicate minerals (Figure 2) (Godoy and Marín, 2019). The alpha diversity of arbuscular mycorrhizal fungi was higher in aluminum-tolerant *Triticum aestivum* compared to the other species; overall, the cereal species had significant effects on the number of spores and the glomalin related soil protein produced by arbuscular mycorrhizal fungi, while both cereal species and aluminum stress affected the roots' mycorrhizal colonization and the hyphal length (Aguilera *et al.*, 2017).

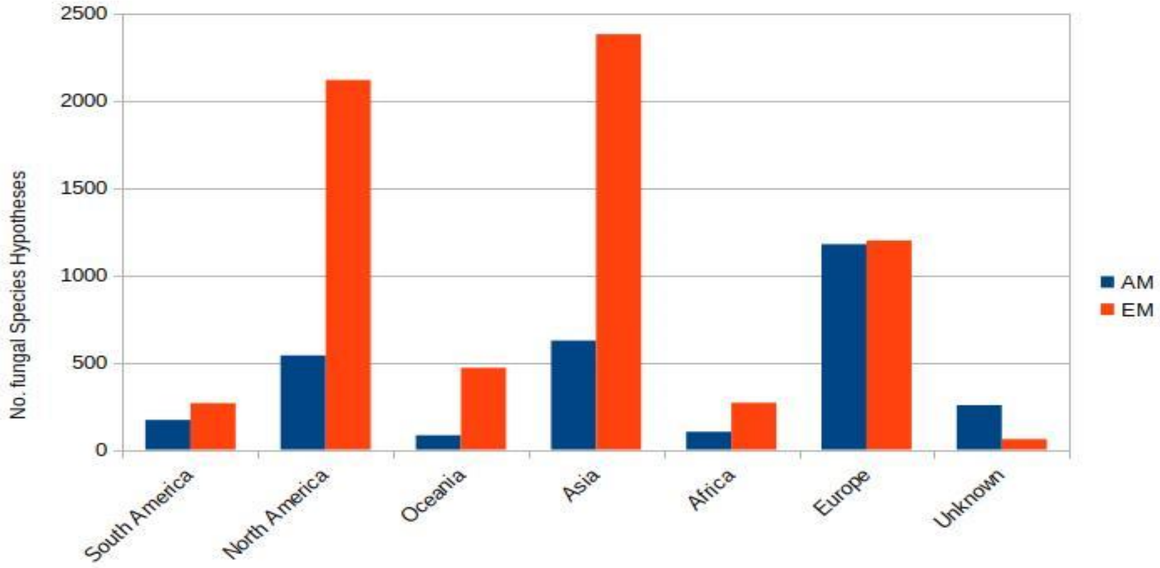

Figure 1: Number of fungal mycorrhizal Species Hypotheses for the different continents, from the database FungalTratis (Põlme *et al.*, 2020).

Mycorrhuizal types: arbuscular mycorrhizal (AM), ectomycorrhizal (EM)

Table 1: Proportion of mycorrhizal types by different plant groups in temperate rainforests of southern Chile

Plant/Mycorrhizal type	AM	EM	ER	OR	NM	Total
Ferns	23 (74.19%)	0 (0%)	0 (0%)	0 (0%)	8 (25.81%)	31
Conifers	7 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7
Angiosperms	157 (75.85%)	7 (3.38%)	10 (4.83%)	4 (1.93%)	29 (14.01%)	207
Total	187 (76.33%)	7 (2.86%)	10 (4.08%)	4 (1.63%)	37 (15.10%)	245

Mycorrhizal types: arbuscular mycorrhizal (AM), ectomycorrhizal (EM), ericoid (ER), orchid (OR), and non-mycorrhizal (NM). Adapted from: Godoy and Marín (2019).

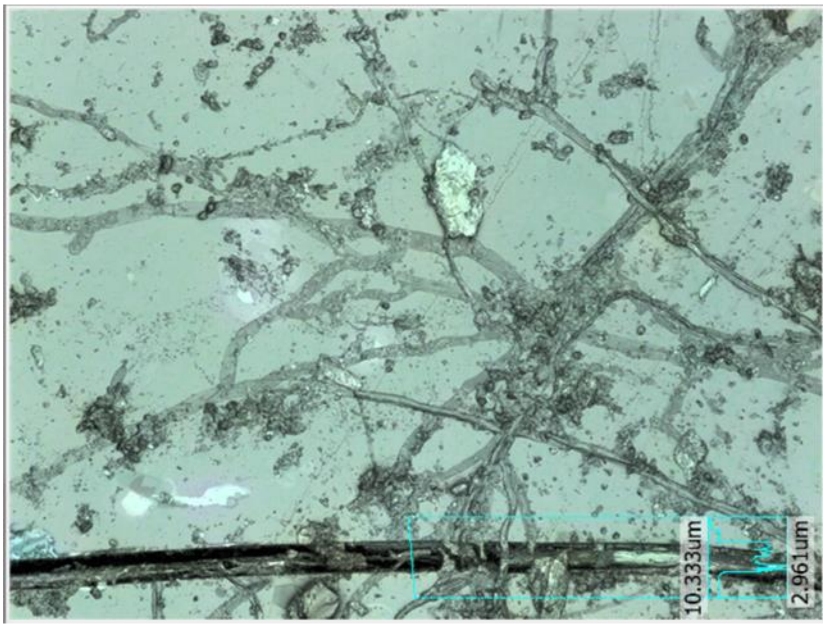

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Figure 2: Channels formed by mycorrhizal fungi on Biotite after one year in a Nothofagus dombeyi forest, Nahuelbuta National Park, Chile

Photo with a Confocal Laser Microscope at 5000×.

Discussion

Despite its modest scientific productivity in comparison to other regions or continents as Europe (Marín and Bueno, 2019), mycorrhizologists in South America have developed a general baseline of mycorrhizal research, but there are still important geographic and research gaps. For example, large plant and mycorrhizal fungi trait databases are fundamental to address biogeographic, ecological, or evolutionary questions, where South American studies and datasets are scarce (Mujica *et al.*, 2019). Overall, the formation of the SAMRN 2.5 years ago – whit significant scientific, technical, and funding limitations in the continent – has already started to fill these knowledge gaps through networking and collaboration. In fact, South American mycorrhizal researchers are becoming more integrated into global-scale monitoring of soil biodiversity and ecosystem services. Thus, activities such as exchanges, partnerships, and future events (e.g. a third Symposium in Leticia, Colombian Amazon, in 2022), are on the immediate horizon to face current and future South American mycorrhizal research challenges.

Conclusions

The plants, soil, and mycorrhizal fungi of entire biomes/countries of South America are understudied (e.g. underrepresentation on molecular databases). Solid knowledge on the distribution of mycorrhizal fungi/types is missing on many (highly diverse) ecosystems. This is partially explained as no continental or multilateral funding is available, but also because training on sampling and molecular, bioinformatic, and statistical methods is missing. As in a global context (Guerra *et al.,* 2020), there is not a full experimental and conceptual integration of mycorrhizal diversity and its ecosystem services. And despite some initiatives, a full picture of the mycorrhizal applications on productive systems in the continent is missing, and also how to transfer and fund this knowledge.

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Soil physicochemical properties, seasonality, plant niche and plant genotype affect bacterial and fungal communities in olive orchard soils

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Abstract summary

Soil microbial biodiversity includes a large variety of microbial communities including bacteria, fungi and archaea living in soil that are essential for nutrient cycling and plant life cycle. The structure and function of these microbial communities are determinant for supporting plant growth and promote plant health, since they act as a natural defence against soilborne plant pathogens. A better understanding of plant-soil microbiome may help to increase plant health and productivity. Therefore, this study has addressed the effects of soil physicochemical properties, seasonality, plant niche and plant genotype as determinants for the assemblages and shifts of the bacterial and fungal communities present in olive orchard soils located at Southern Spain. Results indicated changes in microbial communities mainly according to plant niche and seasonality and to a minor extent according to the olive genotype. Furthermore, the variation of soil physicochemical properties determined the abundance of specific microbial communities. The information obtained in this work contribute to a better understanding on the soil biodiversity related to plant health to maintain and promote sustainable olive agroecosystems.

Keywords: microbial communities, soil quality, soil biodiversity, olive agroecosystem.

Introduction, scope and main objectives

Soil is a non-renewable natural resource within human scale and its preservation is essential for the maintenance of ecosystem services and food security (FAO, 2015). Soil biodiversity involves a great variety of living organisms that reflects a highly natural value contributing to sustain ecosystems function (Giller *et al.*, 1997). Within soil biodiversity, microbial communities play a determinant role in nutrient cycling and soil organic matter regulation, which in turn have a direct influence in nutrient acquisition by plants and potentially in its health status. Unravelling and understanding the function and structure of microbial communities prevailing in soils is becoming a key component of soils due to its role in plant health, productivity and global development (Turner, James and Poole, 2013). To date, despite the many available literature on soil biodiversity, the vast majority of soil microorganisms remain still unknown and their variability at seasonal scale, plant niche, plant genotype and soil physicochemical properties is often overlooked. In this study, we assessed the bacterial and fungal communities associated to roots, rhizosphere and soils close to the rhizosphere of three olive genotypes growing in a same orchard in order to determine the potential influence of seasonality and physicochemical soil properties in their structure and dynamics.

Methodology

Experimental design and sampling. The olive orchard is located in the municipality of Úbeda 'Cortijo Guadiana-Grupo Castillo de Canena' in the province of Jaén, SE Spain. Three of the most commonly cultivated olive genotypes in Spain were selected 'Picual', 'Arbequina' and 'Frantoio'. From each genotype, samples of root, rhizosphere and soil under olive trees were collected during autumn 2018 and spring 2019.

Soil physicochemical properties

Estimated parameters were pH, cation exchange capacity (CE), organic matter (OM), $CaCO_3$, NO_3, P, Ca, Mg, Na, K, Fe, Mn, Zn, Cu, C, N and S, and soil texture.

DNA extraction and next generation sequencing (NGS)

Root, rhizosphere and soil samples were used for DNA extraction using the DNeasy PowerSoil Kit (QIAGEN) following the manufacturer's instructions. Illumina MiSeq platform (PE: 300x2) was used to carry out NGS sequencing using universal primers targeting the 16S (bacteria) and ITS (fungi) rRNA.

Bioinformatic and statistical analysis

DADA2 algorithm was used to eliminate chimeras from raw fastq files. Operational taxonomic units (OTUs) were obtained at 1 percent dissimilarity and then were taxonomically classified using RDP Bayesian classifier against the Silva SSU v.132 database using QIIME2 software version 2019.7. Differences among bacterial and fungal communities were calculated with QIIME2 using alpha-diversity indexes (including Shannon, Simpson, Faith_PD, and Richness) and beta-diversity UniFrac parameters at genus level were assessed using Kruskal-Wallis and PERMANOVA and the effects main factors on soil quality parameters were assessed by ANOVA analyses.

Results

Sequencing data resulted in a total of 649 bacterial OTUs, distributed in 19 phyla and 271 genera while there were 216 fungal OTUs divided in 8 phyla and 106 genera. Globally, *Proteobacteria* was the most abundant bacterial phylum (54.0 percent) followed by *Actinobacteria* (24.2 percent) and *Bacteroidetes* (7.8 percent). Seasonality affected the relative abundance of these phyla. Thus, *Proteobacteria* and *Bacteroidetes* abundance was reduced by 14.5 percent and 2.0 percent, respectively from autumn to spring while *Actinobacteria* abundance increased by 5.9 percent. Unique bacterial genera were associated to specific olive genotypes: 'Picual' (32), 'Arbequina' (46) and 'Frantoio' (20), while there was a total of 115 shared bacterial genera. Also, unique bacterial genera varied according to plant niche from soil (52), rhizosphere (33) and roots (32), whereas there was a total of 79 shared bacterial genera. For fungi, *Basidiomycota* (50.7 percent), *Mortierellomycota* (24.7 percent) and *Ascomycota* (17.2 percent) were the three most abundant phyla in all sample types. A reduction on the abundance of *Mortierellomycota* and *Ascomycota* was also estimated on samples from autumn to spring (*ca.* 4.0 percent both), while the opposite occurred for *Basidiomycota* (23.6 percent *vs.* 27.1 percent). 'Picual' (16), 'Arbequina' (11) and 'Frantoio' (8) displayed different specific fungal genera whereas the core fungal genera were 56. Furthermore, unique fungal genera varied according to plant niche from soil (15), rhizosphere (12) and roots (3), with 17 shared genera.

Soil quality parameters varied according to season of sampling and the olive genotype. Levels of organic matter content (F=14.62; $P < 0.00$), pH (F=10.20; $P < 0.00$), NO_3 (F=4.96; $P < 0.04$), Mg (F=7.37; $P < 0.02$) and Na (F=5.77; $P < 0.03$) differed significantly from autumn to spring. On the other hand, soils sampled under 'Picual' trees displayed a significantly higher level of Ca content (F=11.29; $P < 0.00$) while soils under 'Frantoio' showed a higher significant amount of Cu (F=14.00; $P < 0.00$).

Alpha diversity indexes showed significant differences ($P < 0.05$) in microbiome composition. For bacterial communities, observed OTUs (H=38.79; $P < 0.001$), Shannon (H=35.65; $P < 0.001$), Simpson (H=30.26; $P < 0.001$) and Faith_PD (H=38.89; $P < 0.001$) differed significantly according to plant niche, while Shannon (H=4.72; $P < 0.03$) and Faith_PD (H=7.76; $P < 0.001$) showed significant differences according to season. For fungal communities, alpha diversity indexes displayed significant differences according to plant niche [observed OTUs (H=47.24; $P < 0.001$), Shannon (H=41.44; $P < 0.001$), Simpson (H=31.56; $P < 0.001$) and Faith_PD (H=47.00; $P < 0.001$)]. Principal coordinate analysis of weighted_UniFrac distances (Figure 1) showed that main differences among bacterial and fungal communities (measured as phylogenetic distances) were due to the olive plant niche (*pseudo-F*=11.56; $P < 0.001$) followed by the season of sampling (autumn or spring) as indicated by PERMANOVA analysis that showed significant differences in bacterial communities (*pseudo-F*=2.48; $P < 0.001$) but not in fungal communities (*pseudo-F*=1.59; $P < 0.17$) and with no effect of the olive genotype (*pseudo-F*=0.92; $P < 0.50$).

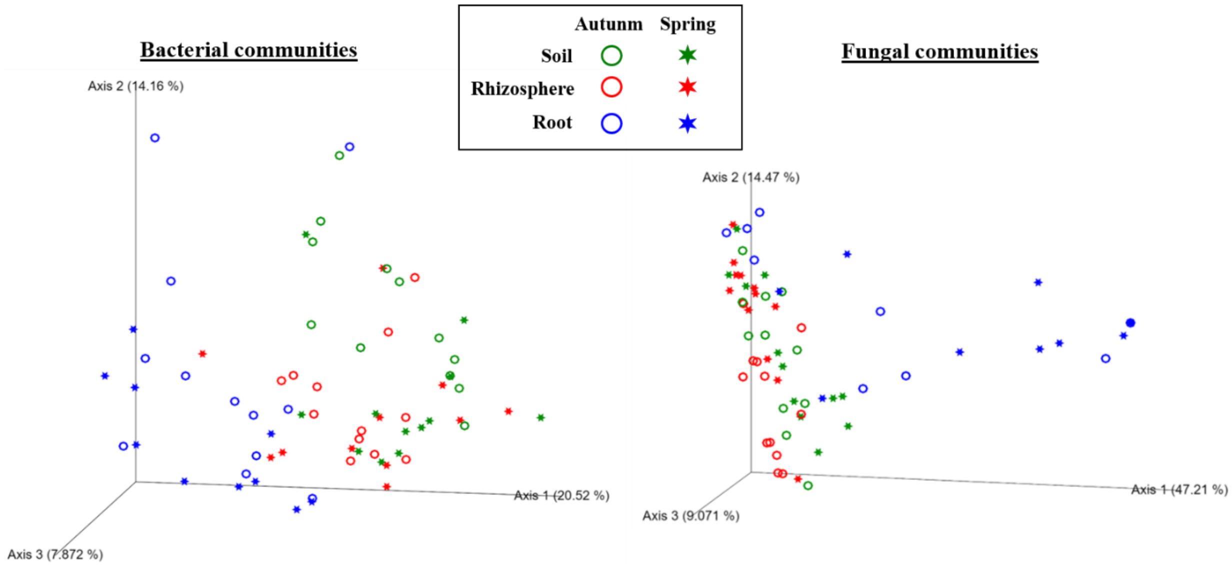

Figure 1: Principal coordinates plots of weighted UniFrac distances of bacterial and fungal communities distributed according to plant niche (soil, rhizosphere and root) and season of sampling (autumn or spring)

Discussion

Functional processes conducted by soil microbial communities have a strong influence on the productivity and health of agricultural systems (Pankhurst *et al.*, 1996). Our results indicate that a great diversity of the microbial populations in agricultural soils from where bacteria richness triplicated that of fungi. These microbial communities were mainly distributed according to plant niche, where the rhizosphere, together with soil associated with the rhizosphere displayed the greatest diversity of taxa as compared to the olive roots. Therefore, this environment is a hot spot of microbial interactions that may result in a battlefield where microorganisms interact with plant pathogens influencing with infection process or soil survival (Raaijmakers *et al.*, 2009). In our study, despite the changes found in microbial abundance proportion, fungal communities showed certain tolerance to the influence of seasonality while bacterial populations resulted significantly affected by seasonality. On the other hand, plant host genotype had a minor effect in the microbiome composition although each olive genotype presented differential microbial communities, which were also influenced by soil physicochemical properties. Plant niche, seasonality, plant genotype, and soil quality parameters are all important drivers of soil microbial diversity that have to be considered in plant-soil microbiome interactions to improve plant health and maintain ecosystem services driven by soil microbial biodiversity.

Conclusions

This work has allowed to determine the influence of plant niche, seasonality and soil physicochemical properties in the microbial diversity associated to different soil ecosystem compartments of different cultivated olive genotypes. Plant niche and seasonality strongly affected the diversity and abundance distribution of these microbial communities while olive genotype showed a relative minor role as driver of microbiome composition. This information contributes to generate new knowledge regarding the effect of environmental and soil quality parameters on the structure and composition of the soil microbiome associated to the olive crop that would promote plant health and soil biodiversity as essential values for soil ecosystem services. In addition, our results raise the need to deeply explore new research focused on the cultivation of the identified bacteria and fungi to determine the major functions that they are performing in the soil agroecosystem and assess their viability as potential biological control agents against olive pathogens inhabiting the soil.

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Biotechnological prospecting studies on microbial strains isolated from different natural biotopes in order to obtain biologically active substances and biomaterials

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Abstract summary

The main objective of this research was to investigate the biotechnological potential of wild-type strains, newly-isolated microorganisms, from various biotopes in Romania, and presents experimental results of enzymes, polysaccharides and antimicrobial substances synthesis in batch fermentations.
Over 150 microbial strains were isolated (*Bacillus*, *Pseudomonas*, *Lysinibacillus*, *Paenibacillus*, *Serratia*, *Cronobacter*, *Cellulosimicrobium*, *Klebsiella*, *Achromobacter*, *Arthrobacter*, and *Galactomyces*) and screened for lipase, amylase, antibiotics and polysaccharides production. *Bacillus mycoides*, *Galactomyces geotrichum* and *Klebsiella oxytoca* were selected for optimization studies. *Bacillus* strains were further investigated for the antimicrobial substances' synthesis.
Enzymes activities and crude exopolysaccharide yield were recorded after optimization studies, as following: lipase – 27 U/mL, amylase – 10.87 U/mL, and biopolymer – 18 g/L (lactose used as substrate); antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* was observed.
As a conclusion, it can be stated that microbiological populations in all their diversity, and depending on ecology, could offer a variety of solutions for a sustainable future.

Keywords: microbial biodiversity, enzymes, polysaccharides, antibiotics, culture collection, biotechnology

Introduction, scope and main objectives

Biotechnology promotes a sustainable future, including for agriculture. Soil microorganisms are essential, as a part of agroecosystems, maintaining soil fertility and ensuring the nutrition of crop plants and, furthermore, they represent a renewable resource for other related industrial processes. (Aonofriesei, 2018)

Enzymes, as economic and environmental-friendly biocatalysts are replacing the conventional chemicals and find various applications, such as: *α-amylases* in starch liquefaction, food, paper and textile industries, and pharmaceuticals; *lipases* in food and feed (dairy products), detergents, cosmetics and pharmaceuticals, polymer synthesis, agrochemical and environmental fields. (Tomulescu, 2015) During the past years, many *polysaccharides* have been discovered and microbial polymers have proven biodegradability and biocompatibility properties, being called "*the sleeping giant of biotechnology*". (Tomulescu, 2016) *Antagonism activity and antimicrobial natural compounds* play an important role in biological control of plant crops. Microbial secondary metabolites with bactericidal and fungicidal effects can be used as biotechnological solutions to combat the plant hyperparasitism (i.e lipopeptides from *Bacillus* spp., phenazines from *Pseudomonas* spp.). (Soare *et al.,* 2017)

This work was aimed to investigate the biotechnological potential of some newly-isolated microorganisms, wild-type strains from various biotopes in Romania, and presents experimental results obtained when enzymes (lipase and amylase), polysaccharides and antimicrobial bioactive substances productions in batch fermentations, were evaluated.

Methodology

Samples of soil, water and vegetal material (pine cones, hay, roots) were collected from different counties in Romania and the isolated microorganisms (bacteria, yeasts and fungi) were deposited in the *CMII-ICCF-WFCC 23.* The Culture Collection is an *associated part in MIRRI since 2014* and holds over 400 microbial strains as producers of pharmaceuticals and similar ingredients, biopolymers, amino acids, enzymes, single-cell proteins, vitamins, bio-pesticides, and bio-stimulants. The strains were screened for lipase, amylase, exopolysaccharides and antimicrobial substances production.

A preliminary identification was done by mass spectrometry analysis, using a MALDI-TOF Microflex LT equipment. 18S rRNA sequencing and BLAST analysis, and 16S rDNA based on PCR and ARDRA technique were applied for a lipase producing yeast and an exopolysaccharide producing bacteria, respectively. (Vassu *et al.,* 2001; Ionescu *et al,* 2013)

Bioprocess parameters effects on the production of enzymes and exopolysaccharides were studied in batch fermentations. Taguchi L9 and L16 orthogonal arrays, RSM-CCRD and ANOVA were applied for optimization studies. Investigations of the exopolysaccharide production, biomass growth and substrate utilization were performed by using Logistic, Gompertz and Luedeking Pirret kinetics models.

The total sugar content of the product was estimated by the phenol-sulfuric acid assay.

Lipase activity was assessed using an adapted method of *Willstäter* (US Pharmacopoeia-35), and a synthetic substrate p-nitrophenol laurate accordingly to Palacios, Busto and Ortega (2014). The protein concentration was assessed by Lowry *et al.*'s method (1951). *Amylase activity* was assayed with 3,5-dinitrosalicylic acid.

Chip electrophoresis was employed to achieve the electrophoretic profile for proteins in amylases, by the use of an Agilent 2100 Bioanalyser equipment.

Products purification was done by ultrafiltration and diafiltration (Pellicon module, Merck-Millipore, PTGC/PLGC membranes of 10/5kDa). The polysaccharide was isolated by precipitation with three volumes of ethanol. (Figure 7)

HPLC was employed to study the bacterial exopolysaccharide structure, by using an ELITE LaChrom Merck equipment (RI L-2490 detector and steel columns: Kromasil 100-5NH2, 250 x 4.6 mm containing an aminopropylsilane filler L8, and Nucleogel Sugar 810 H 300 x 7.8 mm containing a polystyrene/divinyl benzene (PS/DVB) filling in H+).

FTIR was performed by using a FTIR-ATR spectrometer Perkin Elmer and *NMR spectroscopic analysis* was used to evaluate ^{1}H and ^{13}C-NMR spectra, which were recorded with a Bruker Advance III Ultrashield Plus 500 MHz and a DRX400 spectrometer).

Cytotoxicity assay was conducted by using an animal cell line (murine fibroblast - L929-ATCC CRL-6364) and a standardized methodology, which included the addition of tested compounds in different concentrations to cell lines and the use of EMEM Medium.

The antimicrobial activity was tested using an adapted protocol disk diffusion method (Română, 1993). The human pathogens were *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538. Among the tested strains, also newly-isolated microorganisms, were identified *Bacillus subtilis*, *Bacillus pseudomycoides*, *Streptomyces badius*, *Serratia* and *Brevibacillus* sp.

Results

Figure 1: Microscopic evaluation for soil bacteria (Gram staining) isolated from a mining region of limestones and shales, and fungi isolated from spruce cones

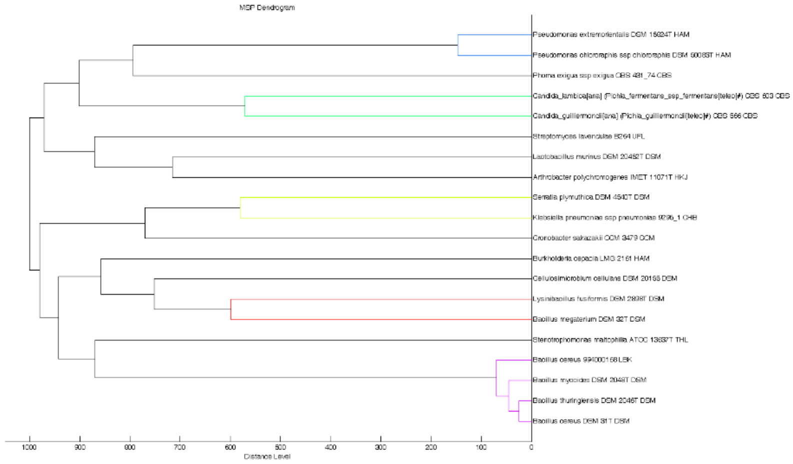

Figure 2: MALDI-TOF MS dendrogram (Tomulescu, 2015)

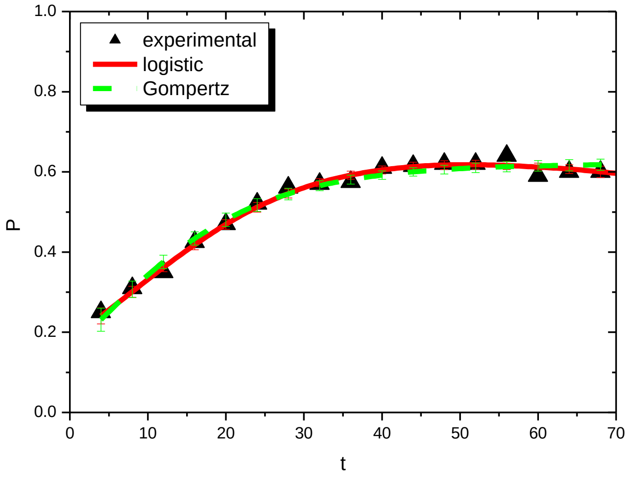

Figure 3: Kinetic profile of the crude exopolysaccharide (P, g/100 mL) – Logistic and Gompertz models

ANOVA for Response Surface Reduced Quadratic model - Polysaccharide						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob>F**	
Model	1.83	7	0.26	6.04	0.0005	***significant***
A-Time	*1.751E-003*	*1*	*1.751E-003*	*0.040*	*0.8425*	
C-Corn extract	*0.034*	*1*	*0.034*	*0.79*	*0.3845*	
D-Glucose	*0.25*	*1*	*0.25*	*5.84*	*0.0244*	
CD	*0.25*	*1*	*0.25*	*5.77*	*0.0252*	
A^2	*0.84*	*1*	*0.84*	*19.48*	*0.0002*	
C^2	*0.30*	*1*	*0.30*	*7.03*	*0.0146*	
D^2	*0.44*	*1*	*0.44*	*10.13*	*0.0043*	
Residual	0.95	22	0.043			
Lack of Fit	*0.91*	*17*	*0.053*	*5.64*	*0.0325*	*significant*
Pure Error	*0.047*	*5*	*9.449E-003*			
Cor Total	2.79	29				

Figure 4a: ANOVA for the quadratic model-exopolysaccharide production

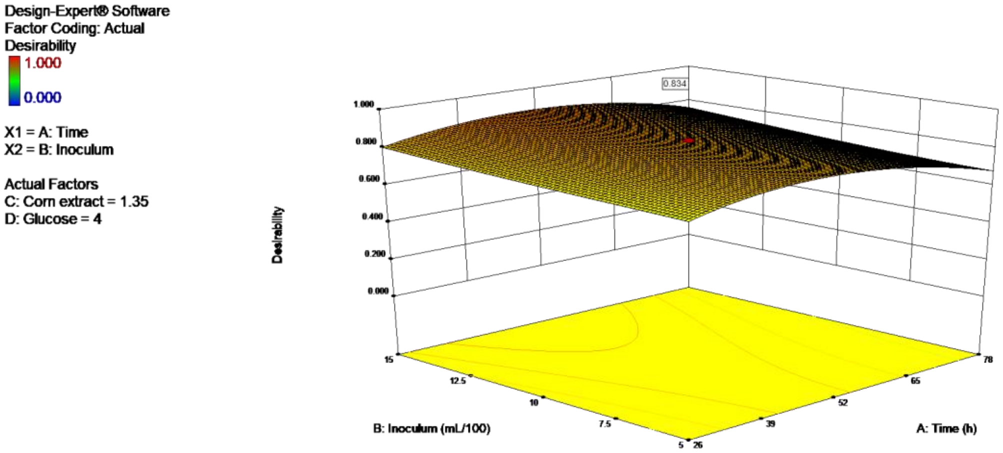

Figure 4b: CCRD-RSM predicted design - exopolysaccharide production using *K. oxytoca* and glucose as substrate

Figure 5: Electrophoretic profile of amylase produced by *Bacillus mycoides*, isolated from soil (Tomulescu, 2015)

Solution	Volume (mL)	Protein concentration (mg/mL)	Enzymatic activity (U/mg)
Filtered solution (on celite) – initial solution	2500	6.88	327.45
Concentrated solution I - Millipore	1650	7.38	315.85
Permeate solution I	850	3.96	262.03
Yield (%)		**70.81**	**68.38**
Losses (%)		**9.6**	**16.0**
Concentrated solution II (diafiltration I)	1850	6.01	273.37
Permeate solution II	650	2.61	133.93
Yield (%)		**64.6**	**54.0**
Losses (%)		**25.59**	**42.0**
Concentrated solution III (diafiltration II)	2000	3.41	128.89
Permeate solution III	500	2.5	129.32
Yield (%)		**61.33**	**28.94**
Losses (%)		**27.43**	**65.79**

Figure 6: Quantitative results obtained after the ultrafiltration of lipolytic product derived from *Galactomyces geotrichum*

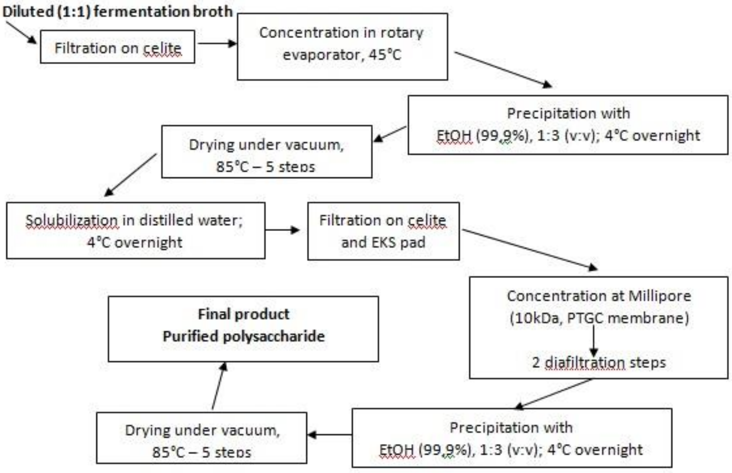

Figure 7: Flow diagram for the EPS isolation and purification process

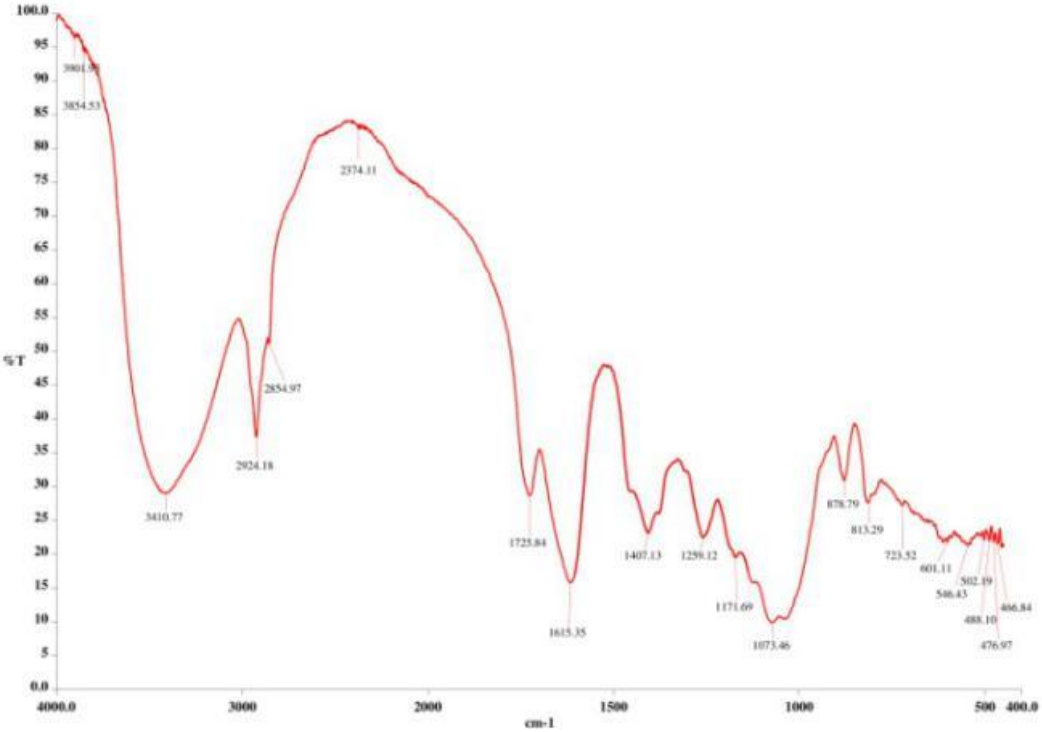

Figure 8: The FTIR spectrum of the purified EPS

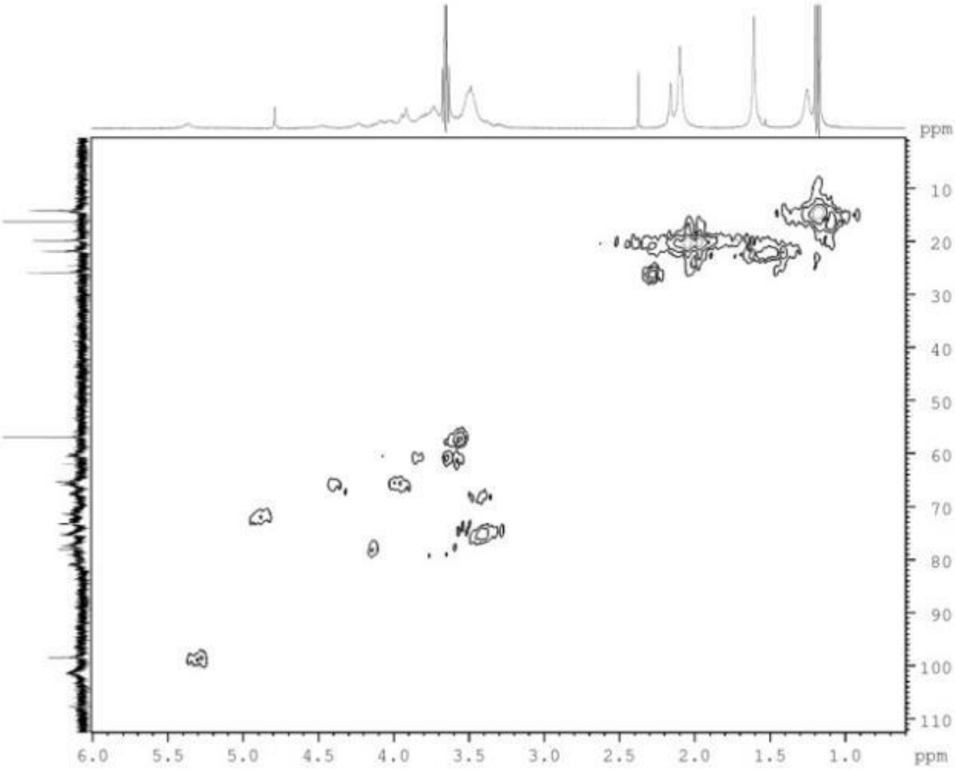

Figure 9: 2D HMQC-NMR spectrum of EPS derived from lactose as the substrate

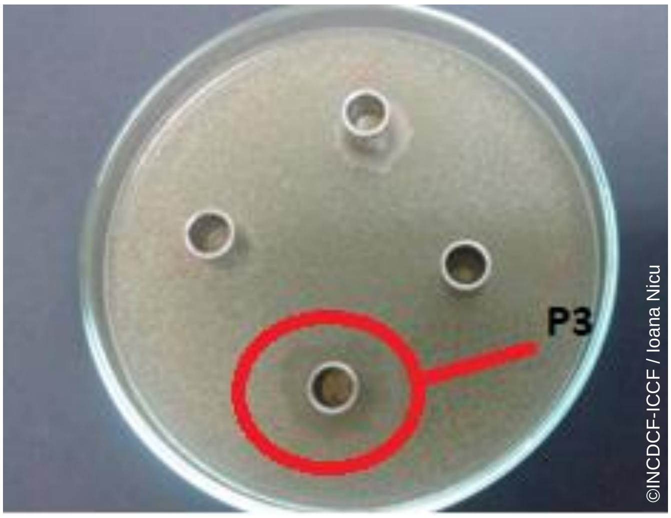

Figure 10: Inhibition zone of 15 mm after 24 hours incubation: the newly-isolated *Bacillus subtilis* and *Staphylococcus aureus* ATCC 6538

Discussion

A total of 104 microbial strains were isolated in the first screening experiment, samples of soil, sand, mud, water, rock green moss, spruce cones and beechnuts, with a microbial diversity representing: 64 percent bacteria, 14 percent yeasts and 22 percent fungi; the bacterial and fungal populations were more diverse in soil and water, Gram$^-$ and Gram$^+$ characteristics corresponding to 34 and 24 strains, respectively. (Figure 1)

54 isolates were considered as potential producers of lipolytic enzymes, and 19 strains presented amilolytic activity. *Bacillus*, *Pseudomonas*, *Lysinibacillus*, *Paenibacillus*, *Serratia*, *Cronobacter*, *Cellulosimicrobium*, *Klebsiella*, *Achromobacter*, *Arthrobacter*, and *Galactomyces* strains were used in batch fermentations. (Figure 2)

Lipolytic activity was determined as 7.46, 6.95 and 6.63 UL FIP/mL for an unidentified strain, and 4.92 and 4.83 UL FIP/mL for the *Lysinibacillus fusiformis*, both isolated from soil, when tributyrin, castor oil or Tween80 were added in the fermentation media. However, optimization studies were conducted using *Galactomyces geotrichum*, in a production medium with glucose, Tween80 and $CaCl_2$. In this case, 39.3 UL FIP/mL were obtained after Taguchi L9 optimization (36 hours fermentation), and 27 U mL^{-1} when p-nitrophenol laurate was used as synthetic substrate (60 hours fermentation). The solution was concentrated and purified with a PLGC membrane (5kDa), but significant losses were recorded. (Figure 6)

A maximum amylolytic activity of 10.87 U/mL using *B. mycoides* was obtained after Taguchi optimization, when malt extract (4 percent w/v) was used as substrate (48 hours fermentation). A positive effect of the citric acid and $CaCl_2$ interaction was observed. *Electrophoretic profile* indicated a molecular weight of 60kDa after comparing the results with the available data of UniProtKnowledeBase. (Figure 5)

The kinetics results indicated values of >0.99 for the R^2 coefficient, in the case of Logistic and Gompertz models, with a maximum production after 56 hours fermentation using *Klebsiella oxytoca* ICCF 419. ANOVA suggested that a quadratic model would be indicated to study the exopolysaccharide production, biomass growth and substrate utilization. The CCRD design was applied (Figure 4A and Figure 4B) and a maximum of 11.03 g crude polysaccharide/L was obtained (when glucose was used as substrate), while an inverse proportionality between cell growth and product synthesis was observed. The main interaction affecting the biosynthesis was determined between glucose and corn steep liquor.

The HPLC showed a sugar content of 86.3 percent, with glucose, rhamnose, fructose, mannose and uronic acids as major components in the exopolysaccharide structure, while bands of OH, CH, COOR, C=O, COO^-, CH and C-O were identified by FTIR. (Figure 8) In the 2D HMQC-NMR spectrum of the polymer, correlations between directly attached carbons and protons could be observed. (Figure 9)

A preliminary pharmacological study was conducted to evaluate the biological activity of the purified polysaccharide produced by *Klebsiella oxytoca* ICCF 419, a newly-isolated strain from roots of *Momordica charantia.* Results showed no cytotoxic effects on fibroblasts and furthermore, cell proliferation was observed (stimulation rate of cell growth of 49 percent). 18 new isolates were screened for antibiotic substances production, of which 10 strains showed activities against the human pathogens tested (inhibition zone diameters of 13- 40 mm). (Figure 10)

Conclusions

Microbial diversity represents a valuable resource for biotechnological applications. More than 150 microbial strains were isolated from different regions in Romania and screened for enzymes, antibiotics and polysaccharides production. *Bacillus mycoides*, *Galactomyces geotrichum* and *Klebsiella oxytoca* were selected for optimization studies to maximize the amylase, lipase and exopolysaccharide yields in batch fermentations. Other studies are ongoing and aim to evaluate their possible applications.

Acknowledgements

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Evaluation of soil health after digestate application using QBS-ar and Solvita®

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Abstract summary

Agricultural practices can cause soil degradation and decline in soil quality. Organic amendments such as green manures, sewage sludge or compost have been shown to increase soil organic matter (SOM) content and impact on soil health. Digestate from anaerobic digestion, has potential to replace inorganic fertiliser use as it contains plant available-nutrients and contributes to the short-term SOM turnover. However, its impact on soil health remains poorly understood.

This paper aims to utilise two contrasting metrics that are posited to evaluate soil heath. To do so digestate was applied to an already established field experiment. Subsequently both the QBS-ar index (Soil Biological Quality index) and Solvita® (Solvita Soil Respiration System) were used to evaluate the impacts of the digestate on soil health.

QBS-ar analysis showed that digestate improved the apparent soil health. Conversely, Solvita® showed no significant differences between digestate-amended samples and control but reported more variation in soil CO_2 flux associated with previously applied field treatments than with digestate application. The correlation found between QBS-ar results and Solvita® was low (19 percent).

Further research on digestate-soil biota interactions is needed to better understand the different responses of QBS-ar and Solvita® in terms of their applicability as indicators of soil health.

Keywords: QBS-ar, Solvita, Digestate, Soil quality

Introduction, scope and main objectives

In order to conserve and enhance soil health, it is often necessary to use fertilisers and amendments (Tisdale and Nelson, 1958). Digestate has the potential to mitigate the use of inorganic fertilizers, since it also contributes to the short-term soil organic matter turnover (Tambone *et al.,* 2010) and it is rich in plant nutrients (Al Seadi *et al.,* 2013). Nevertheless, the effect of the application of digestate to soil and its impact on the environment in terms of soil biodiversity and its effect on soil health are not well understood.

A currently available commercial tool claiming to assess soil health using the soil microbial respiration rate is the Solvita Soil Respiration System (Haney *et al.,* 2015). The microbial respiration rate is evaluated by measuring the CO_2 flux from soil as an indication of N mineralization from SOM (Haney *et al.,* 2015).

Digestate effects on soil have been widely studied, especially in terms of physical and chemical changes (Alburquerque *et al.,* 2012), GHG emissions (e.g. Pezzolla *et al.,* 2012), microbial community (Johansen *et al.,* 2013) but its effects on soil microarthropod communities are not well understood and there is a lack of research related to essential soil mesofauna groups.

The availability of commercial tests claiming to quantify soil health means that it is a pertinent time to test an example of such a test against an already well establish soil health indicator to identify strengths and weaknesses of each potential bioindicator. As such this study aims to (1) evaluate soil heath of an already established field experiment following the application of digestate using QBS-ar (ar - arthropods) index and Solvita® test; (2) compare and contrast QBS and Solvita® results to evaluate whether they are robust methods for the evaluation of soil health.

Methodology

Soil samples were collected from an experimental field located at the Agrii Technology Centre (AgriiFocus) in June 2018.

A long-term trial had already been established at the site in 2012, which consisted of six treatments including one untreated control (Table 1), with four replicates, set up in a completely randomised design. The treatments were: Biosolids, Green Compost, Green Compost (hereafter C50), Farmyard Manure, Chicken Manure (hereafter CM) and Untreated Control (hereafter U), in a spring barley, winter oilseed rape, and winter wheat rotation. Treatments were applied to the individual plots using Richard Western D10 spreader. Application rates are displayed in Table 1.

Table 1: Treatment list. The trial was set up in a randomised block design with four replicates

Treatment	Applied (t/ha)	Total N (kg/ha)	Available N (kg/ha)
Untreated	-	-	-
CM	10	190	57
Biosolid	20	220	33
FYM	43	258	26
Green Compost	25	188	5
C50	50	375	10

A food waste digestate was applied using a boom sprayer perpendicularly to the already established plots at a rate of 60 t ha^{-1} on 1st May 2018. A gap of 12 m was left between the sprayed areas to produce three digestate-free replicates to function as controls.

In June 2018, 24 samples were collected during the winter wheat season using four of the six pre-established treatments. A number of 12 samples was collected from digestate-amended sample points and 12 more from areas with no digestate to function as control samples. These samples included 3 replicates from the pre-treatments U, C50, FYM, and CM. Within 1 m^2 of each sample point composite samples using 4 auger cores (average volume: 235 cm^3 ± 4.6 S.E.) were collected to a10m depth for QBS-ar analysis, and 4 for Solvita®. Composite samples were placed in sealed plastic bags and stored in a cool box. The QBS samples were put in Berlese-Tüllgren funnels.

Microarthropod extraction lasted for 12 days. Observations were made using a dissecting microscope and classified at Order/Class level (Parisi *et al.,* 2005). EMI (eco-morphological index) values were assigned to each arthropod in order to calculate the total QBS-ar value per sample.

Solvita® analysis was undertaken by Lancrop laboratories (Pocklington, York, UK) using the "Soil CO_2-Burst official Solvita instructions".

Statistical analysis was carried out by using R (R Core Team, 2018). Data were analysed using a two-way ANOVA for both QBS-ar and Solvita® results. A Tukey HSD test was used for post-hoc multiple comparison following the two-way ANOVA. Cor.test was used to test for correlation between the two indices, using a Pearson's product-moment correlation.

Results and discussion

The QBS-ar scores resulted from the current experiment showed on average values below 93.7 (Figure 1) suggesting relatively low levels of soil health (Menta *et al.,* 2018). However, soil samples were very dry at the time of the sampling due to an unseasonably dry spring which may explain why low values were found. Microarthropods in soil are, in fact, usually positively correlated with the soil moisture (e.g. Platen and Glemnitz, 2016).

QBS-ar values from the digestate-amended plots were significantly higher compared with control-plots ($p < 0.01$). This suggests that digestate increases soil health as quantified by this metric, regardless the previous management practices. This result agrees with previous studies on earthworm, springtail and mite populations (Frøseth *et al.,* 2014; Platen and Glemnitz, 2016) where a positive effect of a digestate on these groups was observed.

Digestate is a source of OM and so provides a substrate to support arthropod activity. Furthermore, it is likely that that digestate enhanced the environment for arthropods due to increasing soil moisture content as it was approximately 96 percent water by mass. Since digestate was applied at a rate of 60 t ha^{-1}, the quantity of water applied corresponded to 5.76 mm of rain.

Solvita® results showed that the initial treatments were stronger predictors of soil CO_2 flux than the application of digestate ($p = 0.038$; Figure 2). No significant differences were found between digestate-amended plots and control plots ($p = 0.53$). Tukey multiple comparison showed a significant difference between untreated plots and C50 treatment ($p = 0.031$). Accordingly, the Solvita® test found no significant impact of digestate application on soil health. In order to apply the Solvita® test, during the soil preparation, the current procedure requires pulling out more resilient microorganisms: those organisms are likely to have developed in the soil from treatments over several years, which may explain why Solvita® has shown up a stronger relationship to the previous amendments rather than the digestate that had only recently been applied.

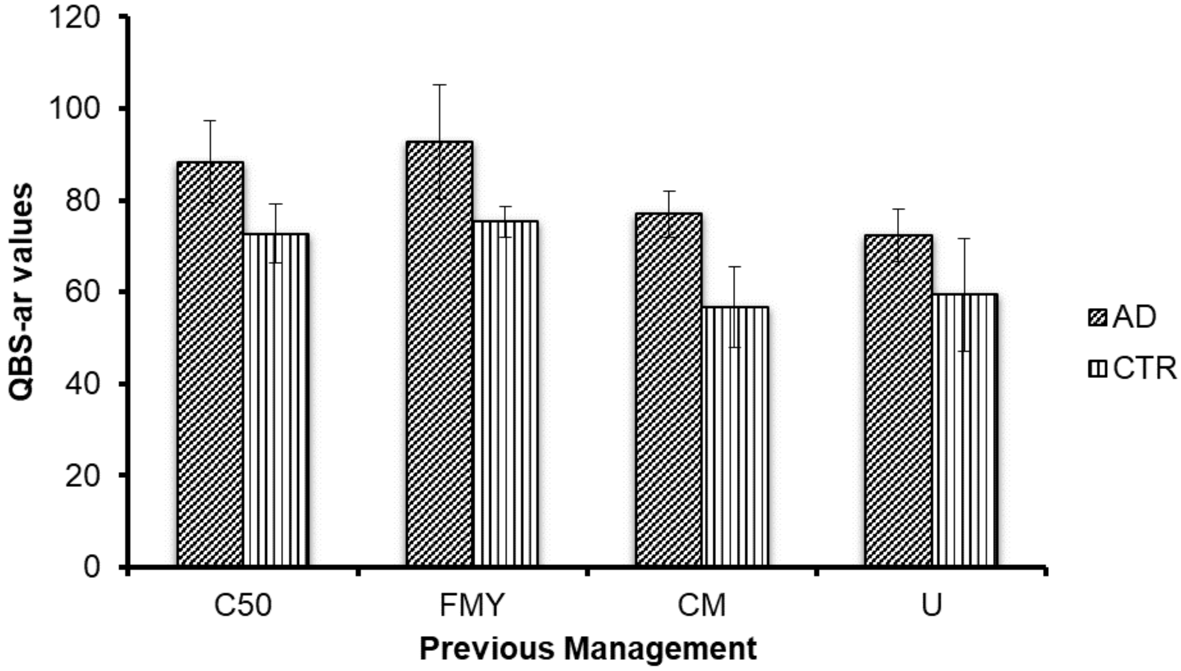

Figure 1: QBS-ar results

QBS-ar values showing both samples with digestate (AD) and without digestate application (CTR). Barplots show means (n=3). Bars show standard errors.

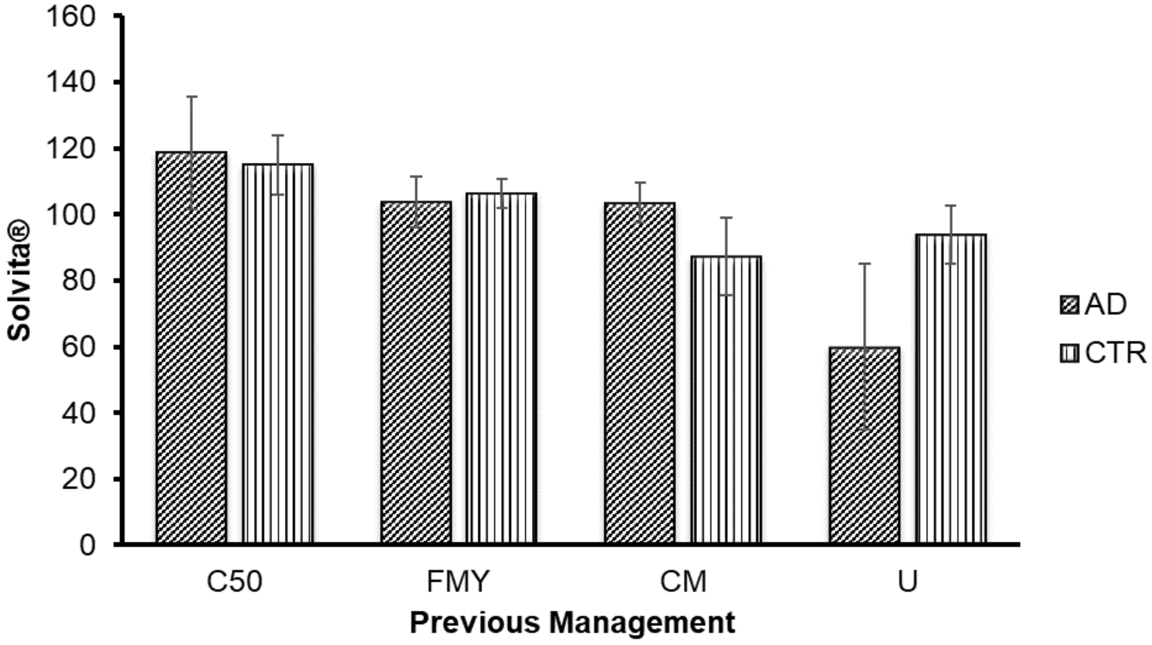

Figure 2: Solvita results

Solvita® values showing both samples with digestate (AD) and without digestate application (CTR). Barplots show means (n=3). Bars show standard errors.

QBS-ar showed significantly higher values in digestate-amended plots compared with non-amended while Solvita® revealed no significant differences following digestate application. A Pearson correlation test found no significant correlation between QBS-ar and Solvita® (p-value of 0.39) suggesting that they have different efficacies in terms of quantifying soil health. A disadvantage of the Solvita® test is that important variables that may significantly affect the CO_2 emissions coming from soil are not entirely considered. These include bulk density, initial moisture content, and total carbon content of soil (Wichuk *et al.,* 2010) as well as others. Soil CO_2 flux can be highly dependent on the content of OM and soil texture (Fungenzi, 2015) and disturbance occurring during transport from the field to the lab may influence CO_2 flux; disturbed soils tend to have higher CO_2 emissions compared with undisturbed soil (La Scala *et al.*, 2006). Moreover, soil CO_2 fluxes can be widely influenced by other biogenic (Kuzyakov, 2006) and abiogenic sources (Shanhun *et al.,* 2012).

It remains unclear whether Solvita® is robust enough to effectively quantify soil health at farmer relevant spatial and temporal scales.

Conclusions

The results showed a positive impact of digestate on QBS-ar values suggesting that digestate application enriched soil microarthropods community diversity. This suggests that digestate can function as a substrate for arthropods and/or it may improve edaphic conditions for soil fauna to survive, such as through increasing soil moisture content. Solvita® results differed from those for QBS-ar, with differences in soil CO_2 flux being associated with previous field treatments rather than digestate application. The correlation found between QBS-ar and Solvita® was low demonstrating that conclusions that would be drawn about the impacts of digestate application on soil health are method dependent.

Further research on digestate-microarthropod interactions is needed to better understand the different responses of QBS-ar and Solvita® in terms of their applicability as indicators of soil health following digestate application.

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Ciliated protists as indicators of soil health:Three case studies from Italy

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Abstract summary

This report aims to provide an overview of the outcomes and the challenges encountered in using ciliated protists as indicator of soil health in the framework of several projects conducted in Italy since 2009. Ciliates in spite of the key roles they play in the microbial loop, still constitute a neglected component of the biodiversity, which is rarely included in biomonitoring plans. In this scenario, the report highlights the potential of ciliates as bioindicators of soil health (in natural, industrial and agro- ecosystems) to evaluate the potential impact of soil disturbance such as those produced by different farming practices (organic vs. conventional) and contaminants. Overall, the results of our surveys conducted in the frame of three distinct projects (*BioPrint, Ciliates in Organic Vineyards & Soil mapping Lombardia*) confirmed the bioindicative potential of soil ciliate communities in discriminating different agricultural management systems, land uses and in detecting different levels of soil pollution.

Keywords: Bioindicators, Soil protists, Community structure, Agroecosystem, Farming practices, Soil health

Introduction, scope and main objectives

Soil biodiversity is a key component of terrestrial ecosystems, being involved in the delivery of several essential ecosystem services such as, among others, nutrient cycling, soil formation, pest and pollution control (Pascual *et al.,* 2015). Thus, soil biodiversity indicators can be used by governments and farmers to monitor soil health and ecosystem functioning under various land uses and farming practices (Turbé *et al.* 2010). In this context, the use of ciliated protists as bioindicators is less common, in spite of the fact that they play key roles in nutrient cycling by feeding on bacteria, fungal biomass and even invertebrates in the plant rhizosphere, thus promoting soil fertility and productivity (Foissner, 1997). Further, soil biomonitoring projects including ciliates offer not only the opportunity to assess the potential of ciliates as bioindicators of soil health but also to explore in detail their diversity, and allowing the discovery of new species (Bharti, Kumar and La Terza, 2015; Bharti, Kumar and La Terza, 2017) (Figure 1).

In the framework of several projects conducted in Italy since 2008, we have investigated the potential of ciliates as indicator of soil health analysing the structure of their communities in both natural, industrial and agro-ecosystems (vineyards, fodders, arable fields) under different level of soil disturbance (Bharti, Kumar and La Terza, 2015; Bharti, Kumar and La Terza, 2017; La Terza *et al.*, 2015). The main aim of all projects was to unveil as to what extent and how, ciliates might contribute to soil bioindication, as well as to generate new baseline knowledge for a more informed use of ciliates as bioindicators of soil health. In this regard, the main hypothesis that were tested through the projects, were the following: i) to evaluate the capacity of ciliate communities to discriminate between different land uses (forests and agroecosystems) with different levels of physical and/or chemical soil disturbance; and ii) farming management practices (organic *vs* conventional); iii) to assess relationships among ciliate communities and abiotic factors.

Methodology

Soil sampling, processing and ciliate communities' analysis

To investigate the diversity of soil ciliated protists, at each investigate site, ten soil samples (0–10 cm depth) were randomly collected with an Edelman auger, mixed to obtain a composite sample (weighing approximately 1 kg), then sealed in a sterile plastic bag and transferred to the laboratory. Ciliate communities were studied by means of qualitative (non-flooded *Petri* dish method) and quantitative methods (direct counting on slide) as described by Kumar *et al.* (2014).

Multivariate analysis was realized using the PAST 2.17c package (Øyvind Hammer, Natural History Museum, University of Oslo).

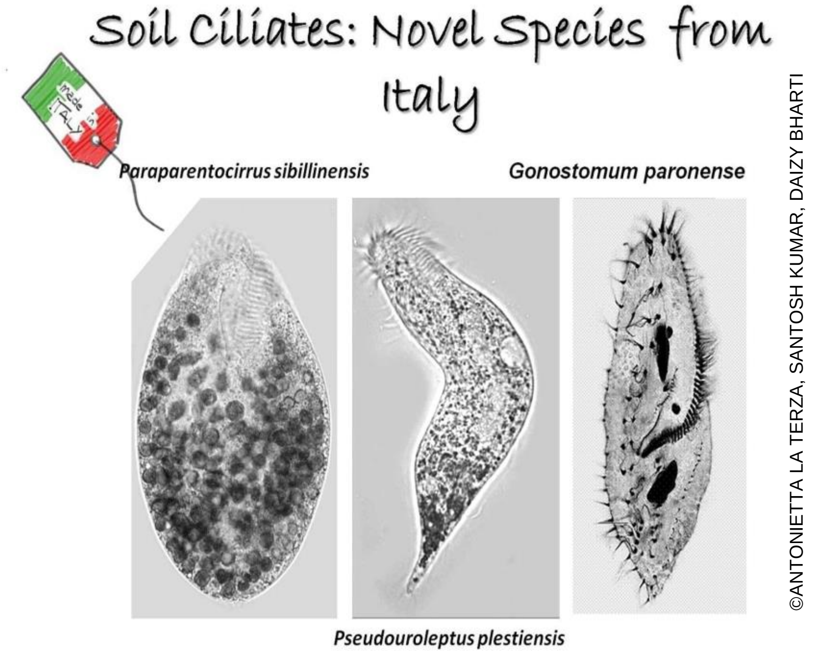

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Figure 1: Novel species of soil ciliated protists from Italy

Paraparentocirrus sibillinensisn. gen., n. sp (Kumar *et al.*, 2014); Pseudouroleptus plestiensis n. sp. (Bharti, Kumar and La Terza, 2014); Gonostomum paronense n. sp. (Bharti, D., Kumar, S. & La Terza, 2015).

Results

First case study - The BioPrint project

In the 2009, in the framework of the BioPrint Project and for the first time in Italy, we investigated the biodiversity and the community structure of soil ciliates from both natural and agro-ecosystems of Marche Region. Soil samples were collected twice from 10 sites with different levels of soil disturbance (5 natural sites: FORest (virgin soils); and 5 agricultural fields: 3 ORGanic (minimum-tillage) and 2 CONventional (sod seeding). Soil chemical-physical (texture, NPK, OM, C/N ratio, soil moisture, and temperature) parameters were also measured. Qualitative analysis allowed us to identify a total of 59 ciliate species representing 29genera and 12 orders (plus 10 species new to science) (Kumar *et al.,* 2014). ORG sites were the richest in species followed by CON and FOR. The mean values for H’ (2.6), d (3.4) and J (0.8) are significantly higher in ORG than in CON (H’=2.1; d=2.7; J=0.7) and FOR (H’=1.7; d=1.95; J=0.6). These results support the “Intermediate Disturbance Hypothesis” (IDH), that slightly disturbed habitats (e.g. minimum-tillage) usually have higher organism diversities than stable ones (Foissner, 1997).

Multivariate analysis show statistically significant differences between natural sites (FORest) and agricultural sites as well as between the ORGanic and CONventional management farming systems. Canonical Correlation Analysis (CCA) analysis show correlations between the distribution of species with environmental parameters; indicating the importance of these parameters in shaping the ciliate communities at different sites (Figure 2).

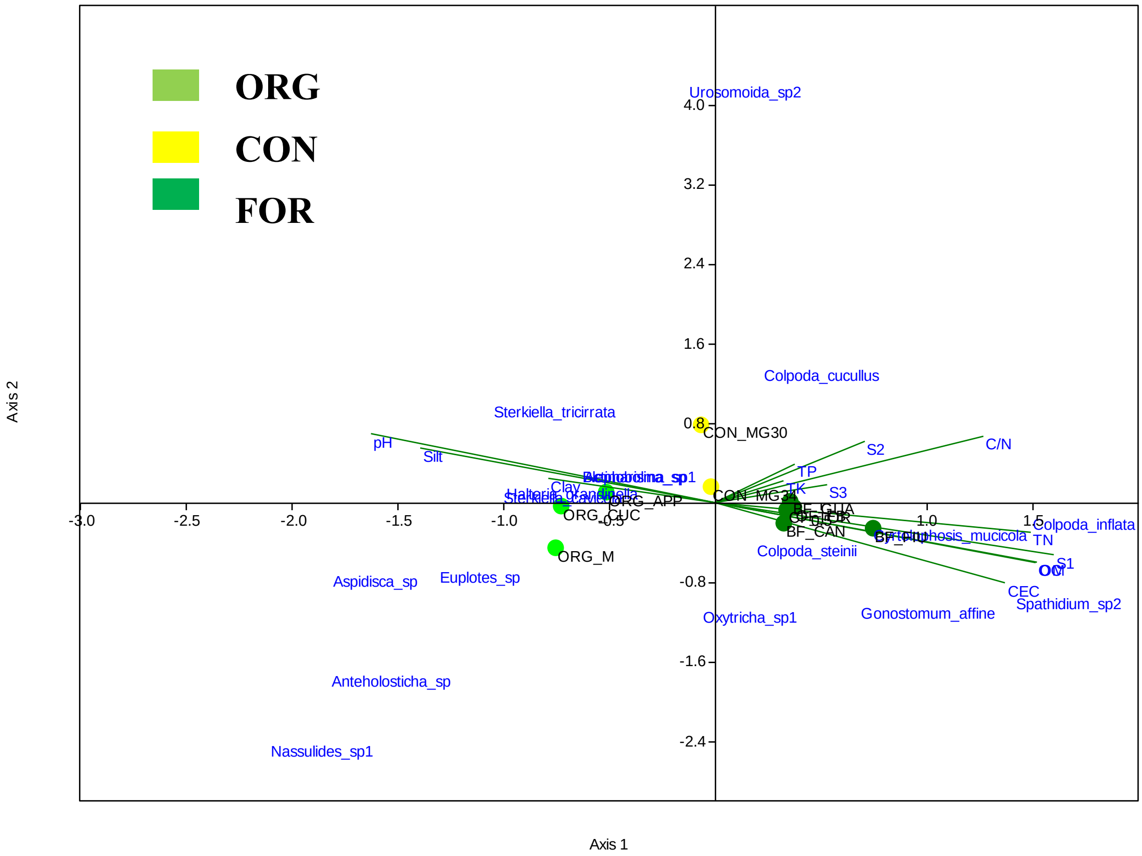

Figure 2: Canonical Correspondence Analysis (CCA)

Relationships between individual ciliate species in the community and abiotic factors at all sites CCA of log-transformed abiotic and species-abundance data.

Second case study -Ciliates in the Organic Vineyards Project

Organic farming employs a set of farming practices that contribute towards preserving soil quality. In this scenario, the aim of this study was to assess the long term effect of organic floor management on soil health by mean of ciliate communities analysis in 3 vineyards which were organically managed (at the time of sampling) for 19 years (V92), 13 years (V98) and 2 years (V09) respectively. The study was realized in a commercial vineyard in the *terroir* of "Verdicchio di Matelica" (Italy), in 2011 with a total of three samplings for each vineyard. Qualitative ciliate analysis allowed us to identify a total of 27 ciliate species belonging to 6 classes, 11 orders, 19 genera. The results of diversity indices (*H'*, J, d) and the multivariate data analysis (nMDS), indicated that the most stable habitat for ciliate communities was the "oldest" (V92) followed by the V98 and the "youngest" V09 vineyards (Figure 3). This effect may be due to the greater soil resilience, possible achieved due to the long-term organic management, of the vineyards V92 and V98 (La Terza *et al.*, 2014).

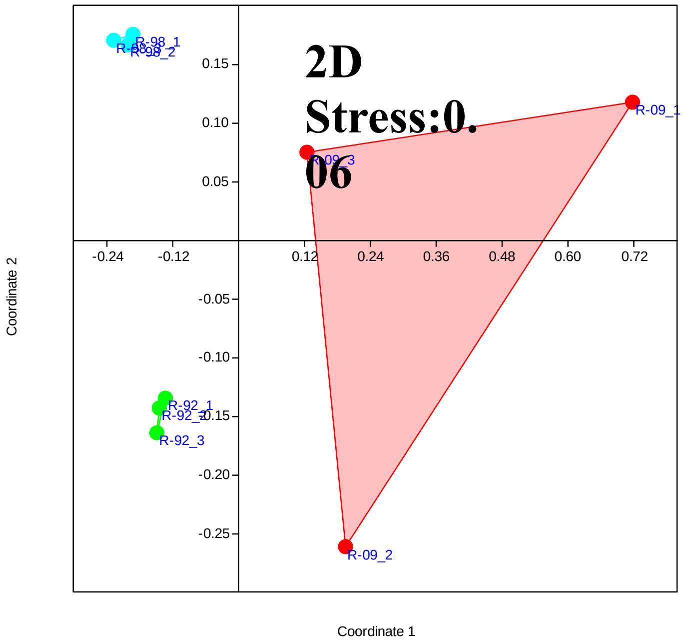

Figure 3: *Non-metric Multidimensional scaling (nMDS)* for spatial taxonomic patterns of soil ciliates for log transformed species-abundance data on Euclidean Distances, for the 3 vineyard, V92, V98 & V09

The *nMDS* analysis evidences a pronounced fluctuation of ciliate community structure in the "younger" V09 vineyard in the 3 sampling periods and, the absence of variations in community structures in the V92 and V98 vineyards.

Third case study- SoilMapping LombardiaProject

In the framework of the *Soil Mapping Project*, the ciliate communities were investigated in four industrial sites (Incinerator of Parona;Site of National Interest, SIN Brescia; Plant of regeneration of exhausted oils, Viscolube; Cement factory of Broni) of the region Lombardia with the main aims: i) to evaluate their potential in discriminating different levels of soil contamination; and ii) to assess relationships among ciliate communities and abiotic factors. Qualitative ciliate analysis allowed us to identify a total of 73 species belonging to 7 classes, 12 orders, and 30 genera, including 12 species new to the science (Bharti, Kumar and La Terza 2015; Bharti, Kumar and La Terza, 2016). The results of diversity indices (*H'*,J,d) and multivariate data analysis, show that the communities associated at the four sites are significantly different; except at the sites of Parona and Viscolube, which were similar for species composition and abundances. Furthermore, multivariate analysis showed correlation between the distribution of species with environmental parameters (and contaminants), indicating the importance of these factors in shaping the ciliate communities at the four investigated sites (La Terza *et al.*, 2015).

Discussion

Each one of the above-described case studies shed light on how ciliate communities can contribute as bioindicators of soil health (Figure 1). In this regard, the *BioPrint project* show the bioindicative potential of ciliate communities in discriminating between natural (FORest) and agro-ecosystems, and different management systems (ORGanic vs CONventional) (Figure 2). In the project: *Ciliates in Organic Vineyards,* the ciliate communities act as a measurable proxy of soil resilience in agroecosystems (and thus, as indicators of sustainable land management) (Figure 3). In final, the *Soil Mapping* project highlight the capacity of ciliate communities in discriminating between different levels of soil contamination in polluted sites.

Conclusions

In summary, these outcomes generated new knowledge and provided baselines for a more informed use of ciliates as bioindicators of soil health. However, further surveys should be conducted in order to better refine and possible standardize sampling protocols, to develop less time consuming methods for soil ciliates analysis and possibly associate the classical methods to the molecular one. Furthermore, these surveys have contributed to "reboot" soil ciliate diversity studies in Italy by updating Italian checklist, identifying more than 25 novel species, and dwelling deeper into the "soil ciliate diversity black box" in natural, contaminated and agro-ecosystems.

Acknowledgements

M.Tiberi, C.Bernacconi & G.Ciabocco "Marche Region Soil Observatory

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Using environmental DNA to assess global soil biodiversity and build a soil quality bioindicator

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Abstract summary

In this study, we propose to build a global indicator for bioassessment of soils based on environmental DNA (eDNA). This tool should be easily applied, inexpensive, and standardizable regardless of climatic conditions. The eDNA metabarcoding method is used to assess the presence of different taxonomic groups (eukaryotes and prokaryotes) considered as soil bioindicators but also to establish global soil DNA signatures without any *a priori* knowledge. This indicator is developed in the context of the restoration of degraded sites, with the purpose of monitoring ecosystem recovery during rehabilitation and remediation actions, for instance in urban environments. For each study area, samples are collected both from reference sites and sites in restoration. The objective is to assess the sensitivity of DNA signature to restoration actions and to inform in which extent the observed signature indicates the degree of restoration in terms of soil quality. Ultimately, a database including different types of soils will be built at the national scale. It will be used to characterize the correlation between DNA signature and ecosystem functions, using 'Machine learning' approaches. This bioindicator can also be used in the agriculture sector, to contribute to the assessment of soil biological quality and to guide cultural practices.

Keywords: soil biodiversity, bioindicator, eDNA metabarcoding, DNA signature, restoration, polluted soils, machine learning.

Introduction, scope and main objectives

In response to environmental but also socio-economic challenges, expenses related to the restoration of degraded or polluted soils have increased in the last 15 years. In addition to obvious public health issues (e.g. reducing the risk of spread of pollutants), the restoration of soils favours the dynamic of vegetal cover and trophic interactions and allows recovering ecosystem functions as well as associated ecosystem services. Ecological restoration generates a gain for the biosphere, but also for humans. Restoration procedures take place according to methods adapted to various operational and scientific objectives, involving technical and financial investments (Bazin and Barnaud 2002). Although these restorative actions have increased in recent years, a large number of studies highlight the lack of suitable and standardized protocols and indicators to assess the restoration of biodiversity, functions and ecosystem services

(Baptist *et al.,* 2018). One of the major challenges is the construction and / or adaptation of dedicated indicators, in connection with the functions and ecosystem services targeted during the restoration of degraded sites, in order to allow effective monitoring of the soil compartment.

Various physicochemical indicators are currently used to assess the quality of a soil (pH, cation exchange capacity, organic matter content, nitrogen and total phosphorus etc.), but these indicators do not allow to assess the level of functionality. The most commonly applied biological indicators (or bioindicators) are based on a descriptive analysis of the richness and abundance of specific groups such as earthworms (or Enchytreids in the absence of earthworms), nematodes, microarthropods or even bacterial or fungal communities (see Bünemann *et al.* 2018 for a summary). However, these taxonomic groups can undergo a strong spatial and temporal variability according to edapho-climatic conditions. The development of bioindicators also requires a sometimes tedious, potentially subjective and particularly costly morphological identification phase. The analysis of environmental DNA (eDNA) from soil samples should make possible to circumvent the technical constraints linked to the morphological identification of the different taxonomic groups listed above. DNA is now recognized as a reliable tool for detecting the presence of species or establishing biodiversity inventories in a given ecosystem (Valentini, Pompanon and Taberlet*,* 2009; Taberlet *et al.* 2018).

Our objectives in this study were:

1. To test the eDNA tool to assess environmental changes in polluted pilot site under restoration.
2. To build a bioindicator based on the whole range of eukaryote diversity in soils, in order to assess the success of restoration of the degraded ecosystem functions.

Methodology

Site

Seven samples were collected in an industrial site being restored. The site is polluted with hydrocarbons and heavy metals. Restoration actions include spreading topsoil as well as gravels to re-establish bird habitats. Five replicates were sampled in non-restored fields, and two in on-going restored fields.

Sampling

For each replicate, 20 pooled subsamples were collected in the surface layer (horizon 0-10cm) of quadrats (1m*1m). From this pool, two soil samples of approximately 20g were taken and preserved dry in silica gel until their analysis in the eDNA laboratory.

Figure 1: Sampling equipment for soil

Laboratory procedures

Extracellular DNA was extracted using a phosphate buffer method (Taberlet *et al.* 2018). Amplification of DNA was performed using the eukaryote primers Euka02 (Taberlet *et al.* 2018) with four replicates per sample. Negative controls were performed at each step of the process. DNA was purified and sequenced in an Illumina Miseq sequencing run (2*125 bp).

Bioinformatic and biostatistics analyses

The OBItools and ROBItools (Boyer *et al.* 2016) were used to assemble, filter and perform taxonomic assignation of sequences (Genbank ®). Statistical analyses included multivariate analyses (vegan, Oksanen *et al.*, 2007), Shannon diversity indices and identification of indicator species (R library: indicspecies).

Results

The composition of taxa in all soil samples shows that, among animals, there is a majority of annelids, protozoans and collembolas. Among plants, monocotyledons and mosses dominate (Figure 2).

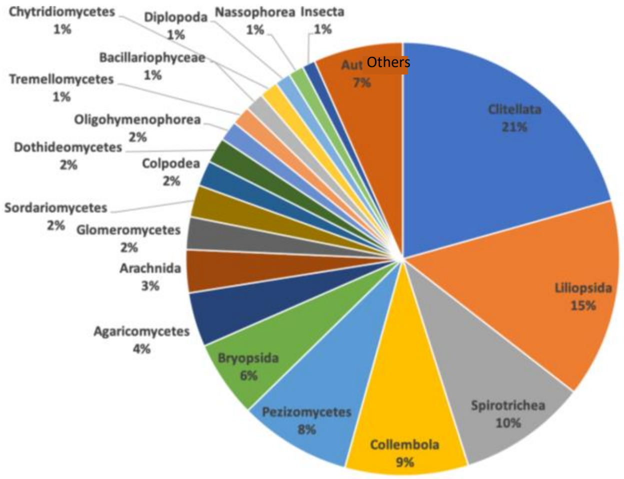

Figure 2: Global composition of eukaryotes in soil samples

The type of habitat (polluted and on-going restored fields) did not influence global soil biodiversity (Shannon indices, results not shown) but a higher proportion of collembolas was observed in on-going restored fields (Figure 3a), which might indicate that this group corresponds to pioneer species.

Soil samples structure mainly according to habitat type (axis 1 in Figure 3b) but also according to the hydrocarbon concentration (axis 2 in Figure 3b). In other words, habitat and hydrocarbon concentration influence the composition of eukaryote communities, but not their diversity.

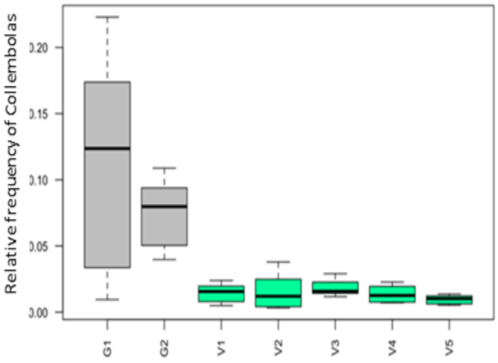

Figure 3a: Relative frequency of collembolas between samples

G1 and G2 correspond to on-going restored fields. V1 to V5 correspond to polluted fields.

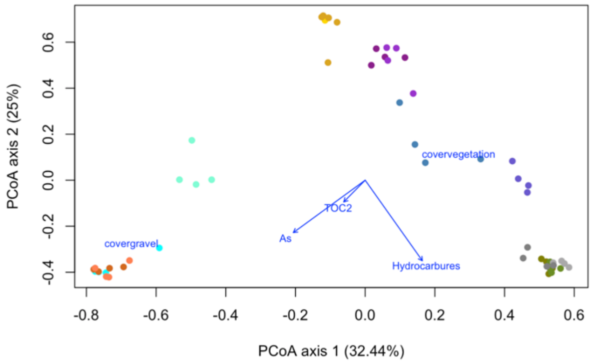

Figure 3b: Structure of samples (multivariate analysis)

"Covergravel" correspond to on-going restored fields, and "covervegetation" to polluted fields.

The focus on collembolas among the metabarcoding results allowed showing that this group responds well to environmental variables, in terms of habitat type or pollution type.

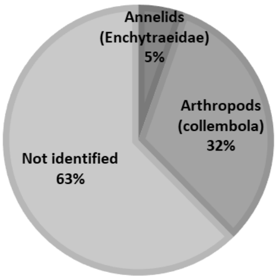

Figure 4a: Taxonomic composition in on-going restored fields

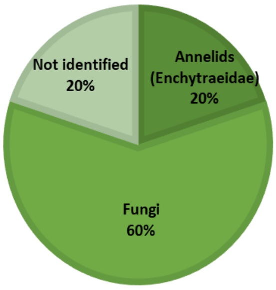

Figure 4b: Taxonomic composition in polluted fields

The statistical treatment of data allowed identifying different 'composite' bioindicators, depending on habitat type, that can testify of the soil restoration without any a priori knowledge (Figure 4). A similar work can be performed on the "pollution' gradient.

Discussion

This pilot study emphasizes the benefit of using the eDNA tool as a global indicator for biological assessment of soils. This tool can be standardized, it is inexpensive and easily applied regardless of climatic conditions.

Ultimately, a database considering different type of soils will be built at national scale. It will be used to characterize the correlation between DNA signatures and ecosystem functions, using 'Machine learning' approaches.

Conclusions

This pilot study showed that a DNA signature can be established from soil samples and that this signature can be used to assess the degree of restoration of soil quality in polluted environments. This bioindicator can also be used in the agriculture sector, to contribute to the assessment of soil biological quality and to guide cultural practices.

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QBS-ar in soil biodiversity monitoring: the experience of Emilia-Romagna Region

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Abstract summary

Land use and management have important impact on soil quality and biodiversity. To monitor the status of the living soil community it is required to have an efficient indicator able to highlight any suffering conditions. Emilia-Romagna Region has adopted the QBS-ar index (Soil Biological Quality Index based on soil arthropods) as indicator of soil biodiversity and soil health, and it started a monitoring plan more than 5 years ago aimed to: i) define the quality status of regional agricultural soils; ii) assess how the different land uses impact soil biodiversity and soil biological quality. The results of this first step is reported here. In 2018, another regional monitoring network has been organised and funded by the CAP 2014-2020 (Measure 20 of regional RDP). The activity is still in progress and it will assess whether farming systems are sustainable and respectful even towards biodiversity.

Keywords: soil biodiversity, soil quality, sustainable agriculture, monitoring network, arthropods

Introduction, scope and main objectives

As required by the EU’s Common Agricultural Policy (CAP 2014-2020), with the Rural Development Programme (RDP) the Emilia-Romagna Region has adopted soil conservation management practices whit the aims of achieving production with less input in terms of pesticides, chemicals, water and lower CO_2 emissions, to protect natural resources and environment. While there is a growing awareness of the need to protect soil from pollution, for healthy food, and from the loss of organic matter, to preserve fertility and increase the soil storage of CO_2, there is still a poor awareness of the importance and the function of soil's biotic component.

The study of edaphic communities has received increasing attention in recent years, however, there is still a limited number of standardized procedures and a lack of reference values to establish the state of soil quality also taking into account the use, type and level of soil disturbance (Turbé *et al.*, 2010).
A bioindicator widely spreading is the QBS-ar index (Biological Soil Quality Index based on soil arthropod community) developed by Parma University (Parisi, 2001; Parisi *et al.,* 2005). It is a metric based on the concept that the number of microarthropod groups morphologically well adapted to soil is higher in high quality soils than in low quality soils. The index values are generally directly

related to the use and state of the soil, allowing to draw conclusions on soil management (Tabaglio *et al.*, 2009).
In 2015 Emilia-Romagna Region and University of Parma have started a monitoring plan aimed to detect QBS-ar index in 58 agricultural soils with different land use and management in order to: i) describe the state of the biological quality of the regional soils; ii) establish reference values to describe the typical characteristics of soils in different contexts of land use; iii) test the use of indicator to highlight soil degradation condition or reduction of biodiversity in relation to the impact of agricultural management.

Methodology

Soil samples were collected in 58 sites located in the plain and on the hill of the Emilia-Romagna Region (northern Italy).

The sites were chosen considering the soil characteristics described by the regional Soil Database with the aim of choosing sites representative of the most widespread soils.

The 58 sites include 3 different land uses: 22 sites in arable land (annual crop), 19 sites in permanent crops (8 in vineyard, 11 in orchard), and 19 sites in grassland. In the latter, three different sub-categories were considered based on the age of the meadow: meadows of alfalfa that remain without tillage for a maximum of 5 years, and are in rotation with annual crops (8 sites); permanent meadows of alfalfa or polyphite grass older than 5 years but under 30 years (6 sites); stable meadows are polyphite grass more than 30 years old (5 sites).

Each site was sampled twice, in spring and autumn, with three clods of soil, arthropod extraction was performed by Berlese-Tüllgren funnel (extraction time 10 days). The specimens were placed in a preservative solution, identified to class level and an ecological–morphological index (EMI), ranged between 1 and 20, was assigned for each taxon. QBS-ar index resulted as the sum of the highest EMI values of the three replications relative to each taxon.

Soil characteristics of the sites were also known, the different types of soil have been grouped into Soil Functional Groups (SFGs) based on the following aspects: texture of the surface horizon, saturated soil condition, presence of organic horizons. Some characteristics of the functional groups used in this work are reported in the Table 1.

Table 1: Mean values of soil parameters of the 58 sites grouped by SFGs

Soil functional groups	**Organic Carbon %**	**Clay %**	**Sand %**	**pH**
A (15 sites)	1.461 ± 0.626	44.68±5.319	11.67±5.762	7.68±0.463

B (8 sites)	1.669±0.275	36.95±1.401	14.4±7.093	7.775±0.429
C (16 sites)	2.067±1.451	30.94±2.383	20.44±7.832	7.697±0.492
D (6 sites)	1.24±0.535	17.67±3.204	21.33±3.67	7.945±0.301
E (11 sites)	1.235±0.499	16.07±5.328	48.3±16.08	7.969±0.191
F (2 sites)	2.49±2.376	8±2.376	83.5±0.707	7.915±0.191

A fine texture, B moderately fine texture with > 35% of clay, C moderately fine texture with < 35% of clay, D medium texture, E moderately coarse texture, F coarse texture

Results

The two-way ANOVA analysis shows that land use is the factor that most affects the QBS-ar values (p value <0.001) while the season does not significantly influence the index value (p value> 0.05). To highlight the differences between different land uses, Tukey’s test was applied (Table 2).

Table 2: Tukey’s test applied to different land use

Land use	**orchard**	**vineyard**	**meadows of alfalfa**	**permanent meadows**	**stable meadows**
orchard	-	-	-	-	-
vineyard	0.093	-	-	-	-
meadow of alfalfa	1.000	0.166	-	-	-
permanent meadow	0.411	0.998	0.540	-	-
stable meadow	1.000	0.242	1.000	0.577	-
annual crop	**0.001**	**0.000**	**0.001**	**0.000**	**0.037**

Value in bold significantly differed from each other (p-value <0.05)

Tukey's test highlights significant differences between annual crops and all other land uses: arable land shows the lowest QBS-ar values among the five categories considered, with values between 41 and 182 and an average value of 105 (Figure 1).

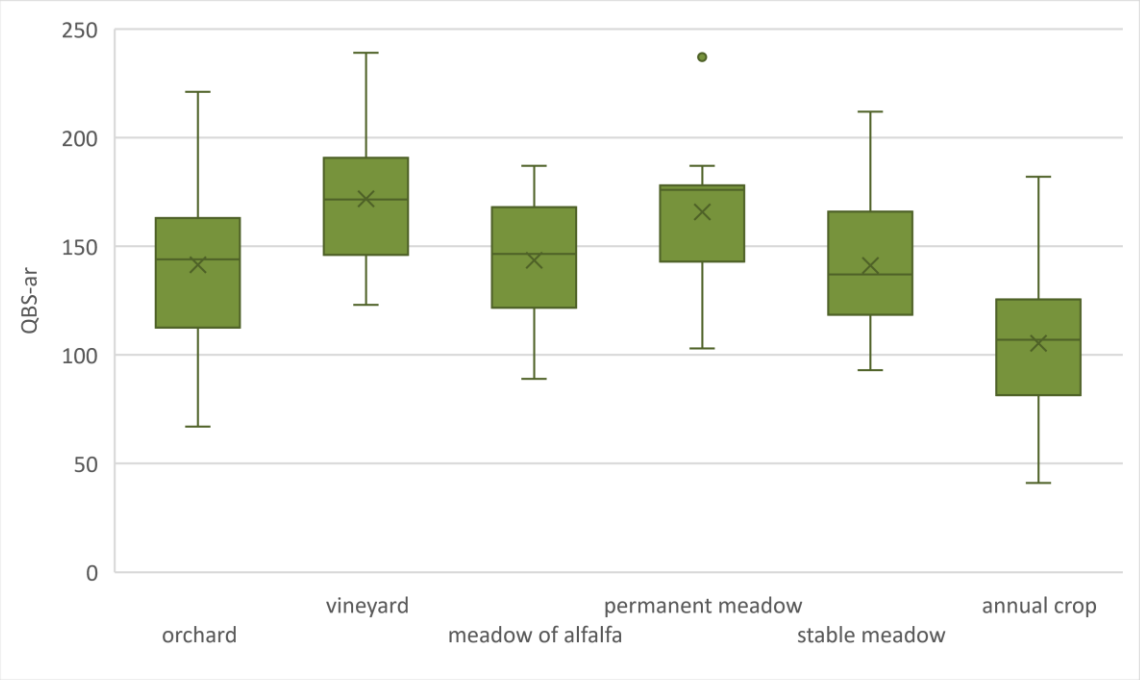

Figure 1: QBS-ar values for different land uses

box plot: X= mean, line=median, box= interquartile range, whisker=minimum, maximum

Meadows reached higher values of the index compared to annual crop (Figure 1) for the absence of tillage and a major protection of the soil surface. Within this category, alfalfa meadow ranged between 89 and 187(average value 143), permanent meadow from 103 to 237 (average value 166), while stable meadow ranged from 93 and 212 (average value 141), but the differences are not significant.

Vineyards have values between 123 and 239, with an average of 172, while orchards have values from a minimum of 67 to a maximum of 221, and an average value of 141. Although these permanent crops were affected by management practices, the grass cover reduces the disturbance of soil and creates an edaphic environment similar to grassland.

To investigate the relation of QBS-ar and soil type Tukey’s test was applied. (Table 3).

Table 3: Tukey’s test applied to different Soil Functional Groups

Soil functional groups	A	B	C	D	E
A	-	-	-	-	-
B	0.998	-	-	-	-
C	**0.040**	0.325	-	-	-
D	0.956	0.880	**0.038**	-	-
E	0.238	0.663	0.997	0.137	-
F	0.500	0.409	**0.027**	0.878	0.063

Value in bold significantly differed from each other (p-value <0.05)

SFGs show different trend of QBS-ar index, though not always these differences are statistically significant (Figure 2). Only moderately fine soils with <35 percent of clay (SFG C) are different from fine soils (SFG A), medium textured soils (SFG D) and coarse soils (SFG F).

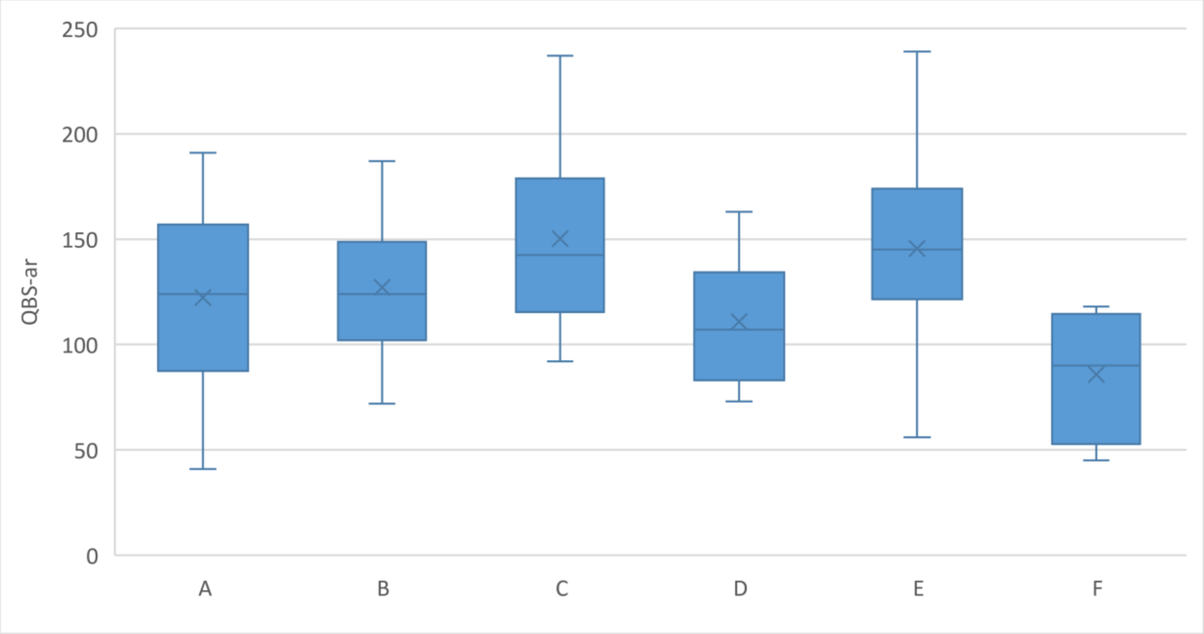

Figure 2: QBS-ar values for different Soil Functional Groups

box plot: X= mean, line=median, box= interquartile range, whisker=minimum, maximum

Discussion

Although there are still no national reference shared values of QBS-ar, the results obtained in this work do not differ from those found in previous studies (Menta *et al.*, 2010; Tabaglio, Gavazzi and Menta, 2008; Tabaglio, Gavazzi and Menta, 2009). Menta *et al.* (2018) reported a reference value of **93.7** as a result of 41 published works worldwide. Compared to this value, all land use categories assessed have a higher average value (figure 1), only arable lands show lower value with 25 percent of the values even lower than 82.5.

The study highlights that the presence of a permanent grass cover, as found in meadows, in many vineyards and orchards, is a key element for the maintenance of a complex arthropod community. Otherwise, more intensive agricultural systems, which require annual soil tillage, make the biological community more vulnerable with simpler and poorer communities in terms of biodiversity and functionality.

As described in the results section, there was no close relationship between the QBS-ar values and the different soil types due to soil management. In this case soil management had a high impact on the edaphic community that didn’t allow the features of the soil to emerge.

Conclusions

This study led to a first important evaluation on the biological quality of the Emilia-Romagna agricultural soils. QBS-ar index can be used to detect soil quality and soil biodiversity, and it can show how different types of soil management can affect soil properties. Conservative practices, such as the minimum or no-tillage, the use of multi-annual crops in rotation with annual crops, the use of cover crop and the grass cover in permanent crops maintain and enhance soil living community and soil functionality.

Thanks to the results achieved in this work, the QBS-ar index has been chosen by the Emilia-Romagna Region as an indicator in soil monitoring activity. In 2018, a regional monitoring network has been organised and funded by the CAP 2014-2020 through Measure 20 of regional RDP. The aim of this new project (123 sites) is to compare soil chemical and biological quality between organic, integrated, conservation agriculture, extensive grassland and traditional farming systems.

Acknowledgements

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Plant productivity enhancement in a simulated Amazonian Dark Earth (Terra Preta Nova)

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Abstract

Amazonian Dark Earths (ADEs) are highly fertile human-made soils, commonly found in Amazonia. These soils have a unique soil biota and a high content of charcoal, organic C, available P and Ca as a result of repeated burning and additions of bone and organic matter (OM) over centuries of Amerindian occupation. "*Terra Preta Nova*" techniques aim to replicate these soils by adding ADE components, but little is known of their single and synergistic impacts on plant productivity. We replicated an ADE by adding five distinct components and studying their single and interactive effects on maize production in the greenhouse. Earthworms (*Pontoscolex corethrurus*), OM (horse manure), biochar (from Brazil nut), ground fish bones and pottery sherds were added to a nutrient poor soil, and after 60 days plant height, above and below-ground (root) biomass, root length, and soil fertility levels were assessed. The main components affecting plant productivity were earthworms, OM and fish bone meal, with significant synergistic effects, while biochar and pottery, although significant, were not as important for plant growth and soil fertility.

Keywords: soil fertility, agricultural sustainability, Amazonia, soil biology, soil amelioration

Introduction

Over centuries of occupation, Amazonian Amerindian populations generated highly fertile Amazonian Dark Earths (ADEs, also known as *Terra Preta de Índio* in Portuguese), by the addition of fresh and burned organic materials (human excrements, animal remains such as fish bones, plant organic matter) and pottery sherds. These soils are highly sought out by present day farmers in the region due to the high plant production potential. They tend to have high soil C, available Ca and P contents, as well as a unique soil biota, including important earthworm populations (Cunha *et al.*, 2016). It is well known that organic materials such as animal manures (Bayu, Rethman and Hammes, 2008) and fish bone meal (Blatt, 1991), biochar (Ding *et al.*, 2016) and earthworms (Brown, Edwards and Brussaard, 2004) can positively affect plant production. However, the impact of pottery sherds on plant production is not well known, though they may contain important nutrient stocks (Rodrigues, 2014). Very few studies have reported results of ADE recreation (*Terra Preta Nova*), on plant production in nutrient poor soils, and none of them evaluated interactions between the different components of ADEs. Hence, the present study was undertaken to evaluate the impact of organic manure, biochar, fish bone meal, earthworms and ceramics on plant production in a nutrient poor Latosol.

Methodology

A clayey yellow Latosol was obtained from Paraopeba-MG, in Central Brazil, and a fractional factorial experiment was installed using 160 pots with 4 kg soil (dry wt equivalent) each. In each pot, each of the five components of ADEs were added or not, generating 32 treatments, each of with was replicated five times (Figure 1, Table 1). A hybrid maize variety was grown in the greenhouse, and 55 days after germination, plant production parameters (height, fresh biomass above and below-ground, root length), soil fertility (exchangeable nutrients, pH), and earthworm survival were recorded. When added to the treatments, earthworms (*Pontoscolex corethrurus*) were 5 individuals, biochar from Brazil nut shells at 10 percent (w/w), ceramic pieces (pottery shards) at 10 g Kg^{-1} soil, partially composted organic matter (horse manure) at the rate needed to reach 2.6 percent C in the soil, and fish bone meal at fertilizer rate of 500 mg dm^{-3} P in the soil. Earthworm abundance, soil C and pottery mass were within values that can be found in ADEs under field conditions. The pots were randomly placed in the greenhouse and a single maize plant per plot kept after germination. Statistical analyses (individual treatment effects and interactions) were evaluated using General Linear Models (GLM) with R (RStudio Team, 2016).

Figure 1: Components of the ADEs used in the Terra Preta Nova experiment in the greenhouse evaluating maize production after 60 days

a) Earthworms Pontoscolex corethrurus; b) Pottery shards; c) Biochar from Brazil nut shells; d) Fish bones before grinding (to produce fish bone meal); e) partially composted horse manure

Results

All of the ADE components used to create the *Terra Preta Nova* had significant impacts on maize production (Table 1). Surprisingly, biochar had a slightly negative impact and ceramics had a major positive impact on shoot mass, while highest positive impacts overall on plant parameters (up to 26 percent increase in shoot mass) were observed with addition of organic matter and fish bone meal, in particular (Table 2). Earthworms positively impacted both shoot and root mass (Tables 1, 2). Several important two-way as well as three-way interactions were detected (Table 1). Four-way interactions were only significant in the absence of OM, with all the remaining components (W x B x P x F). Soil fertility impacts were observed mainly with addition of fish bone meal, but also with OM, with significant increases in available P levels.

Table 1: Results of the GLM tests of significance of main-effects of the different ADE components (factors) and their combined treatments (interactive effects) on maize shoot and root biomass and length after 55 days germination under greenhouse conditions

Treatment	Shoot mass	Root mass	Root length	Treatment	Shoot mass	Root mass	Root length
W	***	***	ns	W x B x P	ns	ns	ns
OM	***	***	***	W x B x OM	ns	ns	ns
P	***	ns	ns	W x P x OM	ns	ns	ns
F	***	***	***	**W x B x F**	**	**	ns
B	*	ns	ns	W x P x F	ns	ns	ns
W x OM	**	***	***	**W x OM x F**	ns	*	***
W x P	ns	ns	ns	**B x P x OM**	***	***	ns
W x F	***	***	**	**B x P x F**	**	ns	ns
W x B	***	*	ns	**B x OM x F**	*	ns	ns
OM x P	ns	ns	***	**P x OM x F**	*	ns	ns
OM x F	***	***	***	W x B x P x OM	ns	ns	ns
OM x B	*	**	***	**W x B x P x F**	***	***	ns
P x F	ns	**	**	W x B x OM x F	ns	ns	ns
P x B	**	***	ns	W x P x OM x F	ns	ns	ns
F x B	ns	ns	*	B x P x OM x F	ns	ns	ns

W=earthworm; OM=organic matter (horse manure); P=pottery sherds; F=fish bone meal; B=biochar. Treatments with significant main effects or interactions are highlighted in bold.

Table 2: Mean values of plant height, shoot and root biomass and root length (and correspondent increase) of maize plants for each component of the ADE used in the *Terra Preta Nova* greenhouse experiment

Treatment	**Plant height (cm)**	**Shoot biomass (g)**	**Root biomass (g)**	**Root length (cm)**
Earthworm				
Presence	**95.7 (+2%)**	**12.2 (+20%)**	**2.78 (+11%)**	12784
Absence	**93.4**	**10.2**	**2.50**	12674
Biochar				
Presence	94.9	**11.8 (-3%)**	2.70	12516
Absence	95.7	**12.2**	2.78	12784
Ceramics				
Presence	98.5	**13.0 (+24%)**	2.94	13488
Absence	94.3	**11.3**	2.60	12266
OM				
Presence	**99.9 (+6%)**	**13.4 (+26%)**	**3.03 (+17%)**	**13889 (+13%)**
Absence	**94.3**	**10.6**	**2.60**	**12266**
Fish bone meal				
Presence	**100.0 (+10%)**	**13.4 (+26%)**	**3.02 (+24%)**	**13789 (+19%)**
Absence	**91.0**	**10.6**	**2.44**	**11546**

Treatments with significant presence effects are highlighted in bold typeface.

Discussion

A spiked interest in "*Terra Preta Nova*" began around 10 years ago, due to the supposed high potential soil fertility and C sequestration effects of biochar (Lehmann, 2009). In fact, the use of biochar and organic matter together had significant interactive effects on plant production parameters. Although biochar use in agriculture has considerably increased, there are still some concerns regarding its possible impacts to soil quality in the long term (Verheijen *et al.*, 2010). Furthermore, in the present experiment, use of biochar resulted in a slight negative impact on maize shoot mass, but root mass and length were not affected. However, several interactions with biochar were significant, showing that it has potential to impact plant parameters depending on what is used together with it. Physical parameters were not measured, and biochar is known to have impacts on soil porosity and water movement (Blanco-Canqui, 2017).

Earthworms are well known plant growth promoters, and *P. corethrurus* is distributed throughout the tropical world, particularly in disturbed regions and agroecosystems, where it can have important effects on soil properties (Taheri, Pelosi and Dupont, 2018). Furthermore, earthworms can ingest biochar (Ponge *et al.,* 2006), and impact organic matter mineralization and nutrient availability, and these may be important reasons why they had positive impacts on maize production in the present experiment (Brown, Edwards and Brussaard, 2004).

Pottery sherds may be important in long-term nutrient release (Rodrigues, 2014), but also in modifying soil physical properties such as porosity and water availability, and in the present case, their addition was important for promoting shoot growth. Little is known of the use of potsherds in agriculture, and they may play a role in *Terra Preta Nova*, particularly in clayey soils with physical restrictions to plant growth.

Organic manures are important for sustainable agriculture in small farms (Bayu, Rethman and Hammes, 2008), particularly where inorganic fertilizers are less available and/or unaffordable. They have important fertilizer value contributing with K, Ca, Mg, N, P, S and B to plant nutrient needs. Fish bone meal and OM were the two most important plant growth promoters in this *Terra Preta Nova* experiment. Fish bone meal also has significant amounts of Ca, P, Mg, S, Fe, Zn, Mn and Cd (Buddhachat *et al.,* 2016), all of which are important for plant growth. The use of high-P and N-containing organic residues is an important strategy for increasing soil fertility and plant production in nutrient-poor soils of the tropics. In acid soils, additional benefits of bone meal use may be long-term impacts on pH and associated nutrient availabilities.

Conclusions

These preliminary results show the potential for wider use of household and farm refuses, such as bones, manures and broken pottery in soil improvement, and the importance of interactive effects in affecting plant growth, factors that need further attention in future soil fertility and management schemes such as Terra Preta Nova technologies, based on the Amazonian Dark Earth concept. However, longer-term and field experiments are needed using these concepts in order to verify these phenomena.

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Soil macroinvertebrates diversity and glyphosate distribution in soybean plantations and surroundings at the Yucatan Peninsula, Mexico

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Abstract summary

In the Yucatan Peninsula, Mexico, soil macroinvertebrates diversity has strongly decreased. At Campeche, although there is an important production of glyphosate tolerant soybeans, no studies have been developed to determine glyphosate and AMPA (its first metabolite) in soils, moreover no information exists of the relationship of this herbicide and soil macroinvertebrates abundance and diversity. Therefore, a systematic sampling was carried out along soybeans and not soybeans plantations in Campeche. Two hundred soil samples and soil macroinvertebrates were collected for the assessment of glyphosate, AMPA, and soil macroinvertebrates abundance and diversity. Glyphosate and AMPA were detected through LC-MS/MS. 68.7 percent of soil samples were above the limit of detection (LOD >0.05 mg.kg^{-1}), and the highest concentrations of glyphosate and AMPA were found in soybean plantations (0.9±2, 0.8±1.1 mg.kg^{-1}); glyphosate and AMPA soil concentrations from non-managed areas were significantly higher (0.3±0.9, 0.2±0.3 mg.kg^{-1}) than in mays crops, where soil macroinvertebrates abundance and diversity were the highest (513.6±754.9 ind.m^{2}, 2.5±1.8 number of taxa groups). The ratio AMPA/soil invertebrates revealed high risk for soil invertebrates in non-managed areas. Formicidae, was the dominant taxa group, while gastropods (snails) were only present in soils from mais crops and non-managed areas where glyphosate and AMPA were not detected.

Keywords: soil macrionvertebrates diversity, glyphosate, soybean plantations, Yucatan.

Introduction, scope and main objectives

Recent studies have demonstrated that glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) are even after years of application present in soils (Silva *et al.,* 2018). AMPA accumulates onsite, attached to the clay particles of the soil (Bento *et al.* 2017) and underlays the risk of offsite transport by water and wind erosion (Silva *et al.,* 2018; Rendon-von Osten and Dzul-Caamal, 2017; Yang *et al.,* 2015).

In the last decade at the Yucatan Peninsula, Mexico, the development of genetically modified glyphosate-tolerant crops (Contardo-Jara, Klingelmann and Wiegand, 2009) has led to the intensive use of glyphosate. Nevertheless, at Campeche (major producer of soybeans), there is a considerable use of glyphosate there are not studies pursued which inform about the amount of glyphosate or AMPA concentrations in soils, and the effects that this herbicide may produce to soil life. Nevertheless, previous investigations have indicated the contamination of humans by this herbicide in Yucatan (Rendon-von Osten and Dzul-Caamal, 2017), no attention has been paid to the sources and dissemination of glyphosate.

This investigation aimed at assessing the concentration of glyphosate and AMPA in soils from the soybean crops into the surrounding vegetation at Campeche. Furthermore, soil macroinvertebrates diversity and abundance were also determined.

Methodology

The study was developed at the state of Campeche, in Hopelchen, Bonfil and Calakmul municipalities. Sampling was carried out by transects in soybean plantations, mais crops and non-managed areas, before the application of agrochemicals. Thus, a total of 200 top-soil samples (0-2cm) were taken for glyphosate and AMPA determination following the methodology given by Bento *et al.* (2016), and 200 25x25x25 cm monoliths were extracted and hand-sorted for soil macrofauna collection according to the TSBF method (Anderson and Ingram 1993), from those monoliths soil was also collected for clay and soil organic matter characterization.

Glyphosate and AMPA determination

First, 0-2cm topsoil samples were immediately frizzed at -20°C, then they were air dried and sieved at 1 mm, next, 2 g of drought soil were weight per sample. Second, glyphosate and AMPA concentrations were determined by LC-MS/MS (Liquid chromatography–mass spectrometry) using an XBridge™ Shield RP C18 column (3.5-µm particle size, 150 mm in length, 2.1-mm i.d., Yang, 2016). Third, multi-level calibrations using solvent standards were implemented for glyphosate and AMPA quantification. Then, glyphosate and AMPA response data was normalized in relation to the corresponding internal standards, and corrected for any effects of ion suppression in the LC-MS/MS measurements. The limit of detection (LOD) for those components was 0.05 mg.kg-^{1}.

Soil organic matter, clay and soil invertebrates' characterization

Previous investigations have highlighted a crucial connection between clay and organic matter with glyphosate and AMPA concentrations in soil (Bento *et al.*, 2016), therefore in this study clay and soil organic matter were determined following the Mexican Norm (SEMARNAT, 2000). Soil invertebrates were characterized by their density (ind.m^{2}) and their diversity (no. species and diversity indexes) per sample.

Calculations

Indexes, glyphosate/ soil macroinvertebrates and AMPA/soil macroinvertebrates was developed in order to assess the amount of glyphosate or AMPA potentially used by the soil macroinvertebrates per m^3, expressed in µg.ind. The indexes are done by de followed equations:

1) $\text{gly/invd ratio} = (\text{gly } (mg.m^3))/\text{invd}(ind.m^3) \times 1000$

2) $\text{AM/invd ratio} = (\text{AM } (mg.m^3))/\text{invd}(ind.m^3) \times 1000$

Where gly is glyphosate, expressed in $mg.m^3$; invd is soil macroinvertebrates density ($ind.m^3$), AM is AMPA also expressed in $mg.m^3$.

Statistical analysis

First, the normality of all variables was evaluated by the levene's test, then if the data didn't follow a normal distribution, was normalized by log (10) (x+1). Second, One-way Anovas were performed in order to find significant differences among glyphosate, AMPA, soil organic matter, clay and soil invertebrates' abundance and diversity per land-use (soybean plantations, mays crops and non-managed areas). Third, a matrix of correlations was carried out to identify significant correlations among the variables.

Results

Significant highest concentrations of glyphosate and AMPA in soils were observed in soybean plantations (0.9±2, 0.8±1.1 $mg.kg^{-1}$, Figure 1a), moreover clay content was also significant highest in soybean plantations (55±12.4 percent), and organic matter was significant high in soils from soybean plantations and non-managed areas; while the significant lowest concentrations of glyphosate and AMPA were found in soils from mays crops (0.05±0.1, 0.07±0.1 $mg.kg^{-1}$), lower than in non-managed areas (0.3±0.9, 0.2±0.3 $mg.kg^{-1}$).Soil invertebrate's significant highest abundance and number of species were observed in mays crops (513.6±754.9 $ind.m^2$, 2.5±1.8, Figure 1b & Table 1). No significant correlations were observed between the concentration of glyphosate and AMPA in soils and the percentage of clay. Instead, a significant spearman correlation was found between the concentration of glyphosate and the percentage of organic matter in soils (R: 0.56 $p<0.05$). Moreover, glyphosate and AMPA concentration in soils were inverse to the number of morpho species and abundance of soil invertebrates, observed by a significant negative correlation between these parameters (R: -0.55, -0.56, -0.43, -0.44 $p<0.05$, respectively). Ants was the dominant group in this study, gastropods were the invertebrates with less presence (Table 1), only present where glyphosate and AMPA were not detected. Ratio glyphosate/invertebrates was significant high in the soybean plantations (Table 1).

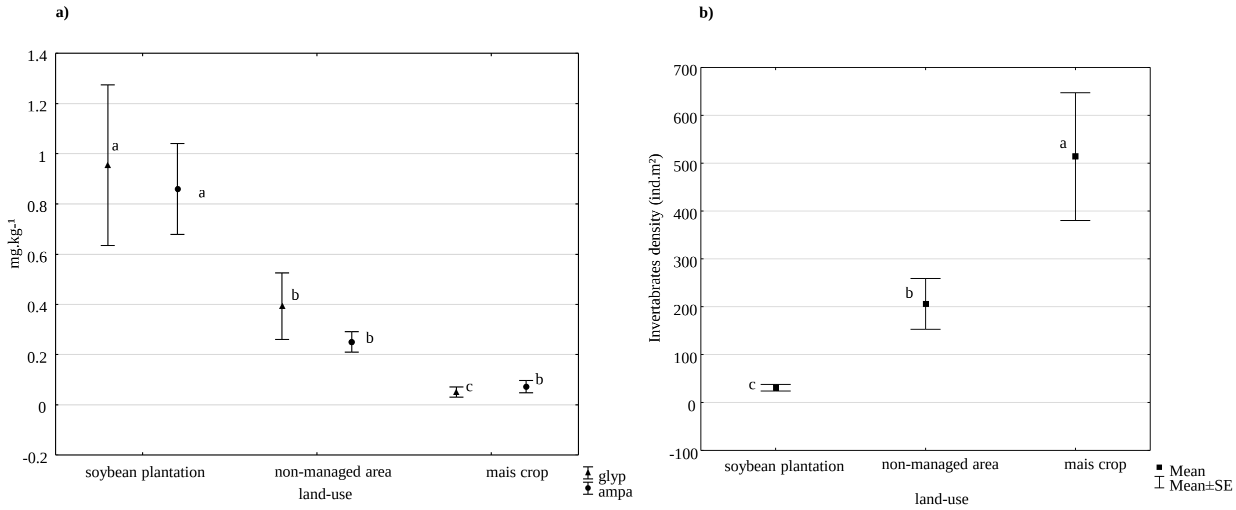

Figure 1: a) Glyphosate and AMPA concentrations in soils per land use, b) soil invertebrate's abundance per land use

Table 1: Soil macroinvertebrates and ratios gly/invd (glyphosate/ invertebrates density), AM/invd (AMPA/ invertebrates density); ew: earthworms, coleo: coleoptera; term: termites; gastero: gasteropoda; others: soil invertebrates from other soil group taxa

Fields		ants	ew	coleo	term	gastro	others	ratio gly/invd	ratio AM/invd
soybean	**Mean**	16.0	1.9	5.6	2.1 b	0 c	3.5 c	7.9 a	10.9 a
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	Maximum	272.0	48.0	64.0	32.0	0.0	16.0	95.6	96.1
	Std.Dev.	40.9	7.3	13.8	7.5	0.0	6.6	20.4	20.3
mais	**Mean**	25.3	1.0	7.8	328 a	647.9 a	38.1 a	0.2 b	0.1 b
	Minimum	0.0	0.0	0.0	0.0	75.0	0.0	0.0	0.0
	Maximum	192.0	16.0	64.0	3328.0	1850.0	250.0	1.8	0.8

	Std.Dev.	49.2	3.9	16.0	783.3	617.4	57.8	0.4	0.2
non-managed area	**Mean**	17.9	4.5	3.2	19.8 b	188.8 b	16.4 b	3.1 b	3.0 b
	Minimum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Maximum	300.0	96.0	32.0	560.0	2625.0	125.0	61.4	19.6
	Std.Dev.	44.9	15.0	8.0	79.8	579.5	27.1	9.4	5.7

Discussion

Glyphosate is a component known to harm humans (Gasnier *et al.,* 2009), soil life (Haney *et al.,* 2000) and wildlife. More attention should be paid on the application of this herbicide. In Campeche, the Yucatan Peninsula, a process of soil biological desertification is taking place, one example is the significant decrease of soil invertebrate's abundance and diversity due to different factors, where glyphosate plays a role, because its concentration is significant inverse to the abundance of soil invertebrates. In this study, it was found 0.8-2.8 taxa groups, while in other investigations conducted in the region, there were found 1-9 taxa groups in managed and non- managed areas (Huerta Lwanga *et al.,* 2008; Sanchez del Cid, 2017). Gastropods are the most vulnerable group in this study, not present in the soybean plantations, and only present in mais and non- managed soils where the herbicide was not detected. While earthworms and termites also scarce in this study were present even with low numbers at the soybean crops. Previous investigations have emphasized the use of gastropods as soil bio indicators, (Druart *et al.,* 2011), and this study can confirm their vulnerability.

Conclusions

Soil macroinvertebrates diversity and abundance in this study are significantly inverse to glyphosate and AMPA concentrations in soil. Gastropods are soil invertebrates susceptible to be indicators of soil quality in soils of the Yucatan Peninsula.

Acknowledgements

To farmers and landowners who allowed us to develop this study

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Modelling decomposition and microbial processes under waterlogging

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Abstract summary

Soil carbon modelling has mostly not described microbial processes mechanistically. Here we use a recently developed model that explicitly take microbial processes and diversity into account. We modify it using both molecular data and carbon flux data to simulate waterlogged conditions. The tests of the model indicate that the modification produce realistic results, but the tests also uncover problems with the original model. We believe this study show a good example of how molecular data can be integrated into model development to produce more mechanistic descriptions of microbial processes in carbon turnover models.

Keywords: soil carbon model, waterlogging, wetland, mechanistic model, microbial process

Introduction, scope and main objectives

Several model of terrestrial carbon turnover in the plant-soil system exist, and these models can be integrated into earth system models (ESMs) to predict the biotic feedback to climate change. One example is the Community Land Model (CLM) (Oleson *et al.*, 2010). Whilst plant processes can be described mechanistically, the description of soil and microbial processes are usually simplistic due to lack of mechanistic understanding. Additionally, most of the theory for soil process description has been developed for agricultural systems. Recently there has been some effort to implement the best available process-based knowledge on soil microbial processes into the CLM (Wieder *et al.*, 2015).

Wetlands are particularly interesting, both because they store large amounts of carbon, and because they are unique habitats (Joosten, Tapio-Biström and Tol, 2012). Historically many wetlands have been drained, but there is currently also a drive to re-wet some previously drained wetlands and bring them back to a state as close as possible to natural. It is not known if carbon content and microbial diversity in re-wetted wetlands will get back to original levels, or how fast this might happen.

Moisture has not received the same attention as temperature in carbon turnover studies, although climate change is likely to affect moisture as well. Those models that have reduced decomposition rates at waterlogged conditions, modify decomposition rate of all pools equally. However, there is good reason to believe that decomposition at waterlogged conditions is fundamentally different from at that at field capacity. The real reduction is not caused by the high water content, but by lack of oxygen. A number of other gases are produced, not just CO_2 (e.g. methane). There is also evidence that microbial utilisation of litter is different e.g. lignin cannot be decomposed without oxygen (Kögel-Knabner 2002).

The aim here is to describe the development and testing of a modified model for decomposition under waterlogging and changing water table, taking into account the best knowledge and new data about the microbial processes and diversity under waterlogging.

Methodology

The MIMMICS model was used for carbon turnover as this model take microbial processes explicitly into account to some extent (Wieder *et al.,* 2015). Microbial populations are divided into two pools r- and K-strategists and they consume litter by reverse Michaelis-Menton kinetics. Otherwise the model uses a similar approach to that used in most soil organic matter models with 2 litter pools and 3 other soil carbon pools. The version of the model used is described in Hartman *et al.* (2019). The soil moisture modifier used in MIMMICS is based on a symmetrical curve with an optimum of 1 at waterfilled pore space at about 0.5 and minimum (0.05) at waterfilled pore space at 0 and 1 (Hartman *et al.,* 2019). An inhouse version with layered soil model and timesteps of one day was used (Le Borgne, 2019).

Incubation experiments were simulated with one soil layer and no plant input during simulation. When a wetland was simulated, a version with soil layers and plant input was used. The top soil layer in the model received both the aboveground litter and some litter from roots, whilst the lower layer received plant input only from roots. Plant input was calculated by BASGRA grass growth model (Höglind *et al.,* 2016). Root input was divided into rhizodeposition and root death. Root death was assumed to be a constant fraction of standing root biomass (0.02 per day) and it was assumed to mainly structural litter. Rhizodeposition was assumed to be proportional to root growth as it is released from growing root tips, and as it consists of soluble carbon it was assumed to be only metabolic.

We attempted to fit the carbon pool distribution in the beginning of the simulation to get the gas flux observed at field capacity in Foereid *et al.* (2018). It turned out to be difficult to reproduce short term variation other than that caused by temperature fluctuations as the model was very sensitive to temperature. In addition, some of the carbon pools only change on timescales longer than the incubation. However, a pool distribution that produced reasonable output was found and used further.

To find out how microbial processes and particularly r and K strategists were affected by waterlogging, data from a number of sites with wetland restoration were examined (Verbruggen and Wilk, unpublished). Soil samples from triplets of pristine, drained and rewetted were collected in each site. The pristine was the most different from the others, and we decided that comparing drained and rewetted is the closest we get to comparing waterlogged and non-waterlogged starting from the same state. An ANOVA analysis with management (rewetted or drained), depth and site (from Poland to Wales) as factors was performed for each variable. The variables were fungi and bacteria measured by plfa, fungi measured by ergosterol (each both on weight and volume basis) and the number of copies of DNA (indication rate of growth).

The model was then tested on the data from Bergman *et al.* (1999). There peat was incubated at waterlogged and at field capacity at 15°C and 25°C and labelled glucose was added in some treatments.

The model was also run for a long time with a drainage introduced at a certain timepoint.

Results

The analysis from the microbial data analysis indicated that there were less microbes in the drained case, and more dominance by bacteria over fungi. We therefor conclude that the main difference in the waterlogged case should be that it had more fungi than at optimal water content. It is also known that waterlogging affects structural litter more than metabolic, at least lignin degradation requires oxygen (Kögel-Knaber 2002). The model was therefore modified so that the moisture modifier affected microbial utilization of structural litter which also reduces fraction of chemically protected becoming active, and death rate of K strategists only. Utilization of metabolic litter and death rate of r strategist was constant as at optimum water content.

The test with the data from Bergman *et al.* (1999) showed that the difference between waterlogged and non-waterlogged was quite realistic, but the model did not respond enough to input of glucose. Both those results and the simulation of the data from Foereid *et al.* (2018) indicate that the MIMMICS model is very sensitive to temperature.

The model predicted the evolution of soil carbon after drainage realistically. Carbon flux was high just after drainage, and it remained above that of the undrained for a long time. Carbon content decreased quickly after drainage.

Discussion

Modelling microbial processes in soil mechanistically is still in its infancy (Crowther *et al.* 2019). Here we show an attempt to use microbial data to adapt a model for waterlogged conditions. However, we also encountered problems with the MIMMICS model. It appears to be too sensitive to temperature, and possibly not sensitive enough to other factors.

Our modification of decomposition under waterlogging produced realistic results. Both in the incubation and in the larger scale simulation the difference predicted between waterlogged and non-waterlogged were realistic. As molecular data in addition to data on rate are used to adapt the model, we believe it is closer to a mechanistic description of the difference between waterlogged and optimum water content than in currently available models.

It may be a bit premature to model decomposition under waterlogging mechanistically, but we still think this study shows how microbial data can be used to adapt models.

Conclusions

We believe this work did not succeed fully because we encountered problems with the original model. Further work should focus on improving the description of microbial processes and finding more and better ways to use the vast amount of molecular data now available to adapt and test the mechanistic description of microbial processes and diversity in models.

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Monitoring soil biological quality in the Veneto region

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Abstract summary

Organisms living in the soil are highly sensitive to soil degradation. Intensive anthropogenic pressures have led to a global decline in biodiversity also in soils with consequences on their functionality. Following the QBS-ar methodology (Soil Biological Quality index), based on the analysis of microarthropods groups present in soil, the regional agency for environmental prevention and protection (ARPAV) carried out a soil quality monitoring plan in the Veneto region for ten years. The QBS-ar index is based on the concept that the higher the soil quality, the higher the number of microarthropod groups morphologically well adapted to the specific soil habitat. Through a network of 10 monitoring stations, more than 240 samples were collected in 18 land uses and more than 800 data were processed. QBS-ar index values above 150 occur in undisturbed environment, therefore it can be taken as quality threshold. In arable crops the index were found below 100, values below 50 indicate poor soil quality. Intermediate values, between 100 and 150, suggest medium biological soil quality.

In agriculture, QBS-ar index may be used to assess the effective impacts of agricultural practices on soil health.

Keywords: biodiversity, monitoring, quality index, Soil Biological Quality

Introduction, scope and main objectives

The community of organisms living in the soil is highly sensitive to soil degradation. Anthropogenic pressures have led to an overall decrease also in soil biodiversity sometimes with serious consequences on their functionality. In 2009 ARPAV (Regional Agency for Environmental Prevention and Protection) started a soil quality monitoring plan in the Veneto region; since 2012 soil monitoring activity has been systematically carried out. The aim of the monitoring plan is to investigate the soil biological quality in the region and to identify reference values according to different land uses. This can be the mean to highlight soil degradation or pollution.

Methodology

The soil microarthropod community was analysed using a soil biodiversity and quality index called QBS-ar (Soil Biological Quality based on soil arthropods, Parisi *et al.*, 2005). The QBS-ar index is based on the following concept: the higher the soil quality, the higher the number of microarthropod groups morphologically well adapted to the specific soil habitat. In "disturbed" soils, microarthropod groups morphologically well adapted tend to disappear and only those less adapted will remain (Menta *et al.,* 2018). The method consists in taking 3 topsoil cubic clods (10 cm^3 each) from every monitoring area. A *Berlese-Tüllgren* funnel was used for microarthropod extraction (with a 2 mm mesh sieve and, above all, a light source; Figure 1). In 10 days, the specimens fall from the soil into the funnel and are collected in a solution of alcohol and glycerine.

The extracted specimens were observed under a stereomicroscope. Soil organisms are classified into biological forms according to their morphological adaptation to soil environments. Degree of adaptation to soil habitat depends on the presence and combination of some morphological characters: miniaturization, anophthalmia, flat body, short antennas and short legs. Each of these forms is associated with a score named "EMI" (eco-morphological index), which ranges from 1 to 20 (depending on degree of adaptation). The QBS-ar index value is obtained from the sum of the EMI scores of all collected groups, based on the principle that is more important the degree of soil adaptation than taxonomy (Parisi and Menta, 2008). If in a taxonomic group, biological forms with different EMI scores are present, the higher value (more adapted to the soil form) is selected to represent the group in the QBS-ar index calculation. The sampling methodology requires the collection of three soil cubes at each sample site, combined into a single sample.

The averages of the number of each taxon individuals and the percentages of total were calculated. The organisms belonging to each biological taxon were counted in order to estimate the average density per square meter (ind/m^2). The number of total taxa found was also considered.

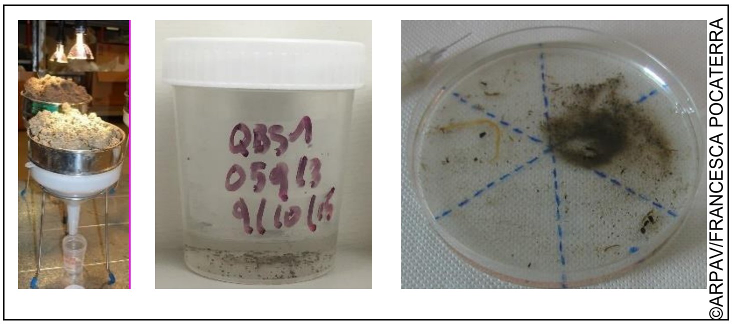

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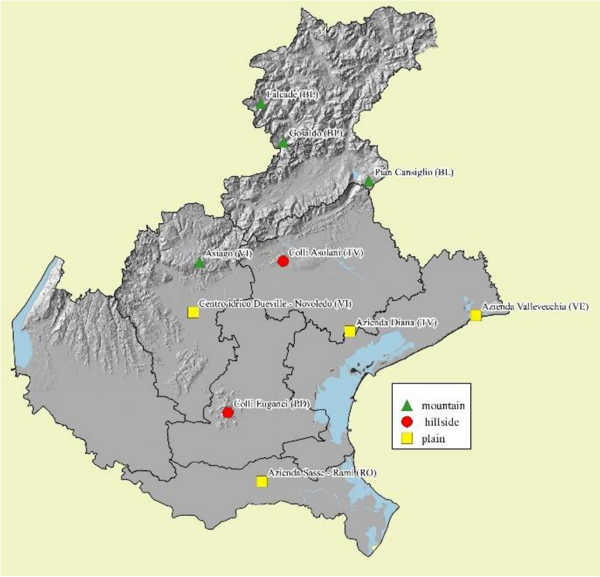
Figure 1: Extraction and microscopic reading

Since 2012, 10 monitoring stations have been set up in the Veneto region (Figure 2), 4 in plain areas, 2 in hilly areas and 4 in mountain areas. All stations are representative of the regional environment for: land use, soil characteristics, parent material and climate conditions. 18 different types of land use (crops or natural vegetation) have been studied.



Environment	**n° station**	**Land Use**
PLAIN	**4**	wheat
		rape
		corn
		soybean
		sorghum
		vineyard
		orchard
		farm tree forest
		alfalfa
		meadow
		coastal pine wood
HILL	**2**	vineyard
		deciduous forest
MOUNTAIN	**4**	meadow
		pasture
		beech forest
		spruce wood
		white fir wood

Figure 2: Monitoring stations in Veneto region, locations and land uses

At each site, information on soil characteristics was derived from semi-detailed soil maps (ARPAV, 2005 and 2020) and samples were collected for organic carbon, soil texture, electrical conductivity and pH analysis. Temperature and soil moisture conditions may influence soil fauna behaviour, so climate data have also been collected from the closest weather stations.

For each area one undisturbed sample was taken in order to measure bulk density and soil moisture.

Statistical processing have been worked out with "Statistica®" software, version 8.0. Significant differences between the land uses, in taxa abundance and QBS-ar index values, were tested using Analysis Of Variance (ANOVA). Statistical tests using parametric methods (test HSD-Honestly Significant Difference di Tuckey, test t) and non parametric (Kruskal-Wallis) were also performed to highlight statistical variability in the QBS-ar index.

Results

Plain environment

In plain areas the following crops were studied in detail: arable crops, alfalfa, meadows, vineyard, orchard (pome fruit), forest tree farming (in previous arable lands), a deciduous forest and a coastal pine forest. Analysis Of Variance (ANOVA) points out relationship between QBS-ar index and land use, post hoc analysis (Tuckey test HSD) was worked out in order to highlight differences between land uses (Figure 3, Table 1).

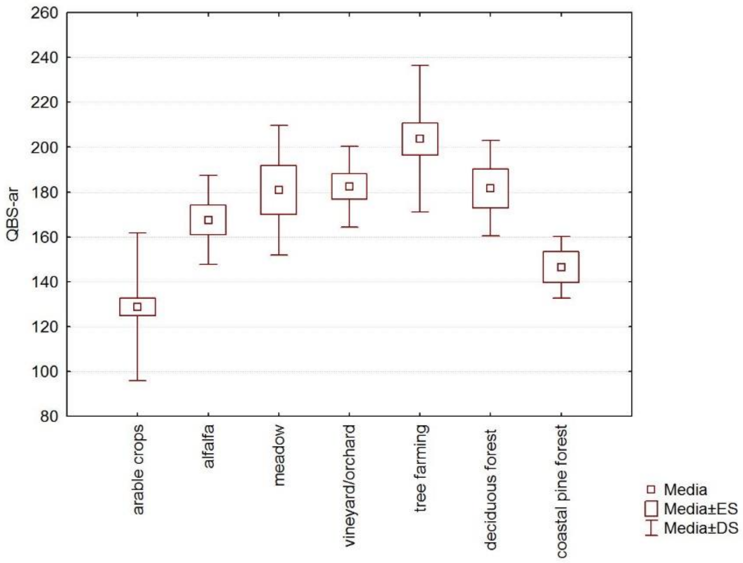


Figure 3: QBS-ar index in relation to land uses in plain areas; 2012-2018

Table 1: Post hoc, Tuckey test, differences in the QBS-ar depending on land use in plain areas

land use	arable crop	alfalfa	meadows	vineyard orchard	foreset tree farming	deciduous forest	coastal pine forest
arable crop		0,095721	**0,022506**	**0,001522**	**0,000026**	**0,040716**	0,982495
alfalfa	0,095721		0,982934	0,945157	0,14873	0,984455	0,958017
meadows	**0,022506**	0,982934		1,000000	0,796638	1,000000	0,680934
vineyard orchard	**0,001522**	0,945157	1,000000		0,700611	1,000000	0,633421
foreset tree farming	**0,000026**	0,14873	0,796638	0,700611		0,870421	0,106394
deciduous forest	**0,040716**	0,984455	1,000000	1,000000	0,870421		0,656195
coastal pine forest	0,982495	0,958017	0,680934	0,633421	0,106394	0,656195	

In red bolt significant differences (p-value>0,05)

It can be seen that among land uses, arable crops differ from all, it has the lowest QBS-ar values (between 80 and 150), whereas meadows (above 150) prove to be a good biodiversity pool (Menta *et al.*, 2011). In orchards and vineyards, the soil between the rows was grass covered therefore showed QBS-ar high values (between 150 and 200), despite heavy machinery passages and phytosanitary treatments. Forest tree farming was found to be the richest habitat (index between 180 and 220) thank to low human impacts and high biodiversity shrub and tree species.

The lowland wood (planted in the 80's on an agricultural area) examined has QBS-ar values lower than forest tree farming due to lack of herbaceous vegetation. Sandy soil texture in "coastal pine wood" is an inhospitable environment to soil fauna. Looking at microarthropod communities (density of taxa per square meter), "Mites" is the largest group, followed by "Collembola" and "Hymenoptera" (Table 2). Larvae of "Coleoptera", "Diplura" and "Chilopoda" are present in low percentages. "Pseudoscorpiones" can only be found in meadows and, rarely, in the vineyard.

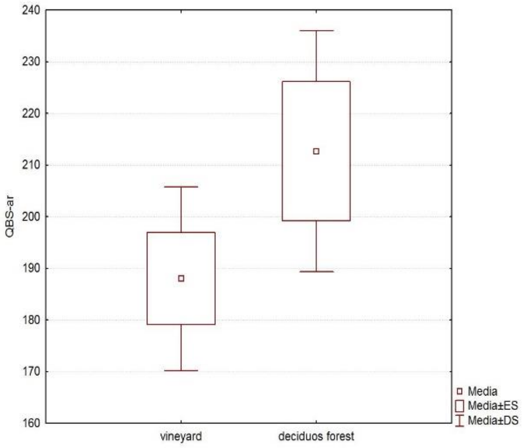
Table 2: Percentage of each taxon in arable crops and meadows

	arable crops	**meadows**
taxa	%	%
Pseudoscorpiones	**0**	**0,6**
Araneae	0,5	0,5
Acari	48,6	**49,5**
Isopoda	0,3	**2,0**
diplopoda	**1,3**	0,7
Pauropoda,	**0,9**	0,3
Symphyla	1,0	**1,8**
Chilopoda	0,7	**0,9**
Protura	**0**	**0,5**
Diplura	1,5	**2,4**
Collembola	**35,4**	17,3
Psocoptera	**0**	**0,6**
Hemiptera	0,4	**1,2**
Coleoptera	**0,7**	0,3
Coleoptera larvae	**2,2**	1,3
Hymenoptera	4,3	**12,3**
Diptera	0,8	**0,3**
Diptera larvae	**1,3**	0,2
Thysanoptera	**0**	**0,8**

The effect of some soil parameters was additionally tested (texture, pH and organic carbon): only coarser soil texture and high soil salinity were found to provide a lower biological quality.

Hilly and mountain environment

In 2018 the monitoring network was implemented with two stations in hilly areas (<700 m asl) and four stations in mountain areas. In hilly areas vineyard and deciduous forest (chestnut, maple, alder and ash) have been studied. Deciduous forest showed higher QBS-ar index (average 213) and number of arthropod, but differences were not statistically significant, probably due to the short period of observation monitoring (Figure 4). Deciduous forest with calcareous substratum (average 240) presents biological quality higher than deciduous forest with acidic substratum (average 190).



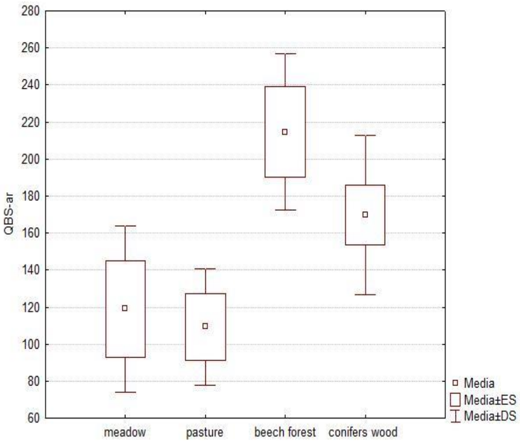


Figure 4: QBS-ar index in relation to land use in hilly areas

Figure 5: QBS-ar index in relation to land use in mountain areas

In mountain areas most common forest land uses were studied: beech forest and conifer wood (over 1100 m asl). In plain areas, meadows and pastures were considered. Despite the few data available being at an initial stage of monitoring it is quite clear that forests (values between 170 and 215) present biological quality higher than meadows (average 115) that are more "disturbed" (livestock grazing for example). Due to the acid litter, soil of spruce wood (170) is less hospitable for the microarthropod community than beech forest (215, figure 5).

Discussion

In plain areas the main factor influencing QBS-ar index is land use: arable crops have the lowest QBS-ar index, number of taxa and density per square meter. Among crops, wheat has the highest QBS-ar index (average 144), soybean the lowest (average 118), corn and rapeseed have intermediate values (average 130). These values were found for usual agricultural practices, but can be modified by the agronomic practices.

In crops less disturbed by farming processes (alfalfa, vineyard, orchard, meadow and forest tree farming) a greater porosity of soil lead to high values of QBS-ar. High biodiversity of plant species makes forest tree farming the richest habitat among agricultural land uses. Lowland woods have intermediate QBS-ar values (average 182 and lower number of taxa) compared to forest tree farming because of litter and the shadow of "high-trunk tree" (limiting the growth of grass). Coastal pine wood has QBS-ar index not very high (average 147) because of thick litter, soil sandy texture, poor undergrowth and problems regarding salinity.

In the hilly areas the highest biological value of natural environments is confirmed compared to the agricultural environment (vineyard). In the deciduous forest probably the pH of the substratum (calcareous or acid) influences indirectly the growing vegetation and directly soil fauna.

In mountain areas most common forest land uses were studied and in plain areas, meadows and pastures were considered. Forests present biological quality higher than meadows as livestock grazing limits arthropod development in soil.

Regarding vegetation, the conifer forests (spruce, white fir) have a lower QBS-ar index value than beech forest, as beech forest litter is more hospitable for microarthropod than conifer wood litter.

Future studies will be focused on how the substratum (calcareous or acid) influences the soil fauna.

Conclusions

Since 2012, the soil biological quality monitoring activity was systematically carried out in four plain area stations. In 2018 two stations were added in hilly areas (altitude between 400 and 700 m asl) and four stations in mountain areas (>700 m asl). All monitoring stations are representative of Veneto region habitats concerning land use, soil and substratum characteristics and climatic conditions.

Reference QBS-ar values have been established in different Veneto region land uses; the index was found to be helpful to highlight potential soil degradation or pollution. As reported in other studies, arable crops have the low QBS-ar values due to the environmental impact of farming. It has been confirmed that meadows are a reservoir of biodiversity. The same biological richness found in orchards and vineyards (despite the heavy machinery treading and phytosanitary treatments) is due to grass cover between rows.

In the agricultural land uses, the coexistence of different habitats has the higher protective value for biodiversity (Romero-Alcaraz and Avila, 2000); in the same direction go practices preventing landscape simplification as farming hedges and wooded areas.

To assess impact of tillage on biological quality, a study has begun on different soil working techniques like minimum tillage or sod seeding, trying to evaluate differences in biodiversity versus conventional tillage.

Acknowledgements

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GLOBAL SYMPOSIUM ON SOIL BIODIVERSITY | FAO HQ | Rome, Italy, 19-22 April 2021

Bioturbations as quality indicators of Typic Argiudolls in the Southeast of Buenos Aires, Argentina: A micromorphological approach

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Abstract summary

The aim this study is to validate a methodology for the quantification of bioturbation on thin sections of Typic Argiudolls of Argentina, for its use as soil quality indicators. Soil samples were taken from surface horizons in plots with different land uses: natural, agricultural, eucalyptus plantation, pines plantation. In each site bulk density, penetration resistance, structural stability, organic matter and pH were determined. For each land use, volume percentages of *total porosity* and *biopores* were determined in the thin sections using two methodologies, deferring on the time spent of image edition and final accuracy. In natural and forested plots the *percent* of *biopores* represented 80 percent of the total porosity, while in A plot the 40 percent, indicating higher biological activity. This activity is linked to the high content of organic matter and high structural stability. Bioturbations would be good indicators of soil quality and, in consequence the methodologies used can be an easy-to-use tool for the determination of bioporosity in soils.

Key words: biological activity, bioturbation, micromorphology, soil quality.

Introduction, scope and main objectives

Biological activity is expressed through bioturbation, which is defined as the reworking of soil components by organisms, plant roots and faunal channels (Meysman, Middelburg and Heip, 2006). There are few studies on the use of bioturbations as soil quality indicators in agroecosystems, which can be carried out through soil thin section analysis.

Typic Argiudolls of Argentina are used for traditional horticultural and agricultural production. Also, it is common to find areas afforested with exotic pine and eucalyptus species. These cultivated soils show a loss of structure, organic matter (40 percent) and clay (50 percent), and low soil biodiversity (Osterrieth, 2001). Previous studies of soil quality indicators in the area showed a decrease of aggregate stability, and an increase of bulk density and penetration resistance in cultivated sites (Alvarez, Osterrieth and del Río, 2012). There are very few references on the micromorphology of the soil porosity under different land uses, and on the relation of the soil biota whit soil structure (Alvarez, Osterrieth and Cooper, 2018).

The aim this study is to validate a methodology for the measurement of bioturbations based on the study of soil thin sections of surface horizons of Typic Argiudolls of Argentina, for its use as soil quality indicator.

Methodology

The study site is located in the Buenos Aires province (Figure 1). Four plots with Typic Argiudolls were selected: *R)* natural, *A)* agricultural, *E) Eucalyptus globulus* forest plantation, *P) Pinus radiata* forest plantation.

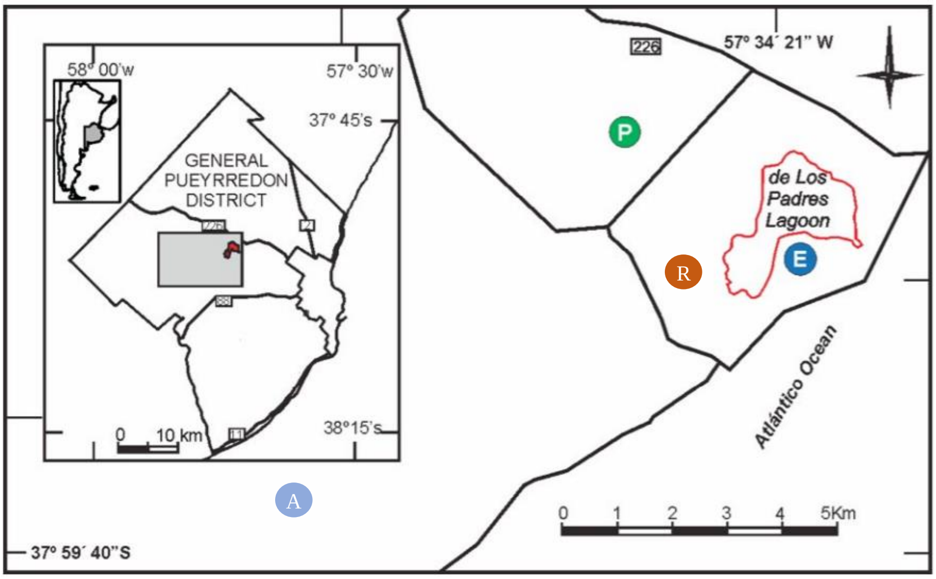

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Figure 1: Map of the location of the study area

R: reserve. E: Eucalyptus globulus forest plantation. P: Pinus radiata forest plantation. A: agricultural. Modified from Alvarez *et al*. (2020)

Five samples were randomly taken at 5cm for the determination of bulk density (BD, Blake and Hartge, 1986) and penetration resistance (PR, Bradford, 1986). PR corrected according to IC = a.Wb (Busscher *et al*., 1997). Structural stability (SS) was measured by the Hénin (1972) method. The pH was measured in a soil paste (1:1). The organic matter (OM) determination was realized by the Walkey and Black (1965) method.

Three undisturbed samples in each site were taken from the upper levels. The samples were impregnated (Murphy, 1986) and a fluorescent pigment (Uvitex OB) was added to the solution. From each impregnated block one vertical thin section, 5x7 cm was obtained. The three thin sections were scanned and edited in Corel Photo Paint ® program. From them, fields of 3.2 cm x 4 cm were analysed.

From each thin section were obtained:

Total porosity: original image was binarized, and the volume percentage of porosity was determined (Poch, 2005), using the ImageJ ® program (Rasband, 2014).

Bioporosity: in original image the biopores were identified and the bioporosity was analysed:

Methodology I (Figure 2)

In those thin sections where the biopores were easily recognizable, an irregular polygon on the outline of the biopore was drawn, using the Corel Draw ® program. In thin sections where almost all volume was bioturbated, subangular and angular aggregates were outlined in the matrix. Images obtained were binarized and the area of pores was calculated used ImageJ ® program (Rasband, 2014).

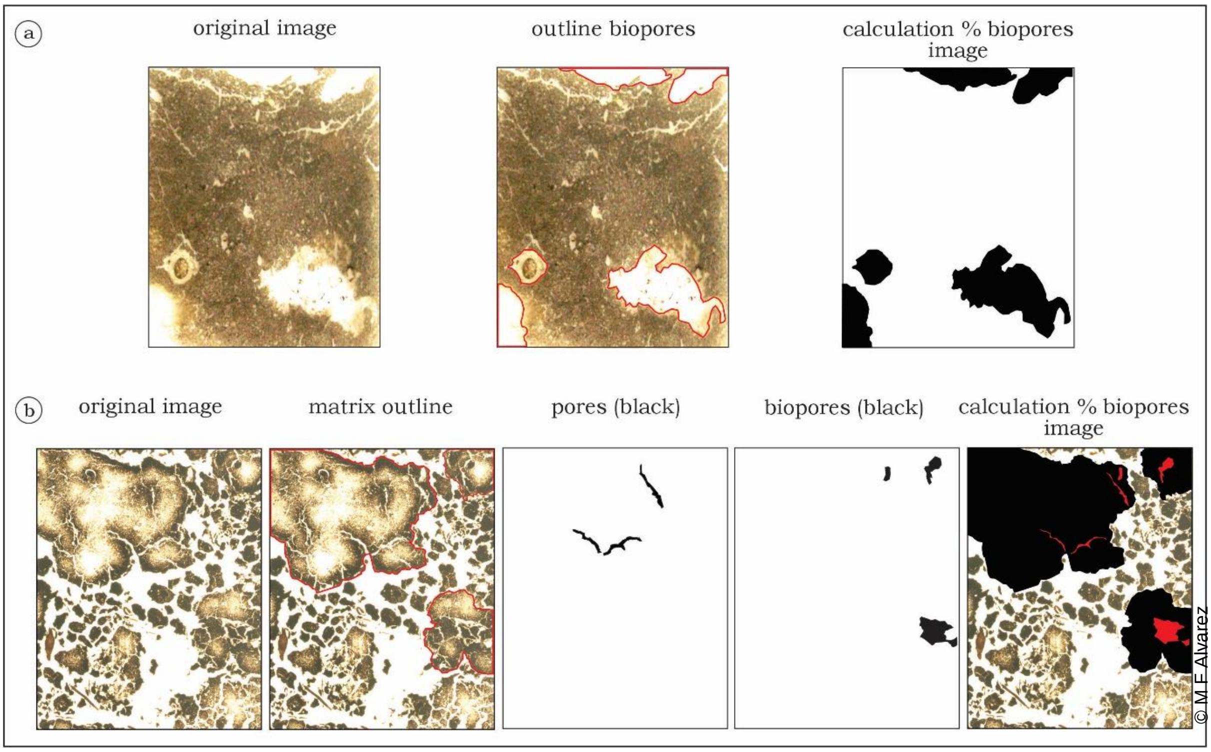

Figure 2: Methodology 1

a: determination from the outline of biopores. b: determination from the matrix outline. Image size: 3.2cm x 4cm.

Methodology II (Figure 3)

The biopores were quantified by point counting (Weibel, 1980). A 20 x 30 square grid was drawn on the image in the Corel-Draw ® program. The calculation of the surface was performed following the stereological principles of Delesse (1847).

A ANOVA, Fisher LSD test ($p<0.05$) using the Statistica ® program was performed to compare the soil properties between different land uses.

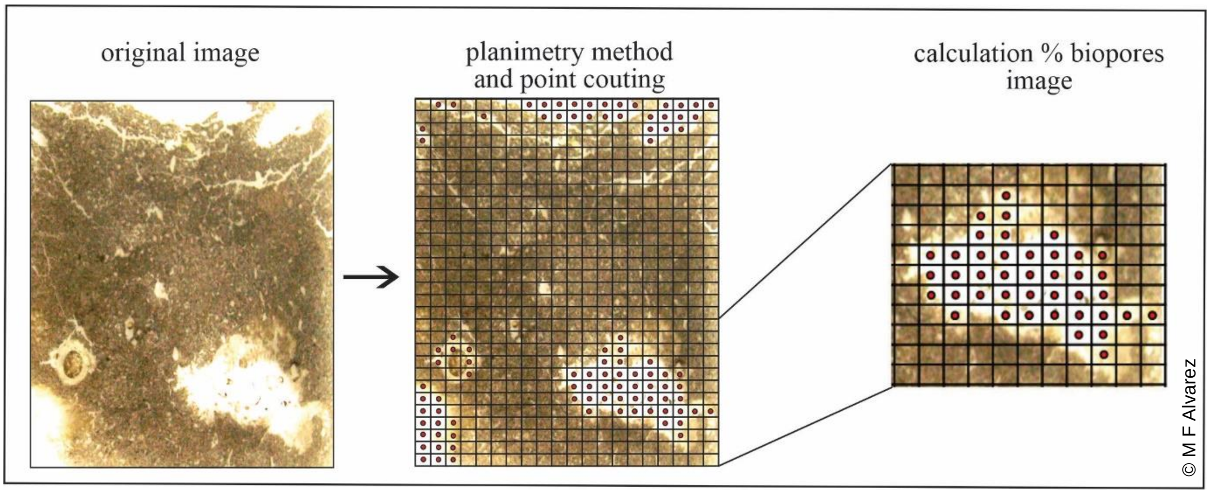

Figure 3: Methodology 2 for the calculation of percentage of biopores by the planimetry and point couting methods Image size: 3.2cm x 4cm.

Results

The E, P y R showed significantly lower values of BD than A, while PR had the opposite behaviour. These plots also showed SS values close to 100 percent, being those of A significantly lower ($p<0.05$) than the other treatments (Table 1).

The OM contents were significantly different ($p<0.05$) between the treatments, following this order: E > R > P > A.

In addition, the R had a slightly acidic pH value, while more acidic values were recorded in the other plots (Table 1).

Table 1: Average values of 5 replications of physicochemical properties in the plots studied

	Bulk density	**Penetration resistance (MPa)**	**Structural stability (%)**	**Organic matter (%)**	**pH(1:1)**
Reserve	**1.02b**	**1.16c**	**100 a**	**11.63 b**	**6.03 a**
Eucalyptus globulus forest planation	**0.73 c**	**1.83 b**	**99.85 a**	**18.90 a**	**5.09 b**
***Pinus radiata* forest plantation**	**0.91 b**	**1.79 b**	**99.38 a**	**9.99 c**	**5.25 b**
Agricultural field	**1.16 a**	**2.02 a**	**89.13 b**	**8.42 d**	**5.05 b**

Different letters indicate significant differences of the variables between plots ($p<0.05$).

R shows a granular microstructure, a high porosity made of interconnected compound packing pores, a high bioturbation degree (Figure 4 a). E and P have a crumb microstructure, high porosity formed by pores mostly as channels and vughs, also due to a high bioturbation (Figure 4 b, c). A showed a more compact structure, a lower porosity formed by planar pores and a lower bioturbation (Figure 4 d).

The two proposed methodologies for the measurement of bioporosity yielded different results (3-7 percent of the biopores) but not significant ($p<0.05$).

E, P and R plots presented significantly higher values of total porosity and biopores than A plot ($p<0.05$) (Figure 4).

In R, E and P the biopores with two methodologies represent a 80-98 percent total porosity, while A the porosity is low and the biopores only represent a 40-65 percent of the total porosity (Figure 4).

	Methodology	Reserve	*Eucalyptus globulus* forest plantation	*Pinus radiata* forest plantation	Agricultural field
Total porosity (TP)		35.43 a	42.65 a	37.49 a	14.39 b
Biopores	1	34.82 b A	41.80 a A	36.51 b A	9.23 c A
Other pores	1	3.40 b	0.85 b	0.98 b	7.89 a
Biopores/TP	1	98.28 a	98.01 a	97.39 a	64.13 b
Biopores	2	28.39 a A	32.89 a A	30.44 a A	6.11 b A
Other pores	2	7.04 a	9.76 a	7.04 a	8.28 a
Biopores/TP	2	80.13 a	81.21 a	77.11 a	42.46 b

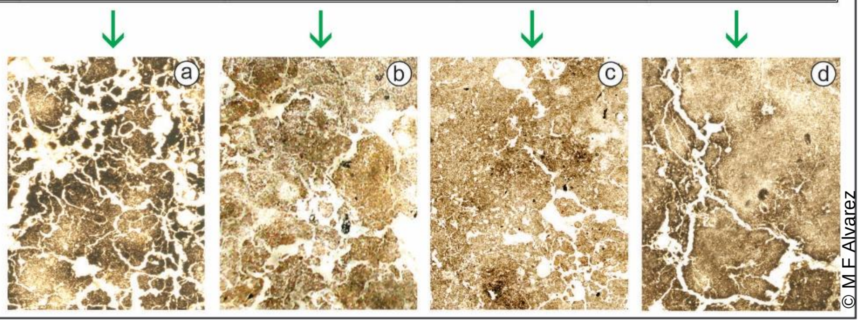

Figure 4: Average values of total porosity, biopore volume, others pores and relation biopores/total porosity percentages

Different lowercase letters indicate significant differences of the variables between plots ($p<0.05$). Different uppercase letters indicate significant differences between methodologies ($p<0.05$). a. Granular microstructure in R plot. b and c. Crumb microstructure in E and P plots. d. Fisures microstructure in A.

Discussion

The biopores of the natural and forested plots represent 80 percent total porosity. It indicates an intense fauna and root activity, reflected in a high aggregation and structuration with stable packing pores. In the agricultural plot the biopores are a 40 percent total porosity, and the rest are planar pores generated after compaction sometimes deformed by fauna and roots, which penetrate in those existing fissures generating channels.

The biological activity in the natural and forested plots is associated with the high organic matter contents. These, in turn, participate in the formation and stability of soil aggregates, which is reflected in their good structural parameters (Figure 4 a, b, c). Earthworm activity is also restricted in soils with acidic pH (Lavelle, 1997), which is reflected in a lower bioporosity of the agricultural plot.

The biological activity is also favored by the physical characteristics of these soils in a feed-back process. The root growth would be more restricted than in the other plots, since bulk density values are greater than 1 g/cm3, while penetration resistance values are higher than 2 MPa, which is also a critical value for root growth (Pagliai and Nobili, 1993).

Regarding the methodologies tested, despite the slight differences of the results, both of them can be used for measuring bioturbation as an estimator of biological activity. However, Methodology 2 requires a lower time of image edition and can be done without special software. Therefore, this technique is preferred for the volume estimation of biopores in these plots.

Conclusions

The study of bioporosity from thin sections allowed to differentiate the biological activity in soils with different uses. Reserve and forested plots had both a higher porosity and bioporosity than the soil of the agricultural plot, which was less porous and with half of the bioporosity than the rest. Hence a greater biological activity can be estimated in R, P and E, in relation to the agricultural plot. This results in a more stable and complex microstructure in natural and forested soils with respect to cultivated ones. Taking into account the results, bioturbations constitute good soil quality indicators of these Typic Argiudolls.

The proposed methodologies for the measurement of bioporosity, in particular Methodology 2, represents a contribution to thin section description and quantification, since it is a simple and nonexpensive tool when evaluating soil quality in biological terms.

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Comparative microbiology evaluation of bacterial biodiversity in rhizospheres of vegetative cover of exotic species of Australia (Eucaliyptus), native to Chile (Quillay) and "badland" soils of Chile

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Abstract summary

This research is part of the CONAF / JICA-Japan Watershed Restoration Project "Erosion Control and Forestry in Watersheds of the semi-arid zone of Chile", it has been extended 1994-2019, three 150 ha pilot areas have been located experimental and has allowed the successful development of nursery, reforestation and soil conservation.

Specifically, a micro-biological analysis of the evolution, biodiversity and quantification of bacterial strains that develop in the rhizosphere of: (1) Eroded soils (xeralf according to soil taxonomy classification), (2) Exotics coverages of Eucalyptus camaldulensis from Australia, (3) Native coverage of Quillaja saponaria endemic species of Chile.

The Eucaliptus camaldulensis, a tree native to Australia of lake albacutia and the Quillay (cryptocaria alba) endemic tree species of Chile have evolved for thousands of years adapting to the various zone-climatic edafo-climatic conditions and "genetic memory", which has led to a parallel evolution of the species bacterial soil. Key elements for bacterial adaptation have been the root exudates released by "Quillay", which allows inferring that the root exudates differ from the Eucalyptus species as they belong to a different ecological and edaphic original environment.

Bacterial biological soil crusts have demonstrated high efficacy to recover eroded and degraded soils. In this context, they contribute to the recovery and re-carbonisation of eroded soils through the induction of potential recovery processes and the generation of pedogenetic eco-system services, support and provision, in the interaction and synergy with soil and water conservation practices.

Keywords: Badland, Soil Biodiversity, Bacteria, Rhizospheres, Evolution, Vegetation Cover

General framework

This research is part of the CONAF / JICA-Japan Watershed Restoration Project "Erosion Control and Forestry in Watersheds of the semi-arid zone of Chile", it has been extended 1994-2019, three 150 ha pilot areas have been located experimental and has allowed the successful development of nursery, reforestation and soil conservation (Francke, 2019).

The project has been transformed into a project of technological transfer, by making feasible and technically and socio-economically soil conservation practices associated with reforestation techniques, nursery of tree and shrub species adapted to arid and semi-arid conditions of extreme droughts with medium annual rainfall 100 mm per year, in the framework of the mega-drought last 2 decades associated with anthropic climate change (Araya, 2015)

- Specifically, a micro-biological analysis of the evolution, biodiversity and quantification of bacterial strains that develop in the rhizosphere of:
- Eroded soils (xeralf according to soil taxonomy classification)
- Exotics coverages of Eucalyptus camaldulensis from Australia
- Native coverage of Quillaja saponaria endemic species of Chile

The biological soil crust (CBS) constitutes a complex component of the ecosystem that encompasses different organisms present in the first soil horizons and shows relative abundance where vegetation cover is scarce in arid and semi-arid ecosystems and can contribute significantly in terms of vegetative cover (Elbert *et al.,* 2012).

Despite the positive effect, the CBS react variably according to the type of vegetation and this technique can be combined with a correct reforestation procedure in degraded soils (Rivera-Espinoza and Dendooven, 2004).

Objectives

General objective

Evaluation comparative microbiological in the rhizosphere of vegetative cover of exotic species of Australia (Eucalyptus), native to Chile (Quillay) of "badland"soils in Chile.

Specific Objectives

1. Evaluate the effects of comprehensive soil restoration treatments through the application of soil and water conservation techniques.
2. Evaluate the microbiological properties of the biodiversity of bacteria in rhizospheres of vegetative cover of exotic, native species and in eroded soils.
3. Lay the foundations to propose a restoration model for the generation of ecosystem services through comprehensive approaches.

Material and method

Material

The area where the project is developed corresponds to the Metropolitan Region, in the central zone of Chile, parallel 33 ° 46´ and 34 ° 04´ South Latitude and meridians 71 ° 12´ and 71 ° 42´ West

Longitude, 38 km Direct distance from the Pacific and 120 km from Santiago. It is located in the semi-arid zone of the inland coastal dryland of the central zone of Chile in the Maipo river basin.

Methodological approach

Table 1 shows the analytical values of base fertility of eroded xeralf granitic soils of San Pedro Melipilla Chile.

Medium to low levels of organic matter are recorded, pH in the weakly acidic range, low electrical conductivity, low levels of N and P, medium and high levels of K, cation exchange capacity that varies from 1.8 (very low) to 16.5 (low).

Table 1: Analytical values of base fertility of eroded xeralf granitic soils of San Pedro Melipilla Chile

Micro-watershed	Horizont	M.O %	pH	C.E mmhos/cm	N ppm	N Total %	P ppm	K ppm	CIC C mol/kg
MC-1	Ap	2.3	6.0	0.45	5	0.065	5	75	15.2
MC-2	Ap	1.2	5.8	0.27	10	0.066	8	38	16.5
MC-3	Ap	2.8	6.0	0.48	7	0.042	4	169	8.5
MC-4	Ap	2.8	6.5	0.63	6	0.092	4	111	1.8

Methodology for soil microbiological analysis

Soil samples are obtained in the rhizosphere of native tree species ("quillay"), an exotic tree (Eucalyptus camaldulensis) and eroded soil samples to establish comparative patterns. Samples are analysed in the laboratory by PCR amplifying the 16S rRNA and subsequently, the analysis is carried out with the PREMIER software for quantitative analysis of the results (Araya, 2015)

For the microbiological study, samples of the rhizosphere of a native tree are obtained, which in this case is quillay (Quillaja saponaria), an exotic tree from Australia Eucalyptus (Eucaliptus cameldulensis) and from eroded soil (without vegetation) to establish comparison patterns.

5 samples of the rhizosphere of 2 trees (Quillay and Eucalyptus) and 5 samples of eroded soil are obtained; the 5 samples of each zone are obtained to homogenize the prospecting of samples. The methodology includes 15 samples in total, are analysed in the laboratory and the nucleotide sequences that identify the respective bacterial families are determined.

The microbiological analysis in the laboratory is carried out based on three logical and successive steps:

- Extraction and cultivation of soil microorganisms.
- Extraction and purification of microbial DNA.
- Extraction and purification of microbial DNA (Department of Biology, Faculty of science, Puerto Rico’s University).

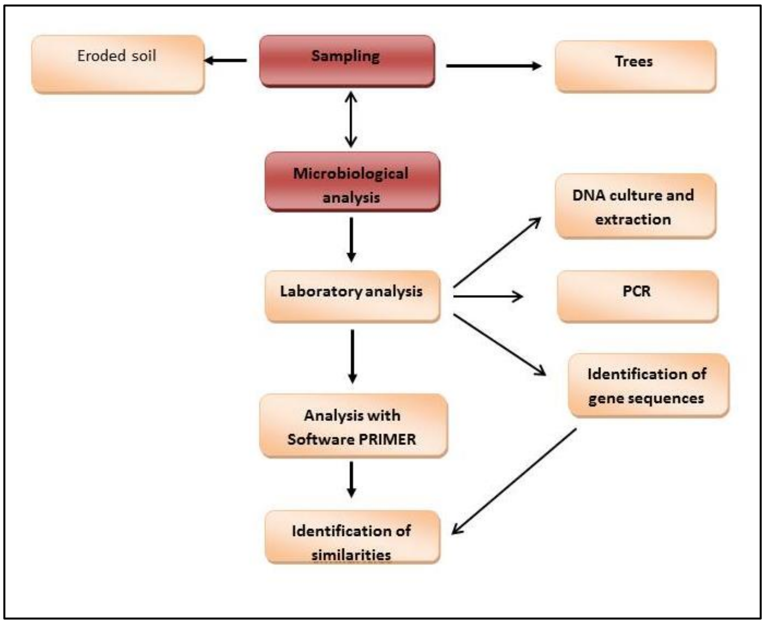
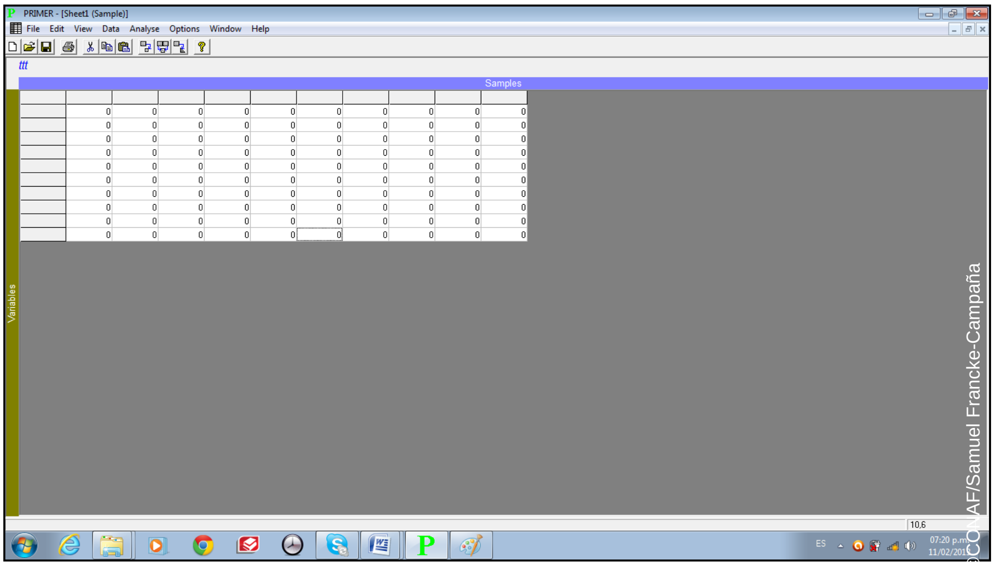


Figure 1: Applied methodological sequence

Premier software template

Multivariate analysis, software analyses information to perform statistical tests and graphical analysis of dendrograms are used. Information about bacteria genes is analysed using PREMIER software.



	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0

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Figure 2: Premier software application

The application of the PCR technique allows to establish genetic differences between one family and another according to differentiation based on the application of an RFLP. The PCR results are analysed with FIRST software, statistical test differences are established and quantified.

Presentation of results

Eroded xeralf soils with gully erosion . Microbasin 4 Control	Reforested area with Quillaja saponaria (native species of "quillay") with infiltration ditches. Micro-basin 1
 ©CONAF/Samuel Francke-Campaña	 ©CONAF/Samuel Francke-Campaña
Reforested area with eucalyptus camaldulensis, infiltration ditches and water micro-reservoir (water reservoir) microbasin 2	Red eucalyptus from Victoria Lake Australia (Eucalyptus camaldulensis)


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Evolution of vegetative coverage in the treatments applied

The evolution of hydrographic basins in the last three decades has been a process characterized by a recovery in terms of vegetative biomass and edaphic conditions, reducing soil loss in reforested areas under soil conservation treatment from 30 ton/ha/year at 100 kg/ha/year, based on evaluation of the USLE model applied to soil conservation works.

Tree biomass formed in the micro watershed under restoration according to the comparative values of NDVI (Normalized Difference Vegetation Index), between1994 and 2010 clearly show a substantial increase in biomass and plant cover Table 2.

Table 1: Microbasin description after NDVI 1990-2010

Microbasin description	Description	NDVI year 1990	NDVI year 2010	increase %
1	Afforestation	0.09	0.21	57.1%
2	Afforestation and erosion control	0.06	0.22	72.7%
3	Afforestation and erosion control	0.08	0.20	60%
4	Control witness	0.07	0.10	30%

For the period under study, the NDVI values vary from 0.09 to 0.21 in microbasin 1, from 0.06 to 0.22 in microbasin 2; 0.08 to 0.20 in microbasin 3 and 0.07 to 0.10 in microbasin 4 control without soil and water conservation treatments (Araya,2015).

During the period of study, the NDVI values are increased in the treatments that include restoration biotechnics and conservation hydrotechnics of soils and waters in high granitic soils and severely eroded by past and intensive agricultural use, unsustainable use of wheat for 150 years at the time of the California “gold rush”.

The quantitative increase in NDVI, clearly indicates that ongoing restoration processes contribute significantly to an increase in vegetative biomass, the recovery and re-carbonisation of eroded soils.

Bacterial microbiological evaluation according to bacterial similarity matrix

Based on the micro-biological evaluation, the amplification of the 16S rRNA is achieved, obtaining communities and not bacterial species. The gene amplification allows establishing the existing variability at the level of bacterial communities in the eroded granitic soils (xeralf) of San Pedro Melipilla, without distinguishing the precise species that coexist there.

Of the 15 samples, 9 achieve acceptable levels of measurement, samples of 54 genes of 16S rRNA are obtained, and the similarity matrix is presented in Table 3.

Table 2: Bacterial Similarity Matrix

	1x	**3x**	**5x**	**6x**	**7x**	**9x**	**12x**	**13x**	**14x**
14x	52.385	60.982	36.250	61.331	61.744	63.350	80.620	72.478	
13x	55.013	63.623	42.357	56.332	57.020	57.916	76.525		
12x	57.471	63.227	42.481	63.654	61.322	60.311			
9x	48.218	58.758	36.706	73.710	79.280				
7x	51.907	62.965	37.461	69.597					
6x	53.151	53.606	44.912						
5x	66.167	51.673							
3x	64.157								
1x									

The soil samples prospected from the rhizosphere of quillay, eucalyptus and eroded granitic "badland" soil (xeralf), establish that there is selectivity in the bacterial populations that inhabit the roots of these trees (Araya,2015)

The results obtained from the bacterial analysis of the soils of the study area show that the population of bacteria is substantially modified when a soil is eroded.

In the same way, the conformation of the rhizosphere of exotic trees (Eucalyptus camaldulensis changes compared to native forests (Quillaja saponaria), is significant in population changes and in the ecology of the rhizosphere.

According to the NMDS (non-metric multidimensional scaling) of bacteria, communities exhibit high similarity when they grow in the same environment. In this case, the bacterial communities of the rhizosphere of eucalyptus, quillay and bare (eroded) soil are significantly different depending on the vegetation cover (attached figure 3).

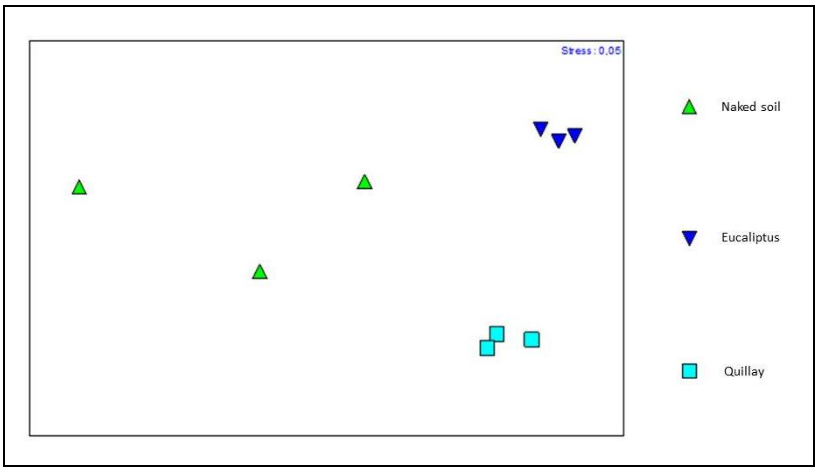

Figure 3: NMDS of bacteria

This implies that the eco-systemic conditions at the root level change widely from one species to another. The quillay as a native species is in water balance between what is available in the soil matrix and the consumption by evapotranspiration.

Evaluation of the degree of genomic variability of the bacterial edaphic communities

Figure 4 indicates the degree of genomic variability between the bacterial communities in the soils of the study area

Samples that have a point in common correspond to similar bacterial communities; This is established by the similarity between the nucleotide sequences of 16S rRNAs. The 9 samples have a high diversity, which is visualized in the bifurcation of the tree dendrográm.

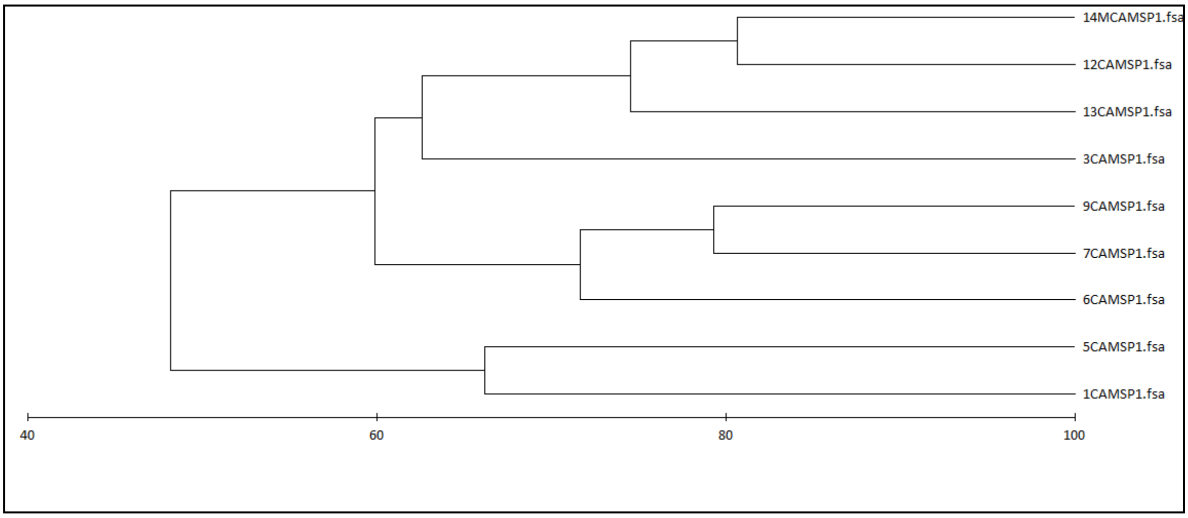

Figure 4: Dendrogram of bacterial microbial communities "genomic variability" of the soils of San Pedro Melipilla Chile

Microbiological evaluation of bacterial activity according to similarity test

Table 3: Percentages of similarity and contribution according to bacterial species

Type of cover	Abundance average (%)	Similarity average (%)	Similarity Sd average (%)	Contribution%
Badland xeralf	66.12	26.41	3.33	43.54
Soil of eucalyptus (exotic species)	62.72	20.94	10.11	28.22
Soil of quillay (native species)	51.30	18.93	9.32	24.73

According to the similarity test, species 92 contributes 43 percent in eroded soil, it constitutes the most abundant bacterial species with 66.1 percent of the soils under study. 9 species represent the sample and 6 of them represent 84.3 percent (Araya, 2015).

On the other hand, in soils with eucalyptus it is observed that the bacterial species (92) contributes 28.2 percent of soil biodiversity and records an abundance of 62.7 percent. 15 species represent the sample and constitute 90.7 percent.

In soils with "Quillay", species 92 contributes 24.7 percent and records an abundance of 51.3 percent, a lower contribution and abundance is observed when compared with eroded soil substrates and eucalyptus. 13 species represent 90.3 percent of the sample.

There is a smaller quantity of species and, therefore, a greater selectivity on the part of the rhizosphere of the "Quillay". This typifies in fact that the selectivity of the quillay at least in bacteria is greater because it probably has fewer species and abundance does not achieve abundance when compared to the soils mentioned.

According to a similarity test, the bacterial communities of the eucalyptus rhizosphere are significantly different from bacterial communities of native species of "Quillay".

Conclusions and recommendations

The Eucaliptus camaldulensis, a tree native to Australia of lake albacutia and the Quillay (cryptocaria alba) endemic tree species of Chile have evolved for thousands of years adapting to the various zone-climatic edafo-climatic conditions and **"genetic memory"**, which has led to a parallel evolution of the species bacterial soil.

Key elements for bacterial adaptation have been the root exudates released by "Quillay", which allows inferring that the root exudates differ from the Eucalyptus species as they belong to a different ecological and edaphic original environment.

Bacterial biological soil crusts have demonstrated high efficacy to recover eroded and degraded soils. In this context, they contribute to the recovery and re-carbonisation of eroded soils through the induction of potential recovery processes and the generation of pedogenetic eco-system services, support and provision, in the interaction and synergy with soil and water conservation practices.

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Comparison between soil biodiversity at Rio da Garça (degraded watershed) and Ribeirão Arrependido (preserved watershed)

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Abstract summary

The aim of this work was to evaluate soil biodiversity at a preserved watershed, Ribeirão Arrependido, with a degraded watershed, Rio da Garça, using the indicators: biodiversity, soil loss by erosion under different types of vegetation cover, measured by water quality index. The two sub-watershed evaluated are part of Rio do Peixe watershed, which has a total area of 1 million hectares. Rio da Garça watershed has an area of 14 thousand ha, it is located close to the source and Ribeirão Arrependido watershed has an area of 5,000 ha and it is located downstream of Rio da Garça watershed, having independent drainage networks. Remaining natural vegetation at these watersheds is composed of Seasonal Semi-deciduous Forest. Indicators analysis of soil loss and water quality were made for: Turbidity, Suspended Solids, Phosphorus and Organic Carbon. Vegetation cover and soil use influenced watersheds soil biodiversity and water quality, being favorable to Ribeirão Arrependido watershed, proving that soil preservation and vegetation cover improvement are beneficial to water production and soil biodiversity at watersheds.

Keywords: watershed, vegetation cover, soil biodiversity, water quality, Turbidity, soil conservation.

Introduction

The most efficient way to evaluate soil preservation is the use of watershed as a study unit, because; at the watersheds that the chemical, physical and biological soil processes occur, in addition to water balance. These chemical, physical and biological soil characteristics are part of these processes and are closely related, that is, if one of them degrades, the other will also undergo degradation. A watershed-monitoring program, aimed at assessing biodiversity related to soil loss and water quality, to achieve this success, should not be carried out for each indicator in isolation. The indicators analysis must be carried out under a context of land use and management in addition to water quality, vegetation cover aspects, soil health and environmental degradation can be good indicators (Attanasio, 2004). According to Castro (1989), who studied similar landscapes near Marília city, stated that the region was deforested more than 80 years ago and, until then, Tropical Latifoliated Forest with tree species such as *Cedrela fissilis, Aspidosperma polyneuron, Gallesia integrifolia, Euterpe edullis* predominated. Forests are considered ideal environments from ecological balance point of view, since in them the mass and energy balance is zero or minimal, while their replacement by another use leads to an increase in outputs, or energy loss (Bruijnzeel, 1990). In Marília region, primitive vegetation is very restricted, located mainly on rugged slopes, whose use for agricultural activities is not feasible due to the steep slopes (Kronka *et al.*, 1993). In this context, urban environment can be considered the opposite of forest use, while the riparian vegetation preservation and application of soil conservation practices would be management forms that aim to reduce the imbalance caused by the change in land use. Knowing water quality values parameters in a region, at watersheds with different uses, is an indicator of environmental balance, being a useful tool for the rational and sustainable management of available resources (Spera, Nizoli and Honda, 2011).

According to Interliche *et al.* 2004, who studied a watershed in Piratinga city, SP, with predominant soil: Red Yellow Ultisol, diversity of epiedaphic fauna is extremely diminished in these areas, and Collembola order is more sensitive to soil uses and management. Additionally, the presence of free-living nematodes in natural environments or with less impact on the soil is an indication of these systems balance, which has been altered by the use and inadequate soil management.

The aim of this work was to evaluate the soil biodiversity by comparing a preserved watershed (Ribeirão Arrependido) to a watershed (Rio da Garça), using as indicators: biodiversity, soil loss by erosion, under different types of vegetation cover, measured by water quality index. The measurement of soil losses and sediment input to the river were assessed by water quality indicators assessed by Turbidity, Suspended Solids, Phosphorus and Organic Carbon, measured by periodic water analysis (Vischi Filho *et al.*, 2020)

Methodology

The work was carried out at two sub-watersheds, components of Rio do Peixe watershed, which has a total area of 1 million hectares. Rio da Garça watershed has an area of 14 thousand ha, it is located close to source. Whose exit is at coordinates: S22 ° 20'5.35 ", W49 ° 54'39.99", Vera Cruz city, SP, Brazil and Ribeirão Arrependido watershed has an area of 5 thousand ha and it is located downstream, with exit at coordinates: S22 ° 19'5.41 ", W50 ° 01'16.23", in Marília city, SP, Brazil (Figure 1.).

Figure 1: Study area location. Brazil, São Paulo State. Rio da Garça and Arrependido watersheds

The two watersheds have an independent drainage network for sample collection purposes. The predominant soil, at the two watersheds, is Abrupt Red-Yellow Ultisol and Litolic Entisol. The remaining natural vegetation at these watersheds is predominantly composed of Seasonal Semi-deciduous Forest, Arboreal Formation and Secondary Vegetation of Seasonal Forest. The water sample collection for soil loss and water quality indicators analysis: Turbidity, Suspended Solids, Phosphorus and Organic Carbon were carried out at the exit of each watershed. Soil biodiversity was evaluated on soil under Natural Forest and Pasture, using the methodology proposed by Islam and Weil (1998) for microbiological analysis, the methodology of Storer and Usinger (1971) for edaphic fauna and Jenkins (1964) for nematodes.

Results and discussion

Remaining natural vegetation at these watersheds is predominantly composed of Seasonal Semi-deciduous Forest, Arboreal Formation and Secondary Vegetation of Seasonal Forest. The city with the most remaining native vegetation is Marília, with 11,031 ha. Ribeirão Arrependido watershed, with a total area of 5,000 ha, is located in Marília and has an area of natural vegetation of 2,058 ha and 9,799 ha of pasture. Rio da Garça watershed, with a total area of 14,076 ha, has an area of remaining natural vegetation of 1214 ha, it is located in Vera Cruz city, has 1,783 ha of remaining natural vegetation and 2402 ha of pasture.

The soil biodiversity assessed in the area covered by natural forest and in the pasture area, vegetation cover predominant in the two watersheds, are shown at Table 1.

Table 1: Evaluation of soil fauna under vegetation cover: cultivated pasture and natural forest

Soil Vegetation cover

	Pasture			Natural Forest		
Soil Fauna	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	Nematodes					
Phytopathogens	12	0	2	4	8	4
Youth	50	12	16	12	8	5
Free life	16	1	3	6	0	0
Total Nematodes	**78**	**13**	**21**	**21**	**16**	**9**
	Microbiological					
CBMS (mg kg^{-1})	350,0	202,6	291,5	339,4	424	312
C-CO$_2$ (mg kg^{-1})	85,4	64,9	78,1	52,1	84	22
qCO$_2$	0,2	0,3	0,2	0,2	0,2	0,1
Cmic/Corg	6,2	3,6	7,5	2,5	3,3	4,2
	Edaphic fauna					
Blatodea	0	0	0	1	1	1
Coleoptera	20	44	34	38	14	32
Collembola	30	34	12	8	38	24
Dermaptera	0	0	0	1	1	1
Diplopoda	0	0	0	0	2	1
Diptera	1	0	0	4	1	5
Hemiptera	0	0	0	0	1	1
Homoptera	0	0	0	0	0	0
Hymenoptera	17	28	35	41	31	36
Isoptera	0	0	0	13	7	7
Lepidoptera	0	0	0	0	1	1
Orthoptera	1	1	1	8	7	7
Quilopoda	0	0	0	1	1	2
Larvas	0	0	0	0	0	0
Acarina	1	2	2	3	0	0
Aracnidea	0	0	0	2	0	0
Gastropoda	0	0	0	0	0	0
Oligoquetas	0	0	0	0	1	0
Total Fauna	**70**	**105**	**80**	**120**	**92**	**118**

*** Adapted from Interliche *et al.*, 2004. R1, R2, R3 = collection point. CBMS = Carbon in microbial biomass. CO in CBMS = Organic Carbon in microbial biomass. qCO2 = Metabolic quotient**

Observing Table 1. It can be inferred that: among individuals of edaphic fauna, Coleoptera, Collembolas and Hymenopterans predominated. On soil under Natural Forest, the number of individuals found was higher than that of pasture. As for the microbial activity, it was higher in the soil under Natural Forest and the number of nematodes was higher on pasture area, which demonstrates the balance of soil on the area covered by natural forest.

At Rio da Garça Watershed, the results obtained with changes in soil and water conservation management practices regarding the improvement of vegetation cover and water quality, occurred by minimizing erosion processes and reducing sediments carried to the watercourse and water quality indicators have proved that. The Water Turbidity evaluated from 2000 to 2007, considered as before the completion of watershed rehabilitation work, presented average values of 192 NUT (Nephelometric Units of Turbidity), against the average values of 104 NUT, evaluated from 2008 to 2016, after carrying out the work at the watershed, a 54 percent drop in average values. The suspended solids evaluated from 2000 to 2007, presented average values of 297 mg l^{-1} (milligrams per litre), against the average values of 132 mg l^{-1}, evaluated for the years 2008/2016, a 44 percent drop in average values. The phosphorus evaluated from 2000 to 2007, presented average values of 0.18 mg l^{-1} (milligrams per litre), against the average values of 0.15 mg l^{-1} evaluated for the years 2008/2016, a 83 percent drop in average values. The Organic Carbon evaluated from 2000 to 2007, presented average values of 9.60 mg l^{-1} (milligrams per litre), against the average values of 5.19 mg l^{-1}, evaluated for the years 2008/2016, a 54 percent drop in average values (Vischi Filho *et al.*, 2020).

At Ribeirão Arrependido watershed the results obtained with the preservation of native forest and also with the changes in soil and water conservation management practices and improvement of water quality occurred by minimizing erosion processes and which were proven by water quality indicators.

The Turbidity of the evaluated water presented, average values of 2.47 NUT, evaluated for from 2008 to 2016, which compared to average values of Rio da Garça Watershed for the same period, had a 97 percent drop in the average values, that is, Arrependido watershed water is 97 percent higher than Rio da Garça watershed water in this regard. Suspended Solids presented average values of 128 mg l^{-1} (milligrams per liter), evaluated from 2008 to 2016, which compared to the average values of 132 mg l^{-1} of Rio da Garça watershed for the same period. It had a 3 percent drop in the average values, that is, the water of Arrependido watershed has a quality 3 percent superior to the water of Rio da Garça watershed at this aspect. The Total Phosphorus presented the average values of 0.02 mg l^{-1} (milligrams per litre); evaluated for the years 2008/2016, against the average values of 0.15 mg l^{-1} of Rio da Garça watershed, for the same period and there was an 86 percent drop in average values in this regard. Organic Carbon presented the average values of 9.60 mg l^{-1} (milligrams per litre), evaluated for the years 2008/2016, against the average values of 7.49 mg l^{-1} of Rio da Garça watershed for the same period, a 22 percent drop in average values in this regard.

Conclusions

Microbial activity was higher on soil under the Natural Forest and the number of nematodes was even greater on the pasture area, which demonstrates the soil balance on the area covered by natural forest.

The edaphic fauna found, Coleoptera, Collembolas and Hymenopterans predominated and on the soil under Natural Forest, the number of individuals found was higher than that of the soil under pasture.

The water quality indicators used proved better water quality of Ribeirão Arrependido watershed, preserved, in relation to the degraded water of Rio da Garça watershed.

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Developing a systematic sampling method for earthworms in and around deadwood

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Abstract summary

There is increasing evidence that earthworm species which live in alternative habitats such as deadwood can be missed by traditional sampling methods, leading to false classifications regarding species distributions and conservation status. Resolving the current lack of a systematic and quantitative methodology for surveying earthworms in microhabitats such as deadwood may also lead to valuable insights into earthworm species ecologies. The aim of this research was to develop and trial a systematic method for surveying deadwood-associated earthworms. Sampling of earthworms within soil, deadwood and soil beneath deadwood was carried out across oak forest stands. Results show that in oak woodlands, omitting deadwood microhabitats from earthworm sampling can lead to incorrect estimates of total earthworm species richness, abundance and biomass. The systematic method presented should be considered as additional and complementary to traditional sampling protocols, to provide a realistic estimate of earthworm populations in woodlands. Adopting this quantitative approach to surveying the biodiversity value of deadwood may enable forest management practices to more effectively consider ecological and conservation impacts.

Keywords: Earthworms, Coarse Woody Debris, Deadwood, Microhabitat, Deciduous Woodland, Oak, Soil, Sampling method

Introduction, scope and main objectives

Deadwood is an important habitat in forest ecosystems; acting as a substrate and shelter for invertebrates, including earthworms (Bunnell and Houde, 2010). Whilst deadwood colonisation processes have been described, very few studies exist which have investigated earthworm: deadwood interactions (Hendrix 1996; Zuo *et al.*, 2018). Furthermore, there is increasing evidence that earthworm species which live in alternative habitats to soil (e.g. micro-habitats such as decaying wood) can be missed by traditional quantitative sampling methods (Schmidt, Shutenko and Keith, 2015; Römbke, Blick and Dorow, 2017). These research gaps are likely due to the current lack of a systematic and quantitative methodology for surveying earthworms and other invertebrates in microhabitats such as deadwood. Resolving this methodological absence may lead to valuable and fundamental insights into earthworm species ecologies and the ecological importance and benefits of deadwood in forest systems. The main objective of this research was to develop and trial a systematic method for surveying deadwood-associated earthworms.

Methodology

The sampling method was trialed across 12 spatially-independent forest stands of common oak (*Quercus robur*), in Alice Holt Forest in England, UK. A square plot of 10 x 10 m was marked out within each forest stand (Figure 1). Each plot was first surveyed for the total volume of coarse deadwood greater than 10 cm in diameter (Bastrup-Birk *et al.*, 2007). From the total available deadwood per plot, five pieces of any size and decay class were randomly selected and sampled for earthworms. This involved relocating the deadwood onto a sheet, and immediately digging a soil pit (0.1 m^2 area and 10 cm depth) where the deadwood had been laying, then excavating this soil onto a separate sheet to hand-sort for earthworms. Whilst processing soil, the deadwood was routinely observed for escaping earthworms. To each pit, 5 litres of mustard suspension vermifuge (concentration of 50 g mustard powder to 10 litres water) was applied to the pits to extract deep-burrowing earthworms, and the pit observed for 15 minutes. Moisture and temperature measurements were taken in soil beneath the deadwood using a delta-T theta probe and a standard kitchen thermometer. Soil samples were collected from the excavated soil for chemical analysis. Soil was then replaced, and attention turned to sampling the deadwood.

Deadwood diameter and length was measured, and the tree species and decay class were estimated based on the criteria of Hunter Jr (1990). This consisted of a 1 to 5 ranking system, whereby 1 is least decayed (freshly fallen) and 5 the most advanced stage of decay (complete incorporation of the deadwood into the soil profile). Deadwood temperature was measured by placing the thermometer beneath any bark present. Any moss and loose bark were removed and inspected for earthworms, and the remaining wood was dismantled and inspected. Organo-mineral accumulations beneath loose bark were collected for chemical analysis. Deadwood was returned to its original location once sampled, with moss and loose bark replaced.

Additionally, five soil pits (0.1 m^2 area and 10 cm depth) were dug in each plot, the soil excavated onto a sheet and hand-sorted for earthworms. Mustard vermifuge was applied to all soil pits and earthworms collected as described above. Soil samples were taken for chemical analysis, and moisture and temperature measurements were taken in the top 10 cm soil adjacent to each pit. Soil bulk density and soil moisture content were analysed by oven drying at 105 °C for 24 h, and soil pH measured in water suspension. All collected earthworms were preserved in 80 percent ethanol, had preserved mass recorded and were identified using the key of Sherlock (2018). Statistical models (described in-text) were applied to data on earthworm abundance and biomass, species richness and diversity, using R Studio statistical software.

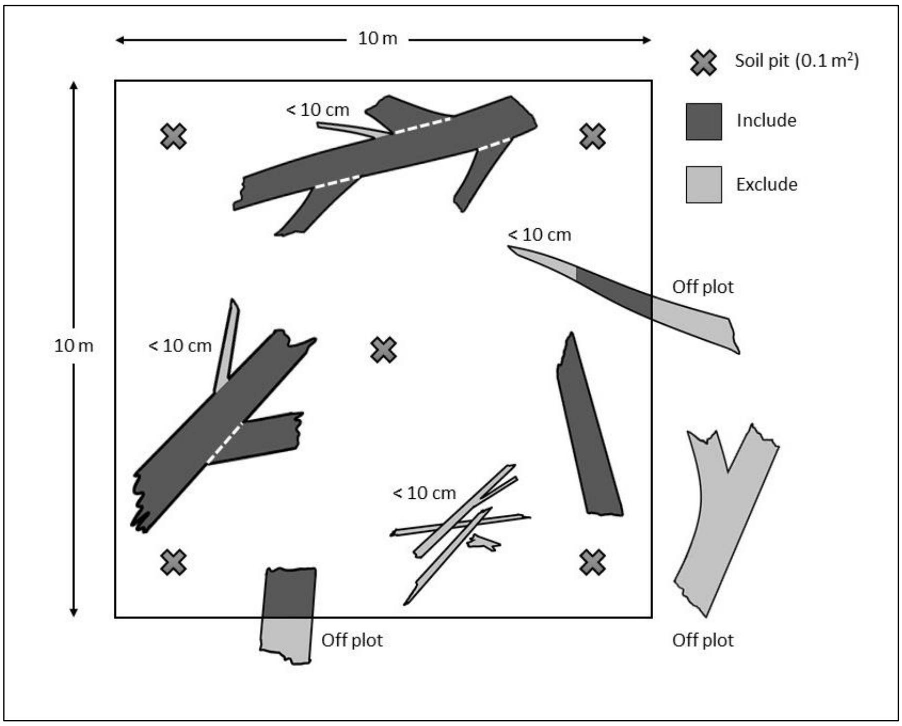

Figure 1: Layout of the sampling method in a 100 m^2 forest plot (adapted from Spetich, 2007)

Dashed white lines within deadwood indicate sections divided into separate pieces. All deadwood ≥ 10 cm in diameter and within the plot were measured for total length and midpoint diameter (dark grey), and five randomly selected pieces sampled for earthworms within the deadwood and in the soil beneath (0.1 m^2 soil pits). All deadwood < 10 cm in diameter or outside the plot were excluded from the survey (light grey). Five standalone 0.1 m^2 soil pits (indicated by crosses) were sampled for soil-dwelling earthworms. Source: Ashwood *et al.* (2019).

Results

In total 1,012 earthworms were collected, representing 13 species. Total earthworm species richness varied by habitat type, with seven species found in deadwood, eleven species within the soil beneath deadwood, and twelve species found in open soil (Table 1). One species, *E. fetida*, was found exclusively within deadwood and one species, *A. rosea*, was found only in open soil. Earthworm species diversity (Shannon-Wiener H) did not differ between the three habitat types.

Table 1: Abundance (individuals m^{-2}, ± SD) of earthworm species found in the three habitats surveyed, arranged alphabetically by scientific name following the nomenclature of Sherlock (2018)

Earthworm species	Habitat		
	Soil	Deadwood soil	Deadwood
Allolobophora chlorotica	19.5 ± 23.8 a	9.8 ± 13.9 a	0.6 ± 1.3 b*
Aporrectodea caliginosa	2.2 ± 3.9	1.0 ± 2.9	-
Aporrectodea longa	0.8 ± 2.9	0.2 ± 0.6	-

Aporrectodea rosea	0.5 ± 1.2 †	-	-
Bimastos eiseni	0.2 ± 0.6 a	0.3 ± 0.8 a	1.9 ± 2.3 b**
Bimastos rubidus	4.5 ± 7.5	6.5 ± 7.8	2.8 ± 3.1
Dendrobaena attemsi	8.8 ± 25.3	6.7 ± 17.8	0.9 ± 2.4
Dendrobaena octaedra	16.8 ± 23.8	12.5 ± 17.2	3.2 ± 4.6
Dendrobaena pygmaea	0.3 ± 1.8	0.2 ± 0.6	
Eisenia fetida	-	-	0.2 ± 0.5 †
Lumbricus castaneus	0.3 ± 1.2	0.3 ± 1.2	-
Lumbricus rubellus	19.2 ± 10.5 a	13.8 ± 9.9 a	1.6 ± 1.4 b***
Octolasion lacteum	0.2 ± 0.6	0.5 ± 1.7	-
Total abundance (Ind. m^{-2})	102.0 ± 63.8 a***	21.33 ± 15.0 b	21.18 ± 10.1 b
Total biomass (g m^{-2})	23.8 ± 9.1 a***	5.0 ± 2.8 b	2.6 ± 1.3 b

Different letters indicate significant differences, * p <.05, ** p <.01, *** p <.001, Kruskall-Wallis non-parametric ANOVA, n = 12. † Unique species to this habitat, - species absent from this habitat. Source: Ashwood *et al.* (2019).

Total earthworm abundance (individuals m^{-2}) and biomass (g m^{-2}) was significantly greater in open soil than in soil beneath deadwood and within the deadwood ($p < .001$) (Table 1). There was an effect of habitat type on the abundance (individuals m^{-2}) of three earthworm species: *B. eiseni*, *A. chlorotica* and *L. rubellus* (see Table 1). The proportion of adult earthworms (percentage of total population) was significantly lower within deadwood than in soil beneath deadwood and in open soil ($p = <0.001$), and vice versa for juveniles (Figure 2). Habitat type influenced soil moisture content (percentage), with significantly greater moisture content in the organo-mineral accumulations beneath deadwood bark than both soil habitats ($p <.001$). Deadwood was also around 1°C warmer on average than the surrounding soil. The deadwood surveys contributed an additional mean 81 earthworms and 209 g earthworm biomass per plot, equivalent to 1 percent and 9 percent of the estimated total earthworm abundance and biomass data respectively per 100 m^2. Sample-based species rarefaction curves indicated that a maximum sampling effort of 8 deadwood samples is required to capture the total earthworm species richness within a plot.

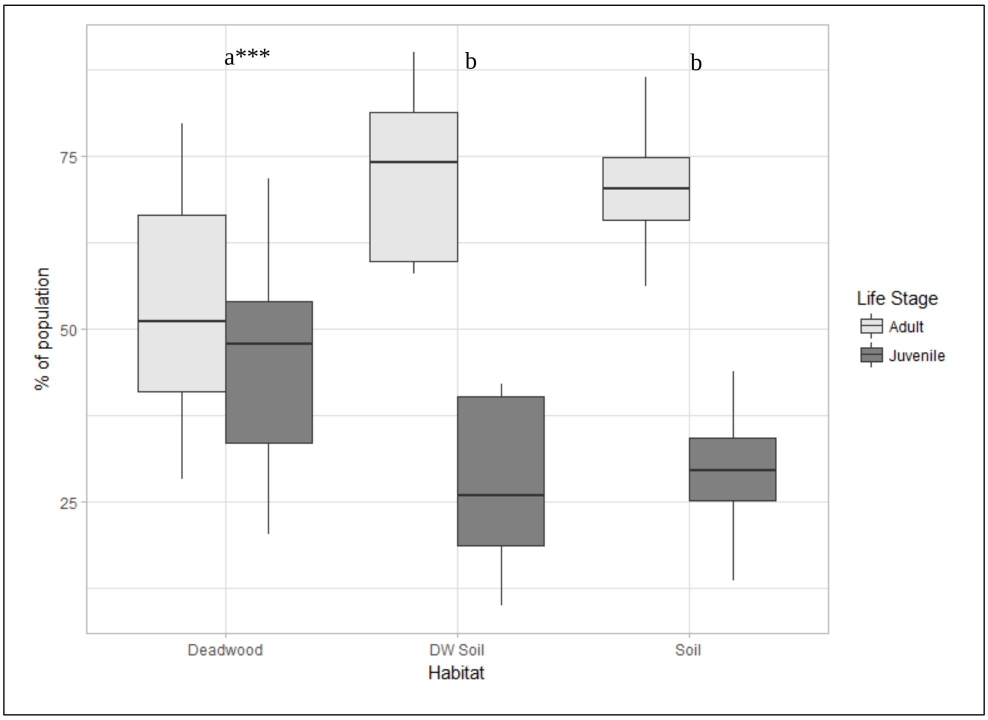

Figure 2: The proportion (% of total abundance) of adult and juvenile earthworms in the three habitats surveyed

DW Soil = Soil beneath deadwood. Generalized Linear Mixed Model (GLMM) with logit link and binomial errors, followed by Chi-squared test, *** p <.001. Source: Ashwood *et al.* (2019).

Discussion and conclusions

Our research demonstrates that omitting deadwood microhabitats from woodland earthworm sampling can lead to underestimates of total earthworm populations and species richness. We also found that the soil beneath deadwood supports much reduced earthworm populations compared to open soil, and a significantly greater proportion of juvenile earthworms dwell within the earthworm communities of deadwood, where temperature and moisture conditions were more favourable. Thus, woodland-based earthworm research which does not account for microhabitat effects may well provide inaccurate population estimates. Based on our results, the systematic earthworm surveying methodology presented cannot replace traditional soil pit sampling alone but should be considered as additional and complementary, to provide a realistic estimate of earthworm populations in woodland systems. As well as improving quantitative earthworm data, such holistic sampling may enable the gathering of fundamental knowledge on different earthworm species life histories and ecological roles (Römbke, Blick and Dorow, 2017), as well as the collection of fine-scale data on other ecologically important invertebrates. Adopting this approach may enable forest management practices to more effectively balance commodity production against the ecological and conservation importance of deadwood retention.

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EUdaphobase – European soil-biology data warehouse for soil protection

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Abstract summary

Assessment of soil biodiversity, i.e., within the context of national and EU directives and policies, must be based on proper baseline and threshold values as well as reliable tools derived from existing supra-regional data on species' occurrences. Throughout Europe, abundant soil biodiversity information exists, whose common potential has yet to be explored or collated together. The COST Action EUdaphobase is therefore developing an existing soil-biodiversity data infrastructure into a pan-European soil-biodiversity data warehouse, establishing a comprehensive knowledge- and database of high scientific and data-management quality for Europe.

This data warehouse will not only aggregate data on species distributions throughout Europe (like other data repositories), but will combine this data with accompanying environmental metadata and species' functional traits, providing a vastly increased value of data re-use for ecological evaluations. Based on the needs of stakeholders and end users, the Action will evaluate and test modern biodiversity modelling approaches for their implementation in semi-automated decision-support tools for soil-biodiversity assessments. A coordinated approach towards assessment of soil health in terms of soil biodiversity and ecosystem services will ensure more efficient and knowledge-based assessment of soil biodiversity, quality and health.

Keywords: Soil biodiversity, Europe, data harmonisation, data warehouse, FAIR principles, data re-use, assessment tools

Introduction, scope and main objectives

European authorities and stakeholders urgently need reliable tools for monitoring and evaluating the environmental condition of soils, particularly within policy assessment in context of national and EU directives. Many soil functions leading to ecosystem services (ESS) are biotically driven (Turbé *et al.*, 2010), so that soil protection requires coordinated efforts for the evaluation of soil biota throughout Europe (Dunbar, Panagos and Montanarella, 2013). However, without proper baseline data and reliable tools for soil-state assessment, it is currently difficult to efficiently address such goals (Glæsner, Helming and de Vries, 2014). Procedures for assessing soil biodiversity as well as establishing baseline values and current states must be based on existing data (Ramirez *et al.*, 2015), preferably accumulated from national or local databanks. International biodiversity databases such as GBIF, PREDICTS or DiSSCo do contain information about species and their distributions, but cannot provide focussed representation of the current status of soil biodiversity. More importantly, they are not operational for ecological assessments or advancing decision-support tools, as they do not include crucial environmental metadata.

The COST Action EUdaphobase is further developing a soil-biodiversity data infrastructure into a pan-European soil-biodiversity data warehouse, establishing a comprehensive database and knowledge portal of high scientific and data-management quality. An aim of the infrastructure is the use of such data in publicly available decision-support instruments for effectively addressing EU-level policy goals concerning soil protection. The focus of the Action is on creating the structures, capacities and procedures necessary for developing an open, publicly available data warehouse for Europe-wide soil biodiversity data and assessment tools. The goal is to establish such a supra-regional pan-European data and knowledge infrastructure, providing both soil-biological data and instruments to EU and national institutions, science and private/public stakeholders for understanding, protecting and sustainably managing soils and their biodiversity and functions.

Methodology

The Action’s infrastructure is based on the EUdaphobase soil-biology data-warehouse platform that includes data from diverse sources (literature, scientific research data, monitoring data, museum collections, etc.) (Burkhardt *et al.* 2014). As opposed to classical data repositories, this data warehouse not only collates, but also harmonizes and integrates heterogeneous data sets, combining biotic occurrence data with georeferenced sites of occurrence and methodological and environmental metadata, and renders all this data reusable for further analyses.

The work plan of the Action follows the basic logical model of information flow from data import by providers, through data curation and harmonization in the data warehouse, to data queries and analyses by end users of data (Figure 1). To ensure highest possible data congruence and data-provider acceptance, the Action is agreeing upon standardized terminologies, implementing international standards for data harmonisation (i.e., AgroVoc standards; EUNIS and CORINE systems, etc.). A first key activity has been the development of an easy-to-use, flexible tool for data upload, harmonization and import into the data platform, allowing data providers to upload their data and metadata as is, without having to reorganize it to fit standard data structures. Data quality-control procedures developed for national data platforms are being expanded into international protocols and an international review board for taxonomic and technical quality control will be established.

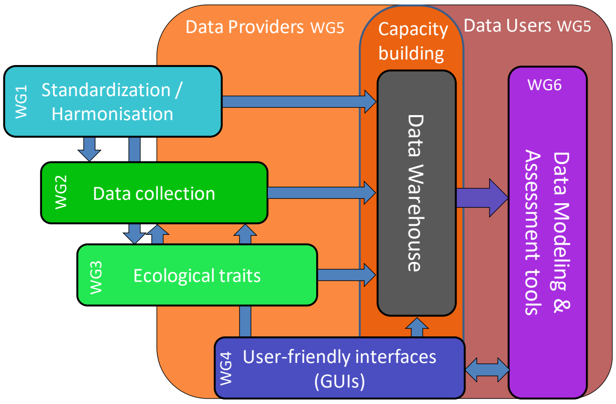

Figure 1: Work plan of the COST Action EUdaphobase

A focal approach is to include morphological, response and effect trait data for soil-dwelling species collated from Action participants' national databases and integrated into the EUdaphobase data infrastructure. The goal is to combine such trait data with observational data on species distributions as well as with indispensable environmental metadata to gain insight into site-specific functional relationships in soils. Available European, national and remote sensing (i.e. satellite) data will be identified that can fill gaps in environmental metadata.

Innovative procedures to operationalize assessments of the state of soil biodiversity will be developed. Specific Action workgroups will work with regional, national and European stakeholders to identify their data-use and -analysis needs. Based on this, specific analytical tools will be developed for applied uses of policy, management and regulatory agencies. These open-access tools of the data warehouse will recognize and visualize (i.e. on maps) the contribution of soil biodiversity to soil functioning related to soil type, use and management practices as well as determine and delineate soil ESS, baselines and set the basis for forecasting changes thereof.

Results

The EUdaphobase consortium presently includes over 70 participants from ca. 30 pan-European countries. The EUdaphobase infrastructure is based on the Edaphobase platform, which uses developed methodologies for integrating biodiversity and environmental data from diverse sources, such as the Essential Biodiversity Variables (Kissling *et al.*, 2018), the INSPIRE guidelines for soil metadata (https://inspire.ec.europa.eu/), DataCite metadata standards for DOI data citations or the DarwinCore tool for biodiversity-data exchange (Wieczorek *et al.*, 2012). Edaphobase implements the FAIR principles (Findable, Accessible, Interoperable, Reusable; Wilkinson *et al.*, 2016) and offers DOIs for data publication.

Edaphobase can currently accept over 600 different variables relating to data source, taxa, sites, soil properties, climate, methodologies etc. The minimal data set has been defined as taxonomic names ("species"), geo-referenced site information and sampling dates ("what", "where", "when"), whereby recommended are furthermore (meta)data on habitat types, land-use and soil parameters as well as sampling and determination methodologies and persons "under what environmental conditions", ("how" and "by whom"). A data policy and data-sharing agreement regulates open access while ensuring the intellectual property rights (IPRs) of data providers as well as implementing recent European data-protection legislation.

Current data-import software can import text, Excel or Access files into the data-warehouse infrastructure. After registering data-provider information, the software records metadata on the data source, the included sites and environmental parameters. Subsequently, the provider's data is mapped to the Edaphobase data structures, converting nomenclatures and vocabularies where necessary. Finally, the software performs basic (pre-import) quality controls. To ensure high-quality data in the infrastructure, further (peri-import) manual quality-control procedures are being tested, and the data provider can (post-import) control the data in the data warehouse before it is opened to public access.

APIs are currently being planned to network Edaphobase with existing trait databases, e.g. BETSI (Hedde *et al.*, 2012) and EcoTaxonomy (Potapov, Sandmann and Scheu, 2019), and procedures are being developed to link trait data to species' sites of occurrence. Edaphobase currently offers basic descriptive data-analysis tools, such as distribution maps (differentiable according to species or habitat/environmental parameters), species' niche-space analyses as well as expected species composition for specific site conditions (Hausen *et al.*, 2017), implementing procedures for automatically querying data from the database for statistical analyses and models. More detailed prognoses of soil biodiversity (distribution maps as well as site-specific point scales) based on species-distribution models are currently being developed. Based on these software procedures, the Action is conceiving more advanced tools similar to, or in conjunction with, decision-support tools such as the recent Soil Navigator (Debeljak *et al.*, 2019).

Discussion

Throughout Europe abundant soil biodiversity information exists, whose common potential has yet to be explored. However, a lack of consensus on taxonomic classification or standardised vocabularies renders their interoperability difficult. A number of recent European projects on soil-biodiversity evaluation (i.e., ENVASSO, EcoFINDERS, LANDMARK) offer cautious optimism that a common knowledge base can be achieved for key soil-organism groups. To solve these problems, instead of organising another round of harmonised large-scale and costly sampling campaigns to generate *new* data, the EUdaphobase Action is establishing procedures for collecting, curating, quality-checking and harmonizing *existing* data. Thereby, the Action aims towards a pan-European data *warehouse* (not a *repository*) specifically structured to integrate different datasets for common data re-use and *post-hoc* synthesis (cf. Inmon, 2005).

End users request procedures for evaluating questions of soil quality relevant for their specific domains. Tools, e.g. for determining biodiversity baselines and thresholds, must use available data relating taxa to sites of occurrence and to the environmental conditions of those sites. By focussing on including environmental metadata, the European data and knowledge warehouse will allow the conception of such evaluation tools for applied uses by policy makers, management and regulatory agencies, consultants (SMEs), NGOs, etc.. Furthermore, the inclusion and combination of species functional traits will allow insight to be gained, through these tools, into site-specific functional relationships in soils and to predict the state of biotically driven ecosystem services.

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Unexpected microbial functions in agricultural soil decontamination from PCB by SMS Spent Mushroom Substrate (SMS)

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Abstract summary

Polychlorinated biphenyls (PCBs) are hazardous soil contaminants for which a bio-based technology for their recovery is essential. The objective of this study was to validate the exploitation of spent mushroom substrate (SMS), a low or null cost organic waste derived from the industrial production of the edible mushroom *Plerotus ostreatus*, as bulking agent in a dynamic enhanced soil landfarming pilot plant, treating ten tons of a historically contaminated soil (9.28 ± 0.08 mg PCB/kg soil dry weight) and designed for the recovery of contaminated agricultural soil. The SMS was exploited at an amendment ratio compatible with the regular practices for the management of agricultural soil. After eight months of incubation, 94.1 percent depletion was recorded. A positive correlation between the increase in bacterial biodiversity and PCB depletion was observed. A molecular approach was adopted for both a taxonomical and functional analysis of bacterial biodiversity and an increase in Actinobacteria and Firmicutes metabolism was positively correlated to PCB depletion. The possible role of bacterial laccase activity was also envisaged. The exploitation of SMS in enhanced landfarming practices resulted to be a valuable management strategy for the re-utilisation of an organic waste deriving from food industry to recover PCB contaminated agricultural soils.

Keywords: PCB, Spent Mushroom Substrate, soil bioremediation

Introduction, scope and main objectives

Pollution from PCBs is particularly dangerous especially when agricultural areas are of concern. PCBs, in fact, can reach humans through the food supply chain. Biological (bioremediation) treatments for the recovery of polluted matrices from PCBs represent the only way forward, in terms of environmental and economic sustainability, to solve widespread contamination of these dangerous contaminants.

PCB detection in the environment is still ubiquitous due to their high chemical stability and affinity for hydro-phobic organic solids (Girvin and Scott, 1997). PCB actually can be frequently recovered also in agricultural soils. Bacterial PCB biodegradation in natural compartments has been reported (Pieper and Seeger, 2008). Generally, PCB congeners with four or more chlorine atoms undergo bacterial anaerobic reductive dechlorination. Lower-chlorinated PCB congeners are subject to cometabolic aerobic oxidation mediated by dioxygenases, encoded by the bphA gene family (Furukawa and Fujihara, 2008). Fungal

PCB biodegradation has also been reported. Fungi with the capacity to transform several PCB congeners in liquid medium were described (Tigini *et al.*, 2009). A few studies investigated fungal transformation capacity in soils. Pleurotus ostreatus produces ligninolytic enzymes described for their capacity to transform a plethora of waste substrates including PCBs (Gayosso-Canales *et al.*, 2012). The basidiomycete is also an edible mushroom whose industrial cultivation is affected by the production of significant amount of spent mushroom substrate (SMS), a lignocellulosic cellulosic matrix, that has to be disposed of, creating a bottleneck in the production chain for mushroom farmers (Chiu *et al.*, 2000). The exploration of new applications for re-utilisation of SMS are desirable. In this context, our aim was to verify the efficiency of SMS from *P. ostreatus* as a bulking agent in enhanced landfarming practice for the decontamination of historically PCB contaminated soil by the exploration of actual agronomical practices: the amendment of up to 10 percent in organic substances for improving soil productivity. The laccase enzymatic activity and the levels of transcription of different bacterial biphenyl dioxygenases were determined.

Methodology

The soil derived from an area in the north of Italy. The texture of the soil was sandy-loam (38 percent silt, 52 percent sand, and 10 percent clay) with total phosphorous, 1.6 percent; total organic carbon, 2.3 percent; total nitrogen 1.5 percent; pH 7.2; PCB contamination up to 9.28 ± 0.08 mg PCB/kg soil dry weight (dw). Any contamination by total petroleum hydrocarbons and polycyclic aromatic hydrocarbons was detected. In a pilot scale experiments a total of 10tons of contaminated soil was mixed in a tank (length x width x height,4,2 × 1,8 × 1,2 m) with 10 percent on a weight base ratio of the SMS. The tank was prepared in March and closed in November and managed as a dynamic enhanced landfarming plant. Collection of representative samples for chemical (PCB content), molecular (taxonomical and functional molecular markers) and biochemical (laccase activity) data was performed as described in Di Gregorio *et al.* (2016) every two months. Analyses for PCB quantification were performed on 15 PCB congeners including six indicator PCBs that are the set of PCBs used to estimate total PCBs to simplify analytical approaches (Babut *et al.*, 2009). Total RNA from soil was purified using the MoBio RNA power soiltotal RNA isolation kit (MoBio Laboratories Inc., USA) following themanufacturer’s instructions. To produce the cDNA template for PCR amplification of the bphA genes and the ribosomal 16S and 18S retrotranscripts, reversetranscription was performed on the total community RNA extracted,using the RNase H activity-less RevertAid premium reverse tran-scriptase (Fermentas, Lithuania) according to the manufacturer’s in-structions. Primers used for the reverse transcription and the quantification of the transcripts for bphA, the total 16S rcDNA and the total 18S rcDNA are listed in (Siracusa *et al.*, 2017). Taxa specific 16S rcDNA primers were used for quantification of the Actinobacteria, Acidobacteria, α- and β-Proteobacteria, Bacteroidetes and Firmicutes. The qPCR reactions were carried out as described in (Pfaffl, 2001). The fractional copy numbers of the amplified genes were calculated as the ratio between the normalized amplification levels of the gene in SMS amended soil and in soil amended with autoclaved SMS and the normalized amplification levels in soil not amended with the SMS. Laccase was extracted from soil samples according to Lang, Eller and Zadrazil (1997) and its activity was measured by the oxidation of 2,2′-azino-di(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Niku-Paavola, Raaska and Itävaara, 1990).

Results

The SMS substrate amendment was mandatory to determine PCB depletion and in particular the 94.1 percent of the total in 8 months. However the soil bacterial community resulted to be also involved in the depletion of the contamination and the assumption derives principally by the observation that if it is true that the depletion of the contamination might be at the base of the recovery of a microbial diversity in soil, in this case the increment in microbial biodiversity was associated to the increase in metabolic activity that are related to PCB depletion such as the increase in the expression of bphA gene isoforms for the bacterial depletion of PCB. At the same time, bacterial laccase activity might be induced and responsible for the depletion of PCBs.

In fact, a positive correlation between a PCB depletion in soil and higher numbers of metabolically active bacterial candidates with particular interest to specific taxa (Firmicutes and Actinobacteria) was observed. Actinobacteria contain well-known PCB degraders like Corynebacterium spp. described as transcribing bphA genes. Thus, the increment in metabolically active Actinobacteria in the presence of the SMS and the concomitant PCB depletion, suggests their involvement in the process. In relation to Firmicutes, the taxon is known to contain species that are involved in the dechlorination of PCB congeners. Both Firmicutes, and Actinobacteria, were previously described as possible bacterial dominant taxa capable of lignocellulose degradation in soils and contaminated matrices. As previously assessed, a positive correlation between the production of a lignocellulosic battery of enzymes and the degradation of recalcitrants in the environment was broadly reported. In this context, the involvement of Actinobacteria and Firmicutes in determining the level of laccase activity measured during the experimentation cannot be excluded. In fact, in contrast to a constant level of laccase activity here recorded, a decrease in the fractional copy number of the metabolically active fungal candidates was observed during the experimentation. This evidence and the previously reported assessment that Firmicutes and Actinobacteria might be capable of lignocellulose degradation in soils, offers new perspective of interpretation of the role of bacteria in PCB degradation in environmental matrices.

Discussion

The reuse of not dangerous supply chain wastes, especially from the food sector, in the context of the circular economy, might be the one of the best options to sustainably recover resources. The use of SMS for the remediation of soils contaminated with PCBs therefore might represent an excellent option in the management systems currently available, allowing the achievement of significant decontamination objectives.

Conclusions

In the frame of the sustainability of the proposed approach, which is inspired by the philosophy of the circular economy, the results here obtained showed both that the SMS is exploitable as a biostimulant bulking-agent for PCB depletion in soil and that it can be exploited at a percentage of amendment, 10 percent on fresh weight basis ratio, compatible with standardized agronomical practices adopted for the improvement of soil quality by mixing with compost or lignocellulosic matrices. At the same time result obtained showed that the increment of the biodiversity of microbial specimen, indigenous to environmental matrices, is correlated to a functional biodiversity that can be actually unexpected with reference with already described and consolidated microbial processes, known to be involved in the maintenance and recovery of the soil resilience.

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The Italian skill network of soil biological quality assessed by microarthropods' community

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Abstract summary

In December 2017, during the 42nd Congress of the Italian Society of Soil Science (SISS), the working group of the QBS-ar index (Soil Biological Quality based on soil arthropods) was established. The aim of this group is to create a network of skillfulness on the well-known and widespread QBS-ar index, conceived by Vittorio Parisi in 2001. The index allows to easily assess soil biological quality analysing the presence of soil dwelling microarthropods. The working group is hosted by SISS and, at present, accounts 57 members expert in this methodology working in 14 different Italian Region, mainly in research public institutions but also in the national System of Environmental Protection Agencies and private bodies. Without any own funds, it already realized 5 workshops and a public seminary at the national scale, a database containing more than 500 files concerning QBS-ar, a SWOT analysis here reported, and a public tender for the graphical Logo.

Keywords: edaphic biodiversity, mesofauna, soil quality assessment, soil management effects, bioindicators

Introduction, scope and main objectives

Since December 2017, the national working group on Soil Biological Quality Index (QBS-ar) aims to be a co-ordination reference for the development, implementation, and standardization of the QBS-ar proposed by Parisi in 2001. QBS-ar provides an assessment of soil microarthropod communities in relation to their peculiar soil adaptation level and can potentially vary between 0 (biological vacuum) and more than 300 (high biodiversity). The method was widely applied throughout Italy at different local levels and it is attracting increasing interest also at the international level (Menta *et al.*, 2018), as it provides fast and reliable assessment of the local soil communities of microarthropods. The working group members work in 14 different Italian regions (Figure 1) and share its achievements twice a year. The 4th workshop, performed on the occasion of the World Soil Day (December 5, 2019) was followed by a public seminary, to disseminate its topics and results, as well as to raise awareness among various stakeholders on the functional importance of soil biodiversity.

In this regard, soils host an immense and still unknown reservoir of organisms that performs pivotal ecosystem functions and services, e.g. soil formation, nutrient cycling and pest control (Pascual *et al.*, 2015). However, an ever-increasing number of studies have shown that anthropogenic activities such as land use changes, compaction, pollution etc., can greatly affect soil living communities, limiting and/or even halting their capacity to provide these critical functions. For these reasons, soil bioindicators are highly effective in to assessing soil functioning and the level of its disturbance (Gardi *et al.,* 2013). In this regard, various soil taxa have been proposed as indicators of soil health and, among them, soil microarthropods (Stone *et al.*, 2016). In order to provide a robust evaluation of the performance of soil bioindicators, it is strategic to develop and standardize appropriate methodological tools to measure (in a harmonized manner) microbial and faunal diversity and thus, to make possible the comparison across different data sets and studies. Several recent EU projects (e.g. ENVASSO, EcoFINDERS, Excalibur etc.) approached these tasks and contributed some ISO standards (Philippot *et al.*, 2012). Nevertheless, most of the developed standards provide insight about the analysis of abundance, structure, and activity of soil microorganisms and, only a few are available for the faunal component of soil. In this regard, ISO standards have been developed for sampling earthworms, enchytraeids, nematodes, macroinvertebrates as well as microarthropods but, in this case, primarily taking into account the most abundant taxa of Collembola and Acarina, and not the less abundant and well-adapted (euedaphic) taxa of Pseudoscorpionida, Protura, Symphyla, Diplura, Palpigrada etc. as instead proposed by the QBS-ar index (Parisi *et al.,* 2005).

In this scenario, the main aim of this network will be to contribute to the correct application and dissemination of this index. In particular the network aims to: i) guarantee the correct QBS-ar use in each application phase everywhere, allowing comparison between sites; ii) create synergies among researchers applying QBS-ar index in soil monitoring programs and projects; iii) gather dataset and publication to promote knowledge in soil microarthropods communities; iv) develop a standardized protocol of QBS-ar application for different climatic zones; v) promote short training courses for beginners or experts; vi) help users to solve troubleshooting during identification.

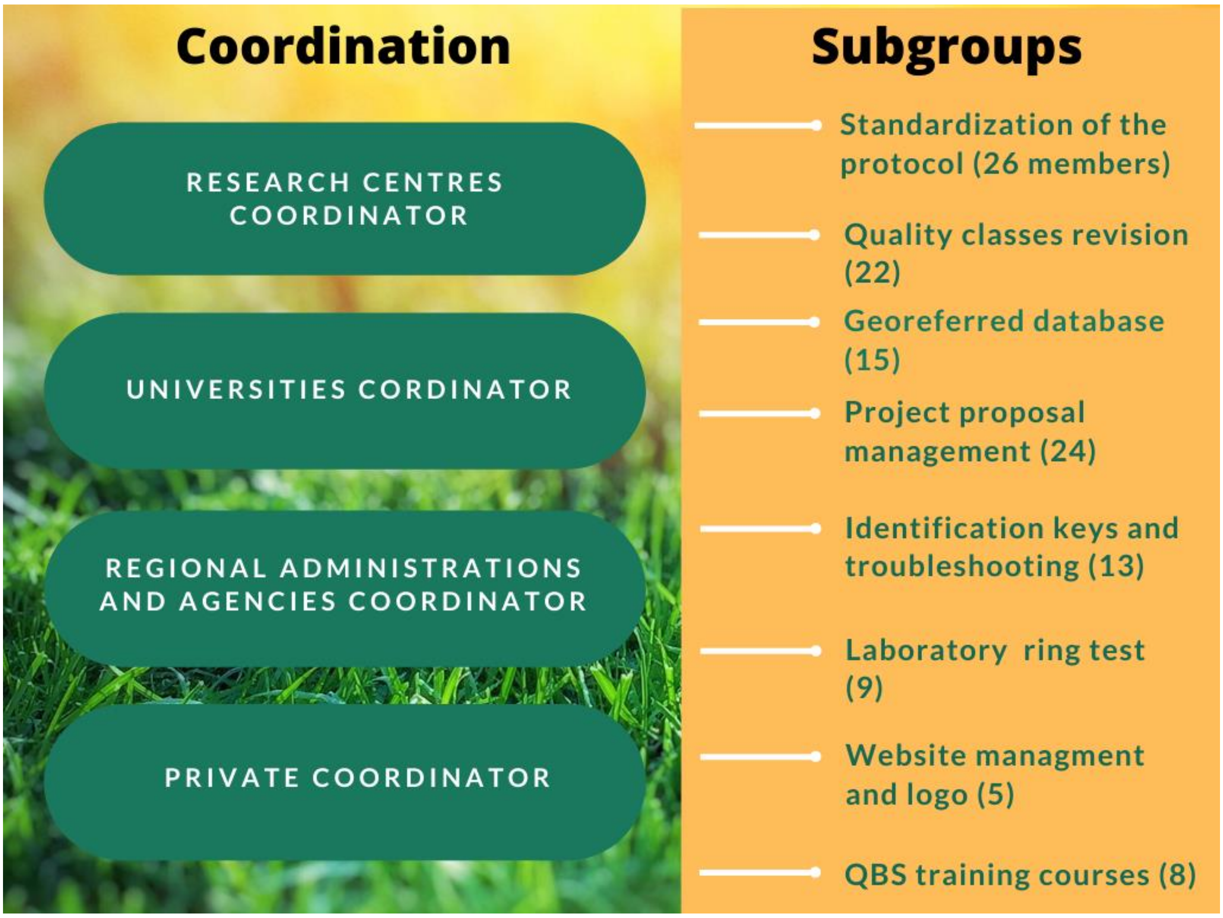


Figure 1: Organizational chart of the working group, detailing the number of members for each subgroup

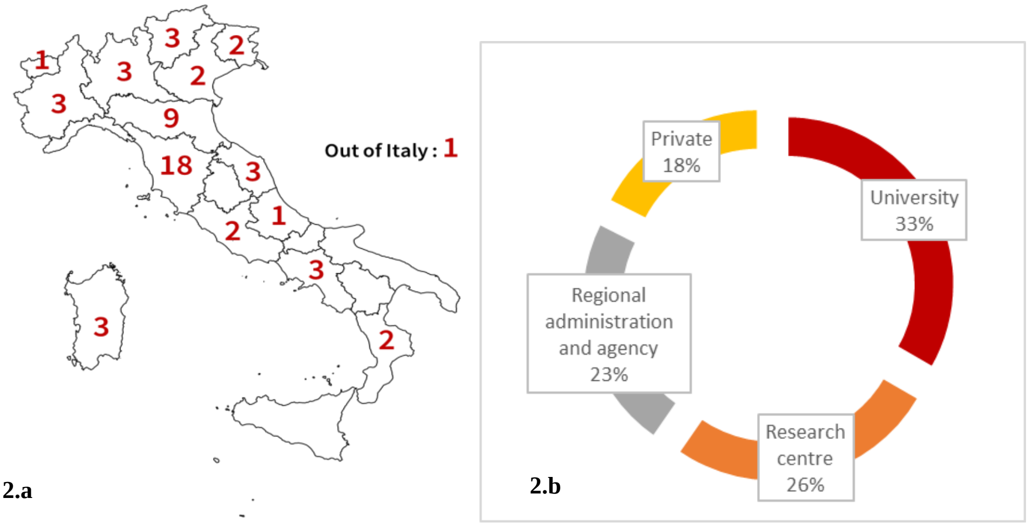
Methodology

The group at present shares a Database with about 500 files on QBS-ar index experiences, organized in 71 folders, aiming to collect the most relevant and affordable publications and descriptions of the method.

The group is organized in a core-team of 4 coordinators (the first four authors of this paper), representative of the national bodies who applied this method: Public Research Centres, Universities, the System of Environmental Protection Agencies and Regional Administrations, and private bodies. Moreover, the group is structured in 8 subgroups, a coordinator and a deputy coordinator were identified for each subgroup. Every member participates from 1 to 3 subgroups (Figure 1).

Achieved objectives

Nowadays the network accounts 57 QBS-ar experts throughout Italy, mainly academic researchers (Figure2). The workflow chart and operational perspective for every subgroup is shown in table 1. The group has already catalogued 232 stereoscope images of microarthropods, assigning them the correct echo-morphological index (EMI) value. The group organized a public tender to have a Logo receiving several tens of proposals. Winning logo, representing a stylized Oribatid mites that goes down to the soil, is reported in Figure 3.

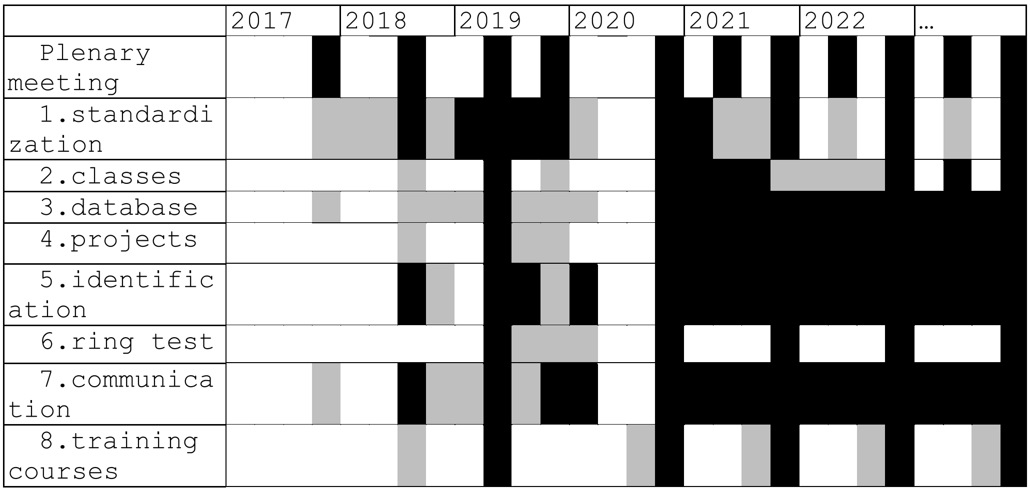


2.a

2.b

Figure 2: Membership distribution on QBS-ar skill network (a) and composition by institution types (b)

Table 1: Group and subgroups activity time▨ schedule (setting phase, ■ operational phase)



	2017				2018				2019				2020				2021				2022				…			
Plenary meeting				■			■			■		■				■		■		■		■		■		■		■
1.standardization				▨	▨	▨	■	▨	■	■	■	■	▨			■	■	▨	▨	■		▨		■		▨		■
2.classes							▨			■		▨				■	■	■	■	▨	▨	▨	▨	■		■		■
3.database				▨			▨	▨	▨	■	▨	▨	▨			■	■	■	■	■	■	■	■	■	■	■	■	■
4.projects							▨			■	▨	▨				■	■	■	■	■	■	■	■	■	■	■	■	■
5.identification							■	▨		■	■	▨	■			■	■	■	■	■	■	■	■	■	■	■	■	■
6.ring test										■	▨	▨	▨			■				■				■				■
7.communication				▨			■	▨	▨	■	▨	■	■			■	■	■	■	■	■	■	■	■	■	■	■	■
8.training courses							▨			■					▨	■			▨	■			▨	■			▨	■

Figure 3: Working group logo that won the call for tender

The number of examined soil sites quoted in 100 publications is more than 2600. The analysis of extant publications shows a sharp increase in number and quality of publications (Figure 4).

The data reported in scientific and technical publications include different objectives, project span and land uses. A meta-analysis showed how: i) the highest average QBS-ar value resulted in orchards, grasslands and forests, ii) lower values occurred in urban parks and soils involved in human degradation, iii) the average value is about 100 (Menta *et al.*, 2018).

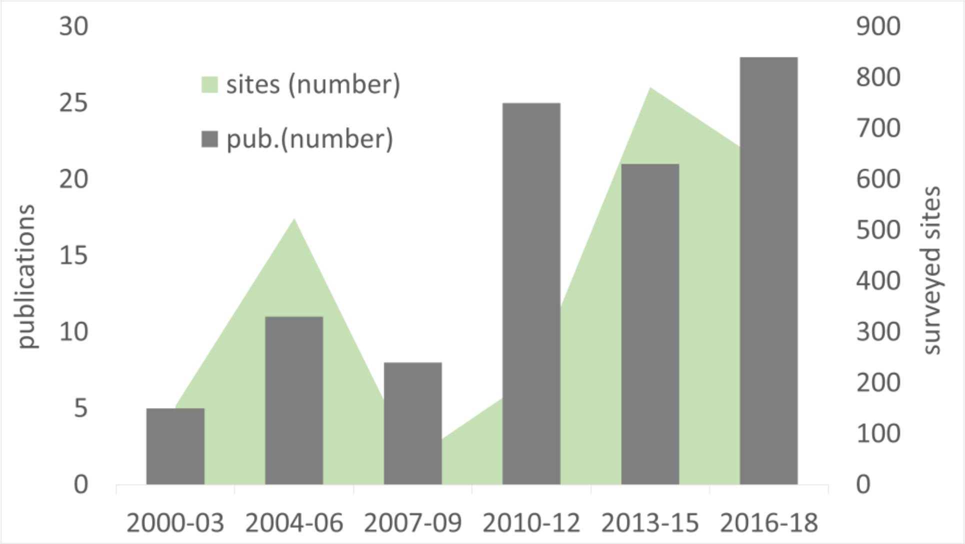


Figure 4: Trend of publications and investigated sites (reported by these publications) regarding the QBS-ar index

The publications are available in the network database

Conclusions

QBS-ar index is an easy-to-learn and cheap tool to describe soil quality and soil biodiversity, it can highlight soil degradation and pollution and can be used to assess the risk of biodiversity loss as consequence of human activities. It responds more quickly than direct measure of soil organic carbon to soil management changes. For these reasons, QBS-ar index can be chosen as indicator in soil monitoring programs to describe the current state of soil quality and to establish local reference values according to the different pedo-climatic conditions, land use and soil management. Many studies have already shown that land use and soil management have the greatest impact on soil microarthropod community, QBS-ar can be used to put in evidence how the different soil management can affect soil quality and biodiversity.

Whereas many studies have been published on QBS-ar application, this network is interested in international collaborations aimed to coordinate the index application in different environments. Moreover, the network has the scope to guarantee the correct QBS-ar use in each application phase everywhere. For this reason, the network members will organize training on the QBS-ar use regularly.

QBS-ar index was recently included within the set of biodiversity indicators in a voluntary certification protocol in agriculture namely Biodiversity Alliance (CCPB, 2017). Maintaining the certification involves checking, usually on an annual basis, the biodiversity parameters of a single farm or an agricultural products supply chain. This monitoring activity takes advantage from the sensitivity of the QBS-ar index in recording the effects on the soil induced by different methods of agronomic management. These statements are fully reflected in the analysis of strengths, weaknesses, opportunities and threats (SWOT) performed by the QBS-ar skill network.

Table 2: SWOT analysis of the QBS-ar method performed by the skill network

Strenghts	Weaknesses	Opportunities	Threats
Robust	Quality Classes to be redefined	Possibility of on-line data inserting	Data Quality Control still absent
Cheap	Generic	Soil Food Webs Insights	Homogeneous Database Implementation
Easy-to-learn, to set up & to implement	Multiple Disturbance Factors	Soil Community Structure Definition	Implementation in not-applicable contexts
Fast in reckoning the final value	Actual representativeness of the sample sites	Correlation with soil resilience to specific stress factors	Mistakes in procedure implementation or in EMI assignment
Data Ecosystem Approach	Not always well-applied outside Italy	Implementation to several scales	Need of milestone sites
Numerical, non-qualitative index	Hard response to forests selective cutting	Easy method's efficacy Communication	Vertical fluctuations, soil humidity and temperature correlations at sampling moment
Short term index, expression of biodiversity	Eventual reference site need (Treatment vs. Control)	Robust Regional Dataset Implementation	Does not allow to check which soil degradation cause and needs other indexes correlation
Soil researchers appreciation	Does not consider specimens abundances	Direct relationship with soil porosity, land use and agricultural practices	Sensitive Species may determine their Faunal Unit absence
Easy to sample and easy to identify Faunal Units			

Represents soil aggregate distribution better than other diversity indexes		

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Ecotoxicological evaluation of lead in distinct soil classes based on acute bioassays with earthworms

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Abstract summary

Lead (Hg) is a toxic metal and well-known by its capacity to cause noxious effects on human health and biota. This work proposes the ecotoxicological evaluation of an artificial soil, Ferralsol, Chernosol, and Gleysol contaminated by Pb at the laboratory, based on acute bioassays with earthworms (*Eisenia andrei*). The bioassays were performed according to standard protocols (ISO). Physical, chemical, and mineralogical characterization of the soils were determined to support the interpretation of ecotoxicological data. The addition of $Pb(NO_3)_2$ induced the reduction of pH levels of all soils. The bioassays revealed the following order of toxicity (LC_{50} mg/kg): Ferralsol (496) > Artificial Soil (3327) > Chernosol (4002) > Gleysol (4438). The high organic matter content in Gleysol seems to be able to decrease the toxicity levels. In Chernosol, the abundance of 2:1 clay mineral suggests the occurrence of metal adsorption, reducing the toxicity. In artificial soil, its mineralogy predominantly composed of quartz and poorly degraded organic matter may have contributed to increasing the toxicity levels. The Ferralsol mineralogy is essentially kaolinite and might have favoured the increase of Pb concentration in soil solution, increasing its toxicity. The results indicated that soil properties played a crucial role in Pb toxicity to earthworms.

Keywords: Toxicity, Lead, Earthworms, Contamination; Biodiversity, Soil

Introduction, scope and main objectives

Lead (Pb) is a highly toxic metal and is known to cause adverse effects on human health and the ecosystem. In the last decades, the number of studies focused on understanding the ecological effects, and their consequences for soil health has been increasing. These studies point out that long-term exposure to heavy metals poses a threat to biodiversity.

The Brazilian legislation that defines the limit concentrations of Pb for soil quality (Brasil Resolution, 2009) is based on values idealized for temperate soil ecosystems and, does not represent particular characteristics of tropical region. The evaluation of toxicity based on bioassays with tropical bioindicators and Brazilian soils is imperative and urgent, in order to assess the feasibility of this legislation in preserving soil biodiversity.

The physical, chemical, and mineral properties of soils play an important role in the behaviour of metals in the soil. Such processes determine the potentially bioavailable concentrations in the soil, the leaching potential, and the geochemical mobility of contamination (Cipullo *et al.*, 2018). The understanding of these processes added to the application of ecotoxicological tests is crucial to assessing the applicability of the current Brazilian legislation, and for the generation of sustainability indicators aiming at (i) the indication of sensitive and priority areas for remediation, (ii) the definition of the most vulnerable ecological receptors and appropriate strategies for the preservation of biodiversity, (iii) the maintenance of ecosystem services, and (iv) the assessment of the most sustainable remediation options given the geochemical behaviour of contaminants and the associated ecological risk.

Bioassays with earthworms (*Eisenia andrei*, *Eisenia fetida*) are widely used to assess toxicity due to metal exposure (Luo, Verweij and Van Gestel, 2014; Sivakumar, 2015). The present work proposes an evaluation of the influence of the properties of different classes of specific toxicity of Pb for earthworms. Therefore, the soils were spiked with Pb, and the bioassays carried out with *E. andrei* in three types of natural soil (Ferralsol, Chernosol, and Gleysol) and artificial soil.

Methodology

Ferralsol and Chernosol were collected in the municipality of Duque de Caxias (RJ - Brazil) (22°41'34.2" S, 43°17'14.5" W) and from Rio de Janeiro (RJ - Brazil) (22°51'22.5" S, 43°30'0.7" W), respectively. Sampling was performed with the aid of an auger, and the material was collected at horizon B.

Gleysol was collected in the municipality of Rio de Janeiro (RJ) (22°52'16.41" S; 43° 42'47.08" W). The sample was obtained at a depth of 10 cm in the transition from horizon A to horizon C.

The physical and chemical characteristics of the soils were determined according to Brazilian Agricultural Research Corporation (EMBRAPA) (1997). The mineralogy of the clay fraction of the samples was carried out by X-ray diffraction. Samples were obtained from the separation of the clay fraction by sedimentation (EMBRAPA, 1997). Were prepared natural, glycosylated, and heated to 550 °C after saturation with KCl slides (Jackson, 1958).

The soils were spiked with Pb, and the concentrations used were (where 0 mg/kg = pure soil): 0, 180, 2000, 3000, 3500, 4000 and 4500 mg/kg for Artificial Soil; 0, 180, 300, 1500, 2000 mg/kg for Ferralsol; 0, 500, 1000, 3000, 4000, 5000, 6000, 7000 mg/kg for Chernosol; and 0, 1000, 3000, 5000, 6000, 7000 mg/kg for Gleysol. The acute test with *E. andrei* was conducted according to the recommendations of (ISO, 1998). The composition of the tropical artificial soil followed the recommendations of Garcia (2004): 70 percent quartz sand, 20 percent kaolin and 10 percent coconut fibre powder.

The LC_{50} was calculated based on the analysis of PriProbit (Sakuma, 1998). The significance of the differences was assessed based on the Kruskal-Wallis test (for $p < 0.05$). The correlations between the parameters were assessed by Spearman correlation.

Results

Ferralsol showed low pH values (4.2), fine texture (58 percent clay), low CTC, and has high levels of Fe (11.3 percent) and Al (26.4 percent). Chernosol is a less acidic soil (6.2), of high fertility and high CTC. Both soils have low levels of organic matter (0.22 percent and 0.34 percent, respectively) since they were collected in horizon B. The Gleysol, on the other hand, has a pH close to neutrality (6.9), high organic matter content (4.88 percent), silty texture and high electrical conductivity (735 µS/cm) compared to Latosol (100 µS/cm) and Chernosol (165 µS/cm).

Ferralsol showed essentially kaolinitic mineralogy (88.6 percent), with the presence of gibbsite (8.9 percent) and goethite (2.5 percent). Chernosol presented a high percentage of vermiculite (60 percent), and kaolinite (40 percent). Gleysol showed a predominance of kaolinite (91.2 percent), followed by illite (4.29 percent), hydromica (3.63 percent), vermiculite (0.60 percent), and interstratified illite/smectite (0.25 percent).

For all classes of soils studied, there was a tendency of decreasing soil pH with the increasing of Pb concentrations. Figure 1 shows the levels of survival and biomass variations of earthworms exposed to different soils contaminated with Pb. The lethal doses of Pb to 50 percent of the organisms (LC_{50}) in different soils, indicates the following increasing order of toxicity: Ferralsol > Artificial Soil > Chernosol > Gleysol. In the case of artificial soil, the significant death of organisms was only detected from the dosage of 3000 mg/kg (LC_{50} = 3327 mg/kg). From the dose of 4500 mg/kg all the organisms died. The toxicity levels in Ferralsol were the highest, and the 500 mg/kg dose was able to cause the death of more than 50 percent of the organisms, on average (LC_{50} = 496 mg/kg). At the 1500 mg/kg dose, all organisms were dead.

The lowest toxicity level was for Gleysol. Only after 5000 mg/kg dose was possible to verify the occurrence of significant mortality (LC_{50} = 4438 mg/kg). At the dose of 7000 mg/kg all organisms were dead. Chernosol had the second-lowest level of toxicity among the studied soils. For this soil, significant death were observed from the concentration of 4000 mg/kg (LC_{50} = 4002 mg/kg), and in doses ≥ 5000 mg/kg all organisms died.

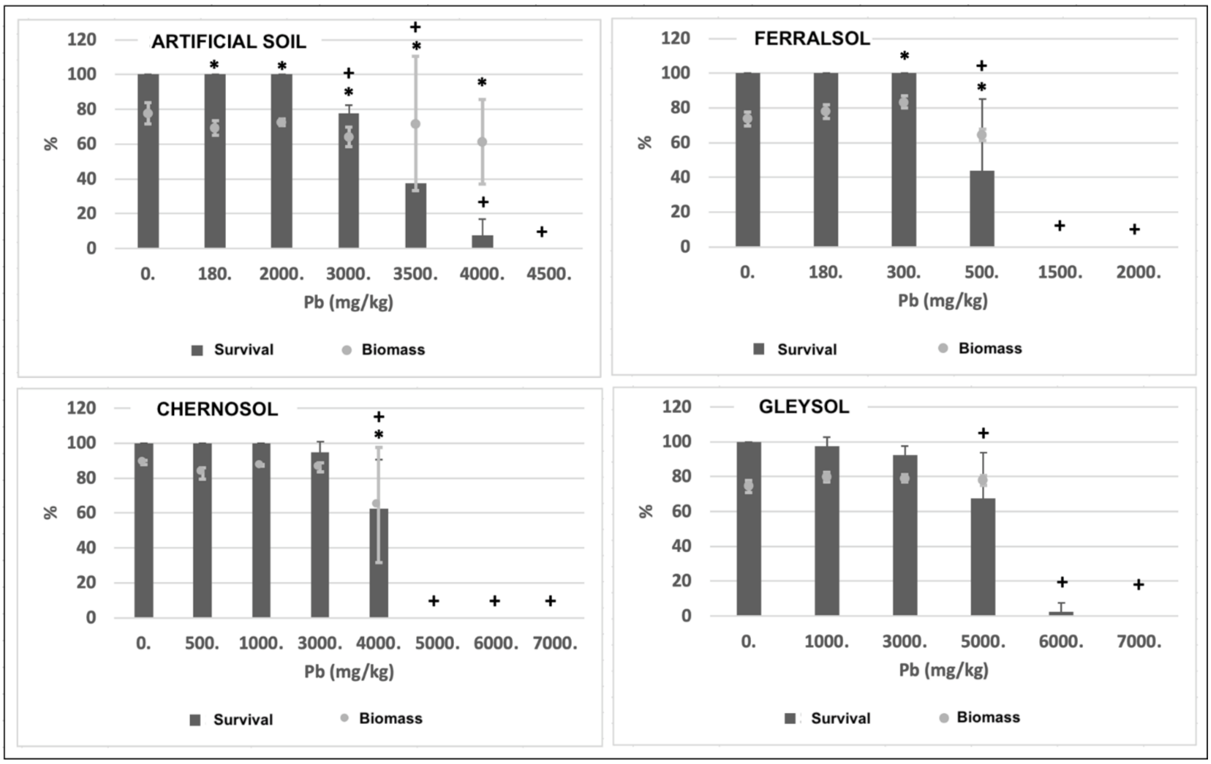

Figure 1: Survival and variations in Eisenia andrei biomass exposed to artificial soil, Ferralsol, Chernosol and Gleysol spiked with lead (Pb)

+ = survival significantly different from the control ($p < 0.05$); * = biomass significantly from the control ($p < 0.05$).

Discussion

In Ferralsol the addition of Pb $(NO_3)_2$ was able to reduce the pH value substantially. For 1500 mg/kg dose, which caused mortality of 100 percent of the organisms, the pH was 2.8. Pearson's correlation indicates that earthworm mortality was positively correlated with Pb concentration ($R^2 = 0.93$). Although the addition of $Pb(NO_2)_3$ is related to the reduction of pH, the Ferralsol is already naturally acidic and, thus, the Pb would tend to become more geochemically available for the solution, even at low doses of $Pb(NO_2)_3$ application. Ferralsol had the lowest LC_{50} value (496 mg/kg) that may be associated with the abundance of kaolinite, a 1:1 clay mineral with low potential for adsorption of metals, and low organic matter content, that could increase the bioavailability of Pb.

Although the mineralogy of Gleysol, similarly to Ferralsol, is predominantly composed of kaolinite, Gleysol showed the lowest toxicity (LC_{50} = 4438 mg/kg) among the studied soils. This finding may be related to the high content of organic matter in the Gleisol (4.88 percent) when compared to Ferralsol (0.22 percent) and Chernosol (0.34 percent), collected in Horizon B. Organic matter has high CEC and is widely known for forming stable complexes with metals, reducing their bioavailability and toxicity to soil organisms. In contrast to Ferralsol, in Gleysol it was possible to detect a significant correlation between the reduction of pH and the reduction of the survival of earthworms ($R^2 = 0.81$). Gleysol has a natural pH close to neutrality, and its reduction by the addition of metallic salt played an important role in increasing the bioavailability of Pb, whose total concentrations also correlates with the increase of mortality ($R^2 = 0.90$).

The low acute toxicity found in Chernosol is possibly linked to its mineralogy, with an abundance of vermiculite, an expansive clay with high CTC, and widely known for reducing the geochemical mobility of metals and their ecotoxicity for soil organisms. Artificial soil is composed of quartz sand. Since quartz has an inert behaviour, it is consistent to assume that the bioavailability of Pb in soil interstitial water is higher, thus increasing the toxicity to earthworms. Although Artificial Soil contains 10 percent of the coconut fibre, this organic matter is very little degraded (and, therefore, less active than that verified in Gleysol), resulting in less potential for metal complexation, including Pb. These findings seem to corroborate the high toxicity of Pb in Artificial Soil in comparison to Chernosol and Gleysol. Similar to the other soils, in Artificial Soil, it was also possible to observe a significant correlation between the pH reduction and the increase in the concentration of Pb ($R^2 = -0.95$), a result of the acidification of the soil by addition of metallic salt.

Finally, it is worth noting that Ferralsol had the highest level of acute toxicity among the studied soils. It is a class of typically tropical soil and the most widely distributed in Brazil. The LC_{50} found in Ferralsol reaches up to six (6), eight (8) and nine (9) times lower than those found in Artificial Soil, Chernosol, and Gleysol, respectively. Toxicity levels were very higher in highly lixiviated soils than in temperate or hydromorphic soils. This fact deserves to be highlighted in the sense that the current Brazilian legislation for soil quality (CONAMA 420/2009), based on guiding values for temperate climate ecosystems, may need to be adapted to the specificities of the tropical region.

Conclusions

Pb was toxic to earthworms in acute exposure, and presented the following order of toxicity by type of soil: Ferralsol > Artificial Soil > Chernosol > Gleysol. The soil properties played a crucial role in toxicity, with emphasis on pH, clay mineralogy, salinity, and organic matter content. In this context, it is worth mentioning that the addition of Pb nitrate to the soil resulted in pH reduction and contributed to the increase of Pb bioavailability. On the other hand, the abundance of expansive clay minerals and organic matter, with high CTC and high potential of Pb geochemical sequestration, was able to mitigate the toxicity of Pb and nitrate salts. In future work, it is suggested to carry out avoidance and reproduction tests with earthworms and collembolans, aiming at a more comprehensive understanding of the ecotoxicity associated with Pb and the generation of data to support the revision of the current Brazilian legislation for soil quality.

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Uncovering linkages between soil fauna and ecosystem function using factor analysis and structural equation modelling

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Abstract summary

Soil mesofauna have historically been grouped together based on taxonomy, trophic position, size, and different diversity index categories (i.e. adaptation to soil environment) in efforts to predict fauna impacts on ecosystem function or to understand fauna responses to environmental factors. These groupings assume that impacts on ecosystem function are tied directly to the fauna’s shared biology and are therefore pre-determined by such set criteria. This research investigates how factor analysis and modelling techniques can be applied to first allow the environment to predict the mesofauna groups and then use those groups to predict ecosystem function. Piecewise structural equation modelling was used on a dataset from a long-term cropping system experiment in New York State, USA. Exploratory factor analysis followed by confirmatory factor analysis were used to create mesofauna groups to incorporate into the cropping system model. These statistical techniques illustrated that taxonomically different taxa can have similar responses to environmental conditions. Incorporating these mesofauna groups into the model revealed linkages between soil health metrics and crop productivity, therefore more clearly connecting soil fauna to specific functions and ecosystem service outcomes. These findings may have important ramifications for how soil biodiversity and functionality is assessed and conserved.

Keywords: Collembola, mites, soil invertebrates, microarthropods

Introduction, scope and main objectives

Soil mesofauna have historically been grouped together based on taxonomy, trophic position, size, and different diversity index categories (i.e. adaptation to soil environment) (Brussaard, 1998; Crossley, Mueller and Perdue, 1992; Menta *et al.*, 2018). These groupings were created in efforts to predict fauna impacts on ecosystem function or to understand fauna responses to environmental factors. However, these groupings assume that impacts on ecosystem function are tied directly to the fauna’s shared biology and are therefore pre-determined by such set criteria. Since these groupings were not designed to consider how mesofauna communities interact in response to different environmental conditions, these grouping approaches may limit our understanding of biodiversity and functional redundancy in soil ecosystems.

Statistical techniques, such as exploratory factor analysis followed by confirmatory factor analysis and different modelling approaches (i.e. traditional structural equation modelling (SEM), piecewise SEM, path analyses), may provide insight into how soil fauna communities respond to environmental changes and influence ecosystem functions (Kozan and Richardson, 2014). This research investigates how statistical techniques can be applied to allow environmental conditions to determine mesofauna groups and inform mesofauna functionality in ecosystems. An example methodology is explained, and further investigations of statistical techniques are discussed.

Methodology

In 2005, the Cornell Organic Grain Cropping Systems Experiment was established in central New York State, USA. Four organic grain cropping systems that varied in crop rotations, fertilizer inputs, tillage practices, and weed control were compared (Jernigan *et al.*, 2020). In 2017, the experimental site was mouldboard ploughed and seeded with sorghum sudangrass as part of a uniformity trial to assess legacy effects of past management practices. Prior to initiating the uniformity trial, soil samples were collected and analysed for soil health indicators. Soil samples were also collected to assess soil invertebrate abundance and community structure at two time points during the uniformity trial. Sorghum sudangrass and weed biomass were sampled at the end of the uniformity trial (Jernigan *et al.*, 2020).

Factor analysis methods were considered to determine how to best group mesofauna in the dataset based on their overall response patterns across the tillage and fertilizer gradients. Exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were then used to create mesofauna groups to incorporate into an overall grain cropping system model. Piecewise structural equation modelling (SEM) was used to quantify relationships between different response variables (Jernigan *et al.*, 2020).

Results

The EFA/CFA process created two fauna groups that had similar response patterns across the dataset. The first fauna group, FaunaF1, is composed of juvenile and adult astigmatid mites (Acaridae) and oribatids in the family Tectocepheidae. While related taxonomically in the order Oribatida, the taxa comprising this group differ considerable in ecology and life history and are therefore often separated into different groupings. The second fauna group, FaunaF2, is composed of collembolans (Onychiuridae and Isotomidae), and the mesostigmatid mite family Rhodacaridae. These taxa are both taxonomically and functionally diverse, and are therefore rarely, if ever, grouped together.

The model revealed different relationships between the two fauna groups and the other response variables. Results from SEM show that soil phosphorus, soil aggregate stability, and soil respiration explained variation in abundance of the fauna comprising FaunaF1 (Figure 1). Aggregate stability, soil respiration, soil moisture, weed biomass, and the fauna comprising FaunaF2 affected the sorghum sudangrass biomass production (Figure 1). Interestingly, FaunaF2 mediated the relationship between soil respiration and crop biomass production (Figure 1).

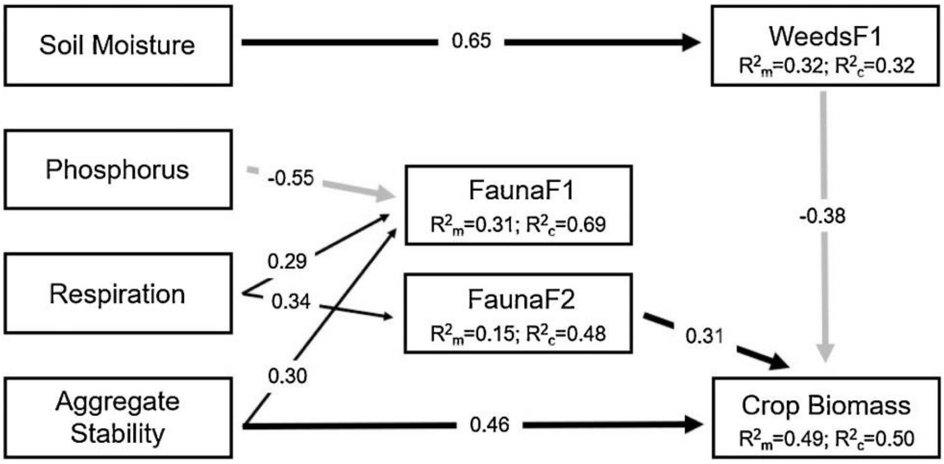

Figure 1: Piecewise structural equation model (SEM) showing how soil health indicators, soil invertebrate groups, and weed biomass group affect crop biomass (sorghum sudangrass biomass)

FaunaF1 is the group factor comprised of phoretic hypopi, Acaridae, and Tectocepheidae. FaunaF2 is the grouped factor comprised of Onychiuridae, Isotomidae, and Rhodacaridae. WeedsF1 is the grouped factor comprised of yellow foxtail, giant foxtail, and yellow nutsedge. Black arrows represent positive relationships, and grey arrows represent negative relationships. Standardized path coefficients are shown for significant relationships. Marginal (R^2_m) and conditional (R^2_c) coefficients of determination are shown for each component model, which describes the proportion of response variance associated with the fixed effects (marginal) and the fixed effects with random effects included (conditional).
Source: Jernigan *et al.* (2020)

Discussion

These statistical techniques, EFA followed by CFA, showed that taxonomically and functionally different taxa can have similar responses to environmental conditions. This method of grouping mesofauna shows promise as a technique that may provide insight into how taxa interact within communities and impact ecosystem functions.

Different approaches to modelling biological communities in the context of environmental parameters exist, however there is not an established approach to identifying correlation structures between species in a diverse dataset (Brown *et al.*, 2014; Wisz *et al.*, 2013). This illustrated technique (EFA/CFA) may fill an important gap in ecological statistical analyses and may serve as an important tool for better understanding how soil fauna interact in response to environmental changes. Our findings may indicate that established functional groupings may not always be sufficient to understand the role of fauna in different environmental contexts.

Within the field of soil ecology there are open questions of how soil fauna communities and their interactions contribute to ecosystem services (Eisenhauer *et al.*, 2017). Incorporating these mesofauna groups into the piecewise SEM revealed linkages between soil health metrics and crop productivity, which connects the soil fauna to a specific ecosystem service outcome. Therefore, this grouping technique in conjunction with modelling methods may provide new insights into how soil biological communities interact to impact ecosystem function and ecosystem services.

Conclusions

Different statistical techniques may provide insight into how soil biological communities respond to changing environmental conditions and how they interact to impact important soil processes. The illustrated grouping method suggests that non-traditional methods of grouping mesofauna may yield new information regarding mesofauna functionality. This dataset identified taxa to taxonomic families, however further research is needed to determine which taxonomic level of identification is best when applying these statistical techniques. Looking forward, future research should consider how mesofauna are initially identified and grouped to best answer the proposed research questions.

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Biodiversity of arbuscular mycorrhizae and chemical properties in soils of the Colombian coffee zone

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Abstract

Diversity and quantity of arbuscular mycorrhizal fungi (AMF) associated to coffee plants rhizosphere (*Coffea arabica*), were evaluated in 28 lots in production of 10 Cenicafé research substations; levels of root colonization and density of spores/g of soil were determined, increasing native inoculum in *Brachiaria decumbens* and *Pueraria phaseoloides* trap crops; chemical analysis of sampled soils was performed. Based on the analysed variables and chemical properties of the soils, multiple linear regression models were estimated, selecting parameters with p-values lower than 10 percent. Twenty HFMA species were identified, which reflects high diversity if we consider that the samplings were carried out in ecosystems with common temperature characteristics and that only one host plant was evaluated. For the total number of AMF species a model was selected where pH, total nitrogen (N), organic matter (OM), iron (Fe) and manganese (Mn) had a significant effect; while for the percentage of colonization the model selected showed a significant effect of the real cation exchange capacity (CEC), iron (Fe) and phosphorus (P). In contrast, the model related to spore density did not show significant parameters.

Key words: Arbuscular mycorrhizal fungi, diversity, soils, mycorrhizal colonization, spore density

Introduction

During the 2018-2019 coffee year, Colombia produced 15,235,000 60 kg bags of coffee from 853,700 ha planted (FNC, 2020); this represented a historical increase in the country's production compared to previous years. Coffee in Colombia is mainly planted at altitudes between 1,200 and 1,800 m on the slopes of the Eastern, Central and Western Cordilleras. The soils of these areas are mostly Andisols, which have high organic matter (OM) and nitrogen (N) contents and high phosphorus retention; they are dark, deep, friable, well-drained, highly porous and have high aeration and moisture retention (Suarez, 1991). However, in the Colombian coffee zone, soils are formed by igneous, metamorphic and sedimentary, heterogeneous, stony, poorly drained and low in moisture retention, which generates difficulties for the adequate development of coffee cultivation. Given the characteristics of the cultivated soils and that coffee does not present a notorious response to phosphoric fertilization in the production phase (Uribe and Mestre, 1976), it has been reported that the presence of the Arbuscular

Mycorrhizal Forming Fungi (AMF) *Glomus manihotis, G. occultum, G. macrocarpum* and *Entrophospora colombiana* in the seedling development stage contribute to the increase of growth, dry weight and absorption of nutrients and water (Sánchez, 1999). In accordance with the above considerations, the purpose of the present investigation (Bolaños-Benavides, 1996) was to estimate the relationship between the diversity and colonization parameters of AMF isolated from Colombian coffee soils with their chemical properties.

Methods

Sample design

A cluster sampling design was employed with sample allocation proportional to the number of lots at the 10 Cenicafé experimental substations located in eight departments of Colombia; the sampling unit was one hectare, within which 30 samples were taken at randomly selected sites (Table 1). With respect to the coffee plant, sampling was done between 0 and 20 cm deep, at three radial distances from the trunk (10, 20 and 50 cm), sampling only coffee roots when there were associations with legumes. In each case, three subsamples were taken to form a composite sample, from which 100 g of soil and 20 g of roots (fresh weight) were used. The root and soil samples were mixed separately and a representative sample of 3000 g soil and 600 g roots (fresh weight) was taken.

Table 1: Substations and amount of soil samples collected.

Substation	Department	Batch	Samples
El Rosario	Antioquia	1	30
Naranjal	Caldas	4	120
Rafael Escobar	Cundinamarca	4	120
Santa Bárbara		8	240
J. Villamil	Huila	2	60
Paraguaicito	Quindío	2	60
La Catalina	Risaralda	1	30
La Trinidad	Tolima	3	90
Albán	Valle del Cauca	2	60
La Sirena		1	30
Total		**28**	**840**

Analysed variables and data processing

Taxonomic identification, evaluation of the amount and diversity of spores in each soil according to their morphology was carried out. It was necessary to increase the native inoculum in trap crops of *Brachiaria decumbens* and *Pueraria phaseoloides* to obtain samples of 250 g of soil with a wide and diverse content of spores per species. For the evaluation of the colonization level (percentage) and spore density of the identified AMF (g/soil), root staining and wet sieving and sucrose gradient were carried out; respectively. Additionally, chemical characterization analyses were performed on the 28 soils sampled following the methodologies of the Agricultural Chemistry

Laboratory of Cenicafé (Carrillo, 1985). Response variables and soil chemical parameters were subjected to a multiple linear regression analysis, selecting the parameters with a p-value lower than 10 percent; processing the data through the free software R (R Foundation for Statistical Computing).

Results

According to the results of soil analysis, they presented pH ranges from 4.2 to 5.4; organic matter content from 4 percent to 21 percent; real cation exchange capacity (CEC) from 8 to 38 $\text{cmol}_{(+)}$ kg^{-1}; total nitrogen (N) content from 0.2 percent to 0.9 percent; phosphorus (P) from 4 to 250 mg kg^{-1} ; potassium (K) from 0.1 to 1.6 $\text{cmol}_{(+)}$ kg^{-1}; calcium (Ca) from 0,6 to 13,8 $\text{cmol}_{(+)}$ kg^{-1}; magnesium (Mg) from 0.1 to 2.7 $\text{cmol}_{(+)}$ kg^{-1}; iron (Fe) from 126 to 1.050 mg kg^{-1}; copper (Cu) from 0 to 39 mg kg^{-1}; manganese (Mn) from 5 to 222 mg kg^{-1}, and aluminium (Al) from 0.1 to 5.0 $\text{cmol}_{(+)}$ kg^{-1}.

A total of 20 species of AMF were identified in the coffee plantations of central Colombia belonging to the six genera of endomycorrhizae; the most frequent being *Acaulospora mellea and Glomus occultum*. According to the multiple regression analysis to establish the relationship of the chemical properties of the soils on the analysed variables, a positive effect of the pH, N and Fe; and negative of the OM and Mn on the total mycorrhizae (total AMF) was observed; while the percentage of colonization (%AMF) was influenced negatively and positively by the content of P in the soil and the CEC and Fe, respectively. On the other hand, the model related to spore density did not present significant parameters. The models obtained are detailed below:

HFMA Total = -12,41 + 3,45pH + 12,46N – 0,55MO + 0,012Fe – 0,028Mn

%AMF = -4,55 + 1,49CEC – 0,33P + 0,13Fe

Discussion

The amount and genera identified in the 28 soils sampled reflect a high diversity of AMF, considering that the sampling was done in ecosystems with common temperature characteristics between 19.4 and 21.4 °C and that only *C. arabica* was evaluated. The selection of pH as a significant and positive parameter for the amount of AMF is related to the condition of acidity characteristic of the central Colombian coffee zone, which would indicate that, by increasing the pH units to a maximum of 5.5, the probability of finding greater mycorrhiza diversity in Colombian coffee plantations increases. In addition, the availability of N becomes decisive to guarantee a greater diversity of mycorrhiza species. With respect to the %AMF, it is important to mention the positive effect of the CEC, which would indicate for this type of acidic soil, that the effective cation exchange capacity (ECEC) should also be considered in order to estimate the variable load of these coffee soils, possibly given the presence of amorphous clays that can directly influence the colonization of the different mycorrhizae in the soil. Considering the models obtained, it is important to highlight the inverse correlation between the percentage of colonization and the P content availablee in the soils, results that are in line with those obtained by Garzón (2016).

Conclusions

According to the results obtained in the present study, the association of AMF to the rhizosphere of *C. arabica* can be ratified and, therefore, they should be considered as natural inhabitants of the coffee ecosystems by virtue of their presence and the variability of the chemical conditions of the soils evaluated. In order to guarantee the conservation of the soil resource as a natural living body, which serves as a support for plants, the importance of the role played by different groups of organisms in processes of solubilization, mineralization, immobilization and humification among others, considered to be of utmost importance in the dynamics of nutrients, should be recognized.

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Patterns of soil biodiversity communities in urban microfarm

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Abstract summary

If green spaces are among the new societal expectations of urban people, they also play a crucial role in preserving biodiversity in urban areas. However, the effectiveness of urban micro-farms in supporting biodiversity, especially soil biodiversity, has rarely been studied.

We investigated the soil biodiversity (micro-organisms, meso- and macrofauna) in rooftop as well as in ground-level urban micro-farms in order to (i) evaluate the soil biodiversity service provided by urban micro-farms, (ii) to determine the key factors that condition the microbial and faunistic soil biodiversity and (iii) to finally study the co-occurrence between microorganisms and fauna.

Surprisingly, urban micro-farms are not supporting high levels of soil biodiversity contrary to others urban agriculture spaces. Due to various soil characteristics (e.g. organic matter), differenced was found between urban micro-farms on rooftop and on the ground concerning the taxonomical structures and compositions of collembolan, macrofauna and microorganisms communities. As it was difficult to find reference values in order to evaluate the biodiversity support, we conclude that there is a strong need for biodiversity studies in urban green infrastructure, especially dedicated for urban agriculture.

Keywords: urban agriculture, green roofs, micro-organisms, mesofauna, macrofauna, Technosol

Introduction, scope and main objectives

With a worldwide urban population projected to reach 5 billion by 2030 (Véron, 2007), the roles and benefits of urban green spaces take a key role in the future of cities, like climate regulation by trees or water flow regulation (Gómez-Baggethun and Barton, 2013). Although the services that urban micro-farms provide to the city are often highlighted (e.g. food supply, landscape integration), this is rarely based on a real assessment (Clinton *et al.*, 2018). Urban micro-farms play also a crucial role in preserving biodiversity in urban areas. Urban micro-farms were defined with four major characteristics: a small surface (<1ha by farmer), often on urban soil, multifunctional (not only food production purpose) and with a strong benevolent implication in the functioning.

The knowledge acquired on biodiversity in cities remains very incomplete and mainly concerns plants (e.g. Muratet *et al.*, 2008), pollinators (e.g. Matteson, Ascher et Langellotto, 2008) or avifauna (e.g. Croci, 2008), rather than soil organisms despite their major role in the functioning of urban soils.

One part of the SEMOIRS project objectives was to evaluate the soil biodiversity service provided by urban micro-farms, to determine the key factors that condition the microbial and faunistic soil biodiversity and finally to study the co-occurrence between microorganisms and fauna.

Methodology

We selected seven urban micro-farms located in the dense urban environment of the Paris region (France) including three on greens roofs, three on the ground and one contaminated site (at soil level – see Table 1). We map each micro-farms and delimit one or two cultivated areas for vegetables. In each cultivated areas, we performed three replicates of sampling for each soil biodiversity groups: micro-organisms, mesofauna and macrofauna. Other data on soil characteristics (fertility and contamination) available in the SEMOIRS project were also used.

As index did not match the basic assumptions of normality and homoscedasticity required for parametric statistics (Wilk-Shapiro test at rejection level of $\alpha = 0.05$), we used the Wilcoxon Rank-Sum test to assess differences in abundance and species richness between microfarms.

We analysed the effects of green roof type on species composition by performing a non-metric multidimensional scaling (NMDS) for each taxonomic groups, using the Bray-Curtis index, to explore dissimilarities between communities. All statistics were performed using R software version 3.2.2 (R Core Team, 2015).

Table 1: Seven microfarms studied for the soil biodiversity; soil type according WRB 2006

Microfarm	Experimental **MF1-Exp**	**MF2**	**MF3**	Contaminated soil **MF4-S.C.**	**MF5**	**MF6**	**MF7**
Age	8	4	6	12	14	6	7
Soil type	Technosol (rooftop)			Technosol (ground)	Pseudo-natural soil (ground)		
Total surface (m^2)	~ 900	~ 698	145	40 000	25 000	~5 819	~ 1100
Total cultivated area (m^2)	~ 200	397	80	~ 1 215	15 000	~ 2000	683
City	Paris	Aubervilliers	Paris	Nanterre	Vitry-sur-Seine	Paris	Paris

Results and Discussion

We noticed a high variability between and within urban microfarms. Composition and biodiversity (abundance and species richness) of the soil biodiversity groups differ between the ground and rooftop microfarms (Table 2, Figure 1). These differences are in relation with the high variability of soil chemical characteristics (more organic matter on Technosol on rooftop). However, the more or less marked differences are not similar between all soil biodiversity groups. We observed more abundances of Collembola and micro-organisms in rooftop than in microfarms on the ground whereas it is the contrary for macrofauna, even if we don’t demonstrate a significant difference for all groups (Table 2).

Table 2: Specific richness and abundances of collembolan in function of urban microfarms type

	Rooftop	Ground
Species number	8,0 ± 1	9,6 ± 3
Density (10^3 ind.m^{-2})	15 ± 10	10 ± 4
Shannon	1,7 ± 0.2	1,9 ± 0.4
Evenness	0.8 ± 0.1	0.9 ± 0.1

No significant differences.

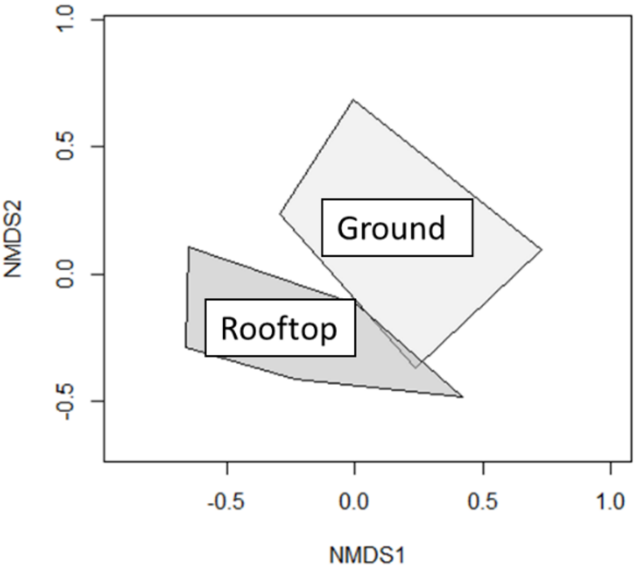

Figure 1: NMDS on collembolan communities in urban microfarms

In order to evaluate the ecosystem service provided by microfarms, we used for comparison references values from urban vegetables gardens or urban and agricultural land uses. We noticed a low biodiversity in the urban microfarms in contrast to the previous studies carried out on green roofs (Joimel *et al.*, 2018) or allotment gardens (Joimel *et* *al.*, 2017). This can perhaps be explained by the dry climatic conditions of the year 2018.

In existing methodologies for the evaluation of soil biodiveristy, a single indicator is often selected (e.g. microarthropods in Calzolari *et al.,* (2016)). In our results, we demonstrated the importance to study various organisms in function of their morphometric classes because their response may be different. Moreover, in the project, we encountered a major obstacle in assessing the biodiversity service provided due to the choice of reference values to compare results and literature. As data are not already available on urban microfarms, we selected three types of references: usage references ("urban agriculture" and "green spaces") and service references ("market gardening"). The multifunctional character of the studied forms tends to compare them to other possible uses of the space where they are located. Comparison with a reference whose main function is the expected service (for example food supply in the case of a market gardening operation), can pose the problem of comparing two systems with different expectations and therefore different levels of services rendered (e.g. yields).

Conclusions

Our methodology has highlighted several limitations to the development of routine tools for the evaluation of ecosystem services by managers of urban micro-farms, particularly with regard to (i) the acquisition of quantitative values. The use of indicators by field actors is desirable and desired. While it is relatively easy for some indicators (level of food production, water consumption, etc.), for others it requires the intervention of specialists (soil microorganism); (ii) there is a strong need for adapted and specific reference systems. Evaluation methodologies require reference values to calibrate the indicators. The lack of knowledge in urban areas is therefore a hindrance to their development.

Acknowledgements

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Earthworm biodiversity from Hyrcanian forests: natural vs. agricultural

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Abstract summary

The role of earthworms in maintaining the soil fertility has been discussed for a long time from Darwin (1881) up to now. They have great ability to change soil composition and plant communities. The presence of earthworms enhance the biological diversity and soil fertility, and alter the soil environmental conditions (Edwards and Bohlen 1996). In order to comparison of earthworm diversity among three different habitats in the North of Iran, 120 stations were investigated. Thirteen species of earthworms identified (*Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea trapezoides* (Dugès, 1828), *A. rosea* (Savigny, 1826), *A. jassyensis* (Michaelsen, 1891), *Aporrectodea longa* (Ude, 1895), *Dendrobaena byblica* (Rosa 1893), *D. hortensis* (Michaelsen, 1890), *D. veneta* (Rosa, 1886), *Dendrodrilus rubidus* (Savigny, 1826), *Eisenia andrei* (Bouché, 1972), *Eiseniella tetraedra* (Savigny, 1826), *Perelia kaznakovi* (Michaelsen, 1910), *Amynthas corticis* (Kinberg, 1867)) belonging to six genera and two families. The earthworm species differed in their eco-morphological characteristics and in relative abundance in each of the habitats sampled. *Ap. trapezoides* was the dominant species in all habitats, namely cultivated land, grassland and forest. *Ap. caliginosa* was abundant except in cultivated soil. The rarest species was *Dendrodrilus rubidus* which recorded only in grasslands. Shannon-Wiener and Equitability indices were calculated in order to assess the inter relationship between habitat and earthworm diversity. Analysis of diversity indices revealed the highest diversity in forest habitats.

Keywords: Earthworm, Habitats, Diversity, North of Iran.

Introduction, scope and main objectives

Earthworms represent the largest component of the animal biomass in soil and are commonly termed 'ecosystem engineers' (Hale *et al.*, 2005). The earthworm is one of the most important members of the soil fauna because they influence the structure, chemical composition and distribution of plant nutrients in soil (Lee, 1985). Many researchers studied the habitat preference of various earthworm species (Scullion and Malik, 2000). The presence of a species in a particular habitat and its absence from other habitats show the species-specific distribution of earthworms in different ecosystems (Tripathi and Bhardwaj, 2004). Most studies in Iran have focused on the identification of earthworms). The preliminary study of Iranian earthworms was conducted by Omrani in 1973; this study included some sporadic surveys from different parts of Iran. After that, more detailed studies were performed about the earthworm fauna of different regions of the country (Latif *et al.*, 2009, Ezzatpanah *et al.*, 2010, Mirmonsef, Malek and Latif*,* 2011, Farhadi, Malek and Elahi, 2013). All the knowledge about Iranian earthworm fauna up to now was reviewed by Latif, Malek and Csuzdi (2016). This study considers the contribution of earthworms to different ecosystems for predicting patterns in earthworm diversity. To study the effect of different habitat on earthworm diversity, the specimens were collected from the north part of Iran, along the northern border of Iran at the southern shore of the Caspian Sea. The plant association present in the studied area is the Hyrcanian deciduous closed forest. The climate of the region is characterized as moderate and humid (Akhani 1998). The species richness and species compositionn of earthworms were investigated in this area.

Methodology

Earthworm samples were collected from the North of Iran. To collect samples, 120 stations were selected. Samples were collected by digging soil and sorting worms by hand. Earthworms were fixed in in 80 percent ethanol. Identification of earthworms was based on morphological and anatomical characters, according to Csuzdi and Zicsi (2003), Sims and Gerard (1999), and Perel (1979). Sampling stations were classified in three categories: cultivated land, grassland and forest. Relative abundance of different earthworm species was studied in various habitats in the current study. Shannon diversity and Equitability indices were calculated. Shannon diversity index was calculated as $H' = -\sum pi \ln (pi)$, where H′ shows diversity index and pi indicates relative frequency of species; and equitability index calculated as $E = H'/\ln S$, where S = total number of species.

Results

A total of thirteen species were found belonging to six genera (Table 1) and two families (Megascolecidae, Lumbricidae). Distribution of earthworm population in different habitats are presented in Table 1.

Dendrobaena pentheri and *Dendrodrilus rubidus* were only found in grasslands, however *Ap. caliginosa* and *Ap. rosea* were abundant in this habitat. *Ap. jassyensis*, *P. kaznakovi* and *D. byblica* were only recorded from forest habitats; and had high relative frequency in the habitat. *D. veneta*, *Eisenia andrei*, and *A. corticis* were only observed in cultivated habitats; *A. corticis* was the frequent species.

The highest value of the Shannon diversity index was recorded from forest habitats (H′ = 1.31) while the lowest diversity index were found in cultivated lands (H′ = 0.712). Only four species (*Ap. trapezoids*, *Aporrectodea caliginosa*, *Ap. jassyensis*, and *Perelia* *kaznakovi*) were abundant in their favorable habitats. Eight species (*Ap. jassyensis*, *D. byblica*, *D. pentheri*, *Dendrodrilus rubidus*, *Eisenia andrei*, *Perelia kaznakovi* and *Amynthas corticis*) were habitat specific species and found in only one type of habitats (Table 1). Regarding to Equitability index, the highest value was recorded in the forest (E=0.431) (Figure 1).

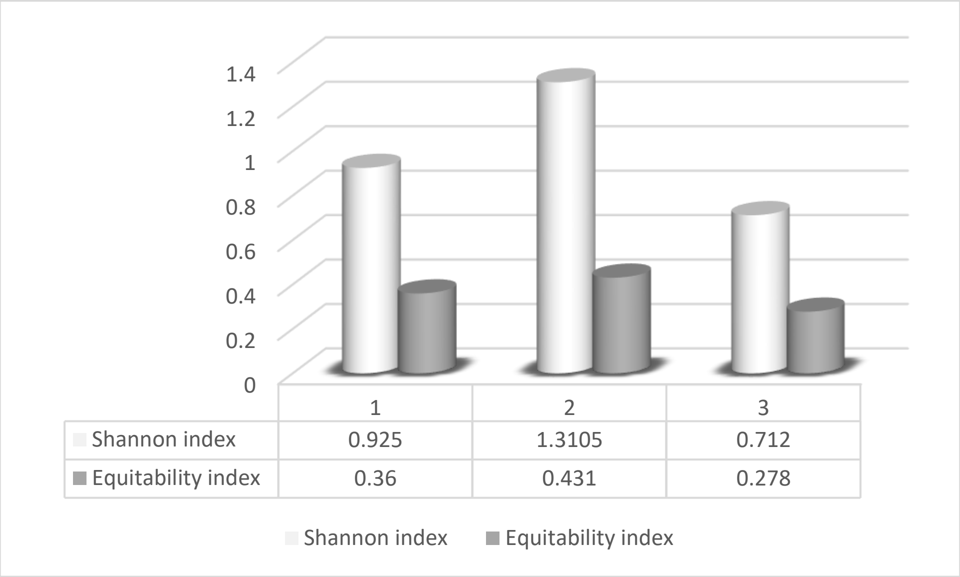

Figure 1: Shanon diversity and Equitability indices of earthworms in the studied habitats

(1=Grassland,2=Forest,3= Cultivated Land)

Table 1: Earthworm population in different habitats. Bold values indicate habitat specific species.

	Grassland	Forest	Cultivated land	Total
Aporrectodea caliginosa	95	52	0	147
Ap. trapezoides0	100	38	25	163
Ap. rosea	24	0	30	54
Ap. jassyensis	0	**127**	0	127
Ap. longa	39	18	0	57
Dendrobaena byblica	0	**128**	0	128
D. hortensis	0	21	30	51
D. pentheri	**33**	0	0	33
D. veneta	0	0	**21**	21
Dendrodrilus rubidus	**14**	0	0	14
Eisenia andrei	0	0	**25**	25
Perelia kaznakovi	0	**117**	0	117
Amynthas corticis	0	0	**87**	87

Discussion

Maximum species diversity in terms of Shannon-Wiener and Equitability indices were found in forest habitats. Edwards and Bohlen (1996) stated that earthworm diversity ranged from 1-15 species, while most earthworm communities contain 3-6 species. Goswami (2015) reported the occurrence of 7-11 species from cultivated, non-cultivated, grassland, garden and sewage soil. In this study, the highest species richness was found at forest habitats. Seven species were found in forest habitats. The highest value of species richness at forest habitat could be due to food abundance and suitable factors compared to other sites. Lee (1985) stated that earthworm diversity is greater in natural systems than the interfered habitat. Cultivated habitats have constant disturbance due to some activities and gives the lowest diversity and equitability indices. Attained organic stability is due to leaf litter and constant moisture. Stable ecosystems have high species diversity than unstable environments (May, 1979). The factors that influence the diversity of earthworm community at a given locality, apart from the type of soil, climate and the available organic resources, are the land use pattern and disturbance (Edwards and Bohlen, 1996). Vegetation maintains soil moisture and soil living organism (Widyastuti 2004). Poor vegetation cover and lack of plant litter in the soil surface tend to reduce the productive habitats. The more productive habitats can support more species (Peet, 1974).

Conclusions

In this study diversity and abundance of earthworms studied in three varied habitats. The combination of ecological factors, species specific distribution Patterns and food preferences may explain the local earthworm composition. High abundance of some species may serve as indicator of various soil properties and plant growth. In this study forest is the best habitat for the highest diversity. Forest habitats have higher proportion of native and smaller proportion of cosmopolitan earthworm species in comparison to other habitats.

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Soil biodiversity from sciences to action – feedback from two decades of soil bio-indicators development as agricultural soil management tool

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Abstract summary

The French Law for the Recovery of Biodiversity, Nature and Landscapes has set a target of reducing the net loss of biodiversity to zero. This biodiversity action plan aims to put this goal into action. One of his aims is to improve our knowledge of soil biodiversity and to develop agricultural and forestry practices that allow it to be conserved restored and developed. This plan can build on several initiatives that were launched since the early 2000’s between the French soil science sphere and the French state to improve knowledge on soil biodiversity and to develop indicators relevant for soil quality monitoring and land use decision The implementation in soil monitoring and participatory observatory networks allowed to gain experience on numerous soil types, land uses and agricultural practices and to establish the first reference values e.g. for microbiological characteristics at national scale. The standardisation of protocols, the industrialisation of soil biological analyses, the emergence of advisory services offer based on soil bioindicators allow these tools to be mobilised by the greatest number of soil managers.

Keywords: biodiversity, soil biological indicators, monitoring networks, standardisation, soil management

Introduction, scope and main objectives

Soil health has been defined as the capacity of soil to function as a living system. Healthy soils maintain a diverse community of soil organisms that help to control plant disease, insect and weed pests, form beneficial symbiotic associations with plant roots, recycle essential plant nutrients, improve soil structure with positive effects for soil water and nutrient holding capacity, and ultimately improve crop production. A healthy soil also contributes to mitigating climate change by maintaining or increasing its carbon content (FAO, 2015). Hence, as stated by Janez Potocnik in European Commission (2010), “Biodiversity loss and climate change are two of the most pressing challenges of our time, and soil biodiversity is part of the solution to both”.

The French Law for the Recovery of Biodiversity, Nature and Landscapes (METS, 2021), published in 2018, has set a target of reducing the net loss of biodiversity to zero. Acting for the preservation of soil biodiversity is one stake of the French Biodiversity Plan. The implementation of this plan is built on initiatives, launched since the early 2000’s to improve knowledge on soil biodiversity and to develop indicators relevant for soil quality monitoring and land use decision.

In this paper, we propose to report these initiatives and their main results. Then, we will discuss how they may contribute to the implementation of the French Biodiversity Plan and draw some perspectives.

Development and testing of soil biological indicators

In the past decades, several calls for research proposals focusing on Ecosystem Services and soil biodiversity were launched by French governmental organisations. In 1998, the French Ministry of Environment has set up the programme "Environmental functions and management of soil heritage" (www.gessol.fr). This programme funded several projects dedicated to the development of soil biological indicators (e.g. Decaëns *et al.,* 2014) and to test their applicability to assess soil functions (e.g. Dubs, 2014). Between 2002 and 2012, the French Agency for Environment and Energy Management (ADEME) co-funded the programme "Bioindicators – biological tools for sustainable soils ", whose objectives were to promote the standardisation of bioindicators to monitor soil quality and to assess the risks for ecosystems and polluted sites. ADEME has published advisory notices on soil bioindicators implementation and recommended key indicators (table 1). Also, the French Ministry of Agriculture (MAA) has published in 2017 an overview of indicators relating to the organic and biological state of soils for farmers (Ministry of the Environment, Energy and The Sea and Ministry of Agriculture, Agri-Food and Forestry, 2017).

In 2018, the French Biodiversity Agency started the research programme "Soil biodiversity and agro-ecology » dedicated to the deciphering of the links between soil management and soil biodiversity. Other national programmes (e.g. SYSTERRA, AgroBiosphere) founded by several research agencies, not directly addressing soil biodiversity, provided relevant knowledge while studying soil contamination or innovative agricultural practices.

At EU level, French laboratories gained expertise and knowledge on the use and interpretation of biological indicators involved in research projects (Envasso, European Commission, 2010b; EcoFinders, European Commission, 2010c; Landmark, European Commission, 2010d).

Table 1: Minimum set of indicators for monitoring agricultural soils (ADEME, 2012)

Monitoring pupose	Indicators	Parameters
Management of sil organic matter	**Micobial**	**Abundance: microbial and fungal biomasses** **Diversity of communities** **Activities: C and N mineralization, ergosterol measurement**

	Fauna	**Abundance and biomass of earthworms** **Functional diversity of nematodes**
Management of agricultural practices	**Microbial**	**Abundance: microbial and fungal biomasses** **Diversity of communities** **Activities: enzymatic measurements linked to C, N, S and P cycles**
	Fauna	**Functional diversity of earthworms and nematodes** **Diversity of collemboal**

Implementation in soil monitoring networks

In 2009 within the FP7 Envasso project it was underlined that biological indicator were missing in quite all EU soil monitoring networks (Morvan *et al.*, 2008) and a list of potential indicators to monitor decline of soil biodiversity was proposed on a tiered approach (Bispo *et al.*, 2009) (Figure 1). Based on the national expertise and the existing protocols, indicators were tested and implemented in the French soil quality monitoring network (RMQS) to spatially assess soil quality across mainland France and overseas territory. Between 2006 and 2012, inventories were conducted at national level, on the soil microbial biomass (Dequiedt *et al.*, 2011) and bacterial communities (e.g. Karimi *et al.*, 2018). At regional scale several biological indicators were also measured (e.g. Ponge *et al.* 2013). A new initiative is underway aiming at inventorying soil microflora and fauna as well as some functional measurements (Imbert *et al.*, 2021) on the RMQS.

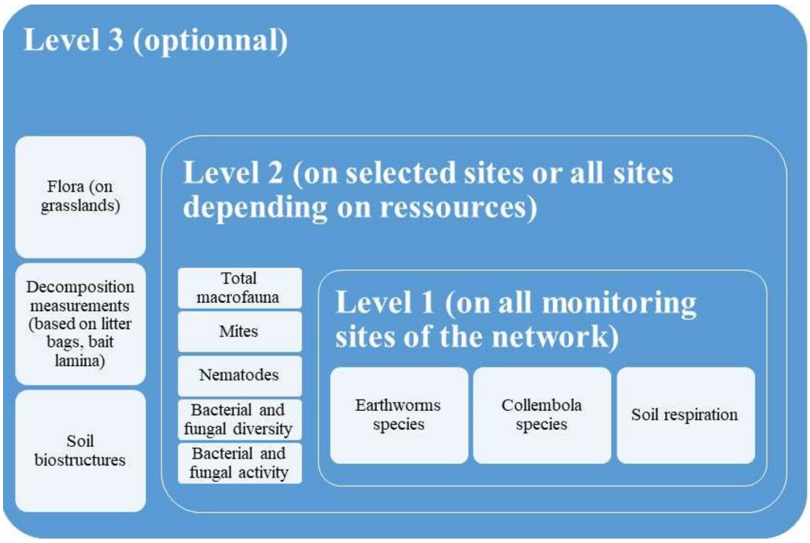

Figure 1: Proposed indicators to monitor soil biodiversity decline in monitoring networks (based on the results of Envasso, see Bispo *et al.*, 2009)

In addition, research organisms have their own long-term monitoring infrastructures (https://www.anaee-france.fr/, IR OZCAR or CA-SYS Agroecology Platform) soil biodiversity are performed on their sites.

During that time, note that participatory observatory networks were also promoted. Volunteer professionals and gardeners are hence encouraged to participate to the observatory programs of earthworms

(OPVT - https://ecobiosoil.univ-rennes1.fr/OPVT_accueil.php) and the agricultural observatory of biodiversity (OAB - http://www.vigienature.fr/fr/agriculteurs). Based on available data, several soil biodiversity indicators are already included in the National Observatory of Biodiversity: earthworms and bacteria.

Transfer through training, standardisation and services offer development

The awareness and training of farmers, agricultural advisers are a key step to consider soil biodiversity. The participatory observatory programmes OPVT and OAB are deploying in training courses of future farmers and agricultural advisers. MAA funded a network about biodiversity and agriculture (http://www.rmt-biodiversite-agriculture.fr/moodle/) with research organisms and different schools of agriculture. The AgrInnov project (2011-2015), funded by MAA and coordinated by INRAE and OFSV (https://www.ofsv.org/), has linked through a participative approach, researcher on soil biological indicators with a network of 250 farmers throughout France. This project has developed and transferred training and dashboard indicators of soil biological quality directly to farmers. The Experimentation and Monitoring Network for Agricultural Innovation takes over from AgrInnov project to train farmers, with the aim of changing their farming systems towards environmental and economic sustainability.

The mobilisation of biological tools as indicators of good soil functioning by agricultural professionals requires also the existence of a supply market to meet the need and also to have protocols that are standardised. ADEME provide financial support to the industrialisation of soil biological analyses and the emergence of advisory services offer based on soil bioindicators for soil managers (agriculture, polluted sites, brownfield management). For instance, the AgroEcoSol project coordinated by the AUREA laboratory aims at developing an advisory service for farmers from 2021 (https://www.ademe.fr/agro-eco-sol).

Soil quality monitoring and development of services offer requires that the methods are widely recognised, reliable, comparable… Standardisation is arguably a best practice. Within the last decade, numerous soil biological indicators were standardised at the international level by the ISO-TC 190 committee (Figure2, from Bispo and Schnebelen, 2018). Also, international standards have been proposed on the basis if methods developed in context of the French research.

The development of an offer also requires being able to interpret the result of a single measurement. Guideline or reference values are needed to statue on the normality of a result (e.g. is the value expected compared to previous measurements or existing knowledge). Considerable databases are needed to do so including biological measurements, but also other factors used to interpret the results as climate, land use, soil type and physico-chemical parameters (Horrigue *et al.,* 2016). As exposed in Figure2, for mainland France, part of the biological indicators already have developed such references (marked "+") whereas others are just under development (marked "+/-") or not yet started (marked "-"). All those indicators can be linked to soil functions which are the way to communicate with land managers and users as farmers (Figure 2).

Group	Indicators	Organisms and/or methods	Standards	Existing references values for France	Link with soil functions
Fauna	Diversity	Earthworms (sampling/extraction)	EN ISO 23611-1	+	Organic matter degradation Biomass production Soil formation Water regulation
		Collembola/mites (sampling/extraction)	EN ISO 23611-2	+/-	Organic matter degradation Biomass production
		Enchytreids (sampling/extraction)	EN ISO 23611-3	-	Organic matter degradation Soil formation
		Nematodes (sampling/extraction)	EN ISO 23611-4	+	Organic matter degradation Biomass production Regulation of pests
		Total macrofauna (sampling/extraction)	EN ISO 23611-5	?	Habitat Organic matter degradation Biomass production Regulation of water and pests
		Fauna (metabarcoding)	-	-	Organic matter degradation Biomass production Regulation of water and pests
	Activity	Bait lamina	EN ISO 18311	-	Organic matter degradation
		Measurement of biostructures	-	-	Soil formation Water regulation Organic matter degradation
Microorganisms	Microbial biomass (based on DNA extraction)	DNA extraction	EN ISO 11 063	+	Organic matter degradation Degradation of contaminants
	Diversity of microbes	PCR analyses based on DNA extraction	ISO 17 601	+	Organic matter degradation Degradation of contaminants
		PLFA analyses	CEN ISO/TS 29843-1 et -2	-	
		Massive sequencing	-	+	
	Global activity	Respiration	NF EN ISO 16072	+	Organic matter degradation Degradation of contaminants
	Enzymatic activities (eg : N, P, S)	Biogeochemical cycles	ISO 14238 ISO/TS 22939 ISO 23753-1 ISO 23753-2	+/-	Organic matter degradation Degradation of contaminants Nutriment cycling

Figure 2: List of indicators with respective methods and standards – links with the soil functions (adapted from Bispo and Schnebellen, 2018)

Discussion and conclusion

The absence of an EU directive on soils leaves a wide range of possibilities to use those works in public policies. The agricultural or forest management practices for the benefit of soil biodiversity and functionality must be encouraged by labels, environmental certifications, by setting up Payments for Environmental Services (PES), soil diagnostics, or also in the 2nd pillar of the Common Agricultural Policy via Agri-environment-climate Measures. In his action plan for biodiversity, France points at that the "PESs will prioritize the development of practices to preserve soils and restore biodiversity" and the state "will promote soil condition diagnoses that enable purchasers and farmers to benefit from information on the condition of the soils they use in order to put in place appropriate management techniques to the benefit of biodiversity, production quality and the environment."

The different research programmes around soil biology have made it possible to acquire a pool of knowledge that can be mobilized to build tools that are both relevant for the assessment of ecosystem functions and services of soils and which meet the expectations of potential users. The standardisation of the protocols on soil biology quality gives a technical implementation framework. The successful mobilisation outside the scientific sphere will go through the improvement of the interpretation frameworks for farm advisory and the explicit identification of their added values compared to physico-chemical analysis. Moreover, we must keep on efforts in training the agricultural communities. This involves knowledge transfer on bio-indicators, through practical guides, training and decision-support tool but also development of participatory research-action involving the farmers. Finally, raising awareness should not be neglected. The Gessol program has developed in 2010 the Happy Families card games "The hidden life of soils" dedicated to soil biodiversity. This card game has been effective to arouse curiosity among the general public (Antoni *et al.,* 2019). Inspired by the successful Canadian initiative "Soil your undies" and by numerous feedbacks from French farmers, ADEME launched in 2019 a large-scale sensitization campaign named #plantetonslip.

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Biofunctool®, a multifunctional approach of soil health related to soil biota activities

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Abstract summary

In a context of global soil biodiversity loss, we need to find effective way to measure the functions supported by biodiversity. This constitutes the main challenge of soil health assessments, particularly in the agroecological transition context. The most shared definition of soil health is based on the ability of soil to function (Karlen *et al.*, 1997) and to provide ecosystem services. However, most methods focus on stock measurements rather than functions. Also, measurements are usually performed in the laboratory, reflecting the potential level of soil functions rather than true field performance. To overcome these methodological limitations, a new framework is proposed to assess soil health based on functional methods that considers the links between abiotic and biotic soil compartments. This method, called Biofunctool®, incorporates nine rapid, cost-effective, and in-field indicators to evaluate three main soil functions: C transformation, nutrient cycling, structure maintenance. The capacity of the set of indicators to assess the impact of land management on soil health will be illustrated in various agroecological contexts in the tropics. Biofunctool® allows to better understand the impacts of agricultural practices on soil functions driven by soil biodiversity and could be in the future included in environmental analyses.

Keywords: Soil health, Soil functions, Soil functional biodiversity, Soil C dynamic, Soil Structure, Nutrient cycle

Introduction, scope and main objectives

In order to move towards a more environmentally friendly and sustainable form of agriculture that preserves biodiversity, we need an effective way to measure the multiple functions (C transformation, nutrient cycling, structure maintenance etc.) provided by this biodiversity. The vision of a multifunctional soil, whose functioning results from the interactions between biotic and abiotic compartments, explains the emergence of the soil health concept (Ng and Zhang, 2019). Doran *et al.* (1994) defined it shortly as "the capacity of living soil to function within the limits of natural or managed ecosystems". However, this functional vision of quality is hardly reflected in the literature (Wienhold, Andrews and Karlen, 2004) and the majority of studies are based on additive vision of soil physical, chemical and biological properties. Some authors, as Kibblewhite, Ritz and Swift (2008) for example, noticed that these additive methods do not allow to assess the soil functioning. Integrated approaches taking into account the functions driven by soil biodiversity should be developed. Those integrated assessment methods do not allow to estimate the specific role of each species but rather assess the result of their interactions. Following this functional vision suggested by Kibblewhite, Ritz and Swift (2008), a set of indicators was developed to evaluate the functions carried by soil biological assemblages. The selection of indicators was made by a panel of experts who had to choose methods from the literature following three criteria. The indicators had (i) to assess one of the 3 functions exposed by Kibblewhite, Ritz and Swift (2008) (carbon transformation, maintenance of the structure and nutrient cycling); (ii) to be implementable in the field to capture the dynamics of undisturbed soil (iii) to be low tech and cost effective to facilitate the transfer to various stakeholders. Based on these different criteria, a set of nine field indicators called Biofunctool® was proposed. This set has been validated over a wide range of pedo-climatic and agronomic contexts (Thoumazeau *et al.*, 2019a; Thoumazeau *et al.*, 2019b; Pheap *et al.*, 2019). Results of the indicators have then been aggregated in an index (Biofunctool® Index - BI), that synthetize the impact of land management tested on soil quality. In this presentation, various case studies will be presented, showing that BI provides a synthetic score of soil functioning that is sensitive to land management and robust in various pedo-climatic contexts.

Methodology

Case studies

The first case study was over various land uses in Chachoengsao province in Thailand. The second case study was designed to compare various agricultural practices in Kampong Cham province in Cambodia. The third case study was achieved in immature rubber tree plantations after logging disturbance in Bongo, Ivory Coast. The different sites are briefly described in Table 2.

Table 1: Site description of the three case studies

	Chachoengsao - Thailand	**Kampong Cham - Cambodia**	**Bongo – Ivory Coast**
GPS position	13°34′; 101°27′	12°12′; 105°19′	5°29′; 3°35′
Soil texture (0-10 cm depth)	Clay: 21% Silt: 21% Sand: 58%	Clay: 68% Silt: 30% Sand: 2%	Clay: 11% Silt: 2% Sand: 87%
Mean annual precipitation (mm.yr^{-1})	1 328	1 577	1 640

Biofunctool® indicators

All Biofunctool® indicators are briefly presented in Table 1. The set was applied in field on the 0-10cm soil layer. The detailed protocols of laboratory preparations and field measurements for the nine indicators are presented in Thoumazeau *et al.* (2019a)

Table 2: Biofunctool® indicators (adapted from Thoumazeau *et al.* (2019a))

Soil function	Indicator name	Measured variable	Biological assemblages	References
Carbon transformation	POXC	Permanganate oxidable carbon	All assemblages	Weil et al., 2003
	SituResp	Soil basal respiration	Micro-organisms	Thoumazeau et al., 2017
	Lamina	Lamina baits	Mesofauna	von Törne, 1990
	Cast	Earthworms cast density	Earthworms	Adapted from Ponge et al., 2002
Nutrient cycling	AEMNO3	Fixed NO3- on ion exchange membranes	All assemblages	Qian et Schoenau, 2002
	NminSoil	Available nitrogen (NO3- and NH4+)	All assemblages	Maynard and Kalra, 1993
Structure maintenance	AggSurf / AggSoil	Aggregate stability (0-2 cm / 2-10 cm)	Macrofauna, fungi	Herrick et al., 2001
	Beerkan	Infiltration rate	Soil engineers	Adapted from Lassabatère et al., 2006
	VESS	Visual evaluation of the soil structure	Soil engineers	(Guimarães et al., 2011)

Statistical analysis

For every case study, statistical analyses were performed with a similar pathway, starting from descriptive statistics on each indicator to the analysis of the BI (Figure 1).

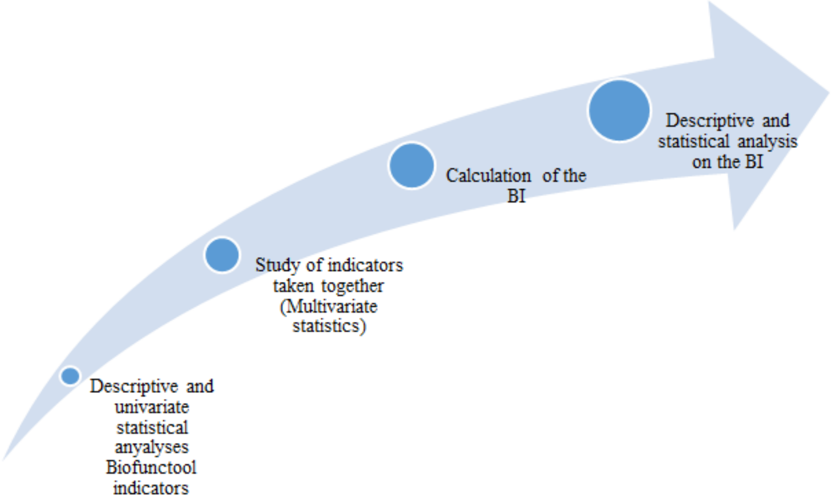

Figure 1: Scheme of data analysis trajectory of Biofunctool® indicators

Results

Impact of land uses changes

The rapid expansion of perennial crops such as rubber is a major threat to biodiversity in Southeast Asia. However, the impact of the conversion from intensively managed annual crops to perennial crops on soil health has not yet been addressed. We assessed in Thailand (Chachoengsao) the land use change impact from a cash crop (cassava) to rubber plantation and to forest on the soil health. BI was (i) not significantly impacted by land conversion (cassava to rubber); (ii) improved with tree ageing after 10 to 13 years of tree plantation due mainly to canopy closure and litter input; (iii) the highest in the forest, reaching twice the score of the cash crop (Figure 2). The three functions measured showed a different trend: the soil structure was mainly affected by land use change whereas the carbon transformation and nutrient cycling were more sensitive to tree ageing.

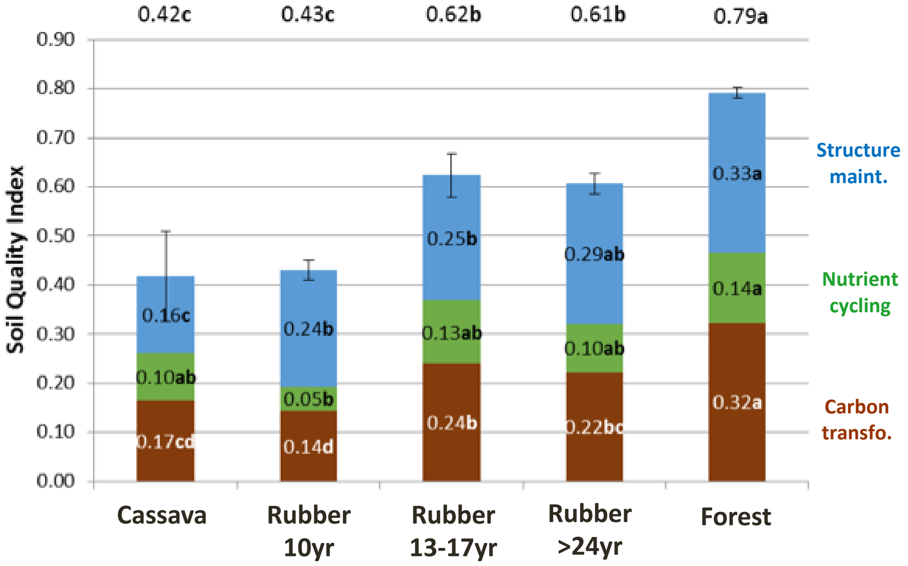

Figure 2: Impact of land uses changes on soil health in a rubber plantation chronosequence (Thailand)

Impact of agroecological practices (conservation agriculture)

Cambodia is a country facing a high level of soil degradation due to agriculture intensification (43 percent). Conservative agriculture (CA), characterized by (i) minimum soil disturbance; (ii) cover crop residues; (iii) use of diversified cropping patterns, has been considered as a way to restore soil fertility (FAO, 2014). The aim of this study was to assess the impact of contrasted agriculture practices on soil health in a long-term experiment in Cambodian uplands. Soil health was twice higher under the CA treatments than under conventional tillage (CT) treatment (Figure 3). Although it was similar in the three CA treatments, the contribution of each soil function to the soil health diverged within the CA (figure 3).

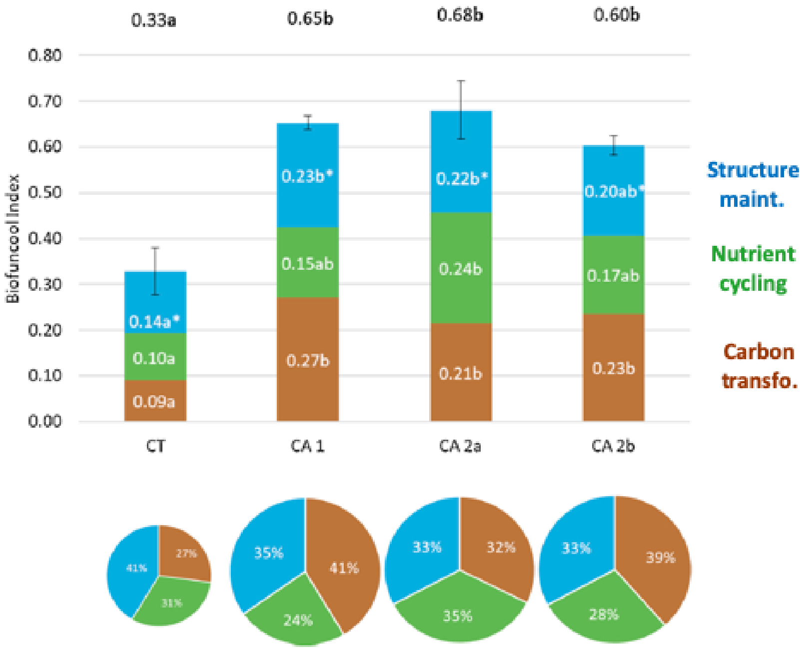

Figure 32: Impact of CT versus CA on soil health (Cambodia) (adapted from Pheap *et al.*, 2019)

Impact of soil disturbance (logging)

Soil functioning resilience after a perturbation constitutes a key scientific issue but remains poorly addressed. The objective of this study, in a rubber plantation in Ivory Coast, was to assess the effect of a gradient of logging residues input on soil health and resilience in the case of logging disturbance (Figure 4). We confirmed a significant recovery of soil health with logging residues 18 months after logging. Moreover, we observed that soil health recovery depended on the quantity and quality of the organic matter added, since legumes alone did not improve soil functions.

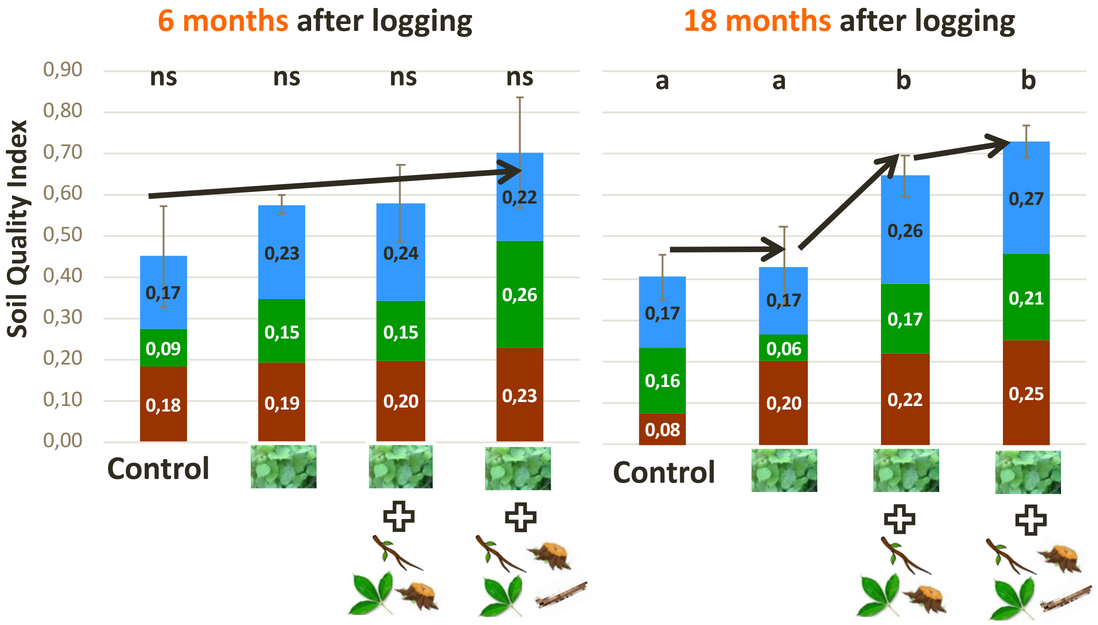

Figure 43: Impact of tree residues on soil health recovery at 6 month (left) and 18 months (right) after logging of mature rubber tree plantation (Ivory Coast)

Discussion

The functional approach of soil health provided by Biofunctool® allows an integrated analysis of changes in soil functioning at the soil system level as affected by land management. This assessment discriminated all the land management contexts explored in this study (land use change in Thailand, comparison of agricultural practices in Cambodia, impact of logging disturbance in Ivory Coast). The examples given in this article illustrate the benefits of the functional approach of soil health provided by Biofunctool® that are (i) multifunctionality; the BI makes it possible to consolidate information provided by all the indicators into a unique score that may be subdivided into scoring for three key soil functions (ii) genericity of certain results; as an example, the positive impact of the age of trees on soil health has been validated in various pedoclimatic contexts (Thoumazeau *et al.*, 2019b) (iii) adoptability: the Biofunctool® approach has been adopted and already used in other agricultural contexts by the different partners associated in these studies (RUA in Cambodia, LDD in Thailand, UNA in Ivory Coast).

Conclusions

This new functional, low tech, cost effective approach of soil health is consistent with the multifunctional role of soil. From an ecological perspective, this functional assessment will acknowledge the linkage between soil functions and biotic assemblages. Combined with others environmental, economic, and social criteria, this functional assessment will allow a better integration of soil biodiversity in the evaluation of agroecological transitions.

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Next generation biomonitoring to assess key species and soil parameters determining the biodiversity in agricultural soils

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Abstract summary

Soil management, and in particular microbial management, can facilitate agroecosystem sustainability, i.e., to better exploit natural resources already present in soil and to reduce the external inputs from agrochemicals (e.g. fertilizers). Thus, we propose a large-scale approach, utilizing next generation biomonitoring that combines DNA metabarcoding of soil biodiversity and agroecological network analysis, to determine key soil drivers and the keystone species influencing soil biodiversity. This knowledge will represent the prerequisite for supporting new sustainable agronomical practices aimed at sustainable agriculture.

Keywords: DNA metabarcoding, Agroecological Network, Microbes, Biomonitoring

Introduction, scope and main objectives

Soil biodiversity, particularly microbes, are playing a critical role in plant health and productivity, and any modifications to soil biodiversity will influence aboveground productivity. Indeed, plant and soil biodiversity management has been extensively demonstrated to be a valuable tool for sustainable agricultural practices, promoting agroecosystem health, environmental quality, and positively benefiting human health (Cavicchioli *et al.*, 2019).

The occurrence (i.e. presence or absence) and abundance of all the components of soil biodiversity (i.e. from microbes to larger metazoans), is associated with a plethora of biotic and abiotic factors such as: i) chemical contaminants; ii) the physicochemical characteristics of the soil; iii) land-use practices; iv) local environmental conditions (e.g., edaphic conditions, seasonality, etc.); and v) interactions with other organisms. In recent years, a DNA metabarcoding approach has been proposed to fully explore soil community diversity (Bohan *et al.*, 2017). Indeed, studies in which similar-size organisms were investigated revealed a positive correlation between abundances and genetic material (Carew *et al.*, 2013; Clarke *et al.*, 2017; Porazinska *et al.*, 2010) and taxonomically informative marker genes now exist for almost all organisms (e.g. 16S rRNA gene in Bacteria, ITS region in Fungi, and COI gene in arthropods) in parallel with well-established and comprehensive databases for a taxonomic assignation. In this respect, we propose a large-scale approach utilizing Next Generation Biomonitoring to quantify soil biodiversity and the complex web of interactions (both abiotic and biotic) influencing it, including ‘ghost interactions’ (i.e. past interactions not immediately visible) via reconstruction of agroecological networks of soil biodiversity. Specifically, the challenge we now face is understanding these drivers of soil biodiversity and how these can be managed for environmental monitoring, agricultural practices to maximize agroecosystem sustainability, and soil remediation.

Methodology

An agroecological community network is composed of the coexisting organisms, individuals that group together more frequently than with the rest, and the keystone species that are in the center of the network. Keystone species are ecologically defined as species that if removed can have dramatic effects on the whole community (Hines, Ray and Borrett, 2018; Layeghifard, Hwang and Guttman, 2018). Biodiversity assessment via DNA metabarcoding is an excellent tool to quantify soil communities due to the high resolution and the massively-parallelized taxonomic identification of taxa within a sample (Bohan *et al.*, 2017). Moreover, advances in sequencing systems, bioinformatics, and computational technologies and data analysis (i.e. mashing learning) can shed new light on the soil biodiversity and functionality at the finer characterization of soil communities. Additionally, the DNA metabarcoding approach coupled with agroecological network analysis have already been demonstrated to be a robust tool to investigate the multilevel community organization community within an ecosystem (Hines, Ray and Borrett, 2018).

Conclusion

The agroecological network will unravel the species-species, species-soil interaction, of the soil diversity associated with the different land use. Soil multifunctionality is the basis for food production, water filtration, nutrient cycling, climate mitigation, and human health. Type, quantity, and quality of soil ecosystem services are strictly dependent on the specific chemical, physical, and biological soil characteristics as well as on the management type. Non-sustainable agricultural practices with significant inputs of agrochemicals and fertilizers lead to changes in soil health, decreasing the capability to provide ecosystem services. The identification of the key drivers and keystone species influencing the soil biodiversity combined with metabarcoding methods will represent the prerequisite for the setup of agricultural practices aimed at sustainable agriculture. Soil microbial management will permit to exploit natural resources better already present in soil and to reduce the use of external inputs.

Acknowledgements

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Soil Protozoa diversity at coal post-mining area at different age of reclamation

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Abstract summary

The reclamation of post coal mining area has been done sixteen years ago in Kutai District - East Kalimantan, Indonesia. The objectives of this study were to assess the diversity of soil protozoa in six different age of soil reclamation at post coal mining area. Reclamation area was planted by fast growing trees such as *Acacia mangium, Paraseranthes falcataria* and *Samanea saman*. Soil samples was done at six different age of reclamation at three different plants area (*Acacia mangium, Paraseranthes falcataria, Samanea saman*). Soil protozoa identification was conducted until morphospecies level. The results found 43 species of protozoa which consist of four groups (Amoeba, Testate amoeba, Flagellates and Ciliates). The occurrence of Protozoa in the area of three tree species (*A. mangium, P falcataria* and *S saman*) at post coal mining area tend to increase by the increasing the age of reclamation.

Keywords: Amoeba, Ciliates, Flagellates, Testate amoeba, post coal mining, succession

Introduction

Reclamation in post coal mining areas involves moving the overburden to its original contour and spreading topsoil over it. After the reclamation process is completed, the reclamation site is then ready to be revegetated. At reclamation sites, soil nutrients are generally limited, soil pH is low, and there are often metal contaminants; therefore, revegetation activity must be carried out with plants selected on the basis of their ability to survive and regenerate or reproduce under severe conditions. Normally the revegetation process is started by selecting plants that are resistant to drought, or fast-growing crops or fodder which can grow with limited nutrients (Komara *et al.,* 2016). Reclamation in post coal mining area at East Kalimantan – Indonesia has been done 16 years ago, by planting fast growing tree and local tree. The efforts on soil restoration are related to input of organic material from trees or vegetation, and the decomposition and mineralization process carried out by soil organisms which served as the key factors in the functioning of the soil ecosystem. Microorganisms play an important role in the support of nutrient cycle in the soil. The key component of biodiversity involved in soil fertility and plant productivity is bacterivores (Bardgett and Cook, 1998; Bonkowski, 2004). Bacterivores are mostly represented by protists (Rønn, Vestergård and Ekelund, 2012). Soil-eating protozoa of soil bacteria are unicellular creatures, which are based on their locomotion and are divided into ciliates, flagellates, amoebas and amoeba testate (Trap *et al.*, 2015). Ciliated protozoa have been studied, flagellates, amoebas and amoeba testate are rarely performed (Frouz *et al.*, 2013). The purpose of this study was to assess the diversity of soil protozoa in six different age of soil reclamation at post coal mining area which planted by three species of tree (*Acacia mangium, Paraseranthes falcataria* and *Samanea saman*).

Methodology

The study was conducted in an overburden field at a post coal mining (00°02′20″ – 00°13′00″ North Latitude and 117°12′50″ – 117°23′30″ East Longitude) in the lowlands of East Kutai District. East Kalimantan Province (approximately 58-200 m above sea level) - Indonesia. The land reclamation has been carried out for 16 years. The climate in this area was A type with average rainfall ranging from 9.65 to 19.38 mm/day. The soils type was Ultisol and Inceptol with the texture of sandy clay loam.

Revegetation was conducted by planting fast growing species (*Acacia mangium, Paraseranthes falcataria* and *Samanea saman*) as pioneers (Komara *et al.,* 2016). Maintenance is carried out by controlling the plants every three months for one year, the dead plants are replaced with the same species. The study site was determined based on the survey of the reclamation process. There are 10 different locations namely premining land (land that has not yet been mined) and reclamation land with the ages of 0, 1, 2, 3,4, 5, 6, 11 and 16 years (Komara *et al.,* 2016). According to the age of reclamation and followed the previous research, six different locations were chosen. T0 is the area that has not been reclaimed, T1 is land that was reclaimed a year ago, T2 is land that was reclaimed 2 years ago and T6 was reclaimed 6 years ago. Based on chrono sequences the study sites were T0, T1, T2, T3, T4, T5, and T6. At each location, a plot of 20mx20m was set and 20 trees of each species (*A mangium*, *P. falcataria* and *S. saman*) was chosen. Soil sample collection was conducted by auger (5cm in diameter) at each tree (Husen, 2007). There were 20 samples from each tree species. All the soil sample from each species collected was mix homogenously. One hundred gram of mix soil was placed in a black plastic bags and conserved in a cooler box for transport to the laboratory for further process. In the laboratory the soil was sorted and cleaned from rocks and rubbish, then filtered and stored in plastic bag. Identification of protozoa requires a longer time, for that reason, the bag is opened and allowed to dry for several days in the refrigerator with a temperature between 10 to 15° C. After drying the bag is closed and stored at 4°C (Adl, Acosta-Mercado and dan Lynn, 2008).

Protozoa identification based on protozoan locomotor. Ciliates are identified following the procedure of Adl, Acosta-Mercado and dan Lynn (2008), Amoeba identification refers to Smirnov and Brown (2004), and Flagellates was carried out according to the procedure of Adl *et al.* (2005). The identification conducted up to morphospecies level and also refer to Smirnov and Brown (2004), Tikhonenkov *et al.* (2012), Bass *et al.* (2009), Ptácková *et al.* (2013) and Lee, Simpson and dan Patterson (2005) as references.

The abundance of active Amoeba and Testate was estimated following (Adl, Acosta-Mercado and dan Lynn, 2008). The soil sample (0.50 g) was placed in petri dish containing 5ml of distilled water. The suspension was observed under phase contrast microscope (200 times magnification). The number of amoebas in petri dish was counted. The active flagellates and ciliates were estimated from the soil subsample following the method of Adl, Acosta-Mercado and dan Lynn (2008). In a petri dish, one gram of soil sample was mix with 5ml distilled water and 15µm of its suspense was pipetted and placed in hemocytometer chamber. Counting chamber was observed under microscope Olympus Cx21 with 400magnification. The number of flagellates and ciliates was count and the abundance was determined by the total number of cells.

Results

Observation in post coal mining area at different age of reclamation found 43 species of protozoa. The diversity composed of four groups that were Amoeba, Testate amoeba, Flagellates and Ciliates. The protozoa composition varied (Table 1).

Table 1: Diversity of protozoa at post coal mining at East Kalimantan

Amoeba	Testate amoeba	Flagellates	Ciliates
Filum: *Sarcodina*	**Filum:** Amoebozoa	**Filum:** Euglenozoa	**Filum:** Ciliophora
1. *Actynophyris* sp	1. *Heleopera rosea*	1. *Bodo* sp 1	1. *Colpidium campylum*
Filum: *Flabellinea*	2.*Heleopera* sp.	2. *Bodo* sp 2	2. *Colpoda* sp.
1. *Vannella platiypodia*	3. *Arcella* sp.	3. *Anisonema ovale*	3. *Litonotus* sp.
	4. *Nebela* sp.	4. *Entosiphon sulcatum*	5. *Sphaerophyra magna*

Filum: *Tubulinea*	5. *Hyalosphenia minuta*	5. *Peranema trichophorum*	6. *Spathidium spathula*
1. *Trichamoeba* sp.	6. *Breviata Anatema*	6. *Neobodo designis*	7. *Vorticella similis*
2. *Hartmannella* sp.	**Filum:** Cercozoa	**Filum:** Cercozoa	8. *Leptopharinx* sp.
3.*Hartmannella Vermiformis*	1. *Cyphoderia* sp 1	1. *Cercomonas* sp 1	9. *Didinium* sp.
	2. *Cyphoderia* sp 2	2. *Cercomonas sp 2*	10. *Halteria Grandinella*
Filum: Percolozoa	*3. Euglypha cilliata*		
1. *Vahlkampfia russeli*	4. *Euglypha compressa*	**Filum:** Choanozoa	
	5. *Trinema* sp	1. Salpingoeca sp.	

The communities of Amoeba and Flagellates always present in the area of *A. mangium, P falcataria* and *Samanea saman* (Figure 1).

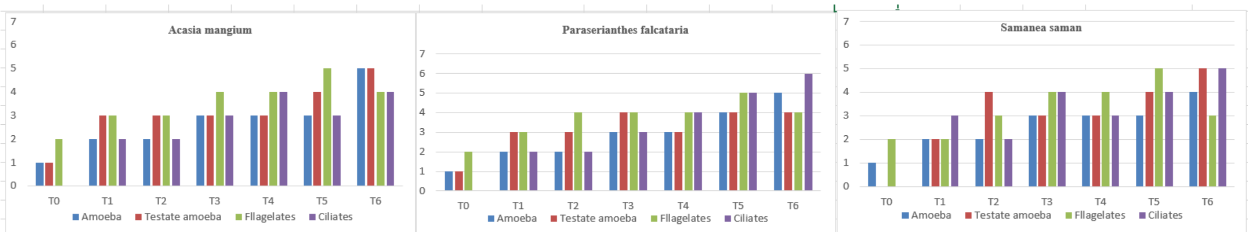

Figure 1: The occurrence of Protozoa at different age of reclamation in the three area of trees (*A. mangium; P falcataria* and *S saman*) at post coal mining area at East Kalimantan

The species that is almost always present in every site of reclamation area is *Anisonema ovale* Kleb which grown at the area of *Paraserianthes falcataria* two years after reclamation. In *Acacia mangium* the present of *Anisonema ovale* Kleb started three years after reclamation.

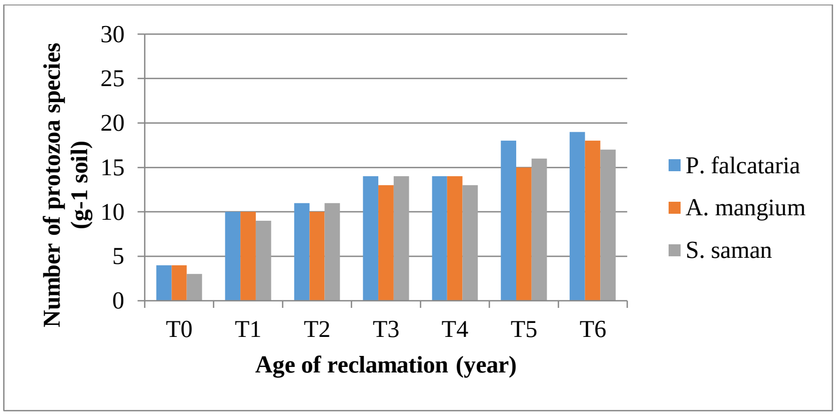

Figure 2: Number of protozoa species from six area of different age of reclamation at three different area of trees (*A. mangium, P falcataria* and *S saman*)

Discussion

In general, the number of species protozoa at the area of one, 2, 3, 4, 5, and 6 years after reclamation tend to be increased (Figure 2). This phenomenon showed that there were succession proses of protozoa community.

Conclusions

After six year of reclamation, 43 species of protozoa found in the reclamation area especially at the area of fast-growing tree (*Acacia mangium, Paraserianthes falcataria* and *Samanea saman*). Following the age of reclamation area, the number of protozoa tend to increase by the increasing the age of reclamation.

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Soil survey in agricultural environments within the Biodiversity Monitoring South Tyrol (BMS)

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Abstract summary

For the northernmost province of Italy, South Tyrol, a permanent biodiversity monitoring system has been set up on the initiative of the South Tyrolean provincial government and under the direction of the Institute for Alpine Environment, Eurac Research. This monitoring not only serves as basic research but also intends to provide a scientific basis for political decisions, especially in with respect to spatial planning, agriculture and nature protection. The Biodiversity Monitoring South Tyrol (BMS) aims to survey species groups that react sensitively to environmental and land-use changes.

For the soil survey within the BMS we aim to investigate how the different habitats differ in species composition and biodiversity living on and beneath the soil surface. Special focus is given to a range of farming systems (e.g. crop fields, apple orchards, vineyards, grasslands). Preliminary results show a change in the community composition between grassland and the more intensively used fields towards a more generalistic community composition.

Keywords: BMS, Biodiversity Monitoring, soil, invertebrates, pitfall traps

Introduction

The establishment of a permanent biodiversity monitoring system for South Tyrol is of great importance for a better understanding of biodiversity patterns in the province of Bozen/Bolzano (Italy), but also for creating a scientific basis for political decisions, especially with respect to spatial planning, agriculture, nature and environmental protection. The BMS assess species groups that are sensitive to environmental and land-use changes. In addition, the BMS should also meet important administrative requirements, such as reviewing the impact of environmental measures taken or serving as a basis for the regular report on the status of species and habitats under the Habitats Directive. Last but not least, long-term biodiversity monitoring can provide well-founded answers to current questions (such as the role of agriculture in relation to biodiversity) and thus contribute to objectifying social and political debates.

Part of the BMS are comprehensive soil surveys. Soils are among one of the most species rich habitats worldwide (i.e. 25 percent of all known species live in or on soil, Orgiazzi *et al.,* 2016), and soil organisms are influenced by abiotic as well as biotic factors. Important factors are pH, soil organic content parameters (SOM), soil texture and carbon to nitrogen ratio. For example, soil texture has a big impact on the activity of soil organisms, and the change in pH influences the metabolism of species and can thereby often be lethal to them (Turbé *et al.,* 2010). For this reason, it is important to carry out a long-term biodiversity monitoring in order to better understand the consequences of changes in abiotic factors (e.g. due to climate change) or the progressive intensification of agriculture, and what strategies need to be taken to preserve soil biodiversity.

A large number of samples from the first year of the BMS survey are still being processed, we thus present and discuss preliminary results regarding ground-dwelling invertebrates and environmental parameters from the different investigated agricultural environments (alpine meadows, meadows with different management intensity, pastures, annual crop fields, apple orchards and vineyards.

Methodology

For the soil survey we installed two pitfall traps on each site twice a year (late spring and autumn) to assess the surface-living animals, and further took soil core samples (spring only) to investigate the distribution and community structure of soil macro-invertebrates in the soils. The animals were stored in 75 percent ethanol and identified under a dissecting microscope. At each site, approximately 100 g of soil material was additionally taken to analyse the physical and chemical soil properties.

During the summer season 2019 (June to October) we investigated 64 individual sites covering a range of habitats, including 37 sites in agricultural environments, such as hay meadows of different management intensity, pastures, alpine grasslands, annual crop fields, orchard and vineyards. The study sites were distributed evenly across the entire Province of Bozen/Bolzano within a broad elevational range from 249 to 2670 m above sea level.

The community data was composed by the abundance of each species scaled to the days of exposure (total trap abundance divided by the days of exposure as individuals per sampling day; activity density [ind./day]). Variation in community composition of selected taxa between the different agricultural sites was expressed as Bray-Curtis dissimilarities, using the function *vegdist()* in the package VEGAN, using the open source statistical software R (R Core Team, 2020). With these dissimilarity values for each site a non-metric multidimensional scaling (NMDS; function *metaMDS()* in the package VEGAN) was performed to visualize the patterns in community composition in the selected agricultural sites. Environmental variables were scaled to a mean of 0 and SD of 1 and plotted ex post on the ordination diagram (using function *envfit()* again in VEGAN.

Preliminary results

Our preliminary results showed no clear differences in activity density [ind./day] and taxa richness of selected groups from caught with pitfall traps in agricultural sites (Figure 1). Vineyards showed the lowest activity density (40.18) whereas meadows showed the highest activity density. We found a correlation between soil organic content (SOM) and elevation (r = 0.67) and thus a possible factor why meadows at higher elevation differed in their species community composition from vineyards, apple orchards and crop fields which are located at lower elevation.

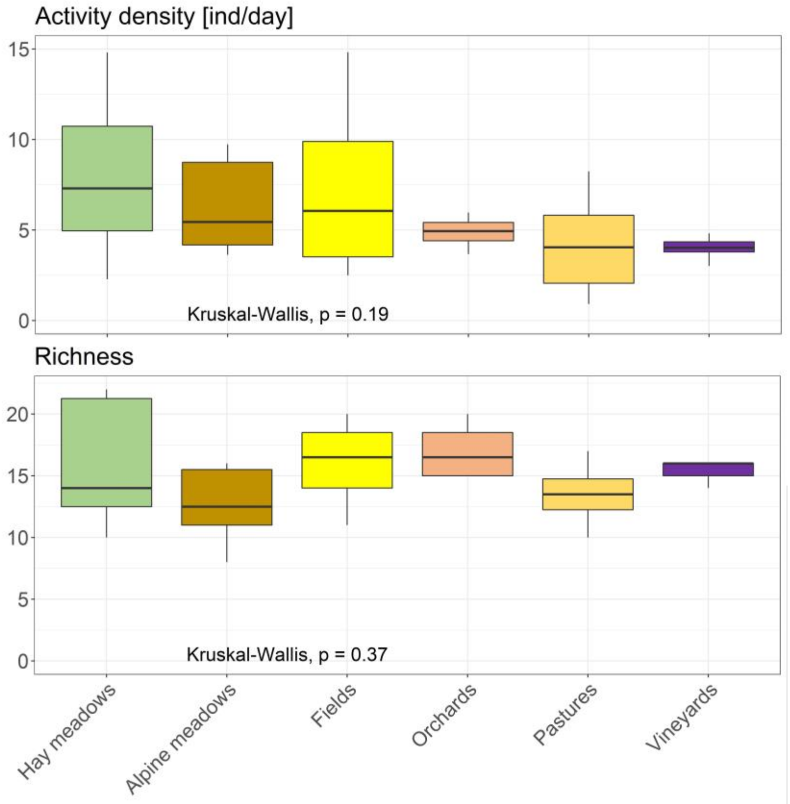

Figure 1: Activity density [ind/day] and taxa richness of selected groups sampled with pitfall traps in different agricultural sites (alpine meadows (n = 6), fields (n = 4), hay meadows (n = 12), orchards (n = 4), pastures (n = 6) and vineyards (n = 5)

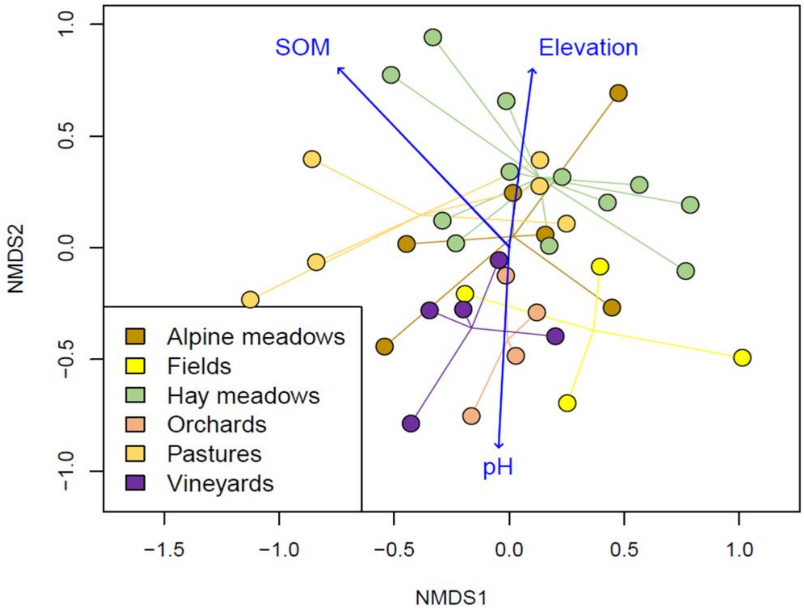

Figure 2: Non-metric multidimensional scaling (NMDS) of selected taxa sampled with pitfall traps in different agricultural sites

Each point represents a site and the environmental parameters pH, elevation and soil organic content (SOM) were plotted ex post on the ordination diagram.

Discussion and conclusion

The Biodiversity Monitoring South Tyrol started only in 2019 and most analyses have still to be conducted. However, here we present first patterns for the different agricultural habitats in terms of activity density, taxa richness and community composition. No significant differences concerning the activity density or taxa richness between the different agricultural practices could be detected so far. Further, the community composition indicated a similarity between intensively used sites versus grassland sites. We found a tendency that intensively used agricultural sites, like vineyards, harbour a lower mean number of taxa, caught per day, which would be in line with the recent literature (Hilpold *et al.*, 2018; Tsiafouli *et al.*, 2015).

Clear differences in community composition between differently used agricultural sites could not be confirmed, but we found hay meadows and pastures to be more similar than apple orchards or vineyards. We expect clearer patterns with the data from the subsequent years, which in turn confirms the importance of a long-term biodiversity monitoring in order to obtain more detailed patterns for a better understanding of the effects of various environmental factors.

From our point of view, it is important to carry out such long-term biodiversity monitoring surveys including many different taxonomic groups and soil analysis in order to gain a better understanding of the current species distribution and ongoing changes such as climate change or land-use changes (mainly intensification of agriculture). These findings are very important not only from a biologist’s perspective, but also for further decision making of the local and regional government to counteract biodiversity loss.

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Assessment of agroecological conditions of terrain and its soil cover using remote sensing data

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Abstract summary

Developing countries and countries which in recent decades had to pass through sometimes multiple changes of ownership of land suffer from lack of relevant actual knowledge of soil resources. Existing soil maps are outdated (30-50 years old) and are not applicable for today needs. This hampers development of their agricultural sector and implementation of modern agricultural technologies

Therefore, these countries have request for actualization of knowledge about soils and their agro productivity. Moreover, this information should be updates for extensive territories, within limited timeframe and with great accuracy. Besides, there is also request for understanding of the dynamics of ongoing changes of soils and agroecological conditions along with changes in methods of land use.

Keywords: sustainable land use, soil maps, agroecological appraisal, remote sensing data

Introduction, scope and main objectives

Speaking about sustainable land use, we must consider three main components. Firstly, these are the matters of preserving and restoring the soil, its biological diversity, content of mineral substances, and hence fertility. The second factor is spatial differentiation and local climate, which determine distribution of solar energy as well as water and mass transfer. The third is economic feasibility. Thus, implementation of sustainable agriculture requires detailed knowledge of soils (structure, ph level, content of mineral elements etc) and agroecological conditions (water balance, solar radiation etc). Selection of crops rotations and agricultural technologies should correspond to the same factors along with economic factors and market requirements.

Methodology

Perhaps the most reliable and the most available data provider for such tasks is remote sensing data (Nawar, Buddenbaum and Hill, 2015; Stasyuk *et al.*, 2017). However, it should be borne in mind that the task of updating soil data and monitoring the current state of soils is complicated by the significant variability of the spectrum of reflection of solar radiation due to human activities.

The proposed report highlights the methodology that, firstly, allows updating soil maps for vast territories in a short time and without large amount of field research. Secondly, it allows to “simulate” (restore) a knowledgebase about soils and processes occurring in them for a specified territory and for the period of about 30 years (history of observations using satellite images). This knowledgebase makes it possible to build forecasts and to monitor ongoing processes.

The core of the technique is the distinguishing (using analysis of a large number of satellite images obtained in different seasons and under different conditions) of spatial invariants that describe landscape-soil differences (Krenke, 2020). The resulting soil differences, supplemented by available historical data and, if necessary, field studies, can be used to build thematic maps of various semantics: from identifying soil properties and the suitability of the territory for specific crops to the applicability of certain agricultural technologies.

The main methodological component of this work is a hierarchical factor analysis and segmentation of the obtained invariant variables in order to highlight stable agroecological conditions. The essence of this technique is to obtain stable spatial objects using unstable reflections from the surface. A hierarchical factor analysis performed for the time series of remote sensing data, describing surface conditions for periods from 4 to 30 years, allows to identify factors of spatial organization that are constant over the observation period (Krenke, Puzachenko and Puzachenko, 2019; Sandlerskiy and Puzachenko, 2009; Puzachenko, Sandlersky and Svirejeva-Hopkins, 2011), and to filter out the components of variability in the original data. The distinguished factors can be associated with the processes that determine the properties of the soil cover. For example, for the differentiation of soil biodiversity, moisture and Ph can be the main governing factor and their spatial differentiation can be described by one or several invariants derived from remote sensing.

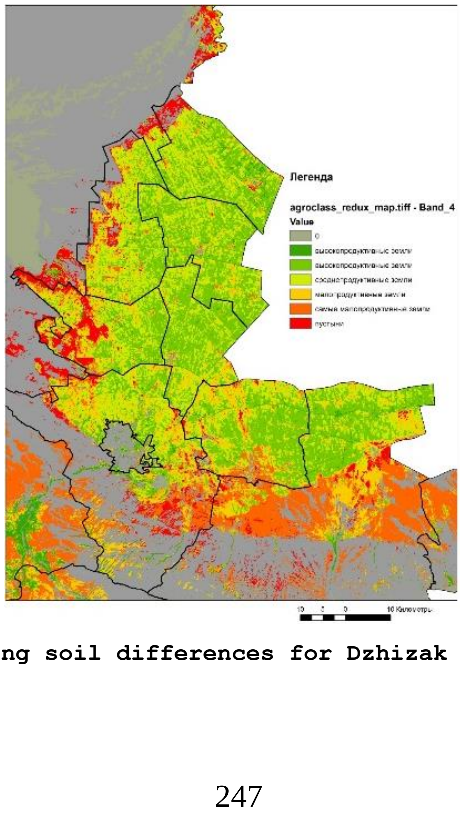

Figure 12: Resulting soil differences for Dzhizak region (Uzbekistan)

Results

Application of the proposed technique makes it possible to quickly develop functional maps, describing both invariant states, i.e., constant terrain characteristics, such as structure and composition of the soil cover and dynamic characteristics that change in time due to the human economic activity and seasonality.

Discussion

Proposed approach will help to overcome the limitations that hamper implementation of sustainable and exact farming in developing countries.

Conclusions

The proposed approach can be used both for global assessments of the state and quality of lands and for solving territorial planning tasks in agriculture, such as intensification of agricultural technologies, application of soil conservation techniques, prevention of soil degradation and soil restoration programs.

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Microscopic "canaries in the coal mine" - environmental sequencing of microbial communities uncovers how changes in climate and land cover affect soil biodiversity and functionality

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Abstract summary

Soil microbial communities represent the greatest reservoir of biological diversity known in the world and sustain the functioning of terrestrial ecosystems. Understanding environmental factors determining the structure and function of the soil microbiome is of pivotal importance to maintain ecosystem functioning and to prevent or mitigate soil degradation and biodiversity loss. Here I synthesize key findings of three soil DNA metabarcoding studies, each tackling a different aspect of studying the effect of environmental factors and land cover on soil fungal communities: 1) an elevation gradient study in Borneo; 2) a secondary tropical forest succession in Costa Rica; and 3) an experimental climate change study in the Alaskan Arctic. In all biomes studied, fungal communities respond strongly to changes in environmental variables, often resulting in alterations in functional profiles, which has potential consequences for vegetation feedback and carbon cycling.

Keywords: Abiotic Factors, Arctic Tundra, Climate Change, Functionality, Fungal Community Turnover, Tropical Forest

Introduction, scope and main objectives

Microorganisms are the foundation of the Earth's biosphere and represent most of the planet's biodiversity. They play crucial roles in ecosystem processes, such as biogeochemical cycling of nutrients, providing functions that ultimately sustain all life.

Primary production and related ecosystem services are highly dependent on soil microbes, including plant mutualists, such as mycorrhizal and root endophytic fungi, and nitrogen-fixing bacteria, as well as pathogens and decomposers. Understanding factors determining the structure and function of the soil microbiome are of great scientific value and has significant potential for application in various areas, e.g., to increase plant nutrient uptake and resistance to biotic and abiotic stresses and to combat problems related to soil degradation, biodiversity loss, climate change, emerging diseases, and sustainability in agriculture.

In this paper, I illustrate the utility of deep parallel sequencing of soil fungal communities to study how the soil microbiome is influenced by vegetation and climate and the recent changes therein.

The projects summarized here focus on 1) the relationships between soil fungal community composition and climatic and edaphic variables as well as vegetation types along elevation gradients in Palaeotropical forests; 2) the richness and community dynamics of soil fungi along secondary succession in Neotropical wet forests; and 3) the effect of experimental manipulations of climate warming on the functional richness of arctic fungi.

Methodology

The study areas included 1) lowland, lower montane and upper montane tropical forests in Kinabalu and Crocker Range Parks in Malaysian Borneo; 2) primary and early- (5-15 y), mid- (15-30 y), and late-successional (>30 y) regenerating secondary tropical forests in the Osa Peninsula of Costa Rica; and 3) arctic tussock tundra habitats subjected to 18 years of experimental passive warming at Toolik LTER in northern Alaska. The study sites and soil sampling procedures are described in detail in Geml *et al.* (2015) and Geml *et al.* (2017). In short, composite soil samples were taken from designated plots representing different habitats and were subjected to PCR and DNA metabarcoding of the ITS2 rDNA region using fungal primers. Soil water content, pH, and C, N and P content were analysed in all the samples. Taxonomic and functional assignments were made based on the curated reference database of UNITE (Kõljalg *et al.,* 2013).

Richness and community turnover of functional groups were compared among habitats using ANOVA and Tukey’s HSD test. Relationships between edaphic factors and functional guilds, and their relative abundance were analysed via linear regressions. We ran generalized non-metric multidimensional scaling (GNMDS) permutational multivariate analysis of variance (PerMANOVA) to determine the effect of land cover or habitat type as well as climatic and edaphic variables on the composition of soil fungal communities.

Results

In the elevation gradient study in Borneo, total fungal richness was generally similar in all three altitudinal forest types, but richness in functional groups showed distinct elevational patterns. GNMDS ordinations revealed strong structuring of fungal communities according to elevation in all functional groups (Figure 1). With respect to abiotic variables, only mean annual temperature (MAT) and soil pH correlated strongly with fungal community composition (p = 0.001) in GNMDS and explained the greatest variations in the total fungal community along the elevation gradient in PerMANOVA. In addition, forest type was a significant source of variation in community composition in all functional groups, explaining between 15.32 percent and 32.58 percent (data not shown).

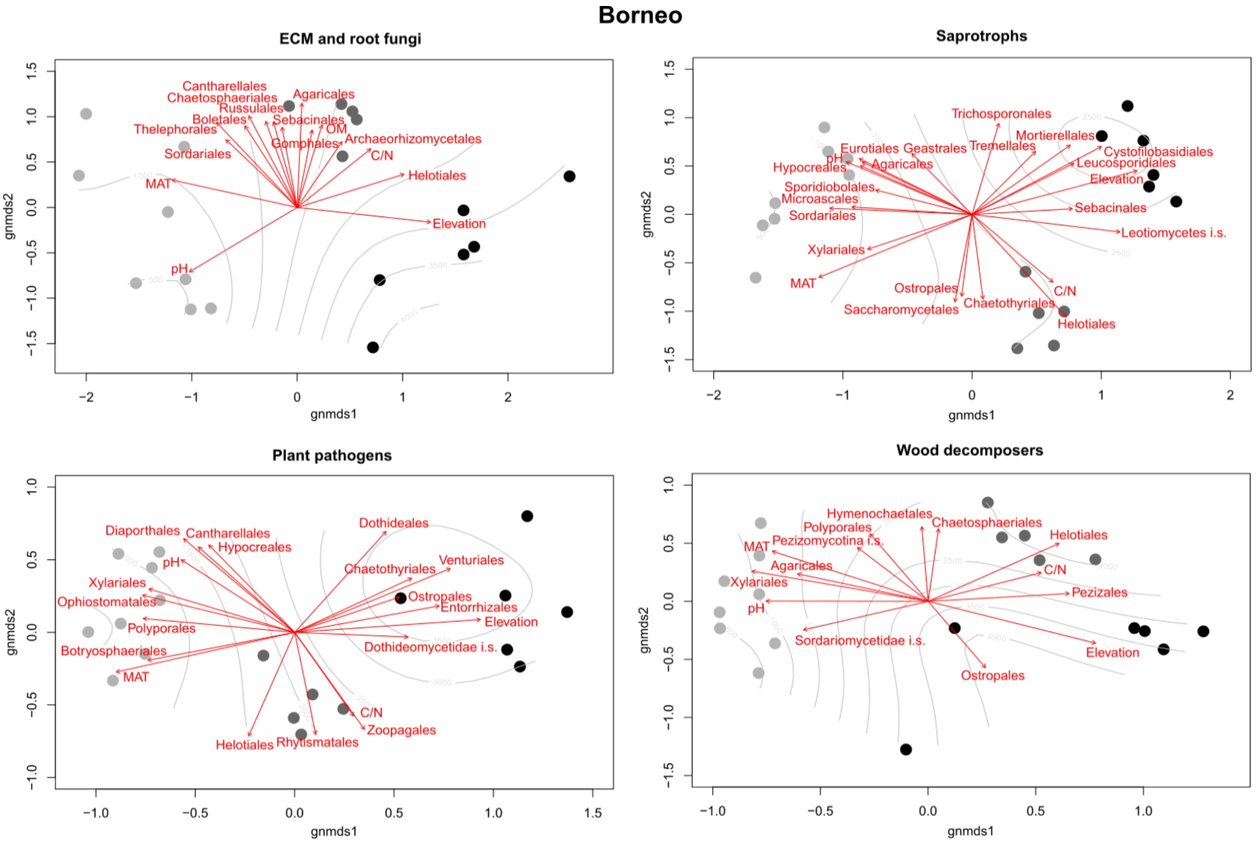

Figure 1: Generalized non-metric multidimensional scaling (GNMDS) ordination plots of fungal communities in the sampled elevational tropical forest types in Borneo, with elevation displayed as isolines

Localities and descriptions of the sampling sites are given in Geml *et al.* 2017. Vectors of environmental variables with significant correlations with ordination axes are displayed. Lowland, lower montane and upper montane forest sites are indicated with light grey, dark grey, and black symbols, respectively.

In the secondary succession study in Costa Rica, primary forest showed significantly higher fungal richness than early- (5-15 years) and late-successional (>30 years) secondary forests, while mid-successional (15-30 years of age) secondary forests were similarly rich in fungal species as primary forests (data not shown). When functional groups were analysed, saprotroph richness was lowest in early successional stands, while no were detected for plant pathogens, wood decomposers and root-associated fungi among the successional stages (Figure 2a). Fungal community composition was strongly correlated with abiotic factors and with each tree community composition (p = 0.002). Total fungal community turnover was significantly lower in primary than in early- and mid-successional secondary forests (p = 0.002), while community turnover among late-successional secondary forests was intermediate (data not shown). At the functional level, community turnover rates were significantly lower in primary than in one or more successional stages of secondary forests in plant pathogens, generalist saprotrophs, and wood decomposers (Figure 2b).

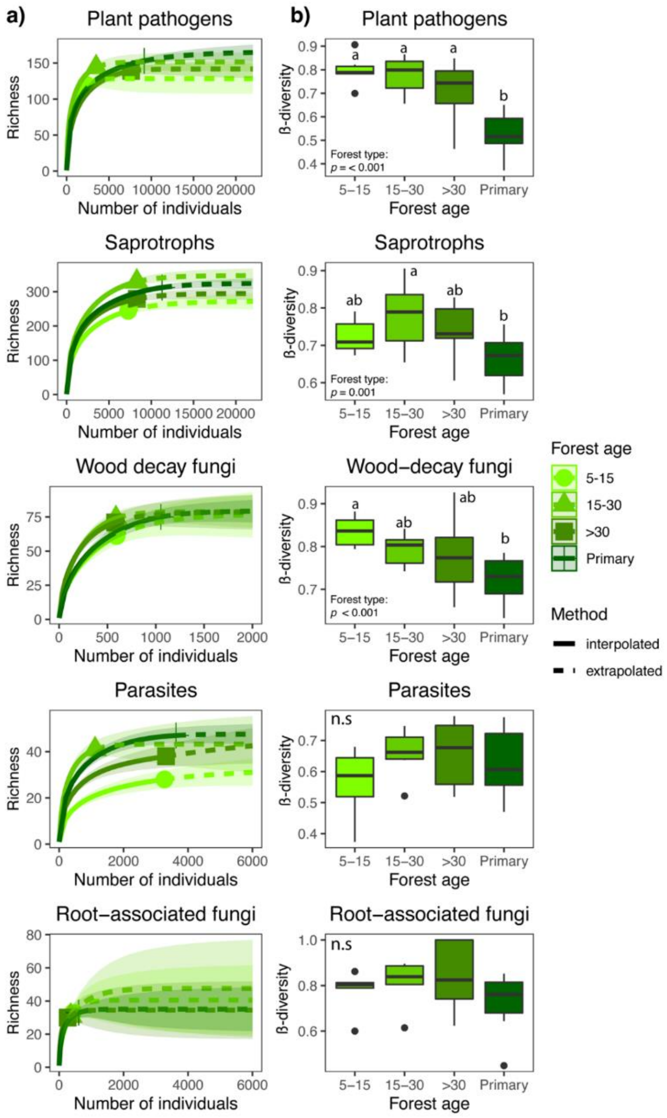

Figure 2: Comparison of richness (a) and compositional turnover, i.e. β-diversity (b), of functional groups of soil fungi in early- (5-15 y), mid- (15-30 y), and late-successional (>30 y) secondary and primary tropical wet forests in Costa Rica

Unbroken and dashed parts of the richness curves denote interpolated and extrapolated values respectively, and the shaded zone around each curve denotes the 95% confidence intervals. Mean values of compositional turnover were statistically compared using one-way ANOVA and Tukey's HSD test. Letters denote significant differences.

In the arctic tundra warming experiment, GNMDS plot revealed strong community turnover in response to the long-term warming treatment in all functional groups of arctic fungi (data not shown). With respect to functional groups, ectomycorrhizal (ECM) fungi represented the most OTU-rich guild and were strongly negatively affected by the long-term warming treatment, similarly to lichenized and litter decomposer fungi, while animal parasites and dark-septate root endophytic fungi were more diverse in the warmed plots (Figure 3).

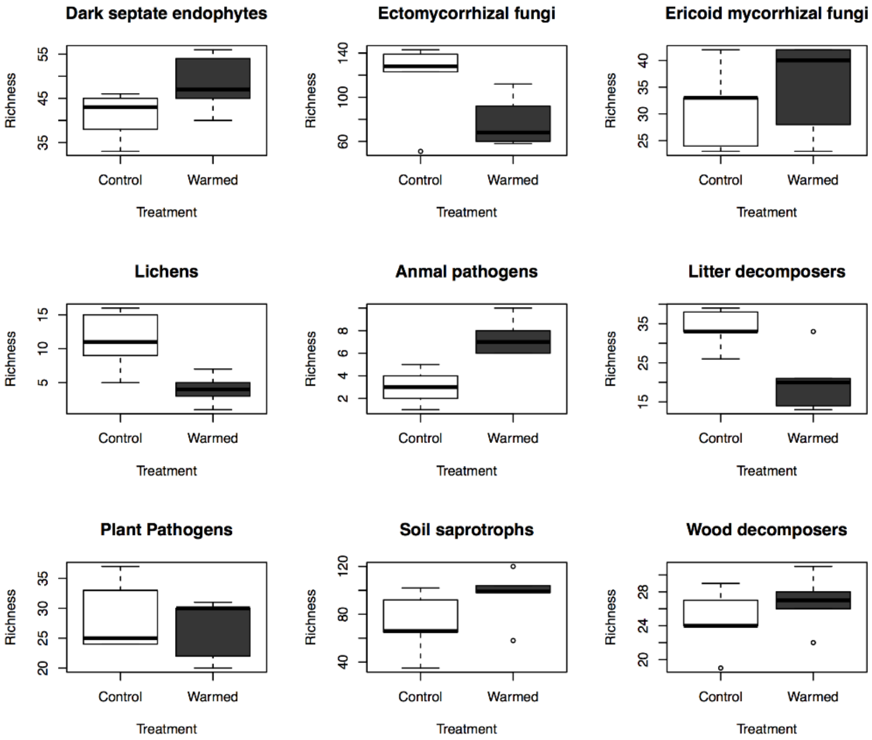

Figure 3: Responses of functional groups of arctic fungi to long-term summer warming in northern Alaskan tundra sites

(re-analysed data from Geml *et al.*, 2019)

Discussion

Along tropical elevation gradients, the data clearly show strong emerging patterns of compositional structure of soil fungi driven by elevation and the resulting environmental filtering according to contrasting climatic and edaphic conditions, and, to a smaller extent, differences in vegetation. Furthermore, the compositional data suggest that fungal communities in the lower montane forests may partly be regarded as intermediate between communities of the lowland and upper montane forests, although with several taxa specific to the lower montane environment.

We found evidence for marked changes in fungal richness between primary and several stages of regenerating secondary tropical forests. In secondary forests, fungal richness appears to be the highest in mid-successional stages, possibly due to presence of both early- and late-successional fungi. The greater compositional turnover of fungi among plots in early- and mid-successional stands than in primary forests may be explained partly by higher stochasticity in colonization following disturbance and by greater variation in environmental variables.

Our results show that arctic tundra fungal communities respond strongly to long-term experimental summer warming. While total fungal richness is not significantly affected by warming, there are strong differences in the responses of various functional groups of arctic fungi to long-term warming. ECM fungi represent the most species-rich and most abundant functional group in the arctic tundra and the strong decrease in their richness in the warmed plots and the compositional shifts likely have implications for ecosystem functionality.

Conclusions

The emerging picture from the above examples is that fungi respond strongly to changes in land cover and in climatic and edaphic factors in all biomes. In all biomes studied, functional groups respond strongly to alterations in environmental variables, often resulting in changes in functional profiles under altered conditions, which has potential consequences for vegetation feedback and carbon cycling. The habitat specificity exhibited by many fungi offers possibilities for monitoring and habitat characterization and we strongly advocate incorporating fungi in biodiversity assessments and conservation efforts.

Acknowledgements

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Soil bioanalysis: a simple and effective tool to access and interpret soil health

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Abstract summary

Soil health is a concept related to the ability of a specific soil to function in order to provide biological productivity, and important environmental services. As in other parts of the world, in Brazil the metrics for soil health assessments in agroecosystems also have been discussed. In the past 20 years, Embrapa´s research group on Bioindicators of Soil Quality has been dedicated to the selection of robust soil health bioindicators to be used in commercial routine soil analyses. The main objective was to provide a simple, effective and practical tool, which would allow onfarm soil health monitoring, so that farmers not only would know exactly what, why, how, and when to evaluate but, most importantly, how to interpret what is being evaluated. Because of these studies, two soil enzymes, arylsulfatase and β-glucosidase (associated with the S and C cycles, respectively) were selected and interpretative tables were developed. The inclusion of these two soil enzymes in routine soil analysis has been termed soil bioanalysis. In this work we describe how the soil bioanalysis was developed and some of its practical applications.

Keywords: arylsulfatase, β-glucosidase, soil quality; Cerrados, Brazil

Introduction, scope and main objectives

Brazil has evolved from a food-insecure country in the early 1970s to one of the most important food producers and exporters in the world. Production has increased steadily and productivity gains have fostered a significant land-saving effect. However, wide variations in the landscape, soils, climate and plant diversity present challenges in applying soil health principles for enhanced management practices. These challenges have been overcome by the application of conservation agriculture, an integrated, holistic farming system that improves soil functioning and consequently soil health, crop growth and yield.

During the past 40 years, Brazilian agriculture, namely in the Cerrado region, has moved faster towards sustainability, with the widespread adoptions of no-tillage (de Freitas and Landers, 2014) and more recently of integrated crop-livestock systems (Cordeiro *et al.*, 2015), which have promoted increased interest in soil health assessments (Balota *et al.,* 2004; Hungria *et al.,* 2009; Mendes, Sousa and Reis Junior, 2015; Mendes, 2016).

Soil health is a broad concept involving aspects related to soil biology, chemistry and physics. It has to do with the ability of a specific soil to function in order to provide biological productivity, and important environmental services, such as carbon sequestration, water storage, bioremediation of pesticides and to promote human, plants and animal health.

As in other parts of the world, in Brazil, the metrics for soil health assessments in agroecosystems also have been discussed. In the past 20 years, Embrapa´s research group on Bioindicators of Soil Quality has been dedicated to the selection of robust soil health bioindicators to be used in commercial routine soil analyses. The main objective was to provide a simple, practical and efficient tool, which would allow onfarm soil health monitoring, so that farmers not only would know exactly what, why, how, and when to evaluate but, most importantly, they would know how to interpret what has been evaluated. Because of these studies, two soil enzymes, arylsulfatase and β-glucosidase (associated with the S and C cycles) were selected and interpretative tables were developed. The inclusion of these two soil enzymes in routine soil analysis has been termed soil bioanalysis. In this work we describe how the soil bioanalysis was developed and some of its practical applications.

Methodology

When: The studies were initiated in 1999.

Where: Thirty field experiments comparing different management systems were evaluated all over the Cerrado region in Brazil during 1999-2019. These studies involved comparisons among no-till (NT), conventional tillage (CT), integrated crop-livestock systems, different crop rotations and successions, fallow and continuous monocrop systems. A set of 24 treatments, in three long-term P fertilization management experiments were used to test several ideas regarding the interpretation of individual values of microbial indicators (Lopes *et al.,* 2013, Lopes *et al.,* 2018; Mendes *et al.,* 2019b).

Soil types: Rhodic Haplustox (a Typic Dystrophic Red Latosol, according to the Brazilian soil classification system) and Typic Quartzipsamment (Neossolo Quartzarenico, under the Brazilian classification, and Arenosols under FAO’s classification).

Soil texture: ranging from 5 percent up to 65 percent of clay content.

Microbial parameters evaluated: Microbial biomass carbon (MBC), basal respiration, activity of soil enzymes: β-glucosidase, cellulase, arylsulfatase, dehydrogenase, acid and alkaline phosphatase.

Diagnostic soil layer: 0-10 cm

Development of an interpretative framework that applied the principles of soil nutrient calibration tests to microbial indicators. In the study presented by Lopes *et al.* (2013), microbial indicators were interpreted as a function of long-term cumulative yields (CY) of corn and soybean and soil organic carbon (SOC) using linear regression models.

Development of the FERTBIO soil sample concept which considers the advantages that standardization of the sampling time and pre-treatment procedures for soil chemical fertility (FERT) and microbiological (BIO) analyses offer to farmers and commercial laboratories (Mendes *et al.,* 2019a).

Development of critical limits specific to post-harvest air-dried soil samples for microbial indicators, under tropical conditions (Mendes *et al.,* 2019a)

Development of a web interface to interpret the results of the soil bioanalyses and to calculate soil quality indexes: the Soil Quality Interpretation Module (Mendes *et al.,* 2021)

Results

Overall, soil respiration data were highly variable, with some studies reporting increased rates under NT, and some showing no significant differences due to tillage. In 26 percent of the studies MBC, showed no detectable differences between the different management systems, whereas enzymes activities, especially β-glucosidase and arylsulfatase showed greater sensitivity.

Some of the main advantages of using the soil enzymes, β-glucosidase and arylsulfatase as microbial indicators of soil health are listed below.

1- They are highly correlated with crop yields and SOM, which are very important parameters for farmers and sustainability of agroecosystems (Lopes *et al.,* 2013; Lopes *et al.,* 2018, Mendes *et al.,* 2019a).

2- They are sensitive to changes in soil and crop management systems and related to soil functioning (nutrient cycling).

3- They are interpretable (we can determine their critical levels). An indicator is only useful if its value can be unequivocally interpreted and reference values are available.

4- They can be used in routine commercial soil analyses, are precise, coherent, replicable and their determinations involve simple and inexpensive analytical procedures, without the production of toxic residues for the environment.

5- They can be performed directly in air-dried soil samples, which makes easier the unification of sampling time and soil handling pre-treatments for soil chemical and microbiological analyses (good for farmers and commercial laboratories).

6- They are highly correlated with microbial biomass carbon (MBC), basal respiration, cellulase, hydrolysis of fluorescein diacetate, dehydrogenase, acid and alkalyne phosphatase.

Discussion

Changes in soil organic matter (SOM) content not always reflect changes that occur in soil due to the adoption of agricultural management systems that enhance or degrade soil quality. Thus, because β -glucosidase and arylsulfatase are associated with the most active and living part of organic matter, the greatest advantage of their use is that they are more sensitive than chemical and physical indicators, acting as early warning indicators.

Soils with increased activities of β-glucosidase and arylsulfatase are more productive, more resilient (able to better tolerate water stress, for instance), with increased capacity for carbon sequestration, reduced persistence of pesticides and reduced emission of greenhouse gases (Mendes *et al.,* 2019a).

The interpretative framework for individual microbial indicators based on the principles of soil nutrient calibration tests established, for the first time, reference values for the soil microbial indicators based on crop yield (CY) (Lopes *et al.,* 2013). The relationships observed between the soil microbial indicators with the CY and SOC content showed that highly productive soils also presented high levels of microbial biomass, respiration and activity levels of the soil enzymes β-glucosidase, arylsulfatase, cellulase and acid phosphatase. Based on the mathematical expressions of these relationships, target ranges for each soil microbial indicator as a function of CY and SOC content were established (<40 percent low, 41 to 80 percent moderate and >80 percent adequate), and interpretative tables were generated.

The development of interpretative tables/reference values using the FERTBIO soil sample concept (Mendes *et al.,* 2019b) also represented an important step in research efforts to incorporate microbial indicators into routine commercial soil analyses in Brazil. This concept standardizes the sampling time and pre-treatment procedures for soil chemical fertility (FERT) and microbiological (BIO) analyses, which is advantageous for farmers and commercial laboratories. Therefore, soil samples are collected at 0 to 10 cm depth at the crops post-harvest stage and β-glucosidase and arylsulfatase determinations are performed directly on air-dried soil samples.

Since July 2020, Brazilian farmers have the opportunity to access soil health by evaluating the activities of β-glucosidase and arylsulfatase. So far, 8 commercial soil laboratories have been capacitated and are performing these soil enzymes determinations as part of their routine in soil analyses.

Conclusions

Since July 2020, Brazil has included two soil enzymes (β-glucosidase and arylsulfatase) as soil health indicators in routine soil analysis. The inclusion of interpretable microbial indicators in commercial soil analyses will be important for farmers to assess whether their management practices are improving, conserving, or degrading soil resources. With the soil bioanalysis (β -glucosidase and arylsulfatase) we are making surveys of soil health at farm´s level in different regions of the country and calculating soil quality indexes.

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Automatic detection of (micro)arthropods in soils

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Abstract summary

Arthropods in the soil have great impact in ecological systems. There is an increasing need to monitor their activity and population growth. This is a time-consuming and financially demanding process. Estimating relative abundances of arthropod populations can be enhanced by using sensors that can detect individuals in the field. We work on developing probes (sensors with traps) which automatically detect arthropods.

The recently developed ZooLog Online monitoring system consists of new probes for different arthropod groups, with soil moisture and temperature sensors joined to them. A data forwarding system is included with a central database and Web interface. The probes transmit sensor data via the internet, thus enabling the visualization of current population numbers of microarthropods and seasonal activity patterns.

Sensors were optimized for arthropods with several lifeforms, with body sizes of 0.5 to 35mm. Detection is based on infrared sensors, but to improve precision and accuracy, photographing on exemplars caught also takes places. In this way even automatic species level identification of soil microarthropods is possible. With this new probe, predictions of the occurrence and diversity of specific arthropod species in the soil can be drawn.

Keywords: mesofauna, automatic detection, microarthropod wireless sensor, infrared sensor, sampling method, activity, monitoring system

Introduction, scope and main objectives

Soil mesofauna, mainly Collembola and Acari, plays a vital role in regulating and enhancing nutrient cycling and decomposition. This group is represented in a high number and diversity in the soil. They live a hidden life and our knowledge about their ecological features and roles is still limited. Especially our knowledge about their activity, which can affect soil the soil processes, is still comparatively scarce.

There is an increasing need to monitor their activity and population growth. This is a time-consuming and financially demanding process. Estimating relative abundances of insect populations can be enhanced by using sensors that can detect individuals in the field. In our previous work we developed such sensors (Dombos *et al.*, 2016; Gedeon *et al.*, 2017). Opto-electronic sensors have low power consumption, but they are less species-specific. This specificity is important and has considerable demand in ecology. The use of cameras, which have improved image analysing techniques for species determination could be a solution, but they have high energy demand and for detection of microarthropods, the current technique is limited. In our work, we combine cameras and opto-electronic sensors.

Methodology

Five prototypes were constructed for catching and automatic detection of different arthropod types: EU- and EPI-EDAPH for the detection of ground living and soil-dwelling arthropods (mostly microarthropods), YF for click-beetles, VARL for monitoring moth species and KLP for detecting western corn rootworms. We further developed our previous EDAPHOLOG probe (Dombos *et al.* 2016) which we had built for detection of surface-living and soil living microarthropods. These probes are based on the idea of pitfall trapping: animals moving horizontally fall down the trap and get caught. We can sink the probe in the top-soil then we can catch not only surface-living, but also soil dwelling microarthropods.

We integrated a camera in the probe. Normally cameras are working continuously and analysing pixel real-time in the chip. Their power consumption is so high that they need wired power supply. That is why we used our infrared (IR) sensor-ring. In our model, when the animal falls down, IR sensor-ring detects it and turns on the camera within 2-3 seconds. Until the individual falls to the photo plate camera starts motion detection, if it succeeded it takes a picture and the camera turns off.

The recently developed ZooLog Online monitoring system consists of new probes with soil moisture, and temperature sensors joined to them, included a data forwarding system with a central database and Web interface. The probes transmit sensor data via the internet, thus enabling the visualization of current population numbers of microarthropods and seasonal activity patterns. We also added a new feature: we built a vacuum device attached to the probe. This pumps up the exemplars from the photo chamber of the probe dug into the soil to a sample container located above-ground.

Results

Sensors were optimized for arthropods with several lifeforms, with body sizes of 0.5 to 35mm. The probe catches and detects the arthropods. In case of microarthropods we achieved a 95 percent detectability level at 0.5- 1.1 mm depending on the shape and colour of the microarthropod species. After detection it takes picture about the given specimens. At final a vacuum unit pump up the arthropods from the photo chamber to the sample container, which can be placed aboveground.

Discussion

While opto-electronic sensors have low power consumption and they are less species-specific, cameras are just the opposite: with high energy demand and improved image analysing techniques for species determination. We combined these two techniques. Photographing trapped arthropods provides an opportunity both to decrease false detections and distinguish among species. With attaching a vacuum unit, we will be able to automatically collect biological materials for further analysis, for example species identification, or DNA analysis. In the real-time measurement, data are forwarded automatically to the server. The end-user gets abundance data of arthropods daily or even immediately from the field.

ZooLog sensor system can be used for local or even national monitoring and for drawing predictions of the occurrence and diversity of specific arthropod species in the soil and aboveground. Automatic estimation of population size can be achieved in the future more routinely. But we need the help of electrical engineers, experts in image analyses. Species selection is the key for improvement: infrared sensors coupled with image analysis seems to be promising method.

Conclusions

By using this newly developed probe, there is a good opportunity to get more detailed data about surface- and soil living microarthropod species. With combining the advantages of the opto-electronic IR sensor and camera trap, a more precise estimation of activity time of soil microarthropods can be achieved. Moreover, by taking pictures of them, the taxonomical shortage of automatic detection of arthropods can be resolved.

Acknowledgements

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Drivers of short-to-medium term litter decomposition across biomes

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Abstract summary

Litter decomposition is an important ecosystem function driving carbon and nutrient cycling in ecosystems, and it is co-depended on diverse factors, whose impact magnitude might change in the course of decomposition process. Disentangling these factors is problematic, especially along large environmental gradients. We combined harmonized data collected within global TeaComposition initiative together with data from two experimental trials to address drivers of early-to-mid-term litter decomposition. We showed that the litter quality was most controlling factor at the global scale, while the effect of climate was only pronounced when inhibiting thresholds were exceeded. At the regional scale, soil and understory cover showed a significant effect, whereas soil microbial community composition and function appeared to have less influence on the short-to-medium-term litter decomposition. Our results highlight the importance of longer-term studies over large temporal and special scales for better understanding of litter carbon turnover. Moreover, obtained common metric provides a strong tool for inter-site comparison within the network as well as within other global networks and can serve as an intermodal link between the ecological research disciplines.

Keywords: Litter decomposition, carbon turnover, tea bags, TeaComposition initiative, harmonized data, networking of networks

Introduction, scope and main objectives

In order to study ecosystem processes as well as to analyse environmental changes and develop appropriate mitigation measures "big data" involving comprehensive datasets collected over multiple temporal and spatial scales and diverse ecosystems is needed. Within the TeaComposition initiative we are collecting harmonized data on long-term litter decomposition process and their drivers globally. As climate, vegetation, and soil are increasingly changing due to global anthropogenic pressures, increasing our ability to understand their independent effects and interactions is vital. By combining long-term studies with experimental trials (mesocosms and phytometer studies) we aim to address decomposition drivers across time and scale. Our specific goals are:

a) To better understand leaf litter decomposition at the global scale (data collected within global TeaComposition initiative)

b) To identify direct and indirect effects of environmental changes on litter decomposition (data collected within PASTFORWARD project)

c) To disentangle effect of vegetation, soil, and climate on litter decomposition (data collected within Phytometer trial)

Methodology

a) Within TeaComposition Initiative, we installed (0-5 cm under the surface) commercially available tea bags, namely fast decomposing Green tea and slower decomposing Rooibos tea, (Keuskamp *et al.,* 2013) at 570 terrestrial sites globally. Tea bags were collected after three, twelve, twenty-four, and thirty-six months of field incubation (Djukic *et al.* 2018). Collected tea bags were manually cleaned from roots and soils dried at 70°C for 48 hours and weighed.

b) To identify the direct and indirect effects of environmental changes on litter decomposition in a temperate mixed deciduous forest, we performed a mesocosms experiment where in ancient and post-agricultural forest soils, decomposition of Green tea and Rooibos tea was exposed to different temperature, light, and nitrogen treatments for the period of 3 and 6 months (Wang *et al.,* 2019).

c) To disentangle the effect of plant community, soil, and climate on litter decomposition, litter decomposing microorganisms, and related functions, we installed multispecies phytometers, consisting of three globally cosmopolitan herbaceous species in local site soils and a standardized substrate, across a pan-European aridity gradient and 18 grassland ecosystems (Wilfahrt *et al.*, 2021).

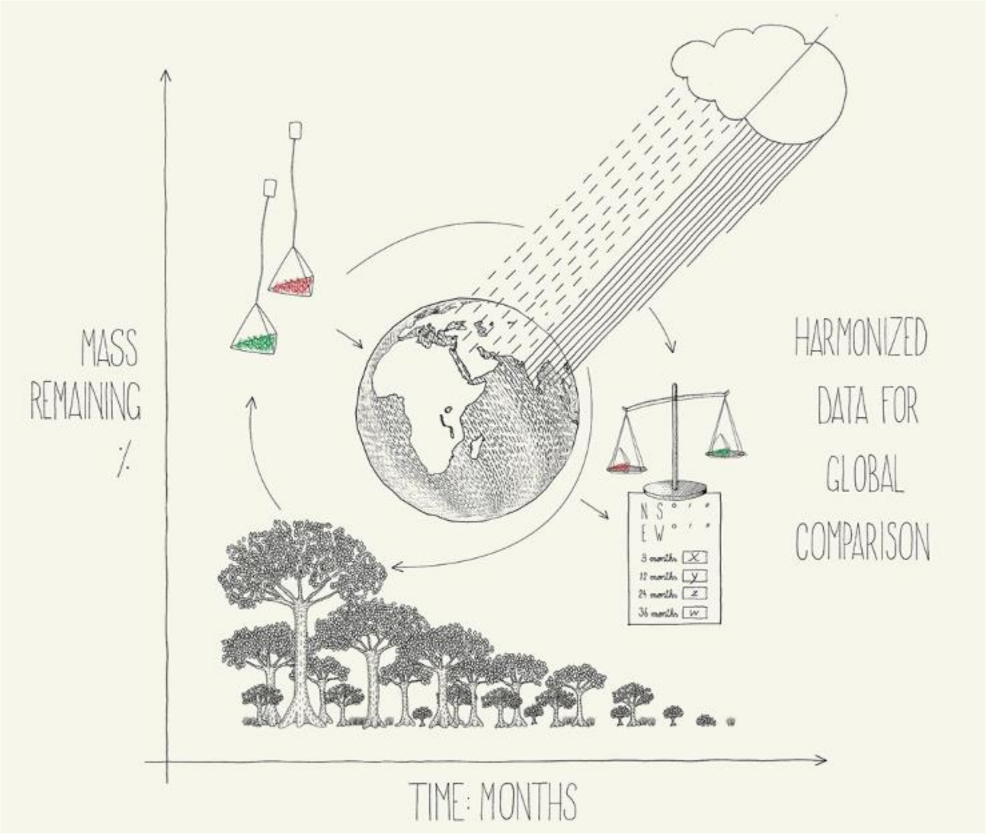

Figure 13: Basic concept of TeaComposition Initiative and Djukic *et al.,* 2018

Results

a) At the global scale, litter quality is the dominant determinant of litter decomposition rates (explaining 65 percent of the variability after 3 months and 38 percent after 12 months of decomposition). The effect of climate, on the other hand, was rather weak after 3 months (explaining 0.5-5 percent of the variance) and became stronger after 12 months of decomposition (explaining 2-13 percent variance).

b) The direct effects of temperature, light, and nitrogen on litter decomposition varied among the two litter types, incubation times, and soil fertility groups in our mesocosms study (Figure 2). The indirect effects of the treatments were consistently negative, as treatments (especially the temperature and light treatments in the early decomposition stage) had a positive effect on plant cover, which negatively affected litter decomposition.

c) Soil microbial communities (bacteria and fungi) and functions (decomposition, basal soil respiration, and soil microbial biomass) were strongly influenced by soil quality and climate factors. Standard and local soil microbial communities differed, especially in terms of richness, but similar climatic factors were found to shape both sets of communities. It was not possible to separate the effect of soil and climate on soil microbial functions, suggesting strong interactive and non-additive effects.

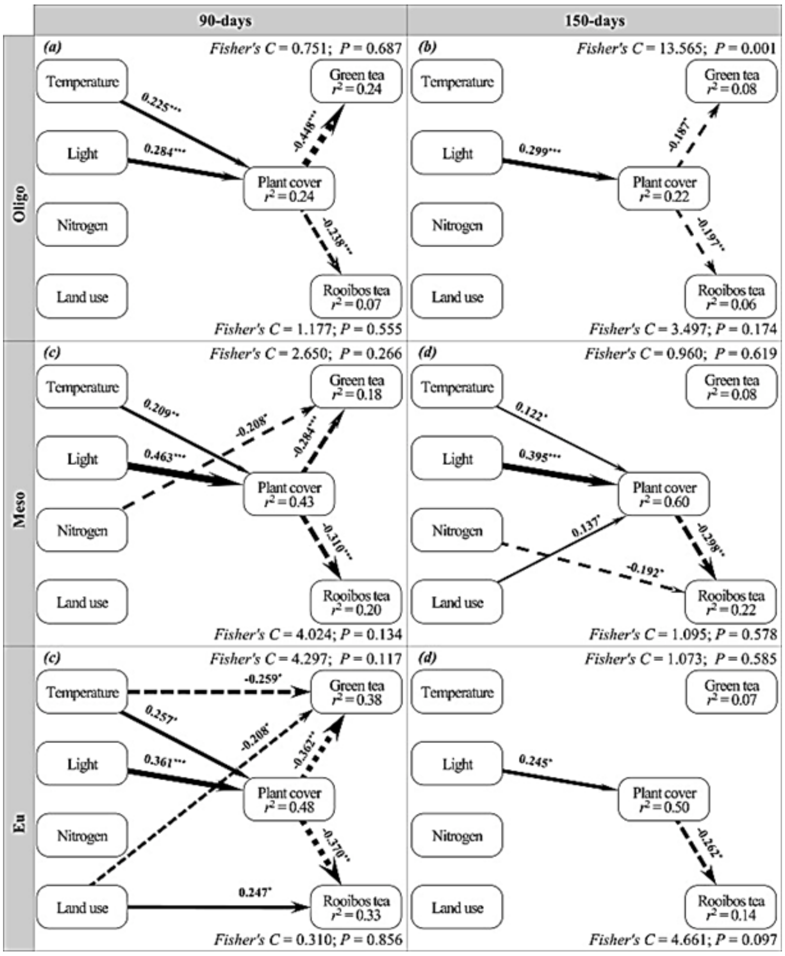

Figure 2: Direct and indirect effects of temperature, light, nitrogen, land use, and understory plant cover on litter mass loss of Green and Rooibos Tea in oligotrophic, mesotrophic, and eutrophic soils after 3 and 6 months of incubation; solid arrows: positive effect, dashed arrows: negative effect; Wang *et al.*, 2019

Discussion

Leaf litter decomposition is highly dynamic process in which controlling factors might change with time (Berg and McClaugherty, 2003). During the short-to-mid-term phase of litter decomposition (~40 percent of mass loss) within our TeaComposition initiative, litter quality is the key driver of the decomposition at the global scale. When comparing the remaining mass after 3 (38 percent to 78 percent for Green and Rooibos tea, respectively) and 12 months (34 percent - to 65 percent for Green and Rooibos tea, respectively; Kwon *et al.*, 2021) only marginal changes in the weight has be observed, indicating strong legacy effect of early stage decomposition on the later process. In contrast, the effect of climate (i.e. temperature and precipitation) increased with time (explained 0-5 percent to 2-12 percent of variation) and was in generally noted when the inhibiting thresholds (temperature <10°C and moisture <30 percent or >80 percent; Prescott, 2010) where exceeded. Furthermore, large variation in litter mass loss within given biomes suggests that local-scale factors other than climate have strong controls on regional litter mass loss dynamics (Djukic *et al.*, 2018).

Indeed, our short-term mesoscosm study revealed a direct effect of soil fertility ($p < 0.001$) and indirect effect of understory cover ($p < 0.001$) especially on early stage decomposition of more labile litter (i.e. Green tea; Wang et at., 2019), indicating that more light need to be shaded on the soil microclimate processes. In this sense, our preliminary results from the phytometer trial show also a strong interactive and non-additive effect of soil and climate on soil microbial communities and functions. However, microbial community composition and function appeared to have less influence on the early stage decomposition of different substrate quality (i.e. Green and Rooibos tea) suggesting that still a sufficient substrate availability precludes the differentiation in substrate use among the microbial groups (Djukic *et al.*, 2013).

Conclusions

Our results indicate that multiple drivers are affecting litter decomposition at different points in time and to different extent. Data from the global TeaComposition study shows large variation in litter decomposition in a given biomes. Therefore, closing the data gaps in less represented areas (e.g. Africa, Russia) is of fundamental importance for understanding decomposition processes. Time and cost efficient tea bag method (Keuskamp *et al.*, 2013; Djukic *et al.*, 2018) enables a crosscutting action between the networks and institutions, facilitating collection of data relevant for understanding of this fundamental ecosystem process (e.g. global soil biodiversity data within Soil BON forum, or nutrients in remaining litter and soil). By doing so, initiative contributes valuably to the United Nations Sustainable Development Goals: climate action, partnership for the goals, and reduced inequalities.

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Ant and termite diversity of the Colombian Amazon soils

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Abstract summary

In tropical soils, ants and termites represent two of the most abundant belowground animal groups with a significant biomass contribution. Together with earthworms, are known as "ecosystem engineers" due to the positive effect of their activity on ecosystems. We present an updated list of ant and termite species that are inhabiting Colombian Amazon soils. Specimens were obtained from TSBF monoliths collected between 2016 and 2019 in 128 different locations. We found 238 ant species distributed in 10 subfamilies and 67 genera, and 97 termite species distributed in two families, seven subfamilies and 36 genera. We reported 32 new termite species records for Colombia and22 new species for science: four new ant species, 13 new termite genera and 18 new termite species. This updated list increases considerably the knowledge about edaphic macrofauna diversity of Amazonia, and specially soil ant and termite diversity of the Colombian Amazon region.

Keywords: Ecosystem engineers, checklist, Formicidae, Termitidae

Introduction, scope and main objectives

From all components of soil macrofauna, ants, termites and earthworms are the most conspicuous. For decades the importance of their role in the structuring and physical conformation of the soil profile has been recognized, as well as their function in decomposition processes of organic matter (Barros *et al.*, 2002; Decaëns *et al.*, 2004; Lavelle *et al.*, 1994). These organisms have been called "ecosystem engineers" for their contribution to soil composition and structure (Culliney, 2013; Decaëns *et al.*, 1999; Lavelle, 1997).

Although it has been possible to establish the importance of these groups in tropical ecosystems, there is a gap in the knowledge of their diversity. In Colombia, for example, there is only a single inventory of soil ants and none of termites (Castro *et al.*, 2018). In this study, we offer new information to increase the taxonomic knowledge of soil ants and termites in the Amazon region of Colombia, to contribute to ecological and biological studies that can be carried out in the future in the region.

Methodology

The study area (Figure 1) goes from the Andean-Amazonian transition in the north to the south of the Colombian Amazon region bordering the borders with Peru and Brazil. The area crosses the Amazon, Caquetá and Putumayo river basins, and include altitudes from 78 to 2275 meters above sea. There, 106 sampling localities were established in 13 municipalities: Belén de los Andaquíes, Florencia, Morelia, San José and Solano, in the Caquetá state; Puerto Leguizamo and La Tagua, in the Putumayo state; and, El Encanto, La Chorrera, Leticia, Puerto Alegría, Puerto Arica, Puerto Nariño and Puerto Santander, in the Amazonas state. Different natural and anthropic land uses were included in the sampling: primary, mature secondary forests, young secondary forests, pastures and indigenous slash-and-burn agricultural plots.

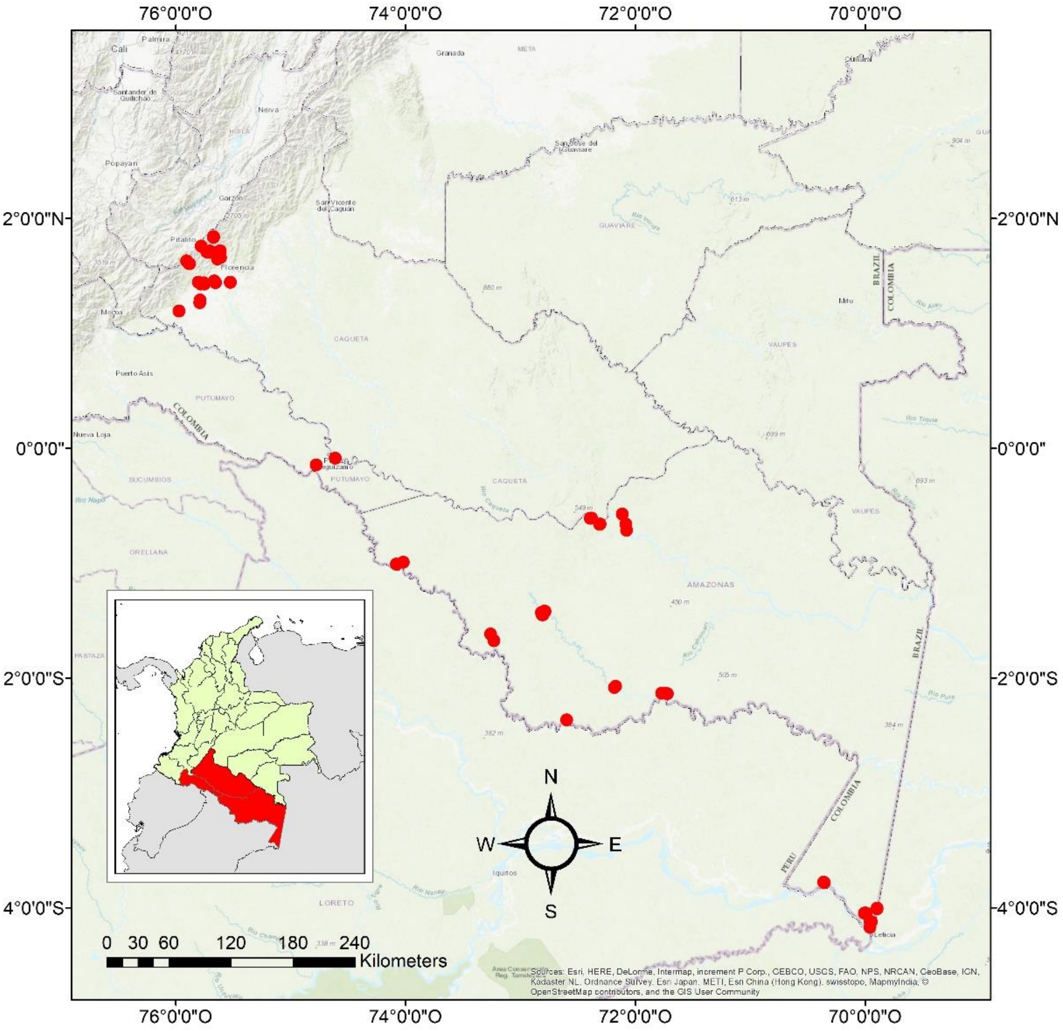

Figure 1: Study area, sampling localities

Soil macrofauna specimens were collected from soil monoliths following the methodology proposed by the Tropical Soil Biology and Fertility Program (TSBF) (Anderson and Ingram, 1993). All specimens were identified using recent taxonomic keys, verifying the species, and in some cases, comparing samples with photos of type material in AntWeb (for termites), and type material from the University of Florida termite collection and Museu de Zoologia da USP (for termites). All data were organized alphabetically by family, subfamily, genus and species in an ant checklist following the nomenclature for ants suggested in the Bolton online catalogue of the ants of the world (AntCat, Bolton 2020) and for termites the Constantino Termite Database (Constantino 2020).

Results

For ants (Hymenoptera: Formicidae), 4318 individuals were identified: 238 species in 67 genera and 10 subfamilies. In terms of richness, the subfamily with the highest number of species was Myrmicinae (105), followed by Ponerinae (47), Formicinae (33), Ectatomminae (18), Dolichoderinae (16), Dorylinae (7), Pseudomyrmecinae (6), Amblyoponinae (3), Proceratiinae (2) and Paraponerinae (1). The genera with the greatest number of species were *Pheidole*, (27 species), *Crematogaster* (16), *Camponotus*, (14), *Odontomachus* (12) y *Gnamptogenys* (8).

The richest genus was *Pheidole* Westwood, 1839 with 27 species, followed by *Crematogaster* Lund, 1831 with 16 species. Other genera rich in species were *Camponotus* Mayr, 1861 with 14 species, *Odontomachus* Latreille, 1804 with 10 species and *Gnamptogenys* Roger, 1863 with 8 species. From all the ant species recorded, 20 species were found in the four soil depths of TSBF monoliths. The most abundant species were: *Wasmannia auropunctata* Roger, 1863, *Tranopelta gilva* Mayr, 1866, *Sericomyrmex bondari* Borgmeier, 1937, *Crematogaster limata* Smith, 1858*, Crematogaster carinata* Mayr, 1862, *Crematogaster brasiliensis* Mayr, 1878, *Crematogaster abstinens* Forel, 1899 and *Brachymyrmex cordemoyi* Forel, 1895.

For termites (Blattodea: Isoptera), 8975 individuals of 97 termite species were identified. They were distributed in two families, seven subfamilies and 36 genera. The richest family was Termitidae (91 species), the family Rhinotermtidae only recorded six species. The subfamily with the highest number of species is Apicotermitinae (37 species), following by Syntermitinae (24 species), Nasutitermitinae (20 species), Termitidae (12 species), Heterotermitinae (3 species) and Copotermitinae (1 species).

Nasutitermes Dudley, 1890 is the richest genus with 10 species, followed by *Syntermes* Holmgren, 1909 with eight species, *Neocapritermes* Holmgren 1912 with seven species, *Anoplotermes* Müller 1873 and *Ruptitermes* Mathews, 1977 with five species each one. The most abundant termite species were: *Anoplotermes meridianus* Emerson, 1925*, Anoplotermes banksi* Emerson, 1925*, Neocapritermes pumilis* Constantino, 1991*, Embiratermes ignotus* Constantino, 1991, *Cylindrotermes parvignathus* Emerson, 1949 and *Disjunctitermes insularis* Scheffrahn, 2017. We highlight two recently described species found in these samples and published for the authors of the present study: *Echinotermes biriba* Castro & Scheffrahn, 2018 and *Rustitermes boteroi* Constantini, Castro & Scheffrahn, 2020.

We found four new ant species for science, from the genera: *Pheidole, Myrmicocrypta, Typhlomyrmex* and *Syscia*. We also found 13 new termite genera and 18 new termite species, from the genera *Disjunctitermes, Anoplotermes, Humutermes,* and *Ruptitermes.*

Discussion

The study done by Wilkie, Mertl and Traniello (2010), which is recognized as the most complete work on ant diversity in the Amazon region, recorded at the Tiputini Reserve in Ecuador 66 genera and more than 300 species from subsoil and canopy. In this work we reported one more genus and 49 percent of these species collecting only in the soil. Although TSBF monoliths are appropriate for collecting these soil ants, which are generally undersampled with other methods of collection, the TSBF method might underestimate army ants and other large ants such as Paraponerinae that were not recorded in the searched Amazon basin area.

Ants are the most diverse soil macrofauna group in the Amazon region (Barros *et al.*, 2003; Mathieu *et al.*, 2005). In the Caquetá state, ants are the densest organisms of the soil macrofauna. In the other two states, ants are only exceeded by termites. Differences in ant and termite densities might be a reflection of the land use in each state. Termites tend to be more abundant in less disturbed ecosystems (Mboukou-Kimbatsa, Bernhard-Reversat and Loumeto*,* 1998; Velásquez *et al.*, 2012), while ants tend to be more abundant in disturbed or degraded ecosystems of the Amazon region. Results presented here increase the knowledge of soil ants from the Amazon region and suggest that ant species richness may increase considerably when sampling effort increases, and combined methodologies are used to capture ants in different habitats.

Apicotermitinae and Syntermitinae are the richest termite families in species. These species belong to humus, litter and organic matter feeding groups (Eggleton and Tayasu, 2001).Nasutitermitinae and Rhinotermitidae were not recorded with higher number of species. All new termite species and genera are Apicotermitinae, a subfamily which is cryptic, and still presents a great challenge for termite taxonomy (Rocha *et al.*, 2019). However, our results conclude that knowing the diversity of these insects can help to understand more the dynamics of these termites in the soil, since they are termites that only feed from humus and litter.

Conclusions

This study increases considerably the knowledge of edaphic macrofauna of the Amazon region. The diversity of soil ants can be even greater as only part of the samples collected in the region are completely processed. Although the TSBF method allows a comparison between macrofauna groups, some ant groups might be subsampled; therefore, increasing the sampling effort and combining collection methods (e.g. Winkler or Pitfall) is recommended. Additionally, increasing the number of points at each sampling site could increase the sampling effort for termites.

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Integrating microbiological quality indicators and soil properties through score functions to assess land use changes in Colombian Andisols

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Abstract summary

The individual interpretation of microbial indicators of soil functionality is often confusing. In addition, the high cost of measuring some of the indicators limit the studies oriented to provide early warnings of changes in soil functionality. We propose a methodology for integrating variables with scoring functions to evaluate the capacity of a set of basic microbial indicators, soil properties and environmental variables (with low measurement cost) to value the functionality of agricultural, mining and undisturbed soils. Based on samples taken from *Andisol* soils in the northeast of the department of Antioquia, Colombia, we found that microbial indicators contribute 34.77 percent of the variation to the entire set of data evaluated and that microbial respiration is the most important factor. Likewise, we observe that the nonlinear scoring equations, are efficient and more sensitive to discriminate the functionality in each of the land uses studied.

Keywords: Microbial Indicators, Land Use Discrimination, Early warnings.

Introduction, scope and main objectives

Microbiological indicators represent a valid alternative to measure soil functionality in different land uses due to its high sensitivity to early variations in soil properties (Balota *et al.* 2014) . However, to obtain a representative image of the different aspects of soil functionality, the assessment should combine microbiological indicators together with other attributes of soil and environmental context. These attributes can respond differently to management practices and, consequently, their results in qualifying soil functionality can lead to confusing interpretations (Gonzalez-Quiñones *et al.* 2011). In addition, microbiological and physicochemical analyses of soils can be expensive, limiting the size of areas studied and constraining the possibility of identifying early warnings due to significant variations in soil functionality.

An alternative to combine microbial indicators with soil attributes is to use geometric means and punctuation functions to integrate variables of different nature into additive functions or indices that calculate simple interpretation values (Li *et al.* 2019). In this regard, diversity, abundance and microbial respiration are variables that greatly affect nutrient soil mobilization (Mendes *et al.*, 2015), serving as good indicators of soil functionality (Bolat, 2019).

We aim at evaluating the capacity of a set of environmental variables, soil properties and microbial indicators, with low measurement costs, to represent the functionality of three land uses in soils known as *Andisoles* from the northeast of the department of Antioquia, Colombia. We want to develop a measure of soil functionality that can generate early and representative alerts in different areas. We build a set of attributes to measure soil functionality based on abundance and microbial respiration, using transformation of variables with scoring functions to integrate different types of variables into a simple additive equation.

Methodology

Our study was made in the municipalities of La Ceja, El Retiro and Envigado. We collected soil samples in nine locations during September and October of 2018, randomly selecting 10 sampling points in each of the nine locations agricultural land uses (Agr), mining projects (Mp), and undisturbed areas (Np). We use a Kestrel 5500 Weather Meter to measure environmental variables Temperature, Relative Humidity (RHA), Heat Index, Dew Point Temperature, Wind Chill Temperature and Atmospheric Pressure (PA). With a Lamotte Salt / pH / TDS / Temp TRACER 1766, we measured pH, TDS and conductivity (COND). We carried out quantifications of Total Organic Carbon and moisture content based on Walkley&Black method and gravimetric method. We measure the microbiological indicators of diversity in CFU types and the abundance of mesophilic bacteria and fungi in CFU/g soil. We quantified the mesophilic bacteria and fungi by serial dilutions plate count method. We determined the Microbial Respiration Rate (MRRat/C) by chloroform fumigation-incubation method.

We built a representative set of variables, following the methodology proposed by Raiesi and Kabiri (2016). First, we integrated some measurements of the same type, using geometric means (GM) into three variables: GM Types Fung./Bact, GM Abundance Fung./Bact, and GM Temperatures. Second, we identified individual variables significantly affected by each land use, using an ANOVA test. We calculated the Importance Coefficients (IC) of each variable with a Factor Analysis (FA). When a factor retained more than one variable, we used correlation coefficients to select the most significant variable. For the Minimum Data Set (MDS), we consider redundant those variables with higher correlated coefficients and lower IQs and excluded them from the analysis. MDS variables were transformed according to linear (A) and nonlinear (B) scoring functions used by Raiesi and Kabiri (2016).

$$A)\quad L_{SF}(Y) = x/x_{max}$$

$$B)\ \ NL_{SF}(Y) = \frac{a}{\left(1 + \left(\frac{x}{x_0}\right)^b\right)}$$

$$C)\ \ SQI = \sum_{i=1}^{n} w_i\, s_i$$

In linear function A, x is the value of the variable and x_{max} is the maximum value observed. For nonlinear function B, a is the maximum value of the function. x is the value of the variable and x_0 is the average value of the variable, b is the value of the slope, which for the "more is better" function was established by the authors as -2.5. In C, SQI is the Soil Quality Index, w is the CI of each variable and s is the value of the variable. We also calculated SQI without including w, considering that all MDS factors had the same relative importance.

Results

According to the ANOVA test, all environmental variables, soil properties and microbial indicators were affected by all uses ($p < 0.05$), with the exception of PA ($p = 0.3959$) and pH ($p = 0.1580$). Figure 1 shows the behavior of the average value of the variables attempted by geometric means in each use. The FA showed three factors with Eingenvalues > 1 that explained 71.87 percent variance of the data (Table 1). The variable with the highest IC in the MDS was MRRat/C followed by TDS and RAH. FA showed that the microbial indicators (GM Types Fung./Bact. and GM Abundance Fung./Bact.) have important load values.

The variables were combined into each factor as shown in Figure 2. The correlation matrix showed a large number of correlations between the variables of each factor (Table 2), so we selected the variables that presented the highest IC. Thus, the variables of the MDS were MRRat / C, TDS and RHA.

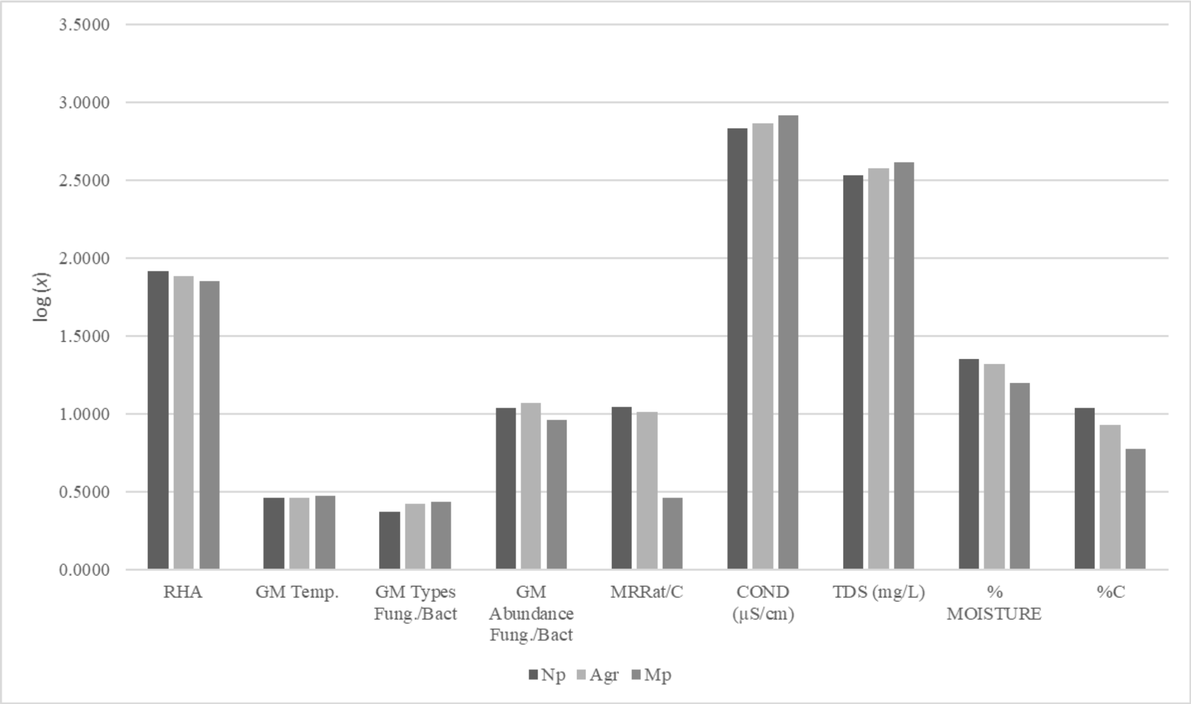

Figure 1: Behavior of environmental variables, soil properties and microbial indicators integrated by geometric means in Colombian Andisols

Table 1: CI and Eigenvectors of a set of variables to measure soil functionality in Colombian Andisols

	Factor 1	**Factor 2**	**Factor 3**	**Communality**	**Specific Variance**
RAH(%)	0.05143	-0.10029	**0.88705**	0.79956	0.20043
GM Temp.	-0.05526	0.04772	-0.84359	0.71698	0.28301
GM Types Fung./Bact.	-0.44932	0.03209	0.15258	0.22620	0.77379
GM Abundance Fung./Bact.	0.73042	-0.19654	-0.14059	0.59191	0.40808
MRRat/C	**-0.86696**	0.10596	-0.21097	0.80735	0.19264
Conductivity(µS/cm)	-0.08549	0.95965	-0.08046	0.93471	0.06528
TDS(mg/L)	-0.11754	**0.96944**	-0.08060	0.96014	0.03986
%Moisture	0.70384	0.27847	0.30760	0.66756	0.33243
%C	0.80971	-0.16461	0.28574	0.76438	0.23560
Eigenvalue	**3.1296**	**1.8449**	**1.4943**		
Variance (%)	**34.773**	**20.499**	**16.604**		
Cumulative variance (%)	**34.773**	**55.272**	**71.876**		

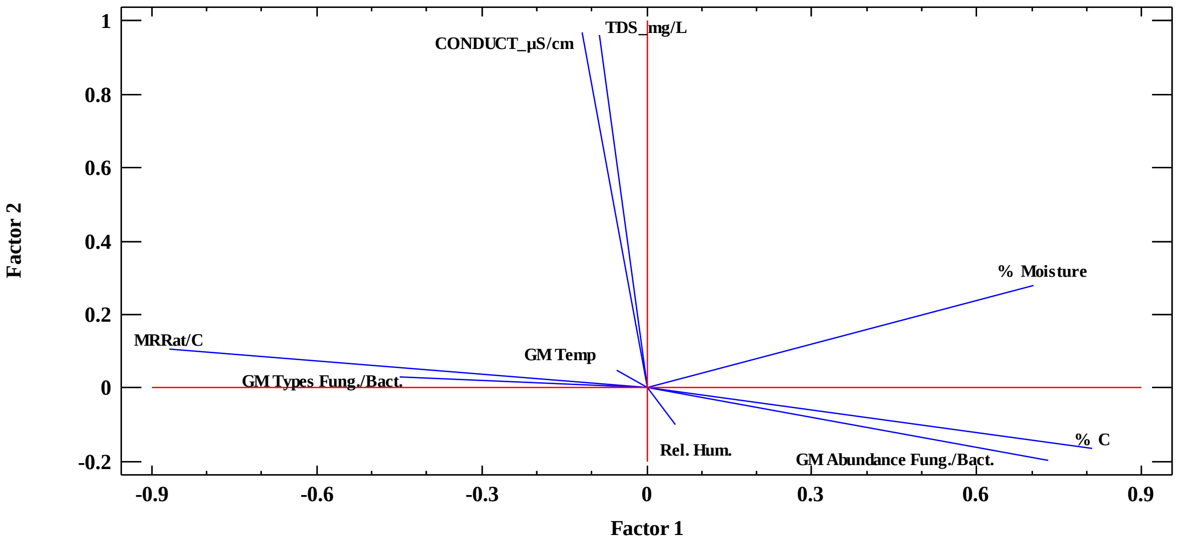

Figure 2: Variables grouped in three factors calculated by FA in Colombian Andisols

Table 2: Correlation coefficients of factor variables in Colombian Andisols

	RAH	GM Temp	GM Types Fung./Bact.	GM Abundance Fung./Bact.	MRRat/C	Conductivity	TDS	% Moisture	% C
RAH		0.0001	0.636	0.3061	0.0023	0.04	0.1578	0.0078	0.002
GM Temp	0.0001		0.5594	0.4913	0.0192	0.3443	0.2951	0.0103	0.0076
GM Types Fung./Bact.	0.636	0.5594		0.0124	0.0164	0.8774	0.9039	0.0615	0.0345
GM Abundance Fung./Bact.	0.3061	0.4913	0.0124		0.0065	0.612	0.7507	0.1672	0.0061
MRRat/C	0.0023	0.0192	0.0164	0.0065		0.2874	0.1954	0	0
Conductivity	0.04	0.3443	0.8774	0.612	0.2874		0.0002	0.0895	0.6701
TDS	0.1578	0.2951	0.9039	0.7507	0.1954	0.0002		0.0171	0.6576
% Moisture	0.0078	0.0103	0.0615	0.1672	0	0.0895	0.0171		0.0011
% C	0.002	0.0076	0.0345	0.0061	0	0.6701	0.6576	0.0011	

Figure 3 shows the behavior of the SQI. In all cases, the Pm use had the lowest index values while the Np use had the highest values. We observed the greatest differences among uses with the SQI calculated with the nonlinear function.

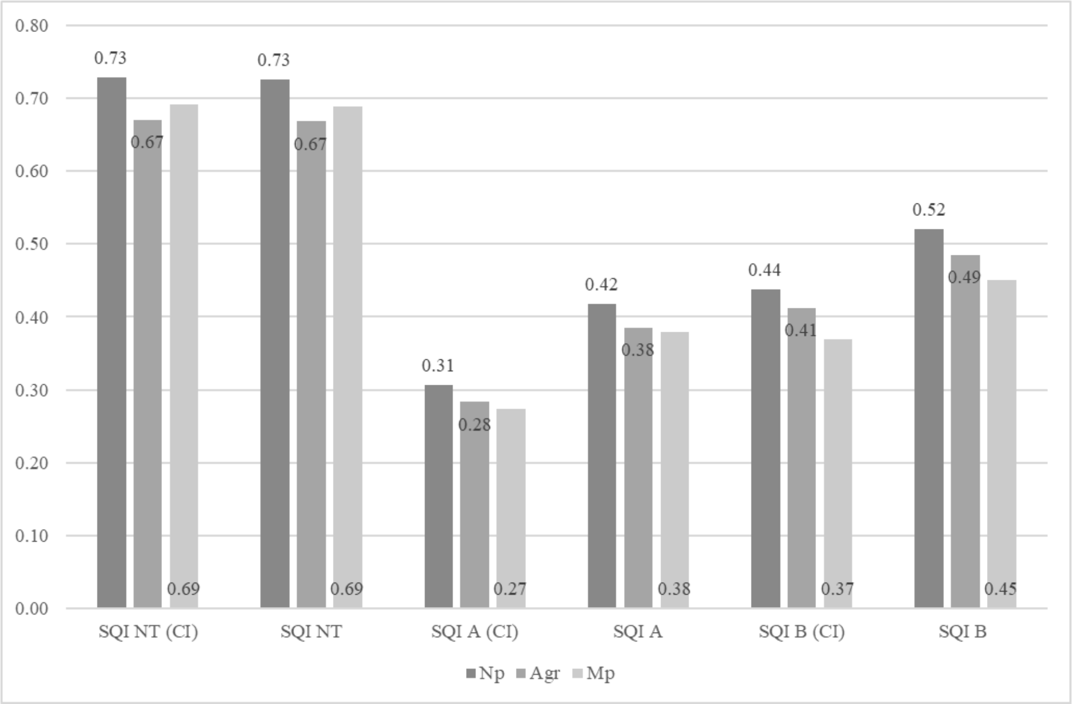

Figure 3: Behavior by land use of SQI. NT, non-transformed variables; (CI) calculated with CI

Discussion

The main impacts observed were the loss of vegetation cover in Pm, and the use of fertilizers in Agr. These impacts explain why the most important factors in the MDS were MRRat /C, TDS and RAH. On one hand, the loss of vegetation cover in Pm affects the supply of organic C in the soil and dramatically decreases the MRRat /C and all microbial processes related to nutrient flow and microbial growth. Furthermore, loss of vegetation cover reduces RAH and moisture contributions to plant component (Procházka *et al.,* 2011). The use of fertilizers, on the other hand, affects the concentration of soluble salts and solids in the soil(Almeida and Serralheiro, 2017). Since Np use did not present these disturbances, its SQIs are all high.

SQIs calculated with nonlinear functions were better at highlighting the differences among the index values for each land use. Furthermore, the SQI calculated with IC, gives a better differentiation among the value for different uses. This improvement is due to the effect of the ICs to increase the sensitivity of the SQI to changes in soil functionality, since the ICs discriminate the influence of each factor evaluated. As expected, Np shows higher values than Agr and Mp. The SQI calculated with the linear function A, however, does not differentiate as clearly the Agr and Mp uses.

Formulating the SQI with MDS based on FA facilitates the clustering of factors of different origin that affect the functionality of the soils. These factors can be measured in low-cost field studies as a first assessment of early changes in soil conditions.

Conclusions

The use of geometric means to generate significant variables is a good alternative to include information representative of the functionality of the soil within an SQI. Likewise, SQI calculated with IC and with non-linear functions can represent better the changes in soil functionality to the evaluated Andisols. SQI calculated with IC and with non-linear functions are more sensitive to disturbances and it can discriminate between different degrees of impact, using variables with low measurement cost. In addition, this kind of index based on indicators of abundance and microbial respiration can be a very useful tool to evaluate large areas with different environmental contexts. We suggest the use of this methodology to evaluate the importance of more robust indicators of microbial diversity and microbial functionality, since these indicators are more important to discriminate early impacts on soil functionality.

This work is a preliminary stage of a research that is evaluating other physicochemical attributes in Andisols, functional microbiological profiles of soil fungal communities through the analysis of sequences of the ITS2 region and designing diversity indicators based in mycorrhiza fungi to include them in assessments of this type.

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Soil Macrofauna Biodiversity in *Paraserianthes falcataria* and *Morus alba* Plant Agroforestry in Bali Island

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Abstract summary

Agroforestry is an activity that supports sustainable agriculture, contributes to increasing the benefits of agricultural productivity in the long term, improving the quality of the environment, and improving the quality of rural community life. Soil fauna abundance is one of the factor that increases soil fertility, its necessary to increase agroforestry land productivity. Soil fauna has an important role in increasing and maintaining soil productivity through its function in the organic decomposition process and optimizing the physical, chemical, and biological characteristics of the soil. This research was conducted to determine the diversity of soil macrofauna on agroforestry land planted with *Paraserianthes falcataria* and mulberry plants. The study was conducted in three areas of Bali, selected as a center for agroforestry development in the Province of Bali. In the study, the *Paraserianthes falcataria* and mulberry agroforestry plot was constructed with a randomized block design with 4 treatments including control treatments without mulberry plants or *Paraserianthes falcataria* monoculture and three treatments of mulberry with 50 x 100 cm, 100 x 100 cm and 100 x 150 cm distance under the *Paraserianthes falcataria* stand including each treatment was 3 times repeated in 3 groups. The results showed that soil macrofauna in 3 times and mulberry agroforestry were found as many as 29 species originating from 26 families and 22 orders. The diversity and richness of soil macrofauna are classified as moderate (H'= 2.21 - 2.58). The dominant soil macrofauna species is *Aporrectodea caliginosa, Lumbricus rubellus, Phyllophaga javana* and *Solenopsis invicta. Lumbricus* is a litter eating earthworm which is quite effective in the decomposition of organic matter can increase soil fertility.

Key words: diversity, soil macrofauna, Morus, Paraserianthes falcataria stand.

Introduction

The Sustainable agricultural development is optimizing the use of renewable resources and unrenewable resources for agricultural production processes by suppressing negative impacts on the environment. Sustainable agriculture is developing towards great benefits for humans, efficient and equitable use of resources with environmental conditions suitable for humans and other species. Agroforestry is an activity that supports sustainable agriculture, contributes to increasing the benefits of agricultural productivity in the long term, improving the quality of the environment, and improving the quality of life of rural communities. Land management with agroforestry aims to maintain the amount and diversity of land production, so that it has the potential to provide social, economic and environmental benefits for land users. This system will provide benefits both ecologically and economically. Agroforestry in Indonesia takes many forms. Grouping forms of agroforestry based on agroecological distribution in the tropics consists of intercropping with trees (tree intercropping), forestry plants and animal husbandry activities (Silvopasture), forest area conservation systems (Protective systems), and agroforestry for wood needs (Agroforestry tree woodlots).

The forms of agroforestry models in Bali consist of mango-based, cashew-based, cocoa-based, coffee-based, coconut-based, mixed-crop agroforestry, intercropping agroforestry, agroforestry-based fodder and agroforestry based on the protection of natural resources. In some areas outside the Bali island agroforestry was developed with other crops, including rubber-based agroforestry in the Jambi area, soy-based agroforestry in the Malang area and zalacca-based agroforestry in the Tapanuli area. The form of the agroforestry model that will be examined is the combination of the main crop of *Paraserianthes falcataria* (forestry) and mulberry (agriculture). So far, the Agroforestry research has focused on agroforestry based on food crops (agrisilviculture). Other types of agroforestry based on honey bees (apiculture), mulberry-based plants for silkworm cultivation (sericulture), based on animal feed for raising livestock (silvopasture) and based on fisheries (silvofishery) are still not receiving much attention.

Various forms of agroforestry that have not been developed yet from mulberry plants (*Morus* sp.). Mulberry plants (*Morus* sp.) Were tested on agroforestry land because they can adapt well to shade-resistant conditions. At 60 percent light intensity, mulberry can grow well. Sunlight conditions on mulberry plants affect the quality of leaves for silkworm feed. In addition, mulberry planted on mixed plants has relatively no effect on nutrient competition. Mulberry plants in nutrient competition are not the main competitors for woody staple crops (forestry plants). Mulberry has a strong root system. Its roots form a very tangled and hard tissue in the soil. Mulberry is ecologically able to suppress sandstorms and conserve soil and water. The level of soil aggregation increases by 25-50 percent each compared to conventional crop patterns.

Soil fauna abundance is one of the factors that increases soil fertility, its necessary to increase agroforestry land productivity. Soil fauna is an important part in the soil ecosystem. The role of soil fauna, among others, can improve soil fertility by physically destroying it, breaking up material into humus, combining decomposed material in the upper soil layers, and forming aggregate stability between organic matter and soil mineral materials. In addition, soil fauna also plays a role in carbon flow, nutrient redistribution, nutrient cycling, and soil structure formation. The existence of macrofauna in the soil is very dependent on the availability of energy and food sources for its survival. With the availability of energy and nutrients, the soil macrofauna development and activity will be well and the reciprocity will have a positive impact on soil fertility. Organic waste discharged into the soil will be used by soil fauna as a source of food. If the amount of organic waste is abundant, the soil fauna will continue to make bio pore and multiply activities, chew and reduce the size of organic waste, and mix it with microbes that can accelerate the weathering process of organic waste into compost and humus compounds that can improve soil conditions.

Based on the background, the limitation of the problem in this study is the study location, the location is only in the agroforestry area in Bali, Indonesia. The object of research is the soil macrofauna that has been found and identified during the study period; the inventory method in the research is the Pitfall Trap method, with a depth of approximately 20 cm; identification of soil insects is carried out to the Family level; abiotic factors measured in this study are pH, temperature, humidity; diversity index measured using the Shanon and Weaver formula; Population characteristics measured include the importance value index (IVI) consisting of density, relative density, frequency, and relative frequency. Therefore, the research objective is to determine the diversity of soil macrofauna in agroforestry land planted with mulberry and *Paraserianthes falcataria* plants.

Methodology

The study was conducted in Bali areas. The chosen location is the centre of agroforestry development in the Bali region. The research location is at 900 meters above sea level altitude. The location temperature is between 20-26°C. The study was conducted in August-October 2019. In the study, the *Parasarianthes falcataria* and mulberry agroforestry demonstration plots were constructed with a randomized block design with 4 treatments including control treatments without mulberry plants or *Paraserianhes falcataria* and three treatments of mulberry spacing 50 x 100 cm, 100 x 100 cm and 100 x 150 cm under the *Parasarianthes falcataria* stand include each treatment repeated 3 times, 3 groups. The agroforestry treatment plot received basic fertilization in the form of cow manure at a dose of 5 tons/h.

The soil macrofauna data technique of collecting uses the monolith method with a hand sortation technique, with modifications to the placement of the research plot. Plots measuring 30 cm x 30 cm with a depth of 30 cm were placed intentionally in each study treatment plot with a diagonal line towards the plot. The number of observation plots for each treatment of mulberry spacing is 9 plots so that the total is 36 observation plots. Soil samples were extracted directly at the study site, then soil macrofauna samples were collected and preserved using 70 percent alcohol for morphological identification in the laboratory.

Descriptive analysis was carried out to determine the description of soil macrofauna using the approach of value importance value index, Shannon-Wienner species diversity index, Margalef species wealth index, Shannon-Wienner species evenness index and quantitative quantitative similarity index.

$$H' = \sum_{i=1}^{n} (\frac{\text{ni}}{\text{N}}) \ln(\frac{\text{ni}}{\text{N}}); \qquad R' = \sum_{i=1}^{n} \frac{\text{s}-1}{\ln\text{N}}; \qquad E' = \frac{H'}{\ln\text{S}}$$

where H′ is the Shannon-Wienner species diversity index, R′ is the Margalef species wealth index, E′ is the species evenness index, *ni* is the number of individuals per species, N is the total number of the entire population, ln is the natural logaritm, S is the number of species. The value of species diversity index according to Shannon-Wienner is defined as H′>3 indicating high abundance species diversity, 1 ≤ H′ ≤ 3 indicates moderate abundant species diversity, H′<1 indicates low species diversity. Margalef species wealth index value is defined as R′> 5 indicates high species richness, 3.5 ≤ R′≤ 5 indicates moderate species richness, R′<3.5 indicates low species richness.

Results

Table 1: Important value Index of soil macrofauna in Paraserianthes palcataria monoculture

No	Species	Family	KR	FR	IVI
1.	*Aporrectodea caliginosa*	Lumbricidae	16,98	20,49	37,47
2.	*Craterostigmus tasmanianus*	Craterostigmidae	5,66	6,83	12,49
3.	*Forficula auricularia*	Forficulidae	1,88	2,27	4,15
4.	*Geophilus flavus*	Geophilidae	3,77	4,55	8,32
5.	*Gryllus assimilis*	Gryllidae	9,43	9,10	18,54
6.	*Lasius flavus*	Formicidae	22,64	20,49	43,13
7.	*Lumbricus rubellus*	Lumbricidae	9,43	6,83	16,26
8.	*Macrotermes gilvus*	Termitidae	3,77	4,55	8,32
9.	*Paederus riparius*	Staphylinidae	1,88	2,27	4,16
10.	*Petaserpes strictus*	Polyzoniidae	5,66	6,83	12,49
11.	*Solenopsis invicta*	Formicidae	15,09	11,38	26,47
12.	*Trigoniulus corallinus*	Trigoniulidae	3,70	4,55	8,32
			100,00	**100,00**	**200,00**

Table 2: Important value Index of soil macrofauna at spasing 50 x 100 cm of Paraserianthes palcataria and mulberry agroforestry

No	Species	Family	KR	FR	IVI
1.	*Achatina fulica*	Achatinidae	2,17	2,94	5,11
2.	*Aporrectodea caliginosa*	Lumbricidae	15,20	13,20	28,40
3.	*Arion vulgaris*	Arionidae	5,43	4,42	9,85
4.	*Campodea staphylinus*	Campodeidae	2,17	2,94	5,11
5.	*Cicindela aurulenta*	Carabidae	5,43	5,89	11,32
6.	*Craterostigmus tasmanianus*	Craterostigmidae	7,61	4,42	12,03
7.	*Geophilus flavus*	Geophilidae	2,17	2,94	5,11
8.	*Gryllus assimilis*	Gryllidae	5,43	7,36	12,79
9.	*Lasius flavus*	Formicidae	16,30	13,20	29,50
10.	*Lumbricus rubellus*	Lumbricidae	8,70	7,36	16,06
11.	*Macrotermes gilvus*	Termitidae	4,35	4,42	8,77
12.	*Petaserpes strictus*	Polyzoniidae	3,26	2,94	6,20
13.	*Phyllophaga javana*	Scarabaeidae	9,78	13,20	22,98
14.	*Solenopsis invicta*	Formicidae	6,52	7,36	13,88
15.	*Thrips aspinus*	Thripidae	2,17	2,94	5,11
16.	*Thyanta calceata*	Pentatomidae	1,09	1,47	2,56
17.	*Trigoniulus corallinus*	Trigoniulidae	2,17	2,94	5,11
			100,00	**100,00**	**200,00**

Table 3: Important value Index of soil macrofauna at 100 x 100 cm spacing Paraserianthes palcataria and mulberry agroforestry

No	Species	Family	KR	FR	IVI
1.	*Aporrectodea caliginosa*	Lumbricidae	12,00	14,80	26,80
2.	*Arion vulgaris*	Arionidae	6,02	4,92	10,94
3.	*Craterostigmus tasmanianus*	Craterostigmidae	6,02	6,56	12,58
4.	*Geophilus flavus*	Geophilidae	4,82	4,92	9,74
5.	*Glomeris marginata*	Glomeridae	2,41	3,28	5,69
6.	*Gryllus assimilis*	Gryllidae	8,43	6,56	14,99
7.	*Lasius flavus*	Formicidae	14,50	14,80	29,30
8.	*Lumbricus rubellus*	Lumbricidae	10,80	9,85	20,65
9.	*Macrotermes gilvus*	Termitidae	6,02	3,28	9,30
10.	*Neobisium carcinoides*	Neobisiidae	1,20	1,64	2,84
11.	*Petaserpes strictus*	Polyzoniidae	3,61	3,28	6,89
12.	*Phyllophaga javana*	Scarabaeidae	12,00	14,80	26,80
13.	*Scalopedra cingulata*	Scolopendridae	1,20	1,64	2,84
14.	*Solenopsis invicta*	Formicidae	6,02	3,28	9,30
15.	*Spirostreptus seychellarum*	Spirostreptidae	3,61	4,92	8,53
16.	*Thyanta calceata*	Pentatomidae	1,20	1,64	2,84
			100,00	**100,00**	**200,00**

Table 4: Important value Index of soil macrofauna at 150 x 100 cm spacing Paraserianthes palcataria and mulberry agroforests

No	Species	Family	KR	FR	IVI
1.	*Aporrectodea caliginosa*	Lumbricidae	19,20	15,50	34,70
2.	*Craterostigmus tasmanianus*	Craterostigmidae	6,41	8,63	15,04
3.	*Geophilus flavus*	Geophilidae	3,85	5,18	9,03
4.	*Gryllus assimilis*	Gryllidae	6,41	5,18	11,59
5.	*Hadronyche modesta*	Atracidae	2,56	3,45	6,01
6.	*Heterometrus cyaneus*	Scorpionidae	1,28	1,73	3,01
7.	*Lasius flavus*	Formicidae	14,10	15,50	29,60
8.	*Lumbricus rubellus*	Lumbricidae	8,97	10,40	19,37
9.	*Macrotermes gilvus*	Termitidae	5,13	3,45	8,58
10.	*Oniscus asellus*	Oniscidae	2,56	1,73	4,29
11.	*Phyllophaga javana*	Scarabaeidae	12,80	15,50	28,30
12.	*Reticulitermes banyulensis*	Rhinotermitidae	6,41	5,18	11,59
13.	*Rhabdomiris striatellus*	Miridae	1,28	1,73	3,01
14.	*Scolia soror*	Scoliidae	2,56	3,45	6,01
15.	*Solenopsis invicta*	Formicidae	6,41	3,45	9,86
			100,00	**100,00**	**200,00**

Table 5: Soil Macrofauna Biodiversity Index

No	Parameters	Monoculture	Agroforestry 01	Agroforestry 02	Agroforestry 03
			(50 x100 cm)	(100 x 100 cm)	(150 x 100 cm)
1.	ni	12,00	17,00	16,00	15,00
2.	N	53,00	92,00	83,00	78,00
3.	H'	2,21	2,58	2,55	2,44
4.	R'	2,77	3,53	3,39	3,21
5.	E'	0,89	0,91	0,92	0,90

Discussion

There 29 species of soil macrofauna are found in this study, originating from 26 families and 22 orders were found in the agroforestry pattern, while in the *Paraserianthes falcataria* monoculture planting pattern 12 species of macrofauna were found originating from 10 families and 10 orders. Several species of soil macrofauna found in the agroforestry cropping patterns include *Aporrectodea caliginosa, Craterostigmus tasmanianus, Forficula auricularia, Geophilus flavus, Gryllus assimilis, Lasius flavus, Lumbricus rubellus, Macrotermes gilvus corpususus, Corpusus.* There are two species of soil macrofauna classified as soil engineers that play a role in the decomposition of organic matter in the soil (Jouquet *et al.,* 2006), namely *Aporrectodea caliginosa* (Lumbricidae) and *Lumbricus rubellus* (Lumbricidae).

Several species of soil macrofauna predominate in *Paraserianthes falcataria* monoculture (Table 1) including *Lasius flavus* (IVI: 43.13 percent), *Aporrectodea caliginosa* (INP: 37.47 percent) and *Solenopsis invicta* (INP: 26.47 percent). Lumbricus species are litter-eating earthworms that are quite effective in the decomposition of organic matter (Anwar, 2009). Earthworms can increase soil fertility and nutrient availability because the decomposition process of organic matter is 2-5 times faster than organic matter without the presence of these organisms (Maftu'ah and Susanti, 2009). The result of dead organic matter decomposition by earthworms provides nutrients available to plants in the form of worm droppings.

Meanwhile, in the agroforestry pattern of *Paraserianthes falcataria* and mulberry at the spacing of 50 x 100 cm, 100 x 100 cm and 150 x 100 cm (Table 2, Table 3 and Table 4) is dominated by *Aporrectodea caliginosa* (Lumbricidae), *Lasius flavus* (Formicidae) and *Phyllophaga javana* (Scarabaeidae) IVI were 34.70 percent, 43.17 percent and 28.30 percent, respectively. Phyllophaga javana is classified as an herbivor which is an important pest especially for some agricultural plants. Manure is highly favored by Phyllophaga javana especially manure from cattle (Brandhorst-Hubbard, Flanders and Appel, 2001). The presence of Phyllophaga javana more in agroforestry patterns is likely related to the provision of manure in agroforestry patterns while monoculture does not add manure.

Soil macrofauna is shown in Table 5 in the agroforestry pattern based on the Shannon-Wienner index of 2.21. This shows that the diversity of soil macrofauna in the cropping pattern is in the medium category as indicated by the Shannon-Wienner species diversity index value greater than 1. The results of the soil macrofauna species wealth analysis (Table 5) show that the soil macrofauna richness (R’) in monoculture area was 2.77, while the agroforestry patterns ranged from 3.21-3.53. The value of macrofauna wealth in both patterns is classified at the same different level, for monocultures classified as low (<3.5), while for the agroforestry patterns that are classified as moderate species wealth (> 3.5). Based on species evenness index shows that the pattern of agroforestry of *Paraserianthes falcataria* monoculture and agroforestry *Paraserianthes falcataria* with mulberry is not much different. This is with a fairly large level evenness of 0.89-0.92 evenly distributed (Table 5).

The level of macrofauna species diversity and richness in the *Paraserianthes falcataria* agroforestry combination with mulberry and the *Paraserianthes falcataria* monoculture pattern are very different. The difference in the number of plant species in agroforestry when compared to monoculture influences the presence of more diverse macrofauna. The diversity of soil macrofauna in the mulberry agroforestry pattern is higher, the closer the planting distance, the greater the diversity index of macrofauna. In addition, other factors are mostly related to the availability and quality of soil organic matter and the remnants of understorey biomass as a food source (Korboulewsky, Perez and Chauvat, 2016). Likewise, there are differences in cultivation treatment such as spacing and agroforestry planting patterns as well as monoculture. The presence of manure input in the agroforestry patterns has an effect on increasing the category of soil macrofauna species diversity when compared with the diversity of soil macrofauna in monoculture patterns that are not given manure.

Agroforestry land management is more intensive such as the existence of weeding and weeding regularly so that it affects the diversity of soil macrofauna. This is as according to Phophi *et al.* (2017), Sasmita, Purba and Yuniti (2019), Halwany (2014) that the diversity of soil fauna has decreased along with the increase in intensive land management in the crop cultivation system.

Conclusions

Soil macrofauna in *Paraserianthes falcataria* and mulberry agroforestry were found as many as 29 species originating from 26 families and 22 orders. The diversity and richness of soil macrofauna species in the *Paraserianthes falcataria* and mulberry agroforestry systems are classified as moderate (H '= 2.21 - 2.58). The dominant species of soil macrofauna is *Aporrectodea caliginosa, Lumbricus rubellus, Phyllophaga javana and Solenopsis invicta. Lumbricus* is a litter-eating earthworm which is quite effective in decomposition of organic matter can increase soil fertility and nutrient availability because the process of decomposition of organic matter becomes 2-5 times faster than organic matter without the presence of organisms. The effect of mulberry spacing on the structure of soil macrofauna communities is relatively moderate at tight spacing, with an average index value of R '= 3.53. The diversity of macrofauna in the soil shows a relatively high correlation with soil organic matter content, dominance of lower vegetation and soil moisture, while surface macrofauna shows a relatively high correlation with penetration of planting distances and sunlight.

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Microbial diversity in forest soil from Vitosha mountain

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Abstract summary

Sampling sites with typical vegetation community for Vitosha mountain – spruce forest and subalpine grassland have been established to analyse the changes in soil microbial diversity. Our study provides the first insight into the microbial content and diversity in soil depth profiles over forest ecosystems – spruce and subalpine grassland in order to demonstrate the relevance of specific soil properties for microbial distribution patterns. The results obtained demonstrate a higher quantity and diversity of soil microflora both in total amount as well as within the identified groups in the surface 5-cm layer of the studied soil. Along the soil profile depth, where the soil humidity and pH decreases, the quantity of microflora issubstantially reduced. The percentage of actinomycetes and micromycetes prevails in soil from spruce forest. On contrary, microorganisms present in subalpine grassland are dominated mostly by non-spore-forming bacteria, being 75 and 67 percent for the surface (0 – 5 cm) and subsurface (5 -20 cm) layers, respectively.

Keywords: Vitosha mountain, Norway spruce, subalpine grassland, soil, microorganisms

Introduction, scope and main objectives

Forest soils are among the most diverse microbial habitats on Earth, in which bacteria are the most abundant group of microorganisms. Soil microorganisms play an important role in the forest environment (Lladó, López-Mondéjar and Baldrian,2017). For Bulgaria, this is especially important as forests cover an area of 3910 kha, which represents 35 percent of the country’s territory (Executive Environment Agency, 2019). Forests in Bulgaria are composed of a large variety of deciduous trees spread on approximately 73 percent of the territory of forest land, while coniferous forest represents the rest 27 percent. Coniferous trees dominate at higher altitudes, but also occur frequently in plantation forests (Yurukov and Zhelev, 2001). Forests represent important carbon (C) sinks in 3 pools – living biomass (above- and below-ground biomass), dead organic matter (dead wood and litter) and soil. Thus, forests are multitude of habitats for huge diversity of organisms. Microbial diversity represents the status of a microbial community, which could be used to predict the transformation trend of the environmental quality and soil nutrient conditions. The main natural disasters in Bulgaria like forest fires, floods, wind throws, disturbances by insects, deforestation influence soil microbial diversity leading to partial or complete destruction of the humus layer of soil (Malcheva *et al.*, 2015).

The composition of forest soil microorganisms, which can be found at high altitudes of the Vitosha mountain has not been studied systematically and deserves more attention. Gaining a better insight into the biodiversity of the microbial community in soils with different land use – coniferous forest and high meadow vegetation - will contribute to a better understanding of their role in forest ecosystems and their relative importance.

Methodology

Soil sampling was performed at two experimental sites as follows – one with forest vegetation of Norway spruce (*Picea abies (L.) Karsten.*) (SS 1) and one with high meadow vegetation (SS 2) in Vitosha mountain (Tables 1 and 2). This sampling area is important because Vitosha mountain is the oldest natural park on the Balkan Peninsula. On the park’s territory, 61 types of habitats have been identified, among them of greatest interest, as far as nature conservation is considered, are the natural spruce forests, the peat areas, the moraines and the caves.

Table 1: Characteristics of sampling sites

Sampling site	Dominant vegetation	Altitude, m	Geographical positions
SS1	Picea abies (L.) Karsten	1520	42°33'48.5"N 23°18'56.9"E
SS2	Subalpine grasslands	1969	42°34'32.4"N 23°17'47.9"E

Table 2: Main soil characteristics

Sampling site	Soil depth	Carbon (C, %)	Nitrogen, (N, %)	pH
SS1	0-5	18.11	0.77	6.16
	5-20	11.09	0.48	5.53
SS2	0-5	16.94	0.77	5.88
	5-20	9.79	0.48	5.18

The sampling sites are spread on the territory of Bistrishko Branishte Reserve, which was established with the aim to preserve in their natural condition high-mountain spruce forests, sub-alpine grass cohabitations, rock formations and stone rivers. Samples for analyses were taken with a sterile instrument from 0-5 cm and 5-20 cm of the mineral soil in sterile paper bags. The sampling procedure was consistent with the procedure adopted in the ICP Manual (UNECE, 2003). All samples were transported and analysed within 48 hours. Microbiological studies included determination of actinomycetes, micromycetes, bacilli, non-spore-forming bacteria, and bacteria, assimilating mineral nitrogen. Appropriate dilutions of soil samples were inoculated on culture media plates containing meat-peptone agar, starch–ammonium agar and Czapek's agar. The agar plates were then incubated for 2–10 days in a thermostat at 27°C and the numbers of typical morphological colonies were counted. The number of tested microorganisms was expressed as colony-forming units (c.f.u.) per 1 g of dry soil (Zvyagintsev *et al.,* 1980).

Results

The results obtained (shown in Figure 1 and Table 3) reveal a higher quantity of soil microflora both as total amount as well as within microbial groups in the surface 5 cm soil layer. Along the soil profile depth, where the soil humidity and pH decrease, the quantity of microflora decreases substantially. In the upper 5 cm – the percentage of actinomycetes prevails – 33 percent (Figure 1A), following by micromycetes. In the deeper (below 5 cm) soil layer of spruce forest, the bacillus content prevails – 39 percent, followed by equal contents of actinomycetes and micromycetes – both with 22 percent of all microflora.

The composition of the bacillus group in the studied soils is characterized by the dominance of *Bacillus megaterium* and *Bacillus cereus* species, which defines their higher durability as spore-forming species and their major role in soil mineralization processes.

A considerable difference in the distribution within groups of microorganisms was observed in soil of Subalpine grasslands sampling sites. In both studied soil layers – 0 -5 cm and 5 -20 cm, the non-spore-forming bacteria prevail – 75 and 67 percent, respectively (Figure 2 A and B). The contents of actinomycetes and micromycetes present are lower in comparison with spruce forest and varies between 15 percent and 20 percent slightly increasing in the lower (5-20 cm) soil layer.

Table 3: Total microflora

Soil depth, cm	**Total microflora, (thousand per gram dry soil)**	
	Picea abies (L.) Karsten	Subalpine grasslands
0-5	2.340	2.280
5-20	0.198	0.714

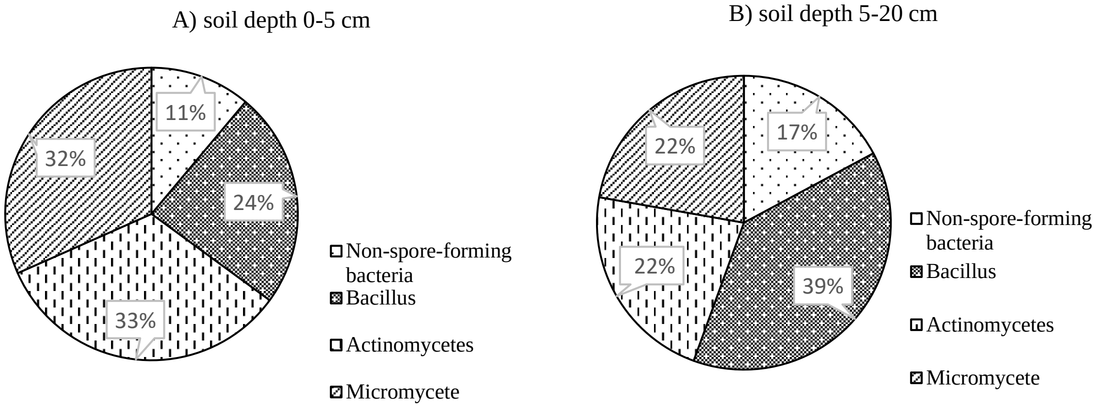

Figure 14: Variability of main groups of microflorae in soil from spruce forest

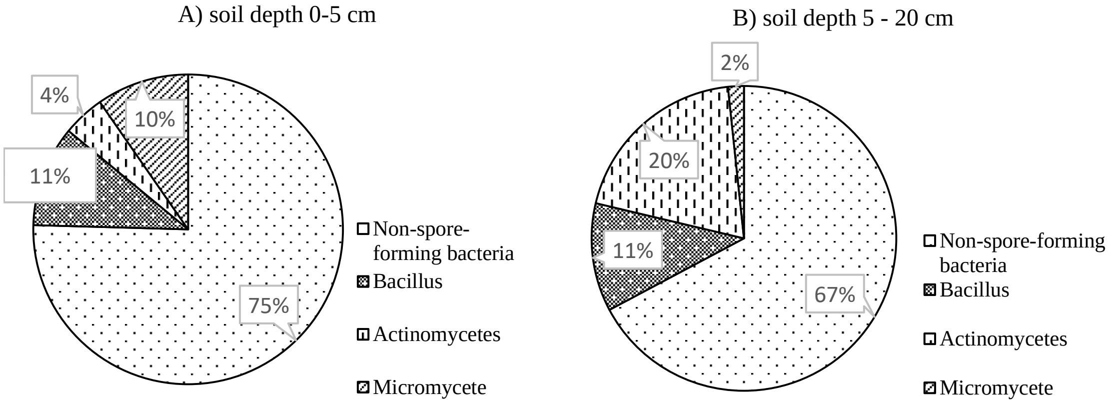

Figure 2: Variability of main groups of microflorae in soil from grassland

The content and distribution of bacteria assimilating mineral nitrogen in soil from both studied territories is presented in Figure 3. The data obtained show that in the surface soil layer (0 – 5 cm) the quantity of these bacteria is orders of magnitude higher in the soil covered with Subalpine grasslands in comparison with the spruce forest. The mentioned quantity decreases considerably in the sub-surface layers (5 -20 cm) of soil from SS 2. The coefficient of mineralization was calculated as the ratio of quantity of nitrogen-fixing bacteria and the sum of the quantities of non-spore-forming bacteria and bacillus. The results obtained for the two sampling sites are presented in Figure 4. As can be seen, the coefficient of mineralization is higher in the surface soil layer from grassland sampling sites, while in the sub-surface (5 – 20 cm) this coefficient is much higher in soil from sampling sites of spruce forest.

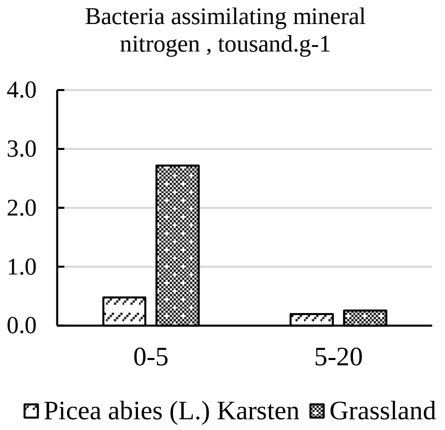

Figure 3: Bacteria assimilating mineral nitrogen in soil from spruce and grassland

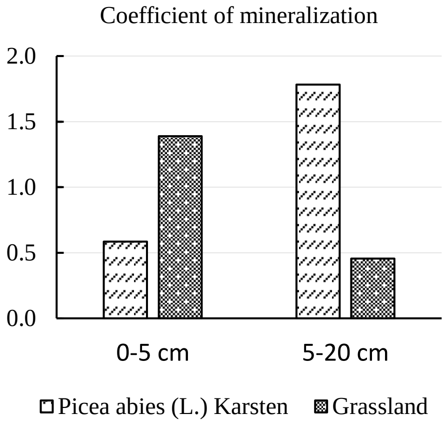

Figure 4: Coefficient of mineralization in soil from spruce and grassland

Discussion

Our study provides the first insight into the microbial content and diversity in soil depth profiles in the investigated forest ecosystems – spruce and subalpine grassland. The study demonstrates a decreasing content in microbial biomass with soil depth accompanied by decreasing organic matter content. This finding is consistent with the results of a study by Yang *et al.* (2016). Variability of main groups of microflora in soil depends significantly on the vegetation. Similarly, the microbial communities of bacteria plus micromycetes, increases greatly in organic soil horizons of stands with a grass-ground cover (Lettl and Hýsek, 1994). Actinomycetes are known as decomposer of a wide array of substrates but are especially important in degrading recalcitrant (hard-to-decompose) compounds, such as chitin and cellulose, and are active at high pH levels. Our results also showed that pH 6.16 in soil of spruce forest enhances the abundance of Actinomycetes and Micromycetes, which in agreement with the findings of Bhatti, Haq and Bhat (2017). It is consistent also with the results of a study by Wei, Zhang and Yu (2009) dedicated to forests from different areas. The distribution of bacteria assimilating mineral nitrogen was explained by habitat type, soil carbon, and soil N contents. The taxonomic composition of soil microorganisms is correlated with spatial variation in climate, plant diversity, pH, disturbance, and many other factors (Nelsona *et al.,* 2016). The functioning of soil microbiocenosis under Grassland is accomplished by the reactive capabilities of the non-spore-forming bacteria, which is accompanied by reducing the rate of mineralization as far as these microorganisms participate in the initial phase of degradation of organic matter (the lowest value of the mineralization coefficient in the lower soil layer for soil below Grassland - 0.46) (Bogdanov, Nustorova and Malcheva, 2015).

Conclusions

The results obtained demonstrate a higher quantity of soil microflora, both as total amount and within groups in the surface 5 cm of the studied soils. In deeper soil layers, where the humidity and pH decrease, the quantity of microflora is significantly diminished. The percentages of actinomycetes and micromycetes prevail in soil from spruce forest. On contrary, the microbial diversity in subalpine grassland is mainly dominated by non-spore-forming bacteria being 75 and 67 percent respectively by for surface (0 – 5 cm) and subsurface (5 -20 cm) layers. Our results show that despite the important role of fungi in forest soils, bacteria accomplish multiple and essential ecosystem roles in the investigated forest environment, including organic matter decomposition, regulation of mycorrhizal symbiosis, and involvement in N cycle processes.

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Enchytraeids in two phytophysiognomies of Brazilian Cerrado

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Abstract summary

This is the first study on enchytraeid diversity in phytophysiognomies of the Brazilian Cerrado Biome carried out with standard sampling and extraction methods. Shrubby woodland (Cerrado sensu stricto, CSR) and gallery forest (MG) was sampled in the Brasilia Botanical Garden Ecological Station and the Brasilia National Park, respectively, with samplings performed at the end of the rainy season in 2017 and 2018. Enchytraeid population density in MG was higher than in CSR, reaching more than 30 thousand individuals/ m^2 in MG and 5 thousand in CSR. Enchytraeid abundance was influenced by soil organic matter and moisture. So far, six genera and six potentially new species taxa were identified. Dominant genera were *Guaranidrilus* and *Hemienchytraeus*.

Keywords: Enchytraeidae, tropics, Oligochaeta, Clitellata, savana, diversity

Introduction, scope and main objectives

Enchytraeids are small oligochaetes living in soil, freshwater and marine environment. Soil enchytraeids have potential as soil quality indicators as they occur worldwide, play a key role in belowground ecosystems and are sensitive to changes in the environment (Pelosi and Römbke, 2016). They play roles similar to earthworms in soil functioning, such as on soil nutrient cycling and soil structure, however at a smaller scale because of their small body size.

Most of the knowledge on enchytraeids is concentrated in the temperate regions, especially Europe. In the tropics, Brazil is the place with more information available, although taxonomic and ecological knowledge is still very scarce and restricted to few locations in the Amazonian and Atlantic Forests (Römbke and Meller, 1999; Römbke *et al.*, 2015). In the Cerrado ecoregion, for instance, genera and species of enchytraeids are practically unknown, although there is some information on population densities in native and cultivated areas. These data, however, were obtained with inappropriate methods (e.g. Silva *et al.*, 2006).

The Cerrado, a tropical savanna considered a hotspot of biodiversity. The 2 million square kilometres of savanna (23 percent of Brazilian territory) have been increasingly taken by the expanding agriculture and livestock activities over the last 40 years, with remaining 56 percent of the area still covered with natural vegetation in 2018 (Mapbiomas, 2019). The Cerrado occupies mainly the central region of Brazil where seasonality is marked by a rainy season from October to March and a dry season during the rest of the year. The loss of biodiversity in the Cerrado biome has been a matter of concern, because endemic species are abundant.

Regarding soil invertebrates in the Cerrado biome, faunistic and ecological information at lower taxonomic levels is scarce, so that studies on enchytraeids are urgently needed considering current threats. We will present the results on enchytraeid population and generic composition in two different phytophysiognomies of the Cerrado. This information will supplement studies that focus on the use of enchytraeids for monitoring soil biological quality, soil biodiversity loss and sustainability of production systems in Brazil.

Methodology

Sampling sites

The study areas included the Brasilia Botanical Garden Ecological Station (JBB) with a total area of 4,518 hectares and the Brasilia National Park (PNB), a Federal Conservation Unit of 43,000 hectares. Both locations were chosen because of their good state of conservation and low anthropogenic influence. Two phytophysiognomies were sampled in each site: Woody Savanna (Cerrado Sensu Stricto, CSR) and Gallery Forest (MG). The climate in this region is tropical savanna climate, *Aw*, according to the Köppen classification), with distinct rainy and dry season. The sampling was carried out in May 2017 and April 2018, at the end of the rainy season. Soil type in CSR areas is a latosol and in MG a gleysol.

Sampling and sample processing

Soil cores of 5 cm diameter and 5 cm depth (including organic and mineral layer) were taken at 10 points distant 10-15 m along a transect at each sampling site in two consecutive years, at the end of rainy season. In MG, because of varying depths of the organic matter layer, two cores were sampled vertically at a greater depth to assure that the mineral soil layer was included in the sample. For comparisons, the average values of the two cores were used. The worms were extracted with a wet funnel device based on methods described in ISO 23611-3/2007 (ISO, 2007) with heating for 2.5 h as described in Niva *et al.* (2015). The enchytraeids were separated from other organisms and counted under a stereomicroscope. Identification of genera was carried out with living worms under a microscope. Soil fertility attributes and moisture (%) were determined for each sampling point.

Results

The enchytraeid population density found in MG was generally higher than in CSR, but there was a marked difference in JBB from the first to the second sampling date, where mean density in MG reached more than 30 thousand individuals/ m^2 (ind/m^2) in the second year, contrasting with only 637 ind/m^2 in the first year. In PNB, on the other hand, there was no significant difference in density between the two years (Figure 1). Generally, the enchytraeid abundance correlated positively with soil moisture and organic matter.

Soil moisture kept the range of 27 to 33 percent in CSR in both locations at both collection dates, while in MG-JBB it raised from 43 to 65 percent from one year to the other and, in MG-PNB, did not change (64-66 percent). The organic matter content in MG was 18 and 16 percent in PNB and JBB respectively, while in CSR it was 3.6 and 5.4 percent. Soil moisture and organic matter were always higher in MG than in CSR.

Eight different genera were recorded. One of them, named spR in the following, is probably new and undescribed. *Hemienchytraeus* was present in all locations and *Guaranidrilus* and spR were dominant in most of the cases (Figure 2). *Achaeta* and *Enchytraeus* occurred at very low abundances, the first was mostly present in samples collected in 2017 and the second, in 2018.

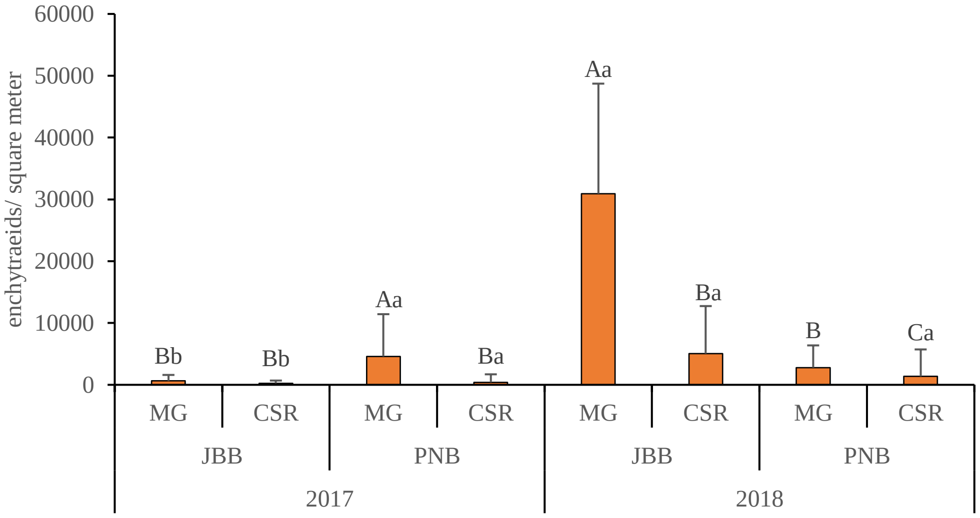

Figure 1: Enchytraeid mean density (individuals/ m^2) and standard deviation in two phytophysiognomies of Cerrado Biome, Brazil, in two collection years

Phytophysiognomies: MG- Gallery forest; CSR- Cerrado Sensu Stricto. Locations: JBB – Brazilian Botanical Garden; PNB: Brasilia National Park. Different capital letters indicate significant difference between years in the same phytophysiognomy and location. Different small letters indicate significant difference between the phytophysiognomies within a year and location (n= 10; non-parametric Kruskal-Wallis test; alpha= 0,05)

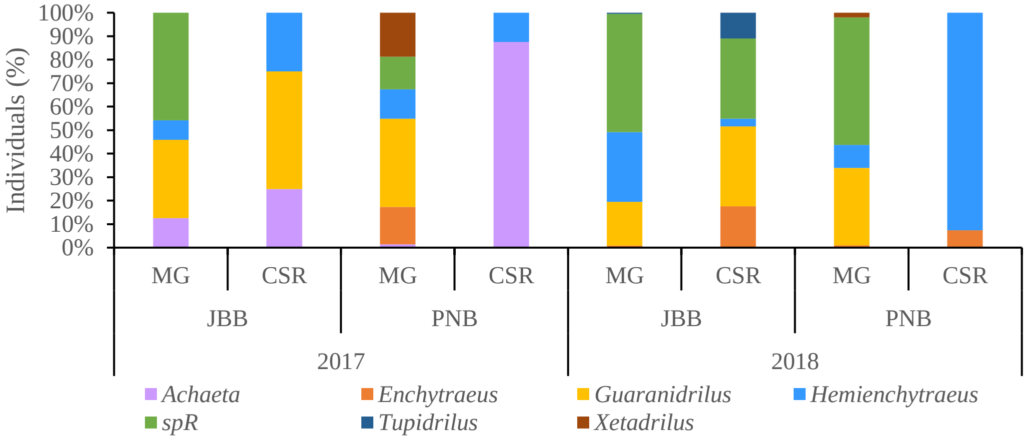

Figure 2: Distribution (%) of enchytraeid genera in each phytophysiognomy of Cerrado Biome, Brazil, in two collection years

Phytophysiognomies: MG- Gallery forest; CSR- Cerrado Sensu Stricto. Locations: JBB – Brazilian Botanical Garden; PNB: Brasilia National Park. spR is a potential new taxon.

The principal component analysis (PCA) performed with population density of each genus, richness, soil chemical and texture variables of the four sampling sites showed that the first and second axis accounted for 62.8 percent of the total variability of the data (Figure 3). The axis 1 mainly separated the phytophysiognomies, and axis 2 separated the locations, suggesting that distribution of soil attributes and enchytraeids were influenced by the sites (JBB and PNB) (Figure 3). The variables which contributed to axis 1 (> 90 percent), shown by the longer arrows, were total N, MO, Sand, H+Al and, negatively, clay (Figure 3). The enchytraeid density (indiv.m) and spR contributed positively to axis 2 (>70 percent), while V, negatively. Genus richness correlated with organic matter and soil fertility attributes.

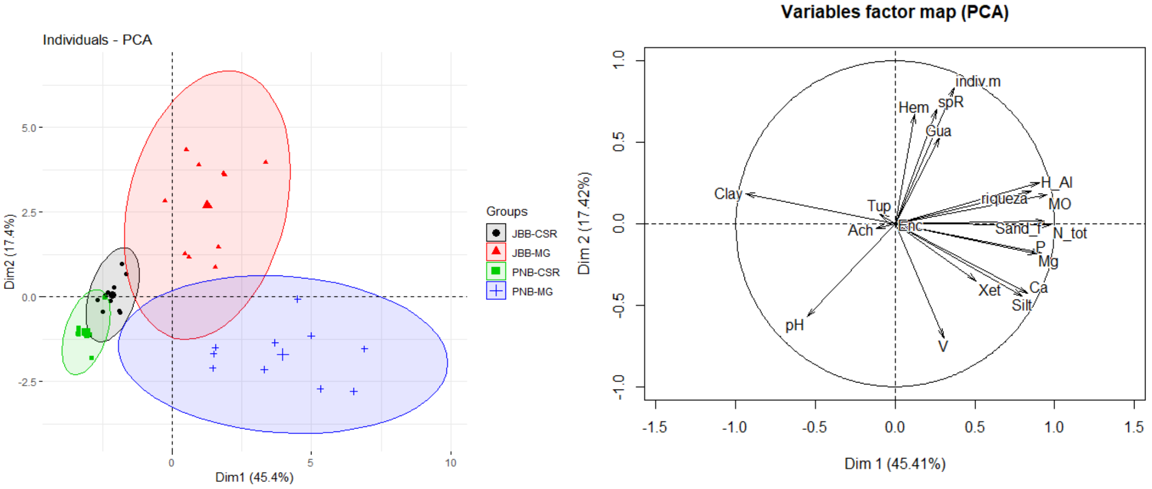

Figure 3: Principal component analysis (PCA) of the variables potentially affecting enchytraeid populations in the phytophysiognomies in each location (A)

Graph (B) shows the correlation of soil variables and enchytraeid data. Phytophysiognomies: MG- Gallery forest; CSR- Cerrado Sensu Stricto. Locations: JBB

- Brazilian Botanical Garden; PNB: Brasilia National Park. Enchytraeidae genera: *Achaeta* (Ach), *Enchytraeus* (Enc), *Guaranidrilus* (Gua), *Hemienchytraeus* (Hem), *Tupidrilus* (Tup), *Xetadrilus* (Xet), spR (potentially new taxon). Other variables: Density (indiv.m), riqueza (Richness), pH, potencial acidity (H_Al), Calcium (Ca), organic matter (MO), magnesium (Mg), total nitrogen (N_tot), phosphorus (P), base saturation (V), fine sand (Sand_f), clay and silt.

Discussion

Very little quantitative and qualitative information about tropical enchytraeids is currently available. Studies in Mata Atlantica and Amazon in Brazil suggest that abundance of enchytraeids in tropical regions might be similar or smaller than in temperate regions (Schmelz *et al.,* 2013). Considering that 10-140 thousand ind/m^2 are expected to be found in temperate forest soils, the mean density of 30 thousand ind/m^2 recorded in MG supports the aforementioned hypothesis. In CSR, however, the density was much smaller (maximum of 5 thousand) (Figure 1). The higher moisture and organic matter content of the gleysol in MG may have favoured the maintenance of enchytraeids compared to the latosol in CSR. The lower abundance of enchytraeids found in 2017 may also have been caused by the lower moisture, due to a severe reduction in rainfall in that year, which agrees with the abundance results. Worms may have moved down vertically to moister strata. In the future, sampling at greater depths may be necessary. The PCA showed a strong association of MO with enchytraeid richness (Fig 3) suggesting a strong correlation of organic matter with enchytraeid abundance.

The genera found in MG and CSR were not different from Mata Atlantica or Amazon (Römbke and Meller, 1999; Römbke, Collado and Schmelz, 2007) except for spR (Fig 2). Similarly, to the forests of other biomes studied so far, *Guaranidrilus* and *Hemienchytraeus* were also abundant genera in these two phytophysiognomies (Figure 2; Fig 3). A preliminary species-level analysis of the specimens, however, revealed six potentially new species, among which, spR may be a new genus with some traits resembling *Guaranidrilus*.

Conclusions

The Cerrado biome harbors enchytraeids at mean densities of up to 30 thousand ind/m^2. So far, this study revealed six genera. Together with a potentially new genus, *Guaranidrilus* and *Hemienchytraeus* were the three most abundant enchytraeid genera in the Gallery Forest and the Cerrado Sensu Stricto. Six species, however, are distinct from the ones known from other biomes and are probably new to science.

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Fungal community assembly in soils of different crops farming in the Puna (North Argentina)

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Abstract summary

Soil microbial ecosystems and its functioning are under the threat of biodiversity loss by the increase of cultivated areas and agronomic exploitation intensity. Among the diverse soil microorganisms, fungi and in particular arbuscular mycorrhizal fungi (AMF) carry out important ecological functions and are considered excellent bioindicators. Even if most ecosystems harbour diverse AMF communities, so far, the information related to AMF biodiversity in ecosystems like the Argentinean Puna, an arid-high plateau where plants experience high abiotic-stresses and unique extreme distinctive environmental conditions are still scarce.

The objective of this research was to analyse in Chaupi Rodeo (Jujuy, Argentina), fungal community assembly in soils of 3 crops species: native corn, bean and native potato, under a familiar land use, without the supplements of agrochemicals but with different histories of crops rotation. Results indicate that AMF spores were most abundant in corn plots, followed by potato and bean. A high AMF diversity was confirmed by Illumina MiSeq data on the same plots. The results suggest that soil fungal, and AMF communities were significantly affected by the rotation histories, with less effect of current crops species. Among soil fungi, Ascomycota phylum was dominant over Basidiomycota, Glomeromycota and Chytridiomycota phyla.

Keywords: Argentinean arid Puna, Andean highlands crops, soil fungi, arbuscular mycorrhizal fungi, land uses, Metabarcoding of fungal communities, IlluminaMiSeq.

Introduction, scope and main objectives

Soil ecosystems and its functioning are under the threat of biodiversity loss by the increase of cultivated areas and agronomic exploitation intensity. Agricultural practices strongly affect soil physical and chemical characteristics, that impact on the microbial communities affecting their abundance, diversity, and activity. Fungi are widely distributed among all terrestrial ecosystems with a huge biodiversity and ecological importance by their principal role in ecosystems processes such as soil carbon cycling, plant nutrition, and phytopathology (Hawksworth and Lücking, 2017). However, the distribution of fungal species, phyla, and functional groups as well as the determinants of fungal diversity and biogeographic patterns are still poorly understood in many environments (Tedersoo *et al.*, 2014; Marín and Bueno 2019). The arbuscular mycorrhizal fungi (AMF) are a relevant group of soil fungi with a worldwide distribution, associated with almost 80 percent of the vascular plants of the Earth. The different land uses, and soil types affect the AMF diversity and their function inside the microbial soil communities and for these reasons, some AMF species may be used as environmental indicators (Oehl *et al.*, 2017). In fact, it is reported (Boivin *et al.*, 2016) that many anthropogenic factors, both in the past and nowadays, can modify and shape AMF communities which could be considered a potentially suitable target for studying regional and local effects on soil microbial diversity. In South American highlands, there are few reports of fungal and AMF Andean communities, either considering spores (Lugo *et al.,* 2008) or genetic diversity in relation to the effects of land uses (Senés-Guerrero and Schüßler, 2016). So far, the information related to fungal biodiversity in ecosystems like the Argentinean Puna, an arid-high plateau where plants experience high abiotic-stresses and unique extreme distinctive environmental conditions are still scarce.

The objective of this work was to analyse in Chaupi Rodeo (Jujuy, Argentina), fungal community assembly, and in particular AMF in soils of 3 crops species: native corn, bean and native potato, under a familiar land use (for their own food provision) without the supplements of agrochemicals but with different histories of crops rotation. Deeper analyses have been conducted in order to evaluate how land uses, soil types and crop rotation affect fungal diversity and distribution in this particular hot-spot area of Argentine Puna.

Methodology

Sampling design / crop species

In the school farming fields of Chaupi Rodeo (Jujuy, AR) (3496-3524 m a.s.l), three plots (30x90 m) cultivated with native corn, bean and native potato, were sampled. Each crop species has 3 plots replicates from which 5 soil sub samples were taken, homogenized, and separated into fractions: a) soil physical-chemical analyses, b) AMF spores extraction and c) molecular analyses. Each soil sample was analysed for pH, % OM, C, N, available P and texture.

Plots of the native most common cultivated crops were labelled as follows: *Vicia faba* (CRP), *Zea mays var.* "ocho rayas" (CRM) and *Solanum tuberosum var. chusqueña* (CRPapa). All plots were under the same agricultural practices of manual tillage or ploughing (hand shovel/ horse) but for all crop species each plot replica has a different history of plant rotation.

Morphological / molecular analyses

The total number of AMF spores/100g of dry-soil were isolated and counted for each sample under dissecting-microscope (Lugo *et al.*, 2008).

For the molecular analysis, prior to DNA extractions, soil samples were sieved through (2 mm mesh size). Genomic DNA was extracted from 500 mg of each soil sample. In order to investigate the total fungal community, the ITS2 region, together with the AMF specific nested approach based on the 18S described by Berruti *et al.* (2017) were chosen. The obtained PCR products were checked, purified and quantified with Qubit, sent to BMR Genomics (Padova, Italy) for Illumina MiSeq platform (2 x 300bp) sequencing.

Bioinformatic analysis

For all data-sets, the sequences were then analysed by means of the microbiome bioinformatics platform QIIME2. Denoising and quality control, including removal of chimeras, were achieved with DADA2 plugin (qiime dada2 denoise-paired) and sequences reads were truncated (>280 bp for forward, >265 bp for reverse reads). Feature tables, for all fungi and AMF, were generated by means of qiime vsearch cluster-features-de-novo plugin using as identity threshold the 97 percent. For the analysis on the total fungal community, the feature table, composed of 633 features, was rarefied at 9657 sequences per sample by means of the --p-sampling-depth method defined in the qiime diversity core-metrics-phylogenetic plugin. The -o-classification method of the qiime feature-classifier classify-sklearn plugin was used for taxonomic assignment. The classifier adopted for the taxonomic assignment was generated using the UNITE Community (2019): UNITE QIIME release for Fungi version 18.11.2018. Ecological roles of the fungal communities at each sampled site were inferred using FUNGuild (Nguyen *et al.*, 2016).

In order to evaluate the possible influences of the different environmental conditions over the populations' distributions the adonis function from the vegan library was used to perform Permanova tests. Permanova tests were run for every metadata, or combinations of metadata using, as dissimilarity indexes, Jaccard and Bray Curtis). Permutest were also run to check the validity of the previous tests by means of the function betadisper of the R package vegan. Bar plots were then generated to visualize the previous statistically significant results with the R package ggplot2.

Results

Morphological observation

Among the three different crop types (CRM, CRPapa, CRP) the number of AMF taxa morphologies was high and variable (20 to 4) and spores were retrieved single, aggregate or forming sporocarps with peridium (Figure 1). The average of spore abundance (a) and the richness among different crop species (b) are shown in Figure 2. The highest value of spore density was observed in corn, followed by potato and bean plots. On the other side, bean plots resulted to retain higher AMF spore's richness than potato and corn ones.

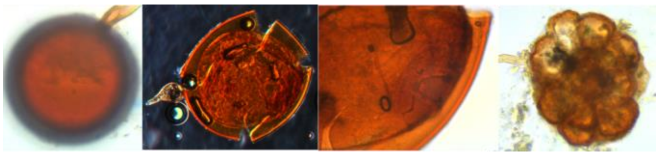

Figure 1: Example of spores' morphology of AM fungi retrieved from Chaupi Rodeo fields

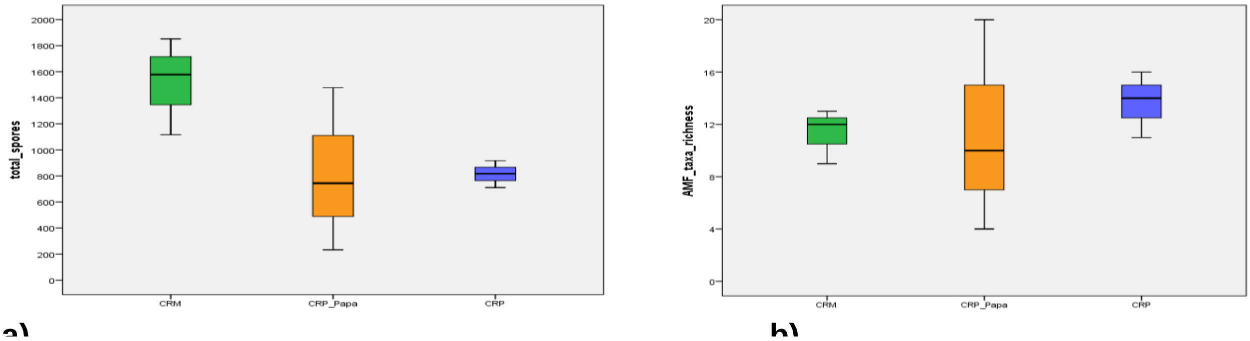

Figure 2: AMF spore abundance and richness in native crop soils of the Chaupi Rodeo settlement in Puna region (Jujuy, Argentina)

(a) Total AMF spore abundance measuring as spore density (number of spores/100 g of dry soil) and (b) AMF taxa richness (number of AMF taxa/100 g of dry soil) within different crop species. References: CRM (native corn, *Zea mays* var. 'ocho rayas'); CRP Papa (native potato, *Solanum tuberosum* var.*chusqueña*); CRP (bean, *Vicia faba*).

Metabarcoding of fungal communities

From cultivated soils of Chaupi Rodeo: a total of 633 OTUs were obtained: Ascomycota phylum was dominant (from 48 percent up to 91 percent) over Basidiomycota, Glomeromycota and Chytridiomycota phyla. The percentage of the different phyla was very variable among the three crop types (CRM, CRPapa, CRP) and also among the 3 plots replicates (Figure 3). Glomeromycota were not always detected and when present they represented 2.3 percent-13 percent of total retrieved sequences.

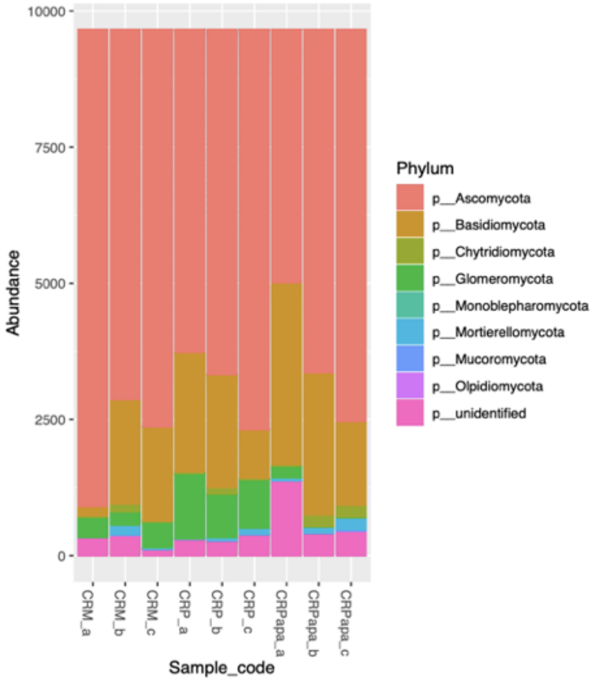

Figure 3: Taxonomic distribution (based on ITS2 target), at phylum level, of fungal OTUs retrieved from the *Zea mays* var

'ocho rayas' (CRM a, b, c), *Vicia faba* (CRP a, b, c) and *Solanum tuberosum* var. *chusqueña* (CRP Papa a, b, c) sampled plots. References: each crop species has three plot replicates named with letters a, b and c, respectively.

Morphological AMF diversity was confirmed by Illumina MiSeq data showing more than 30 molecular OTUs belonged to 3 orders, 8 families (*Glomeraceae, Claroidoglomeraceae, Paraglomeraceae, Diversisporaceae, Archaeospora, Acaulosporaceae, Ambisporaceae and Gigasporaceae)* and 11 genera within Glomeromycotina: *Glomus*, *Claroideoglomus*, *Acaulopora*, *Archaeospora*, *Paraglomus*, *Ambispora*, *Funneliformis*, *Rhizophagus*, *Diversispora*, *Scutellospora* and *Septoglomus* differently distributed among the three crops fields. Taxonomy of AMF OTUs was also refined performing BLAST against the MaarjAM database (https://maarjam.botany.ut.ee/, Öpik *et al.*, 2010). The putative ecological roles of the fungal communities at each sampled site were inferred by FUNGuild that was able to assign function to 389 OTUs (61.2 percent) of 633 ITS2 OTUs. Saprotrophs and Saprotroph-Symbiotrophs relative abundance was greater compared with all the other functional groups, representing 42 percent in CRP, 50 percent in CRM and 56 percent in CRPapa and the 19 percent in CRP, 9.3 percent in CRM and 7.3 percent in CRPapa, respectively. In addition, pathogen fungi were relatively more abundant in potato plots (11.3 percent) respect to the other two crops where they represented around 6 percent for both.

Symbiotrophs, mainly represented by AMF accounted for a minimum of 0.6 percent; 0.8 percent and 3 percent in potato replicate plots to a maximum of 16 percent in beans soil plots. PERMANOVA analyses based on Bray-Curtis and Jaccard measures revealed that AMF communities were significantly influenced (p=0,015) by soil nitrogen (N) and calcium concentration (Ca) (p=0,026). In addition, the preceding crop species do not exert significant influence if considered alone instead together with N and Ca showed significant influence (<0.05) on AMF.

Total fungal communities were not influenced by soil characteristics like N, Ca and Mg, but were influenced by preceding crops (p=0.006) and current crop species (p=0.043), showing significant differences (<0.05) were combined.

Discussion

On the base of results, we can draw some general considerations: 1) the agriculture site of Chaupi Rodeo (Jujuy, Argentina), under a familiar land use without the supplements of agrochemicals but with different histories of crops rotation, retained AMF communities very rich in species. On the base of the recent classification of AMF families into functional groups, as defined by Weber *et al.* (2019), we also found that this particular agriculture sites sited at >3500m above sea level maintains AMF families in the "edaphophilic" guild (*Diversisporaceae*), that improve plant nutrient uptake; families in the "rhizophilic" guild (*Glomeraceae, Claroidoglomeraceae, Paraglomeraceae*), that may protect host plant roots from pathogen colonization; and in "ancestral" guild (*Archaeospora, Acaulosporaceae)*, with lower allocation to either root colonization or soil hyphae than the other two previous guilds (Phillips *et al.,* 2019 and reference therein). These results associated with future more in-depth analysis on the relationship between functional groups of fungi, belonging to the different phyla, will be useful for identifying biological indicators of agricultural environments at high altitude such as Chaupi Rodeo one. Moreover, environments like Argentinean Puna, with scarce resource availability in terms of carbon or nutrients and with stressful conditions, such as drought, the dominance of Ascomycota may reflect a unique distribution pattern of particular taxa belonging to these fungal phyla, in arid and semiarid ecosystems (Treseder *et al.,* 2018). Some ascomycetes taxa may have the ability to tolerate stressful conditions and achieve a more resource use efficiency in harsh environments and to be affected significantly by the by the rotation histories of particular agriculture sites.

Conclusions

Future objective, starting from this work, is the identification of fungal species which can be used as soil indicators of ecosystem health and management with the final goal to use all the knowledge acquired to develop local agriculture management programs for a more sustainable use of arid and semiarid soils in ecosystems with similar characteristics.

Acknowledgements

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The effect of landscape slope on soil organic carbon in domažlice district in the Czech Republic

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Abstract summary

The largest global stock of organic carbon (C) on land is contained in soils and is about twice as large as the atmospheric C stock. Soil properties such as soil organic carbon are used to indicate soil ability to store the nutrients needed for plant growth. The distribution of SOC is influenced by topography and land-use. In this study, we analysed the relationship between SOC and slope in topsoils of the Domažlice region of the Czech Republic. Topsoil samples were randomly collected in Domažlice (n = 67). The relationships of SOC and slope was positive though relatively weak ($R^2 = 0.19$, $p \leq 0.05$). Since the results were very weak, we divided the region's data by land use (arable land, complex systems of cultures and parcels and forests). For arable land, the relationship of SOC and slope showed $R^2 = 0.008$, for complex systems $R^2 = 0.386$, and for forests $R^2 = 0.083$. The results showed that separating the data observed had a positive effect on increased R^2 results in the relationship between SOC and slope. It indicates that the processes controlling the distribution of SOC along the slopes differ between the land-use types.

Keywords: Soil organic carbon, Slope, Land-use, Topography

Introduction, scope and main objectives

About 50 percent of world agricultural land is currently under intensive farming. Soil organic carbon (SOC) is one of the most important soil components as it significantly affects and improves soil chemical and physical properties as well as soil biological activities, biodiversity, and processes. However, the SOC pool can be influenced by natural processes, such as erosion, as well as anthropogenic activities. In this context, rainfall intensity and slope gradient are the most important. SOC loss increases as the rainfall intensity increases. Topography, particularly the slope gradient, is the primary factor affecting the intensity and frequency of erosion and thus may impact SOC stock. Several studies have indicated that erosion and its intensity and frequency increases with increasing the slope as well as flow velocity (Brodowski *et al.*, 2006; Getu, 2012; Reyna-Bowen *et al.*, 2019). However, the relationship between landscape slope and SOC and the relation of SOC in the context of topography, particularly landscape slope has not been well understood. Erosion pattern and original SOC content are two main factors by which different soils can influence SOC loss (Tisdall and Oades, 1982, Muktar *et al.*, 2018; and Yang, 2015.) The purpose of this study was to determine the relationship between SOC and slope in forest and agricultural areas in Domažlice region of the Czech Republic.

Methodology

The study areas are located at Domažlice district, in the south-west of the Czech Republic, respectively. First, the whole datasets for each district were analysed. Then the data were divided into two land-use categories, namely arable lands, and forests were analysed separately. The results of topsoil samples in both districts were analysed. The Linear regression coefficients were calculated to describe the relationships between slope and SOC stock in R and SPSS software. To measure SOC, soil samples were air-dried. The dried samples were then sieved to <2 mm. Subsequently, the SOC of each sample was determined through the oxidimetric modified Tyurin method. Slope data were calculated for the sampling sites using DEM. The SPSS software was also used to determine the correlation matrix between the selected variables.

Description of the study areas and sample collection

The study areas are located at Domažlice (1123 Km2) districts, in the west of the Czech Republic. Domažlice region is mainly agricultural land (53,0 percent) with forest areas (38,2 percent). The elevation of sampling locations ranged between 383 to 691 m for the Domažlice region.

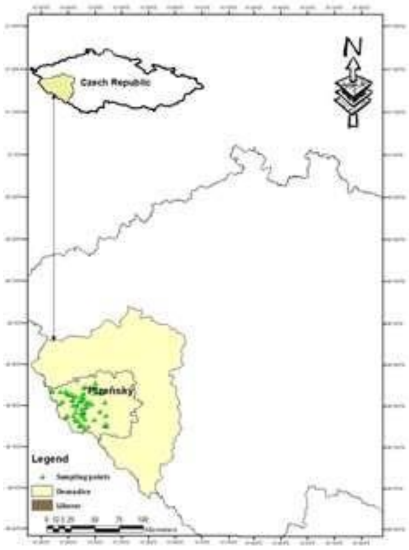
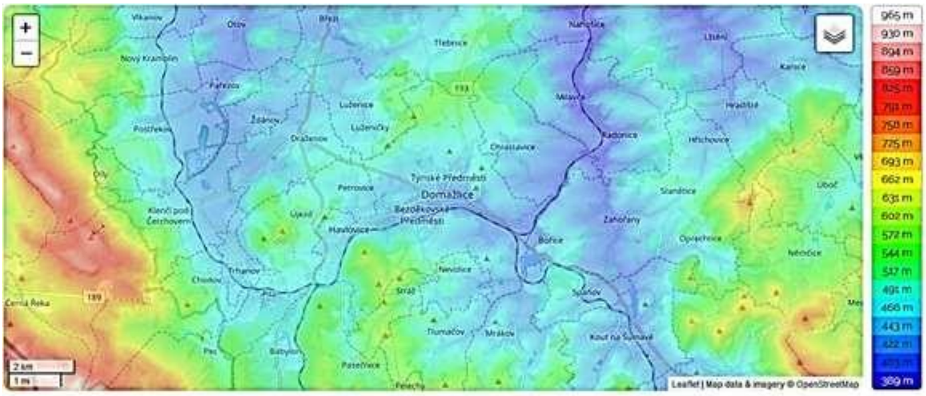


Figure 1: Location of Domažlice districts in the Czech Republic and locations of sampling



Domažlice, okres Domažlice, Plzeňský kraj, Southwest, 34401, Czechia (49.44071 12.92983)

Figure 2: Domažlice district topographic and elevations map

Separation of data and model analysis

Samples were randomly collected from the study areas (Domažlice: 67 samples) (Figure1). The soils were sampled to the depth of 30 cm using a steel soil auger after removing plant debris (e.g. grasses, twigs, etc.) at each sampling location. The collected soil samples were stored in plastic bags.

Laboratory analyses

To measure SOC, soil samples were air-dried at 105°C in the oven. The dried samples were then sieved to <2 mm. Subsequently, the SOC of each sample was determined through the oxidimetric modified Tyurin method (Beretta *et al.,* 2014; Eaton *et al.,* 2008; Francaviglia *et al.,* 2019).

Statistical analysis

Statistical difference between calculated averages was evaluated by one-way ANOVA. After that, the statistical analyses of the data were performed using the R (R-3.5.1) and SPSS (version 11). The SPSS software package was also used to determine the correlation matrix between the selected variables (SOC, slope). Linear regression analysis was used to test the correlations between slope and SOC.

Results

The correlation matrices were performed to determine various relationships between selected variables (e.g. SOC, slope). The relationships of SOC and slope were both positive though relatively weak for Domažlice (R^2 = 0.21, $p \leq 0.05$). Since the results of the relationship between SOC and slope were very weak, we divided the region's data by land-use. The arable land relationship of SOC and slope in the Domažlice district was positive but very weak (R^2 = 0.008 for 35 samples), as the complex systems it was stronger than others (R^2 =

0.386 for 12 samples), and the forests it was very weak (R^2 = 0.083 for 20 samples) (Figure 3,4,5 and6). The data separation increased correlations between SOC and slope. The results could also be used to indicate the effect of land use on SOC. This study found that there was a positive correlation between slope and SOC with (0.444 percent; P<0.01) for Domažlice Region.

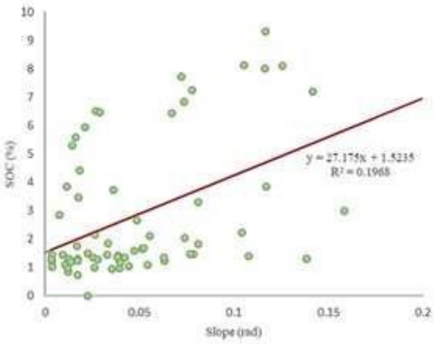


Figure 3: Linear regression of the relationship between slope and SOC content in Domažlice district

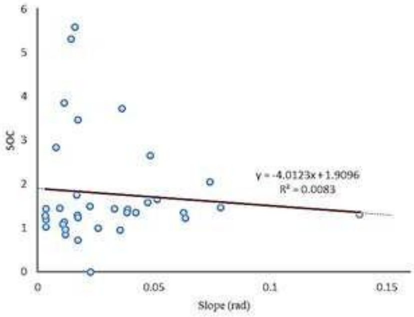


Figure 4: Linear regression of the relationship between slope and SOC in arable lands in Domažlice district

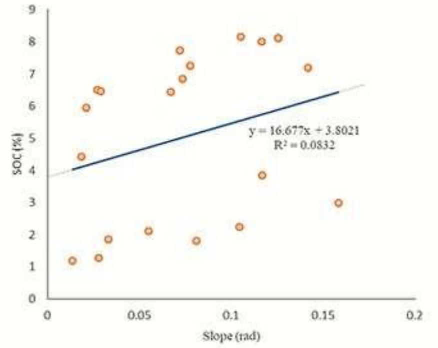


Figure 5: Linear regression of the relationship between slope and SOC in forest areas in Domažlice district

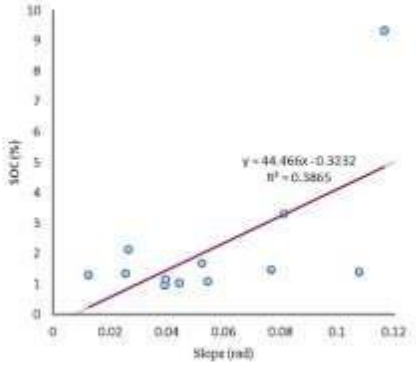


Figure 6. Linear regression of the relationship between slope and SOC in complex systems in Domažlice district

Discussion

The studied district showed a positive and weak linear relationship between SOC and slope. Thus, this study suggested that slope can somehow be used as a general predictor of SOC and increasing slope may decrease soil SOC. The separating of the data observed had a positive effect and increased R2 results in the estimation of the relationship between SOC and slope. Also, results show how much land-use affected the relationship between SOC and slope. Furthermore, aspects slope affects the distribution of SOC by changing the amount of rainfall on a larger scale.

Conclusions

In summary, the relationship between soil organic carbon and slope in the Domažlice district in our study showed a positive but very weak correlation in different land-uses (arable, forests). However, the relationship between SOC and slope was stronger and had a positive correlation. There is some thought about the weak linear relationship between SOC and slope in the Domažlice district. These results meaningfully indicate that with slope increase the SOC will decrease and vice versa. Moreover, the upper position will result in more erosion thus less C content while low (foot) position will have greater sedimentation resulting in increased C content. Also, results show how much land-use affected the relationship between SOC and slope.

Acknowledgements

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Mapping soil biodiversity research: a network analysis approach

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Abstract summary

Soil biodiversity research is attracting an increasing attention on the scientific community due to its relationships with soil erosion, soil desertification, soil productivity and in general to all natural and anthropic processes triggering a changing environment.

The study aims to perform a bibliometric analysis on the soil biodiversity research field to identify how it was structured in terms of the most occurring research terms and their citation impact. A set of scientific papers related to soil biodiversity (n= 825) was retrieved via Elsevier’s Scopus database over the period 1983–2020 using specific keywords. The search was carried out in January 2020. Our co-occurrences term analysis grouped five clusters related to (i) soil paedogenesis diversity (blue cluster), (ii) soil biodiversity related to agricultural productivity (red cluster), (iii) the effect of nutrients and fertilization on soil biodiversity (fuchsia cluster) and (iv and v) relationships between soil micro- and macro-fauna community and soil biodiversity (green and yellow clusters). The co-citation analysis revealed several highly cited research terms across the five clusters, where is noteworthy to mention terms such as “global change”, “terrestrial ecosystem”, “emission” and “food security”. This bibliometric analysis provides an overall picture of the soil biodiversity research field.

Keywords: soil biodiversity clustering, soil science, soil diversity, science mapping, co-occurrence term map, co-citation term map

Introduction, scope and main objectives

Biodiversity is the term given to the variety of life on Earth and the natural patterns it forms. Current biodiversity is the result of the evolution of natural processes and, increasingly, of anthropogenic influence.

"Biological diversity" (CBD, 1992) means the variability between living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and ecological complexes of which they are part: this includes diversity in internal species, between species and ecosystems, for example, deserts, forests, wetlands, mountains, lakes, rivers and agricultural landscapes.

Biodiversity also includes genetic differences within each species, for example, between crop varieties and livestock breeds. Indeed, chromosomes, genes and DNA determine the uniqueness of each individual and each species. In every ecosystem, living creatures, including humans, form a community, interacting with each other and with the air, water and soil around them.

It is the combination, the biodiversity, of life forms and their interactions with each other and with the rest of the environment that has made Earth "our home".

From a functional viewpoint, soil biodiversity can be considered as a dynamic entity. Soil is considered a living entity because it accommodates enormous amounts of microorganisms.

Soil microbes make our planet habitable, it is crucial to better understand how abiotic and biotic factors interact to assemble microbiomes under the given environmental conditions, and how they modulate the intrinsic link between microbial diversity and ecosystem function (Jia, Myrold and Conrad, 2020).

The protection of biodiversity is of strategic importance both internationally and nationally.

Safeguarding biodiversity is fundamental to maintaining food security and the functionality of ecosystems.

The bibliometric approach has already been applied to different research fields such as climate change (Li, Wang and Ho, 2011), geostatistics (Hengl, Minasny and Gould, 2009) and solid waste research (Mesdaghinia *et al.,* 2015).

In recent years bibliometrics has been used in concert with terms analysis (i.e., words) that appear in the title, abstracts and keywords of published papers. Such approach, termed bibliometric mapping or science mapping, has been widely used to acquire a deeper understanding of the research structure itself. For example, it has been applied successfully to different research fields such as chemistry (Boyack, Börner and Klavans, 2008), Mediterranean forests, carbon isotopes (Di Matteo, Nardi and Fabbio, 2016) or climate change (Haunschild, Bornmann and Marx, 2016).

The study aims to perform a bibliometric analysis of soil biodiversity. Science mapping applied at the biodiversity, offers the possibility of obtaining insights into the wide and complex amount of information contained in bibliographic databases, thus, allowing researchers to detect and visualize the main research topics investigated.

Methodology

A cluster can be understood as a research theme in which one or more research topics can be identified. Hereafter, in this paper, we will refer to maps displaying clusters as term maps.

We retrieved a total of 825 papers from Scopus database from 1983 to January 2020 by using specific keywords: soil biodiversity and soil diversity, in the title, abstract and keywords scientific paper.

We used the VOS (Visualization Of Similarity) viewer software to visualize the entire dataset as a network analysis based on the co-occurrences' of terms and co-citations. The idea of VOS mapping technique is that of minimizing a weighted sum of squared Euclidean distances between all pairs of items through an optimization process.

Co-occurrences mean that terms that frequently co-occur within the same papers are located close to each other in a term map.

Co-citation focus on citations meaning how often and by whom a publication was cited as well as which publications are cited together.

Each term is represented by a circle, where its diameter and the size of its label indicate the number of publications that have the corresponding term in their title or abstract. Additionally, using the VOS clustering technique, the software identifies clusters of related terms.

Therefore, the grouping of terms in a term map provides an overview of the main topics in a field. We should note that in term maps colours are used to identify clusters of related terms, while in the citation maps the colours indicate the average citation impact of publications in which the term occurs. More detailed methodological indications are reported in Nardi *et al.* (2016).

Nevertheless, there are some limitations in the term map analysis regarding both the production and the exploration of results. The interpretation of a bibliometric map is not always straightforward. There are mainly two kinds of limitations with the use of such a technique: those generated by the data and those imposed by the map. During the creation of the bibliometric dataset, the record's availability could be limited, and those data could contain a certain amount of noise (mainly due to the arbitrary keywords' choice for the primary search). Another kind of problem that could arise is represented by the synonyms and homonyms (although this is normally solved with an accurate thesaurus realization) (Pallottino *et al.,* 2018).

Results and discussion

Our co-occurrences term map well-defined soil biodiversity research topics. Five clusters have been defined according to the colours: blue, red, fuchsia, green and yellow.

In particular, the blue cluster is characterized by soil paedogenesis diversity. The red cluster is related to the theme of agricultural productivity, while the fuchsia cluster is related to agricultural management practices the effect of nutrients and fertilization on soil biodiversity. The green and yellow clusters are interconnected and essentially explore relationships between the animal communities and the microorganisms (bacteria and nematodes) and soil biodiversity (Figure 1).

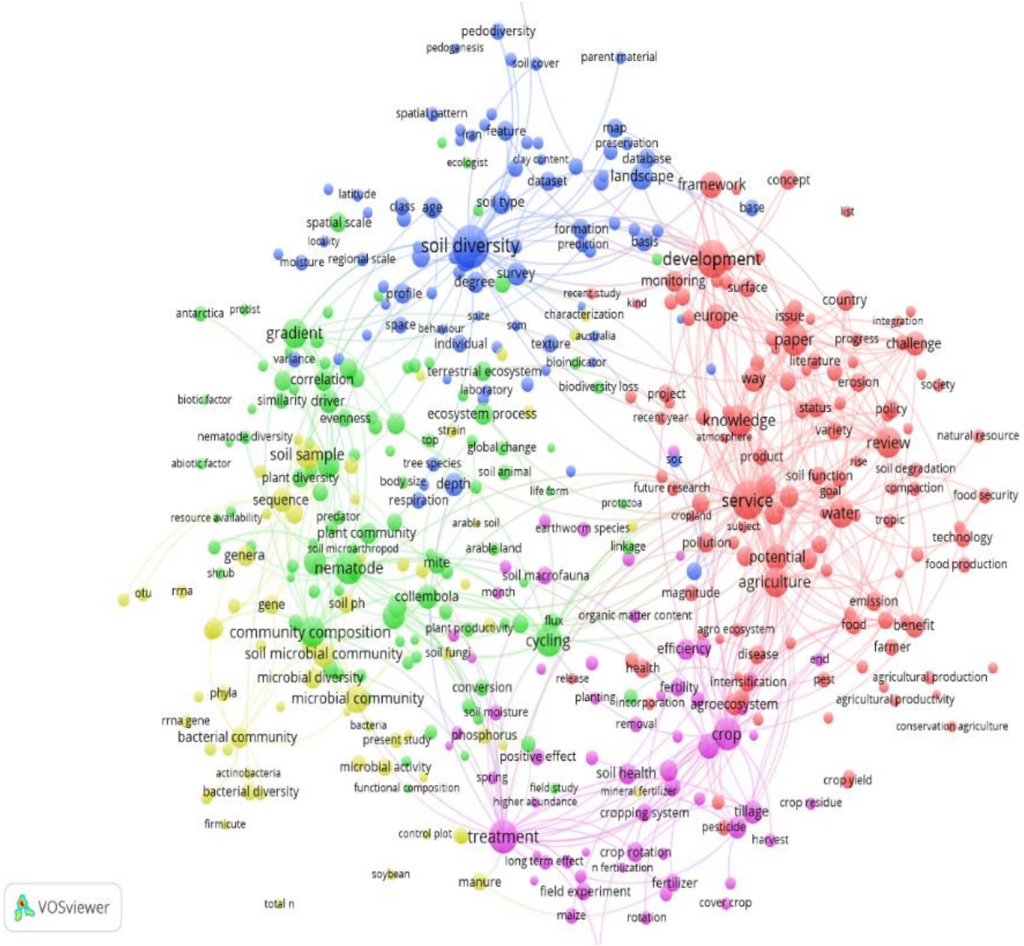

Figure 1: Co-occurrences term map based on world soil biodiversity publications from the time slice 1983–January 2020

Figure 2 expresses the impact of topic soil biodiversity and shows that the most citations in soil biodiversity subject are related.

The most cited red terms are equally distributed in the five clusters: biodiversity, global change, terrestrial ecosystem, progress, food security, conservative agriculture, productivity, fertilization, plant diversity, protist, availability of resources, etc.

The terms mentioned to a lesser extent are blue.

This bibliometric analysis has provided an overview of soil biodiversity over the past 40 years.

Bibliometric results on soil biodiversity indicate in particular that ecological systems and agricultural practices are important for reducing the vulnerability of systems and ensuring diversity.

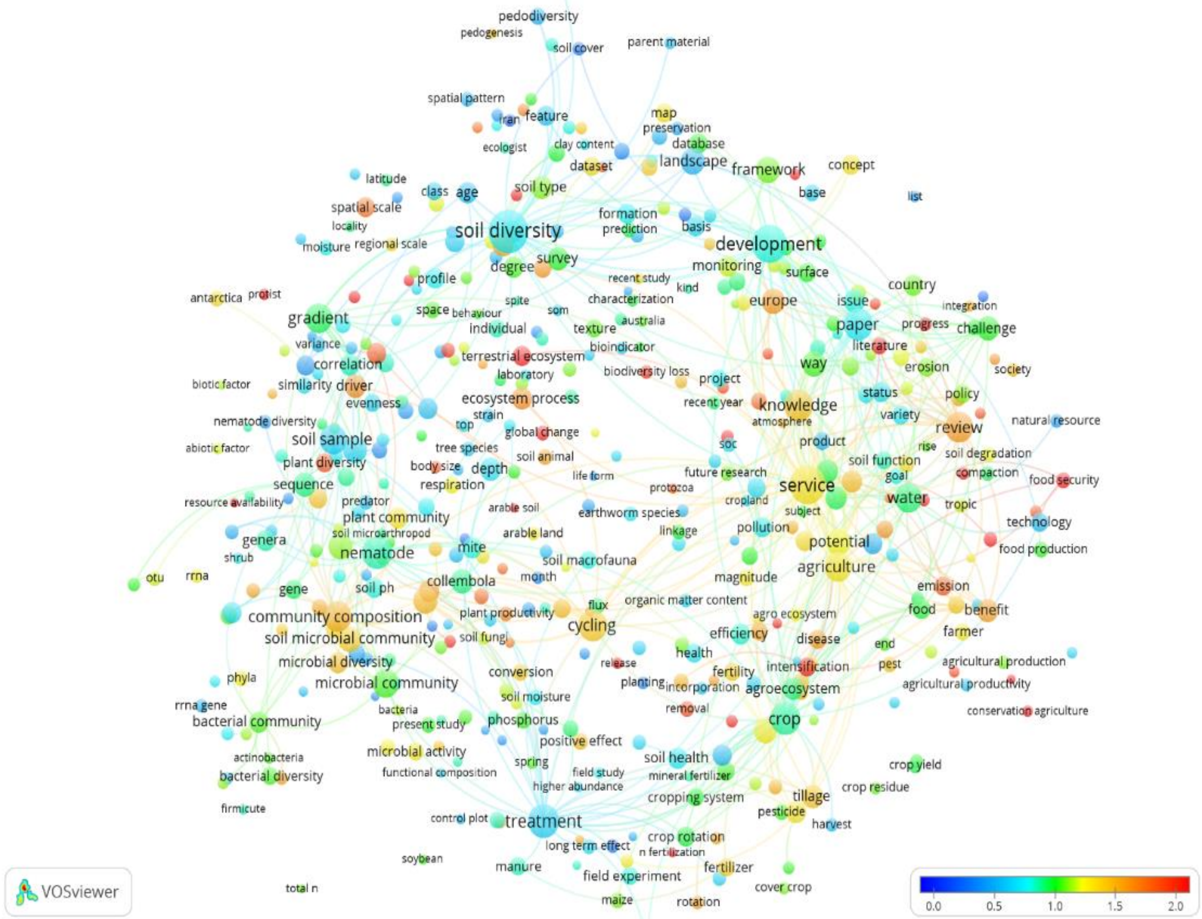

Figure 24: Co-citation term map based on world soil biodiversity publications from the time slice 1983–January 2020

Conclusions

Soil biodiversity research attracts growing interest from the scientific and political community also because of its relationship with many economic sectors that interact and depend on soil biodiversity in various ways.

All terrestrial ecosystems play an important role in supporting soil biodiversity and improving the sustainable use of its resources.

From our exploratory research, a great deal of information emerges in different areas of biodiversity that deserve to be deepened with this method of analysis.

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Regard and protect ground-nesting bees as part of soil biodiversity

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Abstract summary

Ground-nesting bees are not regarded as part of soil biodiversity. Currently, concepts (FAO and EU) include only species contributing to soil quality directly. Microbiota, earth worms, termites, millipedes, moles etc. are regarded as essential species for soil biodiversity. However, the ecosystem service pollination contributes highly to soil biodiversity and health, to food and biodiversity above ground. As 87 percent of all flowering plants depend on pollinators, all ecosystem services provided by these 87 percent of flowering plants depend on pollinators to a high extent and are at risk in case of pollinator decline, e.g. erosion control and nitrogen fixation. Pollinator species can replace each other only to limited extent. 60-70 percent of all wild bee species nest in the ground and spend a long period of their life in soil. The females of some wild species dig hundreds of cavities to lay their eggs, others mine deep and multibranched cavities. Accumulated chemicals in soil cause high risk for these female bees. Tillage and heavy machinery endanger the nests. Soil is an important habitat for the regeneration of ground-nesting bees contributing to soil quality in many aspects. These are two important arguments to include ground-nesting bees in the concept and protection of soil biodiversity.

Keywords: Bumblebees, Cavities, Chemicals, Pollinator-dependent flowering plants, Pollinator-Loss Syndrome, Solitary bees, Tillage

Introduction, scope and main objectives

We reviewed definitions of soil biodiversity and related concepts to conserve soil biodiversity. Ground-nesting bees are currently not regarded as part of soil biodiversity. CBD defines soil biodiversity as "the variation in soil life, from genes to communities, and the ecological complexes of which they are part, that is from soil micro-habitats to landscapes". This definition allows recognition of ground-nesting bees as part of soil biodiversity. However, e.g. the European Community (European Soil Data Centre, n.d.) and the FAO Voluntary Guidelines for Sustainable Soil Management (FAO, 2017) focus on ecosystem services provided by soil biota and list four: driving nutrient cycling and regulation of water flow and storage, regulation of soil and sediment movement and biological regulation of other biota (including pests and diseases), soil structure maintenance and detoxification of xenobiotics and pollutants and regulation of atmospheric composition. The wide and habitat-related definition of CBD is narrowed towards services enhancing soil quality, a functional definition. Therefore, only microbiota, earth worms, termites, millipedes, moles etc. are regarded as essential species for soil biodiversity. Ground-nesting bees are not even mentioned.

However, two aspects make it desirable to include ground-nesting bees in soil biodiversity.

Firstly, soil is an important habitat for ground-nesting bees. Bees are the most important group of pollinators (IPBES, 2016) and wild bees provide most of the pollination services (Nabhan and Buchman 1997). About 20.000 bee species are identified globally, more than 90 percent live solitary. Solitary bees work in a smaller radius than honeybees, in an area of 250-600m (Gathmann and Tscharntke, 2002), they fully depend on the quality of these small habitats for all their needs. Overall 60-70 percent of wild bees nest belowground (Cope *et al.*, 2019). Cultivated areas usually lack aboveground nesting structures used by cavity nesting bees such as trees, shrubs, stems and dead wood. In agricultural lands, therefore ground-nesting bees are more abundant. All species from families Andrenidae and Melittidae are ground nesting bees, also most species from families Halictidae, Colletidae and Apidae (including bumblebees) nest in soil. They are essential pollinators of many wild plant species and of pollinator-dependent crops such as fruit- or nut-producing trees (apple, cherry, almond), cucurbits (zucchini, melon, pumpkin), berries (cranberry, blueberry), oil seeds (canola, sunflower, mustard), aromatic and medicinal plants (basil, thyme, coriander, mint, sage etc.), legumes (faba beans) and forage plants (alfalfa, clover, vetch) (Klein *et al.*, 2007). Ground nesting bees are heavily impacted by agricultural practices. They usually stay in the ground almost a full year, emerging for only 1 or 2 months to reproduce, pollinate and lay eggs. The females of some wild bees dig hundreds of cavities in a small area to lay their eggs, e.g. mining bees of genera *Dasypoda* (Melittidae); *Anthophora* (Apidae), *Andrena* (Andrenidae) dig deep and multibranched cavities. Bumblebees even build colonies of several hundreds of bees in the soil. Tillage and heavy machinery cause risks for these nests. Recently, Goulson (2014) and Willis Chan *et al.* (2019) showed that the accumulation of chemicals in soil can be even lethal for the females digging their nests. 94 percent of the chemical load of neonicotinoids accumulates in soil and water (Goulson, 2014).

Secondly, ground-nesting bees – and other pollinators – highly contribute to soil biodiversity, soil health, food and biodiversity above ground. As 87 percent of all flowering plants depend on pollinators (Ollerton, Winfree and Tarrant, 2011), all ecosystem services provided by these 87 percent of flowering plants depend on pollinators to a partly high extent (Christmann, 2019). Substantially reduced diversity of vegetation influences soil biodiversity – even more, if the abundance of vegetation is additionally reduced. Specific soil organisms depend e.g. on specific roots and the micro-environment these plants create, on a specific spectrum of moisture etc. Pollinator-loss induced loss of flowering plants causes threats for soil biodiversity.

The impacts of pollinator loss can go far beyond (Christmann, 2019; Klein *et al.*, 2018; IPBES, 2016). Pollinator-loss induced simultaneous decrease of all ecosystem services in a certain region can cause interlinked spirals of environmental degradation and, in consequence interlinked poverty spirals (Christmann, 2019). There is no research on potential human reaction to such multifaceted degradation, we do not know, if individuals or groups will react counterproductive or adaptive, collaborative or focused on one's own group and short-term benefits. This increases the risk that such creeping coupled environmental-human degradation spirals can finally turn into a Pollinator-Loss Syndrome (Christmann, 2019).

Methodology

In this opinion piece, we raise two main arguments why ground-nesting bees should be explicitly included in concepts of soil biodiversity and respective protection guidelines and programs: (1) Soil is an essential habitat for ground-nesting bees, (2) ground-nesting bees highly contribute to soil biodiversity as well.

As this is an opinion piece based on literature review, it consists mainly of introduction and conclusion.

Conclusions

It is crucial for the conservation of biodiversity and ecosystem services to sustain ground-nesting bees. It would be advantageous to refer more to the CBD definition of soil biodiversity and to include ground-nesting bees explicitly in concepts dedicated to conservation of soil biodiversity.

Acknowledgements

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First evaluation in south America of the “soil biological quality index QBS-ar”: a pilot study in the Bolivian amazon

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Abstract summary

The QBS-ar index assigns ecomorphological indices to the presence of each main group among the soil microarthropod mesofauna that possess certain morphological characteristics associated with a greater or lesser adaptation to life. In the present study, the first evaluation of this index is carried out for Bolivia and possibly for South America, on Amazon forest soils in 3 rural communities associated with the Amazon nut (*Bertholletia excelsa*) in the transition season (end of the rainy season).

The results, after reviewing fifteen thousand individuals of different groups of microartrhropods, show high QSB-ar index values in slightly altered primary forest soils compared to lower values of the index in neighboring soils that are highly altered by anthropogenic action. Furthermore, there are quantitative differences between index values depending on whether Winkler extractors (higher values) or Berlese funnels (lower values) are used. Although very little is known about the biology of the South American mesofauna, the QSB-ar index could be a low-cost alternative for the evaluation and monitoring of the biological quality of soils in South America, implemented with relative speed, since the ecomorphs are relatively similar between soils of different continents such as Europe, where the index has already been very well calibrated.

Keywords: Mesofauna, Bolivia, neotropical soils, microarthropods

Introduction

Soil biodiversity is variation in soil life, from genes to communities, and variation in soil habitats, from microaggregated to entire landscapes (Turbé *et al.*, 2010). The food webs in which soil organisms participate, play a key role not only in the provision of soil environmental functions, in the form of food productivity, but also in the conservation of diversity below and above the soil surface. Thus, the soil ecosystems host a large part of the world's biodiversity, organized in networks highly complex. Soils and their biological activity also have an impact on the balance climate, since the soil is the second carbon stock at the global level (Orgiazzi *et al.*, 2016).

By far the most abundant group of organisms are soil microbes (bacteria and fungi decomposers of soil organic matter). Soil fauna is formed, for example, by herbivorous nematodes, which feed on roots, and protozoa (amoebae, flagellates, ciliates), which, in turn, they feed on microbes, nematodes (bacterivores, fungivores, omnivores and predators), mites (bacterivores, fungivores, predators), collembola (fungivores and predators), enchytreids and earthworms. Together, these organisms form the food webs of the soil that can be seen as the engine of soil ecosystem processes, such as sequestration of carbon (C) and the nutrient cycle. Also, given the dimensions of the materials they process, soil food webs are considered responsible for substantial components in the cycle global of materials, energy and nutrients (Jeffery *et al.,* 2010). Some authors (Turbé *et al.,* 2010; Orgiazzi *et al.*, 2016) have also classified the organisms soil in 3 functional categories representing different functions, body sizes and densities in soil: Soil chemical engineers, Biological regulators and Ecosystem bioengineers.

(Orgiazzi *et al.*, 2016) have also separated organisms from the soil exclusively by body size. In the classification by body size, the soil mesofauna mainly comprises, organisms whose body size is between 0.1 mm to 2 mm wide.

There are different proposals to evaluate the biological quality of the soil, each one with different knowledge and instrumentation requirements and therefore, with greater or lesser complexity. Some methods combine different approaches (ex. microbial activity or diversity of some groups) or require knowledge of the diversity of organisms at relatively advanced levels (species).

An alternative method is based on evaluating a part of the mesofauna, such as soil microarthropods. The use of soil microarthropods for soil quality evaluation has been proposed using the BSQ-ar index (Biological Soil Qual-arthropods) (Gardi, Menta and Parisi, 2002). In this work we evaluate the QBS-ar index for soils of the Bolivian Amazon, as an example of the adaptation of this index for South America.

Methodology

During the month of March 2019, 3 small rural peasant communities of the department of Pando in the Amazon region of northern Bolivia were visited (Santacrucito, Sinai and El Chorro). In each community, within the forest area where the fruits of the Amazon nut (*Bertholletia excelsa*) are collected, a 350-meter transect was established to obtain soil samples. Winkler extractors and Berlesse funnels were used in the transects. For the Winkler extractors, a sample was obtained every 35 meters of the transect. All the elements that did not correspond to the soil in 1 square meter of surface were removed and the first 5 cm of depth of this soil from that surface was collected on a sieve with a 1 cm mesh. The sieve was subsequently shaken and the soil passing through the sieve was placed in a Winkler extractor. In the case of the Berlese funnels, 7 samples were taken every 35 meters in the transect, removing all the elements that did not correspond to the soil in a 10 x 10 cm surface and, a 5 cm deep sample was placed in a berlese funnel.

For the Berlese funnels, a transect was carried out in the nut collection area and, a transect in the area close to the community (deforested). The samples were left for 2 weeks for the extraction of the microarthropods. Subsequently, the arthropods obtained were counted and classified in each of the large categories for the BSQ-ar index (Figure 1).

The BSQ-ar index assigns ecomorphological indices or "EMI" through the presence of "ecomorphs" within each main group among soil microarthropods that possess certain morphological characteristics (Figure 1) (Gardi, Menta and Parisi, 2002; Menta *et al.,* 2018). Some groups, such as Collembola or Coleoptera, have several ecomorphs and, they also represent different rating values for the EMI. The BSQ-ar index is the sum of the EMI of all ecomorphs in a soil sample and is a measure of the biological quality of that soil (Menta *et al.,* 2018).

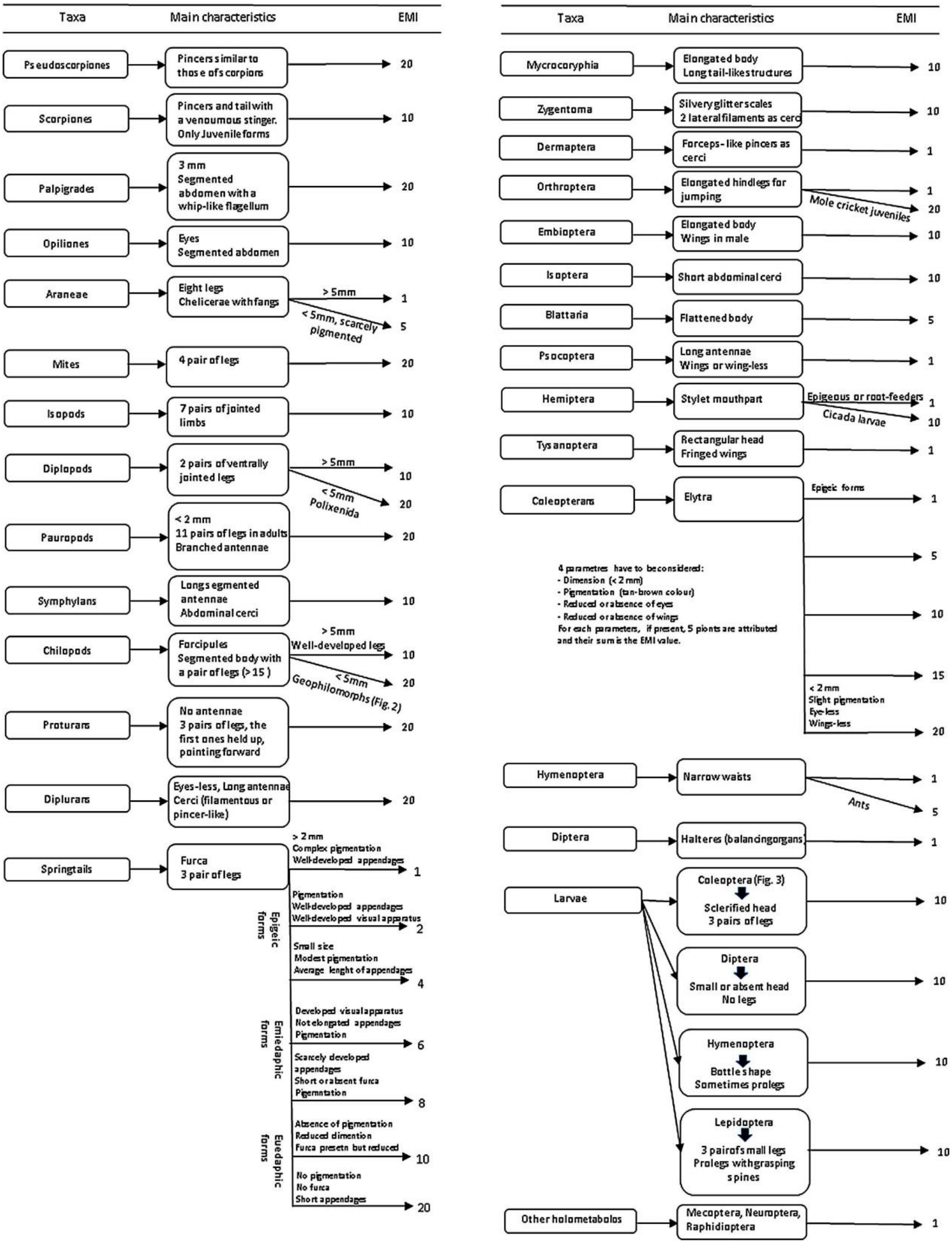

Figure 1: EMI values for the QBS-ar computation (from Menta *et al.*, 2018)

EMI=Ecomorphological Index

Results

By means of Berlese funnels, 5967 individuals have been collected while in the Winkler extractors, 9109 individuals have been obtained (total 15076 individuals). The following groups have not been considered for the analysis because they are not part of the calculation of the BSQ-ar index: (Annelida, Mollusca, Isoptera, Nematoda, Scorpiones, Hemiptera (nymphs other than Cicadidae), Thysanoptera (nymphs), and Blatodea (nymphs and oothecas). Therefore, the final number of samples used for the calculation of the BSQ-ar index was 12785, distributed in different ecomorphs of each taxonomic group (Table 1).

The QBS-ar index for the Berlese funnels that, in general, showed that the deforested areas have lower values when compared to the forest areas in each of the 3 communities (Table 1).

Table 1: Values of Ecomorphological index (EMI) of each group of microarthropods in Berlese funnel

Group	EMI value					
	Berlese in deforested area			Berlese in forest		
	Santacrucito	Sinaí	Chorro	Santacrucito	Sinaí	Chorro
Protura						
Diplura	20	20		20		
Collembola		1	8			6
Microcoryphia						
Zygentoma						
Dermaptera						
Orthoptera		1	1	1	1	
Embioptera						
Blattaria			5			5
Psocoptera		1	1			1
Hemiptera	1	1	1	1	1	1
Thysanoptera				1		
Coleoptera	9	6	14	14	20	10
Hymenoptera	5	5	5	5	5	5
Diptera (larva)				10	10	10
Other holometabolous insects (larvas)	10	10	10	10	10	10
Other holometabolous insects (adults)	1	1	1	1	1	1
Acari	20		20	20	20	20
Araneae				5	5	5
Opiliones						
Palpigradi						
Pseudoscorpiones						20
Isopoda					10	
Chilopoda					5	
Diplopoda	10				10	10
Pauropoda						
Symphyla						
SBQ-Ar index	76	46	66	88	98	104

For the Winkler extractors, the values of the QBS-ar index for the forest areas were 141, 167 and 147 for the Santacrucito, Sinai and Chorro communities respectively.

Discussion

The comparative analysis by means of Berlese funnels, between the samples of forest soils with respect to samples obtained from deforested areas, in all cases, indicate that the samples of forest soils have had higher QBS-ar index values when compared to samples of deforested soils.

There are many proposals for the evaluation of the biological quality of the soil. Many of them have originated from proposals to make assessments at the national level of the biological quality of the soil, mainly in European countries. The technologies related to most of these evaluations, or the type of knowledge related to these evaluations, are not available in Bolivia.

An advantage of the QBS-Ar index is that it does not require a detailed taxonomic classification, only using the concept of "ectomorphs. This facilitates its use by a less specific audience. Groups such as Collembola and Coleoptera, however, require further analysis for the specific determination of the EMI value according to each variant of ecomorphs in these groups. Groups such as Protura, Palpigradi, Polyxenida or Pauropoda, have not yet been registered in Bolivia, but their presence in countries bordering Bolivia, may be evidence of the possibility that these groups are in Bolivia.

In this sense, this first trial to propose an index to evaluate the biological quality of the soil that is adapted to the reality of Bolivia, is promising due to the low cost it implies. A difficulty in Bolivia is related to the ignorance of the biodiversity of the majority of soil microarthropods in Bolivia, their distribution and abundance, and the lack of specialists in soil microarthropod taxonomy. Therefore, the validation and calibration of this index is still necessary in other landscapes in Bolivia.

Ina In summary, a factor that must be developed for a massive use of the SBQ-Ar index, is to solve the taxonomic impediment related to the little development of the study of soil microarthropods in Bolivia, to promote evaluations in a national scale.

Conclusions

The QBS-ar index can be used to evaluate the biological quality of soils in South America as it is an economic methodology. However, more collections and regional and local calibrations are required, according to the change of some groups of the soil mesofauna in the neotropical soils, compared to the soils of Europe where this index is has developed and used so far.

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Theme 2.

Soil biodiversity in action

Keep soil alive, protect soil biodiversity

The issue of soil pollution solved using organic farming#2: Tried-out research of soil biodiversity

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Abstract summary

According to the research of the issue of soil pollution solved using organic farming#1, the case study from Thailand (Chongsermsirisakul and Iamurai, 2018). The work is part of the Bakery for tomorrow and ECO Bakery for Friendly Environments projects, which focus on producing safe food, achieve zero hunger and a healthy environment to support the SDG 2030. Which has focusing on the way to retain and recover the soil for rice planting from the destroyed by agrochemical's usage. The agricultural methods as organic agriculture and crop rotation by legumes planting for organic fertilizers instead.

For this tried-out research, we focusing to study the soil biodiversity, which are the contribution from this organic farming, which is essential to the production and sustainable agricultural productivity including to be the nutrition for the rice such as the soil macro-fauna biodiversity. The organic farming where used legumes plant as the crop rotation method in rice planting.

Those chemical properties of the soil include to the cation exchange of the organic and the physical properties, soil moisture and organic degradation have related with the number and type of the soil macro-fauna biodiversity. We found that the highest value index of biodiversity was the phylum arthropoda order acari family oribatidae as Mites and also: Collemboly, Springtails, Caterpillars and Ants.

Keywords: soil pollution, organic farming, soil biodiversity, safe food, zero hunger research, eco bakery, soil biodiversity fauna

Introduction

In Thailand, there is the increasing problem of soil pollution from the dangerous chemical fertilizer, pesticides usage to increase yields, which has an effect on humans, both the farmers and consumers and on the environment and affects to the soil fauna in the rice planting.

The other crops or plants such as groundnut, which has a short life cycle. They can cultivate plants that use to be the ingredients of bakery such as leguminous. The root can be ploughed and buried as the bio-fertilizer for the rice, which has become the organic matter to improve the soil biological and chemical qualities has been selected to be the organic agriculture and crop rotation method for organic fertilizers instead.

Researchers have launched this concept for testing the conducted research in the land where rice is planted for the soil fauna biodiversity which are the contribution from this organic farming, which has focused to study in this tried-out research.

The soil ecosystem consists of organisms and no organisms. The variances of soil fauna have depended on the soil environment such as nutrient, humidity, temperature, weather and soil properties, those conditions have impacted their growth and propagation increasing. (Kanchanprasert, 2006).

The macro soil fauna and meso soil fauna has the different factions for the soil such as the nutrients circulation processing to the soil: transforms the organic to inorganic matters, cooperated with the microorganisms within the soil. The soil fauna such as the Termites or Millipede (Oribatidae), has the highest of biodiversity index value and Springtails (Entomobryidae) and Ant have the higher and the lowest value is beetle (Ligiidae) for the rice in the next corps and increasing the soil more abundant.

Methodology

By random sampling was carried out at 28 sites, 14 sites of organic farming by legumes planting as the cultivation corps method (1site = 1600 Square Meters) and 14 sites (1site = 1600 Square Meters) of the chemicals farming. The total = 28 sites (22,400 Square Meters) in Pichit Province, Thailand. Biodiversity of macro-fauna in soil tests were compared between pair methods by 4 times of data collecting.

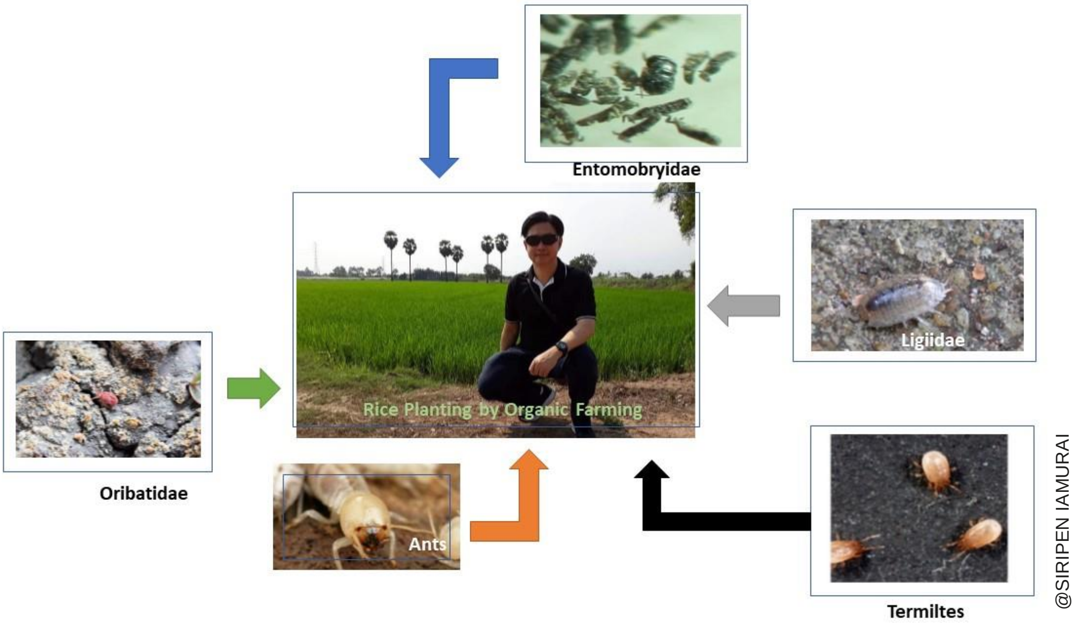

Figure 1: Soil fauna in Organic Rice Planting of the Experimental Research

Results

The results were analysed by The Biological Diversity index value of soil fauna by ShannonWiener's index of Diversity (H ')

Table 1: Types and numbers and biodiversity of soil fauna of 4th testing in organic farming in rice planting

Soil fauna (Phylum/Order/Family)	Number of soil fauna (n)#1st	Biodiversity index of soil fauna#1st	Number of soil fauna (n)#2nd	Biodiversity index of soil fauna#2nd	Number of soil fauna (n)#3rd	Biodiversity index of soil fauna#3rd	Number of soil fauna (n)#4th	Biodiversi index of soil fauna#4t
Phylum Arthropoda Order Acari Family Oribatidae	10	0.29	15	0.30	20	0.29	25	0.26
Order Araneae Family Onnopidae	4	0.17	5	0.16	10	0.2	22	0.25
Order Scolopendromopha Family Scolopendrellidae	5	0.20	7	0.20	12	0.22	16	0.21
Order Lithobiomopha Family Litibiidae	6	0.22	8	0.21	14	0.24	18	0.22

Order Isopoda Family Ligiidae	3	0.14	6	0.18	8	0.17	10	0.15
Order Chordeumatida Family Anthroleucosomatidae	10	0.29	13	0.28	15	0.25	18	0.22
Order Collembola Family Entomobryidae	4	0.17	7	0.20	9	0.18	19	0.23
Order Diptera Family Cecidomyiidae	1	0.06	2	0.08	4	0.11	8	0.13
Order Hemiptera Family Cicadidae	1	0.06	3	0.11	6	0.14	10	0.15
Order Hymenoptera Family Formicidae	6	0.22	8	0.21	10	0.2	15	0.2
Phylum Nematoda Class Phasmida	4	0.17	7	0.20	9	0.18	13	0.18
Phylum Annelida Order Crassiclitellate Family Megascolecidae	10	0.29	11	0.25	13	0.23	19	0.23
Total	64	2.31	92	2.38	130	2.41	193	2.43

The soil fauna of Oribatidae, has the highest value of biodiversity index = 0.30 and Cecidomyiidae and Cicadidae families have the lowest values of biodiversity index = 0.05 from the experimental organic farming sites. We found out that 12 fauna families of the amount 478 samples in 4 times of the organic farming-case study 14 sites and none of all in 14 sites of the chemicals farming.

The Biological Diversity index value of soil fauna by ShannonWiener's index of Diversity (H ') has analysed the plots data, it showed the biodiversity index of soil fauna is worth at 2.30 (Table1).

Table 2: The Statistical Value of Soil Biodiversity from Organic Farming analysed by SPSS

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
Organic Farming	28	1.50	0.51	0.10
Soil Biodiversity	28	0.50	0.51	0.10

One-Sample Test						
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Organic Farming	15.58	27	0.00	1.50	1.30	1.70
Soil Biodiversity	5.20	27	0.00	0.50	0.30	0.70

The data has analysed for statistical significance using t-test based on different sample pairs, with the SPSS program. The mean and SD values were taken with tolerance limits of 5 percent. All data will be evaluated based on Soil Biodiversity of macro-fauna of organic farming. α = 0.05 percent or 95 percent, Z = 0.50, and critical t <0.30 to > 0.70.

Discussion and conclusion

From studying biological diversity of fauna in the soil of rice paddy organic farming by legumes as corps cultivation. It has created the fauna in the soil, which has useful for the rice in the next corps. It’s made and transformed organic decomposition, cation exchange, effected to the soil's physical properties such as increase soil humidity for degradation rate organic objects, adjust pH values, which have related to the increasing of number and type of those fauna in the soil.

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Urban and peri-urban gardening promotes urban and soil biodiversity

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Abstract summary

Urban and peri-urban agriculture (UPA) provides a complementary strategy in order to reduce urban poverty and enhance urban environmental management. From an ecological perspective, urban and peri-urban agriculture presents many advantages and introduces new methods and strategies for sustainable production by providing effective solutions to the need to increase crop performance, food supply and to safeguard biodiversity. The present communication intends to synthesize the benefits of UPA as a nature-based solution to improve urban and soil biodiversity, and food supply as demonstrated by the preliminary results of a research conducted by CREA.

Keywords: Urban and peri-urban agriculture (UPA), Urban gardening, Sustainable soil management, Soil biodiversity

Introduction, scope and main objectives

Urbanization and sustainable agriculture are both major societal challenges. By reducing food miles and connecting people with nature, urban food cultivation has several major advantages. According to an ecological perspective, urban agriculture introduces new strategies in order to increase production sustainability and environmental biodiversity.

The need to adopt eco-friendly cultivation techniques respectful of the environmental sectors - air, water and soil - has meant that in urban gardens the demand for production factors such as energy and machine use become have less and less and have been replaced by conservation techniques. In fact, in micro-gardens soil tillage has been replaced by cultivation in pallets or raised boxes, which are not subject to compaction and, therefore, do not require frequent turning, as in conventional agriculture. On field, the cultivation on raised beds has created the need to use high quantities of humified organic matter to ensure: a) the right soil volume to accommodate the radical plant apparatuses; b) the optimal structure stability. The enrichment in organic matter in natural farming indirectly contributes to improving soil health and to increasing soil biodiversity **(**Edmondson *et al.,* 2015; Quistberg, Bichier and Philpott, 2016**)**.

The most striking feature of urban agriculture, which distinguishes it from rural agriculture, is that it is integrated into the urban economic and ecological system.

Organic and environmentally sustainable UPA should also be included in the political agenda since it is connected to multidimensional co-benefits, such as the support of the local economy, social inclusion, and biodiversity (Rusciano, Civero and Scarpato, 2017; Woods *et al.*, 2016). UPA contributes to ten key societal challenges (table 1).

There are different forms of urban agriculture: private, community and school gardens, vertical farms, rooftop gardens and so on, as shown in figure 2 (Santo, Palmer and Kim., 2016). The growing interest of residents in organic and local food offers a major opportunity to promote the implementation of UPA further (Aubry and Kebir, 2013).

Table 1. Key societal challenges and UPA related action areas (Artmann and Sartison, 2018)

N°	Challenges and Relevant Fields of Action References
1	Climate change (including climate change adaption and mitigation, carbon sequestration)
2	Food security (including food safety, food justice)
3	Biodiversity and ecosystem services
4	Agricultural intensification (including organic farming, sustainable agriculture)
5	Resource efficiency (including energy efficiency, water protection, urban water, life cycle assessment)
6	Urban renewal and regeneration (including abandoned land, brownfields, vacancy)
7	Land management (including land take, soil sealing, soil degradation, soil erosion, green space management, governance, green infrastructure)
8	Public health (including, well-being, quality of life)
9	Social cohesion (including social justice, participation, awareness, civic agriculture)
10	Economic growth (including green jobs, innovation, cost-effectiveness)

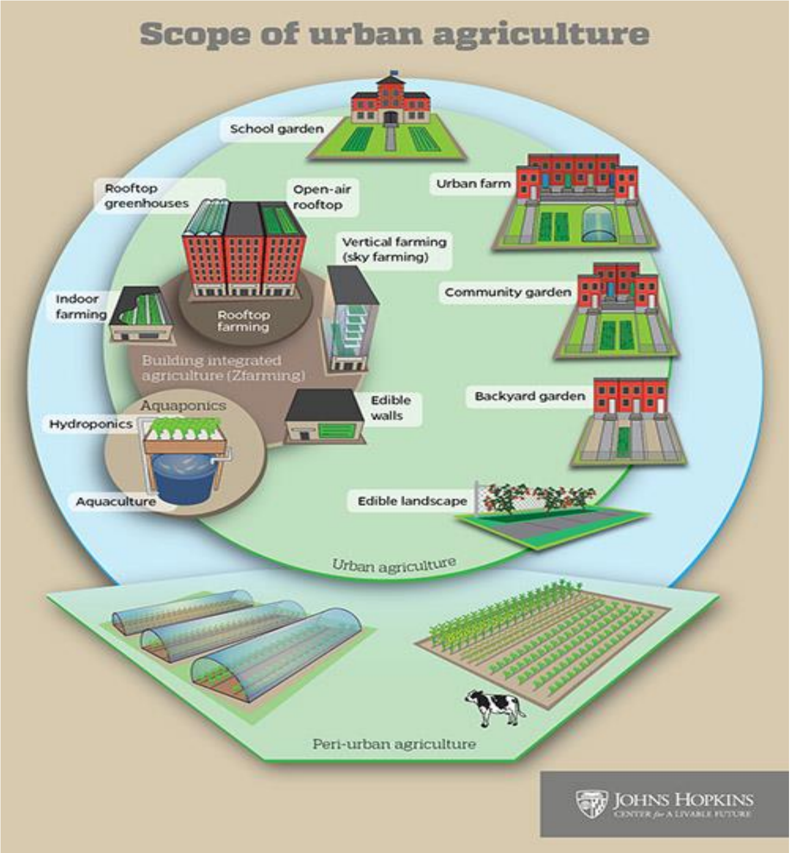

Figure 1: Urban agriculture forms (Santo, Palmer and Kim, 2016)

The management of an urban garden is inspired by the principles of agroecology and natural farming such as synergistic agriculture with the objectives of contributing to food supply, crop yield and safeguarding biodiversity.

Methodology

In 2012 CREA activated a demonstration urban garden in its experimental farm at Monterotondo, managed with natural/conservation farming, that hosts contemporarily several botanic families of vegetables (Figure 2). To increase plant diversity and, consequently, animal and microbial diversity, vegetables are consociated with beneficial plants with attractive effects on pollinators and repellent effects on pests, wich the garden's self-defence capability. The garden is also based on soil self-fertility, through leguminous species intercropping and organic mulching use (straw, shredded wood, crop residues, etc.), to create a superficial humogenic layer which guarantees both the mineralization/humification balance and the right physical, chemical and biological fertility. The garden includes perimetral hedges consisting of perennial plants (*Lamiacee* and *Asteracee*) and fruit trees which serve as windbreaks and anti-pest barriers. Stone walls accommodate insects and slime predators such as small reptiles. A pond ensures the presence of predatory amphibians and birds that feed on mites and insect larvae to increase the garden is self-defence level. In the synergistic garden, by arranging them in a completely randomized way, the following species of vegetable plants have been associated: tomato, roman lettuce, savoy cabbage, parsley, red beetroot and courgette. The same crops were planted in the organic garden, placing them in specialized rows, to compare the results of the two different farming systems (Beni *et al.*, 2018).

Figure 2: The demonstration garden of CREA, an example of a biodiverse peri-urban garden, is managed with conservation techniques and is aimed at research activities and students'/operators' training

Results

The demonstration urban garden provides research activities and students' and operators' training. Since March 2017, it has been recognized an educational-social site by the "National Rural Network" with the involvement of associations, health agencies and neighbouring municipalities.

As far as production is concerned, there is an increase in the synergistic garden compared to the organic one, between 10 and 30 percent. It is equal to 30 percent for the tomato and savoy cabbage. As for the ripening season, there is an advance of about twenty days in cabbage, parsley, lettuce and red beetroot grown in the synergistic garden with respect to the same varieties grown in the organic garden. The results on soil biodiversity are in progress.

Discussion

In cited research, the choice to use this synergistic cultivation system in urban habitat can be a valid alternative to optimize the quality of the crops and to minimize the environmental damages that in conventional agriculture prove to be quite significant (Beni *et al.*, 2018).

The synergistic cultivation method is based on the observation of what occurs in nature; one of the principles on which the method is based is soil non-tillage as soil works on its own through root penetration and the activity of microfauna, mesofauna, macro-fauna and especially of microorganisms.

This principle is connected to the "soil biodiversity" concept. Soil hides an extraordinary number of life forms, an intricate network of interactions involving, over 3000 kg ha^{-1} (Bloem *et al.*, 2003) of living biomass. Agronomic practices modifies these relationships, for example, accelerating cyclical transformations (Rossi and Beni, 2018). This greater dynamism means that agricultural land has a lower stability degree than natural soil.

A study conducted by Oxford University compared the effects of organic farm management on biodiversity versus conventional agriculture (Tuck *et al.*, 2014). The results showed that organic farms have a greater positive effect on biodiversity in the most intensely cultivated territories, providing rich habitats and the absence of synthetic pesticides. On average, organic farming increased species' richness by about 30 percent. This result has been robust over the last 30 years of published studies. The eco-sustainable management techniques adopted in UPA contribute to increasing the soil biodiversity and the natural environment, and to improving the healthier food production (FAO, 2003). The growing interest of residents in organic and local food offers a major opportunity to promote the implementation of UPA further (Aubry and Kebir, 2013).

Conclusions

The realization of an eco-sustainable agriculture is a major challenge to securing the increasing food demand, connected to rapid urbanization. UPA cannot only contribute to food supply but also to climate change mitigation, biodiversity, ecosystems services supply, sustainable agriculture implementation, etc. Thus, it is needed to approach ecosystem-based agriculture in such a way as to avoid agriculture remaining a part of the challenge responsible for ecosystem degradation.

Many studies have been conducted on UPA as value in providing social, economic and environmental co-benefits and ecosystem services, as analysed in a recent review (Artmann and Sartison, 2018) but the valuation of the effects of urban agriculture on soil biodiversity would merit further research.

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How the biological activity of Oligochaeta shape soil aggregation and influence the soil functions

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Abstract

This study aimed to investigate the effects of biological activity on the formation of soil aggregates. The laboratory experiment consisted of a 12-week soil incubation with macrofauna (Oligochaeta) and grass vegetation (*Brachiaria Decumbens* cv). Soil aggregates were separated in types according to their origin, using a stereo microscope (LEICA M125), and physical, chemical and biological analyses were performed on the different aggregates. The results show that the biological activity contributed significantly to soil structure quality and soil functions, where the biogenic aggregates are more stable, have higher nutrient and organic matter content, thus improving soil biological properties. The analysis of soil aggregate types is a good indicator of soil quality, since it encompasses the biological, physical and chemical properties.

Keywords: biogenic aggregates; soil structure, soil quality

Introduction

The importance of soil organisms is recognized in many processes and functions in soils. It regulates the organic matter accumulation, affects the biochemical weathering, and promotes soil horizons mixing and nutrient cycling. Soil structure is also enhanced by the activities of organisms. For example, the invertebrates and the root systems increase soil aggregation, which is responsible for the structural soil porosity and also enhances the organism’s activities (Lavelle *et al.*, 2006).

In the hierarchical aggregate model, aggregates are sequentially formed, i.e. microaggregates are first formed free and then serve as the building blocks for the formation of macroaggregates (Six *et al.*, 2004), which constitutes the physiogenic pathway of soil aggregate formation. On the other hand, the biogenic formation pathway describes how biological activity on soil directly promote the aggregates formation, by the activity of earthworms and plant roots (Pulleman *et al.*, 2005).

Several studies clearly indicate that physical and chemical processes influence on aggregate formation and stability in soils, but few studies investigate the biogenic pathway. This study aimed to investigate the effects of biological activity on the formation of soil aggregates using incubated soil materials. Considering the influence of roots and plants on soil structure and that earthworms are recognized as typical soil ecosystem engineers, we hypothesized that (1) biological activity contributes significantly to soil structure quality and soil functions; (2) the analysis (identification/quantification) of soil aggregate types according to their origin may be applied in establishing a biological soil quality indicator.

Methodology

The experiment consisted of a 12-week laboratory soil incubation with macrofauna (Oligochaeta) and grass vegetation (*Brachiaria Decumbens* cv). Soil samples were collected from the surface layer (0-10 cm) of an Inceptisol (Table 1). Samples were air dried, ground and sieved over a 2 mm sieve. The soil material was positioned inside plastic tubes and a number of 36 earthworms were dispersed in each cylinder, plus 8g of *Brachiaria Decumbens* cv. seeds.

Table 1: Physical and chemical characteristics of soil material

Sand	**Silt**	**Clay**	**pH**	**Ca**	**Mg**	**Na**	**K**	**SB**	**Al**	**H+Al**	**T**	**BS**	**P**	**TOC**
g.kg^{-1}			H_2O	----------------------cmol$_c$ kg^{-1} soil----------------------								%	mg.kg^{-1}	g.kg^{-1}
505	271	223	4.83	3.7	4.3	0.26	0.79	9.03	0.1	8.0	14.65	61	3	21.5

*SB: sum of bases; T: cation exchange capacity; BS: base saturation; TOC: total organic carbon.

After the incubation period, the soil aggregates were separated manually according to morphological fractions (Pulleman *et al.*, 2005), using a stereo microscope (LEICA M125). Physicogenic aggregates were identified by their angular or prismatic morphology. Biogenic aggregates were distinguished by their rounded shape, a result of root activity or passage through the intestinal tract of soil macrofauna, especially the earthworms. Intermediate aggregates were identified by in-between morphology (Figure 1).

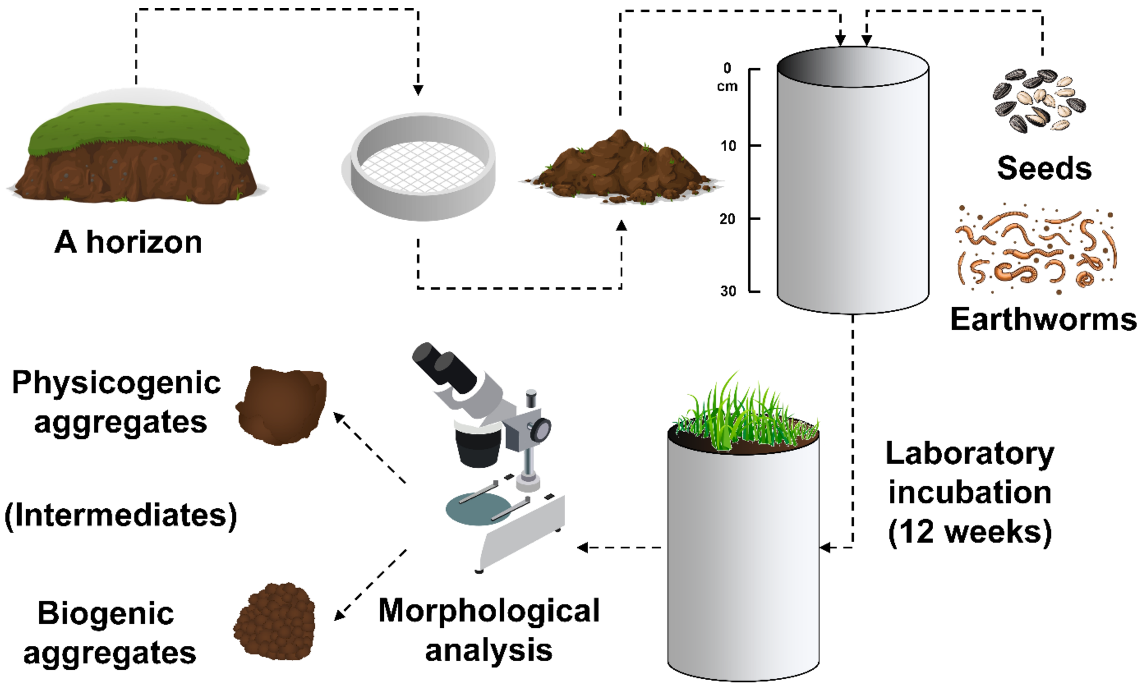

Figure 1: Graphical summary of the experiment design

The aggregates stability in water was evaluated by the aggregate indices: mean weight diameter (MWD) and geometric mean diameter (GMD). The chemical and physical analytical methods are those described by Teixeira *et al.* (2017). The biological properties analysed are the following: microbial biomass carbon (C-MB); microbial biomass nitrogen (N-MB); basal respiration (BR); metabolic quotient (qCO_2) and microbial quotient (qMIC), according to Silva *et al.* (2012).

The X-ray computed microtomography method was applied in representatives biogenic and physicogenic aggregates to measure distribution of pore size (Melo *et al.,* 2019).

Results

The percentage of aggregates ranged from 30.47 to 32.57 percent for biogenic, and from 25.70 to 26.78 percent for physicogenic types. Intermediate aggregates showed the highest percentages at all depths (41.55 to 42.66 percent). The biogenic aggregates showed the highest aggregate stability values (MWD = 3.98 to 4.06 mm and GMD = 3.56 to 3.83 mm); whereas the physiogenic aggregates were the least stable, with MWD between 3.19 and 3.32 mm, and GMD between 3.04 and 3.13 mm (Figure 2).

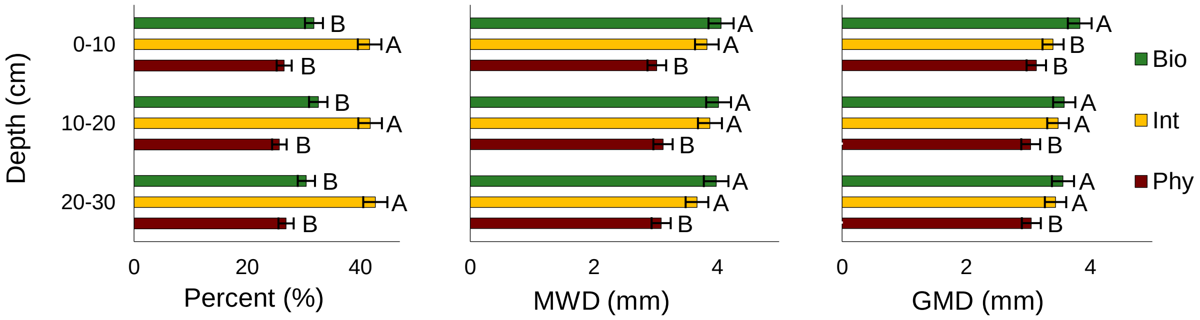

Figure 2: Mean values of soil aggregate fractions, MWD and GMD

***Averages from six replications.**

The biogenic aggregates presented highest values of basic cations (Ca, Mg, K), sum of bases (SB), cation exchange capacity (T), base saturation (BS), phosphorus (P) and total organic carbon (TOC) (Table 2) when compared to physicogenic and intermediate types.

Table 2: Chemical attributes of soil aggregates according to the origin types

pH			**Ca**			**Mg**			**Na**			**K**			**SB**		
H_2O			--- cmol$_c$.kg^{-1} soil ------- --														
Phy	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**
4,47a	4,47a	4,64a	2,7b	2,5b	3,9a	2,6b	2,6b	2,8a	0,04a	0,04a	0,05a	0,02b	0,02b	0,05a	5,41b	5,41b	6,85a
4,48a	4,43a	4,67a	2,7b	2,7b	4,0a	2,4b	2,6b	3,1a	0,03a	0,04a	0,04a	0,01b	0,01b	0,04a	5,14b	5,30b	7,18a
4,62a	4,57a	4,70a	2,9b	3,1b	4,3a	2,8b	2,7b	3,3a	0,04a	0,04a	0,05a	0,01a	0,01a	0,03a	5,65b	5,95b	6,63a
Al			**H+Al**			**T**			**BS**			**P**			**TOC**		
------------------------- cmol$_c$.kg^{-1} soil -------------------------									%			mg.kg^{-1}			g.kg^{-1}		
Phy	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**	**Phy**	**Int**	**Bio**
1,8a	1,8a	1,8a	3,3a	3,5a	3,9a	8,68b	8,86b	10,75a	62b	61b	69a	2b	4b	8a	16,65b	17,95b	28,90a
1,7a	1,7a	1,9a	3,0a	3,2a	3,3a	8,09b	8,45b	10,48a	64b	63b	72a	2b	3b	4aa	12,40b	13,30b	23,60a
0,2a	0,2a	0,3a	3,2a	3,1a	2,8a	8,85b	9,00b	9,43a	64b	66b	70a	2b	2b	2	9,25b	10,50b	21,20a

***Averages from six replications. Phy = physicogenic, Int = intermediate, and Bio = biogenic.**

When comparing biological properties, the biogenic aggregates present the highest values of C-MB, N-MB and qMIC, and the lowest values of BR and qCO_2 (Figure 3). Higher BR values were observed in the physicogenic and intermediate aggregates, and higher qCO_2 in the physicogenic aggregates.

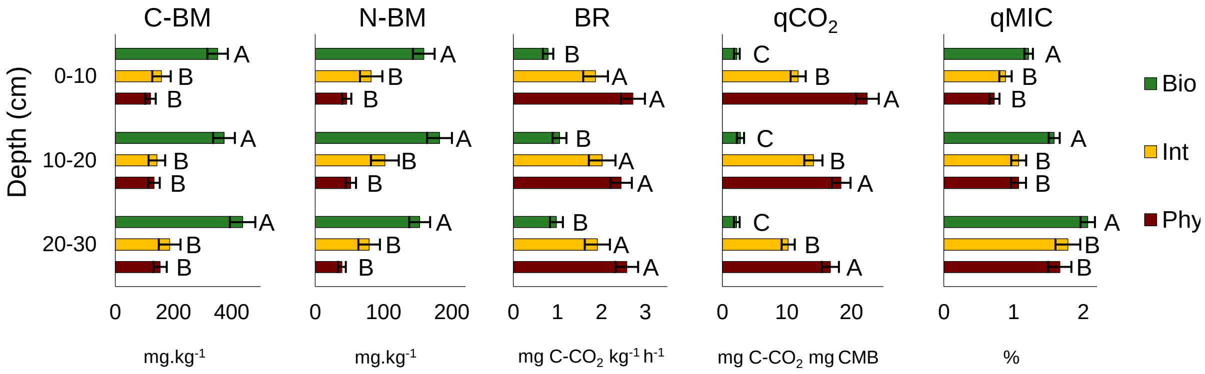

Figure 3: Mean values of soil biological properties

***Averages from six replications**

The porosity evaluation using X-ray computed microtomography showed a higher proportion of large pores in the biogenic than for the physicogenic aggregates (Figure 4).

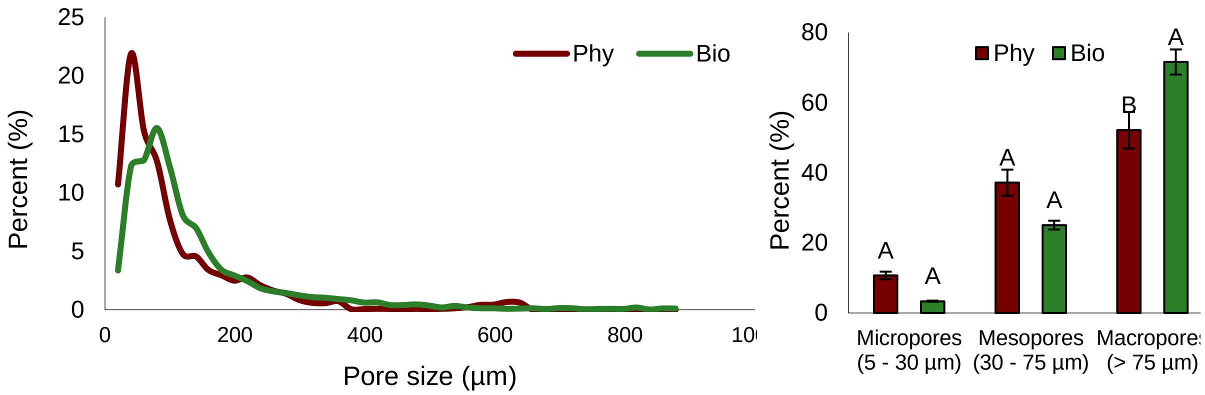

Figure 4: Distribution and mean values of soil aggregates pore sizes

***Averages from six replications**

Discussion

The biogenic formation of soil aggregates represented on average 31.61 percent of the soil aggregate mass. This shows a relevant contribution of the soil macrofauna (Oligochaeta) and plants (roots) in the aggregate formation. Earthworms move soil particles, ingesting them and forming the biogenic aggregates; they are commonly termed 'ecosystem engineers' (Blouin *et al.*, 2013). Plant roots also move particles forcing the soil particles to come into close contact with each other. Also, they contribute indirectly to soil aggregation through the exudation of polysaccharides and other organic compounds, forming sticky networks that bind together individual soil particles and tiny microaggregates into larger macroaggregates (Six *et al.*, 2004). All these different factors are responsible for binding together the small subunits and giving the higher stability of the biogenic aggregates, as observed from the values of MWD and GMD.

The higher nutrient contents in the biogenic aggregates are associated with their processes of formation. Earthworms accelerate the organic materials decomposition by increasing the available surface area of organic matter through comminution. When soil and organic materials pass through the earthworm guts, they are ground up physically as well as attacked chemically by the digestive enzymes of the earthworm and the microorganisms inside the gut. After digestion, some organic compounds are released into the environment in the form of small organic compounds and/or mineral nutrients (Blouin *et al.*, 2013).

As for the biological properties, soil structure also creates the habitat for a myriad of soil organisms, consequently driving their diversity and regulating their activity (Rabot *et al.*, 2018). The higher values of C-MB, N-MB and qMIC found in the biogenic aggregates are associated with the decomposition of organic matter by the earthworms and their effect on nutrient cycling. Models have been used to show the effects of earthworms on the primary production through increased mineralization of organic matter and thus nutrient release (Blouin *et al.,* 2013). Also, the organic matter that is complexed inside the microaggregates becomes inaccessible to the microorganisms and thus is physically more protected from losses (Six *et al.,* 2004).

Aggregate porosity also showed a different pattern between the origin types. In general, the biogenic aggregates presented a higher volume of macropores when compared to the physicogenic aggregates, a result similar to study of Melo *et al.* (2019). After passing through the earthworm gut, the soil ingested is expelled in the shape of pellets. Most biogenic aggregates are formed by joining of these units, thus creating an extensive system of larger pores. This explains the largest amount of macropores observed in the X-ray computed microtomography.

Conclusions

Biological activity undoubtedly contributed significantly to the soil structure quality and soil functions; where the biogenic soil aggregates are more stable, have higher nutrient and organic matter content, thus improving soil biological properties.

The analysis of soil aggregates, according to their origin types is a good indicator of soil quality, since it encompasses the biological, physical and chemical properties. It is a practical, affordable, repeatable, and easily understood and interpreted indicator, which may be used to evaluate if soil conditions are changing according to the adoption of sustainable soil management practices.

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Participatory learning action is important for community action to improve soil-biodiversity

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Abstract summary

Soil is the foundation for diverse form of life, livelihood of human being, earlier traditionally community practices were aligned to ethics, aesthetics, methods, and processes which were maintaining soil-building, soil-biodiversity, soil-health altogether living-soil in their age-old farming systems. Implementation of agriculture practices in bits and pieces manner within the modern agriculture have resulted in fast degradation of land and disturbing the whole eco-system of soil as home of millions of diverse creatures. This in-turn resulting in poor soil-productivity and sustainability. Considering the direct relation between soil and community, VAAGDHARA, tried to revive the age-old approach of "worshiping soil/earth as motherly goddess". The complete process to strengthen the role of farmers in revitalising soil-life, followed the approach of participatory learning and action, in which farmer groups participated in the process of learning and action, which was started with worshiping soil and finally ended with a poem, praise locally called Aarti, in which all farmers thanks the goddess soil for taking case of their families and other forms of life. This paper establishes the need for policies and programs which creates an environment which incorporate community actions as major component in the sustainable soil management.

Keywords: Living-soil, Soil Formation, Circularity, Sustainable Farming System, Indigenous-community

Introduction, scope and main objectives

Like most of the developing countries in India 68.8 percent of population lives in rural area and nearly 160 million families are farmer families (Chandramouli, 2011). The livelihoods of these rural families are directly dependant on soil conditions, which are facing large-scale threat of reducing soil-health, which is normally taken by soil-conservation measures, adding chemical-fertilizers and other inputs. The use of chemicals in farming, such as pesticides and fertilizers, can upset the delicate balance in the soil, supporting one type of organism over another, and disrupting its varied functions, such as the ability to store carbon or water.

But recent studies related to land degradation has brought out the facts that soil-life play important role in maintaining soil-health and demands for immediate actions. VAAGDHARA, is a civil society organization which believes in local solutions for problems affecting local communities. Motivated with the successful implementation of PLA tools for revival of nutrition sensitive farming systems, it evolved tools for applying this technique for engaging farming community to improve soil biodiversity and making it a case for national and international agencies that soil and farmers are dependent on each other, thus community action plays key role in revival of soil-biodiversity and in-turn all its eco-system services. This study has provided us pathway of community action as one of the prominent tools to improve soil-biodiversity and making soils as living soils. As Global soil symposium is a platform which guides countries which are part of the United Nations, therefore we want to share our findings at this platform to for bringing it as part of policies and programs of government and international agencies and also upgrade tools for further improvement.

Methodology

The whole process of community action on revival of living soil through the process of participatory learning and action (PLA) can be grouped under five categories and fourteen sessions as reflected in Figure-1 (Sharma and Joshi, 2019**)**. The aesthetic angle to the whole process is session-1 and session-14 as given in figure-2 and figure-3.

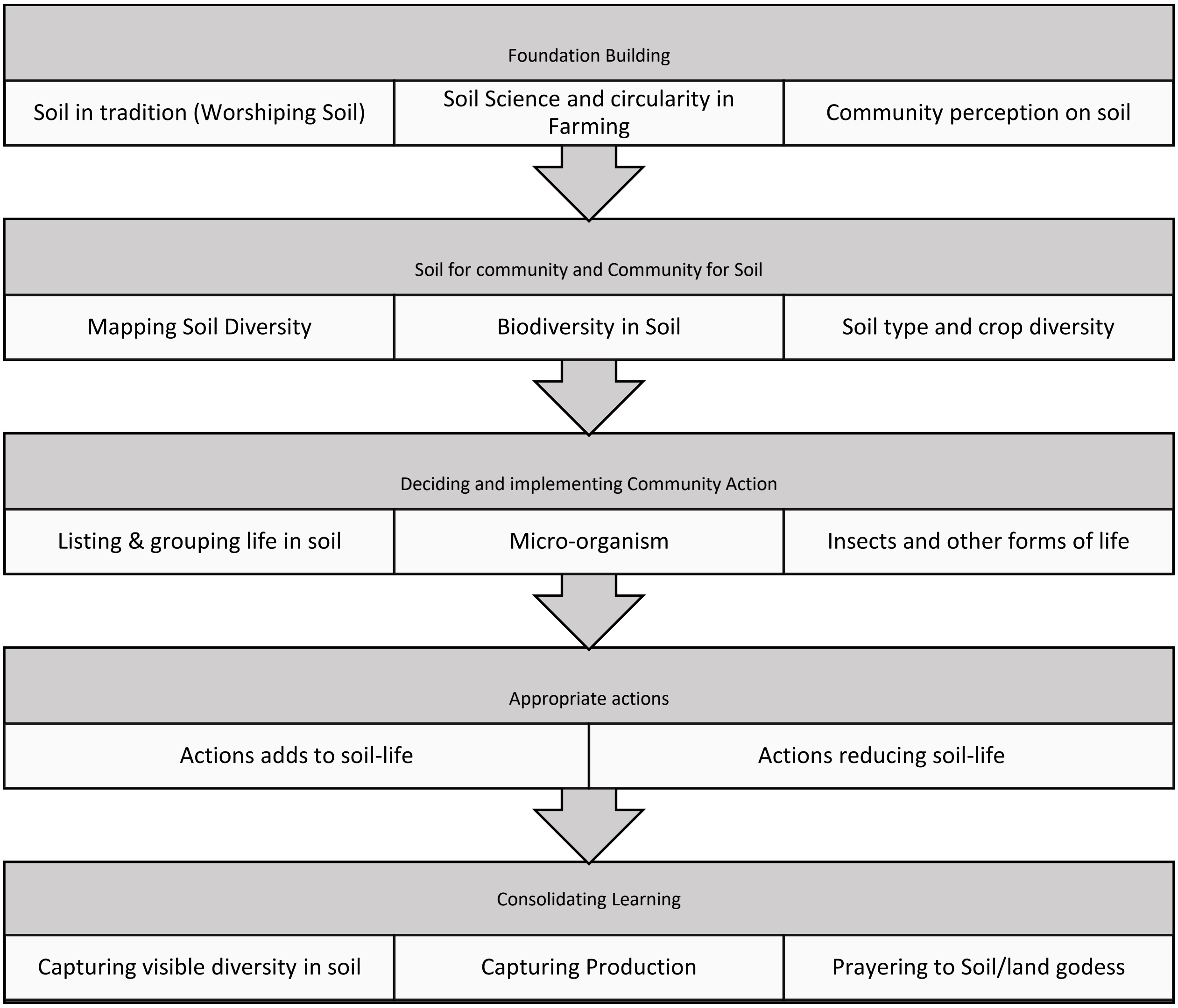

Figure 1: Participatory Learning and Action for revival of living soil

The framework shown in Figure 1, shares the broader steps followed in participatory learning and actions. But at session level and community actions, there are variations learning group level. The community action included adaption of leguminous plants, shrubs, and trees. We translated to Hindi various information videos on soil health, and soil biodiversity, asked community to identify plant and animal diversity they are able to recognize and benefits of them. The PLA exercise also helped in application of various traditional farming approaches, which were organic in nature such as mixed farming, agro-forestry, spray of butter-milk, jaggary solution, liquid-manure.

Figure 2: Worshiping Soil (*Mitti-Puja*)

Figure 3: Prayer of soil

Results

The process of participatory learning and action which started with the community awareness, carried with 30 groups of average 20 farmers each has provided following results;

1. Different communities involved in this process of learning and action resulted in bringing out large number of traditional practices instead of few readymade approaches like distribution of PSB, Trichoderma, Rhizobium, etc. which are targeted simply to increased production of specific crops.
2. PLA approach provides scope for innovations by farming community such application of biological in the field of soil bio-diversity such as inclusion of Legumes in mixed cropping to act as natural fertilizers, crop-rotation, agro-forestry, mulching, plantation in homestead, adapting hedgerows and grassy strips around fields.
3. Government needs to evolve programs for wider involvement of community in soil-building processes.

Discussion

The results of our study brings out that farming communities are in direct interaction with soil, thus have broader role to play in revitalizing soil-biodiversity by making “living soil”. With proper awareness, motivation and training they can play key role the revival process of living soil. The role and the application of soil biodiversity in the field: effective and replicable methodologies, techniques, technologies and practices that are promoting the conservation and sustainable use of soil biodiversity, with a view to upscale those sustainable approaches to improve productivity, accelerate biodiversity conservation and sustainable use of its resources, as well as guarantee equitable participation in productive landscapes.

Conclusions

In developing-world countries nearly 60-70 percent families belong to farming communities, who are in direct interaction with soil-ecosystem, their action affects soil-life and get affected by the soil-life or more precisely soil-biodiversity. Farmers, as custodians of much of the land, can play a crucial role in protecting soil biodiversity, since their choice of tools and techniques has an enormous influence on the factory of life. Mulching, or covering soil, for example with crop residues or compost, helps retain heat, preserve moisture and prevent erosion. Our involvement with indigenous farming community in India to improve soil-biodiversity through participatory learning action pertaining to traditional practices gives way for adaption of this approach by actions of various stakeholders, government and international actors.

Acknowledgements

The Authors acknowledge the farming community of 30 villages in the district of Banswara, Rajasthan, India who participated in the process of participatory learning and action pertaining to living-soil within their program of adapting to sustainable integrated farming system.

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Termites promote resource patchiness in Asia and constitute a model for achieving the sustainable development goals

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Abstract summary

Termites are undoubtedly amongst the most important soil macroinvertebrate decomposers in semi-arid environments in Asia. This communication reviews the key ecological roles played by termite constructions, the so-called termite mounds, in natural and cultivated environments in Asia, a region facing dramatic environmental and societal changes. Through several examples, it shows that termite mounds are under threats in the region due to the unsustainable use of natural resources although they constitute patches of biodiversity and fertility. The past, present and future roles played by termite mounds in the region are discussed taking into account their positive impacts on 6 of the 17 Sustainable Development Goals (SDGs n°1 "no poverty", 2 "zero hunger", 3 "good health", 8 "economic growth", 13 "climate action" and 15 "life on land").

Keywords: termites, mounds, heterogeneity, ecosystem services, sustainable development goals, sustainable agriculture

Introduction, scope and main objectives

The recent IPBES conference in Paris in April-May 2019 raised the urgency to protect biodiversity and to understand the services it provides to human well-being. Biodiversity and resilient ecosystems support livelihoods, enhance food and nutrition security, enable access to water and to health and contribute to climate change mitigation and adaptation. For these reasons, maintaining biodiversity is crucial to ensuring sustainable livelihoods and the worldwide reduction of poverty. However, biodiversity and ecosystem services (ES) are under threat due to a raising demand of our societies, and especially climate change, pollution and unsustainable use of natural resources in agro-systems. Consequently, one of most important challenge for our society relies in our ability to bring out new practices based on the biological potential of ecosystems (i.e., nature-based solutions) and in order to ensure the sustainable management of natural resources. In this context, several priorities come out from the 2030 IPBES agenda for biodiversity and ES. The first of them is the need for a better understanding of the interdependence between biodiversity, the soil-water continuum and human health (IPBES, 2019).

In terrestrial ecosystems, soil biodiversity regulates the cycling of nutrients as well as the dynamic of soil organic matter (SOM), the sequestration of carbon (C) and the emission of greenhouse gases (Wall, Bardgett and Kelly, 2010). Soil biodiversity also modifies the dynamics of water and soil and at larger scale the resistance of cultures to drought and climatic hazards. These services are not only critical to the functioning of natural ecosystems but also constitute an important resource for sustainable agricultural systems. Biodiversity can also positively impact human health either directly when animals and plants are consumed by humans or indirectly through their positive impacts on ecosystem productivity and farmer's incomes. If numerous invertebrates can be consumed by humans and can contribute to increase food safety (Parodi *et al.,* 2018), their influence on soil and water dynamics is limited to organisms from the "soil engineer" functional group (i.e., mainly earthworms and termites) (Lavelle, Bignell and Lepage, 1997). At a large scale, the concentration of nutrients and minerals and the presence of a specific vegetation and animal diversity on termite mounds can have a positive impact on the hydrological characteristics of watersheds (Bargués Tobella *et al.,* 2014). A recent study even suggested that termite mound nests may increase the robustness or resilience of African dryland ecosystems against water shortage and desertification (Bonachela *et al.,* 2015).

The preservation of biodiversity is especially important in South-East Asia (SEA), and especially in the lower Mekong River basin where biodiversity provides food, water, energy, and health security, as well as cultural and spiritual fulfilment to its 60 million inhabitants. The question of the impact of biodiversity on ecosystem functioning along the soil-water continuum is also especially important in this region where people depend heavily on rice for food. This region faces dramatic environmental and societal changes notably due to the rapid economic growth, the achievement of the Chinese "Silk Road and Belt Initiative and the climate change that threat the access to water and its quality in some areas while lead to a pollution and overexploitation of resources in plantations and freshwater ecosystems. In particular, the intensification of agricultural practices leads to a homogenization, simplification and pollution of ecosystems and constitutes a major threat to biodiversity, the preservation of soil and water quality, and consequently human health. Soil conservation is therefore paramount and can partly be achieved by the conservation of soil biodiversity. In this environment, bioturbation is mainly carried out by termites that produce large size mounds covered by trees and shrubs, which contribute to the maintenance of heterogeneity in the landscape.

The objective of this communication is to highlight the diversity of services that can be provided by TM, as hotspot of biodiversity and fertility, and discuss the potential they could play in a changing world.

Methodology

This communication shows results coming from the thesis of Choosai (2010) and Cheik (2019) with study fields in India, Thailand, Vietnam and Cambodia. Studies carried out in India and Vietnam are part of the M-TROPICS project (https://mtropics.obs-mip.fr/).

Results

TM density varies from 10 mounds ha^{-1} in natural forest in India to 6-8 mounds ha^{-1} in Cambodia and <1 mound ha^{-1} in Northeast Thailand. Figure 1 shows that TM are enriched in C, N, clay and exchangeable cations in comparison with the surrounding topsoil.

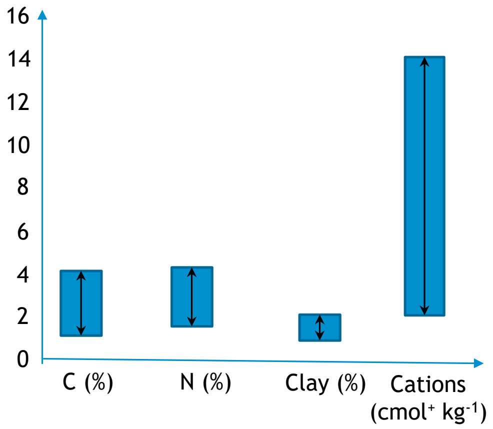

Figure 1: Enrichment in C, N, clay and exchangeable cations in termite mounds and in comparison with the surrounding topsoil environment (data from India, Jouquet *et al.*, 2017)

TM hosts a specific vegetation such as *Streblue asper*, *Azadirachta indica*, *Tamarindus indica*, *Drypetes roxburghii*) and soil invertebrate communities (Figure 2).

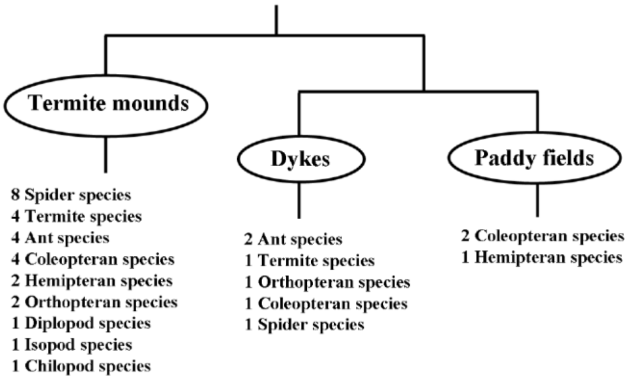

Figure 2: Number of specialist species (according to Indval scores) in the clusters identified by principal component analysis (data from Thailand, Choosai *et al.*, 2009)

Discussion

This communication shows that termite mounds can be compared to patches of fertility and biodiversity because of their higher soil fertility and because they constitute a refuge for plants and other invertebrates during the dry and rainy seasons. The higher amount of clay minerals in TM makes them an interesting amendment for growing vegetables or increasing rice yield while the higher biodiversity within TM provides an access to medicinal plants and edible mushroom and invertebrates. A higher presence of predators (e.g. spiders and lizards) might also control pests, and therefore reduce the amount of pesticides used by farmers with positive impacts on plant and water quality and then human health. Results from Africa also suggest that termite activity and TM soil properties positively influence water dynamic and quality in the soil, as well as plant resistance to drought (Bonachela *et al.,* 2015). Their higher clay and SOM contents are also likely to retain contaminants (e.g. pesticides) and therefore to limit the contamination of water and plants. However, land use changes in the lower Mekong basin result in a rapid disappearance of TM, and with them in a loss of numerous ecosystem services. In Northeast Thailand their density dropped from more than 10 ha^{-1} in the 70th, a density equivalent to the one found in protected forests in Asia, to less than 1 ha^{-1} nowadays. In Laos and Cambodia, Termite mounds are still conspicuous features of the landscapes. However, their density, their utilization by farmers and the potential services they can provide to the population remains challenged by the social changes that Southeast Asian countries face.

Conclusions

Termite mounds provide numerous ecosystem services impacting several sustainable development goals (SDGs n°1 "no poverty", 2 "zero hunger", 3 "good health", 8 "economic growth", 13 "climate action" and 15 "life on land"). However, if these mounds are very abundant in protected environment, the clearing of the forest and the intensification of the agricultural practices in Southeast Asia leads to their disappearance and to the loss of the services they provide to the society. A better understanding and quantification of their effects on ecosystem functioning is therefore needed for the emergence of sustainable, multifunctional and ecologically intensive agricultural practices in the region.

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Soil biodiversity: Why should we care?

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Abstract summary

Soil biodiversity is crucial for 'supporting' primary production, 'providing' food and raw materials, 'regulating' climate and water purification, and for 'cultural' heritage. However, soil organisms remain ignored from predictive modelling exercises, soil management and soil protection policies. This is partly due to the fact that much of the below-ground diversity is almost invisible to our naked eye, but most importantly because their minute existences are believed to be not relevant to solve any of the environmental problems that our Planet and humanity are facing today. Here I give an overview on the beneficial values of soil biodiversity, by providing quantitative data derived from my own work and relevant literature. Through selected examples, I will try to demonstrate that soil biodiversity could hold the solution for many environmental threats to our soils. The overall aim is to highlight the urgent need for designing future soil policies that include compulsory guidelines that specifically protect soil biodiversity globally.

Keywords: soil biota, ecosystem services, environmental threats, EU policies.

Introduction

Soil is a heterogeneous matrix with a porous structure and containing variable amounts of organic materials, food, water and chemicals. Because of this, it provides a wide range of habitats for soil organisms and has become the most diverse habitat on Earth: a single gram of soil may contain millions of individuals (Torsvik *et al.*, 1994). The majority of these organisms are almost invisible to the naked eye (fungi, bacteria, protozoa, mites, collembolans), which has made us to overlook their importance; however, their minute existences play important role in keeping well-functioning ecosystems (both natural and cultivated).

Not only a huge diversity characterises soil biota communities, but also "functional dissimilarity", which means that it is the degree of the functional differences between species what drives ecosystem processes (Heemsbergen *et al.*, 2004). In other words, they differ in their grazing/feeding, burrowing, soil mixing, and casting activities as well as in their adaptations to the surrounding environment. This together with their inter- and intra-specific interactions (e.g. predation) leads to "victories and defeats" across different spatial and temporal scales, and consequently, in different soils and at different moments, different species or groups become predominant and hence, the magnitude and direction of the regulated soil function (Briones, 2018).

Furthermore, research activities in recent years have linked the crucial influence of soil biodiversity to ecosystem services and has prompted a re-evaluation of their intrinsic values beyond non-material goods as in the Millennium Ecosystem Assessment (MA, 2005). When considering the four main broad categories of ecosystem services (provisioning, supporting, regulating and cultural), it becomes obvious that they cannot be delivered without soil organisms. For example, without pollinators or specific mycorrhizal associations, plants cannot grow and without microbial enzymatic activities we will not have many of the antibiotics we know today (provisioning); without burrows and galleries, water cannot flow down the soil profile (supporting), without biogenic structures, soils cannot retain nutrients nor eliminate contamination (regulating), without well-functioning ecosystems, we will not benefit from spiritual, therapeutic nor recreational experiences (cultural).

Despite this increasing awareness on the value of soil biodiversity, no country has a legislation that specifically protects soil biodiversity, which may be an issue considering that global food security is highly dependent on soil organisms. Protecting soils remain an important objective for the European Union (EU), but this goal cannot be achieved without considering soil biodiversity fully.

Here, I will give an overview – mostly based on my own research work, but also from relevant literature – on existing quantitative data that demonstrate that soil biodiversity should be considered as highly valuable source (with both material and non-material values) that must be included in international policies.

Soils are currently at risk and soil biodiversity could help

A recent report produced by European Academies' Science Advisory Council (EASAC) (EASAC, 2018) has identified several threats to European soils, including losses of organic matter, increases in soil compaction, erosion, landslides, soil sealing, contamination, salinization, and with land use and climate change being the main responsible drivers for the observed trends.

Although recent technological developments (e.g. optimizing agricultural management) and new strategies (e.g. 4 per 1000 initiative) have been postulated, they are challenging to put into practice (Montanarella, 2010). Therefore, it might be more cost-effective to improve soil functioning by enhancing soil biodiversity. Indeed, some experimental evidence is proving that soil biodiversity might hold the solution for many of the soil threats identified above. Unfortunately, the protection of soil invertebrates has rarely been a criterion for avoiding changes in land use and in developing climate change mitigation policies.

Decline in soil organic carbon

It is recognized as one of the eight soil threats expressed in the European Union Thematic Strategy for Soil Protection (EC, 2006). The soils in the European Union have been losing soil organic carbon at a rate equivalent to 10 percent of the total fossil fuel emissions

(SoCo, 2009). Future projections are rather pessimistic, and losses between 10 to 14 percent for croplands and 6 to 10 percent for grasslands are predicted even without C input changes (Wiesmeyer *et al.*, 2016).

Solution: It is the bodies of dead organisms (plant remains and soil biota) that return soil organic carbon to the soil and subsequently, a healthy soil provides the largest store of C on earth (FAO, 2015). More specifically, certain soil organisms are more efficient than others in sequestering C. For example, higher fungal:bacteria ratios are usually associated with higher C sequestration (Briones *et al.*, 2014), a typical condition of tundra and boreal regions. In tropical and temperate areas, ecosystem engineers (earthworms, ants and termites) are the main promoters of C stabilisation by creating biogenic structures (casts, galleries, nests and mounds) that form organo-mineral associations (Vidal *et al.*, 2016).

Soil compaction and soil sealing

Land use changes (e.g. urban development) and intensive agricultural practices (e.g. by using heavy machinery) are responsible for observed decreases in soil porosity. On the basis of the data published by the European Environment Agency, the total sealed soil surface area in 2006 was estimated to be around 100,000 km^2 or 2.3 percent of the EU’s territory (EC, 2012), and around 500 km^2 of land are sealed annually (EASAC, 2018). This means that since the mid-1950s the total surface area of cities in the EU has increased by 78 percent, whereas the population has grown by only 33 percent (EEA, 2006).

Solution: Certain invertebrates, such as earthworms and enchytraeids, create galleries and burrows and, by doing so, they let in the necessary supply of air and water. In particular, earthworms are considered to be “the Nature’s plough”, by naturally ploughing our soil and aerating it. The number of burrows in temperate region soils has been estimated to range from 100 to 800 m^{-2} (Lavelle, 1988). Despite accounting for a small fraction of the soil volume, their continuity, stability, and size, greatly increase the preferential flow of air, water, and solutes.

Land degradation due to erosion and contamination

More than half of the world’s arable land is moderately or severely degraded, which is costing the world as much as $10.6tn every year, equivalent to 17 percent of global gross domestic product (ELD Initiative, 2015). According to a relatively recent report, “33 percent of the world’s arable land has been lost to erosion or pollution in the last 40 years” (Cameron *et al.*, 2015).

Solution: The EU Biodiversity Strategy to 2020 calls for restoring at least 15 percent of degraded ecosystems in the Union and expanding the use of Green Infrastructure, e.g. to help overcome land fragmentation. However, restoring soils to their healthy state can only be successfully achieved if we also enhance the active agents in soil formation and maintenance and in the environmental clean-up (soil biodiversity). Some microorganisms secrete a sticky proteins and polysaccharides that have soil-adhesive properties (Costa *et al.*, 2018). Other soil organisms can facilitate plant establishment and consequently, promote the release of root exudates, which in turn increases aggregate stability and decreases erodibility (de Baets *et al.,* 2020). In addition, a broad range of environmental contaminants can be immobilised, metabolised into less toxic compounds, or mineralised by soil biota (microorganisms and soil fauna) and for this reason, several species are widely used as bioindicators of soil quality and level of soil pollution (ecotoxicological tests standardized by ISO and OECD guidelines).

Deteriorating human health

Soil health is directly linked to human health because the majority of the food we consumed is directly or indirectly obtained from our soils, and if toxic substances or pathogenic organisms enter the food chain they can be passed to humans.

Solution

Soil biodiversity is increasingly recognized as beneficial to human health because it can suppress soil-borne pests and provide clean air, water and food (Wall *et al.,* 2015). Thus, exposure to a farm-like house dust microbiome (with high bacterial richness and low abundance of cattle-associated microbes) has been associated with protection against asthma development (Kirjavainen *et al.*, 2019) and immunization with a soil bacterium (*Mycobacterium vaccae*) was found to induce anti-inflammatory responses in the brain and to prevent stress-induced anxiety-like defensive behavioural responses (Smith *et al.,* 2019). Crucially, the majority of approved drugs have originated from nature and soil microbes produce secondary that constitute half of the pharmaceuticals on the market today.

Conclusions

Some soil organisms are good, bad or ugly; however, unpleasant soil organisms represent a very small proportion and they are largely the result of a natural disequilibrium and/or environmental changes, often man-made. Soil biodiversity is now at the centre of sustainability agendas (SDGs, especially SDG15 that addresses terrestrial ecosystems and land degradation), so by protecting soil biodiversity we would also protect soils and enhance ecosystem services. Non-legally-binding agreements and guidelines promote the development of national legislation for soil protection. Policies to protect and value soil biodiversity are urgently needed.

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Application of soil biological quality index to assess the sustainability of soil urban use classes: a case study

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Abstract summary

Soil biodiversity in urban contexts is less considered than in natural areas. In fact, urban soil is mainly perceived as a support that can host human activities while its functions are generally ignored. Urban soils are exposed to anthropogenic disturbance that can cause a reduction in the abundance and diversity of soil communities compared to natural ecosystems.

Therefore, urban soil use and management have crucial importance in urban planning, in order to support a rich biodiversity pool for more sustainable cities.

In this study, soil samples from four urban green types (urban parks, urban forest, roadside green, and arable land) were compared. The biodiversity of soil micro-arthropods communities was evaluated using Shannon-Weiner diversity index (H'); soil quality was estimated by the Acari/Collembola ratio (A/C) and the QBS-ar index. Results are discussed in order to evaluate the influence of urban soil use on soil biological quality and biodiversity.

Keywords: Soil use classes, urban soil, QBS-ar, micro-arthropods

Introduction, scope and main objectives

Urban green areas are primary resources for biodiversity in the city. In addition to recreational functions, they help to mitigate pollution, regulate runoff and improve urban microclimate, while maintaining biodiversity.

Indeed, urban soils can be managed to provide ecosystem services such as supporting plant life and human activities, providing food and raw materials, preserving biodiversity pools and cultural heritage, and also storing and regulating the cycles of organic carbon, water and nutrients (Setälä *et al.,* 2014; Panagopouls *et al.,* 2016).

Despite this, soil is almost totally ignored in relation to its functions and services, especially in the management of urban ecosystems. Soils of urban areas are mostly considered as a support that can host human activities, while their environmental functions are generally ignored by urban planners and managers (Morel *et al.,* 2015). Composition and functions of urban soils and their ability to provide ecosystem services are different from those of natural soils and are often impaired (Morel *et al.*, 2015).

Soil biota play a major role in soil functioning, and among them micro-arthropods have a key role in organic matter decomposition, nutrient element cycling, porosity and infiltration, modifying soil structure and improving its fertility (Menta *et al.*, 2011).

Soil micro-arthropods are sensitive land cover and soil management which may cause a reduction in the abundance and diversity of soil communities.

QBS-ar is one of the indexes that have been conceived and developed in last years (Parisi *et al.*, 2005). QBS-ar in based on the concept that the number of microarthropod groups morphologically well adapted to the soil is higher in high quality soils.

In this study, we analysed soil micro-arthropods presence and ecology and the biodiversity of community from 19 green areas (under different soil use classes) in the town of Carpi (Northern Italy) in order to evaluate their capability to adapt under different urban soil use classes.

Methodology

The sampled areas were located in the city of Carpi (northern Italy, 44°46'59"88 N, 10°52'43"32 E). A total of 19 sites were identified for sampling, belonging to four green areas types: 11urban parks (UP), 1 urban forest (UF), 3 roadside green (RG), 3 arable land (AR). The urban parks were established between 1970 and 2009 and they differ for tree density, presence of play grounds and walkways. On the basis of the vegetation density, urban parks were distinguished in two classes: urban parks with low vegetation density (UPL) and urban parks with high vegetation density (UPH). Agronomical information about the green areas such as age of establishment, management practices, irrigation and fertilization were obtained from historical archives and interviews to technical officers. Soil sampling was done during spring (April-May) 2017, undisturbed soil samples of 10 cm^3 were collected and extracted within 48h for micro-arthropods analysis and QBS-ar index.

A Berlese-Tüllgren funnel was used for micro-arthropods extraction from each of the soil samples. The extracted specimens were observed under a stereomicroscope for identification at different taxonomical levels: classes for miriapoda (Diplopoda, Chilopoda, Symphyla, Pauropoda) and arachnida (Acari, Araneae, Opiliones), and order for insects (Collembola, Diplura, Protura, Hemiptera, Blattaria, Embiottera, Coleottera Dermaptera, Psocoptera) and larvae. The organisms belonging to each biological taxon were counted in order to estimate their density at the sampled depth and to relate the number of individuals of the sample area to a reference unit area (ind/m^2). The biodiversity of soil communities was evaluated using the number of observed taxa (NT), the Shannon-Weiner diversity index (H0) (Menta *et al.*, 2011). Soil quality was estimated by the Acari/Collembola ratio (A/C) and the QBS-ar index.

The A/C index is based on the densities of Acari and Collembola communities: in natural conditions the ratio of the number of mites to the number of Collembolan is larger than one. On the contrary, in case of soil degradation, the ratio shifts towards Collembola and its value decreases (Menta *et al.*, 2011). The QBS-ar index combines the biodiversity of soil micro arthropods community with the degree of soil animals’ vulnerability and provides information on the soil biological quality, which is an indicator of degradation (Parisi *et al.*, 2005; Menta *et al.*, 2018).

Results

A total of 1390 specimens of micro-arthropods belonging to 19 taxa were observed. Hymenoptera (ants) were the most abundant group, representing 44 percent of the extracted micro arthropods. The Collembola and Acari groups represented 14 percent and 12 percent of the total specimens respectively. Coleoptera and Diptera and Lepidottera larvae represented 9 percent of the total specimens, Chilopoda and Symphyla represent 4 percent and 5 percent and were present in 95 percent of the samples. The other groups observed reached an abundance between 0.5 and 1.5 percent of the total number of specimens.

The analysis yielded a clear separation between groups belonging to AR and UF and among these and UPH, UPL, RG ($p<0.001$), which did not show significant differences among them.

Statistical analysis of the indices showed significant differences between almost all soil uses (Table 1). NT significantly higher values for UF while lower values are reported for UPL ($p<0.01$). The QBS-ar index showed the highest mean value in UF samples ($p<0.001$) with significant differences to RG and UPL. A/C ratio results in clear separation between UF and other soil classes uses($p<0.001$). H’ index shows higher values in UF and low values found in UPL ($p<0.05$).

Table 1: Number of taxa (NT) Soil biological quality index (QBS-ar) index, Acari/Collemboli ratio (A/C), Shannon biodiversity index (H’) for each soil use typology

	UPL	UPH	UF	RG	AS
NT	4.5 ±0.48	7 ±0.48	14.5±0.84	6.33 ±0.68	9.33 ±0.68
QBS-ar	70.82±5.44	108.8±5.44	187 ±9.43	86.67±7.70	125.66±7.70
A/C	0.27±0.09	0.34 ±0.09	0.97±0.15	0.29±0.12	0.27 ±0.12
H'	0.90±0.06	1.01±0.06	1.40±0.10	1.05±0.08	1.08±0.08

UPL, urban park with low vegetation density; UPH, urban park with high vegetation density; RG, roadside green; UF, urban forest; AR, Arable land

Discussion

Hymenoptera (ants) were the most abundant taxa, with highly significant presence in AR and UPL. This result is in agreement with other studies, that report as some ant species have successfully occupied urban environments (Yamaguchi *et al.,* 2005; Kamura *et al.,* 2007). Moreover, ants have been often used as short and long term biological indicators for their ubiquity, diversity and abundance (Castracani *et al.,* 2015). Urban forest showed a significant presence of Collembola and Acari compared to the other urban soil use classes, indicating this kind of green areas as suitable habitat of these taxa in urban area (Cole *et al.*, 2006).

The strong differences between soil use classes typologies in the urban area are also highlighted by biodiversity indices and soil quality indicator. UF showed the best values in biodiversity (H’) and ecological equilibrium (A/C), with very high values of biological quality index (QBS-ar). AR, UPH and RG show good values for biological quality (QBS-ar) and E, while the low values of A/C indicate a no ecological equilibrium of these urban green types.

UPL shows the lowest values for QBS-ar, A/C and H’ as compared to the other soil uses classes, the reduction of meso fauna biodiversity it’s probably related to low vegetal density combined with presence of human disturbance. These results could be explained with the importance of tree canopies covering the soil: dense foliage of deciduous species affects either the radiation at ground and indirectly soil moisture, either the litterfall layer, supporting biodiversity and ecological functions of soil.

Conclusions

The abundance of micro-arthropods in UF and UPH demonstrates that these types of urban green show suitable trophic conditions to support micro arthropod communities in urban areas. The higher abundances of Hymenoptera in AR and UPL resulted in low values of H’.

The observed reduction in the number of taxa, quality and biodiversity of soil communities of UPL, compared to UPH could be affected by soil compaction, and low vegetal density. Human disturbances, might result in a decrease in nutrient content and related biological parameters in UPL and RG. AS results, demonstrate that soil tillage has lower disturbing effects on soil biota than anthropogenic factors in urban green areas.

QBS-ar clearly described the sustainable of soil use in urban green areas. The information provided, could be useful to integrating soil management knowledge and practices into the urban green areas management.

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Development of a multi-criteria evaluation of agroecological practices involving soil biodiversity, agronomic performance and farmer perception

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Abstract summary

An experiment has been designed in the Highlands of Madagascar with the aim to produce multi-criteria indicators of performance of agroecological practices. In this experiment, different traditional and innovative practices were tested in field conditions with the assumption that practices that enhance soil biodiversity and soil ecological processes are the one that best promote plant production, yield and sustainability. Both ecological and agronomic performances were confronted with socio-economic performance defined by farmers in order to propose innovative practices adoptable by farmers.

A 2-year field experiment with 16 different practices, replicated 4 times, was designed in the Highlands of Madagascar with upland rice as the main crop. Many soil and plant parameters were measured: 73 parameters to describe soil biodiversity and soil ecological processes, 19 descriptors to measure rice production, nutrition and yield, and 8 socio-economic descriptors.

This study led to the production of a useful indicator allowing to evaluate an agroecological practice on the way it promotes soil health and especially soil biodiversity, crop yield and socio-economical requirements which are the main limits for practice adoption. We measured interesting relations between soil ecological descriptors and agronomic descriptors.

Keywords: Agroecological transition, soil health indicators, soil ecological processes

Introduction, scope and main objectives

Agroecological practices are usually designed following different principles. Some of them (Altieri, 1999) underline the need to increase soil biological activity. Other principles highlight the needs to cross indigenous and scientific knowledge, to be economically and environmentally sound, and based on local resources, to be socially and culturally acceptable, and to enhance farm productivity (Pretty, Toulmin and Williams, 2011). Agroecological transition is particularly important for smallholders in tropical regions where rural societies and food systems generally face many challenges.

Scientists generally recognize the need for an ecological intensification of agricultural production by increasing biodiversity and complexity in agrosystems, to rely more on natural functions, biotic interactions and ecological processes, and to amplify the services provided by living organisms (Altieri, 1995; Barrios, 2007). Generally, at field and farm levels, agroecological practices drive and optimize functional biodiversity aboveground, whereas soil (belowground) biodiversity and functions are rarely managed. The importance of soil functions in the performance of agroecological systems is widely recognized and their restoration appears necessary (Altieri, 1999; Barrios 2007; Brussaard, De Ruiter and Brown, 2007; Bardgett and van der Putten, 2014; Bender, Wagg and van der Heijden, 2016). Unfortunately, due to the complexity of soil functioning and the poor knowledge of its determinism, only little consideration is given to soil when designing agricultural systems. Four basic soil ecological functions are of interest when regarding plant functions: (i) soil organic matter dynamics, (ii) nutrient cycling, (iii) maintenance of soil structure, and (iv) pest regulation (Brussaard, De Ruiter and Brown, 2007; Kibblewhite, Ritz and Swift, 2008). These soil functions directly or indirectly affect plant functions (De Deyn, Raaijmakers and van der Putten, 2004) and are provided by the activity of soil organisms.

The SECuRE project, funded by Agropolis Foundation, aimed to enhance agrosystem services by intensifying soil ecological functions in rainfed rice cropping system in the Madagascar Highlands. The overall objective of the project was to provide Soil Function Restoration (SFR) practices based on local and scientific knowledges, to increase both agronomic, socio-economic and ecological performances of agroecological agrosystems in a tropical context. For this purpose, 16 innovative SFR (4 replications) have been tested following the 5 levers:

1. use original organic inputs with high agroecological performances such as vermicomposts;

2. combine existing organic and mineral inputs promoting plant functions;

3. increase soil heterogeneity by providing various coupled organo-mineral substrates in a stratified way;

4. inoculate mycorrhizae and earthworms (biofertilization) to enhance key soil functions;

5. use crop varieties that best respond to innovative SFR practices.

The impact of each SFR practice on agronomic, socio-economic and ecological performances of the agroecological rice system has been determined in field experiments in order to select those improving rice production, soil health and being easily applicable by small farmers. The objective was to develop, following a participatory and multi-actor approach, innovative indicators adapted to the local and accurate evaluation of SFR practices.

Methodology

SFR practices were experimented using a field trial located in a farm at the vicinity of Imerintsiatosika (20 km west from Antananarivo). In order to understand how each of the 16 practices tested impacted soil ecological functions, carried by soil biodiversity, as well as crop production and quality, several scientific analyses have been performed along the second cultural season, leading to a database of 19 agronomical and 73 soil descriptors. Among those, Biodiversity descriptors have been chosen to focus on the three ecological functional groups: (1) "decomposers" assessed using Biolog® Ecoplates, (2) "regulators" assessed by the number of taxa, the density and several indices reported to the soil nematofauna and (3) "engineers" assessed by the number of taxa, the biomass and density of macrofauna.

The methodology we followed can be visualized on Figure 1.

- descriptors were first selected on the basis of their correlation with one or more SFR conditions;
- mean values of selected descriptors over the four replicated blocks per SFR were homogenised;
- selected descriptors were aggregated into 5 classes for the ecological performance (biodiversity promotion, the soil physico-chemistry, C storage, nutrient cycling and pest regulation) and 4 classes for agronomical performance (plant growth, grain yield, grain quality and forage quality).

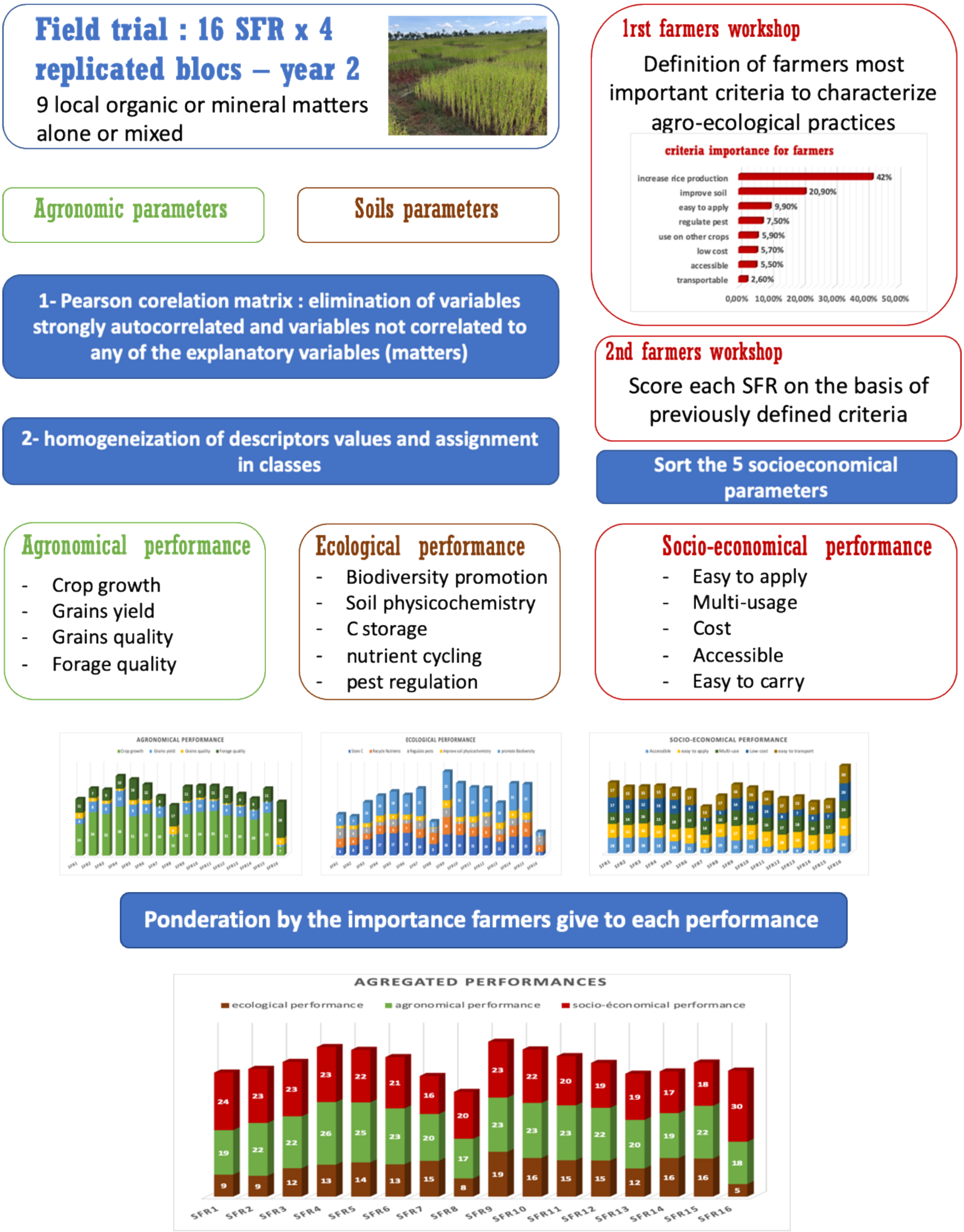

Figure 1: Diagram illustrating the methodological approach followed to generate the agro-ecological indicator, and the type of results obtained

To assess socio-economic performance, two farmers' workshops have been conducted. The first one aimed to identify and sort the main descriptors related to the farmer choice of fertilizers. Descriptors have been gathered into eight criteria, including 5 corresponding to socioeconomic, 2 to agronomic and 1 to ecological descriptors. Each criterion was weighted according to the frequency of citation by farmers. During the second workshop, farmers were invited to rate their perception for each SFR. Workshops were conducted with 7 groups of 5 farmers (2-3 men and 2-3 women). Notes rated for the 5 socio-economical descriptors were weighted by the importance of each criterium determined at the previous workshop and finally summed for each SFR, giving a percentage called socio-economic performance. Agronomical and ecological performances were adjusted by their respective coefficient of importance for farmers determined during the first workshop.

Results

SFR rating the lowest aggregated index is the one based on simple chemical fertilization with NPK (SFR8, Figure 1). This SFR rate at the lowest for each of the three dimensions of the index (ecological, agronomical and socio-economical), even lower than the negative control without any amendment (SFR16). After two years of culture, the mixing of different matters (SFR12 to SFR15, manure + compost + vermicomposts) did not show better results than the traditional cattle manure alone or associated with dolomite, and mixing matters is more constraining for farmers. But their high ecological performance suggests that their positive effect on soil biodiversity and ecological functions can increase with time.

Biodiversity promotion descriptors varied from 3 to 26 percent of the ecological performance. SFR16 showed the lowest value confirming the poor status of this practice. SFR 10 (manure + ashes and earthworm inoculation) best promoted soil biodiversity. From an agronomic point of view, it was as performant as SFR9 (manure + dolomite) but a little less appreciated by farmers. Biodiversity increased with the amount of organic fertilizers or with a mix between organic and mineral fertilizers, and depended on the matters. For example, the Biodiversity index was higher with lombricompost (SFR7) than with compost (SFR6). Lombricompost rated very low by farmers because they barely know this fertilizer in this area, while compost was promoted by the ONG AgriSud International.

When looking at the Pearson correlation matrix between classes and practices conditions, Biodiversity promotion was highly and positively correlated ($P<0.05$) to C storage (0.80), soil physico-chemistry improvement (0.74), crop growth (0.61) and grain yield (0.61) and nutrient recycling (0.56). Conversely, it was negatively correlated with grain quality (-0.81), and accessibility of fertilizers (-0.57). When looking at the practice conditions, Soil Biodiversity was mostly promoted by the quantity of C (0.86), N (0.88), S (0.73) amended as well as the amount of hemicellulose (0.59), cellulose (0.79) and lignin (0.78) of the amendment, and on the C/N of the amendment (0.54). It seems that mixing different matters also promotes soil biodiversity (0.54).

Discussion

This study led to the production of a useful indicator. While the final aggregated value provides an idea of the match between farmer expectations and ecological and agronomical performances, class composition can inform on the criterium to improve in order to increase the global notation.

The participatory approach we followed to develop the socio-economical part of this indicator instead of scientifically quantified parameters (real cost of fertilizers, distance to producers….), introduces the farmer perception into the evaluation. This allows for cross-cutting dialogue between farmers and researchers and help orientate future research in order to build technical solution along with actionable knowledge.

Our study showed that soil biodiversity is especially enhanced in practices with mixing different organic matters or mixing organic and mineral matters, and positively correlated to the amount of C, N, S in the amendments. This confirms previous studies showing the need to feed soil organisms with organic matters (Lavelle *et al.,* 2001). Another interesting result is the positive correlation between soil biodiversity, carbon storage and crop productivity. Nevertheless, the negative correlation between soil biodiversity with rice grain quality reinforces the need to continue improving practices so as to reach this important food function. More communication seems also to be required to improve the farmer perception of agronomic modalities promoting soil biodiversity.

Conclusions

This study led to the production of a useful indicator allowing to evaluate an agroecological practice on the way it promotes soil health and especially soil biodiversity, crop yield and socio-economical requirements which are the main limits for practice diffusion.

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Progress in agro – environmental policy for the protection of soil biodiversity in Cuba

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Abstract summary

This research is a concrete demonstration of the interest of the Cuban government and society as a whole in analysing and projecting the future of rural spaces destined for agricultural activity, from an agro-environmental perspective, where institutions and the environmental issues are key for promoting soil biodiversity as a significant factor for the future of food systems. The methodology was based on the application of an interactive process with a system approach in which 30 government institutions and society in general participated, as well as around 375 people including specialists, researchers, farmers, students, extension workers and decision makers from nine provinces of the country, agreed upon in 13 participative dialogues. The results show the need to increase the integration between different strategies to know the changes and trends of the environment; to continue using scientific knowledge to make the ecosystem approach prevail over sectors, where the Soil, Water and Forest Conservation Areas represent an effective reference for the insertion of the agro-environmental policy. It is clear that decision-makers are called upon to identify policy options to protect soil biodiversity and encourage the adoption of practices to enhance it.

Keywords: Agro environmental policy, biodiversity, soil, agro-ecology

Introduction

The Brazil-FAO International Cooperation Programme, subscribed in 2008 between the Government of Brazil and the FAO Regional Office for Latin America and the Caribbean, identified the need to strengthen agro-environmental policies in the countries of the region as an indispensable step towards achieving the objectives of sustainable development and food and nutritional security. This is the background to the implementation of the Project on Strengthening Agro-environmental Policies in Latin American and Caribbean Countries (GCP/RLA/195/BRA), which aims to promote dialogue and knowledge sharing among stakeholders involved in the formulation and implementation of rural development and natural resource management policies and strategies, in order to move towards an agro-environmental policy. It also has legislation that supports the policies outlined for the use and conservation of biodiversity (CITMA, 2014), which go along with economic and emerging development policies, through remediation, mitigation and adaptation actions, as an indispensable alternative for the sustainable development of soils in the country (Febles González, 2016), where soil degradation and contamination are revealed as some of the most complex problems and difficult to find adequate solutions, without having achieved, to date, definitive or conclusive results that would allow the loss of biodiversity to be halted and endangered species to be protected, through widely and effectively implemented sustainable agricultural practices (Potts *et al.,* 2017), in accordance with the biophysical environments that characterize each ecosystem and where their protection represents a National Security problem (CITMA, 2016). Based on this background, the present work aims to demonstrate the strengths of the agro-ecological movement in Cuba to promote an agro-environmental policy together with voluntary guidelines in sustainable soil management, to protect its biodiversity and the sustainable development of the nation.

Soil degradation in Cuba: Good soils in extinction

Some soils, as well as certain animals and plants, are becoming scarce and threatening to become "extinct". Acting as the foundation of terrestrial ecosystems, soils have an intimate connection with the plants and animals they support. When these soils disappear, so do the plants that used to live in them and that we will not find in other environments (Yang, 2003). It is estimated that around one fifth of Cuba's GDP depends on agricultural activity. In addition, this sector provides about 40 percent of the calories and 37 percent of the protein consumed per day (the rest comes from imported products); and directly employs about one fifth of the economically active population. From the above, it can be deduced that the family economy of four million people depends directly on the performance of the agricultural sphere (Nova, 2014), whose fundamental pillar is the soil under the current conditions of use (Figure 1).

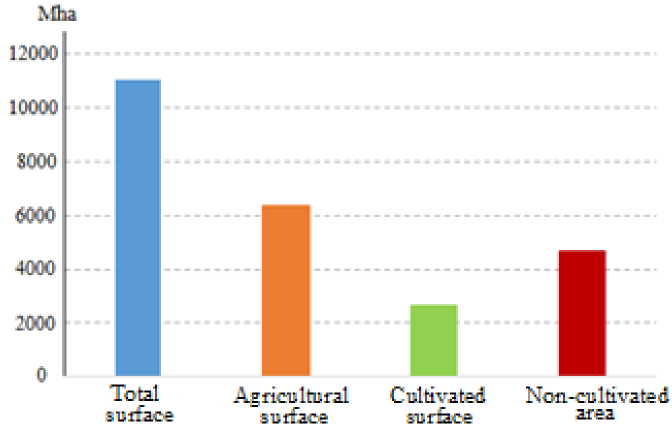

Figure 1: Land use in Cuba in thousands of hectares (MINAG, 2014)

The uncultivated area amounts to 4.646.0 ha (41.71 percent) and if we take into account that 76.8 percent of this area has low productivity categories (30.8 percent low productive and 46 percent very low productive), the situation is particularly tense (Figure 2). In addition, food imports are growing significantly, many of which could be produced nationally under competitive conditions. In this sense, Decree Law No. 300/2012 was enacted on the delivery of idle state lands for free and for a determined period of time to people, so that they may exploit them rationally and sustainably, taking into account the suitability of the soils.

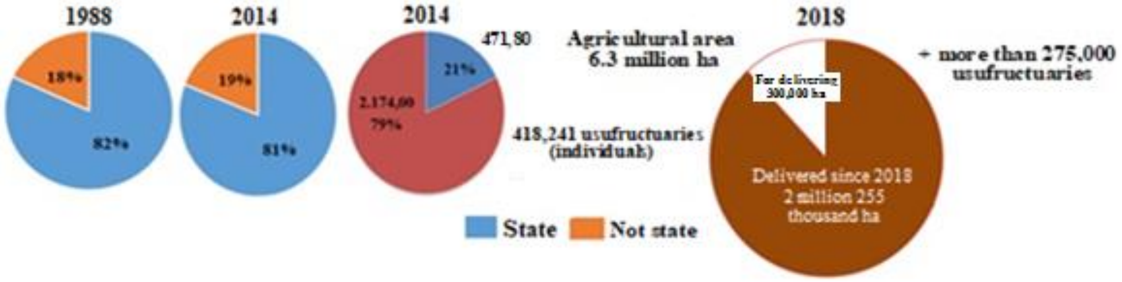

Figure 2: Land use and tenure in Cuba (MINAG, 2019)

The need for agro-environmental protection of soils to maintain their biodiversity

In view of these challenges, the Ministry of Agriculture of Cuba (MINAG) is strengthening its competencies in agro-environmental policies by promoting plans and programs for ecological agriculture, improving institutionality and governance and generating synergies that will make it possible to move towards the objectives of urban, suburban and family agriculture that can improve food security in the medium and long term (FAO, 2014) assumed as a State Plan known as *Tarea Vida* for confronting climate change, approved by the Council of Ministers in April 2017 with a higher scope and hierarchy in terms of mitigating the loss of biodiversity and increasing the capacity to generate environmental goods and services as well as improving the social welfare of people in priority areas.

The agro-ecological movement in Cuba has implemented a reorganization of agricultural production that allows the development of new scenarios taking into account biological, productive, economic, energy and environmental efficiency, with the objective of protecting natural resources, reducing environmental pollution and providing quality food in sufficient quantities to supply the population (Figure 3).

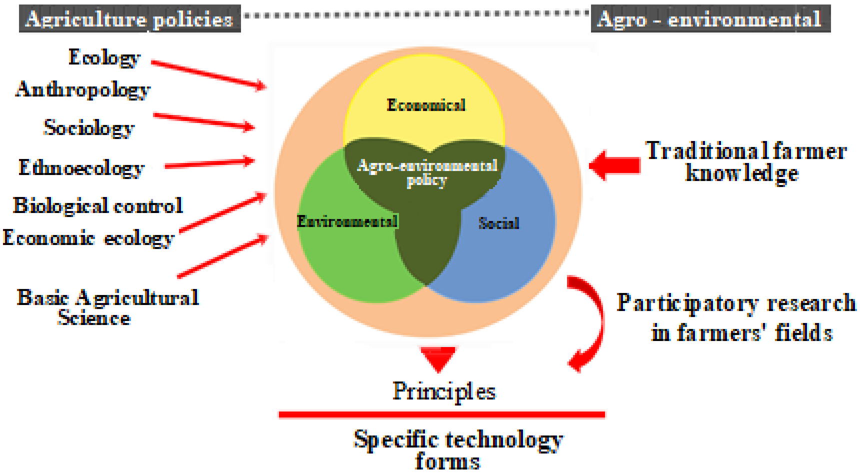

Figure 3: Dimensions of development and agro-environmental policy in Cuba Source: Own elaboration based on Fernández (2006) and Gallopín (2006)

Methods

The methodology was based on the application of an interactive process with a system approach in which 30 government institutions and society in general participated, as well as around 575 people including specialists, researchers, farmers, students, innovators, extension workers, project directors and decision makers, agreed upon in 17 dialogue tables or participatory work sessions (Figure 4) in nine provinces of the country.

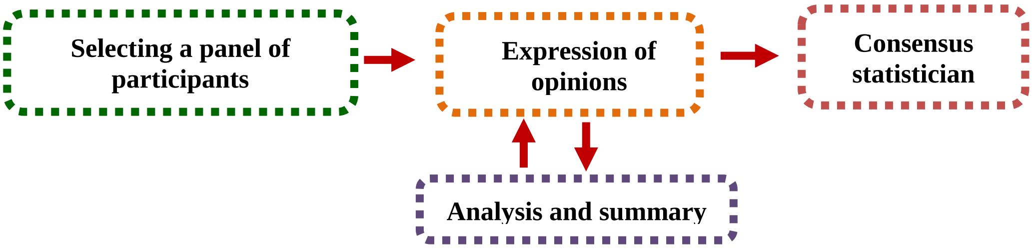

Figure 4: Interactive process with a system approach for building an agricultural policy based on agro-environmental principles

The information gathering contributed to knowing the reality of the environmental, economic, social and organizational culture situation of the group or community in question, recognizing the weaknesses, threats, strengths and opportunities, as well as critically evaluating the capacity to respond to the environment.

Results and discussion

Main criteria provided by the participants in the national dialogue tables

- It is identified as a strength to have a consolidated national agro-ecological movement, supported by the state and civil society, internationally recognized, with willingness, knowledge and experience to increase certified organic production, free of pesticides, antibiotics or other residues, which are materialized in research projects with synergistic effects and agro-environmental approach in favor of food security and sovereignty.

- It is important to recognize the effort that the Cuban State is making to guarantee agricultural and forestry development from an agro-environmental point of view, although the investments and resources demanded by this strategic sector are still insufficient.
- The challenge of the future will be to develop agro-environmental protection that harmoniously and coherently combines agriculture, livestock, forestry and other sub-systems, based on organic and sustainable methods, to achieve synergistic mechanisms and consolidate agro-ecological systems as a higher level for maintaining their biodiversity.

Programs and projects that exemplify agro-environmental soil policy to maintain soil biodiversity – Soil, Water and Forest Conservation Polygons

The development programs and agro-environmental projects of the MINAG in Cuba include productive, social and environmental objectives, oriented towards professional training, scientific and technological research and support to the productive sector, in which the Soil, Water and Forest Conservation Areas stand out for their integrality and synergetic approach with the agro-environmental policy, through the implementation of the FAO project (TCP/CUB/3002), under the principles of Conservation Agriculture for sustainable land management and maintenance of biodiversity, which allows the development of ecosystem services such as carbon sequestration, oxygen generation, water purification, agro tourism and in general quality food products that play an increasingly important role in the development of society, supported by three national programs: Cuban Program for Soil Improvement and Conservation; National Action Plan to Combat Desertification and Drought and the Disaster Management Plans, which allow for increasing the resilience of production systems and communities.

In this way "The polygon", while serving as a demonstration area, is a place that remains with the systems of measures implemented, where it is possible to measure the impacts generated in the short, medium and long term to promote capacities and address the effects of climate change on biodiversity and according to the views of farmers in several provinces of the east of the country (Las Tunas - Granma - Holguin), express that: "*in the polygons the vegetable cover has grown*" *[...]* and the farmers have seen the results of these practices "*in their pockets*" *[...]*; "*here there was nothing, only the soil and it was bad*" *[...]*, indicated a farmer in the province of Pinar del Río in the west of Cuba.

At present, there is a National Group that coordinates 189 polygons (35 provincial and 154 municipal), 51 of which are in areas of implementation of the *Tarea Vida*, covering 32,281 ha in 1,916 farms located in different soil and climate regions and in the most important crops. A set of agro-environmental indicators has also been created

(Figure 5) to assess the environmental, social and economic impacts that contribute to enhancing the ecological services provided by ecosystems.

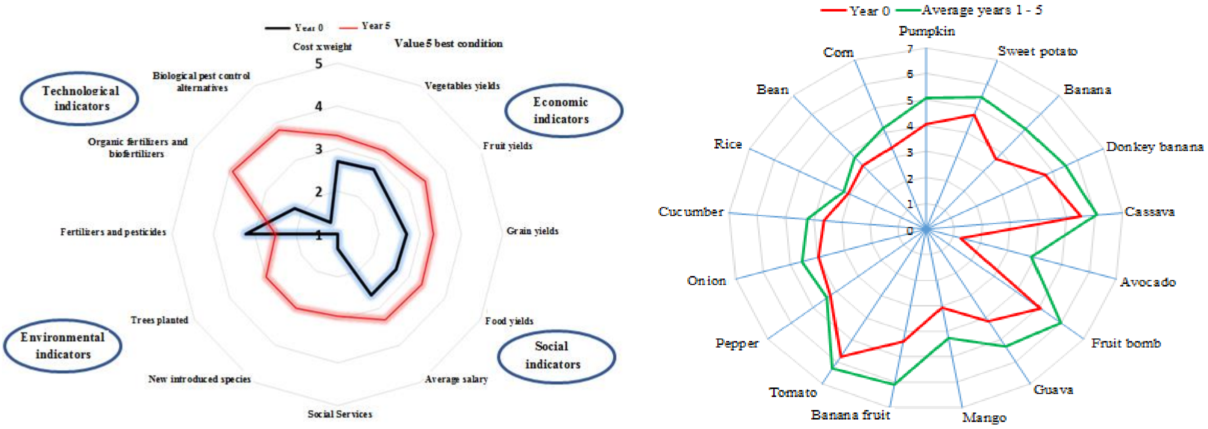

Figure 5: Evolution of sustainability indicators in 17 sites in the country subject to sustainable land management practices

(Value 5 best condition). Source: Aguilar *et al* (2015)

In a general assessment of the experience, the positive trends of the established impact indicators, the reception and consolidation in the producers and the integration between the institutions that manage the soil, water, forests and their direct link with the producers are maintained and increased, in addition to the growing recognition of the Cuban initiative by national and international organizations.

Conclusions

This research is fully in line with the country's current priorities, and confirms the political will to gradually change the type of agriculture towards conservationist agriculture where each unit of land is assigned the most appropriate and efficient use so it is capable of making the concepts of sustainability a reality, as the only way to achieve Food Security and consequently increase soil biodiversity.

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Communicating the importance of soils to human health: New options and opportunities

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Abstract summary

As soil scientists we have a lot of useful information to offer society. However, the general public is often not aware of the importance of soils, often known as “dirt”. In fact, when the general public thinks of soil the perception is often negative. Soil scientists need to find more effective ways of reaching out and extending our field’s message beyond its academic borders. Doing so requires that we find 1) one or more key links that will connect people to soil and 2) an effective method/platform to spread that message. Concepts such as soil health, soil security, or soil ecosystem services that build on ideas the general public already fundamentally understands may facilitate communication. Social marketing through social media platforms may provide the needed method/platform. However, there are unique challenges and unknowns that must be overcome when using these relatively new marketing options. The presentation will discuss such issues in greater detail.

Keywords: social marketing, social media, effective communication, soil health, soil security, soil ecosystem services

Introduction, scope and main objectives

All of our knowledge goes unused if people are not aware of or do not understand it. As scientists we spend a lot of time communicating with each other, but are not always so effective at communicating outside the scientific sphere (Weingart, 2011). To make informed decisions about a topic people have to be aware of it (Chikowero, 2015). Once aware of a problem or an issue people are more likely to engage with that issue (Prokopy *et al.*, 2008). However, few people seem to recognize the links between soils and human health. This certainly isn’t because of a lack of communication between scientists; a number of recent papers, books, and book chapters have addressed this issue in the literature of multiple scientific and human health fields. Given the abundance of scientific communication coupled with the relative lack of public recognition, the logical conclusion is that the scientific community is profoundly failing to communicate the importance of the soil - human health connection to the broader public.

Before people will connect with a soil message, they need to see soil as something that is important in their lives (Underwood and Morrison, 2011). At present the public perception of soil is often negative, in fact, the public perception of soil is often "dirt" rather than "soil", something that is reinforced in sayings such as "dirt poor", "dirt bag" "soiled", and "mudslinging" (Henry and Cring, 2013). To change perceptions of soil it is important that we do two things: 1) find a way to make a positive connection between people and soil and 2) find a way to reach people with this message. If the negative image of soil can be changed, and people learn that soil is important to their health, they should then theoretically behave in ways that will improve soil conditions and thus their own health (Brevik *et al.*, 2019). This project explores new opportunities to bridge the communication divide that so often exists between scientists and the public.

Concepts for a positive connection

Making a positive connection between the general public and soil involves presenting a viewpoint of soil that people who are not intimately vested in soil can connect with. Brevik *et al.* (2019) proposed soil health and soil security as two concepts that show promise in this regard. There are several advantages to using the soil health concept. One is that the idea of human health is already implicit in widely accepted definitions of soil health, and the connection between soil health and human health is already documented. Commonly used soil health definitions also incorporate the concepts of improving air and water quality, and these are goals that already enjoy wide-spread public support. Soil health already has international acceptance by agricultural interests and policy makers, and some farmers recognize that links exist between the health of their soils and the health of those who consume products produced on those soils. Therefore, soil health shows promise as a concept to connect people to soil.

The soil security concept is much more recent than soil health and does not yet have the same recognition (Brevik *et al.*, 2019). However, soil security seeks to take advantage of the recognition that concepts such as food security, water security, and energy security have gained, particularly among policymakers (McBratney, Field and Koch, 2014), and links have been identified between soil security and human health. Soil security also incorporates social aspects into the concept, which makes it appealing as a possible way to connect people to soil. The term "ecosystem services" (ES) was introduced in the 1990s and has rapidly gained widespread acceptance in many of the natural sciences; however, including soils in ecosystem services evaluations was not common until the 2000s (Baveye, Baveye and Gowdy, 2016). Soils have been linked to a wide range of ES, including those that support human health. The importance of ES are now widely accepted within the scientific community, but at present there is some evidence that there is little recognition of ES by the urban public (Collins, Cook-Monie and Raum, 2019), and Bagstad *et al.* (2016) found that the public had a limited ability to perceive the importance of ES provisioning regions. Therefore, while soil security and soil ES are concepts that have potential to engage the public, each also appears to need more public exposure to do so most effectively.

Ways to communicate

Having a message that will resonate with the public is only one part of the picture. Another major aspect is how that message will be communicated and perceived. There are many options for this, including social marketing and social media. Both should be effective ways to communicate soil information to a diverse public audience.

Social marketing applies marketing techniques and principles with the goal of influencing public behavior in a way that benefits society. In traditional marketing the goal is to convince people to make a purchase; in social marketing the goal is to illicit a specific behavioral change. Regarding the soil concepts previously discussed (soil health, soil security, and soil ES), the ultimate goal of a social marketing campaign would be to create behaviors that promote soil health, soil security, and soil ES. This promotion may not be direct. For example, the willingness of consumers to pay a premium price for products produced in a way that promotes and protects soil security/health/ES could convince farmers and ranchers to adopt such practices (Chen, 2017). Some early efforts at social marketing for soil purposes are being attempted (Brevik *et al.,* 2019), time will tell whether or not they end up being successful.

Social media has become a powerful platform for communication in the modern world, and it comes in many different forms. Social media is expanding rapidly, making popular social media outlets effective platforms for marketing efforts (Shareef *et al.*, 2019). However, marketing on a social media platform has important differences from marketing on traditional platforms. Social media views often occur through the recommendations of peers, rather than randomly like on a billboard or television. Unlike traditional marketing, where the content of the message is most important, the context of the message (who it comes from) is more important on social media (Shareef *et al.*, 2019). In other words, when a social media marketing message comes from a source the recipient trusts, the recipient is more likely to accept the content of the message, and vice versa; informal messaging is more likely to be persuasive in the social media environment than traditional formal marketing. This introduces unique challenges to generating an effective social media marketing campaign, but also the opportunity to reach people on budgets that can be much smaller than those required for traditional marketing outlets (Yakin and Eru, 2017).

There are some current attempts to market soil science through social media. Examples include the "Soils Matter" blog (https://soilsmatter.wordpress.com/) run by the Soil Science Society of America (SSSA), the Twitter feeds run by SSSA (@SSSA_soils) and the International Union of Soil Sciences (IUSS) (@IUSS_ORG), and the Facebook pages run by SSSA and IUSS. There are also a series of YouTube videos developed or supported by SSSA (e.g., https://www.youtube.com/watch?v=vDL6F6GkAzI), Soil Science Australia (e.g., https://www.youtube.com/watch?v=S7I-yEUZ1j4), and the YouTube channel run by the Soil Health Institute (https://www.youtube.com/channel/UCeBuJZT0GiS-iVxaPNfqkww). Several professional soil science societies have LinkIn accounts. Some measurements of the effectiveness of these efforts can be made. The SSSA blog now averages over 35,000 views per month, each YouTube video displays the number of views it has received, Twitter tells how many times something has been retweeted, and LinkIn accounts display the number of followers that a professional society has. However, much like social marketing efforts, the long-term effectiveness of marketing through social media has yet to be determined.

Conclusions

As soil scientists we do a good job talking to each other, but we need to engage in more effective communication with those outside our field. This engagement involves finding 1) a message that will catch people's attention and 2) an outlet that can reach a large number of people in an engaging and cost-effective way. Concepts such as soil health, soil security, or soil ecosystem services provide possible messages, and social media provides a possible outlet. However, more work is needed to determine the best way to structure social media marketing efforts.

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How much soil diversity is restored after a cattle ranching pasture is abandoned for its natural regeneration in the Amazon region?

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Abstract summary

Soils with diverse biological communities are considered healthy soils. Intensive deforestation for livestock production in the northwest of the Colombian Amazon has significantly reduced native forests. This study evaluates two soil biological communities through a chronosequence between 0 to more than 40 years of natural regenerating process after degraded pastures were abandoned. The soil macrofauna was collected and quantified from 0.25 × 0.25 m monoliths in five points on a plot of 60 x 60 m, at three different depths: litter, 0-10 cm, 10-20 cm and 20-30 cm. Soil samples for arbuscular mycorrhizal fungi were collected from the topsoil at the side of each monolith. Abundance and species richness were evaluated at each forest age. Diversity was measured by rarefaction. We identified 28 taxonomic groups and 791 morphospecies of soil macrofauna, and 44 species of arbuscular mycorrhizal fungal species (as virtual taxa). The two biological communities had different composition in each successional stage. Soil macrofauna species richness was correlated with the age of the succession forest, but not the soil macrofauna density. Abundant and higher numbers of arbuscular mycorrhizal virtual taxa in the soil indicated higher degree of disturbance. Results indicate that degraded pastures could restore their soil biological communities naturally through the time, but some changes in the biological communities are unrestored.

Keywords: Arbuscular mycorrhizal, Soil biology, soil macrofauna, taxonomic groups.

Introduction, scope and main objectives

Soil macrofauna contribute to organic matter cycling and the efficient return of nutrients to plants (Ackerman *et al.*, 2009; Six *et al.*, 2004). Arbuscular mycorrhizal (AM) fungi contribute significantly in the efficient phosphorous (P) acquisition of plants through mycorrhizal associations (Smith *et al.*, 2011), especially in limiting P environments such as Amazon soils (Quesada *et al.*, 2010). Both, soil macrofauna and AM fungal communities are highly sensitive to land use (Lavelle, 1997; Pauli *et al.*, 2011; Sepp *et al.*, 2018). Deforestation of natural forest to transform it in pastures is common in Amazonia, but it is poorly known if biological communities restore themselves naturally after pastures are abandoned for their natural regeneration (Suárez *et al.*, 2015).

Taking advantage of the potential that soil macrofauna has as an indicator of soil quality, and the permanently presence of AM fungi associated to plant communities present in the Amazon region, we propose to evaluate how the diversity of these two biological communities are affected along a natural regeneration of degraded pastures which were abandoned after cattle ranching use. We expect to contribute to better understand the changes that occur into the soil and how those changes might be useful in restoration processes.

Methodology

This study was conducted in the Caquetá state, Northwestern of the Colombian Amazon. We evaluated five different forest ages: 1) Pastures that had recently livestock activity; 2) Young secondary vegetation from natural regeneration between 0 and 10 years; 3) Secondary vegetation from natural regeneration between 10 and 20 years; 4) Secondary old forest between 20 and 40 years; and 5) Mature forest more than 40 years old. Six plots of each of each forest age were sampled for a total of 30 plots.

Soil macrofauna sampling was done following the methodology of Tropical Soil Biology and Fertility Program (TSBF) (Anderson and Ingram, 1993), which is based on the capture and collection of soil organisms (visible to the human eye), to determine presence and population density in a known volume of soil. On each forest a plot of 60 x 60 meters was limited. There, five TSBF monoliths of 25 x 25 cm was made (Figure 1). In each monolith, samples were collected at four different depths: litter, 0 to 10 cm, 10 to 20 cm and 20 to 30 cm. Due to inconveniences in the conservation of the samples, this study did not collect earthworms.

For AM fungal sampling, a topsoil (0-15 cm depth) sample of approximately of 100 g was collected at the side of each TSBF monolith. Communitarian soil DNA was isolated using commercial kits for DNA extraction and latter amplified with WANDA and AML2 specific PCR primers for AM fungi (Dumbrell *et al.*, 2011; Lee *et al.*, 2008), followed by Illumina sequencing method to obtain AM fungal sequences. Sequences were cleaned and analysed following Davison *et al.*, (2012) recommendations. Sequences were analysed against INSDC and Maarj*AM* databases to identify specific OTU´s of AM fungi as virtual taxa (VT).

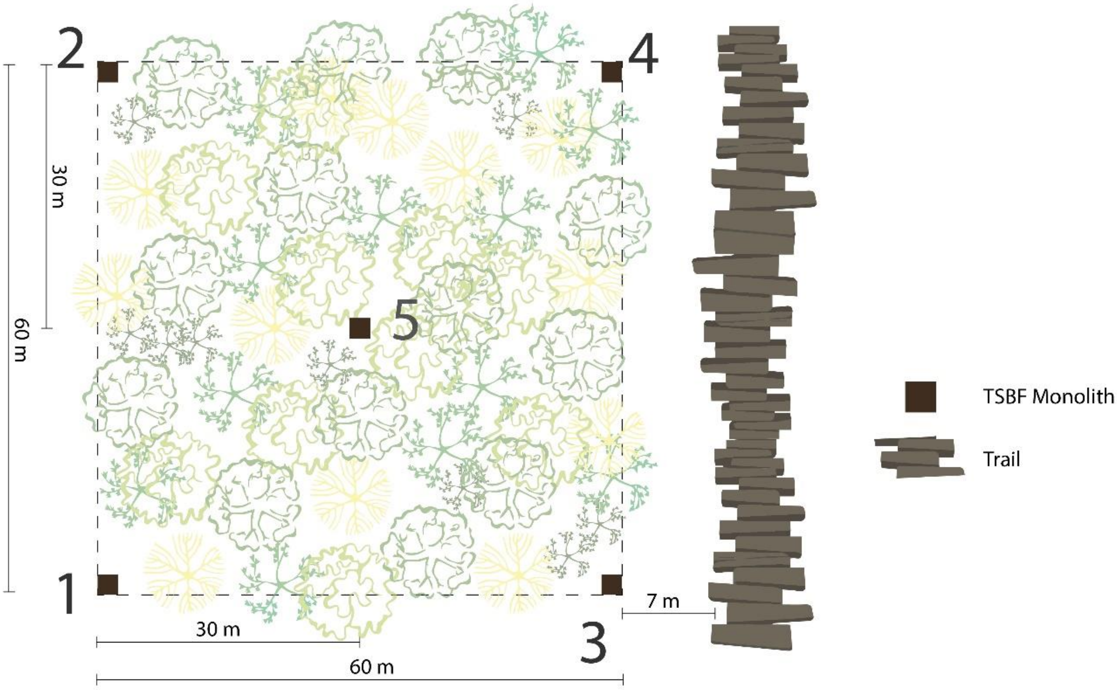

Figure 1: Sampling method

Soil macrofauna density (number of individuals per square meter) data and species richness data per taxonomic group, were analysed by ANOVA. A Tukey test was used when significance differences were obtained. Analysis was done using platform R (R Core Team, 2020). Diversity analysis were done through rarefaction and extrapolation of curves with iNETX package (Hsieh *et al.*, 2016) in (R Core Team, 2020). Differences in AM fungal diversity and abundance were analysed by a Kruskal Wallis test, using platform R (R Core Team, 2020).

Results

Soil macrofauna community

We obtained 13.685 individuals from 28 taxonomic groups of two phyla: Arthropoda and Onychophora. Individuals were from nine classes (Arachnida, Chilopoda, Collembola, Diplopoda, Entognatha, Insecta, Malacostraca, Onychophora and Symphyla), 37 orders, 124 families and 106 different genera. A total of 791 morphotypes were identified. Form them, 133 were identified at the species level. Formicidae (ants) with 34,6 percent was the taxonomic group with most occurrences, followed by Isoptera (termites) with 14,8 percent. Immature forms (larvae) represented the 8.9 percent of total soil macrofauna (Table 1).

Table 1: Density and relative abundance of soil macrofauna taxonomic groups in a natural regeneration chronosequence

Taxonomic group	**Density (ind/m²)**						**Relative abundance (hits)**					
	Pasture	**0-10**	**10-20**	**20-40**	**> 40**	**Total**	**Pasture**	**0-10**	**10-20**	**20-40**	**>40**	**Total**

Isoptera	18.13	558.13	656.56	757.81	495.94	2486.56	94	84	60	90	14	342
Formicidae	113.44	336.88	396.88	201.88	279.69	1328.75	178	166	180	157	119	800
Larvae	10.00	12.81	28.44	17.19	20.94	89.38	59	35	41	45	26	206
Chilopoda	0.63	14.38	15.00	15.63	18.75	64.38	42	31	36	37	2	148
Diplopoda	2.50	5.94	17.50	13.75	14.06	53.75	29	17	35	39	7	127
Coleoptera	4.69	14.69	12.81	8.75	12.81	53.75	37	45	34	25	14	155
Araneae	5.63	6.25	11.25	11.88	13.44	48.44	39	19	31	34	17	140
Hemiptera	3.44	6.56	13.75	9.69	3.44	36.88	9	18	27	23	9	86
Diplura	0.31	3.44	5.63	5.63	7.81	22.81	20	7	13	15	1	56
Opiliones	0.00	5.94	2.50	7.50	5.63	21.56	16	16	8	21	0	61
Isopoda	1.56	1.56	5.31	3.44	2.50	14.38	8	5	12	7	2	34
Blattodea	1.56	1.88	2.81	4.06	3.75	14.06	12	4	9	11	5	41
Pseudoscorpionida	0.00	1.25	5.00	4.06	3.13	13.44	9	4	13	9	0	35
Acari	0.00	0.63	2.81	2.50	1.56	7.50	4	2	8	7	0	21
Orthoptera	0.94	0.63	0.63	1.56	0.94	4.69	3	2	2	4	3	14
Archaeognatha	0.00	0.63	0.31	0.63	1.88	3.44	4	2	1	2	0	9
Dermaptera	0.31	0.94	0.00	0.31	1.25	2.81	4	2	0	1	1	8
Symphyla	0.00	1.25	0.31	0.63	0.31	2.50	1	3	1	2	0	7
Collembola	0.00	0.31	0.00	1.56	0.00	1.88	0	1	0	3	0	4
Schizomida	0.00	0.00	0.31	0.31	0.94	1.56	3	0	1	1	0	5
Scorpiones	0.00	0.63	0.00	0.31	0.00	0.94	0	2	0	1	0	3
Onychophora	0.00	0.31	0.00	0.31	0.00	0.63	0	1	0	1	0	2
Thysanoptera	0.00	0.00	0.00	0.63	0.00	0.63	0	0	0	2	0	2
Ricinulei	0.00	0.00	0.31	0.31	0.00	0.63	0	0	1	1	0	2
Psocoptera	0.31	0.00	0.00	0.00	0.00	0.31	0	0	0	0	1	1
Diptera	0.31	0.00	0.00	0.00	0.00	0.31	0	0	0	0	1	1
Trichoptera	0.31	0.00	0.00	0.00	0.00	0.31	0	0	0	0	1	1
Hymenoptera	0.00	0.00	0.00	0.00	0.31	0.31	1	0	0	0	0	1
Total	**164.06**	**975.00**	**1178.13**	**1070.31**	**889.06**	**4276.56**	**572**	**466**	**513**	**538**	**223**	**2312**

Termites and ants were the dominant groups. Ants were more abundant in forest between 10-20 years old. Termite was the group with the highest density in soils. Termite density was correlated with the age of the forest, presenting the highest density and highest species richness in mature forests.

The relative abundance of soil macrofauna is significantly lower in the pastures, but similar in young and mature forests (Figure 2). Soil macrofauna species richness increases with the age of forest regeneration but without significant differences in successional forest older than 10 years old (Figure 3).

The depth with the highest soilmacrofauna density was 0-10 cm (where organic matter is higher). The soil macrofauna density in pastures at litter and 0-10 cm is very similar, but with the natural regeneration through the time, the soil macrofauna density increases initially at the 0 -10 cm depth, and in older stages at 10-20 cm and 20-30 cm depths also.

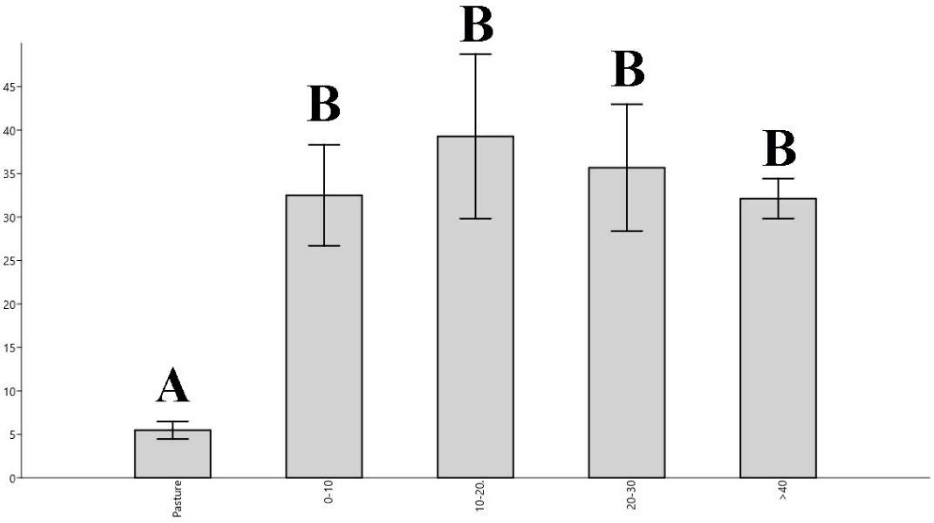

Figure 2: Abundance (individuals/ m2) of soil macrofaunal groups in the chronosequence

Different letters above the bars reflect statistical differences using Tukey test (p < 0.05). Line bars represent standard deviation.

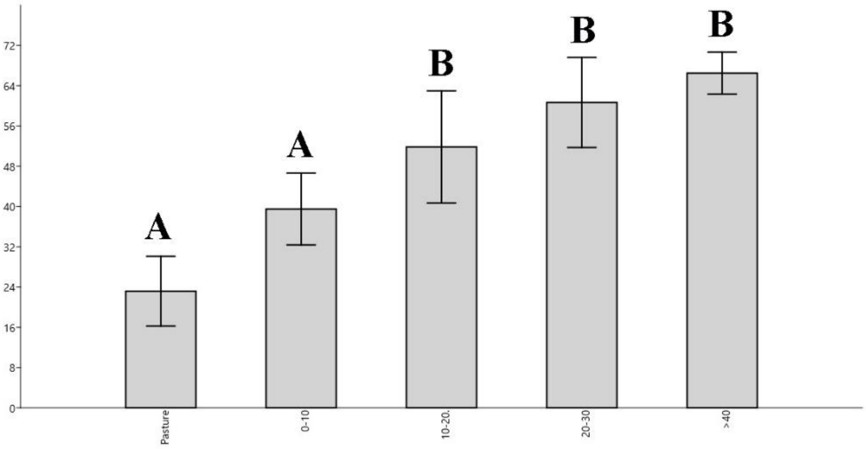

Figure 3: Soil macrofauna richness in the chronosequence

Different letters above the bars reflect statistical differences using Tukey test (p < 0.05). Line bars represent standard deviation.

We found that the species diversity was clearly different at the five forest ages of the chronosequence. As expected, the lowest species diversity occurred in pastures. The highest species diversity occurred in mature forest with more than 40 years old (Figure 4).

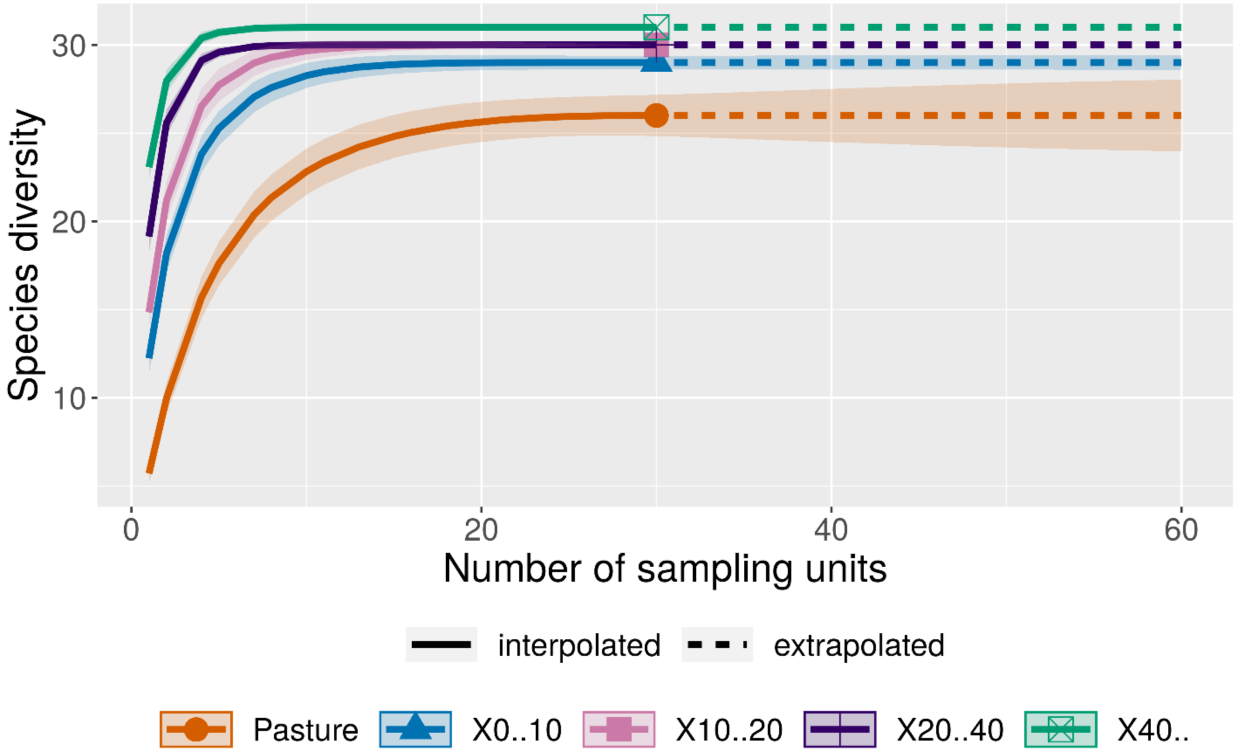

Figure 4: Rarefaction and extrapolation sampling curves in a natural regeneration chronosequence

Arbuscular mycorrhizal fungal community

A total of 44 VT was recovered in the study area. VT corresponded to *Acaulospora* (6 VT), *Archaeospora* (2 VT), *Ambispora* (1 VT), *Claroideoglomus* (1 VT), *Gigaspora* (1 VT), *Glomus* (31 VT), *Paraglomus* (1 VT), and *Rhizophagus* (1 VT) genera. The genus *Glomus* (71 percent of total richness) was the most abundant, followed by the genus *Acaulospora* (14 percent).

Pastures and young secondary forests presented higher abundance values of AM fungi than mature forests, with significant differences between pastures and forests with more than 40 years of age ($p = 0.002$). AM fungal community of pastures was dominated by the genus *Glomus*. Other AM genera appeared early in the natural succession process, maintaining this genera diversity though all successional and mature forests.

Rarefaction curves indicated that sampling was not enough to estimate the richness for this microbiological community (Figure 5). AM fungal communities being different at the five forest stages of the chronosequence. AM fungal diversity, as VT richness, was significantly more diverse in pastures and young secondary forest soils than in mature forest soils ($p = 0.034$).

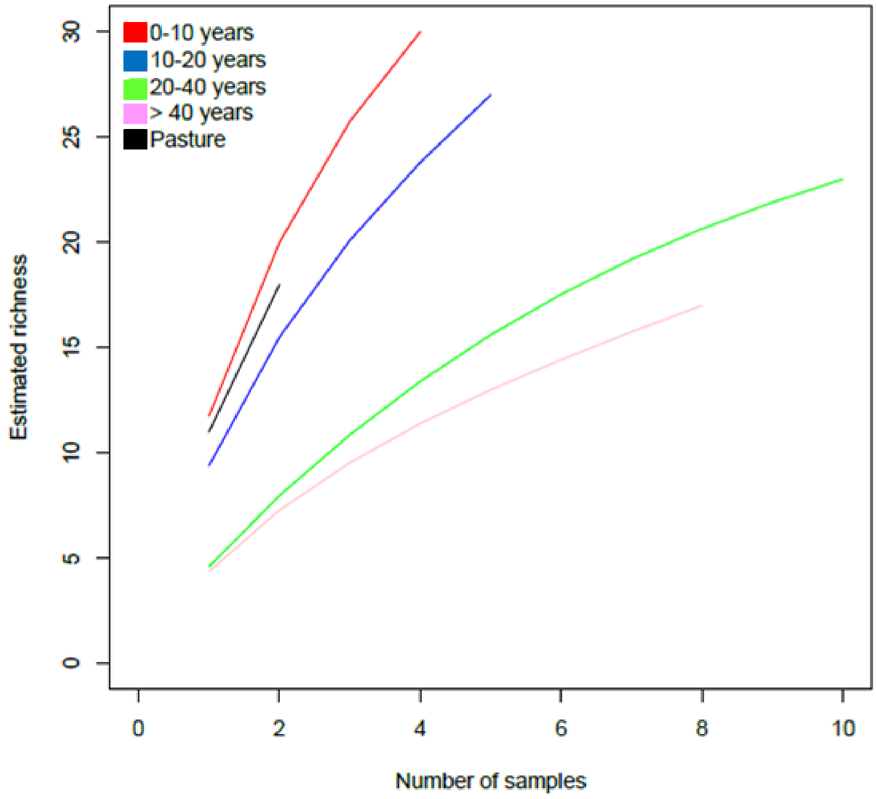

Figure 5: Rarefaction curves for arbuscular mycorrhizal virtual taxa richness in a natural regeneration chronosequence

It is interestingly to observe, that there are dominant VT in pastures that endure in the different stages of the regenerating chronosequence, and also dominant VT that only appeared in older stages of the regenerating chronosequence, being indicators of mature forests (Table 2). We also found one VT that was present in mature forest but not in pastures or regenerating forests (*Acaulospora* sp. VT102), indicating that there are some VT that are never recovered through the time.

Table 2: Richness (as VT average), abundance (as number of sequence reads average) and most representative arbuscular mycorrhizal fungi in a natural regeneration chronosequence

	Pasture	**0-10 years**	**10 -20 years**	**20-40 years**	**> 40 years**
No. VT	11 a	12 ab	9 ab	5 ab	4 b
Abundance	2,874 a	932 a	2,164 a	163 b	108 b
			Most representative VT		
	Glomus VT126	*G. decipiens* VT39	*Glomus* VT223	*Paraglomus* VT444	*Glomus* VT359
	Glomus VT60	*Glomus* VT89	*Paraglomus* sp. VT444	*Glomus* VT70	*Glomus* VT126
	Glomus VT89	*Claroideoglomus* sp. LH-C101	*Glomus* VT89	*Glomus* VT126	*Glomus* VT292
	Glomus VT70	*Glomus* VT79	*Glomus* VT253	*Glomus* VT359	*Paraglomus* VT444
	Glomus VT398	*Glomus* VT292	*Acaulospora* sp. VT15	*Claroideoglomus* sp. LH-C101	*Glomus* VT79

Note: Letters indicate the significant differences among chronosequence plots. The five more representative VT are ordered according to the abundance of reads in each range of ages. VT that were in pastures and persist through the years are in yellow. VT that are present in mature forest and appeared in old secondary forest are in green.

Discussion

Soil macrofauna and arbuscular mycorrhizal fungi of Amazon soils could be recovered naturally by the natural regeneration of degraded pastures that are abandoned after their cattle ranching use. However, the two biological communities follow different patterns along the natural restoration process.

Ants and termites are the most abundant and diverse groups, where termites are more sensitive to soil changes (Amazonas *et al.*, 2018) than ants. As described before by de Souza *et al.*, (2016), soil macrofauna abundance and species richness are not correlated through the time. In our work after 10 years of natural regeneration, the soil macrofauna abundance is restored, but soil macrofauna richness require a longer period to rich similar values to those found in mature forests. However, some authors indicated that soil macrofauna communities could be restored in a shorter time when active processes are performed (e.g. using agroforestry systems; Barros *et al.*, 2002; Barros *et al.,* 2003). The soil macrofauna of old secondary forests is never as diverse as in non-disturbed Amazon forest, indicating that some species might disappear permanently. The consequences of the loss of those species is still unknown.

AM fungi being more abundant and diverse in pastures and young secondary forest soils than in mature forest soils. Results are the reflect of the type of sample used to study AM fungal communities. The soil contains less AM fungal biomass due to the obligate root endophyte condition of mycorrhizal fungi (Helgason and Fitter, 2009), and consequently, a lower concentration of AM fungal DNA that is reflected as a more abundant and diverse AM fungal community in plant roots than in the soil (Saks *et al.*, 2014; Varela-Cervero *et al.,* 2015). Because of that, the AM fungal community into the soil only represents a fraction of the total AM fungal community hold into the plant roots. However, in pastures, disturbed ecosystems and early successional stages of the Amazon region, the production of AM fungal spores is greater (Leal *et al.*, 2013; Stürmer and Siqueira 2011) in response to the stressing conditions of soil, a change in the plant composition offering more host plants to AM fungi to colonize, and a change in the soil pH where pastures and disturbed soils have a less acidic pH which favors AM fungal sporulation.

Contrary to soil macrofauna, abundant and diverse AM communities into the soil reflects a high degree of ecosystem disturbance. AM fungal communities of the five stages of the chronosequence were different. Pasture were dominated by the genus *Glomus* while successional and mature forests had more AM fungal genera. Not all the AM fungal species had the same affinity for all plants (Davison *et al.,* 2020) and their efficiency as symbionts allocating P into plant roots is different too. As it is not possible to know the status as symbiont of the AM fungal species lost to estimate the consequences for the ecosystem or for the nutrition of particular plant species.

Conclusions

Degraded pastures that are abandoned for their natural regeneration could restore their biological communities naturally through the time. Restoration of biological communities take more than 20 years to reach similar species richness than non-disturbed Amazonian forests, but species composition will never be the same. Changes in soil macrofauna and AM fungal composition could have consequences that have not been estimated yet.

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Mass multiplication of native soil mesofauna for re-introduction in the degraded agro-ecosystems

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Abstract summary

Soil organisms are essential components of agro ecosystems. There is a worldwide concern regarding the impact of modern farming practices on soil biological fertility due to loss of soil biodiversity. Organic and bio-dynamic farming are being promoted to conserve biodiversity. However, these practices support existing soil biota in the degraded agro ecosystem and empirical evidence of beneficial effect on soil mesofauna diversity is less. The aim of this investigation was to evaluate the locally available suitable media for mass multiplication of native soil mesofauna. Farmyard manure (FYM), coconut coir peat (CP), soil and various combinations with household vegetable wastes harboured higher mesofauna abundance throughout the year compared to the peak mesofauna abundance period in the undisturbed natural ecosystems. Major taxonomic group of soil mesofauna thrived well and diversity was almost similar to natural ecosystems.FYM and equal proportion of CP+FYM+soil documented higher abundance of mesofauna. Higher diversity was observed in 75 percent CP+25 percent FYM combination. Soil alone supported less abundance but more diversity of organisms compared to other media.

Keywords: Native mesofauna, Mass multiplication, Coir-peat, FYM, Vegetable waste

Introduction

Soil organisms’ plays a major role in the improvement of soil physio-chemical properties. Decomposition of organic materials is largely a biological process, with three major determinants of the rate of the process soil organisms, the physical environment and resource quality (Swift, Heal and Anderson, 1979). Mesofauna are present in the top layer of the soil plays important role in the decomposition of organic materials. The activity of these organisms depends on the biotic and abiotic factors of the particular location. Long term use of several cultivation practices and change in climate (prolonged drought, flood, soil erosion) also affected soil fertility and sustainability due to loss of soil biota. Organic and bio-dynamic farming are being promoted to enhance soil fertility. However, application of well or partially decomposed farmyard manure or green leaf manures support only existing soil biota in already degraded agro-ecosystems (Kumar, 2004). This supports the earlier findings which indicated the variation in compost biota from fresh heap to fully decomposed stage. Earlier, Keesing and

Wratten (1998) highlighted the importance of indigenous faunal components in ecological restoration in agricultural landscapes for better sustainable function. Mesofauna exist in soil pores and are poor movers. Earlier research also suggests the addition of mesofauna rich soil to the agro-ecosystems along with FYM enhanced the abundance and diversity of soil biota (Kumar, 2004). However, mesofauna rich soil is very limited and is not practical also. The activity of soil organism in the natural ecosystem depends on the soil moisture and temperature in the presence of abundant food (Kumar *et al.*, 2011). With this background, the present investigation was aimed to develop suitable media for mass multiplication of mesofauna with easily applicable and locally available media (coconut coir peat, FYM, household vegetable wastes) without altering physico-chemical properties of the soil after application.

Methodology

The investigation was carried out at the Zonal Agricultural Research Station, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India. The site is located at an altitude of 929 m MSL. The soil is classified as oxic Haplustalf. The soil is lateritic derived from granite gneiss under subtropical semi-arid climate. The topsoil pH is 5.03 with the textural class of sandy-loam. Bi-modal distribution of rainfall accounting 928mm.

a) The experiment was set up with thirteen treatments and three replications.

Each pot size was 30cm width at the top, 20cm width at the bottom and 30cm height. The commonly available soil, coir peat (CP), farmyard manure (FYM) and different combinations of these media were evaluated for mass multiplication of native soil mesofauna. Appropriate quantity of media was filled to each pot (Table 1). The previously known mesofauna rich soil was collected from the undisturbed natural grassland, mixed forest and *Leucaena leucocephala* plantations and mixed well. About 400g of such soil was placed on the surface of the media in each pot in the first week of November 2017. Household vegetable wastes (250g) were placed on the surface of the media in each pot at a weekly interval. These pots were watered (1litre/pot) daily. The samples (200g) were drawn from each pot at the monthly interval after introduction (MAI) up to 1 year and two months once after 1 year for mesofauna extraction.

Table 1: Details of the treatments

No.	Treatment	Quantity (Kg/pot)
1	Soil alone	4.6
2	Coir peat alone	0.7
3	FYM alone	3.0
4	75 % Soil + 25 % CP	3.44+0.18
5	50 % Soil + 50 % CP	2.3+0.35
6	25 % Soil + 75 % CP	1.14+0.53

7	75 % CP + 25 % FYM	0.53+0.74
8	50 % CP + 50 % FYM	0.35+1.5
9	25 % CP + 75 % FYM	0.18+2.24
10	75 % Soil + 25 % FYM	3.44+0.74
11	50% Soil + 50 % FYM	2.3+1.5
12	25 % Soil + 75 % FYM	1.14+2.24
13	33.3 % Soil + 33.3% CP + 33.3% FYM	1.38+0.21+0.9

b) Back yard plot

A 25sq.m plot was levelled and spread vegetable wastes on the surface of the soil. About 4kg of mesofauna rich soil (as mentioned in 7cabove experiment) was spread on the surface of the plot and watered daily. Vegetable wastes were spread at weekly interval. Four soil samples (400g) were drawn (0-15 cm) at monthly interval upto 12MAI and two months once after 1year for mesofauna extraction. Weeds growing in the plot were retained to enhance the plant diversity.

Mesofauna extraction

The mesofauna were extracted from the samples using Rothamsted modified Mc Fadyen high gradient funnel apparatus. Extracted mesofauna were collected in a vial containing 70 percent ethyl alcohol after 48 hours. A stereo binocular microscope (35X magnification) was used for sorting out the mesofauna into different taxonomic groups. The abundance of mesofauna is expressed in number per 400g soil.

Statistical analysis

To test for the effect of the experimental factors (media and sampling period) in the variables analysed, a two-way ANOVA with interaction term was performed using SPSS statistics 23.0 software. Values are given as mean ± SD in experiment b. Simpson diversity indices were worked out in each treatment (Simpson, 1949).

Results

a) Soil mesofauna abundance

The significant difference in the mean mesofauna abundance was observed among the media (Figure1). FYM harboured significantly higher mesofauna abundance than the rest of the media. Equally no significant media effects on soil mesofauna abundance were observed among the combination of media except in soil alone and equal proportion of soil+FYM. Latter two treatments were on par in abundance and soil alone had least abundance. However, it possessed higher abundance than the mesofauna peak abundance period in the natural ecosystems. FYM followed by media with an equal proportion of soil+CP+FYM and CP alone. Latter two media were on par in abundance with the rest of the combinations.

Abundance varied from 113.84 (18MAI) to 238.60 (9MAI) mesofauna /400 g soil during experiment period. Significant difference in abundance was noticed among the period of sampling.

Diversity of mesofauna

The mean mesofauna diversity index was significantly higher in the media with 75 percent CP+25 percent FYM and was on par with equal proportion of soil+FYM (Figure 2). Least diversity was observed in the media with equal proportion of CP +FYM. However, it was on par with majority of the media combinations including soil, FYM and CP alone media. Further, it was almost equal to peak diversity in natural ecosystem. The dominance of different taxonomic groups in the best and least media is given in the table 2. Significantly higher diversity index was noticed even after 24MAI compared to 12 MAI.

b) Back yard

Abundance: The mean mesofauna abundance varied from 56.75 to 101.75/400g soil (Figure 3). The peak and least abundance were noticed at 18 and 1MAI, respectively.

Diversity: The mean diversity of mesofauna was maximum at 18 MAI compared to 12 and one MAI (Figure 4) but very close to diversity in natural ecosystems. Soil organism’s dominance are given in the table 2.

Table 2: Percent composition of soil organisms across media at 18 MAI

Sl. No.	Taxonomic group	Occurrence of soil organisms (%)			
		Natural ecosystem at peak activity *	Best media (FYM alone)	Least preferred media (soil alone)	Back yard
1.	Cryptostimatids	39.09	5.0	2.44	16.39
2.	Mesostigmatids	8.12	8.0	2.44	13.66
3.	Other acari	5.08	21.0		
4.	Podurids	3.55	19.0	30.49	9.84
5.	Entomobryids	6.09	5.0	3.66	8.19
6.	Onychiurids	24.88	4.0	34.15	37.16
7.	Symphylans				6.01
8.	Pseudoscorpions			1.23	
9.	Isopods			2.44	1.64
10.	Psocids	1.01			1.09
11.	Centipede larvae	2.03		1.23	2.19
12.	Earwigs			2.44	1.09
13.	Spiders	0.51			0.55
14.	Hemipterans			2.44	0.55
15.	Dipterans maggot	0.51	8.0	2.44	
16.	Coleopteran grubs	3.04	12.0	2.44	1.64
17.	Ants	6.09	18.0	9.76	
18.	Earthworm immature			2.44	

**L. leucocephala* plantation

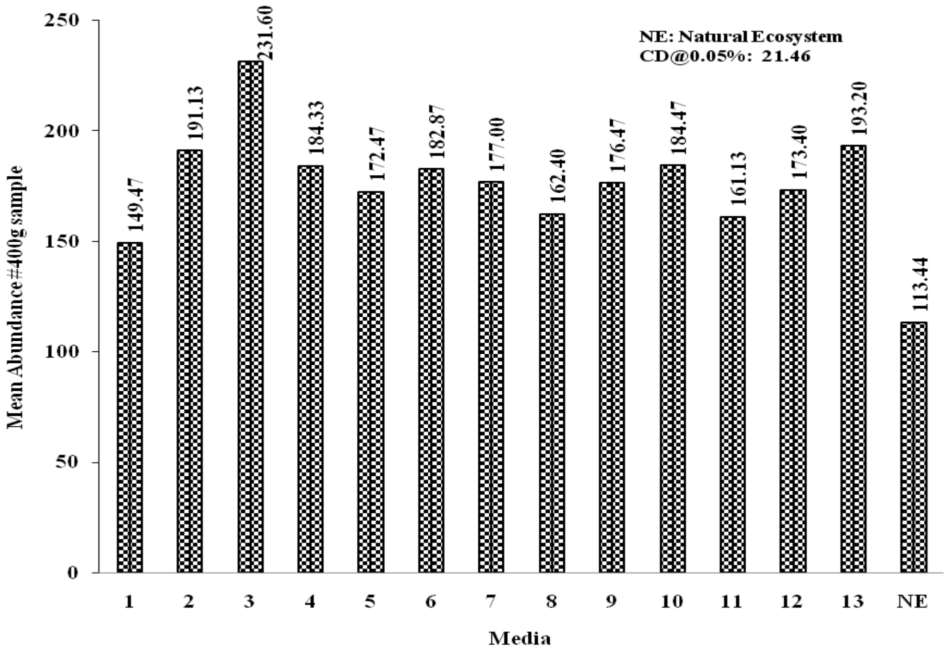

Figure 1: Mean mesofauna abundance in different media

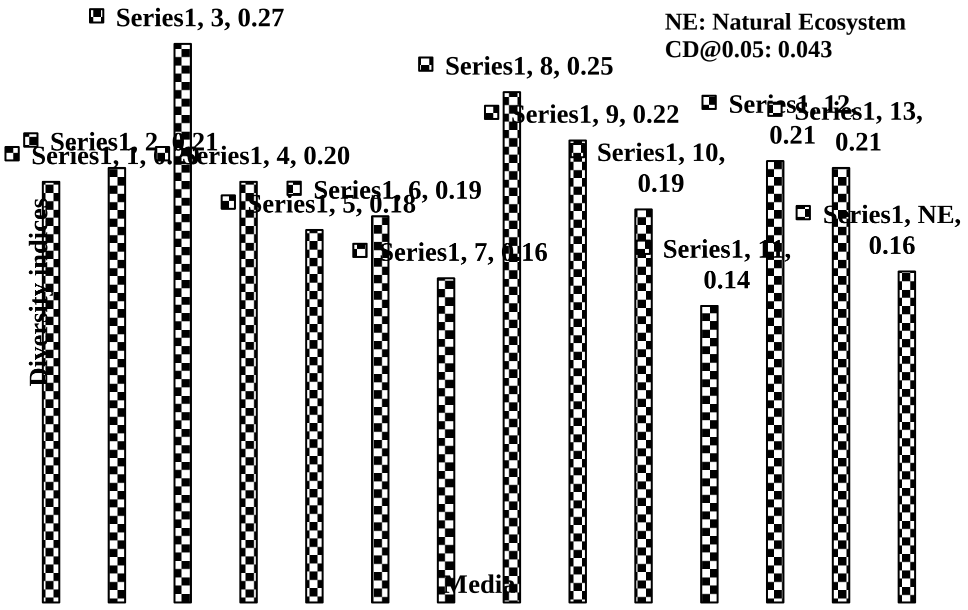

Figure 2: Diversity indices in different media

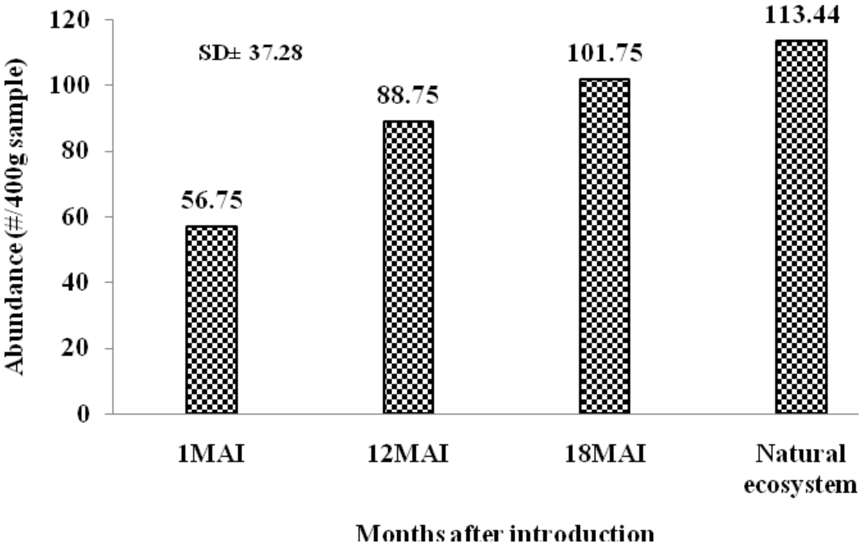

Figure 3: Abundance of soil mesofauna in backyard soil samples

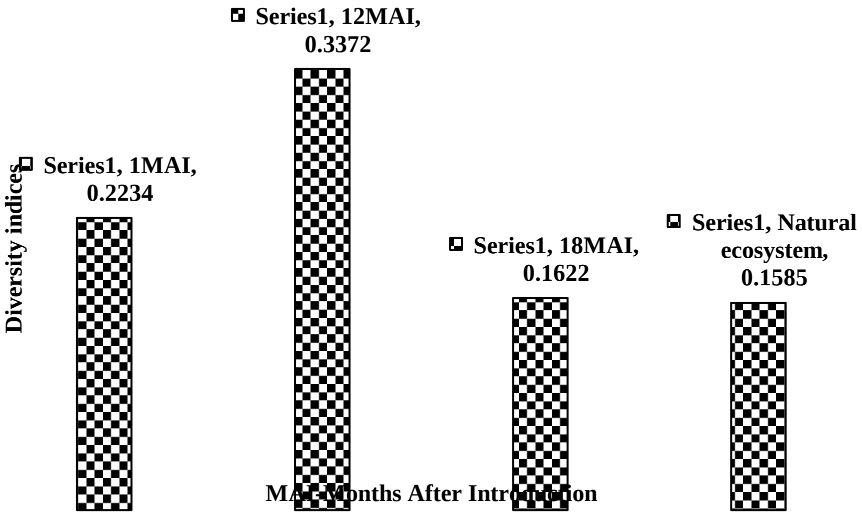

Figure 4: Diversity indices in backyard soil samples

Discussion

This study have demonstrated the benefits of mass multiplication of native soil mesofauna for re-introducing in the degraded agro ecosystem for enhancing richness and abundance. Higher mesofauna abundance in all the evaluated media was observed throughout the year compared to peak activity at the end of rainy season in the natural ecosystems. This is related to an improved microclimate (moisture and temperature) and access to food without habitat disturbances are known to positively influence the diversity and abundance of soil mesofauna communities. FYM media favoured major mesofauna viz., Acari, Collembola and attracted more of dipterans and coleopterans. This media is known to support microflora which resulted in more of mycophytic mesofauna. The predatory ants and mesostigmatids might have affected the population of prey. Accumulation of vegetable waste residue is important factor in ameliorating temperature and moisture content especially in FYM and CP based media, thus providing a more stable environment for mesofauna compared to soil alone. However, soil provided a stable population of many taxonomic group similar to natural ecosystems but less abundance than FYM and CP.

Conclusion

This study assessed how the native mesofauna can be mass multiplied in Coir peat, FYM, soil and combinations of media by utilizing household vegetable wastes. Results showed that 1) all media harboured higher mesofauna abundance throughout the year compared to mesofauna of natural ecosystems at peak activity period. Coir peat is suitable for urban dwelling. An agency can collect the mass multiplied mesofauna rich media and distribute to the farmers or can be used in terrace or backyard gardens. 2) Soil mesofauna diversity can be maintained in an institute to protect the indigenous soil mesofauna for future use. 3) The farmers can use backyard or farmyard manure heap to multiply native soil mesofauna. Mesofauna rich coir peat or FYM or combination of these two can be easily applied in the degraded land without adverse effect on the soil properties. The diversity of mesofauna and micro flora can be replenished by introducing small quantity of topsoil and litter of the undisturbed ecosystems.

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Cultural ecosystem services of soil biota and possibilities of their use

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Abstract summary

Until recently, cultural ecosystem services (CES) provided by soil biodiversity remained virtually unknown. We have reviewed multilingual and multisubject literature on cultural benefits provided by soil biota in European forests. We found 226 papers mentioning impact of soil biota on the cultural aspects of human life. According to the reviewed literature, soil organisms contribute to all CES, although their weight (expressed as a number of references found) was different for individual CES and individual organism groups. Soil biota is an important supplier of CES but they are generally not well understood by the general public. We propose the measures to raise public awareness of soil biota and the significance of its diversity by using knowledge gained from the CES review and the CES themselves.

Keywords: soil biodiversity; soil ecosystem services; Europe; public awareness

Introduction, scope and main objectives

The idea of ecosystem services (ES) was originally coined to quantify the benefits that natural ecosystems generate for human society (Westman, 1977). The aim of this effort was to raise the public awareness for the value of biodiversity and conservation of ecosystems. And, though the ES paradigm has recently begun to receive criticism for its anthropocentrism and monetization of ecosystems (Silvertown, 2015), it is still a good tool to explain importance of biodiversity to politicians, decision makers and the general public. Cultural ES (CES) stand out among other ES in the intangibility of their benefits and the "non-use values" for most of them (Burkhard *et al.*, 2014). On the other hand, many researchers recognize them as one of the strongest arguments for ecosystem conservation (Hernández-

Morcillo, Plieninger and Bieling, 2013), and CES are even used as a counterargument in the discussion against the utility of ES (Schröter and Oudenhoven 2016).

Until recently, CES provided by soils were understood in a rather vague way, with occasional comments that soils are archives of archaeological heritage and notes of spiritual-religious meanings of soil, or they were seen as secondary, derived from another ES provided by soils. It was even stated that "Soils … contribute to cultural services although to a rather minor degree…" (Lavelle *et al.,* 2006). Thus, the perception of CES from soils was rather biased towards abiotic structures and processes, in contrast to the usual classification and assessment of ES in which biota play the main role as service provider (Van der Meulen, Braat and Brils, 2016). This is surprising given the estimate that about 25 percent of the species on earth live in the soil (Jeffery *et al.,* 2010), not counting plant root biodiversity. In our review we aimed to identify the CES of soil biota (limiting to European forests) and to highlight the importance of belowground diversity on human culture and well-being.

Methodology

Based on 11 types of CES (following MEA, 2005) we have evaluated six groups of belowground biota: roots, fungi, microorganisms, mesofauna, macrofauna and megafauna. For literature analysis, we conducted a reference search in Clarivate Analytics Web of Knowledge and Google Scholar based on a system of keywords in 13 languages. Additional literature and in some cases examples from other sources were found by snowball search and expert suggestions.

Results and discussion

The reference search resulted in 226 papers, which were further examined. Soil organisms contributed to all CES, although their weight (expressed as numbers of references found) was different for individual CES and individual organism groups. Based on the reviewed literature, the highest impact was found for cultural diversity and the lowest for aesthetic values. Of all soil organisms, fungi had the highest impact on CES, while microorganisms and mesofauna had the lowest (Figure 1).

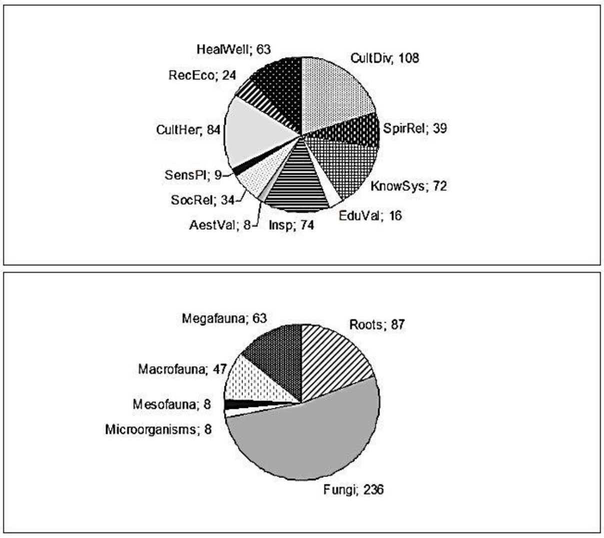

Figure 1: Distribution of references according to (a) cultural ecosystem services (CES) and (b) organism groups

Acronyms of CES are as follows: CultDiv – Cultural diversity, SpirRel – Spiritual and religious values, KnowSys – Knowledge systems, EduVal – Educational values, Insp – Inspiration, AestVal – Aesthetic values, SocRel – Social relations, SensPl – Sense of place, CultHer – Cultural heritage values, RecEc – Recreation and ecotourism, HealWell – Health and wellbeing (from Motiejūnaitė *et al.,* 2019).

These "cultural divisions" can largely be explained by the fact that most of the CES is based on folk perception of nature, i.e., on the cultural recognition of biota, which is governed by the salience of different taxa, which Hunn (1999) classified into phenotypic, perceptual, cultural, and ecological categories. Hence, folk recognition of organisms is based on: 1) economical salience; 2) morphological/behavioural salience; iii) ecological/geographical salience; iv) size salience. The latter is especially evident: microscopic species are "invisible" and therefore non-existent.

In any case, our review has shown that soil biota is an important supplier of CES and these CES are less controversial than those based on landscape values. However, CES derived from soil biota are generally not well understood. Problems in the evaluation and understanding of CES derived from soil biota by the general public can be due to several reasons: 1) different values and understanding of CES by beneficiaries; 2) temporal and spatial fluctuations of CES and their regionality; 3) people generally do not associate CES with soil.

In order to improve the situation, we propose several measures: 1) using CES as promotional tool to draw public attention to soil biodiversity, its significance and its interactions; 2) creating new and expanding existing educational materials to show soil organisms and their connection to soil; 3) promoting and supporting citizen science activities in the field of soil biodiversity recording (in biodiversity recording platforms and individual projects).

Conclusions

Identified CES will not only supplement evaluations and mapping of ES (including CES) provided by soils, but may also help to raise public awareness of soil biota and the significance of its diversity. The importance of soil biota and its diversity needs to be given more emphasis in educational programmes and public awareness campaigns. It is particularly important to close the gaps in public knowledge about soil biota interactions and their importance for soil quality.

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Vermicompositng: An alternative and complimentary eco-friendly tool for sustainable waste management and soil fertility enhancement

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Abstract summary

It is well known that the fish wastes have been used as organic fertilizer and nutrients for both agricultural purposes and for rehabilitation of degraded areas. Vermicomposting is essentially organic composting through earthworms and an eco-biotechnological process that transforms energy rich and complex organic substances into a stabilized vermicomposts. The composting process kills the pathogens due to the heat generated during the thermophilic phase; the organic compositions in waste will be converted into stabilized humic substances through mineralization and humification with a significant reduction in volume. The vermicompost was prepared from fish waste using three earthworm species namely *Eudrilus eugeniae, Perionyx excavates,* and *Eisenia fetida* which was analysed on 30th day for its physico-chemical parameters postulates the composting efficiency of earthworm species. The composted fish waste was found to have improved the green gram (*Vigna radiate)* growth and its soil NPK content was improved. Earthworm nutritional content was good in contents which can be substituted with the meat or in drug discovery. Due to its innate biological, biochemical and physiochemical properties, vermicompost may be used to promote sustainable agriculture and also for the safe management of agricultural, industrial, domestic and hospital wastes which may otherwise pose serious threat to life and environment.

Keywords: Vermicompost, earthworm, nutrition, fish waste, soil biodiversity

Introduction, scope and main objectives

Man knows earthworms ever since he started soiling his hands and called them as “intestine of earth”. The earthworms are natural resources of fertility and life, so they are popularly called as “friends of farmers”. But now it is a time for us to think and work on the utility of earthworms in the area other than farmer’s friend. Earthworms are beneficial macro-invertebrates that contribute to soil fertility through their activities. In addition to their natural functions, such organisms are a source of animal protein in the diets of wild animals and indigenous people (Edwards, 1997). Organic manuring is an important component of low input sustainable agriculture without sacrificing the level of productivity. It can be supplemented to fertilizer applications partially substituting but not completely replacing it. The soil organic matter and nitrogen levels are important for crop production. An odourless innocuous and stable organic amendment can be obtained by composting, and its use for improving soil structure and soil organic matter has been reported worldwide.

India is the third largest producer of fish in the world next only to China and Peru and it ranks second in the production of Inland fishes. Fish sludge contains macro and micronutrients, especially high levels of nitrogen and phosphorus. Fish production has increased from 0.75 million tons in 1950to 6.90 million tons in 2016-2017, registering a compound growth rate of 4.53 percent per annum which has been the fastest growing one in respect of any item in the food sector. In the present investigation three species of earthworm namely *Eudrilus eugeniae, Perionyx excavates,* and *Eisenia fetida* was subjected for decomposing fish waste whose maturity was analysed with the following issues (i) Determination of physic-chemical parameters of vermicomposted fish waste (ii) Nursery application of vermicompost for green gram (iii) Analysis of untreated and post harvested soil (iv) Nutritional estimation of fish waste ingested earthworms compared with control.

Methodology

Eudrilus eugeniae, Perionyx excavates and *Eisenia fetida* were collected from Nonconventional Energy Resource and Development (NERD) Society, Vadavalli, Coimbatore, South India. A wooden box of size 60 x 40 x 30 cm was collected from fruit shop near Lawley Road, Coimbatore, South India. Bottom of this wooden box was completely covered with a wet sac. A layer of straw was laid down above this; a layer of pretreated fish waste and cow dung was laid and inoculated with earthworms. Finally a layer of goat dung was laid down. The wooden box was covered with a sac at the top in order to protect and prevent the escape of worms. The bed was occasionally mixed and water was sprayed frequently for 30 days. The compost was collected on 30th day of inoculation and sun dried for analysis like physico-chemical parameters like Bulk density, Particle density, Water holding capacity, Organic carbon, Humic acid, Nitrogen, Phosphorus and Potassium. Then vermicompost was applied to pot culture sown with green gram and compared with chemical fertilizer containing NPK. The efficiency of vermicompost as organic manure was elucidated using plant height, biomass and yield characters. The pre and post harvest soil also evaluated for its NPK content. Finally, Carbohydrate, Glucose, Total lipids, Free fattyacids, Triglycerides, Protein and Total free aminoacids were studied to explore the nutritional content in pre and post fish waste ingested *Eudrilus eugeniae, Perionyx excavates* and *Eisenia fetida.*

Results

The fish waste subjected for vermicomposting was analysed for its maturity on 30th day of composting whose results are given in table 1.

From physic-chemical results it is evident that the *Eudrilus eugeniae* was efficient in composting fish waste than other species used. Table 2&3 gives the Plant height and biomass of green gram. In that the height and biomass of green gram plant was more or less similar in all three-compost applied when compared to chemical fertilizer. Yield characters like pod, seed numbers and pod length were also postulating the efficiency of vermicompost. Table 4 indicates the NPK content of pre and post harvested soil of green gram on 0 to 65th day of harvest. Finally, it is evident from Table 1 that the proximate composition and nutrient composition of earthworms were higher and the content of carbohydrates, glucose, total lipids, free fatty acids, triglycerides, protein and total free amino acids, respectively.

Discussion

The degradation of our natural resources and environmental pollution are major problems in soil productivity. Vast area of lands is degraded, some may be irreversibly due to wide range of degradation process like accelerated erosion and desertification while others owing to declining of organic matter, depletion of soil fertility and biodiversity. Degradation of soil is expressed in terms of its direct impact on crop productivity, its quality and environmental pollution and indirect on human as well as animal health. Soil productivity is the capacity of soil to produce a certain yield of crop plant under optimum set of conditions and management practices. Soils provide physical support to plants, animals andhuman infrastructures (Dominati, Patterson and Mackay, 2010).

The induction of plant growth by vermicompost might be due to its rich fish nutrients and organic nature. It provides appreciable amount of NPK and micronutrient for root and shoot growth (Bhawalkar, 1992). The maximum plant height recorded by green gram in the present study might be due to supply of required nutrients from the applied composts. Generally the physic-chemical parameters have some components, which enter the plant during early stages of growth and have some additional sources of polyphenol that act as respiratory catalyst for plants and metabolism. In present investigation the nitrogen, phosphorus and potassium content and these values are comparable with the compost prepared [N (1.38 percent), P (0.77 percent) and K (1.20 percent)] from leaf litter (Umamaheswari, 2005). The increase in NPK content might be due to solubility effect of certain organic acids released during the decomposition of organic matter present in the vermicompost. A similar finding has been reported by Hangarge *et al.* (2004) who have indicated that application of either organic alone or combination of organic and inorganic fertilizers proved to be better in enhancing the availability of NPK and organic carbon content in soil.

Although many years ago the inclusion of earthworms in the diet of humans has been reported, little is known about the nutritive value of these edible invertebrates (Wallace, 1853). Evaluation of the nutritive value of earthworm becomes important as it could form a base for new food/feed product of considerable nutritive value. The use of earthworms is believed to extend beyond the nutritional value.

Traditionally, many claim that these worms have medicinal properties. Therefore, the study was conducted to determine selected nutritional properties of the earthworms. Furthermore, the studies of Albarran (1996); Dynes (2003) and Vielma-Rondon *et al.* (2003) had shown that not only could earthworm serve as a rich protein source but also as a source of essential amino acids, especially lysine which is limiting in many basic foodstuffs and that the amino acid composition of earthworm meal is very similar to that of fishmeal and potentially superior to that of meat meal.

Conclusions

Vermicomposting is a cost-effective and eco-friendly waste management technology which takes the privilege of both earthworms and the associated microbes and has many advantages over traditional thermophilic composting. Vermicomposts are excellent sources of biofertilizers and their addition improves the physiochemical and biological properties of agricultural soil. Vermicomposting amplifies the diversity and population of beneficial microbial communities. The study also concludes that earthworm fed fish has good nutritional impact which can be substituted with animal and human diet.

Table 1: Determination of physic-chemical parameters of vermicomposted fish waste using Eudrilus eugeniae, Perionyx excavates and Eisenia fetida

PHYSICO-CHEMICAL PARAMETERS	VERMICOMPOST SPECIES		
	Eudrilus eugeniae	***Perionyx excavates***	***Eisenia fetida***
Bulk density (g/cc)	0.50	0.39	0.36
Particle density (g/cc)	1.01	0.94	0.94
Water holding capacity (%)	56.0	55.21	55.78
Organic carbon (g %)	9.20	8.93	8.89
Humic acid (g %)	5.00	4.97	4.99
Available Nitrogen (g %)	1.11	1.03	1.00
Available Phosphorus (g %)	1.00	0.98	0.99
Available Potassium (g %)	1.00	0.91	0.96

Table 2: Nursery trials on green gram (Vigna radiate) height and biomass applied with vermicompost and chemical fertilizer

TREATMENTS	PLANT HEIGHT			FRESH WEIGHT			DRYWEIGHT		
	DAYS AFTER SOWING								
	25	45	65	25	45	65	25	45	65
C- control	27.60	32.20	37.73	0.54	0.87	1.42	0.12	0.70	0.20
Chemical fertilizer	29.60	35.40	42.86	0.86	1.20	2.28	0.63	1.15	0.40
Eudrilus eugeniae	29.40	30.20	39.20	0.67	1.08	1.89	0.60	1.00	0.44
Perionyx excavates	27.10	31.80	39.10	0.56	1.02	1.85	0.47	0.98	0.38
Eisenia fetida	25.00	34.60	39.60	0.71	1.23	1.65	0.51	1.11	0.31
	D**	T**	DT**	D**	T**	DT**	D**	T**	DT**
SED	3.80	7.28	12.61	0.12	0.24	0.42	0.67	0.12	0.22
CD (P < 0.05)	7.59	14.54	25.18	0.25	0.48	0.84	0.13	0.25	0.44

Table 3: Determination of yield characters of green gram (Vigna radiate)

TREATMENTS	NUMBER OF PODS/PLANT	POD LENGTH (cm)	NUMBER OF SEED/POD
C- control	2.0	3.50	4.0
Chemical fertilizer	7.5	3.85	9.0
Eudrilus eugeniae	9.5	5.20	10.5
Perionyx excavates	6.0	4.80	8.5
Eisenia fetida	6.0	4.80	9.0

Table 4: Analysis of untreated and post harvested soil

TREATMENTS	pH	N	P	K
Preharvested soil	7.48	120.0	6.0	215
POST HARVESTED SOIL				
C- control	6.52	139	8.0	478
Eudrilus eugeniae	6.64	187	8.0	500
Perionyx excavates	6.72	167	6.0	476
Eisenia fetida	6.95	164	6.0	500
Chemical fertilizer	6.80	171	8.0	500

Table 5: Nutritional value of earthworm before and after composting fish waste

Parameters	*Eudrilus eugeniae*		Perionyx excavates		*Eisenia fetida*	
Composting period	Before	After	Before	After	Before	After
Carbohydrate	3.21	04.34	3.00	04.01	3.56	04.66
Glucose	02.00	02.33	2.00	02.11	02.23	02.42
Total lipids	0.31	0.48	0.36	0.45	0.32	0.42
Free fattyacids	0.25	0.31	0.27	0.30	0.26	0.31
Triglycerides	0.19	0.23	0.15	0.22	0.17	0.23
Protein	4.85	05.21	4.33	05.81	4.48	05.00
Total free aminoacids	1.96	2.31	1.00	2.00	0.96	2.01

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Marine soil biodiversity as a key factor for ecosystemic health

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Abstract summary

Marine soil hosts a plethora of organisms, which are grouped using the term "benthos". Benthic communities are fundamental to marine ecosystems. Autotrophs (fitobenthos) perform photosynthesis and provide energy, while heterotrophs (zoobenthos) are valuable for carbon and nitrogen cycles (Grime, 1979). These organisms are patterned depending on the depth they are found at, the salinity, the water temperature and the size of the bottom clasts.

Zoobenthic communities are essential for a variety of ecosystem services, including faster decomposition of organic detritus, which is their main source of nutrition. These organisms process the detritus and return important components to the marine environment, providing energy sources for other species. This leads to an increase of organisms able to consume these nutrients, which in turn are then food sources for larger species as well as fundamental links of food webs, as some of the benthic invertebrates are also predators. An alternation in the levels of benthic biodiversity can lead to dramatic effects on an entire ecosystem. On a general level, a rich biodiversity is a marker for the good health of the marine ecosystem. In fact, benthic communities are often used to describe underwater environments (marine, fresh and brackish ones).

Keywords: Underwater soil, Marine soil, Benthos, Benthic communities, Ecosystem health, Biodiversity

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Introduction, scope and main objectives

The aim of this presentation is to summarize the main concepts and knowledge about biodiversity in underwater marine soils. There will be a summary of the terms used to describe animals and plants benthic communities, with an exposition on the importance of having a high biodiversity for both environmental and economic reasons.

Methodology

The knowledge reported in the presentation has been built during the academic career of the candidates, with a particular highlighting on the experimental thesis works done during the university cycle of studies (Barbour *et al.*, 1999; Castelli, Lardicci and Tagliapietra., 2004; Toso, 2019). Some integrations are reported from the main scientific papers on the topic.

Results

Benthic communities

Benthic communities are defined as the set of species that live in close contact with the seabed and interact directly or indirectly with each other, in a marine, freshwater or brackish environment (Barbour *et al.,* 1999; Toso, 2019). These organisms can be both animal and plants and are classified according to their size in micro-, meio- and macrobenthos (Grime, 1979; Dauer, 1993). Benthic populations can represent real biodiversity hotspots, with a very high number of species in a very small portion of seabed (Toso, 2019). Fitobenthic communities can also be at the base of ecological successions similar to those of higher plants on the mainland, with the presence of real climax species (e.g. meadows of the marine angiosperm *Posidonia oceanic* (Linnaeus) (Delile, 1813), or forests of Phaeophiceae algae) (Airoldi and Beck, 2007; Toso, 2019).

Biodiversity as health indicator

Many papers and studies have reported a clear relation between the trend of biodiversity in benthic environment and the health status of the entire system in contact with the seabed communities (Dauer, 1993; Barbour *et al.,* 1999; Borja, Muxika and Rodriguez, 2009). In particular, zoobenthic communities have been used for years to describe the health status of underwater coastal environments with excellent results in terms of replicability, costs and ease of sampling (Dauer, 1993; Castelli, Lardicci and Tagliapietra., 2004). Parameters such as number of species (richness), species distribution (evenness) and the presence or not of indicator species for ecological status of the environment were considered (Borja, Muxika and Rodriguez, 2009). Same results have been found in brackish and freshwater ecosystems.

Discussion

A good status of benthic communities is fundamental for all the effects on the trophic chain, including organisms farmed or fished by humans (Smith *et al.,* 1998; Borja, Muxika and Rodriguez, 2009). Nowadays aquaculture is a sector in high expansion due to the limited fishing stocks and many culture techniques in lagoons or in open sea involve stages closely related to the seabed (e.g. *Ruditapesphilippinarum* (Adams & Reeve, 1850) (cited in Airoldi and Beck, 2007). A constant monitoring is therefore essential for the preservation of the seabed, habitats as important as they are fragile, both from an economic point of view for the health and growth of the organisms caught and bred, and from a strictly environmental point of view (Airoldi and Beck, 2007; Borja, Muxika and Rodriguez, 2009). Benthic ecosystems have in fact a role key in the recycling (consumption) and in the remineralization (bioturbation) of the organic substance, in the metabolization of the chemical compounds present in the sediments or at the water-sediment interface, as well as in the primary productivity, always present although lower than the phytoplanktonic one (Dauer, 1993; Borja, Muxika and Rodriguez, 2009).

Conclusions

Pursuant to European Union Water Framework Directive (WFD, 2000/60/EC), the member states of the European Union are headed towards achieving a good state of all water bodies, including marine waters up to a mile from the coast. Therefore, it can be deduced that a maintenance, or even an increase in the monitoring operations of the benthic communities can guarantee an ever-greater knowledge of the health of the water bodies. The purposes of this monitoring will have managerial and conservation aspects, considering the exceptional importance of these environments.

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Effect of shifting cultivations on bacterial communities in Hurulu forest of Sri Lanka: a metagenomic approach for diversity estimation

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Abstract summary

Soil bacterial communities contribute to maintain the health and productivity of terrestrial ecosystems. Anthropological soil disturbances can alter soil microbial community composition and structure which can lead to a chain-reaction on entire ecosystem. Current study used a metagenomic approach for measuring the level of diversity in soil bacterial populations as affected by disturbances due to shifting cultivation in 'Hurulu' nature reserve, a dry mixed evergreen forest in Sri Lanka. Soil samples were collected in triplicates from 0-5 cm depth from deep forest, re-generating forest after abandonment from shifting cultivation and an area under shifting cultivation for two years. Total soil DNA was extracted and V3-V4 region in 16SrRNA gene was sequenced using Illumina MiSeq platform with bacteria specific primers. DADA2 pipeline and MG-RAST server were used for sequences analysis. Soil sample from deep forest had the highest α bacterial diversity and samples from regenerating forest soil demonstrated the lowest α diversity as inferred by Shannon and Simpson indices. Orders, Actinomycetales, Bacillales and Burkholderiales were commonly found in all samples. *Bacillus cereus* was the dominating Bacillales in all the locations. *Bacillus pumilus, Bacillus megaterium, Bradyrhizobium japonicum* and *Bradyrhizobium canariense* showed divers distribution among samples. Results of this investigation indicated that forest disturbances adversely affect the diversity of soil bacteria.

Keywords: Soil disturbances, Metagenomics, Dry-evergreen forest, 16SrRNA, DADA2 and MG-RAST

Introduction, scope and main objectives

Bacteria are among the most numerous soil inhibiters and their relationship with other organisms shape the characteristics of each ecosystem. Studies indicated that bacterial communities get drastically affected by soil disturbances such as agriculture, fire and flood. Investigating the damage occur form anthropological disturbance on soil bacterial communities and the extent they recover is valuable to formulate and implement future conservation strategies. Soil biodiversity of dry forested ecosystems, which spread over 22 percent of area in Sri Lanka, where the total forest cover is 26.6 percent, has not been studied in detail.

This study focused on Hurulu dry-mixed evergreen forest in Sri Lanka, which is declared as an International Biosphere Reserve and contribute to approximately 1.5 percent of forest cover of the country. Only about 50 percent of the Hurulu forest is relatively undisturbed from anthropogenic activities and the remaining extent shows stresses enforced by socio economic factors on the forest reserve and include regenerating forest and areas that have been used for crop cultivation (Jayasuriya, Kitchener and Biradar, 2011). Conventional culture based microbiological techniques severely underestimates the true soil microbial diversity and it is time consuming (DeLong and Norman 2001; Angel *et al.,* 2010). Molecular techniques like16S rRNA gene based metagenomic approach has been effectively used in estimating soil bacterial diversity (Kato *et al.,* 2015).

Hypothesis: Forest disturbances reduce the soil bacterial diversity in Hurulu dry mixed evergreen forest in Sri Lanka.

Objectives: To determine the species composition of soil bacterial communities inhabiting Hurulu International Biosphere Reserve in the Dry Zone and to assess the effect of forest disturbances on soil bacterial diversity.

Methodology

The Hurulu International Biosphere Reserve of Sri Lanka was used as the study site. A transect across the forest including relatively undisturbed forest, regenerating forest after abandoning shifting cultivation and an area under active cultivation were selected for sampling to assess the effect of forest disturbance on soil bacterial communities in this forested ecosystem. For sampling, 10 x 10 m quadrats were identified in triplicates from each land-use category and soil samples were collected at 0-5 cm depth using an auger. DNA extraction was done using MoBioPowerSoil DNA extraction kit using manufacture’s guidelines. Sample libraries were prepared targetingV3-V4 regions of the 16SrRNA gene using bacteria specific primers. The output sequence files were demultiplexed and used in the form of FASTQ. Sequences files were analysed by using DADA2 pipeline and MG-RAST server. The forward reads were trimmed as 250th base and reveres reads were trimmed at 200th base and chimera were removed in data processing. Sequences were subjected for rarefaction analysis. After merging forward and reverse reads and chimera removal, taxonomic annotation was done using SILVA_ SSU sequences data base at 97 percent sequence similarity, p-value threshold at OMEGA_C = 1e-40 and OMEGA_A = 1e-40. Shannon and Simpson indices and alpha diversity were calculated for each sample set and two representative samples for each land-use were selected for Principal Coordinates Analysis (PCoA) and construct ordinate graph with MG-RAST server to calculate β diversity. Three abundant orders (Actinomycetales, Bacillales and Rhizobiales) were subjected to further analysis for the genus level and species level information generation.

Results

Highest number of sequence reeds was shown by samples from undisturbed forest (166,358 sequences) and the least number of sequences was observed from re-generating forest (141,984 sequences). The sequence recovery after initial quality controlling was higher than 70 percent for all the sampling sites. Rarefaction curves that illustrate the species diversity on each sample started plateauing after 2 x 10^4 reads indicating that species coverage obtained from each sequence runs were optimum and species richness for each site was exhaustingly sampled (Figure 1).

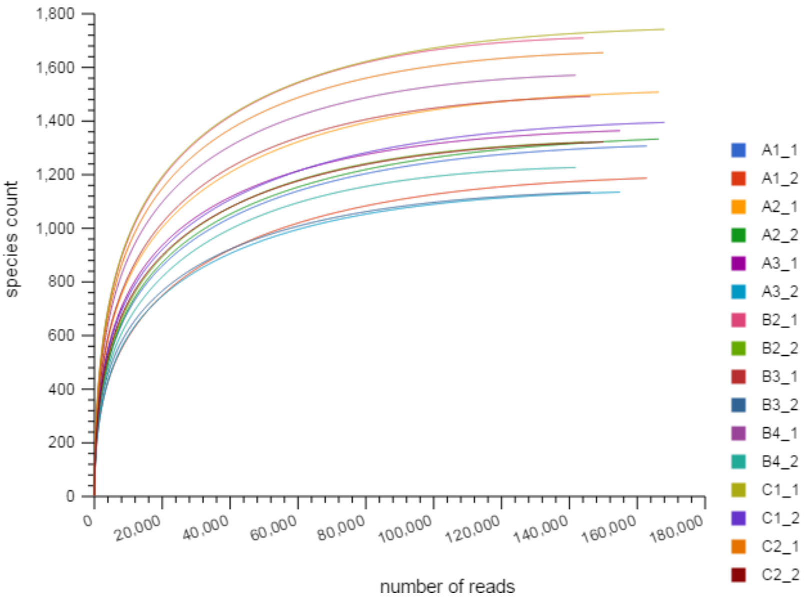

Figure 1: Rarefraction curves developed for soil bacteria in undisturbed forest (A1-A3), Regenerating forest (B2-B4) and cultivated land (C1-C2) using sequences from both forward and reverse reads (denoted by _1 and _2, respectively, after sample ID) in 16S rDNA analysis

In undisturbed forest soil samples, Shannon index ranged from 6.92 to 7.00 and Simpson Index ranged from 0.998 to 0.999. In regenerating forest soil samples Shannon Index and Simpson index ranged from 0.60 to 1.25 and 0.40 to 0.61, respectively. In cultivated area, Shannon Index and Simpson index ranged from 1.05 to 1.44 and 0.63 to 0.68, respectively. The bacterial orders Actinomycetales, Bacillales, Clostridiales, and Burkholderiales were common among all samples. The abundance of members of Actinomycetales were higher in disturbed soils (cultivated area and regenerating forest) compared to undisturbed forest. *Bacillus pumilus* and *Bacillus megaterium,* were relatively abundant in disturbed soil. A member of order Rhizobiales, *Bradyrhizobium japonicum* found to be dominated in the undisturbed sample whereas *B. canariense* found to be dominated in the soil from cultivated land. This indicates that bacterial diversity is high in locations having comparatively stable up-ground diversity than that of re-generating forest soil where up-ground diversity fluctuates.

Discussion

Here we repot for the first time, metagenomics-based soil bacterial diversity in Sri Lankan dry mixed evergreen forest. The α diversity in each sample was estimated by using Shannon and Simpson diversity indices. Shannon index (H’) demonstrated the species richness and evenness in a given sample whereas Simpson diversity index (1-D) considers the dominance of each species over others. The results indicated that soil bacterial diversity can differ even in the same ecological area based on the level of disturbance. The investigation highlighted that the sampling sites with relatively higher forest cover showcased higher species richness, evenness and less domination.

Bacillus megaterium and the *Bacillus pumilus* were found in only in soil samples from area under shifting cultivation. *Bacillus pumilus* is resistant to environmental stress conditions and has been often reported in soil and brackish water environments. A study conducted in Netherlands demonstrated *Bacillus pumilus* can present in relatively higher proportion in agricultural soil and long-term arable lands than other species of the genus *Bacillus* (Garbeva, Van Veen and Van Elsas, 2003). Results indicated that the shifting cultivation influence on Hurulu forest soil facilitated the growth of different *Bacillus* species than those in undisturbed forest soil.

Bradyrhizobium japonicum was predominately recorded in undisturbed forest soil samples. Genus *Bradyrhizobium* and *B. canariense* were recorded from area under shifting cultivation but soil samples from re-generating forest after abandonment from shifting cultivation showed the lowest number of individuals from the genus *Bradyrhizobium*. Both *Bradyrhizobium japonicum* and *B. canariense* are nitrogen fixing bacteria. Study conducted in Los Tuxtlas tropical forest of Mexico indicted that the shifting vegetation or aboveground diversity significantly affected on diversity and availability of genus *Bradyrhizobium* (Ormeño-Orrillo *et al.,* 2012*).* The differences of above ground vegetation may have contributed to the differences observed in diversity of genus *Bradyrhizobium* in Huruly dry-evergreen forest in Sri Lanka. Bacterial diversity in regenerating forest soil is less than both undisturbed deep forest and cultivated area in the forest boundary. And this may be partly attributed to less diversity in aboveground plant species in regenerating forest that dominated by pioneer plants compared to other two land-uses.

Conclusions

The study indicated deep forest soil with minimum anthropological soil disturbances harboured higher bacterial diversity at genus level and less domination of single genus over the others in soil bacterial communities. Disturbing the forest ecosystem in Hurulu dry mixed evergreen forest in Sri Lanka for cultivation reduced soil bacterial diversity.

Findings of the current study can execute on forest conservation and restoration attempts of degraded forests. Minimizing the soil disturbances and forest removal from ecosystems can maintain higher bacterial diversity in soil which affects beneficially for balance environment with significant longevity and productivity.

Acknowledgements

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Recovering soil ecosystem services and functions at a burned native forest in the Mediterranean zone of central Chile by the use of organic amendments

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Abstract summary

Global climatic conditions have exacerbated the occurrence of fires, particularly in Mediterranean biomes. In Chile, during the 2016-2017 summer, a megafire affected more than 5,000 km^2 in the nation's south-central area, compromising sclerophyllous forests characteristic of the Chilean mediterranean ecosystem. This work aims to evaluate the effect of organic amendments (swine manure, poultry manure and compost) on the recovery of soil biological conditions including basal respiration, microbial biomass, microbial metabolic coefficient and the coefficient of carbon mineralization, and some physicochemical conditions. Results indicated that soils microbial related process responded differently following eight month of treatment establishment. Related to the type of organic amendment, soils receiving swine manure evidenced the highest microbial basal respiration and microbial biomass, followed by those receiving poultry manure. However, greater mineralization rates and thus presumably shorter periods of C sources consumption were related to such organic amendments. Soils treated with compost accumulated the most organic carbon and nitrogen, ensuring a long-term nutrient release, thus a longer time of C storage.

Keywords: restoration ecology, soil respiration, microbial biomass, wildfires.

Introduction, scope and main objectives

Fires have historically played an important role in the composition and distribution of terrestrial ecosystems (Pausas and Keeley, 2009). However, these events also represent an important pressure that induce land degradation worldwide, especially in Mediterranean biomes (D'Ascoli *et al.*, 2005). These ecosystems are recognized as one of the world's most susceptible environments to fire occurrence, principally due to their climatic conditions (Bodí *et al.*, 2012). They have also been indicated as environments highly susceptible to the effects of Climate Change, which make them one of the most fragile biomes on Earth (Fletcher and Zielhofer, 2013). The most devastating fires ever recorded in Chile occurred during the 2016-2017 season, affecting a close to 600,000 ha in central Chile (CONAF, 2017).

Land burning can compromise belowground conditions that are essential to support aboveground life (Certini, 2005). This is critical if the edaphic properties altered are precisely those enhancing plant establishment and successions after the occurrence of these events (Pérez *et al.,* 2004). Soil biological conditions including diversity, functions, and their services are all affected by land burning. Thus, understanding how biotic properties affected by fires can be recovered in degraded lands is central to the restoration and resilience of forest ecosystem functions (D'Ascoli *et al.,* 2005). This work aims to evaluate the effect of organic amendments (swine manure, poultry manure, and compost) on the recovery of soil biological conditions including basal respiration, microbial biomass, microbial metabolic coefficient and the coefficient of carbon mineralization, along with some physicochemical properties. We hypothesize that biological conditions in soils receiving fresh manures will show and immediate, but transient responses to these amendments as compared to those in soils amended with compost.

Methodology

Research site

the study was conducted at a private land (34°36.502'S; 71°42.281'W) in the east front (dryland zone) of the coastal mountain range of the O'Higgins region. This is covered by native sclerophyllous forests, with the canopy dominated mainly by *Quillaja saponaria*, *Lithraea caustica*, and *Peumus boldus*, followed by *Trevoa trinervis*, *Azara serrata* and *Colliguaja odorifera* in the understory. According to local residents, in the last 30 years this site has been mainly used by small farmers for livestock pasture and wood charcoal production and had not experienced fire events until January of 2017.

Experimental design

In early June 2018, the application of organic amendments and vegetation establishment were implemented to evaluate the effect of them on soil biological conditions. Organic amendments were manually incorporated over the upper 30 cm of soil (Figure 1), following rototilling at the same depth. All treatments, except the control and reference, were covered with a mulch layer (1 cm approx.) consisting of a wheat and oat straw mix. For the present work, plots established in 2018 with organic amendments and seedlings were revisited for further study. Thus, there were six treatment evaluated, five at a burned area (50 x50 m) and one located at an unburned area (20 x 20 m) within the research site at a distance of approximately 500 m. Treatment consisted of: T0, reference (unburned area); T1, control (with no treatment); T2, no amended (only mulch cover); T3, compost (200 m^3 ha^{-1}, plus mulch); T4, poultry manure (200 m^3 ha^{-1}, plus mulch); T5, swine manure (200 m^3 ha^{-1}, plus mulch), each of them distributed in four parcels (3 x 1.5 m).

Figure 15: treatment establishment

Sample collection and analysis

In January 2019, sampling was conducted. At each parcel, five sub-samples were taken at the corners and the centre of them, at a 6 cm depth following the removal of organic debris (1 Kg each sub-sample approx.). Soil sub-samples were carefully mixed in the field to obtain a composite sample per plot. For every sample an aliquot was kept under 4 °C to measure microbiological parameters, and the rest was dried at room temperature. Air dry samples were used to determine: Aggregate Stability (AS) (samples sieved between 4.00 and 0.25mm) and physicochemical analyses (samples sieved at 2 mm) including: EDTA-extractable trace elements, pH, electrical conductivity (EC), soil organic carbon (SOC), carbohydrates (Ch), total N, available P. Microbial measurements included Basal Respiration (BR) and Microbial biomass (MB), determined by substrate-induced respiration (SIR) with glucose. Both were performed in an automated impedance-meter (BacTrac 4200 Microbiological Analyser, Sylab, Austria). These values were used to calculate: 1) the metabolic coefficient (qCO2 =BS/MB) and 2) the coefficient of carbon mineralization (C min.coef = BR/soil organic carbon).

Statistical analysis

To test whether the soil characteristics differed among treatments, we fitted Generalized Linear Mixed Models (GLMM) using the soil properties analysed as response, followed by multiple comparisons performed with the Tukey test ($p < 0.05$). The relationship between the soil parameters measured regarding the treatments applied was analysed using principal component analysis (PCA). Two steps were taken to test the effects of soil physicochemical parameters and the different treatments on BR, MB, Cmin.coef and qCO2. First, stepwise regressions with all physicochemical parameters (but excluding treatments) in both directions were performed. Then, in new models, the treatment was included as a predictor before the model resulting from the stepwise regressions. All analyses were performed in RStudio v.3.6.2 (RStudio Team, 2019)

Results

The soils under study showed variable responses in physicochemical conditions following fire occurrence and the incorporation of treatments. Amended plots showed the highest OC contents, with those receiving compost having the greatest values (5.19 percent), followed by poultry manure (4.73 percent) and swine manure (4.14 percent) plots. The same pattern was observed for N contents, where soils amended with a more stable material, such as compost, had significantly higher values (0.54 percent) than those receiving fresh amendments in the form of poultry manure (0.47 percent) and swine manure (0.36 percent).

Soils microbial related process responded differently following eight months of treatment establishment (Figure 2). Based on the comparison between soils from the ecosystem of reference and those from the burned area, it is evident that basal respiration (Figure 2.A) was significantly disturbed by fires, while microbial biomass carbon (Figure 2.B) resemble to pre-fire conditions even without the application of an organic amendment. Soils receiving swine manure showed the highest microbial basal respiration and microbial biomass, followed by those receiving poultry manure. Soils amended with compost showed values similar to poultry manure amended soils. The later resulted in a significant increase of respiration as compared to control plots and plots covered with mulch, but not in microbial biomass.

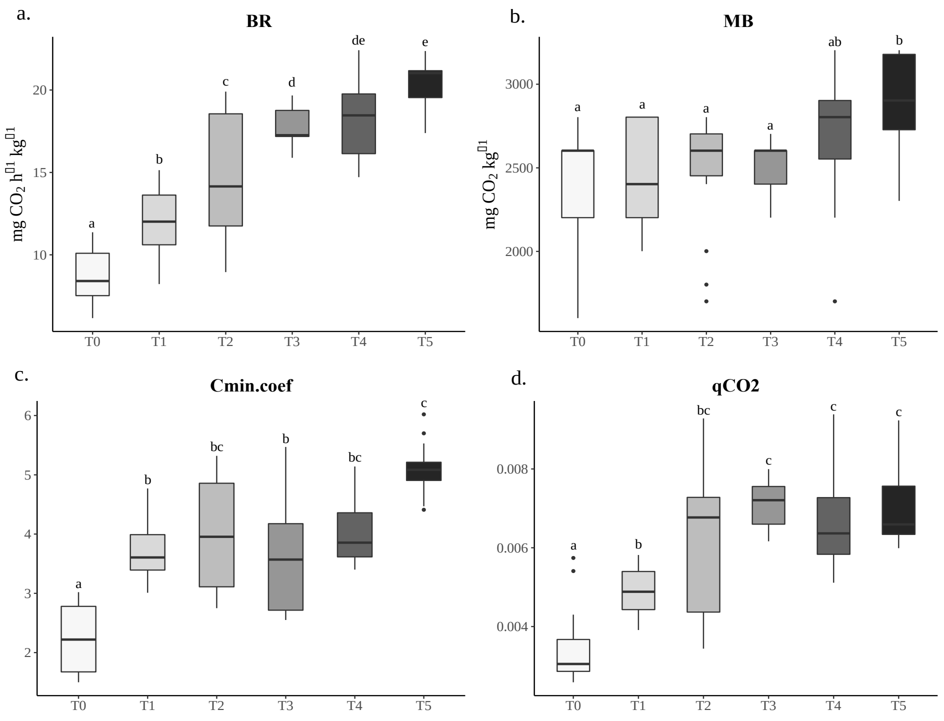

Figure 2: Soil biological conditions studied

Metabolic coefficient and carbon mineralization rates were significantly lower in soils from the reference site than burned soils (Figure 2.C and 2.D). Out of burned soils, all of those receiving organic amendments had significantly greater metabolic coefficient values than control soils. Carbon mineralization rates and microbial metabolic coefficient varied among burned soils, with the greatest value observed in soils amended with swine manure.

PCA analyses (Figure 3), based on soil physicochemical variables, showed amended soils clearly separated apart from those at the ecosystem of reference and burned soils without amendment. Micronutrients including Zn, Cu and Fe along with N were the variables that explained the most this ordination.

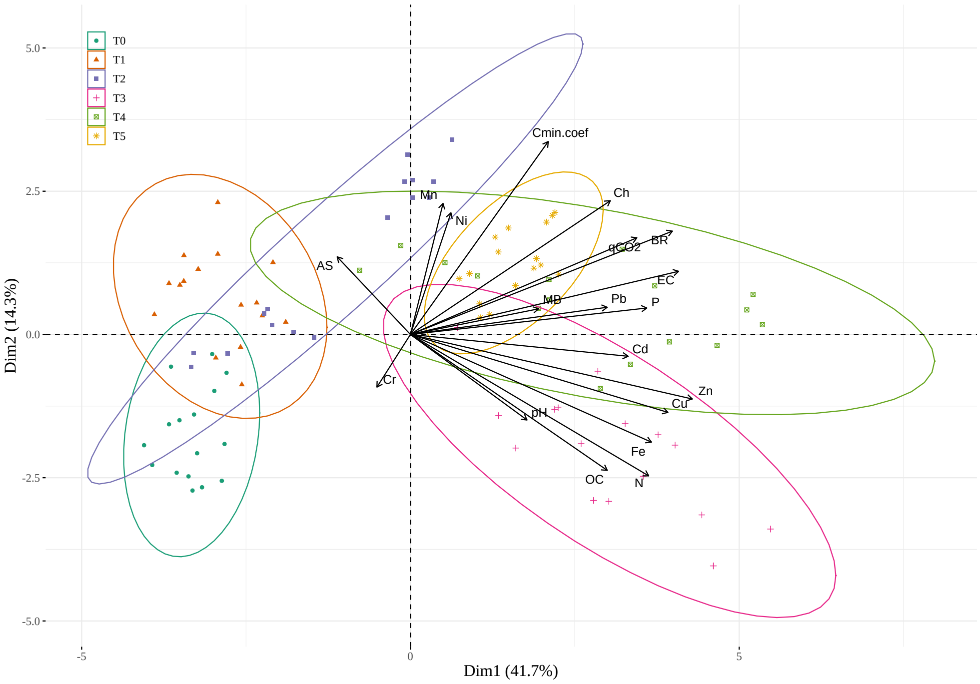

Figure 3: PCA analysis performed for soil physicochemical variables

The organic treatment had a significantly higher effect than the soil physicochemical parameters on these four microbial parameters. The EC had an effect on all measurements except for qCO2, while some micronutrients (Fe and Mn) and potential pollutants (Pb and Cd) also affected some of the microbial parameters.

Discussion

Soil microbial biodiversity and functions are recognized as the main drivers of post-fire ecosystem recovery: however, the effects of fires on them are less understood than those on soil physicochemical properties -which are less sensible to these events (Prendergast-Millerr *et al.* 2017). In this study, we clearly found higher effects on biological than in physicochemical characteristics. Out of the organic amendments used, those based of rather fresh materials (manure) as compare to more stable materials (compost) resulted in more dynamic conditions in soils, well above the ecosystem of reference, that represent a more stable (indeed, less disturbed) environment.

The incorporation of carbon sources at a fire affected soil enhanced the presence of heterotrophic microorganisms, as observed by CFU counts (Marín and Rojas, 2020) and stimulated microbial activity and abundance, as observed here via respiration and biomass. Soil *priming* effect has been shown to positively respond to organic inputs in arid environments, which in turn influence soil C cycling (Bastida *et al.,* 2019). This influence likely contributed to plant colonization and succession at amended plots (data not shown). Under a global change scenario, soil restoration should consider the effect of proposed practices on soil ecosystem services as C sequestration, directly in its matrix and indirectly promoting vegetation reestablishment. Thus, future work should contemplate the type of organic inputs utilized, to precisely enhance soil functioning as a C reservoir in the long term.

Conclusions

Out of the organic amendments evaluated, fresh materials (manure) showed greater influence in the biological conditions studied, as compared to compost amendment. Following eight months of treatment establishment, all amended soils appaired to remain in an active transition phase above the ecosystem of reference. Future studies, considering global change climatic conditions, should consider the effect of diverse organic inputs to promote, in addition to short-term reestablishment of soil functions and services, the effect of them on soil ecosystem services as important as C sequestration.

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Understanding impact of soil biology on crops: the key to sustainability of farming systems

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Abstract summary

Objectives: accelerate adoption of Sustainable Soil Management Systems by farmers, by increasing useful knowledge of farmers about agricultural soil.

Study: Several farmers associations have been reviewed, successfully engaged into Sustainable Soil Management systems, in diverse places (Europe, Africa, South and North America).

Different education materials are used by them:

-to raise awareness of their member farmers,

-to train them to improved Sustainable Soil Management practices.

Key findings: Most of the educational material used by these associations is about soil biology, on the contrary of conventional farmers, more focused on inputs and technologies.

All this material comes from scientific publications in specialties of soil science, soil biology, agronomy, plant physiology and plant nutrition.

The successful use of scientific knowledge by farmers associations relies on a technician transforming the diverse monodisciplinary scientific findings into a pedagogic package linking to a system of practices farmers can appropriate, in an easy to understand visual material.

These technicians need a double competency in agricultural systems and sciences.

Recommendations are made to accelerate transfer between scientific publications and farmers.

Keywords: Sustainable Soil Management, acceleration of adoption by farmers, soil biology, appropriation by farmers of scientific findings, education material.

Introduction

Situation of Sustainable Soil Management actual implementation at farmers level

It is very variable between continents and regions, but can be highly improved, especially in Europe, Africa, Asia and Middle East.

Scientific findings about soil biology and its importance for sustainability on earth are continuously increasing, as well as the awareness of the need to improve practices to achieve Sustainable Soil Management. However, knowledge and know-how among farmers and practitioners still need important improvement at global scale.

Objective

This study aims at contributing to accelerate adoption of Sustainable Soil Management Systems by farmers, by increasing their access to useful knowledge about agricultural soils.

Study

Several farmers associations have been reviewed, successfully engaged into Sustainable Soil Management systems, in diverse places (Europe, Africa, South and North America).

Methodology

An evaluation has been made about their farming systems, their technical performance, their success in dissemination, the keys for success and issues they meet.

An inventory has been made of the education materials they use:

-to raise awareness of their member farmers,

-to train them to improved Sustainable Soil Management practices.

A qualitative survey has been made with detailed interviews with farmers, individually or in focus groups, about their objectives, their needs and wishes to improve their operations.

The results are more qualitative than quantitative, due to the variation between individual situations, especially between countries, which do not enable to make statistics. However, some trends can be found with high level of confidence, because repeated by many farmers.

Results

Most technicians and conventional farmers are focusing on technical aspects as inputs and technologies (machinery, genetic, fertilizers, chemicals, data monitoring).

On the contrary, the farmers looking for Sustainable Soil Management in the dedicated associations declare being more concerned about biological processes, interactions between soil and crops, conception of systems (rotations, associations of plants, of animals) … in the search for more resilient and robust systems enabling them to save effort and money.

The farmers reviewed in the study are using the principles of Conservation Agriculture as described by FAO (2014): simultaneous use of the three pillars: no-tillage/permanently covered soils/rotations

The farmers report that, when they apply properly these principles, with some experience and know-how, after several years (five to ten, depending on starting conditions) they get improved and improving results on yields, resilience towards drought or humidity, weeds, some pests, organic matter, erosion, run off.

Using diverse tools to evaluate the quality of their soils, they attribute the improvement to a better soil biology, also named “soil health”.

It is difficult to qualify exactly what it means because of multiple criteria. Earthworms are appreciated for their visibility and for the work they do. But more and more, fungi and balance fungi/bacteria are looked for, despite not easy to evaluate, and lack of access to easy to use and reliable methods. Pfeiffer chromatography is under evaluation in our network in Europe. It helps understand fungi/bacteria balance and soil health, and link to practices when we compare fields.

This high interest of farmers in soil biology (some people say it is a passion) is confirmed by the themes treated during the activities of these associations: field tours, testing, workshops, training programs.

It is well reflected in the inventory of the educational material they use: most of it is about soil biology.

Soil biology is in the heart of the agroecosystem (Pfeiffer, 1984; Arroyo *et al.*, 2018).

Farmers know this, and want to know everything possible about it, and learn how to use it.

Unfortunately, despite progresses, soil biology is still broadly unknown, especially about the useful functions for pests and weeds control.

And access to recent findings is unequal across countries and needs a lot of time and effort to get them.

All the material used by farmers associations includes findings coming from scientific publications, covering all specialties of soil science, plant physiology and plant nutrition, agronomy, all related to soil biology.

At least what they can access to with reasonable effort.

Most of the scientists in fact ignore what farmers are doing with their publications, and farmers do not know the authors.

The publications are not used as they are published.

Their findings are digested, interpreted, integrated into a corpus of information and recommendations farmers can appropriate and use for practical action.

This work is nearly never done by farmers themselves.

Their associations hire a technician for this, or use a private advisor, when they can pay, or partner with an institution providing extension services. The ones progressing the faster are the ones with their own employee, because of better focus on farmers objectives to get results.

Discussion

Scientific studies are most often focused on a particular subject, a single part of the agroecosystem, in a linear study of a single factor or phenomenon.

Piling up mono-factorial results of diverse disciplines does not recreate the system with its complex interactions.

Scientists in agronomy often complain that they have no methodology well adapted to the study of systems. They try with pluri-disciplinary studies and models, but the models are usually weak to describe and predict the complex reality of living ecosystems.

Farmers think in a systemic way. They are complementary of scientists.

The best studies on farming systems are made by joining both capacities: scientists as specialists, and farmers as integrators and systemic thinkers.

Many scientific studies are descriptive of a phenomenon.

Farmers need more: to understand the relation between a result and the actions (often multiple and simultaneous) to undertake to get it.

For all these reasons, technicians working for farmers look for the scientific findings they can access, transform this into material adapted to the needs and mindset of farmers. Some even say “translate”.

The diverse monodisciplinary scientific findings are integrated into a package reflecting the cropping system and articulated around it. To describe a system of practices and recommendations farmers can appropriate.

In addition, the published text is replaced or complemented by illustrations, figures, pictures, graphics, to make the material visual, easier to understand, and attractive.

A technician is necessary for this successful use of scientific knowledge by farmers associations.

These technicians need a double competency in agricultural systems and sciences.

In some cases, farmers are themselves educated as engineers, graduated in agronomy.

These ones can bridge the triple competencies of scientific approach, project engineering, and farm management. They are unbeatable in all aspects of development and dissemination of new systems. This high degree of education of farmers is the reason for Argentina or North America being able to make changes in farming systems so quickly compared to other geographies.

Conclusions

Sustainable Soil Management Systems use less inputs, but increase their efficiency, and are based on the optimization of the conception agroecosystems, and of the efficiency of their natural functions, like maximization of photosynthesis, of nutrition, of soil biology functions, of natural pests and weeds control (Jones, 2009; Oehl *et al.*, 2011; Provençal, 2017).

They are knowledge intensive. They need from farmers, advisors, supporting scientists, a different and smarter mindset.

The key to accelerate implementation and dissemination of SSM is knowledge and access to it in an easy and usable way.

Recommendations are made by these associations to accelerate transfer between scientific publications and farmers:

- put all scientific publications in free access in one single global depository, with index, to enable everyone to download. In FAO (UN) web.

- the formats must be designed to make usage of the publication as easy as possible: pdf, word or PowerPoint, graphics and pictures, easy to download, to modify, and integrate in a document for training.

- open data free of charge

- advertise widely when a new publication is added.

- make publications easier to read, more understandable. Systematically request an educational / communication version. Many publications are presented with a PowerPoint, but this one is not available.

- open the possibility of publication to less "scientific" format publications. Many engineers and practitioners, even farmers, have findings worth being known, and useful for farmers and for others, but not published in "peer reviews" papers, thus useless for the global community.

- intensify research on soil biology, fundamental and applied.

- orient the research programs towards strategic issues, as sustainable crop protection, increase of biomass (to sequester more carbon), understanding biological "useful" functions of soil…

- integrate diverse international research and development programs / projects / initiatives into a coherent integrated and comprehensive program, avoiding overlapping, gaps, and looking for synergies.

- put the results on farms as the key objective.

- associate farmers to research programs, from their conception to their interpretation and valorisation.

- put a farmer's college in all institutions treating of agriculture, as full partner in full responsibility.

This total connection and full integration between scientists, agronomists and farmers has been the key of a farming revolution in some countries. It can be applied in all places, for the benefit of scientists as well as of farmers and citizens.

It can be recommended and initiated by GSP and other partners treating of soils, soil biodiversity, and SSM.

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Soil biodiversity in action at ecosystem level

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Abstract summary

Genomics, transcriptomics, and proteomics are powerful tools for the assessment of soil microbial diversity and functionality, but they are time- and cost-intensive, and therefore hardly applicable when the sample number is very high. We tested alternative time- and cost-effective high-throughput (in terms of high sample number) methods. This included a combined approach consisting in a multiplexed enzyme assay, a dsDNA-based quantitation of soil microbial biomass and the assessment of extracellular (eDNA) and intracellular (iDNA) fractions of the total soil DNA pool along an altitudinal gradient at South- vs. North-exposed sites in the Italian Alps (450 soil samples).

Non-Metric Multidimensional Scaling (NMDS) based on Euclidean Distances revealed the factor „site‟, and thus the „exposure‟ (North vs. South) and altitude of sampling, as the most prominent environmental factor in shaping the soil microbial properties defined by enzyme activities and dsDNA-microbial biomass (46 percent variance).

Our proposed approach improved our knowledge in the context of microbial soil diversity and function and, rather than being an alternative to costly high-end methods, it is capable to pre-select samples for deeper analysis.

Keywords: climosequence, soil layers, microbial richness, enzymomics, functionality, microannelids, large sample set

Introduction, scope and main objectives

Soil microbial diversity is crucial for ecosystem functioning. It is indeed a multi-faceted subject requiring powerful analytical tools, adequate statistically sound sampling and big-data management capability. Genomics, transcriptomics, and proteomics generate enormous data sets and specific information for each sample. However, they are time- and cost-intensive, especially when in-depth analysis of large sets (hundreds of samples) is needed. Although bioinformatics is now developed, it is a challenge to use such huge data sets for improving the understanding of soil biodiversity and functionality without getting drowned in such an "ocean" of data, and possibly conclude, at last, that differences among samples are small. The availability of time- and cost-effective high throughput (in terms of high sample number) analysis methods is thus of utmost importance. Examples of such techniques are a multiplexed enzyme assay (Cowie *et al.*, 2013; Bardelli *et al.*, 2017), the dsDNA-based quantitation of soil microbial biomass (Fornasier *et al.*, 2014) and the assessment of extracellular (eDNA) and intracellular (iDNA) fractions of the total soil DNA pool (soil metagenome; Gómez-Brandón *et al.*, 2017), where the ratio given by the two DNA fractions is used as a proxy of microbial activity.

In this work, we show how the above concepts and techniques were combined and exploited for an in-depth investigation at ecosystem level and what benefits were gained.

Methodology

We focused on an Alpine soil climosequence in Northern Italy (Bardelli *et al.*, 2017), being an ecosystem with high sensitivity and responsiveness to environmental changes. Five pairs of North- and South-facing sites along an altitudinal gradient (from subalpine to alpine setting) were used in this climosequence-approach (Figure 1) and three soil layers (0-5, 5-10, 10-15 cm) from each site were investigated, achieving a total of 450 soil samples (10 sites × 3 plots × 5 sub-samples × 3 soil layers).

At the same sites, an *in-field* mesocosm experiment was set-up in order to evaluate the Coarse Woody Debris (CWD) decay dynamics and the effects on soil layers over time (2 and 3 years at the subalpine and alpine scenarios, respectively) (Bardelli *et al.*, 2018a; Bardelli *et al.*, 2018b), collecting a total of 234 samples for each matrix (soil and CWD blocks).

Soil and CWD samples were analysed with a) multiplexed enzyme assay: 16 hydrolytic enzyme activities (Cowie *et al.*, 2013); b) dsDNA based microbial biomass estimation (Fornasier *et al.*, 2014); c) sequential extraction of eDNA and iDNA (Ascher *et al.*, 2009; Gomez-Brandon *et al.*, 2017). Chemical and (micro)biological variables, soil microbial biomass (Substrate Induced Respiration; SIR), abundance of soil and wood microbiota (quantitative real-time PCR), and microannelids (*Enchytraeidae* family) serving as soil pH-indicators.

Statistical elaboration of data included robust methods, such as non-parametric tests and Non-Metric Multidimensional Scaling (NMDS), for a clear and rapid view of the results.

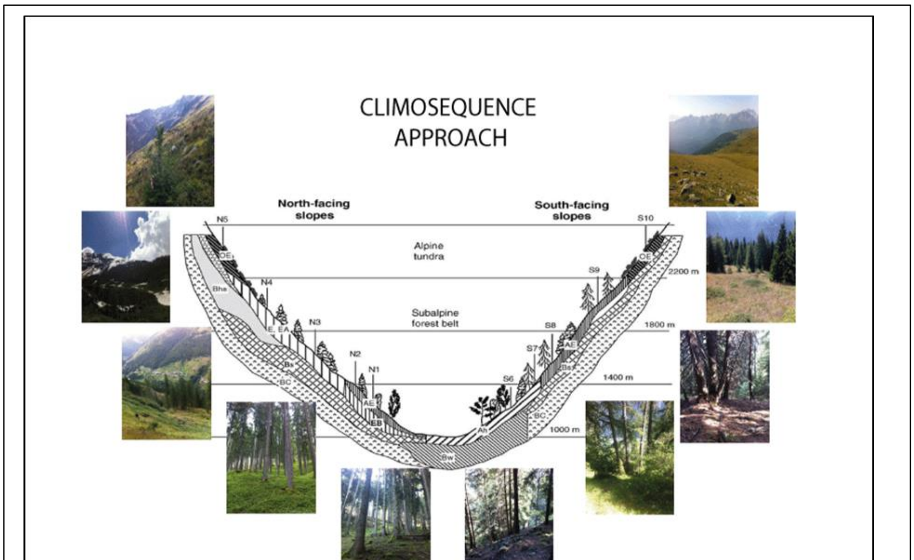

Figure 1: Overview of the study area comprising five pairs of North (N_1-N_5) and South (S_6-S_7)-facing sites along an altitudinal gradient from 1200 to 2400 m above sea level (a.s.l.) in the Italian Alps (Trentino Alto Adige, Italy)

The photos (J. Ascher-Jenull) were assembled by T. Bardelli on the modified scheme of Egli *et al.* (2006).

Results

Specific findings derived from the high-throughput methods (performed with 450 samples) were:

- SIR-soil microbial biomass was higher at the North- than at South-facing slope, whereas soil dsDNA-based microbial biomass did not show any exposure-effect;
- Higher soil eDNA/iDNA ratio, indicative of lower microbial activity, was recorded at the North-facing slope;
- An increase in the C-related enzyme activities in the CWD blocks was accompanied by an increase in wood fungal abundance and microbial biomass (dsDNA) at the end of the observation period, suggesting a higher activity of the wood-inhabiting fungi at this stage of decay;
- Soil beta-glucosidase- and acid phosphomonoesterase-activities were more pronounced at the North- than at the South-facing slope and this was linked to the more acidic, moister and cooler conditions observed at the northern slope, as also confirmed by the higher abundance of *Enchytraeidae* (strong acidity indicator species);
- The wood and soil bacterial communities became more similar in terms of richness, diversity, evenness and composition with progressing decay;
- Soil microbial domains responded differently to exposure in terms of abundance:

  bacteria (South- > North-facing sites; altitude-independent);

  fungi (North- ~ South-facing sites);

  and archaea (North- > South-facing sites; altitude-dependent);
- NMDS based on Euclidean Distances (Figure 2) revealed the factor „site‟, and thus the „exposure‟ (North vs. South) and altitude of sampling, as the most prominent environmental factor in shaping the soil microbial properties defined by enzyme activities and microbial biomass (dsDNA yield) (46 percent variance). In addition to the sampling site, also the „soil depth‟ was found to

significantly affect the soil properties defined by enzymatic activities and soil dsDNA content (17 percent variance).

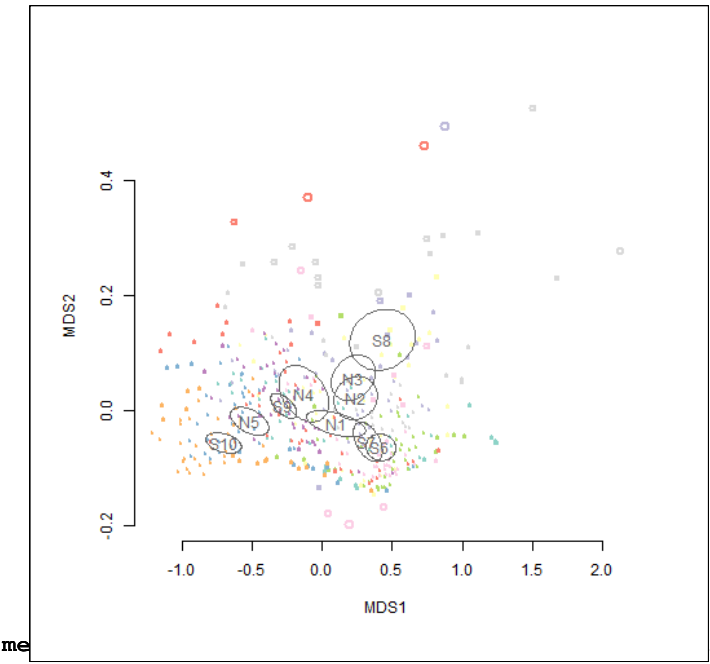

Figure 2: Non-me zymatic activities and microbial biomass estimated by dsDNA contents

The multidimensional character of the data was compressed into two dimensions based on Euclidean distance. Convergent solutions were found and the lowest stress was 0.133 (R2=0.867). Site scores were coloured according to sampling sites (see Figure 1). The radius of the circles correlates to the accuracy of component prediction. Ellipses embracing sample groups were drawn at a confidence interval of 95% around group centroid.

Discussion

The effect of exposure was enzyme-specific, altitude- and time-dependent for soil and CWD samples along the climosequence. This underlines the importance of performing multiple enzyme assays on a large sample set, in order to avoid misinterpretation (e.g. of nutrient cycles). The results of soil microbial biomass measurements differed among methods. Substrate addition in SIR stimulates the activity of predominant and fast-growing soil microorganisms; in contrast, the dsDNA-approach proposed by Fornasier *et al.* (2014) directly measures the extractable dsDNA of the overall autochthonous soil microbiota, independent from their growth strategy and metabolic status.

The higher soil eDNA/iDNA ratio reflects a lower potential microbial activity due to lower degradation and/or accumulation of eDNA recorded at the North-facing slope. For these evidence, the ratio is suggested as a powerful "low cost alternative" for a generic screening of microbial communities and their response to changing environmental conditions.

Conclusions

In order to get a comprehensive picture of both the microbiota and complexity of biogeochemical cycles occurring in a specific ecosystem, our approach emphasized the relevance and pinpointed the advantages of using two distinct classes of "high-throughput" methods:

The high-end class includes Next Generation Sequencing (NGS), able to generate huge, very detailed data sets and specific information for each sample. The high-throughput class of methods includes as a multiplexed enzyme assay, eDNA/iDNA ratio and dsDNA-microbial biomass, which are able to process hundreds of samples in a very short time at minimal cost. These, rather than being an alternative to costly high-end methods, serve as a tool to select samples for deeper analysis. They are able to improve our knowledge in the context of soil biodiversity and function, if statistics are properly applied throughout the investigation process and not just after data production.

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Soil biodiversity for agricultural production and environmental integrity

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Abstract summary

Soil biodiversity is a regulator of ecosystem functioning. However, the extent to which soil biota directly contribute to agricultural production and how soil management affects this is not well understood. While lab studies can uncover crucial, basic relationships, the transfer to applied settings is difficult. Studies at the field scale have to deal with a variety of confounding factors that make presenting direct benefits to practitioners challenging.

We conducted studies of different levels of complexity. A lysimeter study provided evidence that a reduction in soil biological complexity impairs N cycling and crop yields. A sample survey across an agricultural landscape showed that the abundances of different soil organism groups relate to nutrient loss and plant yield. In an applied field experiment, we show that symbiotic soil fungi can substantially support agricultural production. Cumulatively, these studies provide compelling evidence that soil biodiversity can directly support agricultural production and environmental integrity.

Future research has to identify specific management strategies that promote soil biological benefits for agricultural sustainability. Moreover, effective communication strategies are needed to successfully transfer such research results to stakeholders, such as policy makers and farmers. We present an example of a citizen science project focusing on soil-related knowledge transfer to the Swiss public.

Keywords: N cycling, soil fauna, metagenome, Arbuscular mycorrhizal fungi, Tomato, Corn, #SoilYourUndies, Citizen Science

Introduction, scope and main objectives

Soil organisms perform a vast variety of processes and regulate the functioning of ecosystems (Bardgett and van der Putten, 2014; Wagg *et al.*, 2014). In agriculture, soil organisms receive little recognition. Agricultural management generally imposes threats to many components of soil communities leading to reductions in their biodiversity, abundance and, potentially, their functioning. It remains unclear to what extent soil biota contribute to agricultural production and how different management strategies affect the magnitude of these effects. Since 12 percent of the global land area is used for crop production

(FAO 2011), the way soils are managed could play a pivotal role in promoting soil biodiversity- or in putting it at risk.

Evidence is increasing that soil biodiversity plays fundamental roles in ecosystem functioning, which could also greatly increase the sustainability of agricultural production and support crop yields (Bender, Wagg and van der Heijden, 2016). However, to convince land-managers and policy makers of considering soil biodiversity as a valuable resource that can support agricultural production systems, compelling scientific results created under practically relevant conditions are needed. Moreover, such results must also be communicated effectively to potentially find implementation in policy and practice.

We present results of three different studies investigating the effects of soil biota on crop yields and agricultural sustainability. The studies are performed at different experimental scales and levels of complexity, but all provide evidence for the crucial role of soil biota for agroecosystem functioning. Finally, we present a citizen science project aiming at effectively transferring such research results to the public, practitioners and policy makers with the aim to implement scientific evidence in society.

Methodology

In Experiment 1, we manipulated soil communities in outdoor experimental lysimeters planted with maize (Figure 1 A) and tested effects of the simplification of soil communities on crop yield, nutrient recycling and nitrogen losses through leaching and denitrification. Leachate was collected and analysed for nutrient concentrations over the entire growing season. N losses as gaseous compounds in the form of N_2 and N_2O were assessed after a fertilization pulse in situ using the ^{15}N gas flux method over a time span of 10 days. Soil metagenome sequencing was used to investigate the biological basis of soil nutrient cycling processes.

Experiment 2 consisted of a sampling survey including 30 sites across an intensively managed agricultural landscape in northern California and investigating the effects of land-use intensity on soil biological communities in tomato production systems (Figure 1B). Soil arthropod, nematode, and microbial communities were investigated, and it was tested whether increased land use intensity affected soil communities. Intact soil cores were extracted from the same fields and used for a greenhouse experiment assessing ecosystem functions provided by these soil communities.

In Experiment 3, the contribution of the plant-symbiotic arbuscular mycorrhizal fungi to tomato yields in differently managed crop rotations was investigated in a long-term, replicated field trial (Figure 1C). A non-mycorrhizal tomato mutant and its wildtype progenitor were planted into four different tomato-corn rotations and AMF abundance in roots, as well as tomato yields were assessed. The difference between tomato yields from both tomato genotypes gives an indication about the extent to which AMF contribute to tomato yields depending on agricultural management. Root DNA was sequenced to assess AMF community composition in dependence of long-term agricultural management.

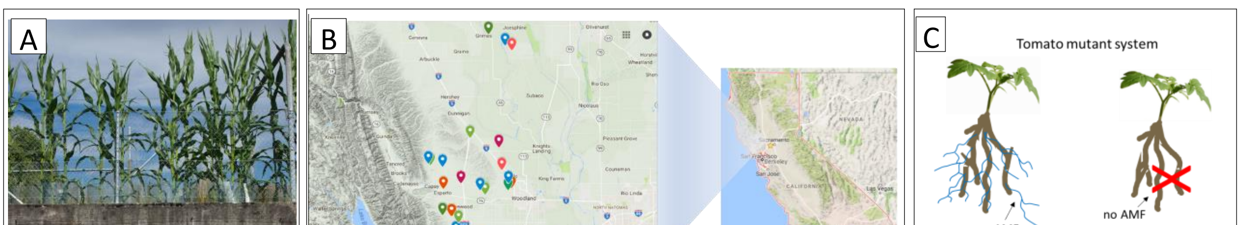

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Figure 1: Experiments were conducted at three different levels of complexity

(A) Lysimeter study growing maize in two different soil community treatments (Experiment 1); (B) field sample survey investigating soil communities and their functioning in a gradient of management intensity across 30 field sites (Experiment 2); (C) AMF-forming tomato wildtype and tomato mutant with reduced AMF associations planted in 4 different tomato-corn rotations in a replicated long-term system trial at the Century Experiment, UC Davis, CA (Experiment 3).

Results

Experiment 1: In lysimeters with simplified soil communities, corn yield was on average 13 percent lower, while plant uptake of N and P was reduced by 20 percent and 58 percent, respectively. In contrast, N losses though leaching where increased by 65 percent with simplified soil communities and emissions of the greenhouse gas N_2O and of N_2-gas were about two-fold higher. Soil DNA sequencing confirmed significantly reduced soil microbial diversity and significant differences in genes encoding for enzymes involved in the production of N_2O and N_2 that support the observed differences in gaseous N losses.

Experiment 2: With increasing land use intensity, we observed reduced abundance for several groups of soil organisms as well as reduced microbial diversity. Assessment of nutrient leaching losses from intact soil cores in the greenhouse showed that N leaching was significantly enhanced with increasing management intensity. Microbial biomass and the overall abundance of nematodes was significantly positively related to tomato yields assessed in the greenhouse. There was a significantly negative relationship between the ratio of fungi to bacteria in soil with Nitrate leaching losses.

Experiment 3: Tomato yields differed significantly between different crop rotations and were highest in a Tomato-Corn-Alfalfa rotation. This system also showed a distinct AMF community composition compared to the other rotations. When AMF were excluded by planting the non-mycorrhizal tomato mutant, the yield loss in the Tomato-Corn-Alfalfa system was highest, amounting to a 33 percent yield reduction, while no effect of AMF on yield was observed in the organic rotation.

Discussion

The results of each of the experiments show that soil biological communities are important regulators of ecosystem functioning. Soil biological processes support crop yields and can significantly reduce the amounts of nutrients lost to the environment, thereby contributing to improved water quality and the mitigation of climate change.

The fact that the results from all three studies point in a similar direction shows that results generated in artificial model systems can represent processes that are transferable to field situations. The lysimeter study provides a proof of concept that soil biota is integral for maintaining nutrient recycling processes in soil. A reduction in their abundance and diversity shifted the nutrient balance away from crop uptake to loss to the environment. While it has been shown before that increasing agricultural management intensity can reduce the abundance and diversity of soil organisms (Tsiafouli *et al.*, 2015), the consequences of these reductions are less clear. The field survey across 30 sites differing in management intensity (Experiment 2) could provide correlative confirmation of the results generated in the lysimeter study in an applied, real-world setting. The abundance of different organism groups was positively related to plant yield and N leaching decreased with increasing fungi/bacteria ratio. However, the relationships between soil biota and ecosystem functioning were decoupled from management intensity. How agricultural management exactly affects the contribution of soil organisms to agricultural sustainability is not well understood.

In Experiment 3, however, we could show that the agricultural management strategy strongly affected the contribution of one specific group of soil organisms, the arbuscular mycorrhizal fungi, to crop yield. Surprisingly, AMF did not show an effect on tomato yield in the organic rotation. However, the other 2 systems that included winter cover crops in the rotation showed >30 percent yield reductions when AMF abundance was reduced. Overall, tomato yield and AMF contribution were highest in the system including three continuous years of Alfalfa in the rotation. This system also showed a distinct AMF community composition to the other systems. Further research is needed to identify specific management strategies that serve to maximize the soil biological contribution to agricultural production.

While the research community generates compelling scientific evidence for the pivotal role of soil communities and their diversity to ecosystem functioning and agricultural sustainability, this message is not widely received by policy makers and farmers. Effective communication strategies are needed to make compelling scientific results heard. By involving citizens in research activities with the potential to gain substantial media attention can be an effective communication and outreach strategy.

Conclusions

Cumulatively, our results highlight the fundamental value of soil biological communities for sustainable agricultural production and environmental integrity. Future research should focus on identifying and developing agricultural management systems that can maximize benefits provided by soil biodiversity. Moreover, effective communication strategies for scientific results need to be implemented to increase the adoption of soil life promoting practices and to preserve the valuable resource of soil biodiversity.

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Soil monitoring using arthropod adaptation to soil: the case of QBS-ar index

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Abstract summary

Soil quality has been long-time ignored despite its importance in the provision of ecosystem services. A universally accepted formula to measure soil quality does not exist yet. The QBS-ar (Soil Biological Quality index based on arthropods) has been conceived and developed in recent years. It has been applied in a huge number of cases in Italy, and European and non-European countries have recognized the potential of this index. This work aims to highlight the skill of this index to define Soil Biological Quality under different soil management conditions, basing on the QBS-ar dataset collected by the UNIPR team from 2001 to 2019.

Keywords: soil fauna, soil arthropods, bioindicators, soil degradation, land management

Introduction, scope and main objectives

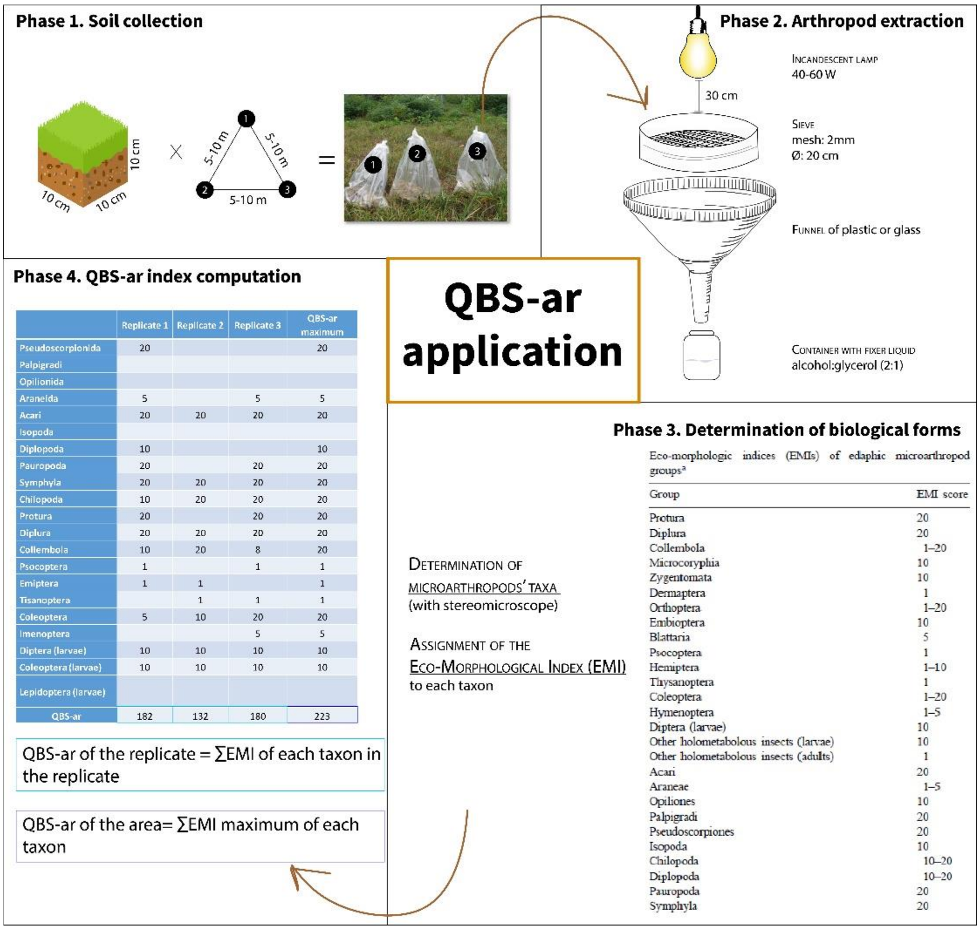
The ability of soils to perform ecological functions is often referred to as soil quality. Soil functions, and thus ecosystem services they provide, mostly depend on biodiversity. Therefore, a thorough knowledge of soil biodiversity is essential for the understanding of soil ecological functions and the linkages occurring between them (Lavelle *et al.*, 2006). Soil quality has been long-time ignored (Havlicek, 2012), despite its importance in the provision of ecosystem services, such as food production, carbon sequestration, flood control, detoxification, biodiversity conservation, etc. (Cortinovis and Geneletti, 2018; Menta *et al.*, 2018a). One main approach to investigate soil quality uses indicators (Bastida *et al.*, 2008; Devillers *et al.*, 2009). Only a limited number of these applications attempted to generalize their results and converted them into indices for extensive applications. A universally accepted and applicable formula to measure soil quality does not exist yet (Bastida *et al.*, 2008; Menta and Remelli, 2020), and no fully efficient bioindicator toolbox for soils is currently available (Havlicek, 2012). Most difficulties in applying the existing indices are related to the poor standardization of the methods; in addition, problems associated with the spatial scale at which they can be applied hamper their use (Bastida *et al.*, 2008). The QBS-ar (Soil Biological Quality index based on arthropods) has been conceived and developed in recent years (Parisi, 2001; Parisi *et al.*, 2005). Soil arthropods are one of the most important components of soil-living communities and play a key role in maintaining soil quality. It is a well-known fact that soil arthropods are involved in many processes such as organic matter translocation, breaking and decomposition, nutrient cycling, soil structure formation, and consequently water regulation (Mulder *et al.*, 2011). In addition, some groups are highly sensitive to changes in soil quality because they live, feed and reproduce in the soil, and are extremely adapted to specific soil conditions (Parisi *et al.*, 2005; Parisi and Menta, 2008). QBS-ar is a metric based on the number of microarthropod groups that are morphologically well adapted to soil. Higher is the number of well-adapted groups, better soil quality is. QBS-ar joins the biodiversity of soil microarthropod community with its sensitivity, resulting in a good indicator of land degradation. This index is rather inexpensive, both in terms of equipment required, in sampling and sample analysis effort. All these features allowed QBS-ar index to be considered as a standard protocol for measuring soil fauna across Europe LTER sites ExpeEr Ecosystem Research Program (Experimentation in Ecosystem Research, proj. no. 262060) (Experimentation in Ecosystem Research (ExpeER), undated; Firbank *et al.*, 2017) and it is reported in the European Commission DG ENV 2010. In addition, QBS-ar was taken as a model for the development of two biological indices: one based on collembolan community (QBS-c, Parisi and Menta, 2008) and the second one based on earthworm community (QBS-e, Paoletti *et al.*, 2013). The high number of applications in Italy, European and non-European countries (see Menta *et al.*, 2018b) highlights its potential. Parma University (UNIPR) team is the historical trainer in the application of this index, due to its experience in its application (from 2001 to present) in a wider spectrum of land use managements throughout Europe and South America. In December 2017, during the 42nd Congress of the Italian Society of Soil Science (SISS), a working group for the QBS-ar index was established.

The aim of this work is to highlight the skill of this index in describing Soil Biological Quality under different soil management conditions, basing on the UNIPR dataset collected from 2001 to 2019.

Methodology

QBS-ar

QBS-ar considers soil microarthropods (Phylum Arthropoda), ranging between 0.2 and 2 mm. Euedaphic arthropods are particularly sensitive to soil degradation and are unable to survive in or move away from disturbed soils. The QBS-ar is based on the assumption that the number of arthropod groups well adapted to soil is high in soils characterized by good “quality” (understood as good stability, high organic matter content and good biodiversity level). Figure 1 summarizes the main phases of the QBS-ar application.



Eco-morphologic indices (EMIs) of edaphic microarthropod groups[a]

Group	EMI score
Protura	20
Diplura	20
Collembola	1–20
Microcoryphia	10
Zygentomata	10
Dermaptera	1
Orthoptera	1–20
Embioptera	10
Blattaria	5
Psocoptera	1
Hemiptera	1–10
Thysanoptera	1
Coleoptera	1–20
Hymenoptera	1–5
Diptera (larvae)	10
Other holometabolous insects (larvae)	10
Other holometabolous insects (adults)	1
Acari	20
Araneae	1–5
Opiliones	10
Palpigradi	20
Pseudoscorpiones	20
Isopoda	10
Chilopoda	10–20
Diplopoda	10–20
Pauropoda	20
Symphyla	20

	Replicate 1	Replicate 2	Replicate 3	QBS-ar maximum
Pseudoscorpionida	20			20
Palpigradi				
Opilionida				
Araneida	5		5	5
Acari	20	20	20	20
Isopoda				
Diplopoda	10			10
Pauropoda	20		20	20
Symphyla	20	20	20	20
Chilopoda	10	20	20	20
Protura	20		20	20
Diplura	20	20	20	20
Collembola	10	20	8	20
Psocoptera	1		1	1
Emiptera	1	1		1
Tisanoptera		1	1	1
Coleoptera	5	10	20	20
Imenoptera			5	5
Diptera (larvae)	10	10	10	10
Coleoptera (larvae)	10	10	10	10
Lepidoptera (larvae)				
QBS-ar	182	132	180	223

QBS-ar of the replicate = ∑EMI of each taxon in the replicate

QBS-ar of the area= ∑EMI maximum of each taxon

Figure 15: Main phases of the QBS-ar application

For more details see Parisi (2001) and Parisi *et al* (2005)

UNIPR dataset on QBS-ar

From 2001 to 2019 UNIPR collected n. 1917 soil samples in eight different land uses:

- Agro-silvo-pastoral system
- Urban area
- Grassland
- Cropland
- Orchard
- Shrub
- Uncultivated area
- Woodland

Soil samples were collected in Italy, Spain, and Chile. The QBS-ar index was applied following the procedure showed in Figure 1. The woods are separated in 8 sub-categories in relation to the plant species, and agriculture ecosystem comprises 9 meadows and 3 orchards.

Results

QBS-ar highlights differences in soil biological quality between the eight land uses considered (Figure. 2). Agro-silvo-pastoral environment and crop show lower values when compared with the other land uses. Besides, woodland and shrub show the highest index values (more than 200). Grassland and orchard show similar values to uncultivated areas and urban gardens.

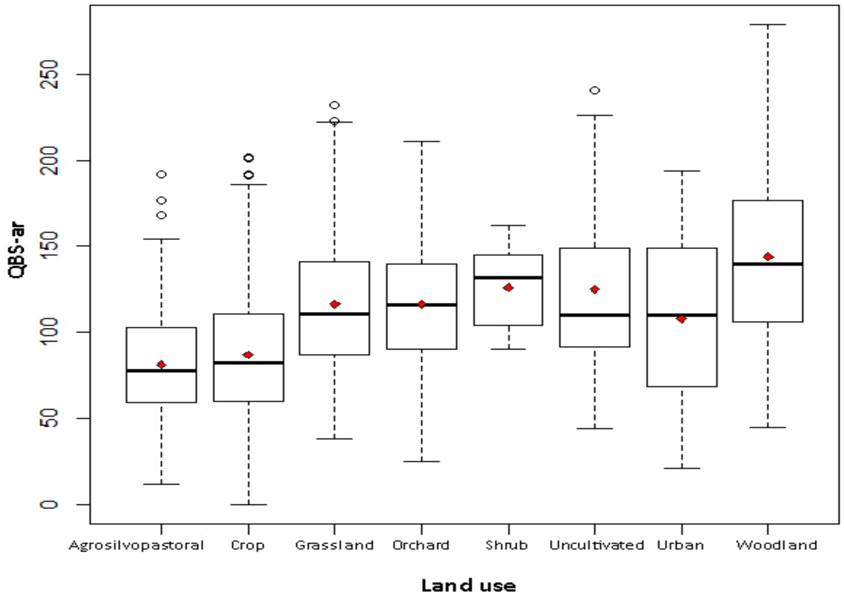


Figure 26: QBS-ar values plotted according to land use

Average (red point) is visualized for each box plot.

Within the sub-category Woodland (Figure 3), oak wood seems to provide better condition for soil arthropods, while poplar and pinewood show the lowest QBS-ar values. Chestnut and beech wood highlight good level of soil biological quality.

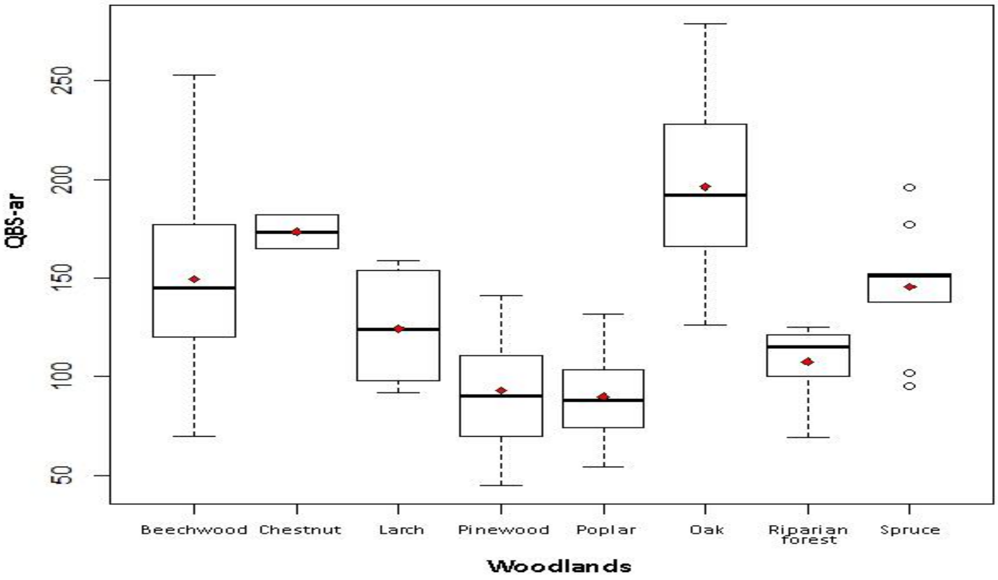


Figure 37: QBS-ar values plotted according to wood

Average (red point) is visualized for each box plot.

Considering the complex agricultural ecosystems (Figure. 4), conventional management systems (ploughed and maize) show the worst condition; beside peach highlights an important difference compared to the other permanent crops such as vineyard and pear, reaching values comparable to high-quality woodlands.

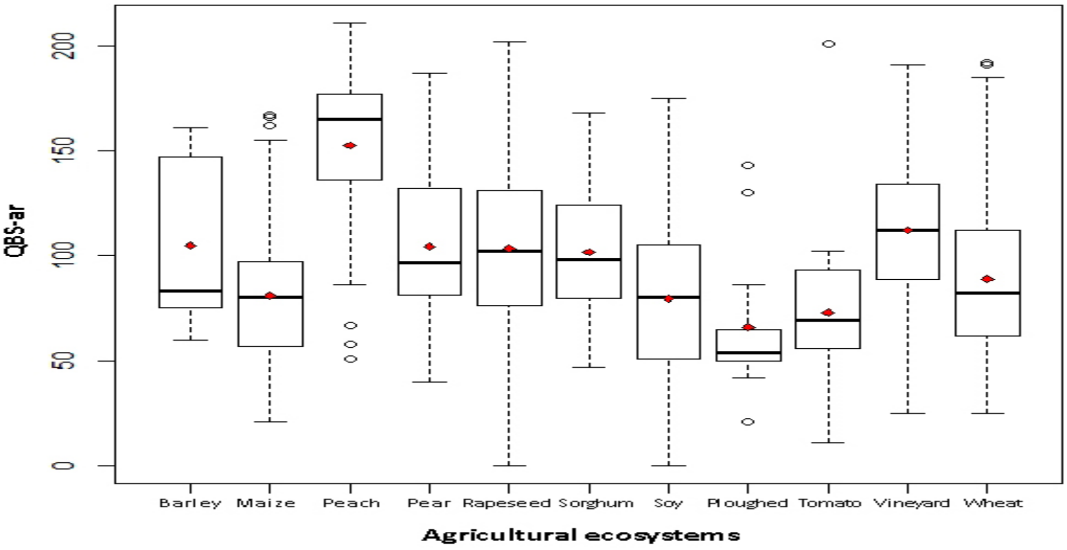


Figure 4: QBS-ar values plotted according to agricultural ecosystems

Average (red point) is visualized for each box plot.

Discussion

Since its first application, QBS-ar has been recognized as a good indicator of soil quality. Its effectiveness depends on its ability to discern between high- and low-quality soils. Moreover, the benefit of the index relies in the possibility to link soil quality with the agents that reduce it. The results showed in this paper confirmed that land use affects QBS-ar values significantly, even though a clear pattern that links QBS-ar to land use has not yet emerged completely. Still, evidence indicates that degraded and highly managed soils are characterized by lower QBS-ar values.

Conclusions

The insertion of QBS-ar index in a set of ecological protocols suitable for widespread adoption by the ecological community (Firbank *et al.*, 2017) can be considered a good opportunity to improve the application of this index at an international scale. The authors invited researchers that are conducting -or have already concluded- studies on QBS-ar to report the published data to the WG-QBS-ar SISS. The aim is to create an international network that will contribute to apply QBS-ar correctly and to insert this index in soil monitoring programs.

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Re-carbonization of soils with intensive agricultural use with the presence of pine forests and provision of fungal ecosystem services (suilus luteus and suilus granulatus). Chilean experiences

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Abstract summary

The establishment and management of the forest associated with the management of humic roots (decomposition rate C/N, C/P, C/S) especially moor and moder type influence the formation of fungi (mushrooms), mainly due to an association of the condition of forest structure and climatic aspects.
A multiple use forest management should be contemplated in order to achieve the re-carbonization of previous intensive agricultural soils through vegetative pine covers that consider factors the density and initial spatial distribution of the plantation, successive forest management interventions (thinning and pruning), so that the closing of cups does not occur and the luminosity is favoured in such a way as to induce the provision of fungal eco-systemic services.
Research is currently being carried out, to evaluate the effects of soil conservation practices such as selection of the species of broadleaf and shrubs (lupines-N-org.) through the application of soil and fauna strains, the improvement of mineralization and humification conditions through whitewashing (CaO) with basal applications of low doses of MgO and P2O, according to litter thickness, and promote fertilization practices that do not favor excessive stimulation of humification and mineralization processes of re-carbonization and bioturbation of soil flora and fauna.

Keywords: Recarbonization, Degraded, Soils, Pine,Forest ,Fungal, Ecosystem Services

Management of humic covers and fungi biodiversity in forest soils

The sequestration and management of organic carbon is essential to re-carbonize soils from previous intensive agricultural use. When carbonizing soils with Pinus radiata plantations, an organic horizon O is generated and the sub horizons (OF, OF, OH), organic carbon is sequestered and fixed in ranges of 22.3-44.4 ton/ha after soil site (Schlatter, Grez and Gerding, 2005), which plays a fundamental role in the nutrition and fertility of forest soils.

In this way the forest soils are re-carbonized, initially it is formed by the fine branches, pine acicles and fungal hyphae that make up the leaf litter (forest mulch), and in turn contribute to the soil's biodiversity.

Humus moor
Humus Moder
Humus Mull

Figure 1: Forest Floor (Francke, 1988)

Humic forms and biological activity of the soil

The establishment and management of the forest, associated with the management of humic roots (decomposition rate C / N, CP, C / S) especially moor and moder type influence the formation of fungi (mushrooms), mainly due to an association of the condition of forest structure (canopy cover, density, distribution), climatic aspects (rainfall regime, air temperature, wind speed, cloudiness and humidity), among others. On the other hand, in bacterial covers type mull predominate bacterial strains (see Table 1).

Table 1: Interrelations of humic forms of forest types, biological activity and the C/N ratio (Francke, 1988)

C/N ratio	**Humic form**	**Ad/ ▲AC**	**Biological Activity Micro organisms**	**Fertilidad**	**Tipo de Bosque**
<10	Mull	>▲D	High	High	Broad leaves
<10-20	Moder	▲D= ▲AC	Medium	Medium	Mixed
>20	Moor	>AC	Low	Low	Conífers

▲ D = decomposition rate
▲ AC Accumulation Rate

Pine mycorrhizae and mushroom productivity

Mycorrhizal associations

It is widely recognized that the symbiotic relationship between fungi and trees generates significant benefits in the establishment and subsequent growth of introduced forests, this condition being of greater importance when forest soils present marginal soils conditions.

As a result, all Pinus radiata plants produced in national nurseries are inoculated with mycorrhizal fungi, with the aim that future forestry plantations develop this symbiotic relationship.

The growth and development of these fungi is dynamic, and the productivity of the mushrooms is determined by a multiplicity of environmental factors, associated with the climate, the organic horizon of the soil, the humic formation, the structure and management of the Pinus radiata forest.

The main edible mushrooms produced in the country correspond to the species Suillus luteus and Suillus granulatus, constituting more than 90 percent of fungal exports. These fungi develop in the forests of Pinus radiata from 6 to 20 years, with yields of 300 kg / ha / year and 35 kg / day of extraction.

In attached illustration 3, Suillus Luteuus (boletus) is presented, and in illustration 4 and 5, Suillus granulatus (pinkish granulated of pink pine) is presented, both constitute the most relevant edible fungi in Chile. The mycorrhiza that generates these mushrooms develops in forests of Pinus radiata forming a symbiotic association with the roots of this tree.

Suillus luteus, boletus-pine

It hat constitutes a mycorrhizal species of pines, mainly of Pinus radiata. A convex pool a little reddish brown, its surface a bit viscous. In the lower part it has fine ocher yellow tubes, which become more brown colours. It is distributed geographically from the central zone to the southern zone and bears fruit in the autumn season and in early winter (see below Figure 2).

Figure 2: Suillus luteus (Hongos de Chile, 2021)

Suillus granulatus, granulatus of pine

It is a mycorrhizal species of pines. It has a yellow brown convex pool. Its surface is dry in dry times, but it can be a bit viscous in rains. It has in the lower part thin pale-yellow tubes that become more reddish as it ages. It is a cosmopolitan species found in Chile from the central zone to the southern zone. Its fructifications can be found in the autumn season and in early winter (see below Figure 3 and 4).

Figure 3: Suillus granulatus, granulatus of pine (Hongos de Chile, 2021)

Figure 4: *Suillus granulatus,* granulosa pine (Hongos de Chile, 2021)

Soil and water conservation practices that affect mycorrhizae and mushroom production

Fertility and soil biodiversity in forest soils

The determinants of fertility and biodiversity of forest land can be influenced by soil conservation practices, as shown in Table 2 attached.

Table 2: Management of humic covers and effects on the biodiversity of flora and fauna of forest soils through soil conservation practices (Francke, 1988)

***Variable/* factor of soil**	**Practical soil conservation practices**
Provision of nutritional elements	• The objective is that the state of the soil and nutritional offer reaches an optimum level again, so that new supplies of nutrients are not necessary through: • Mineral fertilization, plantation with deep-rooted species, establishment of species rich in nutritive elements with good decomposition of its litter, tillage of the soil to remove the compacted strata with flora and fauna of the soil. • No practices to stimulate mineralization processes should be carried out.
Base saturation	• Mineral fertilization. • Green fertilizer. • Choice of the broadleaved species. • Application of soil flora and fauna strains. • Improvement of mineralization conditions through fertilization with CaO, MgO and P2O, according to litter thickness.
Humus	• Mineral fertilization. • Soil tillage work. • Choice of the species. • Application of soil flora and fauna strains. • Forest treatments and care.
Porosity	• Measures to favour the activity of soil microorganisms through soil tillage and drainage work.
Cultural care of the forest	• Creation and maintenance of an orderly development of the forest (thinning, thinning, pruning). • Avoid over-site coverage, damage to the ground (compaction) in the forest harvest, scattering of forest crop remains (bark, branches and leaves).

Experiences of sustainable forest management and mushroom production in the maule region, Chile

The objective of multipurpose management of the forest should consider, depending on the density and initial spatial distribution of the plantation, successive interventions of thinning and to a lesser extent pruning, to prevent the closing of glasses and maintain a luminosity percentage greater than 23 percent. The heterogeneity of the forest cover and the presence of openings and empty spaces in the treetops ensure greater mushroom productivity compared to homogeneous and closed canopy forests.

If the objective of the plantation is to implement a silvopastoral system, it is very compatible with mushroom production, it must be regulated during grazing times and avoid soil compaction, due to animal overload.

The age at which thinning interventions are performed may vary, depending on the dynamics of forest growth. For the case in which the canopy closure occurs before the age of 7 of the forest, it will be advisable to advance this activity in order to maintain the productivity of fungi.

Table 3: Multipurpose Management Scheme for timber and edible mushroom production (INFOR, 2019)

Dominant height (m)	**Age (years)**	**Residual density (trees/ha)**		**Cultural activities**	**Technical Specifications**
0	0	1250	0	Establishment	Use good genetic material and intensive establishment forestry. It is necessary to analyse the use of chemical products and/or fertilizers if the commercial objectives point to organic mushroom markets.
	1-2	1250	0	Weed and/or fertilization Control	The height of the weed, and undergrowth. Use fertilization when appropriate and sow fungal spores when plants do not have nursery inoculation. This is achieved by spreading leaf litter collected from pine forests in formation.
6-7	6	1250	700	Pruning 1	Pruning up to 2 to 3 meters. Select according to criteria of growth, shape, quality and luminosity of the soil. Do not exceed 35% tree height. Sort waste in the planting line, leaving the inter row clear. Encourage the formation of herbaceous (non-shrub) strata in inter-row strips, as it favours the formation and protection

Dominant height (m)	**Age (years)**	**Residual density (trees/ha)**		**Cultural activities**	**Technical Specifications**
					of mushrooms against the effects of wind.
6-7	6	700	550	Thinning	Waste or semi commercial Thinning of all unpruned trees. Sort waste not marketed in belts over the row. Schedule activity in those seasons where there is no fungus production (winter or summer). Flip those individuals that improve the luminosity of the forest (the formation of holes of light considerably increases the production of mushrooms). Avoid closing glasses.
14-18	9	500	200	Commercial thinning	Commercial thinning Turning of all the trees without raising of glass and those necessary to improve the luminosity of the forest. Sort waste not marketed in belts over the row. Schedule activity in those seasons where there is no fungus production (winter or summer). Avoid closing glasses.
24-38	16	400	100	Commercial thinning	Commercial thinning Turning of those trees that improve the brightness of the forest. Sort waste in belts over the row. Schedule activity in those seasons where there is no fungus production (winter or summer). Avoid closing cups and encourage the development of herbaceous strata, but not shrub.
28-32			400	Final Harvest	Harvest of all trees. Waste management in belts in contour lines.

Conclusions and recommendations to practice

The sequestration and management of organic carbon is essential to re-carbonize soils from previous intensive agricultural use. By re-carbonizing soils using Pinus radiata roofs, especially through the organic horizon O and sub-horizons (OF, OF, OH), organic carbon is sequestered and fixed in ranges of 22 to 44 ton / ha, which plays a role fundamental in the nutrition and fertility of the forests.

In this way, forest soils are re-carbonized, initially conforming to the fine branches, acicles and fungal hyphae that make up the leaf litter (forest mulch) and in turn contribute to soil biodiversity.

The typical forest humic forms when re-carbonizing soils for forest use and with their corresponding transitions correspond to Mull, Moder and Moor. Moor-like humic forms are humidified to Moor and humic-type humic forms are humidified to mull through the interaction of forest management and intrinsic soil biodiversity.

In these humic and modern humic roofs, ectomicorric fungi that correspond to the species Suillus luteus and Suillus granulatus develop, which develop in the forests of Pinus radiata from 6 to 20 years.

The most important edaphic-ecological factors that influence mycorrhization and mushroom production correspond to light intensity, temperature, the percentage of soil moisture, fertility and pH.

From the forestry point of view, the level of vegetation cover of the forest constitutes one of the determining factors in the production of mushrooms, as well as the regulation of the percentage of luminosity that reaches the ground (a minimum of 23 percent), the level of undergrowth and type of litter and type of forest management.

The litter, forest mulch (10 to 15 cm high), contributes to the protection and proliferation of mushrooms, increasing the resistance to soil evaporation and dehydration of mycelia. Especially important is the depth of the litter in the protection of mycelia.

A multiple use forest management should be contemplated in order to achieve the re-carbonization of previous intensive agricultural soils through vegetative pine covers that consider factors the density and initial spatial distribution of the plantation, successive forest management interventions (thinning and pruning), so that the closing of cups does not occur and the luminosity is favored in such a way as to induce the provision of fungal eco-systemic services (suilus luteus and suilus granulatus).

The structure and temporal planning of silvicultural interventions are compatible with the production of edible fungi, because the initial density is not high and the successive thinnings allow a good luminosity of the undergrowth.

Research is currently being carried out, to evaluate the effects of soil conservation practices such as selection of the species of broadleaf and shrubs (lupinus) through the application of soil and fauna strains, the improvement of mineralization and humification conditions through whitewashing (CaO) with basal applications of low doses of MgO and P2O, according to litter thickness, and promote fertilization practices that do not favour excessive stimulation of humification and mineralization processes of re-carbonization processes by budding oxidative bioturbation of soil flora and fauna.

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Soil functioning relates to land use in a sustainably managed agro-sylvo-pastoral ecosystem

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Abstract summary

The sustainable and diversified land use in the Barroso Globally Important Agricultural Heritage System (GIAHS) led to a rural mosaic of agricultural fields, pastures and natural ecosystems. We aimed to assess whether soil functional biodiversity matched this aboveground landscape complexity. Using soil nematode communities as bioindicators, we characterised soil processes in the Montalegre region of the Barroso GIAHS in Portugal, and further related soil functioning with three distinct land use types: agriculture, pasture and natural forests. Nematode communities revealed high structure and enrichment throughout, but agriculture and pasture soils were apparently more fertile ($p<0.01$). More nutrients were allocated to bacterial-feeding than to fungal-feeding nematodes, depicting dominant bacterial decomposition in all land-use types, but the stronger nutrient flow in agriculture site originated from herbivory suggested a larger pressure of plant-parasitic nematodes in these sites. These were less stable sites, where the regulatory potential of higher trophic levels on the soil food web was significantly lower than in pastures or natural forests. Overall, belowground functions mirrored aboveground trends and support the sustainability of this ecosystem.

Keywords: Decomposition, fertility, Globally Important Agricultural Heritage System (GIAHS), metabolic footprint, nematode bioindicators, soil food web

Introduction, scope and main objectives

In Globally Important Agricultural Heritage Systems (GIAHS), the rural landscape has been shaped by human activities for millennia, providing sustainable, diverse and resilient agroecosystems. These are dynamic systems, unique due to their local environmental conditions, but also because of the human traditions, culture and knowledge they harbour. The role of sustainable practices leading to highly diverse landscape mosaics of human livelihoods, farming and surrounding natural systems is key in recognising and safeguarding GIAHS (FAO, 2019). However, little is known about the local belowground processes that ultimately maintain these ecosystems.

Soils host a wide variety of organisms that provide key ecosystem services, such as decomposition and nutrient cycling, detoxification of chemicals and purification of water, and the regulation of pests and diseases. Among these, nematodes are notoriously abundant, making up to an estimated grand total of $4.4x10^{20}$ nematodes in topsoil worldwide, that together hold and turnover about 0.3 gigatonnes of biomass (van den Hoogen *et al.,* 2019). This is a highly diverse group represented in all trophic levels in the soil food web, apart from producers. Due to their ubiquity and abundance in soils, their ease of extraction and classification into functional groups, and their rapid response to varying soil conditions, they have been proposed as bioindicators of soil functioning (Bongers and Bongers, 1998). The analysis of soil nematode communities has since been a useful tool to interpret soil processes in a range of ecosystems subjected to various land-uses and to different sources and levels of disturbance (Ferris, Bongers and de Goede, 2001).

Here, soil nematode community analyses are applied to provide a characterisation of soil processes in the landscape mosaic in the Montalegre district of the Barroso sylvo-agro-pastoral ecosystem, a GIAHS in the North of Portugal. These include indicators of soil food web structure and complexity, nutrient enrichment, and the magnitudes of activity of different compartments of the soil food web, involved in decomposition, herbivory or regulation (Moore and Hunt, 1988). Our main goal was to provide a wide ecological characterisation of soil functioning in the Barroso GIAHS. Specifically, we aimed to relate patterns arising from nematode community analyses with three different but sustainable land-use types in this region: agriculture, pasture and the surrounding natural environment.

Methodology

The study area was in the Montalegre district of the Barroso GIAHS, a mountainous area with temperate climate, that combines natural forests with a managed agro-sylvo-pastoral system closer to inhabited areas along rivers. Small fields growing rainfed horticultural crops and cereals in rotation with grazed fallows are interspersed with meadows. These become marsh-like due to traditional gravity irrigation, that prevents freezing in Winter, providing permanent pastures for beef cattle, the main activity. Cattle graze freely in the permanent pastures, providing organic input into these soils throughout the year. Local people collect shrubs from the understory of nearby natural forests for animal bedding that, after pile-composting, are incorporated into agricultural fields (FAO and ADRAT, 2018).

Sample sites were selected comprising agriculture, pasture and adjacent natural forests up to ca. 5 km downstream of the spring of the Cávado River and two of its contributaries, Cabril and Rabagão. Two agricultural sites were sampled, in putatively different stages of a rotation, namely an actively growing turnip field, and a recently harvested maize field. Pasture sites (7) were extensive cattle-grazed meadow/marshes. Natural sites (6) were oak-dominated forests. Sampling was done between December 2018 and January 2019; composite soil samples, 15 in total, were taken at a depth of about 10 cm.

Nematodes were extracted from 100 ml of soil from each sample site through the tray method, and obtained suspensions were concentrated by sieving. Up to 150 nematodes were identified to family level using an inverted microscope. All nematodes were quantified in each sample. Family abundance was analysed using NINJA (Sieriebriennikov *et al.*, 2014) to obtain the Enrichment Index, indicative of the proportion of bacterial and fungal-feeding colonisers and enrichment opportunists, and the Structure Index, indicative of the proportion of persister nematodes, often in higher trophic groups, revealing food web complexity (Ferris, Bongers and de Goede, 2001). Metabolic footprints taking into account both the abundance and the metabolism and biomass of different nematode families were calculated for enrichment and structure components. These were also calculated for each nematode trophic group (bacterial-feeder, fungal-feeder, omnivore, plant parasite or predator), and are indicative of nutrient flow and allocation to different components of the soil food web (Ferris, 2010). Results for agriculture, natural or pasture sites were further compared using General Linear Models in SPSS for Windows v. 22.

Results

Soil nematode communities in this area had overall high structure index values, consistently over 75 percent across sites. Agriculture and pasture soils were similarly nutrient-enriched, with an enrichment index value significantly higher than that of natural soils. However, the nematode enrichment footprint of agriculture sites was smaller than those of pasture or natural sites ($p<0.01$). Larger structure metabolic footprints were obtained for natural and pasture soils than for agricultural soils ($p<0.01$). Further, the asymmetry (diamond-shape) of the rhomboid suggests an imbalance between enrichment and structure components in agriculture sites, with pasture and natural sites having more balanced enrichment and structure footprints (Figure 1).

Nutrient flow through the food web across nematode trophic groups, as shown by the magnitude of their individual metabolic footprint, differs between the three land-use sites. Fungal-feeder footprints are small throughout, and the bacterial-feeder footprint is significantly larger in pasture, followed by natural sites, with the smallest values obtained in agriculture sites ($p<0.01$). In the latter, the herbivory channel is dominant ($p<0.05$). Nutrient allocation to higher trophic levels is reflected on the omnivore and predator footprints, larger in natural and pasture sites.

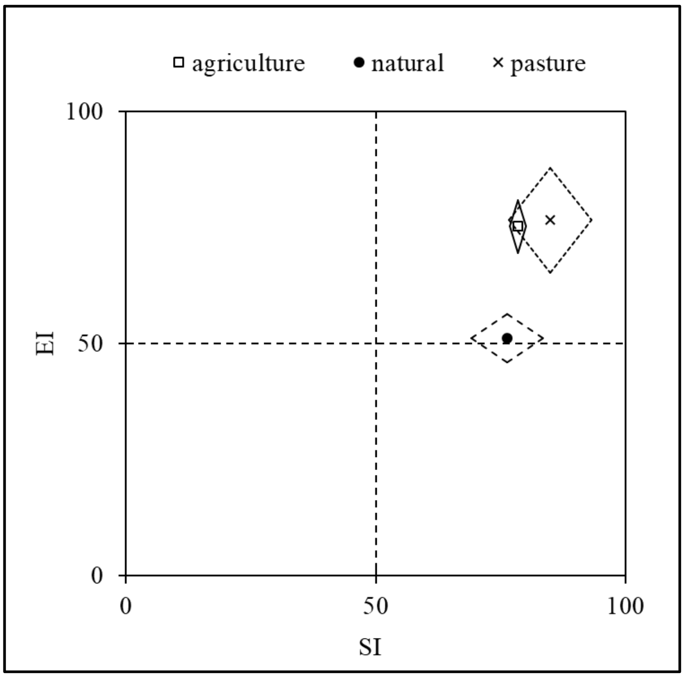

Figure 1: Faunal profiles of soils in agriculture, natural, or pasture sample sites; EI – enrichment index; SI – structure index

Rhomboids represent functional footprints (represented with a *k* value of 1.5), with enrichment and structure footprints shown in the vertical and horizontal axes, respectively; values are means of agriculture (n=2), natural (n=6) or pasture (n=7) sites.

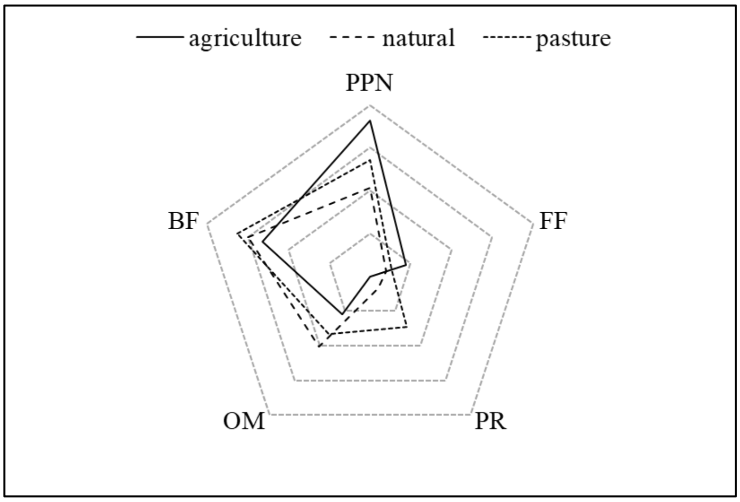

Figure 2: Magnitude of metabolic footprint profiles inferred from nematode communities, depicting nutrient flow through BF – bacterial-feeders, FF – fungal-feeders, OM – omnivores, PPN – plant-parasites and PR – predators

Values are mean of agriculture (n=2), natural (n=6) or pasture (n=7) sites.

Discussion

Soils in the study area had an overall highly complex and regulated, and moderately enriched nematode community, typical of relatively undisturbed environments (Ferris, Bongers and de Goede, 2001). Both agriculture and pasture were more nutrient-enriched – depicting higher fertility - than natural sites, which agrees with the traditional fertilisation practices used in this closed system (FAO and ADRAT, 2018). Although crop rotation and fallows are commonly used in the small fields, they are still impacted by agricultural practices: the larger structure footprint in natural and pasture sites indicate more nutrients are allocated to longer-lived, persister nematodes with a regulatory role in soil (Figure 1). This is supported by the observed trend for a larger metabolic footprint of higher trophic levels in pasture and natural sites relative to the agriculture sites, revealing a greater flow of nutrients through omnivores and predators that prey on other nematodes (Figure 2).

Having the smallest enrichment footprint, soil biota of agriculture sites are not as responsive to rapid nutrient enrichment and therefore nutrient-use efficiency is lower in these sites. The asymmetric functional footprint in these agriculture sites (enrichment vs structure footprint) indicates a lower food web regulatory potential by higher-level trophic groups (Figure 1). This may support the observed large allocation of nutrients to plant-parasitic nematodes in agriculture sites, where the largest herbivory footprint relative to that of all other trophic groups indicates most of the nutrients in the soil food web were obtained from plants. It also implied that plant-parasitic nematode pressure is larger on crop plants than in natural vegetation (Figure 2), even though nematode-induced yield losses have not been reported. With only two agricultural fields sampled, we cannot extrapolate these findings to the agricultural settings of the region, where other crops are commonly grown.

Although the herbivory footprint was similar in agriculture and pasture, most nutrients in the pasture food webs were derived from bacteria, indicating a higher activity of the bacterial decomposition channel. In all sites, bacterial decomposition dominated over fungal decomposition, with the fungal-feeder footprint being unexpectedly small throughout (Figure 2). In fact, stable soil environments with organic input usually have a larger proportion of fungal decomposition (Ferris, Bongers and de Goede, 2001). We speculate that the animal origin and frequency of organic input may lead to an N-enrichment of soil organic matter, thus reducing its C:N ratio, and enabling the faster decomposition by bacteria.

Conclusions

Traditional sustainable practices that have shaped the above-ground landscape of the Barroso GIAHS are evident in the belowground functioning of this ecosystem. The long-term anthropogenic distribution of nutrients from natural forests to agricultural fields and man-made permanent pastures, highly dependent on animal husbandry, has enriched agriculture and pasture soils, where the soil food web drives the rapid decomposition of organic matter, increasing fertility. Among the three land use types assessed, agriculture was, expectedly, the most disturbed, with a larger proportion of nutrients in soil food webs originating from belowground herbivory, and a smaller regulatory effect of higher trophic levels.

Acknowledgements

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Diversity and abundance of the entomopathogenic fungi *Metarhizium* spp. at high sampling resolution in typical Swiss permanent grassland

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Abstract summary

Inoculation and conservation biological pest control strategies based on entomopathogenic fungi attract more and more interest as tools for the implementation of sustainable agriculture. *Metarhizium* spp. are entomopathogenic fungi most prevalently found in the soil and they are widely used as biological pest-control agents. In order to enhance the natural regulation potential of *Metarhizium* spp., a better understanding of the ecology and behaviour of these fungi is needed. In a spatiotemporal approach, the present study has examined abundance and diversity of *Metarhizium* spp. in three Swiss permanent grassland types. Fungal abundance, as well as the genotypic diversity using microsatellite markers was assessed. *Metarhizium* spp. abundance and genotypic diversity varied across sites as well as within site. Extensive permanent grassland showed the highest *Metarhizium* spp. abundance but harboured the least diverse population while *Metarhizium* spp. populations of the orchard and the low-intensive grassland were more diverse but less abundant. Multiple correlation analyses between fungal abundance and soil physicochemical parameters showed that phosphorous is the most important factor supporting *Metarhizium* spp. abundance in the grassland fields investigated. Further research should include additional factors like host abundance in order to elucidate the mechanisms and processes influencing fungal communities at habitat-level.

Keywords: Permanent grassland, fungal abundance, genotypic diversity, spatiotemporal variability, microsatellite marker, community composition

Introduction, scope and main objectives

Metarhizium spp. are insect-pathogen fungi most prevalently found in soils in many different habitats and they are widely used as biological control agents (BCA) for pest control in agriculture. They have been identified worldwide in natural as well as in non-natural habitats (Meyling and Eilenberg, 2007). Presence, abundance and population structure of these fungi in the environment depend on many abiotic (e.g. pH, carbon-, potassium- or nitrogen content) and biotic factors (e.g. host presence, vegetation). In an agricultural context this may include the land-use type, with factors like crop-type, -management and -protection. So far, studies addressing *Metarhizium* spp. occurrence and diversity have focussed on differences among habitats and/or cropping systems (Garrido-Jurado *et al.,* 2015, Meyling and Eilenberg., 2007) and investigations on lower scale distribution, i.e., within habitats are missing. In a recent study comparing abundance and diversity of *Metarhizium* spp. in 30 grassland, arable land, and forest plots in Switzerland, *Metarhizium* spp. abundance was significantly higher in grassland plots as compared to arable land and forest plots. (Fernández-Bravo *et al.*, unpublished data). However, variation of the within plot data was very high, which highlighted the need for detailed investigations of spatial and temporal within plot variability at higher sampling resolution.

In order to address this aspect, we have performed a study with the following aims: 1) investigate the abundance, diversity and population structure of *Metarhizium* spp. with increased sampling resolution; 2) assess the spatial and temporal distribution of *Metarhizium* spp. and 3) evaluate the effect of physical, chemical and biological parameters on *Metarhizium* spp. populations in three typical Swiss grassland fields.

Methodology

Experiments were performed in three different permanent grassland plots located within a radius of 400m at Agroscope Reckenholz, Zürich (Switzerland). The first plot was characterized as a permanent extensive grassland, the second as a 4 years low intensive grassland and the third as an orchard. Land-use, chemical as well as physical soil parameters at the sites were measured. Each of the 3 plots (10 x 10 m) was divided into 25 subplots (2 x 2 m). One soil core was taken from each subplot in each plot. Sampling was performed 3 times in one year (autumn, spring and summer). *Metarhizium* spp. detection and isolation was carried out following the protocol developed by Strasser, Forer and Schinner (1996). Abundance of *Metarhizium* spp. was assessed by determining colony forming units (CFUs) per gram of soil.

DNA was extracted from each isolate and multi locus genotypes (MLGs) determined using 15 microsatelite markers (Mayerhofer *et al.,* 2015).

Metarhizium species identification was performed with one randomly selected isolate per MLG. The 5' portion of translation elongation factor 1 alpha (EF-1α) was amplified, sequenced and the sequences aligned with *Metarhizium* spp. reference strains described by Bischoff, Rehner and Humber (2009).

Population diversity and community structures were investigated with the R statistical program using the Poppr and Vegan packages.

Results

Metarhizium spp. abundance was determined in each soil sample. CFU values per gram soil dry weight (CFU g^{-1} soil DW) ranged from 66.7 to 21,600 (Figure 1). Across all the three sampling time points, the extensive grassland showed significantly higher mean CFU abundance than the other two sites ($p<0.01$). Except for the summer sampling, means CFUs g^{-1} soil DW were higher in the orchard than the low-intensive grassland. At each sampling time point, CFUs g^{-1} soil DW were significantly affected by the site ($p<0.01$). In spring and in summer, CFUs g^{-1} soil DW from the orchard and the low-intensive grassland did not differ significantly from each other ($p=0.34$) while the extensive grassland was significantly different from the orchard ($p < 0.01$) as well as from the low-intensive grassland ($p<0.01$) (Figure 1).

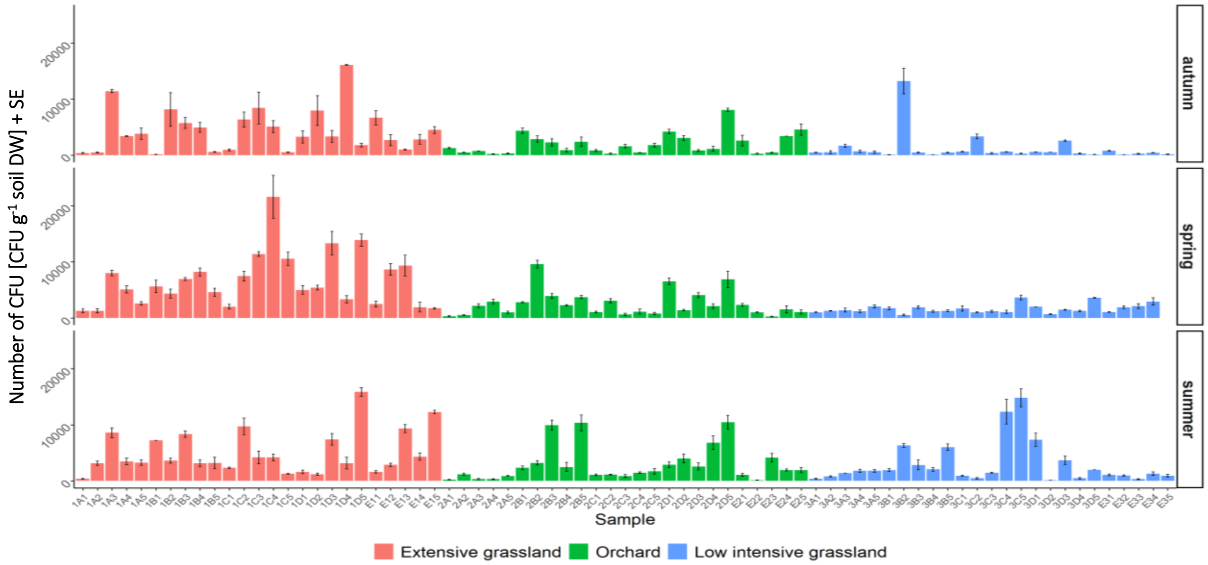

Figure 1: Abundance of *Metarhizium* spp. in soil samples collected from three grassland plots at three sampling time points

Columns represent means of colony forming units per gram soil DW (CFU) ± standard error of the mean (SE) of three replicate plates per soil samples.

The correlation of *Metarhizium* spp. abundance with soil physical and chemical parameter such as: carbon mass, humus mass, calcium-, potassium-, and magnesium content, available phosphor, total phosphor , pH, sand, silt, clay and, total nitrogen. Potassium and silt content as well as available phosphate correlated negatively ($\rho=-0.77$, $\rho=-0.73$, $\rho=-0.87$; $p<0.01$), whereas sand content correlated positively ($\rho=0.59$, $p<0.05$) with *Metarhizium* abundance. All the other soil parameters measured did not significantly correlate with *Metarhizium* abundance.

For *Metarhizium* spp. diversity and population structure analyses, only the isolates obtained in autumn and spring were considered. 299 isolates were collected in total, i.e., 126 in autumn and 167 in spring samplings. The MLG based population structures at the three plots for both sampling time points are shown in Figure 2. In total, 29 different MLGs were identified. In autumn, 19 MLGs were detected of which three MLGs (7, 8, 13) occurred at each site, two occurred only in the orchard and the low-intensive grassland and 14 occurred at a single site only. In spring, 20 MLGs were detected of which the same three MLGs (7, 8, 13) occurred again at all three sites. Seventeen MLGs were site specific in spring. EF-1α sequence analyses allowed allocation of the 29 MLGs to three species, i.e., *M. brunneum*, *M. guizhouhense* and *M. robertsii* (Figure 2).

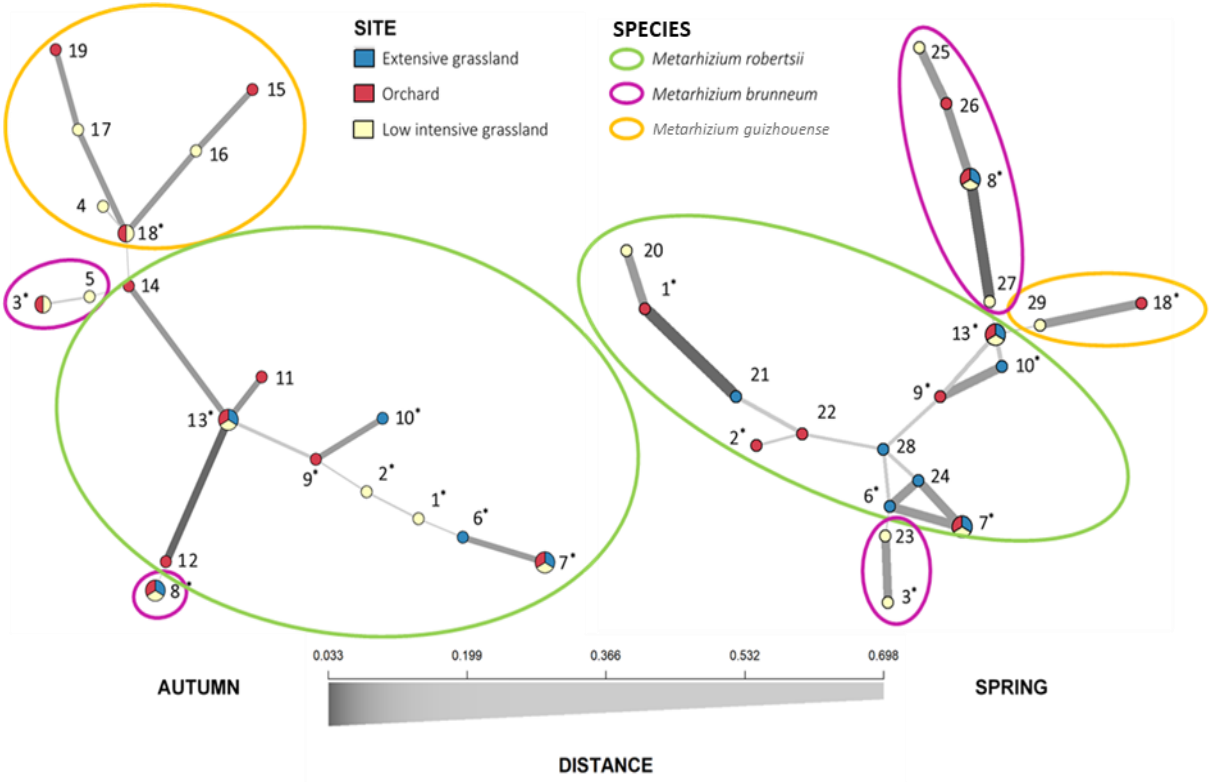

Figure 2: *Metarhizium* spp. population structure in autumn (left) and spring (right) displayed as minimum spanning networks based on adapted Bruvo's genetic distance

Nodes represent genotypes and node-colors indicate sites at which they were detected. Thickness and shading of the lines linking nodes represent the genetic relation. Genotypes marked with a * are present at both sampling time points. Colored circles indicate species allocation.

Discussion

In this study, we have investigated the abundance and genetic diversity of *Metarhizium* spp. with high spatial and temporal resolution in three grassland plots. Results have demonstrated that abundance and particularly the diversity studied were different, not only among the plots, but also among sampling points and sampling times, that is, every grassland site harbored different populations of *Metarhizium* spp. even though they are located within a radius of 400m. This fact is clearly demonstrated by the high number of site-specific MLGs found, not only in each site, but also in each sampling point and sampling time. Indeed, only three MLGs of the 29 obtained in total were found in the three grasslands sites at each sampling time.

It is well known that environmental factors are one of the major drivers of presence and abundance entomopathogenic fungi in the environment. In order to further investigate this aspect, 13 soil physical and chemical parameters were included in our analysis to elucidate variation in *Metarhizium* spp. abundance in the three grassland sites. The factors potassium, silt and sand content as well as available phosphate had a significant effect on *Metarhizium* spp. abundance. Interestingly, in a recent study in which abundance and diversity of *Metarhizium* spp. in three different land-use types (arable land, grassland and forest) were investigated, different factors, i.e. C:N ration, bulk density and basal respiration explained most of the variation found (Fernandez-Bravo *et al.* unpublished data). These observations confirm that soil physicochemical parameters have strong effects on *Metarhizium* communities. However, it appears that the different factors affect the fungus in a site and/or time specific way, as the factors explaining most of the variation are different depending on the comparisons made.

It is evident that assessment of the effects of biotic factors need to be included in future studies. Particularly the effect of the abundance and diversity of insect hosts in the soil has to be considered, a factor that has been neglected so far. An associated study performed in the same plots and using soil cores collected in parallel to this research is currently conducted in order to explore in detail the relationship between *Metarhizium* spp. communities and soil micro-arthropod populations (Fernández-Bravo and Enkerli, unpublished data).

In summary, we conclude that the effect of some soil physicochemical parameter partially explain the variation of *Metarhizium* spp. communities in the three different grasslands selected. It will be important to include particularly biotic factors in future studies to further improve our understanding of the development and population dynamics of *Metarhizium* spp. in the soil. In addition, since the variation even within single plots can be substantial, further studies on even lower spatial scales might be necessary to understand the distribution of the fungus in soil.

Conclusions

- All grassland types harbored *Metarhizium* spp.. The abundance varied within site, across sites and across time.
- 29 genotypes belongings to 3 *Metarhizium* species were identified in the different populations.
- Soil physicochemical parameters are relevant determinants of *Metarhizium* spp. abundance. Soil available phosphorus is negatively correlated to the abundance.

Acknowledgements

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Effect of fairy ring fungi on topsoil micromorphology in Pyrenean grasslands

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Abstract summary

La Bertolina grasslands hosts many types of fungi that stimulate vegetation, generating a margin of increased growth in the form of a ring, which is called a fairy ring (type II). Studies have been carried out to determine the growth and the genomics of the species that generate them, however the effect that the sequences of appearance of the rings may have on the soil is unknown.

Three Fairy Rings were sampled in four different points, designated in a radial transect across each ring. The soil profile was described and analysed; and 24 thin sections were made from the surface horizons, scanned and analysed using the petrographic microscope.

The results show that neither the composition nor the microstructure of the surface horizons vary depending on the time the fairy ring appeared on the sampled spots. Nine types of fungal structures were identified (five types of spores, sclerotia, perithecia and hyphae). While their relative abundance obtained by visual estimation does not relate with the position within the rings, other biological traits as mesofauna droppings are, more frequent in the zone of highest vegetative growth (R13 and R19), with respect to the central and outer zones of the rings, which may respond to the higher moisture content in these areas.

Keywords: Soil micromorphology, Fairy Rings, biological components of soil, fungal structures, microstructure, pedofeatures.

Introduction, scope and main objectives

Fairy rings fungi are very important species in grassland soils fungal diversity, because of their strong impact on niche creation and nutrient cycling. They can affect soil properties such as pH and salinity, induce hydrophobicity, as well as mineralize the organic C and increase the reserves of K, P and Ca in the inner zone of the circle in comparison with the external unaffected grassland (Bonanomi *et al.,* 2013).

Soil micromorphology through the study of thin sections of undisturbed soil provide information on the microstructure, aggregates, pedofeatures and biological characteristics, as well as contents and relative distribution of organic matter, fungal activity, fauna activity at a microscopical scale, among others (Bosch-Serra *et al.,* 2017). This technique has many possibilities of use, which has never been applied to the study of soils under fairy rings.

The aim of this study is to determine the effect of the appearance of Fairy Ring on the micromorphological characteristics of grassland soils in the mountainous region of the Catalan Pyrenees.

Methodology

Study sites

The study was conducted in La Bertolina, an area of ecological interest, located in the Eastern Pyrenees (42°05'56" N, 1°39'40" E) (Municipality of Navés, Catalonia), 1276 m.a.s.l. (Figure 1). This grassland is extensively grazed by cattle (0.44 LSU ha-1) from May-June to November (Marí, 2017). The parent material corresponds to slope colluvia of polygenic calcareous conglomerates.

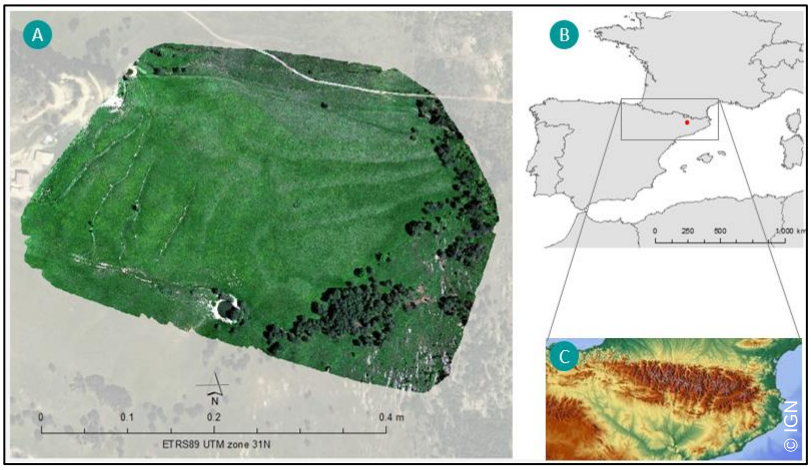


Figure 18: A) Experimental area La Bertolina. B) Red dot locates La Bertolina and the box the Pyrenean region and C) is the Digital Elevation Model of the Pyrenees Region. Source: IGN.

The survey was conducted in March 2019, in pre-Fairy Rings campaign, before the rings were visible in the vegetation. Selection of the rings (1, 2 and 3) and sampling in this grassland area was made possible by geo-referencing of these fairy rings from the mosaic aerial images from previous studies.

Profile description and physicochemical analysis

The representative soil of the area was described following SINEDARES criteria (CBDSA, 1983). The different horizons of the profiles were analysed following the methods described by Porta *et al.* (1986). Soil samples were air-dried and sieved to 2 mm. The following analyses were undertaken on all samples: pH (1:2,5), electrical conductivity (EC)

(1:5), carbonates, soil organic matter (SOM), phosphorous and particle size distribution.

Soil micromorphological analyses

Three Fairy Rings (1, 2 and 3) were sampled in four sampling points, designated in a radial transect across each ring: 1) In the center of the ring ("Center"); 2) In the geo-referenced 2013 ring zone ("R13"); 3) In the geo-referenced estimated apparition zone 2019 ("R19"); and 4) outside the ring, without any possible ring influence (> 2m outside the ring), ("Out").

Twelve undisturbed blocks (20x10x10 cm) were taken from the soil surface (one at each sampling point); were air-dried and impregnated with polyester resin with a fluorescent dye (Uvitex©) and two thin sections were obtained from each undisturbed soil block, for a total of 24 horizontal thin sections of 5×13 cm in size. They were studied using an Olympus petrographic microscope (BX51). True color scans of the sections were made with a high-resolution Epson scanner (Figure 2).

The guidelines of Stoops (2003) and Loaiza *et al.* (2015) were followed for the thin section description. The plant residues were described according to Nicosia and Stoops (2017) and Ismail-Meyers *et al.* (2018).

Description of the different excrements and other organic components was made following Stoops (2003), Loaiza *et al.* (2015) and Nicosia and Stoops, (2017). The excrements and biological traits were classified according to the relative abundance.

Fungal structures were exhaustively described according to morphological criteria and tentatively classified at the taxonomic level of order. Their relative abundance was visually estimated after the exploration of the entire area of each thin sections, from a reference thin section with the highest content of fungal structures (approximately 5 percent of the total surface or apparent volume). Six classes based on the visual estimation of relative abundance with respect to the occupied percentage of the solid fraction were established.

Results

Soil macromorphology, analysis and classification

The modal soil profile described in the field, was composed by four horizons: A1 (0 – 2 cm), A2 (2 – 10/12 cm), A3 (10/12 – 27 cm) and Bw (27 – 55/999 cm) and classified as Typic Ustorthent (Soil Survey Staff, 2014) or Regosol (FAO, 2015).

The soil is well drained, slightly stony, moderately deep, with a strong reaction to HCl 11 percent due to the presence of $CaCO3$, basic (pH: 7.8 - 8.5), non-saline, with organic matter content of 8,7 percent and phosphorus content (Olsen) = 12,3 ppm in the surface horizons. The texture of the A1 and A2 horizons is loam, while in the A3 and Bw horizons it is sandy loam.

Soil micromorphological analysis

The groundmass of all the sections has a single spaced porphyric c/f related distribution. The micromass is light brown in colour, mottled, with a weak crystallitic micritic b-fabric. The organic material is randomly distributed throughout the thin section, while the presence of charcoal, fungal structures, and pedofeatures vary in abundance and in distribution.

The upper centimetres of the A1 horizon show a moderately developed crumbly and/or laminar structure, which changes in depth to a moderately developed subangular block structure.

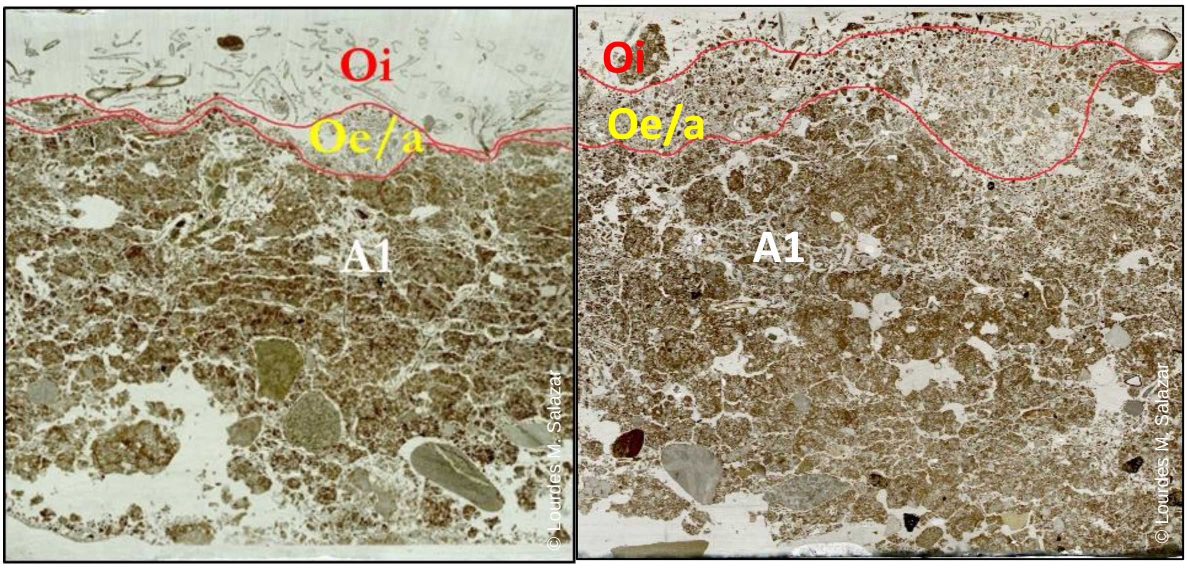


Figure 2: Thin sections scans using parallel polarizers

Left) laminar structure and subangular blocks. Ring 3, R13. Right) crumbly structure and subangular blocks. Ring 1, R13.

Fungal structures

Nine types of fungal structures were identified (five types of spores, sclerotia, perithecia and hyphae). The relative abundance of equinulated spores (Figure 3 A) (order *Agaricales*) seems to be related to the zones of appearance of the rings, because only appeared in the zones "R13" and "R19. The orange spores (Figure 3 B) (order *Glomerales*) seems to be related to the zones of appearance of the rings due to its symbiotic functionality and its relatively greater abundance in the zones of the rings "R13" and "R19".

The relative abundance of brown rough spores (Figure 3 C) and brown square spores (Figure 3 E) (order *Eurotiales* respectively), as well as brown smooth spores (Figure 3 D), perithecia (Figure 3 F) and hyphae (Figure 4 A) (order *Pleosporales* respectively), neither large sclerotia (Figure 4 B) nor small sclerotia (Figure 4 C) does not seem to be related to the zones where the rings appear.

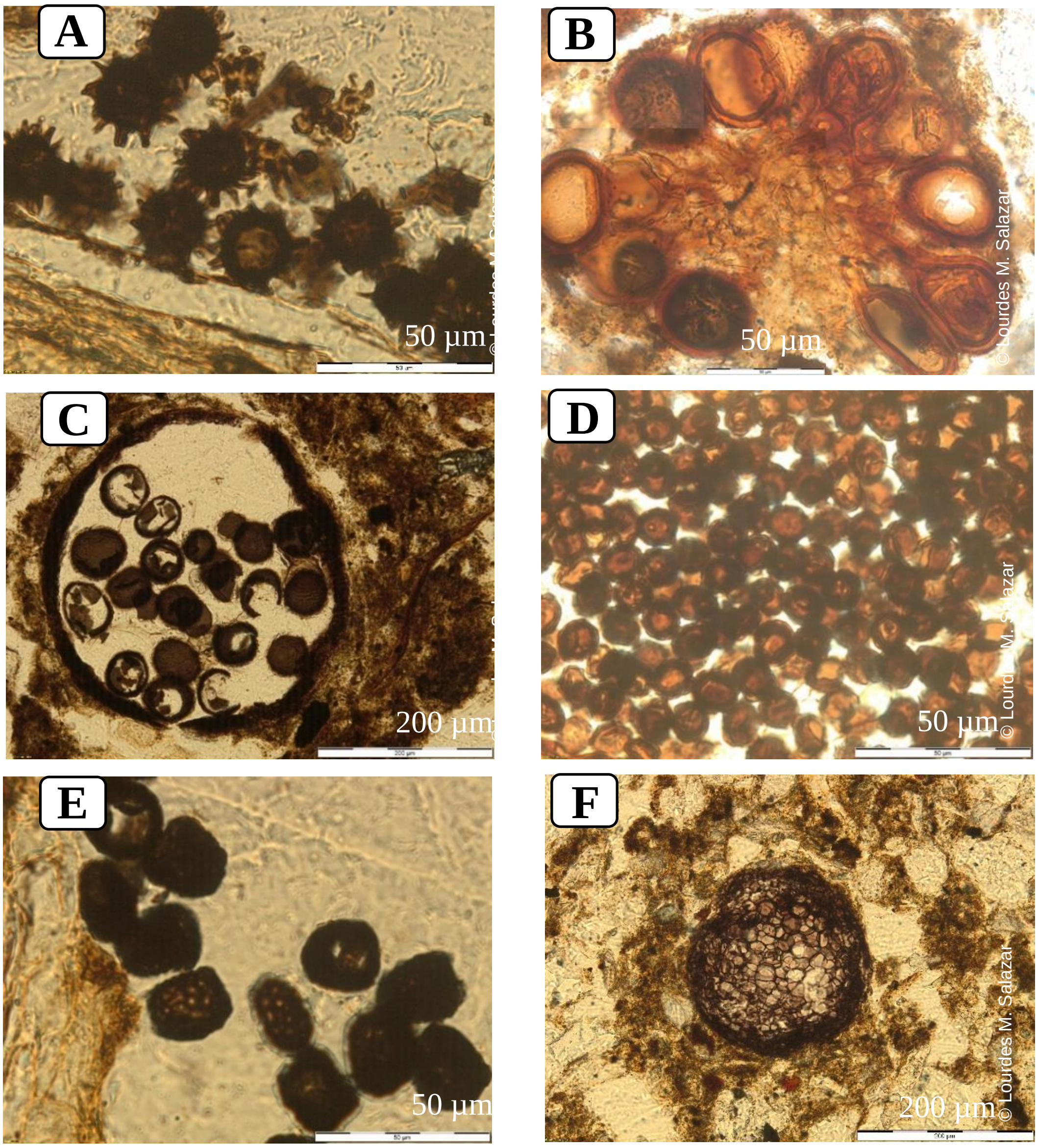
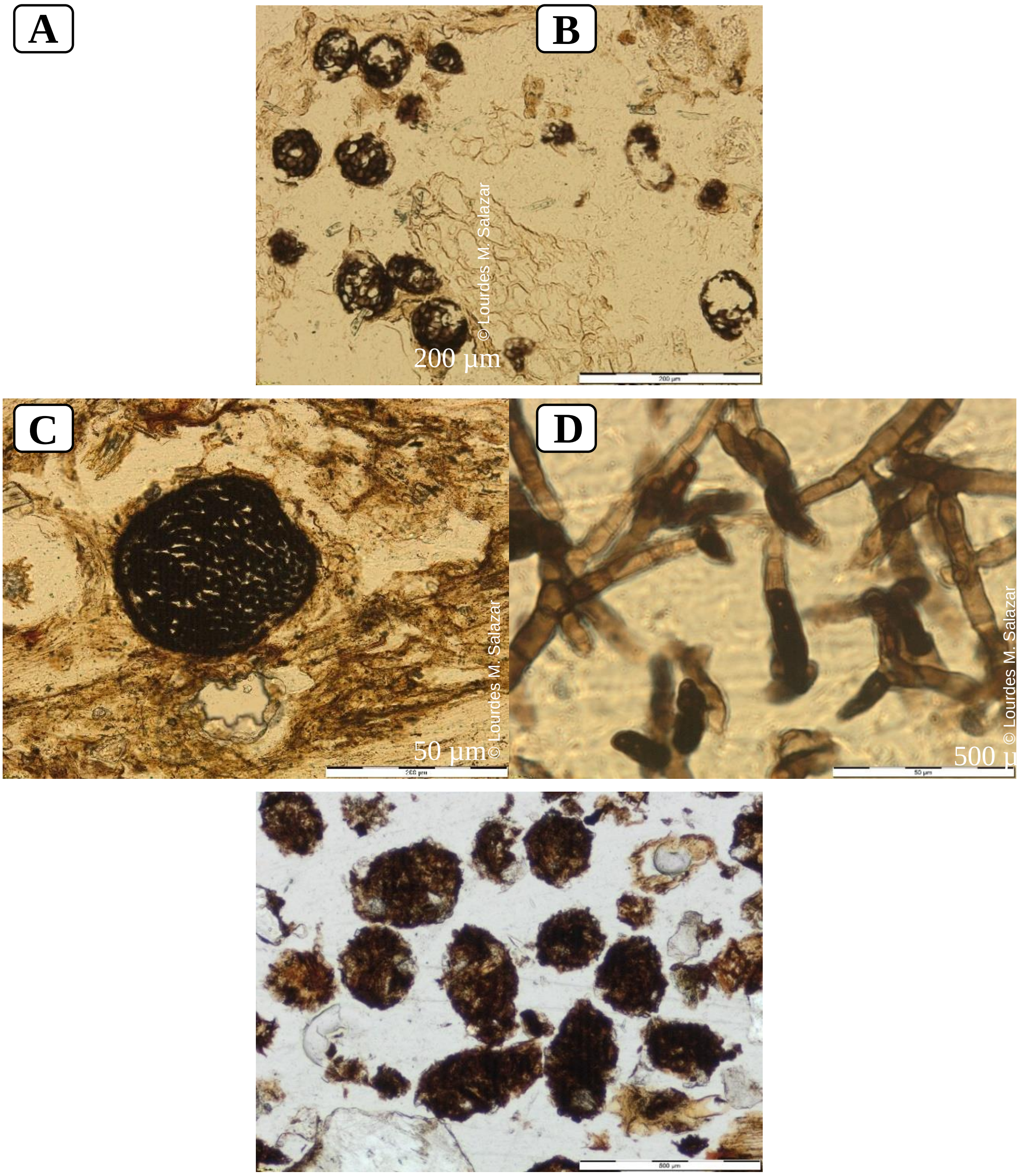


Figure 39: A) Equinulated spores. Ring 3, R13, PPL: parallel polarizers. B) Orange spores. Ring 3, R13, PPL. C) Brown rough spores and structure in which they are contained. Ring 2, Out, PPL. D) Brown smooth spores. Ring 2, R13, PPL. E) Brown square spores. Ring 3, R13, PPL. F) Large sclerotia. Ring 1, R13, PPL.

Pedofeatures

Were identified excrements of micro and mesofauna, as mites (Figure 4 D), enchytraeids (Figure 4 E) and isopoda/diplopoda/arthropoda (Figure 4 F).

Other features founded were biospheroids and charcoal.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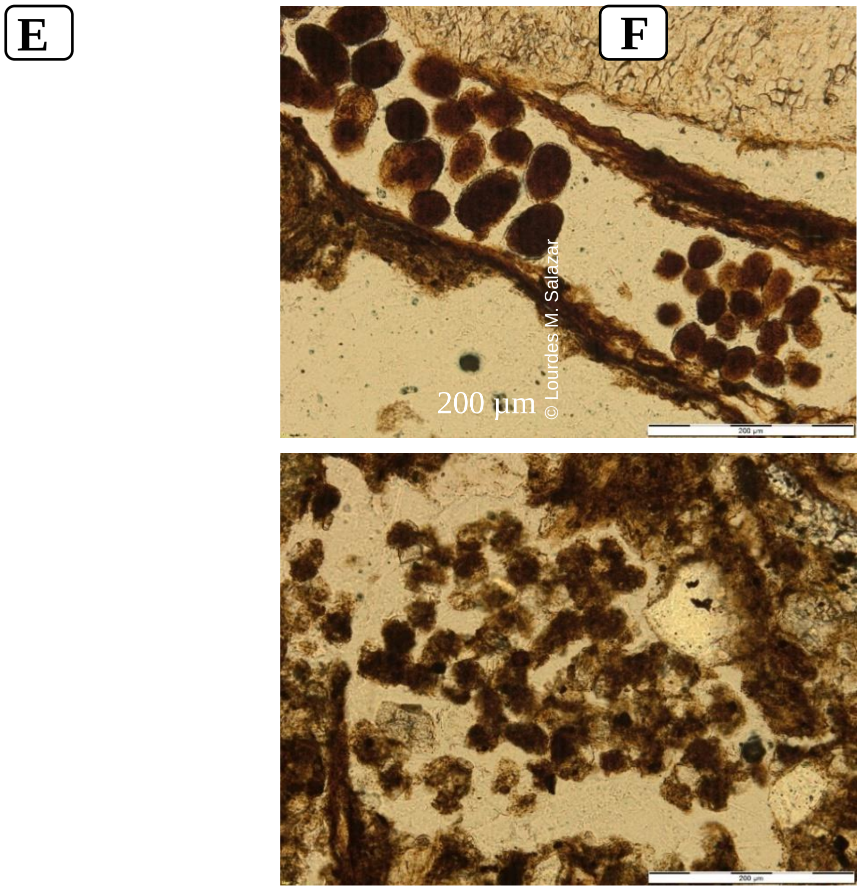

Figure 410: A) Small sclerotia. Ring 2, Out, PPL. B) Perithecia. Ring 1, Center, PPL. C) Ascomycota fungal hyphae. Ring 2, R13, PPL. D) Large droppings. Ring 1, R13, PPL. E) Mite droppings. Ring 2, Out, PPL. F) Enchytraeid droppings. Ring 2, Center, PPL.

Discussion

Neither the composition nor the microstructure of the surface horizons depends on the position of the ring zones, so we attribute the development of the laminar structure to grazing cattle trampling.

Large excrement were more frequent in the zones "R13" and "R19", which may respond to a higher availability of nutrients in the soil due to higher fungal activity; resulting in the enhanced vegetative development and therefore the mesofaunal activity.

Equinulated spores and orange spores are the only structures that seem to have some relationship with the zones of appearance of the fairy rings, due to their symbiotic functionality and relatively greater abundance in the internal zones of the rings. Nevertheless, when comparing the relative abundance between rings, it is observed that there is more variability between them than between the areas within them. On the other hand, it would be very difficult to find a distinct distribution pattern of the features, since the grazing season lasts until November and there has surely been some local redistribution of the fungal structures within each ring, masking the original differences. It is possible that a sampling when the rings are visible would allow to observe a greater quantity of structures clearly related to the rings.

Conclusions

The micromorphological analysis of topsoils under fairy rings on pastures from the Catalan Pyrenees has proved useful to identify fungal structures (five types of spores, sclerotia, perithecia and hyphae). Regarding the groundmass of the topsoil, the fungal activity seem not to affect the soil porosity since fungi are not soil engineers. Nevertheless, the more frequent large excrements (mesofauna) in the spots where the rings appear could be related to the higher nutrient or water availability due to the fungal activity. The lack of micromorphological differences point to some other mechanisms (chemical, biological) that may trigger the appearance and growth of the fairy rings.

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Thirty years of different amendment practices: effects on microbial soil turnover

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Abstract

This research considers the agronomic validity of the recovery of both bovine and vegetal biomasses in agriculture. The effect of different amendment practices on soil microbial turnover was studied in long term fertilization experiences.

The results showed that applying the same kind of organic fertilizers for long periods did not produce a particularly stimulating effect on microbial turnover.

Keywords: Soil biodiversity, soil metabolism, organic fertilization, long-term fertilization.

Introduction, scope and main objectives

Soil amendment practices play an important agronomic and environmental role in that, by improving the structure of the soil, they contribute to reducing erosion, increasing the retention of water and nutrients thus allowing an increase in production agricultural. In particular, organic soil improvers, such as zootechnical effluents, perform an important function in the turnover of the organic substance of the soil, also presenting a nutritional effect which, although modest and variable depending on the composition of the materials, is often characterized by a positive residual effect.

In the present research we wanted to evaluate the long-term effects of various amendment practices on the microbial turnover of the soil with the aim, on the one hand of enhancing and optimizing the recovery of effluents from livestock breeding and, on the other, of reaching an understanding of the transformations. These practices can induce changes in the microbial community of the soil and possible effects on the organic carbon and nitrogen cycle and on the dynamics of the nutrients of the soil.

Methodology

The trial was conducted in Italy, in an agricultural site which has been managed for thirty years with pluriannual fertilization plans with biomass (bovine slurry or manure) and crop residues (burying straw or stalks).

The added biomasses doses are: 7.5 t ha^{-1} of dry matter after corn and 6.0 t ha^{-1} of dry matter after wheat, which have been administered according to the following scheme: untreated soil (T); soil + straw/stalks (PS); soil + slurry (LQ); soil + manure (LT). The data analysed relate to the soils sampled for each thesis after thirty years of treatment.

For each thesis, the C content of the biomass was determined according to the fumigation-extraction method proposed by Vance *et al.* (1987) and soil respiration by titrimetric modifying a method proposed by Jenkinson (1976). The CO_2 measurement has been made at set times (1, 2, 3, 4, 6, 8, 10, 12, 14 days). The metabolic quotient q (CO_2) has been elaborated (Anderson and Domsch, 1990).

The potentially mineralizable nitrogen (Benedetti and Sebastiani, 1996), the potential nitrification test (Landi, 2004) and the potential ammonification test (Benedetti, Tittarelli and Rossi., 2004) were determined on the test sample according to the Stanford and Smith technique modified by Benedetti, Alianiello and Dell'Abate (1993). The forms of mineral nitrogen from the leaches were determined by Autoanalyzer Tecnicon II in accordance with Wall *et el.* (1975) for N-NH_4 +, with Kamphake *et al.* (1967) for N-NO3- and through a modification of the Griess-Ilosvay procedure for N-NO_2- (Keeney and Nelson, 1982).

In the nitrification test, values are expressed as percentage of nitrified N compared to the addition of 250 mg / kg of N from ammonium sulphate. In the ammonification test, values are expressed as percentage of ammonified N compared to the addition of 250 mg / kg of N from lactic casein.

All results are expressed on an oven-dry soil basis (105 8C, 24 h) and are the mean of three replicate analyses.

Statistical analysis of variance (ANOVA) was made, Duncan test was used to identify significant differences between treatments.

Results

The soil respiration values were incremented by the addition of organic substance in the form of bovine slurry or manure (table 1). The highest respiration values were detected in the soil treated with LQ, thus highlighting the persistence over time of the acceleration effect on the mineralization process of the organic substance administered. This soil also presents the highest specific respiration value, shown in table 1 as a metabolic quotient q(CO_2). The addition of organic matter causes an increase in biomass in all treatments (table 1). The examination of biomass values (table 1) allows a distinction to be made between zootechnical waste and crop residues. In particular for the latter, it is necessary to consider the anabolic work associated with the increase in biomass as a consequence of the immobilization induced in the short term.

Table 1: Soil C-biomass (BC), respirometric activity (CO_2), metabolic quotient q(CO_2)

Treatment	**CO_2** (mg C-CO2 kg^{-1}soil 14 $days^{-1}$)	**BC** (μgC_{mic} g^{-1}soil)	**q(CO_2)** (mg CO2- C g^{-1} Cmic h^{-1})
T	572 b	42 d	0.04
LT	834 a	88 b	0.07
LQ	898 a	63 c	0.12
PS	542 b	141 a	0.006

Different letters indicate significant differences at $P \leq 0.05$

The administration of organic matter to the soil also affects nitrogen metabolism. As for the activity of bacteria responsible for nitrogen mineralization (table 2), the percentage of ammonified nitrogen is significantly greater in all the fertilized theses compared to the control T while the nitrifying activity seems to be stimulated more intensely in the soils treated with bovine slurry (LQ) or manure slurry (LQ). The study of potential mineralization (table 3) did not reveal any significant differences between the theses. The concentration in total N (table 3) was significantly higher in the soil treated with bovine slurry and manure.

Table 2: Nitrification and ammonification test

	Potential Nitrification Test			**Potential Ammonification test**			
	weeks			weeks			
Treatment	**2**	**4**	**total**	**2**	**4**	**6**	**total**
T	19 c	3 b	22 b	15 b	2 b	2 b	19 b
LT	49 a	5 a	54 a	32 a	3 a	3 a	38 a
LQ	47 a	3 b	50 a	34 a	2 b	3 a	39 a
PS	24 b	3 b	27 b	31 a	2 b	4 a	37 a

Different letters indicate significant differences at $P \leq 0.05$ (Values are expressed respectively as% of nitrified N with respect to the addition of 250 mg / kg of N from ammonium sulphate and as% of ammonified N with respect to the addition of 250 mg / kg of N from lactic casein)

Table 3: total N in soil and % N potentially mineralizable (30 weeks)

Treatment	**N tot** (mg Kg^{-1})	**% N mineralized** (30 weeks)
T	610 b	4.0 a
LT	893 a	4.8 a
LQ	781 a	4.3 a
PS	646 b	4.4 a

Different letters indicate significant differences at $P \leq 0.05$

Discussion

The microbiological indicators as microbial biomass carbon (BC), soil respirometric activity, the metabolic quotient (qCO2), the biologic index of fertility (BIF) (Dilly *et al.*, 2018; Renzi *et al.*, 2017) as well as the nitrogen mineralization processes in soil (Florio *et al.*, 2015; Benedetti *et al.*, 1998; Rossi *et al.*, 1998) can be useful in the evaluation of the impact of the different management techniques on the stability of the microbial community and soil alterations or contaminations. In order to evaluate long-term variations of soil metabolic cycles it is essential to have long-term agronomic tests to deepen the knowledge of the changes induced over time by the adoption of sustainable fertilization techniques in agriculture (Luo *et al.*, 2014).

After thirty years of amending practices, the addition of organic substance in the form of bovine biomasses, repeated over time, has increased respirometric activity. Burying crop residues has a slightly depressive effect on respiration which corresponds to the highest biomass value and the lowest metabolic quotient of all theses. This indicates a state of stress that persists over time, while in other cases the low respiration values found may indicate that the system has reached a stable condition or a metabolic adaptation to the greater contributions of organic substance. A flattening of microbiological activity was also found for potential nitrification and ammonification, which show very low overall values.

Conclusions

The agronomic practices conducted consistently on each parcel for 30 years have undoubtedly led to an improvement in the organic carbon content in the fine parcels compared to the control (T), especially as regards bovine manure. However, prolonging the same fertilization practices in the long run, it could cause a change in the microbiological turnover of the soil not attributable to an increased biological fertility. By comparing the indices of microbial activity taken into consideration with the corresponding biomass values, it is possible to hypothesize a metabolic adaptation of the microbial population to the greater availability of organic matter in the theses amended with respect to the soil as is. In fact, in these theses there was no real increase in metabolic efficiency. It is useful to underline that the observed trends of the microbiological activities derive from the superimposition of the long-term effects on the short-term effects of the amendment practices in question, which makes the interpretation of the phenomena not immediate.

It would, therefore, be desirable to adopt eco-sustainable fertilization techniques that also provide for the alternation of the source of organic carbon made with biomass and any mineral additions in order to avoid, in the long run, a slowdown in soil metabolism and an excessive specialization of microbial soil biodiversity.

In conclusion the study suggests that long-term fertilization treatments produced a specialization in the composition of microbial community generating probably a simplification of the richness of microbial biodiversity. In order to preserve soil biodiversity, it is useful to manage soil fertility according to sustainable agricultural practices.

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Restoring the soil while preserving functions: a winning approach by exploiting microbial biodiversity

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Abstract summary

The mining activity often causes drastic changes in the soil profile, altering the equilibrium among the factors that influence the self-regulation capacity and resulting in a loss of soil biodiversity, soil composition and negatively affects ecosystem functionality. The contamination is reflected in the surrounding territories, contributing to their degradation and the limitation of their use for human activities and agricultural use. The remediation of these areas is a priority. Phytoremediation is a sustainable approach to contain and reduce environmental damages, since it respects and improves over time the soil biodiversity and soil functions. The effectiveness of phytoremediation can be enhanced by plant growth promoting bacteria (PGPB). The experience at the mining site of Ingurtosu (Sardinia, Italy) is here reviewed. The abandoned mine was chosen as experimental field trial to test an assisted phytoremediation, designed by creating a toolbox with native plants and bacteria. Aim was to improve soil functions needed to stabilise metals leaching in soil. The toolbox was selected on an ecological basis, associating the endemic pioneer plant *Euphorbia pithyusa L.* with an autochthonous PGP bacterial consortium. The positive effects on the plots treated with microbial bioaugmentation were evident over time, improving soil microbial biodiversity, soil functions and triggering a spontaneous revegetation. The field trial has been monitored over 7 years (2011-2018) and is still under observation.

Keywords: Microbial biodiversity, Bioremediation, Bioaugmentation, Soil recovery

Introduction, scope and main objectives

Soil degradation has assumed great importance in recent decades and the agri-food system is strongly affected by its impact. The growing demand for food collides with the conditions of severe soil degradation. The persistence of inadequate agricultural and forestry practices, industrial activities and ongoing climate change, suggest that soil degradation is destined to grow. Pollution contributes to a good extent to this phenomenon and requires to be faced through an assessment on ecosystem scale. It is known that pollution spreads from the areas of origin to the surrounding territories, in particular those downstream, which are often destined for agricultural use.

The maintenance of the available agricultural areas depends on the measures we manage to mitigate degradation of contaminated areas.

An interesting case is represented by abandoned mining areas, where environmental contamination chronically occurs, by release of metals through leaching, air dispersion or erosion of metals contained in the mine wastes or in contaminated sediments. In the European Union, the problem of soil degradation, estimated at around 38 billion euros per year, is moreover intensified due to the high population density. The remediation of these areas is therefore a strategic goal for European policies.

The remediation measures must be compatible with the conservation of the soil functions.

Chemical-physical technologies return, in the majority of cases, a substantially sterile soil, after treatment. Soil is therefore deprived of its functions of recycling elements in nature, primary for the survival of life on the Planet and peculiarly performed by microorganisms. To date, although it is not yet fully mature, bioremediation is the only technology capable of restoring the soil while preserving its functions.

Microbial biodiversity plays a key role in bioremediation technologies. The question is: how to use this biodiversity? The implementation of a "knowledge-based" functional bioaugmentation is based on a rational selection of the inoculum, identifying the autochthonous (or allochthonous) functional groups suitable for enhancing the metabolic functions necessary for remediation.

The 7-year-long experience at the mining site of Ingurtosu (Sardinia, Italy) is reviewed. The case study concerns an abandoned mine of sphalerite and galena, in Sardinia, where a field trial was set up in October 2011 (PF7 UMBRELLA project), continued within the regional project S.ME.RI. (2014-2015) and is still under observation.

Methodology

Ingurtosu mine site (Sardinia, IT) was one of the largest and most productive of sphalerite and galena mines in Sardinia, today it is part of the Geological and Mining Park and in 1997 it became one of the UNESCO networks of Geo-parks. The mine is inserted in a highly natural environmental and landscape context. The peak of productivity was the period between the 19th and 20th centuries, and the plants closed in 1969.

The scarce prevention during the extraction activity and following closure led to a considerable environmental impact. Under the European Project UMBRELLA, in September 2011 a field trial was set up in the abandoned mine (Figure1) in order to design a phytoremediation process, assisted by plant growth promoting bacteria (PGPB).

A preliminary characterization of the field was carried out through a hydro-geo-chemistry survey, a bioprospection for microbiology (Fig 2) and botany, heavy metal content and mobility analysis.

Following a greenhouse experiment (Wernitznig *et al.,* 2014) a toolbox was established by the endemic pioneer plant *Euphorbia pithyusa L.* associated with a bacterial consortium (UI), composed by ten selected native strains, metal-tolerant and good PGP (Figure 2; Table 1).

The field experiment considered 9 conditions tested in triplicate on the 27 subplots it was divided (Figure1). The different treatments were applied singly or in combination: bioaugmentation with bacterial consortia, mycorrhizae and the commercial mineral amendment Viromine™, a by-product of the bauxite industry which is widely used for environmental remediation processes due to its metal-trapping capacity. About three years later (December 2014), a second bioaugmentation was performed with a modified consortium (USMI), obtained with new PGP strains isolated from the microbial community that had spontaneously evolved in the meantime (Figure 3). In addition, the native species *Juncus maritimus Lam.* was added to the association.

The field management was minimal, minimal irrigation and bacteria dispersion were performed with the water taken from the Rio Naracauli, which flows under the experimental site, and which carries a load of 40 kg of zinc per day.

Sporadic inspections have been carried out from 2015 to the 2018,

Survival of plants in relation to soil metabolic activity, microbial biodiversity (Sprocati *et al.,* 2014b) and other biochemical and microscopic parameters (Medas *et al.,* 2015) were the parameters observed.

Microbial community was monitored over the time through biochemical (EcoPlate, BIOLOG System™) and molecular (PCR-DGGE, Denaturing Gradient Gel Electrophoresis) profiling.

The cultivable bacterial fraction was isolated from the rhizosphere, identified by sequencing of rDNA16S and characterized for PGP functions: auxin production, N_2 fixation, PO_4 mobilization and production of siderophores.

Results and discussion

An early assessment carried out 5 months after the first inoculum proved that subplots, which underwent bioaugmentation, retained the best metabolic activity with a high functional diversity, supporting the decision to proceed with the field trial (Sprocati *et al.,* 2014a).

In the first two years, a general gradual decrease in the plants survival was observed. In the plots treated with bioaugmentation the plant survival was 20 percent higher than controls. Later on, as a result of a very dry season, without any irrigation, the control plants survival fell close to 100 percent, while 20 percent of the plants assisted by bacteria survived and recovered over time, unlike the controls (Figure4). These differences in the survival and resilience of plants were reflected in the physiological soil profile -at the community level- presenting a much higher metabolic activity, measurable over time, in the plots treated with bioaugmentation (Sprocati, 2015).

The results of molecular and biochemical profiles in soil samples collected from June 2014 to March 2016 indicated that the bioaugmentation contributed to the development of a more specialized community, consisting of few species better adapted to the extreme conditions.

At a distance of 7 years from the beginning of the trial, many of the introduced plants *E. pithyusa* and *Juncus maritimus* are established and other new spontaneous plants have settled (mainly *Rumex* sp. and *Helichrysum* sp.).

Conclusions

The phytoremediation approach aimed at enhancing the growth-promoting functions of plants in the soil of the mine, through bioaugmentation with autochthonous PGP bacteria. The results obtained with only two bioaugmentation applications, show that a positive process was triggered, improving the soil metabolic activity, favouring the creation of a stable soil-plant-bacteria system, the survival of introduced plants and a spontaneous revegetation with endemic plants. The experience developed during Umbrella project (2009-2012) in the experimental field of Sardinia, together with the other experimental fields across Europe (Wells, Germany, Poland, Romania, Sweed) had led to the definition of a first attempt of guideline for the application of bioaugmentation for assisted phytoremediation. The continuation of the experience that took place in Sardinia up now, provides a useful indication for defining better guidelines.

Figure 1: the Ingurtosu Mine site (Sardinia, Italy) and the in situ experimental camp

The field experiment considered 9 conditions tested in triplicate on the 27 subplots.

Microbial load	9*10^6 CFU/g soil^{-1}
Colony morphotypes	41
N$_2$fixer	90 %
PO$_4$ mobilizer	44 %
Siderophore producer	63 %
Auxine producer	32 %
Soil extract (metal resistant)	54 %

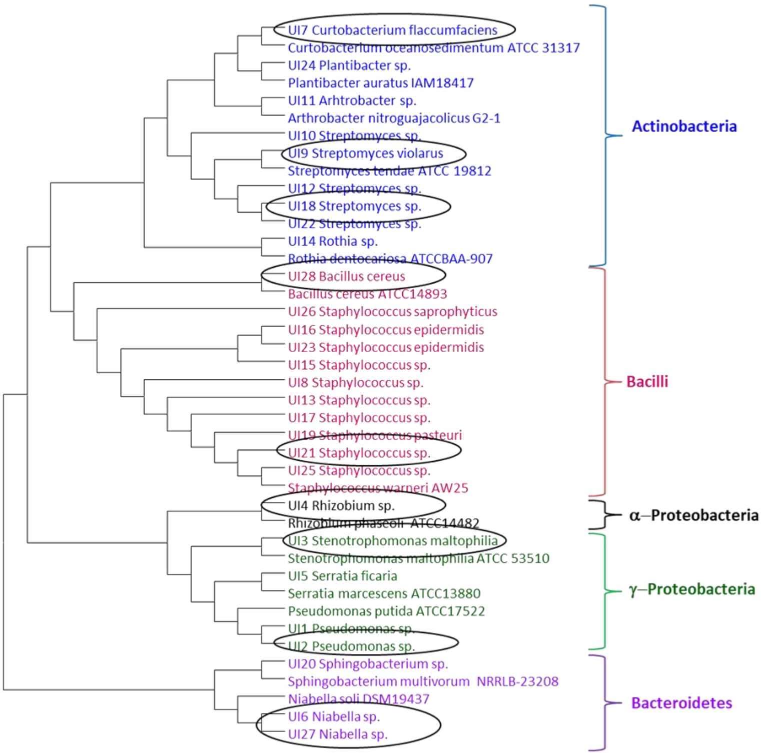
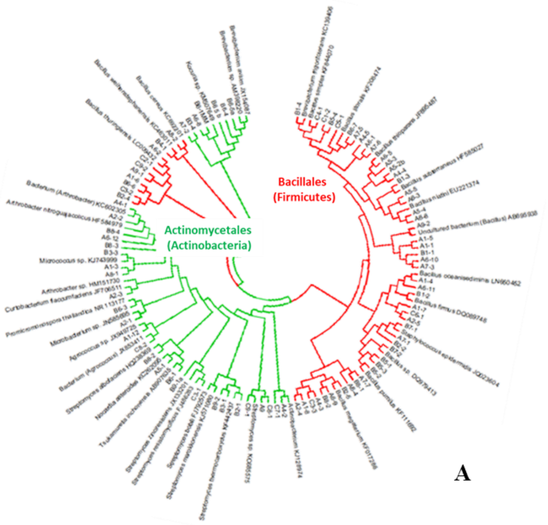


Figure 2: Phylogenetic three of the bacterial strains isolated from Ingurtosu mine site

In the table the composition of PGPB.

Table 1: Bacterial consortium UI, composed by ten selected native strains, metal-tolerant and good PGP, used for the bioaugmentation inoculum in the 2011

Bacterial Strain	**N$_2$ fixation**	**PO$_4$ mobilisation**	**Siderophore production**	**Auxine production**
Pseudomonas sp.	+	+	+	+
Stenotrophomonas maltophilia	+	–	+	+/–
Rhizobium sp.	+	–	+	++
Niabella sp.	+	–	+	+
Curtobacterium flaccumfaciens	+	+	+	+/–
Strept. violarus	+	+/–	+	+
Streptomyces sp.	+	+	+	–
Plantibacter sp.	+	+	+	+
Niabella sp.	+	–	+	+
Bacillus cereus	+	+	++	+



Colony morphotypes	**94**
N_2fixer	82 %
PO_4 mobilizer	35 %
Siderophore producer	93 %
Auxine producer	21%

B

Bacterial Strain	**N_2 fixation**	**PO_4 mobilisation**	**Siderophore production**	**Auxine production**
Bacillus aryabhattai	+	+	+	+
Micrococcus sp.	+	+	+	+
Streptomyces thermocarboxydus	-	+	+	-
Bacillus safensis	+	+	+	-
Streptomyces resistomycificus	+	+	+	+/-
Streptomyces bobili	-	+	+	-
Kocuria rhizophila	+	+	+	+
Streptomyces sp.	+	+	+	+/-

C

Figure 3: Phylogenetic tree of the bacterial strains isolated from Ingurtosu mine site

At the right the microbial community composition of PGPB and in the table the Bacterial consortium USMI of selected PGP bacteria used for a second bioaugmentation inoculum in 2014.

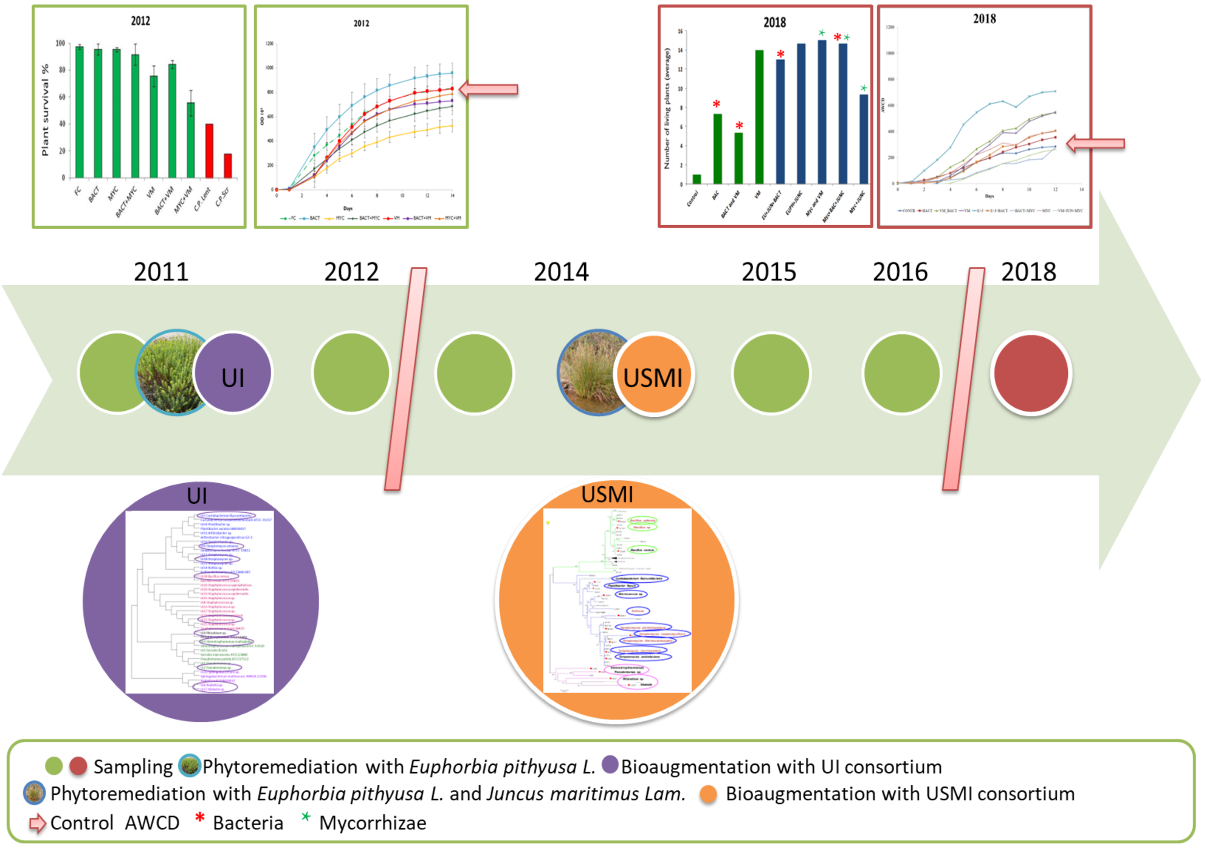


Figure 4: Timeline of interventions and monitoring done from 2011 to the present in Ingurtosu field experimental camp.

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Utilisation of grassland vegetation is favourable to the development of earthworm communities

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Abstract summary

Earthworms contribute to the major soil processes which determined most of the ecosystem services of grassland. The quantity and quality of the vegetation are key factors in maintaining earthworm communities, however effects of different herbage utilization on earthworm remain largely unknown. In this context, the aim of the present study was to determine the long-term effects of (i) herbage utilisation, (ii) animal presence, (iii) animal grazing intensity and (vi) grazing species (sheep or cattle) on the vegetation and earthworm communities in mesophile permanent grasslands. Vegetation and earthworms were sampled in a 15-years-old experiment in ACBB Theix site (Massif central, France) in beginning of April 2019. Abandoned and mowed grasslands were compared with pastures grazed at low or high intensities by cattle or by sheep, but at low grazing intensity only. Regarding vegetation, herbage utilisation by grazing or by mowing significantly decreased plant standing biomass (at least 1.7 times) compared to the abandoned treatment. The differences in plant standing biomass were associated with a significant change in plant community structure. Regarding earthworm communities, vegetation utilisation by grazing and mowing significantly increased earthworm biomass, total richness and Shannon index compared to the abandoned treatment. Except for the pastures grazed by cattle with a higher herbage utilisation level, earthworm abundance was at least 2.0 times as high in the grazing with low herbage utilisation level or mowing treatments as in the abandoned treatment. Earthworm communities were significantly different between grazed and mowed treatments notably due to changes within strict-anecic and endogeic earthworm. Overall, the utilisation of the vegetation by animals or by mechanical export is beneficial for earthworm communities. Results of each parameter will be presented and discussed as well as the perspectives about grassland management in Europe.

Keywords: Permanent grassland, grazing, cattle, sheep, mowing, vegetation

Introduction, scope and main objectives

Earthworms are widespread in temperate climates and contribute to the major soil processes, which determined most of the ecosystem services of grassland, such as herbage provision or water and nutrient regulation (Blouin *et al.*, 2013). Several studies highlighted that grassland management strongly modified earthworm communities through effects on vegetation, soil structure and animal grazing faeces (Cluzeau *et al.*, 1992; Curry, 2004; Schon *et al.*, 2017). Among grassland management, herbage utilisation is a key factor in the development of earthworm communities since it serves as both a refuge and a food source for earthworms (Curry, 2004). However, previous studies on the effect of herbage utilisation on earthworm communities focused on animals or mowing but none have investigated these two effects at the same time (Schlaghamerský, Šídová and Pižl, 2007; Curry *et al.*, 2008; Schon *et al.*, 2017). In this context, the aim of the present study was to determine the long-term effects of (i) herbage utilisation, (ii) animal presence, (iii) animal grazing intensity and (vi) grazing species (sheep or cattle) on the vegetation and earthworm communities in mesophile permanent grasslands.

Methodology

Study site

The study site consists of upland grassland plots located in Theix (Massif central, France) and is part of the long-term observatory ANAEE_F SOERE-ACBB (Systems of Observation and Experimentation in Environmental Research - Agroecosystems, Biochemical cycles and Biodiversity). The climate is semi-continental with an annual rainfall of 754 mm and a mean annual temperature of 8.8°C (2009-2019). In 2005 five treatments were established: (i) abandonment (AB), (ii) mowing regime (M), (iii) grazing by sheep with low herbage utilization (SH-), (iv) grazing by cattle with a low herbage utilization (CA-) and (v) grazing by cattle a high herbage utilization (CA+). None of the treatments involved fertilization. The AB plots were neither grazed nor mowed whereas the M plots were mowed three time a year and the grazing method consisted of five annual rotations. Each treatment was repeated in 4 blocks, resulting in a total of 20 plots.

Sampling and analysis of plant and earthworm communities

In spring 2019, in each plot, in three different square meters spaced at least 5 m apart, plant species abundance was visually assessed, then plant standing biomass and earthworms were both sampled at the same location.

Plant standing biomass was sampled using an electric shear, and placed into individual bags. In the laboratory, these samples (senescent and green material combined) were weighted after drying at 60°C for 48 hours.

Earthworms were sampled according to the ISO 23611-1 protocol modified. This protocol combines a chemical and a physical extraction. The chemical extraction consisted of three applications of 10 L of a formaldehyde solution on the square meter with increasing concentrations, each followed by 15 minutes of earthworm collection. The physical extraction consisted of the hand sorting of a 25 cm × 25 cm × 20 cm block removed from each quadrat. Collected earthworms were stored in pill bottles. In the laboratory, the sampled earthworms were identified to the lowest possible taxonomic rank (species or genus), assigned to a functional group (epigeic, epi-anecic, strict anecic or endogeic) and weighted.

Results

Aboveground plant communities

Herbage utilization by grazing (SH-, CA-, CA+) or by mowing (M) significantly decreased plant standing biomass (at least 1.7 times) compared to the abandoned (AB) treatment (282.2, 265.3, 109.2, 46.3 and 478.8 gDM m^{-2} respectively). Moreover, plant community structure in the grazed (SH-, CA-, CA+) and mowed (M) treatments was significantly different compared to the abandoned (AB) treatment.

Regarding the effects of animal presence, plant standing biomass was at least 2.4 times as high in the grazed (SH-, CA-, CA+) treatment as in the mowed (M) treatment. Moreover, plant community structure was significantly different between the grazed (SH-, CA-, CA+) and mowed (M) treatments.

Regarding the effects of grazing species and herbage utilization intensity, plant standing biomass was at least 2.4 times as high in the pastures grazed by sheep or cattle with low herbage utilization (SH- and CA-) as in those grazed by cattle with a higher herbage utilization (CA+). Among the grazed treatments (SH-, CA-, CA+), plant community structure was significantly different in pastures grazed by sheep and cattle with low herbage utilization (SH- and CA-) compared to those grazed by cattle with a higher herbage utilization.

Earthworm communities

Herbage utilization by grazing (SH-, CA- and CA+) and mowing (M) significantly increased earthworm biomass, total richness and Shannon index compared to the abandoned (AB) treatment. Except for the pastures grazed by cattle with a higher herbage utilization (CA+), earthworm abundance was at least 2.0 times as high in the grazing (with low herbage utilization: SH- and CA-) or mowing (M) treatments as in the abandoned (AB) treatment. Moreover, earthworm community structure was significantly different between grazed (SH-, CA- and CA+) and mowed (M) treatments compared with the abandoned (AB) treatment.

Regarding the effect of animal presence, total earthworm abundance, biomass, species richness, evenness and Shannon index were not significantly different between grazed and mowed treatments. Nevertheless, epigeic abundance was significantly higher in the low herbage utilization grazing (SH-, CA-) treatment as in the mowed (M) treatment. Quite similarly, epi-anecic abundance was significantly higher in low herbage utilization cattle grazing treatment (CA-) as in the mowed (M) treatments.

Regarding grazing species and herbage utilization intensity, epi-anecic abundance was significantly higher in the low herbage utilization cattle grazing treatment (CA-) than in the higher herbage utilization (CA+) treatment, however no difference was observed on the others earthworm community parameters.

Discussion

In agreement with previous studies, we observed different plant community structure according to the herbage utilisation (Krahulec *et al.*, 2001; Kohler *et al.*, 2004; Louault *et al.*, 2017). This is likely due to the reduced competition for light following the destruction of the tall and late flowering plant species, allowing the development of different plant species (Louault *et al.*, 2005).

The positive effect of herbage utilization on earthworm biomass and richness measured in our study could be related to the modification of the plant communities (litter and root modification) and/or grazing animals faeces as both a protection and food resource (Wright, 1972; Curry *et al.*, 2008; Eisenhauer *et al.*, 2009).

Conclusions

The present study clearly illustrated positive effect of herbage utilization either with animals or with mechanical export on different earthworm community parameters such total biomass or richness but also on plant diversity. However, the herbage utilization by animals or by mechanical export has different consequences on the structure of plant and earthworm communities, which could alter the contribution of earthworms to soil functioning and associated ecosystem services.

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A large database on functional traits for soil ecologists: BETSI

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Abstract summary

Functional approaches are gaining considerable traction in ecology. Expanding the functional trait-based conceptual framework to soil organisms has been slowed by the lack of standardized semantic and methods. The Biological and Ecological Traits of Soil Invertebrates database (BETSI, https://portail.betsi.cnrs.fr/) is a European database dedicated specifically to soil organisms' traits. This open database now gathers 129 185 entries on 44 413 species and 56 traits coming from about 2000 literature references and is linked to a thesaurus defining each trait. In this presentation, we will give an overview of the database and of its multiple associated research projects. With more than 20 articles, four PhD thesis conducted on various taxonomic groups and land uses/soil types, and multiple collaborative projects, e.g. on soil invertebrates feeding preferences or earthworm distribution in France, the database offers many opportunities to improve our knowledge on the functional facet of soil biodiversity. Most publications using or citing BETSI aimed to clarify the relationships between functional traits and habitat or environmental stressors. We will further highlight the main perspectives and future directions on functional traits research in soil ecology in order to advance the monitoring and conservation of soil biodiversity.

Keywords: functional traits, soil fauna, database, standardisation, cooperation

Introduction

The functional approach provides quantitative and generic predictions in order to understand how organisms interact, respond and affect their environment. Moreover, in the era of Big Data and FAIR principles (Data need to be Findable, Accessible, Interoperable, Reusable), there is growing interest and need for data integration and accessibility in order to facilitate scientific networking, discovery and innovation (Jones *et al.*, 2006; Wilkinson *et al.*, 2016). Consequently, many databases on various taxa's functional traits were created: plants

(TRY database (Kattge *et al.*, 2011)), fungi ('FUNGuild' (Nguyen *et al.*, 2016)), the 'Reptile Trait Database' (Grimm *et al.*, 2014) or the 'Coral Trait Database' (Madin *et al.*, 2016). As soils host a quarter of our planet's biodiversity and provide a variety of ecosystem services, it is crucial to expand the functional trait-based conceptual framework to soil organisms.

Currently, existing databases for functional traits of soil fauna focus on single taxonomic groups (Antbase: AntBase.net, Carabidae of the World: Carabid.org, ColTrait for Collembola, DriloBASE: taxo.drilobase.org) or on soil invertebrates but with no trait data (Burkhardt *et al.*, 2014). Until now however, no single database gathered functional traits of soil invertebrates across taxonomic groups. BETSI, a database dedicated to soil invertebrates' functional traits in Europe was created to fill this gap. The aim of the present presentation is to describe the functioning, the innovations, the current and potential future uses of BETSI.

BETSI database functioning

The BETSI database currently gathers data on 13 traits (morphological traits and ecological preferences) for 9 taxonomic groups: Arachnida (Aranae), Chilopoda, Coleoptera, Collembola, Diplopoda, Hymenoptera (Formicidae), terrestrial Isopoda, Oligochaeta and Orthoptera. With 129 185 entries on 44 413 species and 56 traits coming from about 2000 reference, BETSI is currently the largest database on functional traits of soil fauna.

BETSI is designed to host different types of trait data inputs - in their definitions and in their trait values - and is able to provide the users with harmonized trait data outputs. Indeed, to ensure semantic homogeneity on traits or ecological preferences, BETSI query trait or ecological preferences names from a controlled vocabulary organized into a hierarchy: the thesaurus for soil invertebrate trait-based approaches (T-SITA) http://t-sita.betsi.cnrs.fr/BETSI_vizIndex.jsp (Pey *et al.*, 2014). The T-SITA reflects the consensus of a community of soil zoologists on trait and ecological preference semantic properties (Pey *et al.*, 2014). To date, the T-SITA gathers 298 definitions for traits and ecological preferences.

Moreover, traits in BETSI were split into attributes by a fuzzy coding approach (Pey *et al.*, 2014). Attributes were not always exclusive, but may present affinity scores for each trait of one functional trait. Such data transformation allows values to be computed for functional traits derived from a variety of sources, mainly arising from identification keys.

An interface to the BETSI database is available at the following address (https://portail.betsi.cnrs.fr/, Figure 1).

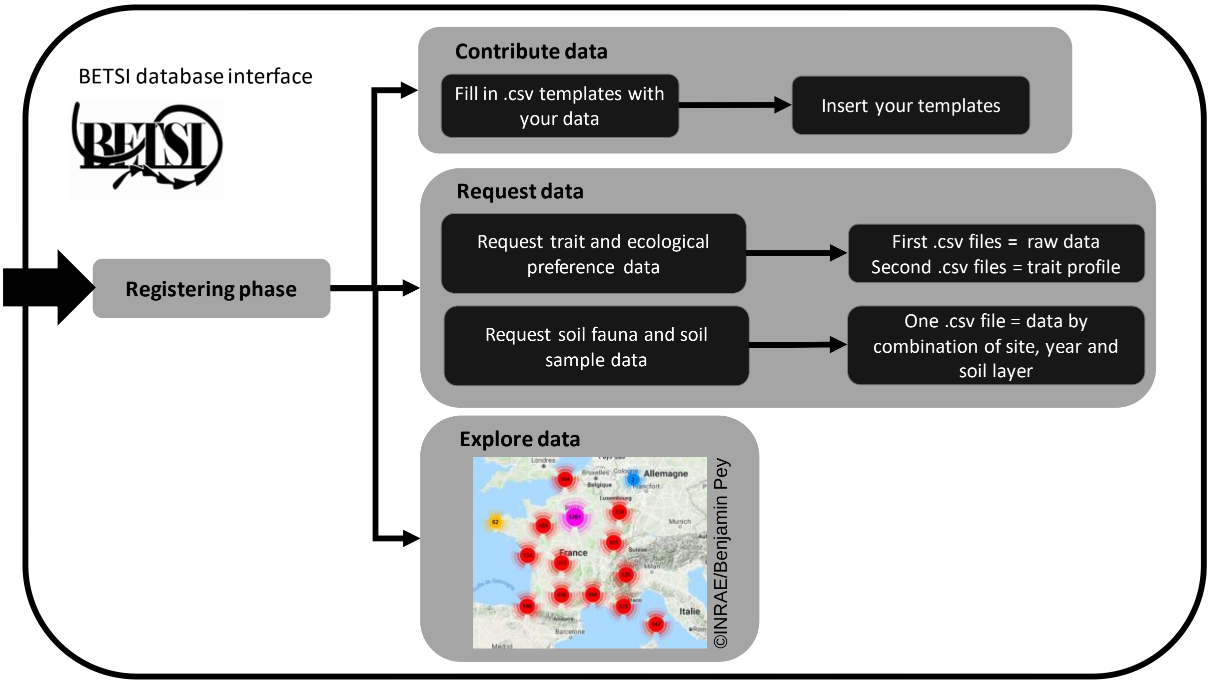
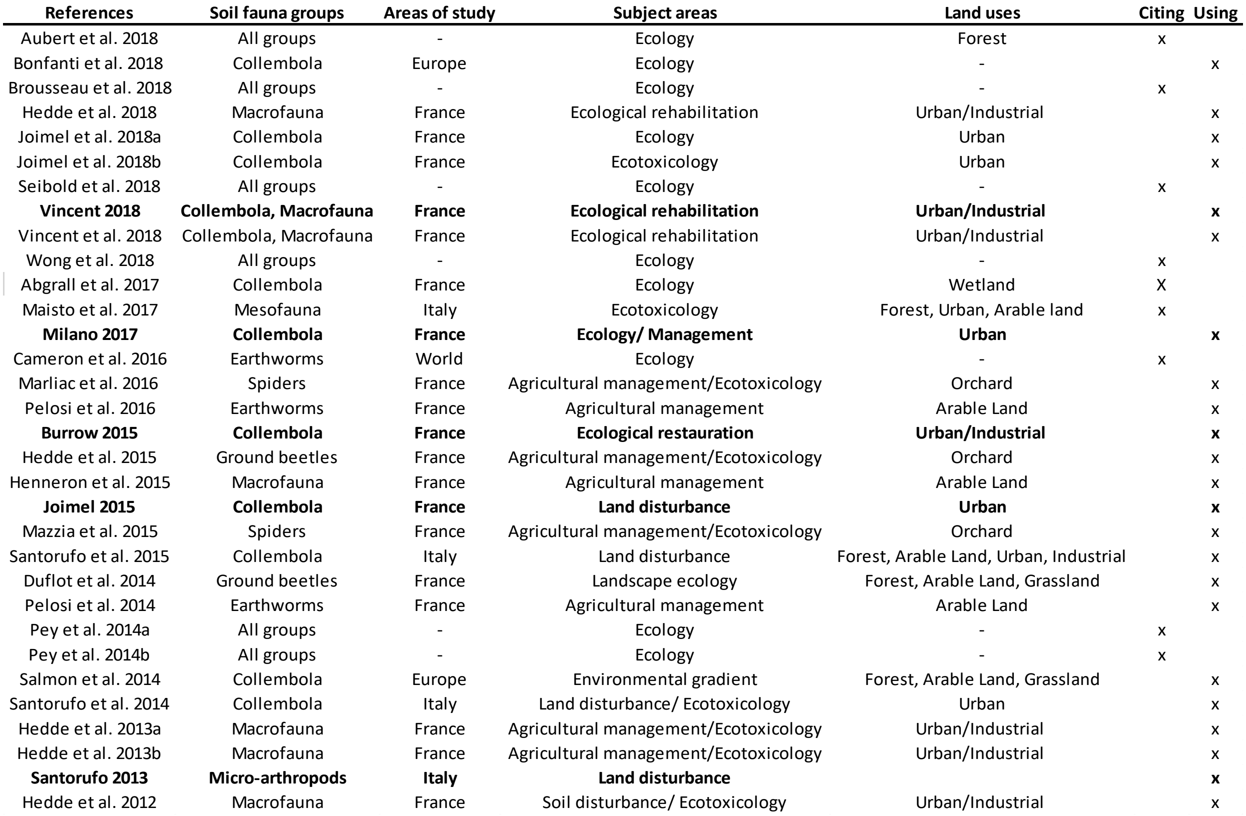


Figure 1: General workflow of user interaction with BETSI

Current uses and opportunities

BETSI allowed the publication of more than 20 articles, four PhD thesis conducted on various taxonomic groups and land uses/soil types or ecological gradients, and collaborative projects on soil invertebrates feeding preferences, or earthworm distribution in France (Figure 2). These studies mainly focused on Collembola (44 percent), and on macrofauna (41 percent), particularly on spiders, ground beetles and earthworms. Until now, the BETSI database contains information on a restricted number of traits on few - but highly representative - European taxonomic groups of soil invertebrates, and Orthoptera due to time constraints. However, new members and collaboration promise increase knowledge on other important group such as gastropods, ants or nematodes. BETSI is supported by an international research open network (www.reseau-tebis.fr) that has been gathering in a 3-days congress every year in a French research institution since 2014.



References	Soil fauna groups	Areas of study	Subject areas	Land uses	Citing	Using
Aubert et al. 2018	All groups	-	Ecology	Forest	x	
Bonfanti et al. 2018	Collembola	Europe	Ecology	-		x
Brousseau et al. 2018	All groups	-	Ecology	-	x	
Hedde et al. 2018	Macrofauna	France	Ecological rehabilitation	Urban/Industrial		x
Joimel et al. 2018a	Collembola	France	Ecology	Urban		x
Joimel et al. 2018b	Collembola	France	Ecotoxicology	Urban		x
Seibold et al. 2018	All groups	-	Ecology	-	x	
Vincent 2018	**Collembola, Macrofauna**	**France**	**Ecological rehabilitation**	**Urban/Industrial**		**x**
Vincent et al. 2018	Collembola, Macrofauna	France	Ecological rehabilitation	Urban/Industrial		x
Wong et al. 2018	All groups	-	Ecology	-	x	
Abgrall et al. 2017	Collembola	France	Ecology	Wetland	X	
Maisto et al. 2017	Mesofauna	Italy	Ecotoxicology	Forest, Urban, Arable land	x	
Milano 2017	**Collembola**	**France**	**Ecology/ Management**	**Urban**		**x**
Cameron et al. 2016	Earthworms	World	Ecology	-	x	
Marliac et al. 2016	Spiders	France	Agricultural management/Ecotoxicology	Orchard		x
Pelosi et al. 2016	Earthworms	France	Agricultural management	Arable Land		x
Burrow 2015	**Collembola**	**France**	**Ecological restauration**	**Urban/Industrial**		**x**
Hedde et al. 2015	Ground beetles	France	Agricultural management/Ecotoxicology	Orchard		x
Henneron et al. 2015	Macrofauna	France	Agricultural management	Arable Land		x
Joimel 2015	**Collembola**	**France**	**Land disturbance**	**Urban**		**x**
Mazzia et al. 2015	Spiders	France	Agricultural management/Ecotoxicology	Orchard		x
Santorufo et al. 2015	Collembola	Italy	Land disturbance	Forest, Arable Land, Urban, Industrial		x
Duflot et al. 2014	Ground beetles	France	Landscape ecology	Forest, Arable Land, Grassland		x
Pelosi et al. 2014	Earthworms	France	Agricultural management	Arable Land		x
Pey et al. 2014a	All groups	-	Ecology	-	x	
Pey et al. 2014b	All groups	-	Ecology	-	x	
Salmon et al. 2014	Collembola	Europe	Environmental gradient	Forest, Arable Land, Grassland		x
Santorufo et al. 2014	Collembola	Italy	Land disturbance/ Ecotoxicology	Urban		x
Hedde et al. 2013a	Macrofauna	France	Agricultural management/Ecotoxicology	Urban/Industrial		x
Hedde et al. 2013b	Macrofauna	France	Agricultural management/Ecotoxicology	Urban/Industrial		x
Santorufo 2013	**Micro-arthropods**	**Italy**	**Land disturbance**			**x**
Hedde et al. 2012	Macrofauna	France	Soil disturbance/ Ecotoxicology	Urban/Industrial		x

Figure 2: Review of the literature using or citing BETSI database. In bold are theses

Most publications using or citing BETSI aimed to clarify the relationships between functional traits and habitat or environmental stressors (Hedde *et al.* 2012). They focused on environmental stressors such as soil contamination by metals (Joimel *et al.*, 2018), uses of pesticides in relation with agricultural management, changes in agricultural practices such as tillage (Pelosi *et al.*, 2014), or effects of land use. BETSI also incorporates behavioural traits, making it possible to study biotic interactions. Moreover, BETSI gathers enough data to explore functional biogeography questions (Bonfanti *et al.*, 2018).

Functional traits can also be used to help understanding the impacts of biodiversity on ecosystem processes and services (Wood *et al.*, 2015). For instance, a clear theoretical framework using traits exits for linking plant diversity to ecosystem processes (Lavorel et Garnier,, 2002). However such a framework is not adapted to soil invertebrates whose dynamics and effects on ecosystem processes are driven by a multitude of complex interactions (mainly trophic) (Gravel, Albouy and Thuiller, 2016). In this context, a new framework has recently emerged, reinterpreting foodweb interactions via the prism of functional traits (Gravel, Albouy and Thuiller, 2016). As BETSI already hosts many traits used in the functional foodweb description for soil invertebrates, it could be the cornerstone of the storage of trait data used in these approaches.

Conclusions

BETSI is designed to be a collaborative and an interactive database that already offers great opportunities for trait-based approach in soil ecology. BETSI has been evolving over the years thanks to its support by the TEBIS network of soil biodiversity researchers.

Most of data are represented by information available in synoptic books (species level data) rather than by actual measurements (individual level data). We aim to continue data collection to enhance data on functional traits of soil organisms.

Within the on-going European COST Action EUdaphobase that aims to improve soil biodiversity monitoring and conservation, the functional trait-based framework has been identified as a crucial approach. A pan-European soil-biology data warehouse hosting data on species traits and distribution, will improve the potential of functional trait approaches to assess soil biodiversity response to global changes at broad scales.

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Recent successes and persistent challenges in restoration of degraded dryland soils

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Abstract summary

Biological soil crusts (biocrusts) are diverse assemblages of soil microorganisms that reside on the soil surface across dryland ecosystems globally. Due to the importance of biological soil crusts communities to the ecological functioning of dryland ecosystems, there is keen interest in restoration of these communities after soil surface disturbance. However, active biocrust rehabilitation has remained extremely difficult to achieve in field settings, both because of questions surrounding how to create biocrust inoculum and how to ensure the success of that inoculum in the environment. In a series of multi-factorial experiments, we examined the effects of biocrust inoculum type and soil stabilization strategies on biocrust development in disturbed soils at cool and hot desert sites in the Western United States. The study was carried out in a factorial design of inoculum type by soil stabilization strategy on two soil types. We report on biocrust development and soil stabilization after two years. We discuss our success in creating biocrust inoculum and results from the multifactorial experiments in the context of conducting larger scale biocrust restoration in dryland ecosystems.

Keyword: biocrust, dryland ecosystems, restoration, Western United State

Introduction, scope and main objectives

Degradation of the global land surface negatively impacts the well-being of over 3.2 billion people on the planet. Land degradation encompasses the many human-caused processes that drive the decline or loss in biodiversity, ecosystem functions or ecosystem services that cannot fully recover without human intervention. Dryland soils around the world are particularly vulnerable to land degrading activities which are driven by a broad range of human land use such as unsustainable agricultural practices and extractive industries. In highly degraded ecosystems, rehabilitation of soil health is an important first step in the restoration process. One of the more remarkable and diverse communities of organisms present across the drylands of the world that are associated with soil health are those associated with biocrusts, which cover approximately 12 percent of the terrestrial land surface. As plant cover declines in arid and semi-arid regions of the world, the soils in the interspaces of plant canopies are often colonized by communities of microorganisms (e.g. cyanobacteria, mosses, lichens, and fungi) that develop in the top few millimetres of soil surfaces. These surface residing organisms in turn support populations of heterotrophic bacteria and fungi. Biocrusts are an essential functional component of dryland systems of the globe. They are often associated with increased soil nutrient and water retention—resources that are highly limiting to plant productivity in these ecosystems. But most importantly, biocrusts stabilize soil surfaces against wind and water erosion. Due to the functional importance of biocrust communities to the ecological functioning of dryland ecosystems there is keen interest in restoring these communities. The objective in this presentation is describe our collective efforts to cultivate the diverse array of biocrust organisms for restoration of degraded dryland soils.

Methodology

In this project we established a biocrust nursery to supply inoculum for biocrust restoration, identified successful field application methods of biocrust inoculum in a series of field trials, and evaluated soil and plant responses to biocrust restoration in multi-factorial field experiments. Biocrust inoculum was developed from locally collected biocrust organisms and either cultivated as a mixed community under a greenhouse environment or from lab cultures of isolates of the major pioneer cyanobacteria (*Microcoleus* spp., *Scytonema* spp., *Nostoc* spp.). These two sources in addition to field collected biocrusts constituted the supply of inoculum for a restoring biocrust communities on disturbed soils. We then used these three sources of inoculum to evaluate biocrusts recovery after two years in recently disturbed soils. In a series of multi-factorial experiments, we examined the effects of biocrust inoculum type and soil stabilization strategies on biocrust development in disturbed soils at cool and hot desert sites in the Western United States. We inoculated experimentally disturbed soils with 3 types of biocrust inoculum: field collected, which was biocrust that was collected at the site, mixed, and redistributed across plots; local biomass inoculum that was grown in the greenhouse under optimal soil moisture, temperature, and nutrient conditions from small samples collected in the field; and mixed isolate inoculum that was created in the laboratory from cyanobacterial cultures collected at the site. Plots were also prepared with two soil stabilization strategies. Straw checkerboards, in which straw served as silt fences. In a second stabilization approach, polyacrylamide (DirtGlu) was applied to the soil surface in plots of the same size. The study was carried out in a factorial design of inoculum type by soil stabilization strategy on two soil types. We measured the biocrust community composition, chlorophyll a, and exopolysaccharides which taken together are indicators of biocrust recovery.

Results

We report on optimal moisture, nutrient, and soil substrate conditions to grow biocrust inoculum from field collected samples which can be completed over an approximately 8 week period (Velasco *et al.,* 2017, Bethany *et al.,* 2019, Velasco *et al.*, 2020). More time consuming culture based methods from isolates of early pioneer cyanobacteria were also developed successfully developed.but are currently limited by time and resources in scaling up the biomass to a level for landscape scale restoration (Giraldo-Silva *et al.,* 2019a, Giraldo-Silva *et al.,* 2019b). We show that the level of biocrust inoculation (10, 20, 40 percent surface cover of inoculum) did not strongly determine the long-term recovery of the biocrust community (Antoninka *et al.,* 2019). This suggests that even small amounts of biocrust inoculum added to degraded sites may enhance recovery. Shading of the soil surface has consistently shown to be effective in enhancing the recovery of the biocrust community (Chock *et al.,* 2019, Fick *et al.,* 2019, Antoninka *et al.,* 2019). Shading likely decreases water stress by increasing soil moisture through decrease surface evaporation and also directly by decreasing UV stress. In highly degraded sites where soils are actively eroding, the addition of synthetic soil stabilization agents appeared to have no inhibitory effect on biocrust recovery. Thus, the use of soil stabilization products to increase soil surface stability before biocrust inoculation may work to prevent biocrust inoculum being buried by high mobile and eroding soils. Addition of three inoculum types (field collected, greenhouse grown, lab developed) showed mixed results. Field collected biocrusts show modestly higher biocrust growth relative to greenhouse grown local biocrusts and lab grown mixed isolates. Again, addition of polyacrylamides to stabilize soils exhibited similar soil stability to intact biocrusts with no evidence of inhibiting biocrust recovery. What is clear is that significant barriers still exist to biocrust recovery under stressful field environments. This is likely due to resource limitation and more specifically to water and UV stress as demonstrated in our early field trials. Future work on successfully should focus on maintaining adequate water balance for biocrust recovery and the possibility of using natural shade structures in the field such as shrubs and other perennial plants. Inoculum placement on cooler, wetter north facing aspects of these natural shade structures may also further promote more rapid biocrust recovery.

Discussion

Our research has yielded effective methods to grow biocrust inoculum both from small field collected samples and cultured isolates of early successional cyanobacteria, mosses, and lichens. We have shown that inoculation of soils with lab and greenhouse growth biocrusts enhance biocrust recovery. Barriers and challenges still exist in biocrust recovery with inoculation under field settings and this is likely due to resource limitation to biocrust growth and recovery and more specifically water availability.

Conclusions

We have developed novel approaches to developing biocrust inoculum for restoration of degraded dryland ecosystems. Biocrusts play a functional important role in dryland ecosystems influencing soil stability, nutrient availability, and hydrology. Thus, rehabilitation of these biotic communities will benefit these ecosystems and the services they provide. Our future challenge is scaling these approaches to larger landscape scale restoration approaches.

Acknowledgements

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A coordinated research enterprise on agricultural soil microbiomes and soil ecosystems across Usda research locations.

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Abstract summary

My presentation will highlight advanced soil ecosystem research efforts being conducted by scientists within the US Department of Agriculture (USDA), Agricultural Research Service (ARS). Research questions around soil ecology and the microbiome of the soils on which we rely to produce food, feed, fiber and fuels is a global concern. It is my hope that my talk will catalyze international dialog and collaboration around this important research topic.

Keywords: Soil Ecosystem, Agricultural Soil Microbiomes, Soil Ecology

Introduction, scope and main objectives

Found primarily within the Soil and Air National Program, ARS soil ecosystem scientists have historically - and are holistically - investigating soils to deeply understand them and to use the gained knowledge to develop management and systems approaches that lead to optimal agricultural outcomes. Their investigations span a range of topics needed for a holistic understanding of soils, including investigations of inorganic/organic nutrient state and flux - as influenced by local geology, climate flux, cropping systems, and management practices. Their studies also include evaluations of the biological and genetic diversity and associated functionalities of soil micro- and macrobiomes; In addition, our research investigates how soil ecosystems and their relevant phenomena are influenced by - and influence - cropping systems and their productivity and sustainability. From the aspect of understanding the interfaces and fluxes between soils, agricultural operations, and the atmosphere, we seek to understand the myriad interaction between soils and agricultural systems with the atmosphere - and what soil management practices can be developed to ameliorate greenhouse gas generation, improve carbon sequestration, and enhance nutrient retention. ARS soil ecosystem science seeks to use powerful and novel investigative strategies to improve environmental agroecosystem quality, provide beneficial ecosystem services, and lessen agricultural impact on the environment. Our research efforts span an incredible diversity of agricultural soil ecosystems, as well as degraded, marginal, and native, unimpacted lands. ARS is committed to supporting enhanced efforts of our scientists and engaging our global community to enable advances in soil science to address and minimize adverse changes in agroecosystems arising from changes in climate, pest distribution, and other emerging impacts.

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Intercrop management as a tool to increase soil microbial diversity on rainfed almond cultivations

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Abstract summary

Differences on microbial communities (fungi) and alpha diversity on an intercropping tree system of almond with vineyard and wheat in an arid and temperate agricultural area in the northern of Almeria province (Spain) were studied. Microbial DNA was extracted with a commercial kit, sequenced with the MiSeq platform (Illumina) and taxonomy and diversity indices were obtained using QIIME2 software. Statistical differences were found among the different crop management practices, demonstrating that the combination of almond tree with another plant produces more fungal diversity, specially the combination of almond and wheat, where we found the highest number of taxa associated, with several fungi species (*Paraboeremia selaginellae, Cladosporium cladosporioide*) and genera (*Didymella, Tetracladium*) only or mainly exclusively on this cultivation.

Keywords: Microbial diversity, almond, vineyard, wheat, intercropping practices

Introduction, scope and main objectives

The European Research project "Crop diversification and low-input farming cross Europe" (Diverfarming) aims to develop and test different diversified cropping systems under low-input practices to increase land productivity and crops quality.

Based on this premises we selected as case study almond tree intercropping system on the Almanzora Basin, close to Oria town (northern province of Almeria, Spain). The climate of the region is arid (346 mm per year) and temperate (18°C annual mean) with cools winters which makes this area more suitable for rainfed crops like fruit trees (García-Lorca, 1998). The typical management system on the area consist on tree lines with barren soils among them. However, erosion processes and loss of biodiversity on soils are important issues on the area. The adoption of an intercrop system can help with this problematic thus increase the economical profit of farmers.

Our objective on this work is to study the differences on the microbial diversity (fungi) of soils under an intercropping system (almond tree, vineyard and wheat) on the arid area of the Almanzora basin (northern province of Almeria, Spain).

Methodology

Soils of three different cultivations (almond tree, almond tree with vineyard, almond tree with wheat) were sampled up to a depth of 10 cm. The experimental design consisted of three parcels per crop with three replicates per each one, which accounted a total of 27 samples. Microbial DNA was extracted with Qiagen commercial kit and amplicons were sequenced with the MiSeq platform (Illumina) and finally, the sequences of the fungal ITS1 region using the ITS5 - ITS2 primers were compared with Kronos ITS database to obtain the taxonomy and diversity indices using QIIME2 software (Bolyen *et al.,* 2019).

Statistical analyses included one-factor permutational multivariate analysis of variance (PERMANOVA) conducted in Primer (ver. 7, Quest Research Ltd, Auckland, New Zealand; Anderson *et al.,* 2008; Clarke and Gorley., 2015) and LDA Effect Size (LEfSe) analysis with Calypso software (Zakrzewski *et al.,* 2016).

Results

Fungi communities were clearly different on the three different crop management practices (Tables1 and 2). Analysing the alpha diversity indices (Table 3), crops including another plant from almond showed higher diversity values, being the combination of almond and wheat the one with the highest values for all the indices.

Table 1: one-factor permutational multivariate analysis of variance (PERMANOVA)

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Crop system	2	3522.4	1761.2	3.0002	0.001	997
Res	23	13502	587.03			
Total	25	17024				

Table 2: Pair-wise test PERMANOVA

Groups	t	P(perm)	perms
Almond - Almond with vineyard	1.5321	0.001	975
Almond - Almond with wheat	1.7989	0.001	974
Almond with vineyard - Almond with wheat	1.9248	0.001	983

Table 3: Diversity indices (mean deviation ± standard deviation)

Crop	ASVs	Faith	Shannon	Pielou
Almond	249 ± 39.66	44.63 ± 5.01	5.17 ± 1.35	0.64 ± 0.16
Almond + vineyard	287 ± 38.08	46.89 ± 4.58	5.32 ± 0.57	0.65 ± 0.06
Almond + wheat	305 ± 15.84	49.81 ± 1.14	5.68 ± 0.63	0.68 ± 0.07

The previous was also corroborated by the LEfsE analysis, which revealed the fungi taxa that were significantly different on their abundances on the different crops systems (29 for almond with wheat, 25 for almond with vineyard, 16 for almond alone) (Figure 1). The almond + wheat crop system showed the highest number of taxa with more significant differences on their abundance. Indeed, several fungi species (e.g. *Paraboeremia selaginellae, Cladosporium_cladosporioide*) and genera (e.g. Didymella, Tetracladium) were found exclusively or almost exclusively on this combination of crops (Figure 2).

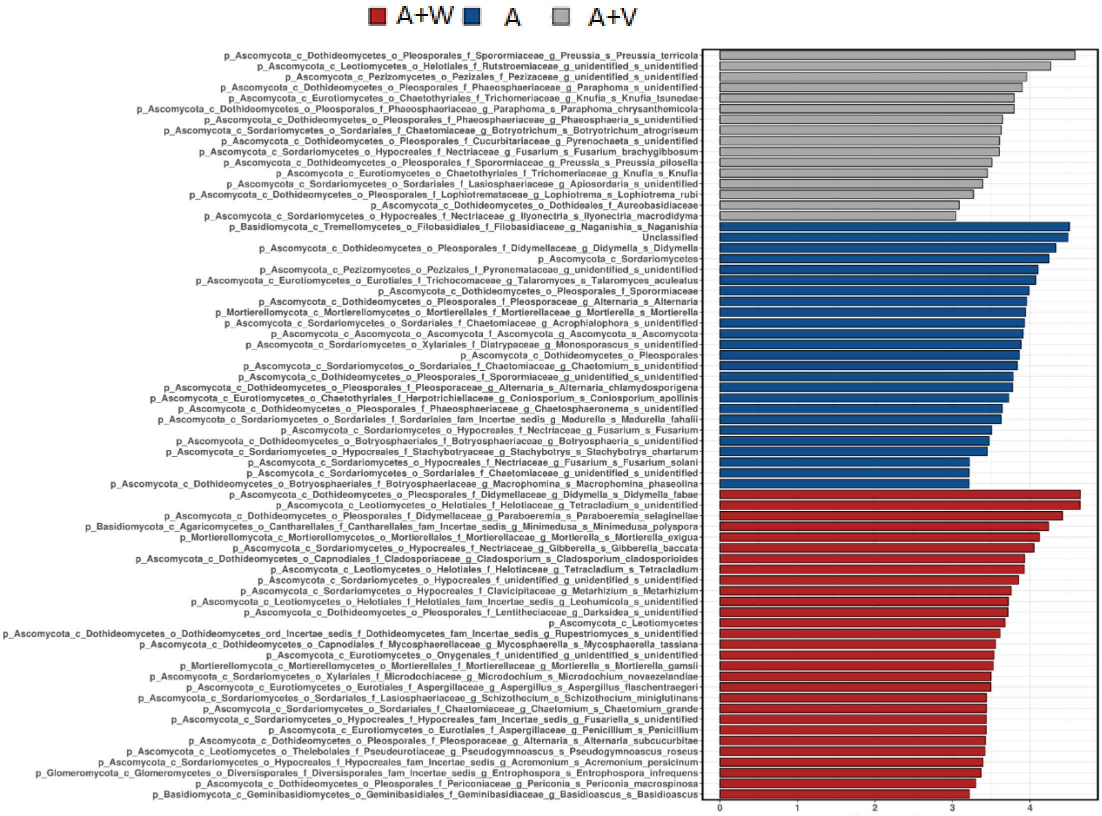

Figure 111: Taxa more associated to different crop systems based on the LEfsE analysis

A+W: Almond + Wheat; A: Almond; A+V: Almond + Vineyard

(p: phylum; c: class; o: order; f: family; g: genera, s: species)

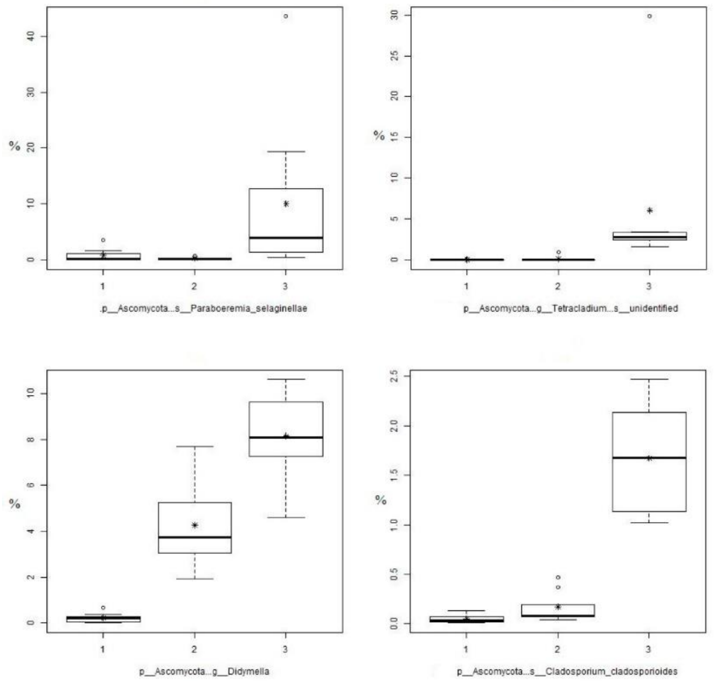

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Figure 212: Fungi taxa more present on the almond with wheat crop system (p: phylum, g: genera, s: species)

Discussion

Bacteria and soil fungi are an important part of the microbiological properties of soils and play an important role in agricultural ecosystems as these microorganisms can promote plant growth facilitating nutrient absorption, stress tolerance, disease prevention, and carbon capturing. Meanwhile studies focused on bacteria are more frequent, however studies focused on fungi are more scarce.

Lacombe *et al.* (2009) found a higher abundance of fungi in intercropping systems compared to monocropping systems. In addition, Bainard *et al.* (2011) found more diversity in intercropping systems. These findings agrees with our results as we found that fungi microbial communities are significantly different when vineyards and wheat are introduced as intercrops on almond trees cultivations, being thus more diverse, showing the alpha indices this gradient: almond + wheat > almond + vineyard > almond. This is of great interest as several studies have shown the effect of fungal diversity on plant productivity (e.g. van der Heijden *et al.*, 1998; Wagg *et al.*, 2011).

Then, introduction of other plants in monocrops can have beneficial effects in cultivations and physical (e.g. protection against erosion), chemical (e.g. structure development by roots) and biological (increase in microbial diversity) properties of soils. An important number of taxa were related to the different crop systems showing the number of taxa again the trend almond + wheat > almond + vineyard > almond. This suggest that each combination of crops can favour certain taxa which in turn can have important implications in the development of more sustainable crop systems (Bagyaraj and Ashwin, 2017).

Conclusions

The introduction of vineyard or wheat in almond tree cultivation produced significant differences in fungi microbial communities and an increase in the fungal diversity. This increase in the diversity can improve the biological soil properties and several authors suggest that also can have important implications in crop productivity. Besides, we found which taxa were more associated with the different crop systems being the system almond with wheat the one with the highest number of taxa related. This study can result in a start point for further research on the interactions of microorganism – plants associated to crops and the effects of intercropping in increasing the soil biodiversity.

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Effect of warming-induced shrub encroachment on soil fungal communities in Western Greenland

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Abstract summary

Climate warming in Greenland is resulting in the expansion of shrub vegetation across wide areas of tundra, that is replacing other soil ecosystems. These vegetation shifts have consequences for terrestrial ecosystem structure and functioning and may involve climate feedbacks. In particular, fungi are the most abundant and one of the most diverse components of arctic soils, where they play an important role in the availability and recycling of plant nutrients. Nevertheless, few studies have focused on the factors driving the diversity and functionality of fungal communities in these ecosystems. We used DNA metabarcoding to compare fungal assemblages in three soil habitats (bare ground, biological soil crusts and vascular vegetation coverage) in West Greenland. Fungal richness increased with the increasing coverage complexity, but bare grounds and biological soil crusts plots had the highest number of indicator OTUs. Differences in fungal community composition and distribution of functional guilds identified were mainly due to the type of coverage, but also correlated with edaphic factors (mainly pH and water content). These results suggest (i) possible losses of local fungal diversity connected to the expansion of shrub vegetation and (ii) possible interactions among members of different functional guilds, likely due to nutrient limitations, with potential effects on elements recycling.

Keywords: Functional Guilds, Metabarcoding, Global Warming, Edaphic Factors, Environmental Filtering

Introduction, scope and main objectives

Arctic regions have been experiencing some of the highest rates of warming, with an average increase of temperature of about 0.1 °C per year since the 1980s (ACIA, 2005), particularly evident in Greenland (Bevis *et al.,* 2019). Rising of temperatures is causing an overall greening with a significant expansion of shrubs vegetation all over the Arctic (e.g. *Betula nana* and *Salix pulchra*) at the expense of vegetation dominated by bryophytes and lichens (e.g. Vowles and Björk, 2019).

Given the role of fungi in degradation of organic matter and nutrient recycling and their intimate relationships with plants in mutualistic symbioses, as mycorrhizal or other endophytic interactions, that can allow more efficient nutrient and water uptake and modulate resistance to abiotic and biotic stresses, they could be viewed as sentinels and amplifiers of global change (Vincent, 2010). One of the most widespread hypotheses is that shifts in fungal communities composition are likely to affect soil carbon and nitrogen cycles and promote the breakdown of organic matter (Zhang *et al.,* 2014; Treseder *et al.,* 2016), increasing the release of greenhouse gases to the atmosphere (Commane *et al.,* 2017), amplifying climate feedbacks.

In this optic, we compared soil fungal communities in three different habitats in Western Greenland. These habitats represent a gradient of vegetation complexity: bare grounds (BGs), biological soil crusts (BSCs), dominated by bryophytes and lichens, and soil covered with a vascular vegetation (VV). Therefore, the main objectives of the present study were (i) to understand how fungal communities' richness and composition and the distribution of different functional guilds relate to different habitats and to biotic and abiotic variables, and (ii) to gain insights into the landscape-level dynamics of vegetation and soil fungi in this area.

Methodology

Sampling was carried out in July, 2017 in the area of Kobbefjord, Nuuk (64°08′ N, 51°23′ W). Twenty 2m^2 plots were sampled: 5 in BGs, 6 in BSCs, and 9 in VVs. In each plot, three replicates of soil samples were collected aseptically at a depth of 5 cm.

Soil water content, pH, and C, N and P content were analysed in all the samples. DNA was extracted from soil and the ITS1 region was PCR amplified using ITS1F and ITS2 primers, as described in Smith and Peay (2014). The equimolar pool of uniquely barcoded amplicons was paired-end sequenced (2×300 bp) on an Illumina MiSeq platform. Sequences were processed with Amplicon ToolKit v.1.2.1 (Palmer *et al.*, 2018). The OTU table was normalized by rarefying the number of sequences to the smallest library size (33.870 reads). Taxonomy was assigned to OTUs based on the UNITE+INSD reference database dynamic Species Hypotheses. OTUs with > 90 percent similarity to species hypothesis with known functions were assigned to one of the following guilds: animal pathogens, ectomycorrhizal (ECM) fungi, ericoid mycorrhizal (ERM) fungi, lichenized fungi, mycoparasites, plant pathogens, other root-associated fungi (non-ECM fungi, non-ERM fungi and root endophytes) and saprotrophs. Functional assignments were made by FunGuild (Nguyen *et al.*, 2016) and manually checked. Total fungal richness, richness of functional guilds and their relative abundances were compared among habitats using ANOVA and Tukey's HSD test and among edaphic factors using linear regressions. We ran non-metric multidimensional scaling (NMDS) on the Hellinger-transformed OTU table. Permutational multivariate analysis of variance (PerMANOVA) was carried out on Bray-Curtis distance matrices of Hellinger-transformed OTU tables to determine the effect of soil physicochemical characteristics and the type of coverage on the observed variance.

Results

The quality-filtered and rarefied dataset contained 2661 OTUs. Total richness showed a slight increase ranging from BG to vegetation covered soil plots (Figure 1A). However, BG plots showed the highest number of indicator species (16 percent of OTUs) compared to the other habitat types.

1105 OTUs were assigned to mentioned functional guilds. ERM fungi, mycoparasites, plant pathogens, animal pathogens and saprotrophs showed a richness increase from BG to VV plots (Figure 1C and F-I). ECM fungi richness increased in VV plots respect to BSCs, with a not significant difference between VV and BG plots (Figure 1B). Lichenized fungi showed the highest richness in BSCs (Figure 1E). When significant, the trends were the same also for the relative abundance of the components of the guilds (data not shown).

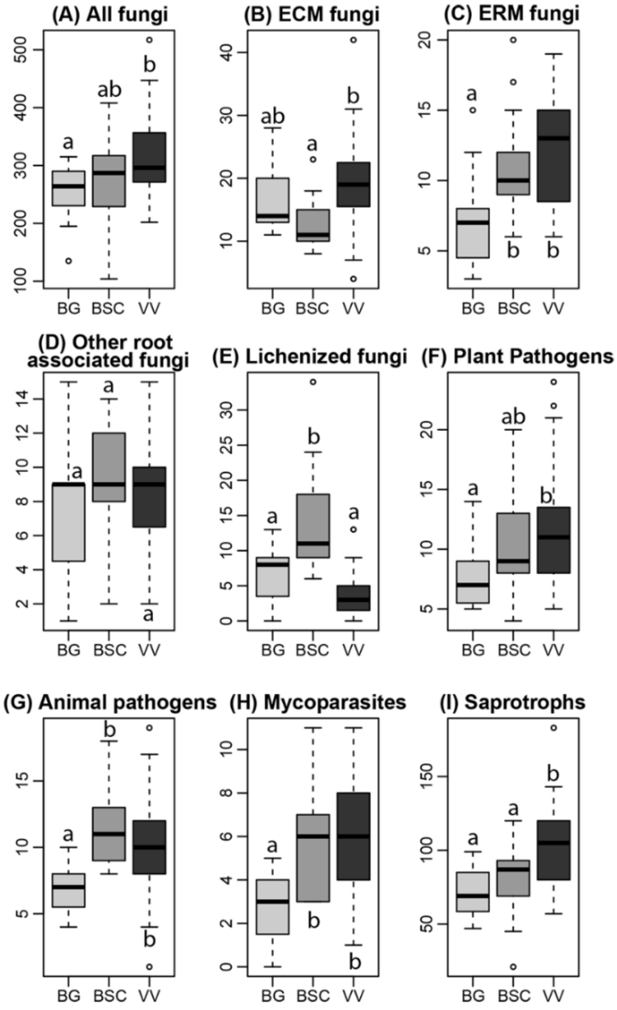

Figure 6: Richness of the total fungal communities (A) and of functional guilds (B-I), in each habitat

Letters indicate significant differences in one-way ANOVA Tukey's HSD test (significant for $p<0.05$) (Canini *et al.,* 2019).

Water and P content were generally positively correlated with the richness of the total communities and of the functional guilds studied, instead pH had an opposite effect. C and N content and their ratio were significative for few groups and were generally positively correlated with the richness.

The composition of the total fungal community changed significantly among the three different habitats, as visualized in the NMDS ordination (Figure 2).

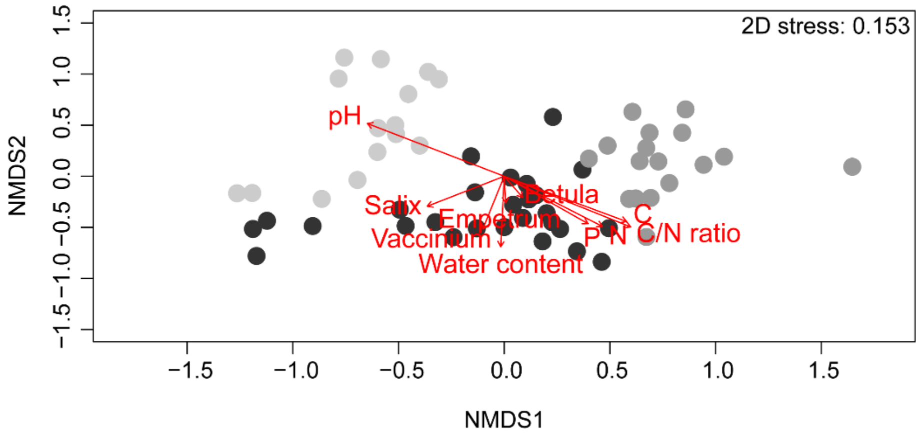

Figure 2: NMDS ordination of the differences in composition of fungal communities in the habitats

(light grey, BG; dark grey, BSC; black, VV) (extracted from Canini *et al.*, 2019)

The type of habitat resulted to be the strongest parameter influencing the variance of fungal communities, followed by the pH (Table 1). All the parameters resulted significant when considered independently, but when combined, depending on their influence, C and P content were not independent from the other parameters in shaping the community.

Table 1: Proportion of variation in fungal community composition explained by soil variables added sequentially (from the first to the last) in a model, depending on their independent influence on the variance

Variable	**Variance (%)**	***p***
Habitat	**18.145**	**0.0001**
pH	**3.697**	**0.0004**
C/N ratio	**2.886**	**0.0017**
C	1.355	0.3958
N	**3.708**	**0.0001**
Water content	**2.119**	**0.0302**
P	1.779	0.1001
Residuals	66.311	

Significant values in bold.

Discussion

This study is the first to characterize fungal communities in a Western Greenland landscape. The strong structuring at small spatial scales reported for the three habitats analysed confirms patterns observed in Eastern Greenland (Grau *et al.*, 2017) and in other Arctic landscapes (e.g. Mundra *et al.*, 2015; Geml *et al.*, 2016). One of the most alarming result is the greater richness and relative abundance of saprotrophic fungi in VV plots, likely due to greater soil moisture and possibly to an increase in plant litter biomass. The increase was primarily driven by litter decomposer and wood decaying fungi (mainly basidiomycetes). Among parameters tested, soil pH had a strong affect, reported all over the Arctic, as it can influence the availability of different nutrients.

Conclusions

Our results support the idea that plants strongly regulate fungal community composition through their role in substrate supply (e.g. litter, root turnover, exudates) and by modifying the physical environment in the active soil layer. They also suggest possible losses of local fungal diversity. We highlighted some possible interactions among the components of different functional guilds, mainly regarding the degradation of organic matter and nutrient cycling. These outcomes should be taken into account in the context of global change and the connected encroachment of shrubs occurring in the Arctic, which could lead to substantial changes in soil communities, with possible effects on the degradation of the organic matter, as well as C and N fluxes to the atmosphere.

Acknowledgements

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Yatesbury house farm is growing soil

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Abstract summary

This paper reports on a study carried out on-farm by an organic farmer. The initial motivation for this work was the need to formalize the research that happens on many farms every day, but mostly goes unreported, as these results do not stand up to scrutiny due to lack of experimental rigour. However, we have established a replicated experiment, testing the hypothesis that returning organic crop residues to the soil will lead to a marked improvement of soil health, soil function and ultimately crop productivity. We have seen an increase of soil carbon beyond 4 per 1000, which is reflected in the overall negative greenhouse gas emission of the whole farming system. There were beneficial effects on the depth distribution of organic carbon, as well as on soil mesofauna populations. This study highlights the potential of on-farm research and its direct relevance for improving the sustainability of modern farming in capturing carbon for climate change mitigation and adaptation.

Keywords: soil biodiversity, soil health, organic farming, soil organic carbon, soil mesofauna

Introduction, scope and main objectives

I am a farmer and a scientist. our farming system at Yatesbury House Farm demonstrates that mixed farming, which integrates cattle and cereals with woodland, hedges and field margins, can annually sequester 10 times more carbon (C) than it is emitting as a result of farm operations.

This work looks at a small part of our farm system, with the goal of bridging the gap between farming and science. One of the key objectives is to look at maximising the value of science for modern farming, but also to increase the relevance and importance of farming and farmers when engaging with the scientific community. My research is motivated by the development of forward-thinking farming methods, the main hypothesis underlying this research is that feeding the life in the soil with highly diverse leys will improve soil function and have multiple benefits for the crop, farm, wildlife, water catchment and beyond. Soil health thus directly contributes to the sustainability of plant or animal production, biodiversity and eventually, because of the global impact of agriculture, the Earth.

Methodology

In the 1990s, our farming system systematically removed all the crop residue to supply fodder for livestock farms elsewhere. This meant very limited food was remaining for the soil biota after the crops were harvested. Starting in 1998, we reintroduced legume-based leys to fix nitrogen from the air and to restart soil development. The leys were grazed in-situ, by introducing a cattle herd. Very quickly, we improved upon this approach by using high diversity leys with 23 species and 33 varieties.

Landwise soil survey

Landwise, a University of Reading-led project of which we are a part, is looking at Natural Flood Management in the Thames River catchment. Part of the project is carrying out a broad field survey across the Thames region to assess the likely benefits of soil improving measures; to analyse the current state of flood risk; and research the implications of land use change. SOM tests were done using standard loss on ignition test @ 400^0C.

High biomass rotation, on farm doctoral research

In 2014, we established a long term on-farm experiment (Fig 1) to determine whether retaining crop residues and planting cover crops to feed soil biota leads to a measurable improvement in the long-term health of the soil compared to the practice of removing and selling crop residues off farm. The principal comparison in the experiment looks at the difference between 'enhanced rotation' (cover crops; incorporation of all crop residue) with a 'standard rotation' (no cover crops; removal of crop residue). We also included a positive reference (continual diverse ley) and a negative reference (continual fallow; maintained via cultivation 3 times per year). Table 1 shows the details of our crop rotation practice and its application within the experimental design.

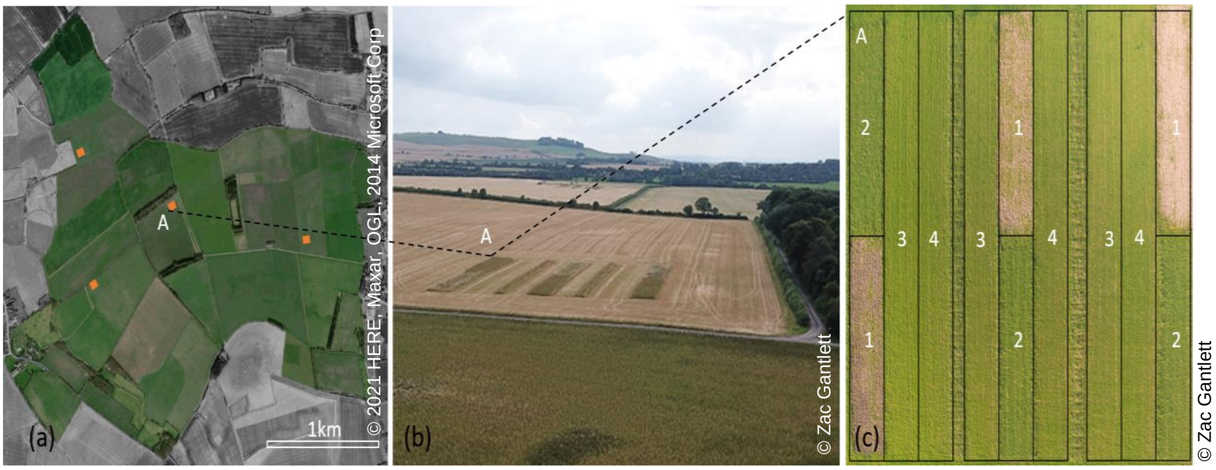


Figure 113: (a) Aerial Map of experimental field locations (orange squares) (b) drone image of Croft field showing plots within field setting (c) drone image of

the layout of 3 replicate blocks of 4 treatment-plots (full-size-plots: size 8 m x 80 m) within a single field (Croft Field)

Treatments: Negative reference (1); Positive reference (2); Standard (3); Enhanced (4), (the 2 reference treatments share one full-size-plot)

Table 1: Plot rotation, Fields in rotation position in year 1, standard and enhanced treatment examples

Rotation Year	Rotation Crop	Field in 2014	Standard Biomass- Input Treatment	Enhanced Biomass-Input Treatment
1	Diverse ley	Long Barrow	Mowed for hay/silage	Topped after 15th June to promote lignin production and reduce weed seed set
2	Diverse Ley		Grazed	Grazed
3	Cereal: Spelt or Wheat or Oats	Hut Field	Remove straw	Chop & incorporate straw
4	Cereal: Spelt or Oats		Remove straw	Chop & incorporate straw
5	Bean whole crop silage	Fifty Acres	Harvested as forage silage	Cut and mulched as green manure nothing harvested
6	Spring Beans		Fallow over winter	Green cover over winter
7	Spring Oats under sown with diverse ley	Croft Field	Harvest as whole crop	Chop & spread straw and green material

FCT carbon audit

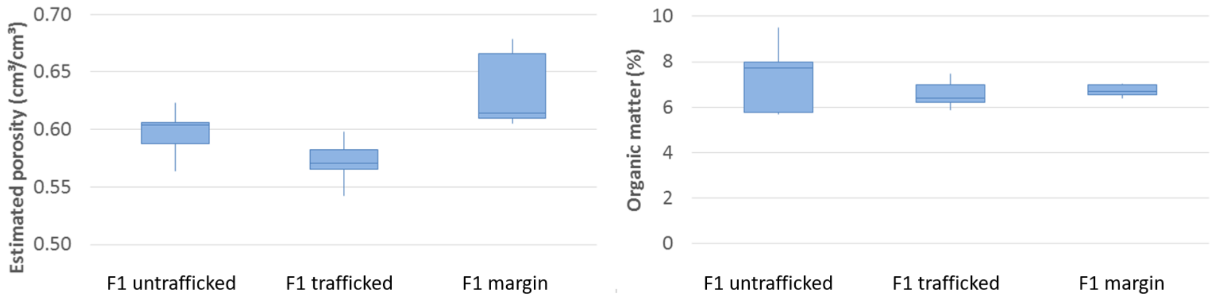
In Spring 2019, we carried out a farm carbon audit using the Farm Carbon Toolkit. The toolkit is a free online self-assessment tool. The change in soil organic carbon was established by comparing the SOM at any two points during the history of fields on our farm, presented as an annual soil organic matter (SOM) change necessary for the toolkit.

Results

Landwise field survey

Preliminary results from the Landwise project, Spring 2019 are shown in Figures 2 below. In Field 1, Figure 2 a) the organic matter levels in all field areas are the same where the margin would expect to be higher given no tillage and permanent plant cover; porosity varied as expected. Field 2 and 3, Figure 2 b), showed higher organic matter and porosity in the permanent pasture compared to the woodland.

a) F1= Cropped field; free draining loamy over chalk



b) F2= Permanent Pasture field and F3= Woodland; both impeded drainage loamy/clayey over chalk

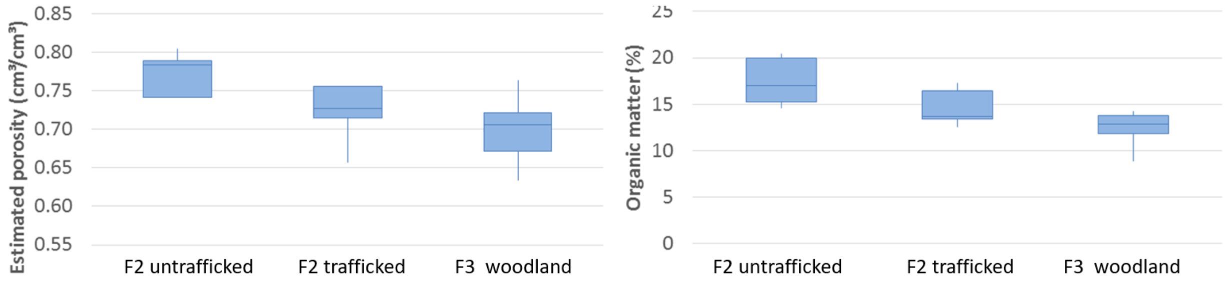


Figure 2: SOM and Porosity in one cropped field, one permanent pasture and one woodland

High biomass rotation, on farm doctoral research

Figure 3 shows mesofauna counts, giving an impression of change in soil life from the different treatments shown in Table 1.

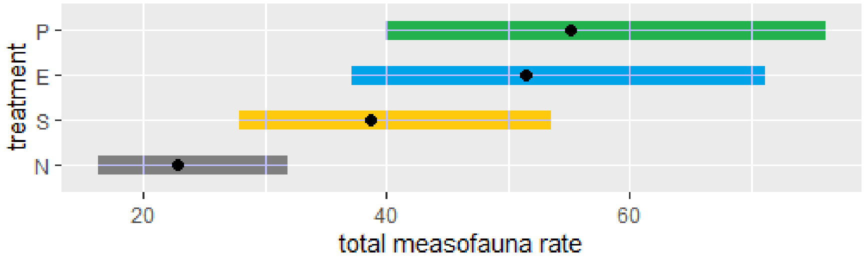


Figure 3: Total mesofauna counts, plot of predicted means in three graphs, by treatment, with 95% confidence intervals

Soil Organic Matter changes are shown in Figure 4. Soil organic matter increased in the crop rotation whether crop residues were retained or not. The five years diverse ley resulted in a larger increase in soil organic matter, and bulk density and aggregate stability compared to the other treatments. The amount of organic matter in the top 100 mm of soil increased by between 1.21 percent and 3.14 percent yr^{-1} in the biomass input treatments and at the 100-300 mm depth by between 0 percent and 1.57 percent yr^{-1}. These outcomes easily surpass the COP21 target of 0.4 percent annual increase in soil organic carbon stock, at the 0-100 mm soil depth with no loss or greater at the 100-300 mm depth.

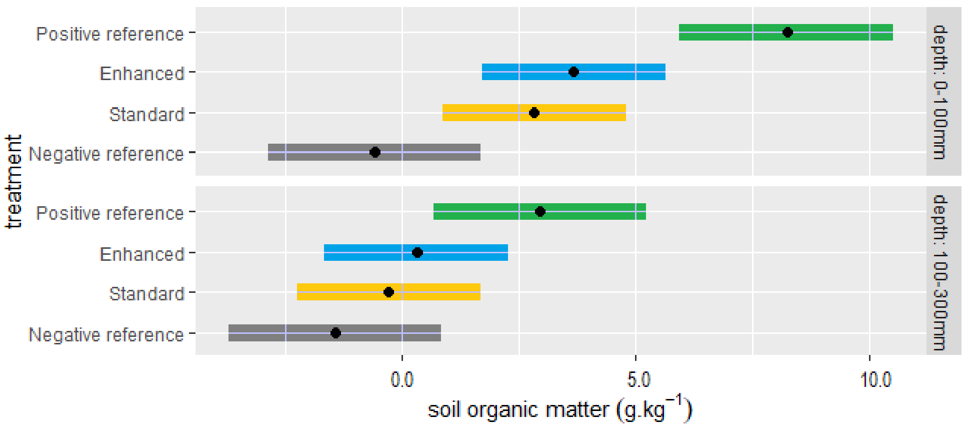
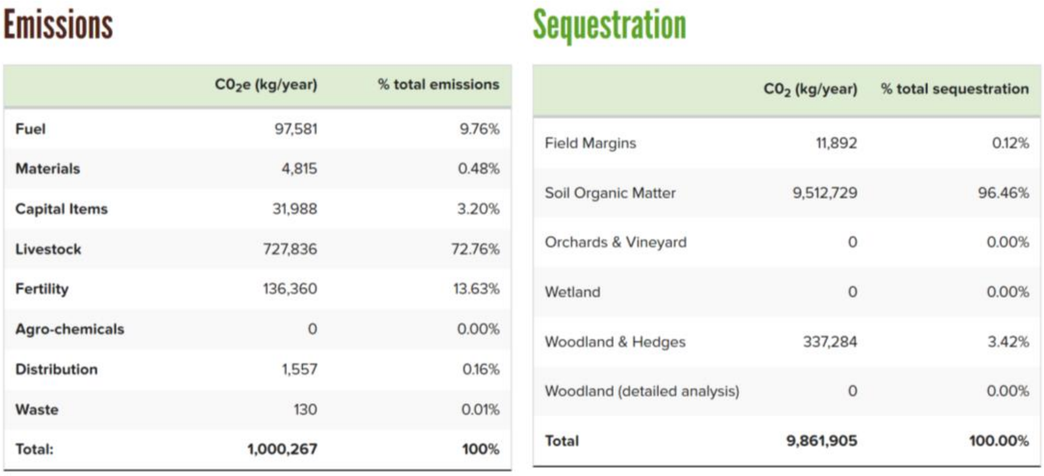


Figure 4: Change in SOM between 2014 to 2019 (g.kg^{-1}) in four plant biomass treatments: enhanced (retention of all crop residue in situ), standard (removal of residues, business as usual), positive reference (5 year ley), and negative reference (tilled fallow)

Dots show predicted means by treatments and depth, bars represent 95% confidence intervals.

Carbon audit

Figure 5 shows the emissions and sequestration of greenhouse gases in 2019. 75 percent of the emissions were from cattle. Fertility in the Figure refers to Nitrous Oxide emissions from crop residues, given in carbon dioxide equivalent. C sequestration from 50ha of woodland, 19km of hedges and the field margins equate to about half the cattle emissions. SOM is currently increasing by 0.25 percent per annum across the cropped land, approximately doubling in each field. Farm carbon sequestration is 9.9 times the emissions.



Emissions

	CO$_2$e (kg/year)	% total emissions
Fuel	97,581	9.76%
Materials	4,815	0.48%
Capital Items	31,988	3.20%
Livestock	727,836	72.76%
Fertility	136,360	13.63%
Agro-chemicals	0	0.00%
Distribution	1,557	0.16%
Waste	130	0.01%
Total:	**1,000,267**	**100%**

Sequestration

	CO$_2$ (kg/year)	% total sequestration
Field Margins	11,892	0.12%
Soil Organic Matter	9,512,729	96.46%
Orchards & Vineyard	0	0.00%
Wetland	0	0.00%
Woodland & Hedges	337,284	3.42%
Woodland (detailed analysis)	0	0.00%
Total	**9,861,905**	**100.00%**

Figure 5: Carbon Audit in 2019 for the whole of Yatesbury House Farm

Discussion

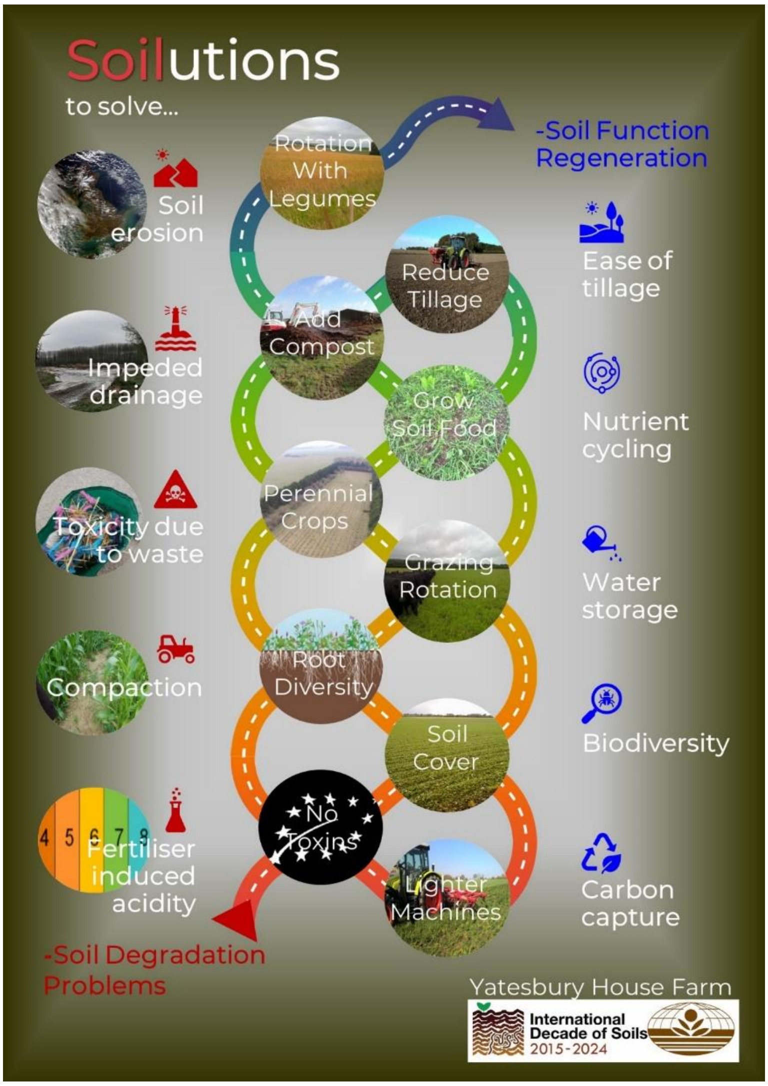


Figure 6: Soilution poster

Yatesbury House Farm soil has grown its SOM as the carbon audit demonstrates. The doctoral research results show that 2-year and 5-year diverse leys provide favourable conditions for SOM increase and mesofauna development. The soil organisms stick together soil particles with polysaccharides, polyphenols and glycoproteins (Gougoulias, Clark and Shaw, 2014) to build their homes. This protects organic matter from decomposition (Six *et al.*, 2002), protects minerals from being eroded, and facilitates the spread of water and air throughout the soil. This life in the soil therefore has a critical role in both organising the soil and stabilising the soil so that it can increase in volume. These processes produce the soil functions (Figure 6) that farmers, homeowners, gardeners and in fact everyone relies upon.

Diverse ley mixtures of legumes, grasses and herbs, are critical to the system. Each species occupies a different space in the soil, each prefer different conditions, and each provide different functions. Functions such as nitrogen fixing by legumes, soil structure improvement by deep rooting species (often termed bioturbation, enabling water storage and drainage), nutrient cycling by taking up leachable nutrients held in the soil water and capture of carbon through the miracle of photosynthesis and work of soil organisms to protect the carbon in the soil.

Döring *et al.* (2013) confirmed that diverse leys have many positive outcomes. These diverse leys were initially in place within the rotation for 2½ years, with field beans as a green manure in the rotation. As a result of the doctoral research, we are keeping the diverse leys in place for to 3½ years to increase the carbon storing potential and reducing the cost of seed.

Machmuller *et al.* (2015) showed that converting cropping land to intensive grazing can fix significant quantities of carbon. Reeder and Schuman (2002) showed that grazing pastures stores more carbon compared to non-grazed pastures.

Conclusions

The increases in SOM found in the carbon audit and backed up through the Landwise survey and doctoral research are due to three parts of the farming system, feeding soil life through adding biomass from ley cropping and crop residues, spreading of soil organisms through the functioning of the cattle and caring for the soil by reducing compaction, reducing tillage (no ploughing) and diversity of plants. From this work we can conclude that Yatesbury House Farm is growing soil, both by increasing soil organic matter and increasing soil meso fauna. Growing soil means farms can be part of the solution to climate change. The method here can be adopted to all cropped land particularly that with livestock.

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Use of Biochar-*Lupinus*- *Bradyrhizobium* as an alternative to improve the vegetation cover of High Andean soils contaminated by heavy metals

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Abstract summary

The Andean Mountain range, due to geodynamic processes, is a scenario of mineralization generating sites with significant mineral wealth, historically exploited, and many countries constituted an economic source. However, mining activity has also generated toxic waste dumped on the soil causing the imbalance of its components; in this situation new bioremediation techniques are being studied. The objective of this research was to observe the effect of Biochar (BIO), the inoculation of symbiotic strains of *Bradyrhizobium*, and the interaction of both; on the growth of *Lupinus mutabilis* in substrate of heavy metals contaminated soil, and in greenhouse conditions. The results showed that the inoculated treatments had significantly better growth of aerial parts than the control, manifested in the aerial dry weight, aerial length and foliar coverage; and the treatments with biochar showed greater growth of the root zone of the plants. Likewise, the treatments with biochar showed significantly less concentration of lead, chromium and iron in the aerial parts and greater concentration in the root zones. It is concluded that the biochar + *Lupinus* spp + *Bradyrhizobium* can be used to improve the vegetation cover of soils contaminated by heavy metals in High Andean areas.

Keywords: Biochar, Lupinus, Bradyrhizobium, biorremedición

Introduction, scope and main objectives

Agricultural soils are being affected by high concentrations of metals, such as As, Cd, Cr, Cu, Pb, Ni, Zn; that exceed the maximum limits allowed and cause a potential risk (Jiang *et al.*, 2019). Other sources of soil contamination are the mining industries and mineral processing. The Andean Mountain range, due to geodynamic processes, is a scenario of mineralization generating areas with significant mineral wealth, historically exploited that have generated toxic waste.

There are several biological remediation techniques, such as phytostabilization, this process reduces the mobility of pollutants by preventing their migration to groundwater or air (Barton *et al.*, 2005); and some of the species with good characteristics are the lupines such as *Lupinus albus*, *L. angustifolius*, *L. uncinatus*, *L. mutabilis* (Vázquez *et al.*, 2006; Ehsan *et al.*, 2009; Hinostroza, 2018).

On the other hand, biocarbon (bochar) is also recommended in soil management, as described by Cernansky (2015); It works as a carrier of microorganisms, and the addition to the soil can increase the population of mycorrhizal fungi and *Rhizobium* infection levels (Amonette, Energy and Billings, 2009).

It is knowing that rhizobium-legume symbiosis improves soil fertility, and in many cases, it has high resistance to biotic and abiotic stresses, which gives it a potential capacity in soil and vegetation phytoremediation (Carpena *et al.,* 2006).

This investigation uses of the biochar and symbiotic strains of *Bradyrizobium* in order to determine their effect on the growth of *L. mutabilis*, and their role in the mobilization of some metals from a contaminated soil to the plant.

Methodology

The experiment was carried out in the greenhouse of LEMYB - UNALM, between the months of June to September of 2019; in which the minimum and maximum temperatures was between 15 and 21°C respectively.

The substrate used (RECU) was part of the soil collected from a High Andean mineralized area located in the department of Ancash (Peru) at 4280 m of elevation. It showed acid pH and high concentrations of iron, lead, cadmium and chromium in relation to agricultural soils and according to the quality standard referred in the Peruvian regulations D.S. N ° 011-2017-MINAM (Table 1).

Table 1: Characterization of the altered soil (RECU) and agricultural land

Samples	**pH**	**E.C.**	**$CaCO_3$**	**O.M.**	**P**	**K**	**Fe**	**Cu**	**Mn**	**Zn**	**B**	**Pb**	**Cd**	**Cr**
Units	**ue**	**dS/m**	**%**		**ppm**									
Altered soil (RECU)	3.2	2.67	0	0.94	1.8	59	1030	11.9	5.6	5.95	0.49	527.7	2.54	144.13
Agricultural Soil (SNAT)	6.89	1.14	0.5	2.38	210	106	103	4.95	112	11.9	0.66	-	-	-
D.S. 011-2017-MINAM	-	-	-	-	-	-	-	-	-	-	-	70	1.4	-

pH: Hydrogen potential; C.E.: Electric conductivity; O.M.: Organic matter, P, K: macronutrients; Fe, Cu, Mn, B: micronutrients; Pb, Cd, Cr: heavy metals

L. mutabilis seeds, two strains of *Bradyrhizobium,* and rice husk´s biochar (subjected to a pyrrolytic oven for 3 hours at 500 °C) were used. The soil and the biochar were passed through a 2 mm mesh screen.

The experiment had seven treatments: Control (RECU); Ctl+BIO (RECU + 15 percent of biochar), L4 (RECU+seeds inoculated with LSHZ-L4 strain), L4+BIO (RECU + 15 percent of biochar + LSHZ-L4 strain); L6 (RECU+LMHZ-L6 strain), L6+BIO (RECU + 15 percent of Biochar + LMHZ-L6 strain); and the control SNAT (agricultural soil + inoculation of both strains). Each treatment had three repetitions; and each experimental unit (pot) had three plants.

The surface of the *L. mutabilis* seeds was disinfected with 70 percent alcohol for three minutes and 1 percent of sodium hypochlorite for two minutes before snowing. For the inoculation, bacterial strains were multiplied separately in YEM culture medium (Yeast-Mannitol Extract Broth) (Vincent, 1970) until reaching 10^8 CFU/ml. the selected treatments were inoculated with 1.5 ml of the corresponding strain on each experimental unit. 15 days later it was re-inoculated with the same volume of inoculant.

Variables measured in plants

After 65 days, the leaf cover (COB) was measured in an Acinterlab phenotype. The plants were harvested at 67 days and the aerial length (L.AER), aerial fresh weight (PFA), aerial dry weight (PSA), fresh root weight (PFR), dry root weight (PSR), number of root nodules (NDT), and some qualitative data such as the appearance of branches, roots and nodules were measured. Also, the nutrient concentrations (N, P, K, Fe) and heavy metals (Pb. Cd and Cr) were analysed in plant tissue.

Statistical analyses

The RStudio program (1.0.143) was used for exploratory analysis, variances ($p = 0.05$), principal component (ACP) and correlation analysis between the evaluated variables.

Results and discussions

Effects of treatments on plant growth

The characteristics that describe the growth of plants showed highly significant differences between the treatments (Table 2). The inoculated plants showed greater growth of the aerial and root parts than the control plants; the treatments with Biochar and inoculated also showed better characteristics than their respective control (CTL+BIO). The inoculated treatments (without Biochar) especially L4 strain allowed better growth of the aerial part; and the treatments with Biochar and inoculated, of the root parts. Evidently the alkaline pH of the biochar (pH = 8.5) improves slightly the acidic conditions of the substrate RECU and consequently allowed the best root growth.

Table 2: Effect of inoculation and addition of biochar to the substrate, on the growth of *Lupinus mutabilis* (p=0.05)

Treatments	L. AER (cm)	PFA (g)	PSA (g)	PFR (g)	PSR (g)	NDT	COB (cm^2)	N (%)	P (%)	K (%)
Control	42.99 bc	5.44 b	0.89 c	1.98 cd	0.24 d	0 b	183.54 bcd	21.72 b	0.71 e	8.99 d
Ctl+BIO	40.84 c	4.51 c	0.74 d	1.78 d	0.26 cd	0 b	153.2 d	15.75 d	1.26 c	4.21 e
L4	45.83 ab	6.03 b	1.07 b	2.02 cd	0.3 bc	2.78 ab	251.02 ab	24.76 a	0.64 e	16.86 b
L4+BIO	44.37 bc	5.34 bc	0.85 c	2.65 abc	0.33 ab	5.11 ab	169.1 cd	18.47 c	1.1 d	14.24 c

L6	46.41 ab	5.99 b	0.94 c	2.26 bcd	0.26 cd	3 ab	231.3 bc	19.11 bc	1.12 cd	87.58 a
L6+BIO	46.39 ab	6.1 b	0.89 c	3.03 ab	0.37 a	3.78 ab	225.15 bc	25.33 a	1.42 b	10.19 d
SNAT	49.16 a	8.58 a	1.2 a	3.27 a	0.34 ab	17.11 a	312.09 a	26.25 a	2.41 a	3.85 e

L. AER: aerial length; PFA: aerial fresh weight; PSA: aerial dry weight; PFR: fresh root weight; PSR: dry root weight; NDT: number of root nodules; COB: leaf cover; N; P; K: Concentration of Nitrogen, Phosphorus and Potassium in aerial part

Inoculated plants showed nodules in primary and/or secondary roots; however, in inoculated treatments and with Biochar, plants had greater number of nodules than treatments without biochar. The number of nodules were significantly higher in SNAT than the rest of treatments; so, the acidity and/or high metal concentrations would be preventing the formation of nodules.

A higher concentration of N was evidenced in treatments with Biochar and inoculated with L6 strain, but this nutrient decreased with L4 strain. However, biochar could be suppressing the uptake of potassium by plants, so there was a higher concentration of K in inoculated treatments than in those who also had biochar.

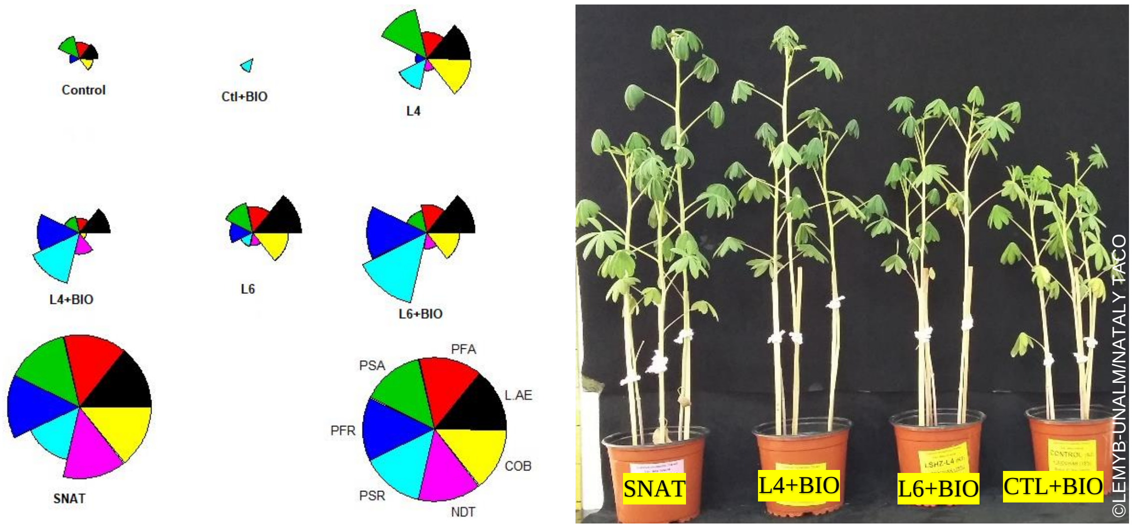

Figure 1: Representativeness of the variables evaluated by treatments

Presence of metals in the root and stem

The treatments inoculated and with biochar showed greater concentration of lead, chromium and iron in plant´s root (123 percent, 180 percent and 186 percent respectively more than their respective control; and 48 percent, 217 percent and 76 percent more than the inoculated treatments without biochar); in contrast, less concentration of lead, chromium and iron in aerial part (83 percent, 84 percent and 90 percent respectively less than the control+BIO; and 90 percent, 80 percent and 95 percent less than inoculated treatments without biochar).

The L6 treatment without biochar showed lower concentration of lead and iron in roots (49 percent and 23 percent respectively) than the control and in the aerial parts (77 percent and 75 percent respectively) than the control.

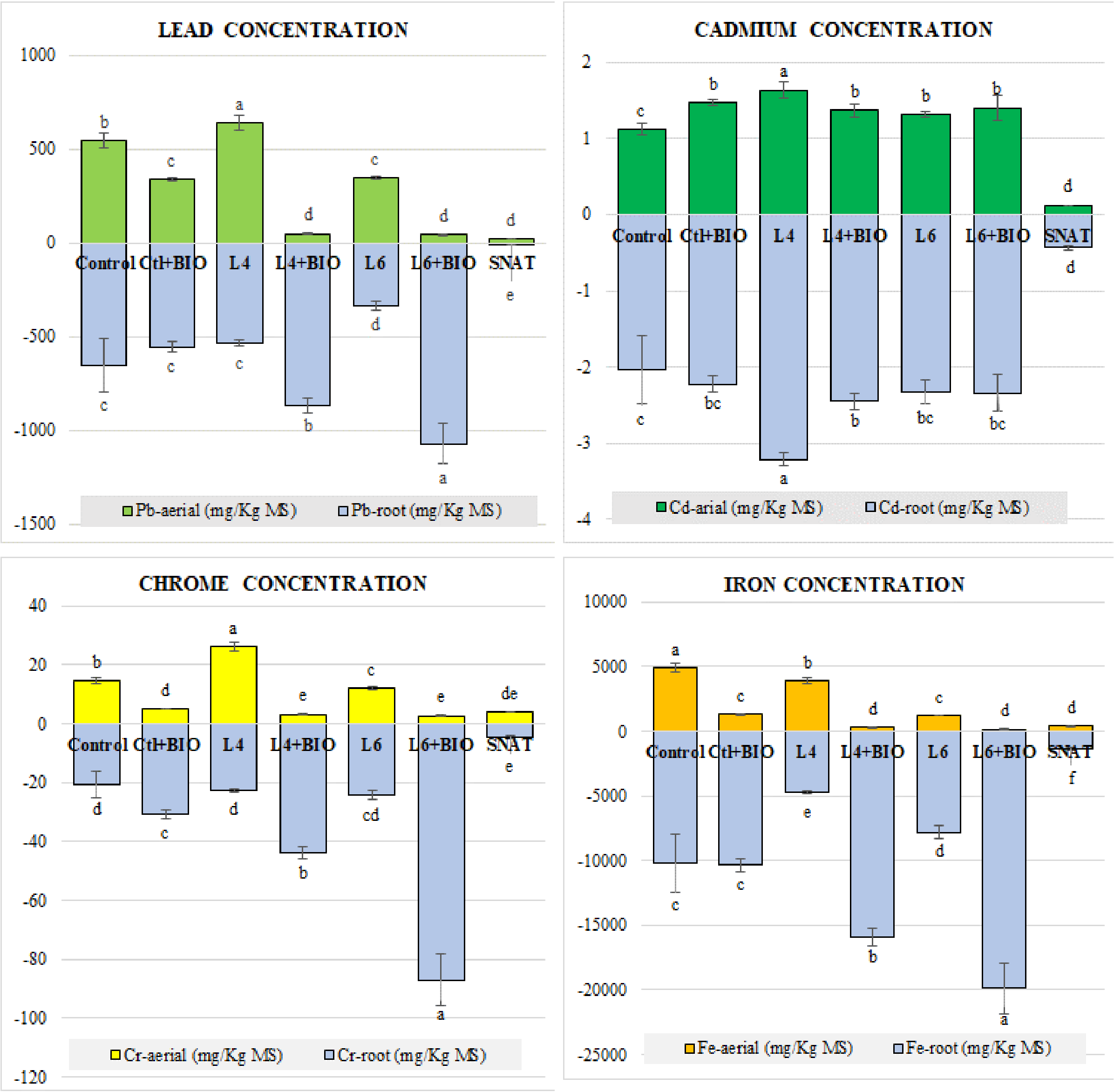

Figure 2: Concentration of Pb, Cd, Cr and Fe in aerial and root parts by evaluated treatments (p=0.05)

Conclusions

The treatments with and without biochar and inoculated with *Bradyrhizobium* showed significantly greater growth of aerial and root parts of plants, than their respective controls.

The treatments that were inoculated and with biochar allowed significantly higher concentration of lead, chromium and iron in the root of plants, and lower concentration of these metals in aerial parts. The LMHZ-L6 strain allowed lower concentrations of lead and iron in aerial and root parts.

It is recommended to carry out field tests applying this biotechnology that includes *Bradyrhizobium* strains and an organic support as an alternative to improve the vegetation cover of soils contaminated by heavy metals and acid conditions in high Andean areas.

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The change in biological activity is a good indicator of soil organic matter change

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Abstract summary

In a *Quercetum petraeae-cerris* forest in north-eastern Hungary, we examined the effects of litter input alterations on the quantity and quality soil biological activity. Treatments at the Síkfőkút DIRT (Detritus Input and Removal Treatments) experimental site include doubling of either leaf litter (DL) or wood (DW) (branches), and removing all aboveground litter (NL), all root inputs by trenching (NR), or removing all litter inputs (NI). The goal of this international project is to assess how rates and sources of plant detrital inputs control the accumulation and dynamics of SOM and nutrients in forest soils over decadal time scales, and how ecosystem function is affected by, and affects, these changes in SOM dynamics. Soil microorganisms react very quickly to changes in environmental variables of the soil. Therefore, in our experiment, the activity of the enzymes they produce was determined to compare the effect of different treatments. Current findings have showed a significantly higher β-Glucosidase and Phosphatase activity in case of soils of detritus addition treatments and control treatment than in detritus withdrawal treatments in spring months. Furthermore, DW treatment showed significantly higher phenoloxidase activity compared to the other treatments, which is explained by the high lignin content.

Keywords: enzyme activity, biological activity, soil organic matter, DIRT, lignin

Introduction, scope and main objectives

Soil's variable organic matter supply, quality and quantity determine not only the physical and chemical properties of soils, but also their biological properties (He *et al.*, 2009; Juhos *et al.*, 2019). Organic matter (SOM) forms the C and N reserves of soil, is involved in pH regulation, cation exchange, structure formation, and is a key substrate for soil microorganisms (Kotroczó *et al.* 2014; Tóth *et al.*, 2011).

Microbial enzymes in the soil play an essential role in catalysing processes required for organic matter decomposition and nutrient turnover. Soil enzyme activities are “sensors” of soil organic matter decomposition since they integrate information about microbial status and soil physicochemical conditions. Most organic C and N compounds are too large to be absorbed by microbes or plants, so bacteria and fungi produce enzymes extracellularly to break down organic matter into useful forms. Therefore, soil enzymes are good indicators of microbial activity and soil fertility. There is currently great interest in the use of extracellular enzyme activities as biological indicators of soil quality, because they are relatively simple to measure, are sensitive to environmental stress and respond rapidly to changes in land management (Yakovchenko, Sikora and Kaufman, 1996). Extracellular enzyme activities can also be directly affected by factors such as temperature, moisture, pH, nutrient availability and chemical properties of the litter. Phosphatases, β-glucosidase and phenoloxidase play important role in organic matter mineralization in soil. Phosphatase enzymes can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils. Phosphatase activity is related to soil and vegetation conditions, responds to changes in management, and can be related to seasonal changes in soil temperature and moisture (Speir and Cowling, 1991). β-Glucosidase is active in the first phases of degradation of organic compounds that reduce the molecular size of organic structures, thus facilitating future microbe enzyme activity (Sardans, Peñuelas and Estiarte, 2008). β-Glucosidase is produced by many diverse fungi, including the wood-rotting basidiomycetes (both white- and brown-rot). Some organisms use extracellular phenoloxidases to degrade lignin and humus to gain carbon and other nutrients. More generally, extracellular phenol oxidases are deployed by both fungi and bacteria to mitigate the toxicity of phenolic molecules and metal ions, and aid in antimicrobial defence.

Our objective was to examine seasonal dynamics of soil phosphatase, phenoloxidase and β-glucosidase activities and to determine the effects of detrital manipulations on these dynamics.

Methodology

Síkfőkút DIRT Project (SIK) was established in 2000 as the part of US ILTER DIRT (Detritus Input and Removal Treatments) network. The study area comprises 21ha in the south part of the Bükk Mountains in Hungary (N 47°55' E 20°46'), 325 m altitude. The mean annual temperature is 10°C, and means annual precipitation is 553mm. Six treatments were established according to ILTER DIRT (Nielsen and Hole, 1963) (Table 1).

Table 1: The applied DIRT treatments in SIK

Treatment	Method
Control (C)	Normal litter inputs are allowed.
No Litter (NL)	Aboveground inputs are excluded from plots.
Double Litter (DL)	Aboveground leaf inputs are doubled by adding litter removed from No Litter plots.
Double Wood (DW)	Aboveground wood inputs are doubled based on measured input rates of woody debris fall.
No Roots (NR)	Roots are excluded with impenetrable barriers extending from the soil surface to the top of the C horizon.
No Inputs (NI)	Aboveground inputs are prevented as in No Litter plots, belowground inputs are prevented as in No Roots plots.

The soil samples were taken randomly, from the top 15 cm layer. The phosphatase, phenoloxidase and β-glucosidase activity was measured for one year in each season according to Sinsabaugh *et al.* 1999.

Results

The soil phosphatase and β-glucosidase enzymes measured showed similar seasonal dynamics. Both enzymes showed the highest activities in spring coincident with high soil moisture and, presumably, high root activity.

In soil enzyme activity, there was a significant difference between glucosidase and phosphatase enzymes activity between treatments. In case of phenoloxidase enzyme we found a significant difference between treatments and DW treatment. There was no significant difference between glucosidase enzyme in June and September (Table 2). In contrast, there were significant differences between treatments in the early spring (March). The highest activity was observed in this period in the case of doubling treatments (DL, DW) and in the soils of C plots. Withdrawal treatments (NL, NR, and NI) showed lower activity than the Control. A similar trend was observed for the phosphatase enzyme (Table 3). For this enzyme, of the withdrawal treatments, the NL treatment showed higher activity than the NR and NI treatments but did not reach the values measured for the doubling treatments. In the case of the phenoloxidase enzyme, the activity values measured with the DW treatment were outstanding (Table 4).

Table 2: Glucosidase activity in SIK

	Glucosidase							
	March		**Jun**		**Sept**		**March**	
C	2.3733	*b*	1.7868	*a*	1.8758	*a*	2.9887	*c*
DL	2.2307	*ab*	1.6843	*a*	1.7343	*a*	2.2887	*abc*
NL	1.4970	*ab*	1.4451	*a*	1.6174	*a*	1.4988	*ab*
DW	2.5876	*b*	1.8877	*a*	2.5325	*a*	2.4305	*bc*
NR	1.0246	*a*	1.0942	*a*	1.1038	*a*	1.1345	*a*
NI	1.4454	*ab*	1.1329	*a*	1.3694	*a*	1.4053	*ab*

Table 3. Phosphatase activity in SIK

	Phosphatase							
	March		**Jun**		**Sept**		**March**	
C	13.1809	*ab*	14.9159	*c*	13.6611	*a*	16.7176	*ab*
DL	13.1103	*ab*	13.4965	*bc*	15.0411	*ab*	20.3283	*b*
NL	9.6985	*a*	12.4979	*abc*	12.3192	*a*	15.5959	*ab*
DW	16.6995	*b*	15.9529	*c*	21.6149	*b*	21.0025	*b*

NR	8.3477	*a*	9.4485	*ab*	11.2727	*a*	14.4563	*a*
NI	9.4191	*a*	8.8021	*a*	11.1448	*a*	13.7713	*a*

Table 4. Phenoloxidase activity in SIK

	Phenoloxidase							
	March		**Jun**		**Sept**		**March**	
C	0.2692	*a*	0.2990	*a*	0.1676	*a*	0.2832	*a*
DL	0.3147	*a*	0.2699	*a*	0.1427	*a*	0.1837	*a*
NL	0.1727	*a*	0.2719	*a*	0.2002	*a*	0.1170	*a*
DW	0.3814	*a*	0.3308	*a*	0.3262	*a*	0.7424	*b*
NR	0.2765	*a*	0.3129	*a*	0.2740	*a*	0.2368	*a*
NI	0.2797	*a*	0.2657	*a*	0.2590	*a*	0.4042	*a*

Discussion

Enzymes of soil microorganisms are excellent indicators of soil degradation and build-up processes and are therefore well suited for rapid signaling of changes (Lajtha *et al.,* 2018). According to our result in soils of DL and DW treatments had significantly higher β-Glucosidase and Phosphatase activity than in the detritus withdrawal treatment condition. Similar findings were made by Krakomperger *et al.* (2008) in experiments with organic matter manipulation. In DL and DW treatments the presence of hot spots of microbial activity was amplified with increasing amount of plant debris, including litter and deadwood; so naturally the microbial activity of soils was enlarged. High activity was observed mainly in the early spring. During this period, the soil has a higher moisture content and the microbes have a more ideal temperature. It was previously reported that soil moisture content has an effect on and positively correlate with microbial activity in a certain moisture range (Fekete *et al.,* 2011). Lower activity was clearly observed in the soil of the withdrawal treatments. This was due to the lack of input of different quality organic matter.

In the case of DW treatment, the outstanding phenoloxidase enzyme activity clearly confirms that difficult decomposable SOM (e.g. lignin) is persistent in the soil. As a result, lignin-degrading microorganisms growth. In our opinion, the reason for this is that wood debris represents a rich and reliable source of organic matter which is hard to transform, mainly due to its impermeability and high lignin content (Brown and Chang, 2014).

Conclusions

Compared to control, long-term organic matter withdrawal produced greater change than doubled organic matter input. Overall, it was found that the quality and quantity of SOM caused significant changes in soil microbial activity. Not only the quality but also the quantity of SOM entering the soil had a significant impact on soil organisms.

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Recovery of microbiological status with organic amendments on soils affected by mining activity in a decadal temporal scale

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Abstract summary

A restoration with compost from urban waste as organic amendments was carried out in totally degraded soils from an opencast mine in Almería (SE Spain). Bacterial communities were studied after ten years of restoration. Also, bacterial communities of adjacent natural soils were selected and studied as a quality reference to know the naturalization state of the soils. Different chemical parameters (pH, total organic carbon "TOC" and total nitrogen content "TN") were analysed. Microbial DNA was extracted, amplified *in vitro*, and V4-V5 regions (400-500 bp) of the 16S rRNA were sequenced with the MiSeq platform (Illumina). Finally, the sequences were compared with the SILVA database at the genus level using the QIIME2 software. A Generalized Linear Model (GLM) analysis was carried out to discover which treatments had the greatest influence on these bacteria. Interestingly, these bacterial genera were almost completely influenced by compost treatment and, in addition, as reflected in experimental plots, soil bacterial taxa were exclusive or almost exclusive in compost restored soils and natural soil. These results suggest that long-term restored soils with compost have microbial communities similar to the surrounding natural soils. Such communities may be good descriptors of the recovery of degraded soil.

Keywords: soil restoration, compost, metagenomics, chemical parameters.

Introduction, scope and main objectives

The use of organic amendments in degraded soils by opencast mining is key to ensure the success of restoration, mainly in fragile ecosystems such as those located in semi-arid climate, due to the erosion produced by scarce torrential rains, high radiation and low vegetation cover (Luna *et al.*, 2016). Limitations on the physical and chemical properties of soil combined with low microbial activity in semi-arid areas complicate natural restoration in these regions (Juwarkar and Jambhulkar, 2008). The use of organic amendments could be a solution to accelerate the soil naturalization processes and, therefore, its restoration, since they improve the physicochemical and biological properties of soils (Rodríguez-Berbel *et al.*, 2019). Soil microorganisms could be excellent indicators to evaluate quality recover in restoring soil, because they play a vital role in soil structural formation and contribute to plant establishment and organic matter transformation (Bender *et al.*, 2016; Zink and Allen, 1998), as well as being directly related to biogeochemical cycles (Adak and Sachan, 2009). Moreover, soil microbial communities reflect better soil quality indicators than physico-chemical soil properties (Deng *et al.*, 2019). The study of soil microbial communities can provide important information on the condition of soils after restoration, in order to determine the organic amendments that can promote bacterial growth by simulating the reference soils considered to have the highest soil quality thresholds.

The objective of this work was to study the bacterial communities at genera taxonomic level to determine if application of organic amendment (compost from urban waste) approached in the long-term to reference state (natural soils) after its addition in restored soils of a limestone quarry in a semi-arid climate.

Methodology

The restoration was performed ten years ago on degraded soils of a limestone quarry located in the Gádor range (Almería, SE Spain) in a semi-arid Mediterranean climate. The experimental plots were installed in a completely degraded soil (Figure. 1A), and then organic amendment compost from urban waste (CW) was added. In turn, control plots (NA) were established without amendments applied and adjacent natural soils (NS) were selected as quality reference. More information about the study area is found in Luna *et al.* (2016).

Six soil were sampled up to a depth of 10 cm and bacterial communities and several chemical parameters were analysed (pH, total organic carbon "TOC" and total nitrogen content "TN"). Microbial DNA was extracted with a commercial kit and V4-V5 regions (400-500 bp) of rRNA 16S were amplified *in vitro* (PCR), then amplicons were sequenced with the MiSeq platform (Illumina) and, finally, the sequences were compared with SILVA database at to genera level using QIIME2 software.

Data of relative abundance of bacterial taxa were analysed with Generalized Linear Models (GLMs) to find out if treatment influenced each soil bacterial taxa. In addition, Pearson’s correlations (r; $p < 0.05$) was used to determine the significant correlations between soil bacteria at the genera level and chemical soil parameters.

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Figure 1: Study area

A) Totally degraded soils where the experimental plots were located. B) Soils recovered after ten years of restoration.

Results

A total of 162 soil bacterial taxa with a relative abundance of more than 0.1 percent for all the samples were studied.

Generalized Lineal Models showed that soils treatment with compost amendment (CW) was the one which most influenced ($p< 0.05$) the highest number of bacterial taxa (59 percent of taxa) meanwhile 14 percent of genera were mainly influenced by natural soils (NS). In addition, the boxplots revealed that there were some bacteria taxa that were common and exclusive or almost exclusive in CW-treated soils and in natural soils (Figure.2).

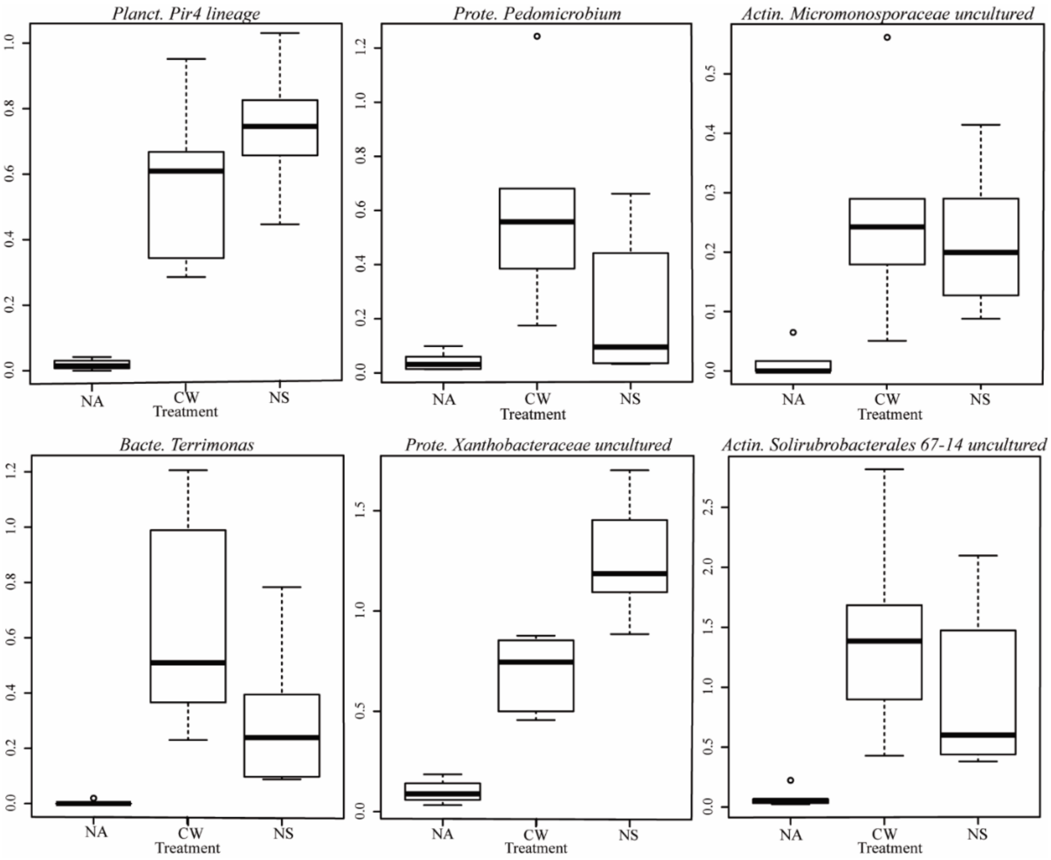

Figure 2: Boxplots of soil bacteria at genera level distribution among the three types of experimental plots, being exclusive or almost exclusive of compost treatment soils and natural soils

(CW: Compost plots; NA: No amendment plots; and, NS: Natural soil).

Pearson's correlation analysis of soil bacteria taxa that were common between CW-treated soils and natural soils (*Pir4 lineage*, *Pedomicrobium*, *uncultured* [Family: Micromonosporaceae], *Terrimonas*, *uncultured* [Family: Xanthobacteraceae] and *uncultured* [Family:

Solirubrobacterales 67-14) and chemical soil properties showed significant ($p < 0.05$) and positive correlations with TOC and TN and significant negative correlation with soil pH (Table 1).

Table 1: Significant correlations ($p < 0.05$) between soil parameters and soil bacteria

	pH	TOC	TN
Planc. Pir4 lineage	*-0,761*	0,852	0,736
Prote. Pedomicrobium	*-0,615*	0,802	0,805
Actin. Micromonosporaceae uncultured	*-0,629*	0,784	0,717
Bacte. Terrimonas	*-0,731*	0,725	0,770
Prote. Xanthobacteraceae uncultured	*-0,636*	0,709	0,378
Actin. Solirubrobacterales 67-14 uncultured	*-0,651*	0,680	0,747

Negative correlations are in italics. TOC: Total Organic Carbon content; TN: Total Nitrogen content.

Discussion

Compost treatment (CW) promoted in the long-term soil restoration, showing TOC and TN values similar to natural soils (NS) and, soil pH values were significantly lower than in other types of soils (data not shown). Organic amendments have contributed in different studies to increase and improve the microbial proliferation of the soil (Bastida *et al.*, 2008; Tejada *et al.*, 2006). In addition, the type of organic matter and plant residues influence the composition of soil microbial communities (Kramer and Gleixner, 2008).

The soil treated with compost was the most influential in the bacterial taxa, leading us to believe that this organic amendment favoured the bacterial proliferation due to the improvement of the chemical properties of the soil which in turn favored increased plant growth by Luna *et al.* (2016) five years after restoration (Figure 1B). Interestingly, these bacterial taxa were present almost exclusively in soils amended with compost and natural soils (Figure 2), and may have been the organic amendment of compost was the one that came closest to the reference soils, confirming again that it is possibly due to the vegetal stabilization that favors rhizospheric niches and also to the improvement of the chemical properties of the soil (Bastida *et al.*, 2016).

Several common bacterial taxa among CW-treated and NS soils also showed significant positive correlations with soil TOC and TN (Table 1). Besides, bacterial taxa as *Pedomicrobium* was observed in soils rich in organic matter (Lima *et al.*, 2015) or as *Terrimonas* that was found in developed soils near our study area (Sánchez-Marañón *et al.*, 2017), corroborating that compost-treated soils improved their quality in ten years. Similarly, these bacterial taxa also strong negative correlations with soil pH (Table 1). Lauber *et al.* (2009) established that soil pH plays a crucial role on soil microbial composition which drive changes in soil bacterial taxa.

Conclusions

These results suggest that long-term restored soils with compost treatment have established microbial communities similar to surrounding reference natural soils. These results show that compost management of urban waste is a suitable method to recover microbial communities of degraded soils in a temporal scale of a decade.

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Volatilee organic compounds produced by selected antagonistic rhizobacteria against soil-borne phytopathogenic fungi

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Abstract summary

The aim of this study was to elucidate the antagonistic potential activity of TvPs1.6 and TvPs2.4 strains, rhizobacteria isolated from common bean plant, based on the identification of volatile organic compounds produced to control mycelial growth of *Sclerotinia sclerotiorum*, *Fusarium oxysporum* and *Rhizoctonia solani*. These fungus represent the most prevalent soil-borne fungal pathogens on legume fields in Peru causing damping-off and common blight of plants. TvPs1.6 and TvPs2.4 strains produced 42 volatile organic compounds detected by SPME/GC–MS analysis. From them, compounds with relatively high abundance were D-Limonene, Dimethyl disulfide, methyl butyrate and ethyl 3-methylbutanoate, methyl 3-methylbutanoate and ethyl 3-methylbutanoate.

Keywords: Disease control, rhizobacteria, Volatile organic compounds

Introduction, scope and main objectives

Fungal pathogens cause most of the diseases occurring in agricultural and horticultural setups. The diseases cause by the fungal pathogens is the major causes of yield crop losses and diminished crop quality. These organisms are capable of infecting different tissues of the plant causing symptoms and/or signs such as leaf spot, rust, wilt, blight, canker, damping-off, root rot and dieback. Over time, various agro-chemicals have been developed to promote plant growth and to minimize the impact of pathogenic fungi on plant health and yield. However, due to the adverse environmental effects by overuses chemical fertilizers and pesticides, pesticide resistance and the strict regulation of their use, there is an urgent need to further reduce the use of pesticides and to identify more sustainable crop protection strategies. Many studies describe the potential use of Plant Growth Promoting Rhizobacteria (PGPR) to control a wide range of fungal phytopathogens through their antagonistic capacity against diseases. PGPR produces metabolites such as iron-chelating siderophores, antibiotics, volatile biocides and lytic enzymes. The aim of this study was to elucidate the antagonistic potential activity of TvPs1.6 and TvPs2.4 strains, rhizobacteria isolated from common bean plant, based on the identification of volatile organic compounds produced to control mycelial growth of *S. sclerotiorum*, *F. oxysporum* and *R. solani*.

Methodology

Volatile compounds production by sealed plate method

Bacterial isolates were cultured in Tryptic Soy Broth (TSB) at 28° C and shaken at 150 rpm overnight up to an OD_{600} of 0.9-1.2. 100 µL of the bacterial fermentation broths were spread onto the bottom dish of a petri plate containing Tryptic Soy Agar. A 5 mm agar plug of freshly growing mycelium was taken from the margin of the colony using a sterile cork borer and placed in the centre of a fresh Potato Dextrose Agar plate. It was then immediately inverted over the bacteria plate and sealed with Parafilm®. The plates were incubated at 25° C for 4 to 5 days (Fernando *et al.,* 2005). Plates with only the agar discs of test fungi were used as controls. Antagonistic activity of bacteria through the production of volatile compounds was evaluated against the soil-borne plant pathogens *S. sclerotiorum*, *F. oxysporum* and *R solani*. The diameter (mm) of the fungal colony was measured until the mycelium of the controls extended over the entire plate. The tests were independently performed on two separate samples of each bacterial isolate. The fungal growth inhibition was quantified using the percentage inhibition formula: $((R-r) \times R^{-1} \times 100)$.

Analysis of volatiles by Solid Phase Microextraction-Gas Chromatography–Mass Spectrometer (SPME-GC–MS)

SPME/GC-MS was used to characterize the volatile fraction of the two best antagonistic strains TvPs2.4 and TvPs1.6 by the production of volatile organic compounds (VOCs) against *R. solani, S. sclerotiorum* and *F. oxysporum*. The SPME fibre used to collect volatile compounds was 2 cm divinylbenzene/ carboxen on polydimethylsiloxane (Supelco, Fluka). To extract the volatile compounds, a 2 ml bacterial suspension (16 hours old) was grown in 25 ml of TSB in a 100 ml headspace vial at 28° C for 24 hours. The SPME fibre was inserted into the headspace vial just above the inoculated medium and allowed to equilibrate for 30 min. GC-MS analysis was performed using a Agilent Technologies 7890-GC gas chromatograph in combination with a Agilent Technologies 5975C MS mass spectrometer. A capillary GC column, J&W 122-1545.67659 DB-5ms, (60 m x 250 µm x 0.25 µm), was used to separate the volatile compounds. Helium was used as the carrier gas under a constant flow of 1 ml min^{-1}. The SPME fibre was inserted and desorbed at 70° C for 1 min into the injection port of the injector. The injector temperature was finally maintained at 300° C. The working temperature of the column was set as follows: 35° C for 3 min at the beginning, increased to 110° C for 10 min and finally to 300° C at 30° C for 1 minute. AMDIS software was used to visualize GC–MS data, and NIST08 MS-libraries were used to identify the VOCs by fragmentation patterns.

Results

Production of volatile antifungal compounds

In order to determinate the involvement of volatile compounds in the antifungal activity against to phytopathogens, rhizosphere bacterial were tested by sealed plate’s method. TvPs1.6 and TvPs2.4 strains inhibited the mycelial growth of *S. sclerotiorum* (91 percent and 97 percent), *R. solani* (54 percent and 47 percent*)* and *F. oxysporum* (31 percent and 29 percent) respectively. The mycelium of *F. oxysporum* turned transparent and presented no pigmentation. *R. solani* and *S. sclerotiorum* presented mycelium growth inhibition (Figure 1).

Analysis of volatiles by Solid Phase Microextraction-Gas Chromatography-Mass Spectrometer (SPME-GC-MS)

VOCs emission profiles measured by SPME/GC-MS analysis varied in strains. 42 VOCs were detected and the emission profiles of eight of them differed according to the type strain (Table 1). Analysis of the VOCs from TvPs2.4 consistently revealed four peaks with relatively high abundance (22.57, 18.65, 15.41 and 7.84 percent) for D-Limonene, Dimethyl disulfide, methyl isovalerate and ethyl 3-methylbutanoate respectively. In the case of TvPs1.6, revealed four peaks with relatively high abundance (38.89, 16.10, 7.52 and 5.84 percent) for Dimethyl disulfide, D-Limonene, methyl 3-methylbutanoate and ethyl 3-methylbutanoate respectively.

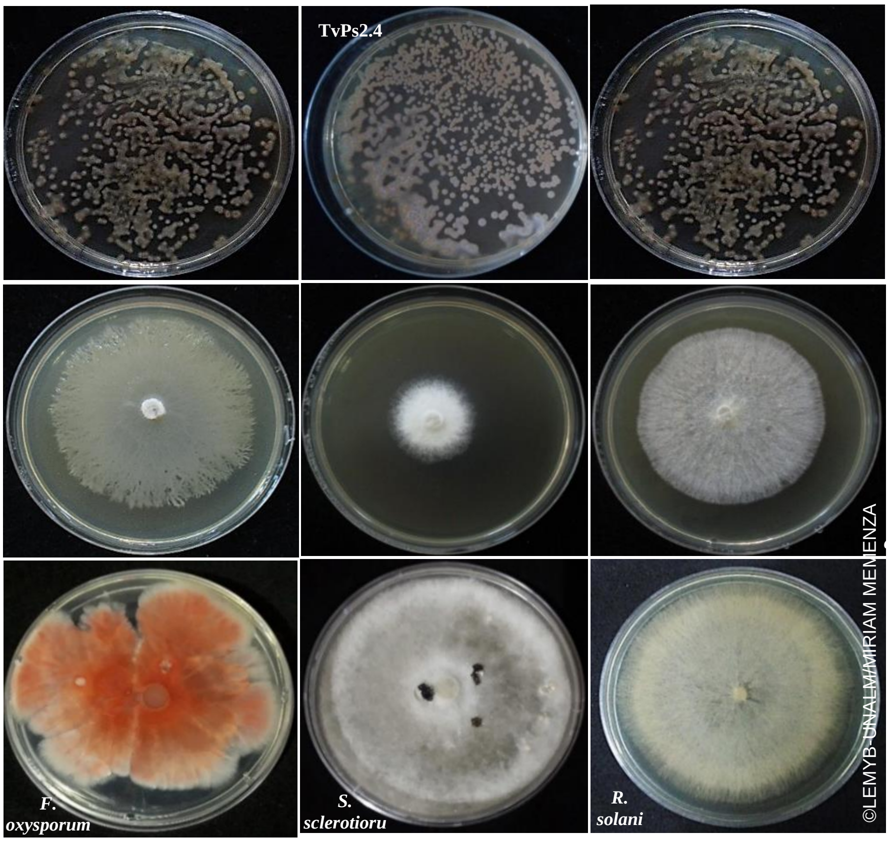

Figure 1: Inhibition of micelial growth of *F. oxysporum, S. sclerotiorum* and *R. solani* (second row) by volatile organic compounds produced by TvPs1.6 and TvPs2.4 strains (top row)

Control plates with the mycelium of the fungus grown on PDA medium (bottom row).

Discussion

TvPs1.6 and TvPs2.4 strains showed the antifungal activity against *R. solani, S. sclerotiorum* and *F. oxysporum* by the production of VOCs. SPME/GC–MS analysis revealed 42 VOCs produced by these strains, among them five peaks were detected with relatively high abundance for D-Limonene, Dimethyl disulfide, methyl butyrate and ethyl 3-methylbutanoate, methyl 3-methylbutanoate and 2-nonanone. There are studies that demonstrate that VOCs of soil bacteria can influence the growth of phytopathogenic fungi (Alström, 2001; Wheatley, 2002). The VOCs are generally lipophilic substances with high vapor pressure, which freely pass-through biological membranes and are released into the atmosphere or in the soil where the producers are (Pichersky *et al.,* 2006). Dimethyl disulfide produced by *Streptomyces globisporus* JK-1 showed antifungal activity against *Penicillium italicum* on *Citrus micracarpa* at a concentration of 100 µL L^{-1} in treatment containers (Li *et al.,* 2010). D-limonene induces expression of PDF1.2 gene and resitance against *Colletotrichum higginsianum* in *Arabidopsis* ecotype Col-0 (Fujioka *et al.,* 2015). The acetic acid, 2-nonanone, d-limonene and dimethyl disulfide with minimal inhibitory quantity of 4.19, 4.92, 17.20 and 31.38 mg, respectively, produced by different strains of *Pseudomonas* spp. resulted the most active compounds in reducing mycelium growth arising from the fungal plug of *S. sclerotiorum.* Observations at light microscope of the *S. sclerotiorum* mycelium exposed to VOCs of the *Pseudomonas* spp. strains, showed hyphae morphological abnormalities compared to the control treatment. In fact, in all the treatments, hyphae observed by optical light microscope, appeared thinner and characterized by the presence of vacuolization of cytoplasm when compared to the control. Besides, VOCs of *Pseudomonas* spp. isolates caused hyphae with numerous mitochondria with iper-vesiculated and hypertrophic ridges and multi-vesiculation and cytoplasmic accumulation of material (protein or lipid) (Giorgio *et al.,* 2015). The activity of such molecules is related to their ability to cross lipid layers of cell membranes in relation to their lipophilicity, which depends on the acyl chain length appearing optimal when 9–14 carbons long. VOCs exerted anti-microbial activity for their capacity to penetrate the microbial cell interfering with transport of metabolites and maintenance of potential at membrane level.

Table 1: Chemical composition of volatiles obtained from cultures of the PGPR antagonistic strains using solid-phase microextraction (SPME)

Retention time (min)	Molecular weight	Compound	Abundance (Relative) [a]	
			TvPs2.4	TvPs1.6
4.96	102.13	methyl butanoate	1.02	0.46
5.15	90.21	Unknown ($C_4H_{10}S$)	1.08	
5.17	74.16	Unknown (C_3H_6S)		1.17
5.66	94.2	Dimethyl disulfide	18.65	38.89
5.88	116.16	methyl isovalerate	15.41	
5.90	102.13	methyl 3-methylbutanoate		7.52

6.09	130.21	Unknown ($C_7H_{14}O_2$)	0.38	0.48
6.17	116.16	ethyl butyrate	0.30	
6.26	104.24	Unknown ($C_5H_{12}S$)	1.98	2.14
6.95	130.19	ethyl 2-methylbuttyrate	0.98	0.67
7.02	158.24	ethyl 3-methylbutanoate	7.84	5.28
7.11	152	Unknown	10.77	
7.12	118.22	Unknown ($C_5H_{10}OS$)		6.73
7.76	146.18	3-methyl-1-methylethyl butanoate	2.01	2.82
9.01	222	Unknown	5.86	
9.02	132.61	Unknown ($C_6H_{12}OS$)		7.42
9.10	172.27	propionic acid 2-methyl-hexyl ester		0.62
10.12	158.81	Unknown ($C_9H_{18}O_2$)		0.36
10.22	136.23	β-Pinene	0.55	
10.66	240	Unknown	0.55	
10.67	15.24	2-methylpropyl 3-methylbutanoate		0.80
11.33	136.23	Terpinolene	0.84	
11.60	134.21	o-Cymene	2.65	1.65
11.78	136.24	D-Limonene	22.57	16.10
11.97	154.25	eucalyptol	1.93	1.10
12.07	158.24	3-methylbutyl butanoate	2.15	
12.08	158.81	Unknown ($C_9H_{18}O_2$)		4.83
14.04	142.24	2-Nonanone	1.03	0.66
15.90	152.23	α-Thujone	0.83	
21.70	555.13	Unknown ($C_{35}H_{68}O_4$)	0.62	0.31

[a] **The abundance (relative) represents the percentage amount of each compound in total area relative to all listed compounds detected for one strain**

Conclusions

TvPs1.6 and TvPs2.4 produced 42 volatile organic compounds detected by SPME/GC-MS analysis. From them, D-Limonene and Dimethyl disulfide have been reported their antagonistic activity against different soil-borne pathogens. The results of this preliminary screening of PGPR from the rhizosphere of the common bean plant in Peru form a starting point to optimize the production of bioactive extracellular compounds that inhibit the mycelial growth and sporulation of harmful phytopathogens. In time, these PGPR could improve yields in the field, reduce the use of chemical pesticides and fertilizers, and thus reduce environmental pollution and contribute to the further development of sustainable agriculture.

Acknowledgements

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Soil biodiversity teems with life but faces pollution. Are we acting correctly in agro-ecosystems?

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Abstract summary

We present a systematic review study on how biodiversity research is currently performed and how representative was holistic biodiversity studies compared to the ones focusing on individual group-biodiversity. Most of the known studies tend to focus on single biota groups/family presence interaction rather than multiple ones. Research efforts remain siloed and thus a holistic understanding of relationships between the organism's groups shaping biodiversity is still challenging. In this work, we prove the effectiveness of biodiversity-based solutions on the mitigation of soil biodiversity loss in agroecosystem posed by (?)pollution. The application of symbiotic microorganisms to the soil during agriculture activities fosters the increase of microbiological diversity and vitality at the rhizosphere level thus supporting sustainable development in agriculture.

Keywords: biodiversity, knowledge gaps, land-use, pollution, agro-ecosystem, symbiotic microorganism, sustainability, holistic approach

Introduction, scope and main objectives

Soils are globally considered as the reservoir of biodiversity but at the same time are negatively affected by anthropic activities-alterations to the environment (Geisen, Wall and van der Putten, 2019; Nuti and Giovannetti, 2015). As soil biodiversity sustain agro-ecosystem functioning and increases resilience-resistance of soil and plants from stresses ensuring a functional food system, assessing the status quo on how soil pollution does affect biodiversity in agricultural soil becomes fundamental.

Here we present a systematic review study on how biodiversity research is currently performed and how representative were holistic biodiversity studies compared to the ones focusing on individual group-biodiversity. To test whether reduced biodiversity in soil could be mitigated and soil biodiversity restored, long-term field experiments with biodiversity-based solutions (microorganisms) were used This work aims at understanding main drivers for soil biodiversity loss, knowledge gaps and foster the discussion on practical responses to reverse them.

Methodology

Systematic approach to known literature on the impact of pollution on biodiversity of agro-ecosystem has been performed and analysed. Land use and source of pollution were used as discriminant factors for biodiversity representativeness in agricultural soils. Empirical experiments on mitigation of biodiversity loss were performed with multiple agricultural crop fields via long-term field experiments with symbiotic microorganisms inoculated in the rhizosphere during the phenological cycle of plants. Microbial activity and diversity in soil were measured via respiration activity, culturable and non-culturable methods while for macro-invertebrates the method of count of individuals was adopted.

Results

The theoretical known complex biodiversity in soil represented by the high diversity of soil biota groups is not rationally mirrored by the selection of research studies. Most of the known studies tend to focus on single biota groups/family presence/ interaction rather than multiple ones. Despite urgency and great importance, research efforts remain siloed and knowledge advancement is limited. Therefore, a holistic understanding of relationships between the organism's groups shaping biodiversity remains lacking. Moreover, despite the clear efforts on the quantification of land use and pollution impact on biodiversity loss, the non-holistic approach applied to soil biodiversity research hampers the understanding of the changes in biota groups interactions of agro-ecosystem under stress conditions. It is rare but there is experimental evidence that mitigation of soil biodiversity loss is feasible if applying symbiotic microorganisms in agro-soils with a result of the consequent increase of microbiological vitality at the rhizosphere level.

Discussion

The sustainable management and assessment of soil biodiversity are pivotal to agriculture development that should point at more resource-efficient and resilient systems. This is, however, not possible if the non-holistic assessment of soil biota is performed in case of biodiversity loss and functioning restoration. Additional investigation on how relationships between crops and fungi-microbial biota in agro-soils could increase soil biodiversity and reactivate the communication between the two via increased plant nutrient uptake and defense against pathogens assessed.

Conclusions

The currently available approaches and data can be considered inadequate for assessing soil biota diversity and its functions. A more holistic assessment should be defined where not only the effect on land use and ecological impact of pollution are incorporated, but also biocoenosis distribution in the agro-ecosystem. Modern developed methods such as DNA screening could be incorporated into monitoring processes for specific soil biota groups normally found in soil such as beetles and mites yet not assessed. The application of symbiotic microorganisms to soil during agriculture activities can be considered as good practice to reduce and mitigate the adverse effect of intense agricultural activities on soil biodiversity.

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How much the Soil Biodiversity is known in Mexico?

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Abstract summary

Mexico is a mega-diverse country it contains 12 percent of the 1.8 million species described worldwide despite occupying only 1.5 percent of the Earth's surface. Although its soil biodiversity is poorly known, and most efforts focus on some specific groups. From bibliometric analysis of the web and databases available mainly from CONABIO (Nacional Council on Biodiversity-Mexico or Comisión Nacional para el Conocimiento y Uso de la Biodiversidad) and specific studies, we confirm that the soil biodiversity and its services are poorly known in México. There are specific areas of Mexico that are sampled and other not at all. In México, up to now, only 8,899 species have been registered in the REMIB-CONABIO database as specific soil dwellers, of which only 62 species are fungi. Arthropoda was the Animalia group with 98 percent of the number of species mostly insects (Coleoptera). The main conclusions are that we need more surveys in none sampled areas. Taxonomists also, are needed and these must relate the organism they identify to the soil as their habitat even if they spend only part of their life span in the soil, like majority of insects.

Keywords: microbiota, macrofauna, mesofauna, below ground diversity

Introduction, scope and main objectives

Mexico is a mega-diverse country it contains 12 percent of the 1.8 million species described worldwide despite occupying only 1.5 percent of the Earth's surface (CONABIO, 2006). The spatial patterns of this diversity have been explained in very general terms by physiographic, climatic, biological and cultural factors. However, our ecological understanding of global biodiversity patterns, for example gradients of latitudinal diversity, relies heavily on the distribution of taxa that live above ground. However, it has been shown that some organisms have patterns of global diversity different from organisms on the ground like earthworms (Phillips *et al.,* 2019) or Nematodes (van den Hoogen *et al.,* 2019). Although these patterns can be biased as there is not enough information.

Mexican soil Biodiversity is poorly known, and most efforts focus on some specific groups. Some are studied for their importance in agriculture such as nitrogen fixing bacteria, VAM fungi or nematodes. For the mesofauna and the macrofauna, we have some good data on collembola, ants, beetles, diplopods or earthworms because we have specialists on specific groups. In some protected natural areas, some groups of soil organisms are being studied more deeply like coleoptera. However, the diversity of these groups is not yet well known in different land uses or in different ecosystems. The objective of this work was to have a broad overview of how much the soil biodiversity of Mexico is known.

Methodology

1-Biblioometric analysis in the webs and observation of the existing data bases from Conabio mainly.

2- Analysis of results from soil biodiversity samplings made with the methods of Moreira, Huising and Bignel (2008)

Results and discussion

The bibliometric analysis made in the Web of Science (WOS) with the words: soil and biodiversity, showed that only 3.4 percent of the results were from Mexico and it was 508 records. It is only since 2006 that we have more than 20 publications per year. The main collaborators for these papers beside Mexican authors are Americans, Brazilians and French. The journals with more articles were Southwestern Entomologist, Mycorrhiza and Applied Soil Ecology. With a search made in Google with the words Biodiversity, Soil and Mexico in Spanish we could get 20 times more records than on WOS, which is quite substantial but at this moment all this information has not been debugged or analysed. But from some preliminary observations we can say that in the 32 states of Mexico only few groups have been inventoried in most of them, which are the ants and termites. And there are 6 states that have 0 or very few records. One of the states that is subsampled is the biggest (Chihuahua).

In México, the National Biodiversity Commission (CONABIO) promotes specialists to do inventories to complete the National Biodiversity Information System (SNIB). Up to now, 8,899 species have been registered in the REMIB-CONABIO database as specific soil dwellers, of which only 62 species are fungi. Arthropoda was the Animalia group with 98 percent of the number of species. This group was represented in 10 classes (mostly insects), 37 orders, (mostly Coleoptera).

The specific example of the project conservation and sustainable management of the belowground biodiversity (CSM-BGBD) showed that we are missing taxonomist as many organisms could not be identify particularly the ants, coleoptera and nematodes, because the lack of knowledge but mainly because the lack of time to do all the work (Figure 1).

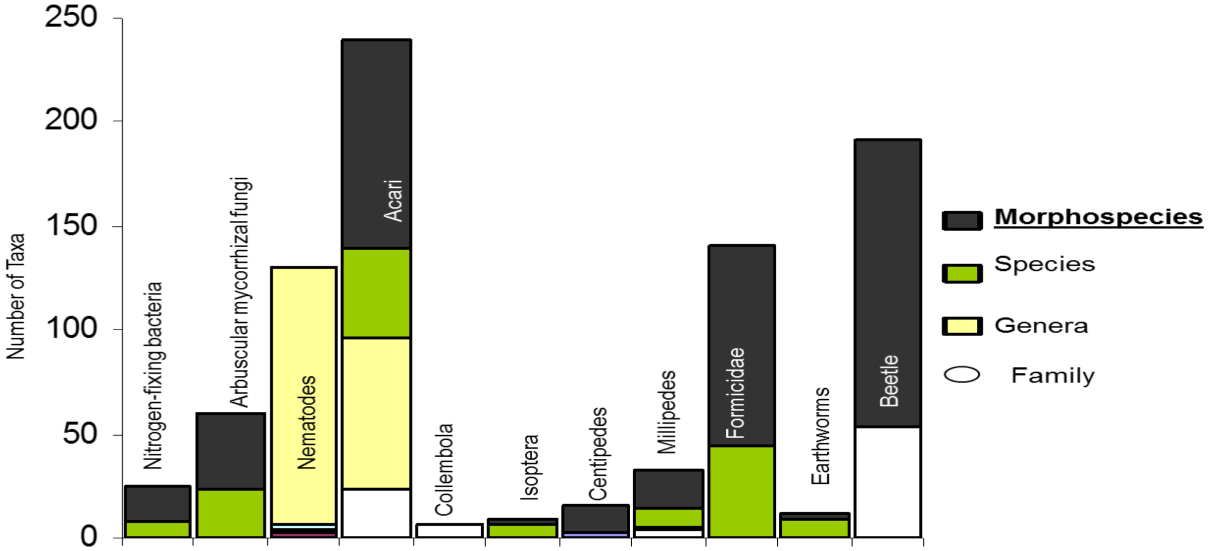

Figure 17: Number of taxa sampled in different land use in Los Tuxtlas Biosphere reserve in veracruz Mexico

The original vegetation was the rainy tropical forest, the other landuses were pasture, maize field and secondary forest. These results are from 89 points sampled.

Conclusions

Our general conclusions for Mexico are that the study of soil organisms focuses on emblematic or charismatic groups, as for the above ground organisms. We need to stimulate to study other groups and have more taxonomists to identify them. The information should be more centralized and systematized in CONABIO's databases and in these databases mention the habitat as soil dweller even if it is only in their larval state. We need to make systematic inventories throughout Mexico and especially in the states that are poorly sampled.

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Recovery of soils biodiversity on reclaimed drilling pads of oil-gas wells in East Ukraine

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Abstract summary

The number of microorganisms belonging to the main ecological-trophic and taxonomic groups and the number and biodiversity of invertebratesin soils drilling sites reclaimed in different years have been investigated. Measures for technical reclamation of soils violate the structure of soil microbial coenosis, reduce the number of soil microflora and number and biodiversity of invertebrates. Over time, the number of microorganisms belonging to the main ecological and functional groups and soil invertebratesis gradually restored to the level of the background soil. In case of soil contamination by pollutants associated with the production, the restoration of indicators to the level of the background will not be observed for decades. The degree of restoration of soil biota state depends on the quality of the reclamation, the duration of its effects, the acquired properties of the soil.

Keywords: soil microorganisms, soil invertebrates, ecological and trophic groups, biodiversity, reclaimed soils

Introduction, scope and main objectives

Land reclamation is an obligatory part of petroleum production operations. It is aimed at rehabilitation of disturbed lands and comprises two consecutive steps – technical stage (treatment of the pad after well completion, retrieval and disposal of topsoil onto the pad, and site flattening) and biological stage (planting of cereals and legumes crops in order to restore soil fertility). However, biological stage of reclamation is often disregarded in Ukraine and soil is returned into agricultural use immediately after technical stage.

Technical reclamation alters soil physical and chemical properties affecting soil biota environment and decreasing biodiversity of soil microorganisms and invertebrates. Microbial component of biocenoses is considered in priority under monitoring of terrestrial ecosystems. Soil microorganisms provide many ecological functions of soil and wide range of ecosystem services (FAO, 2019) essential for sustainable functioning of natural ecosystems and managed agricultural ecosystems. Soil microorganisms and invertebrates are integral edaphic components, very sensitive to changes in soil quality.

The objective of the study is to assess impacts of technical reclamation on soil biodiversity (microflora and invertebrates) and estimate its recovery rates.

Methodology

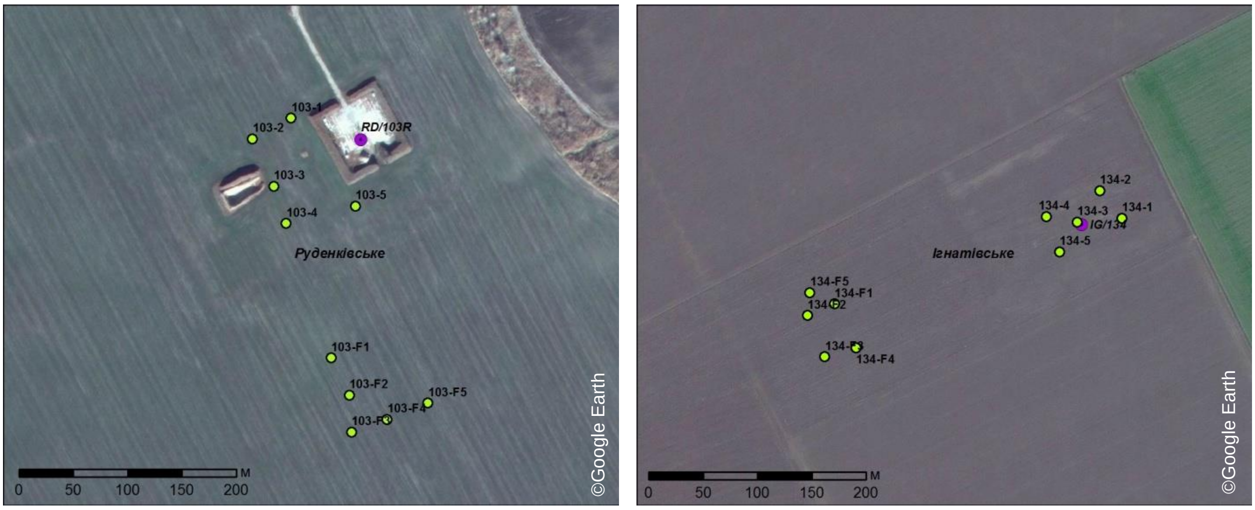
We studied reclaimed and background soils on drilling pads on Ignativka and Rudenkivka fields in southern part of Eastern oil-gas-bearing basin of Ukraine. Terms of drilling and soil reclamation were different for all studied boreholes (Table 1).

Undisturbed background soil of the study area is represented by ordinary chernozems. On each well site we sampled topsoil (0–25 cm depth) on reclaimed and background sites. Background soil was sampled at the distance of 500 m from the reclaimed plot. We take five samples from each plot. All wells and background sites were located in arable lands with the black soil (Figure 1).

Table 1: Belonging sites to the fields and the age of technical reclamation

Borehole	Terms of drilling and reclamation, years ago
Borehole 23, Ignativka field	35
Borehole 134, Ignativka field	7
Borehole 103, Rudenkivka field	3
Borehole 140, Ignativka field	Less than 1

a) borehole 23 b) borehole 140



c) borehole 103 d) borehole 134

Figure 1: Location of soil sampling points on boreholes sites

The effect of technical reclamation on the soil structures illustrated below (Figure 2).

a) borehole 140 c) borehole 103

b) the background for borehole 140 d) borehole 134

Figure 2: The appearance of the soil surface in the on the territory of well-drill sites

Analytical measurements were done by standard techniques in accredited laboratories.

Number of soil microorganisms of main eco-functional and taxonomic groups was determined by sowing dilutions of soil suspensions on dense nutrient media with fourfold repetition: each soil sample was sown on each nutrient medium in 4 parallel Petri dishes (ISO 7847: 2015).

Summary biological index (SBI) was calculated using relative values method.

Soil invertebrates were collected by soil excavation. Twenty samples (25×25×30 cm) were taken at each study (reclaimed soil) and control (undisturbed soil) plots in late April 2016. They were associated with the location of soil analysis samples: 4 excavations around one place of soil analysis. The soil was sorted out in the field; the invertebrates found in each sample were preserved separately in 75 percent alcohol or 10 percent formalin (earthworms) and identified in laboratory conditions.

Results

Soil properties

At the sites reclaimed 1 and 3 years ago, density of topsoil was slightly higher comparing to the background and reached in average 1.5 g/cm^3 and 1.4 g/cm^3 respectively, corresponding to moderate and low level of agrophysical degradation. Enrichment rates of heavy metals in mobile form were elevated in reclaimed soil resulting from alteration of soil structure and composition. However, metals concentration did not exceed the limits. Density of soils reclaimed 7 years ago was 1.1 g/cm^3 at former drilling pad and at background site. This is considered optimal for such soil type. Heavy metals concentration similarly was close at reclaimed and background site indicating the stabilization of soil condition. The oldest sites reclaimed 35 years ago had topsoil density 1.4 g/cm^{3}that corresponds to low level of agrophysical degradation. The soil has highly variable concentration of Ag, Ba, Pb, Ca, Mo, Zn derived from drilling fluids. The highest concentration of heavy metals was detected in the centre of the former drilling pad.

Number of microorganisms of main ecological-trophic groups and microbial cenosis structure

Microbiological analysis has shown certain discrepancies between studied sites with different terms of drilling and soil reclamation (Table 2).

Table 2: The number of microorganisms from main ecological-trophic and taxonomic groups (average values for 5 samples from each plot)

Sampling sites	Nitrogen assimilating bacteria, mln. CFU/g		Actinomycetes, mln. CFU/g	Fungi, th. CFU/g	Olygotrophs, mln. CFU/g	SBI, %
	organic	mineral				
Borehole 103	25.34	46.35	7.21	19.01	54.98	65
Background for 103	19.22	41.00	13.59	20.68	36.64	63
Borehole 134	37.10	42.62	11.85	53.07	41.57	78
Background for 134	23.68	42.16	9.40	35.65	23.17	61
Borehole 140	14.26	19.75	11.14	22.26	14.12	43
Background for 140	27.36	59.02	14.12	36.83	33.00	79
Borehole 23	18.98	21.23	10.73	11.87	26.40	66

Background for 23	14.27	35.28	14.35	25.61	37.60	89

Note: CFU – colony forming unit

Recently reclaimed soil (borehole 140) had much lower microorganisms number comparing to background values: organotrophic bacteria by 48 percent (1.9 times), mineral nitrogen consumers – by 58 percent (2.4 times), fungi – by 40 percent (1.7 times), oligotrophic – by 57 percent (2.3 times). Deviation rate of microorganisms in reclaimed soil corresponds to moderate level of biological degradation.

The number of microorganisms at the site reclaimed 3 years ago (borehole 103) did not differ significantly from background values except for actinomycetes (table 2). Their number was twice lower in the reclaimed soil (49 percent or 1.9 times).

Similarly, no significant difference in microorganisms' number was found on the site reclaimed 7 years ago (borehole 134).

In the soil reclaimed 35 years ago (borehole 23) microorganisms' number for almost all studied groups was significantly lower than in background soil. For instance, for mineral nitrogen consumers it was 32–47 percent lower. Such soil condition can be defined as moderate level of biological degradation.

Number and biodiversity of soil invertebrates

At the study sites invertebrates of four classes were considered. The family *Enchitraedae* was the richest in individual numbers. Invertebrate density correlated negatively with soil density ($r = 0.363$, $p = 0.0003$), the highest individual abundance being recorded from sites with the density 1.1–1.35 g/cm^3. Correlation of animal density and soil moisture was statistically insignificant ($r = 0.0578$, $p = 0.4063$).

In the first post-reclamation year, the soil was nearly empty. Even at the third year after disturbance, the invertebrate density was much lower than at the control sites (Table 3). It has recovered by the 7th year due to the high enchytraeid number. These worms multiply rapidly under favourable hydrothermal condition sand occupy vacant ecological niches in the absence of competitors. The first inhabitants of reclaimed soil are the larvae of phytofageous beetles. The lumricids recolonized the soil very slowly. Invertebrate alpha diversity was extremely low in the first years after disturbance and recovered in the seventh one (Figure 3).

Table 3: Average density of soil invertebrates (ind. /sq. m)

Groups of invertebrates	Time since reclamation		
	1 years (borehole 140)	3 years (borehole 103)	7 years (borehole 134)

	reclamed	background	reclamed	background	reclamed	background
Class Insecta: *Scarabaeidae*	0	0.8	2.4	1.6	0	2.4
Elateridae	0	0.8	0.8	7.2	0	2.4
Chrysomelidae	0	0.8	0.8	0	0	0
Class Chilopoda	0	8	0	1.6	4.0	4.0
Class Diplopoda	0	8	0	0.8	3.2	0.8
Class Olygochaeta: *Lumbricidae*	0.8	8	0	4.8	0.8	24.8
Enchytraeidae	0	94.4	0.8	86.4	89.6	51.2
Total	0.8	120.8	4.8	102.4	97.6	85.6

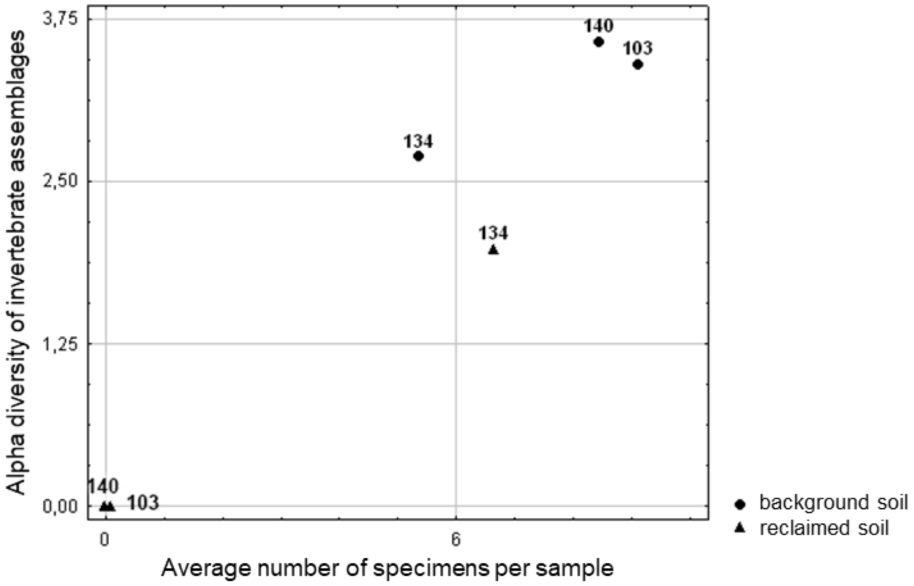


Figure 3: Biodiversity of invertebrate (Shannon index)

Discussion

Microbiological and zoological indicators of reclaimed soil condition depend on quality of soil reclamation and duration of residual effects of soil disturbance.

Even in case of proper reclamation, soil microorganisms undergo essential alteration as it was revealed on the borehole 140 reclaimed less than a year before the sampling. This is detected by double decrease in number of almost all studied microorganisms’ groups. Such effects resulted from inevitable topsoil compaction and mixing caused by heavy vehicles during the site flattening.

Though, microbial indicators of the reclaimed soil tend to rehabilitate quite soon. The same investigations at the boreholes where reclamation was done 3 and 7 years ago indicated no significant difference between reclaimed and background sites. The exception was found only for actinomycetes which number appeared to decrease at the points with relatively high concentration of heavy metals in reclaimed soil. Sensitivity of actinomycetes to heavy metals pollution and irrigation of soil with saline water has been reported in many papers

(Andreyuk *et al.,* 2001; Kolesnikov, Kaseev and Valkov, 2001; Kolesnikov *et ali,* 2013).

Drilling pad that reclaimed 35 years ago turned to have the worst conditions of microbial cenoses mainly due to high concentration of heavy metals most likely derived from drilling fluids spillage. In particular, at the most polluted soil points, the number of actinomycetes decreased up to 28–32 percent comparing to background values.

Microbiological indicators had close positive correlation with humus concentration and negatively correlated with soil compaction rate.

Certain correlation was also observed between microbiological indicators and data of invertebrate fauna distribution.

Conclusions

Technical stage of land reclamation has a detrimental effect on the soil biota. Number of soil microorganisms and invertebrate fauna has positively correlated with soil humidity and negatively with soil compaction and heavy metals concentration.

Re-colonization of disturbed soil begins in the second year by the larvae of phytophagous insects. The biodiversity recovery is slow and approaching initial values after seven years of reclamation. The most vulnerable are the earthworms. The Oligachaeta worms (*Lumbricidae, Enchitraeidae*) are recommended as indicators of the soil reclamation adequacy.

Microbiological and zoological indicators immediately react to changes of chemical and physico-chemical properties of soil and adequately reflect alterations rate. Therefore, there is a need to include them in the system of indicators for reclaimed soils monitoring.

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Urban soil biodiversity: A multi-city comparison

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Abstract summary

In an increasingly urbanized world scientific research has shifted towards the understanding of cities as unique ecosystems. In cities there is an inherent diversity of soil conditions from remnants of naturally developed to fully engineered soils. They represent a variety of habitats for soil organisms from marginal to optimal conditions. Urbanization has been considered a major cause of local species extinction and a process leading to biotic homogenization.

Soil organisms provide many important ecosystems services, but disservices, as well. Understanding the mechanisms driving structure, function and temporal changes of soil biota at multiple scales, and incorporating this knowledge to management practices is vital in maintaining multifunctional green spaces.

This presentation consists of two parts. First, I briefly summarize the current state of urban soil biodiversity and point out knowledge gaps. Then I will describe the results of a pilot study comparing urban soil properties and soil biota in five cities located on four continents. This project tested hypotheses about ecosystem convergence and biotic homogenization.

Keywords: urbanization, land use, disturbance, earthworms, microarthropods, microbial community

Introduction, scope and main objectives

Urban soils support more life than predicted solely based upon their physico-chemical properties (Joimel *et al.*, 2017). Similarly, to natural environments, our knowledge on urban soil biodiversity is hindered both by lack of effort and taxonomical knowledge, yet species level information is needed to test hypotheses such as biotic homogenization (McKinney, 2006). Additionally, a great discrepancy in research focus exists among soil invertebrate taxa. Ground beetles, ants, isopods and earthworms have received disproportionately more attention than enchytraeids, nematodes and microarthropods, or lesser studied groups, such as Protura and Diplura (McIntyre, 2000, Szlavecz *et al.,* 2018).

The Global Urban Soil Ecology and Education Network (GLUSEEN) was initiated in 2014 to study urban soil ecological systems and to engage citizen scientists. In pilot study, a multi-city comparison was conducted to explore the effects of land use on soil abiotic and biotic properties within the city and at global scale. Three questions were addressed: How does urban land use/soil habitat type affect abundance and diversity of soil biota; 2) How do soil properties affect soil community structure, and 3) How similar are urban assemblages across regions?

Methodology

Based on two disturbance and three management levels, a matrix of habitat categories with underlying soil characteristics was established (Pouyat *et al.,* 2017). In this study three soil habitat types were sampled:

1) Low disturbance–Low management (REM: remnant soil)
2) High disturbance–Medium management, (TURF: turf/lawn soil)
3) High disturbance–Low management (RUD: ruderal, massive or fill soil)

A semi-natural habitat type (REF: reference soil) outside the cities was assigned for comparison. Sites were unmanaged and represented undisturbed native soil and biome typical of the region.

Five cities, Baltimore, USA; Budapest, Hungary; Helsinki and Lahti, Finland and Potchefstroom, South Africa participated in the pilot study (Figure 3). Each habitat type was replicated five times, resulting in a total of 100 sites. Data on edaphic properties and on microorganism, arthropod, and earthworm communities have been collected.

Earthworms were sampled using hot-mustard extraction method. Microarthropods were surveyed only in Baltimore, in spring and summer 2018. Ten soil cores per site were pooled, and extracted in modified Tullgren funnels. Earthworms were identified to species level except for immature specimen. Collembola and Acari were identified to family level; all other microarthropods were identified to order.

For microbial community assessment soils were extracted following the MOBIO Laboratories Inc. Powerlyzer™ Powersoils® DNA isolation kit protocol, with some modifications (Epp Schmidt et al 2017). Q-PCR for the combined bacterial and archaeal community was performed using the primers F515 and 806R of the V4 region. Fungal community were quantified by targeting the ITS region using ITS1F and 5.8S. Amplicon sequencing targeted a section of the 16S rRNA using primers 515F+adapter and 806R+adapter and the ITS1 region in fungi using primers ITS1F+adapter and ITS2+adapter. The QIIME pipeline was used in processing sequence libraries.

Most statistical analysis of the data were carried out using SYNTAX and R packages: *vegan* (*betadisper, envfit*) *multcomp*, *MASS*, *stats and lawstat*.

Results

The earthworm survey resulted in 19 species with the highest richness (16) in Baltimore. Remnant forests and lawns supported the highest earthworm biomass. Soil properties explained neither earthworm community composition nor abundance. Evaluating all cities together, earthworm communities were significantly structured by habitat type. Communities in the two adjacent cities, Helsinki and Lahti were very similar, but Budapest clearly separated from the Finnish cities. Earthworm community structure in Baltimore overlapped with that of the other cities.

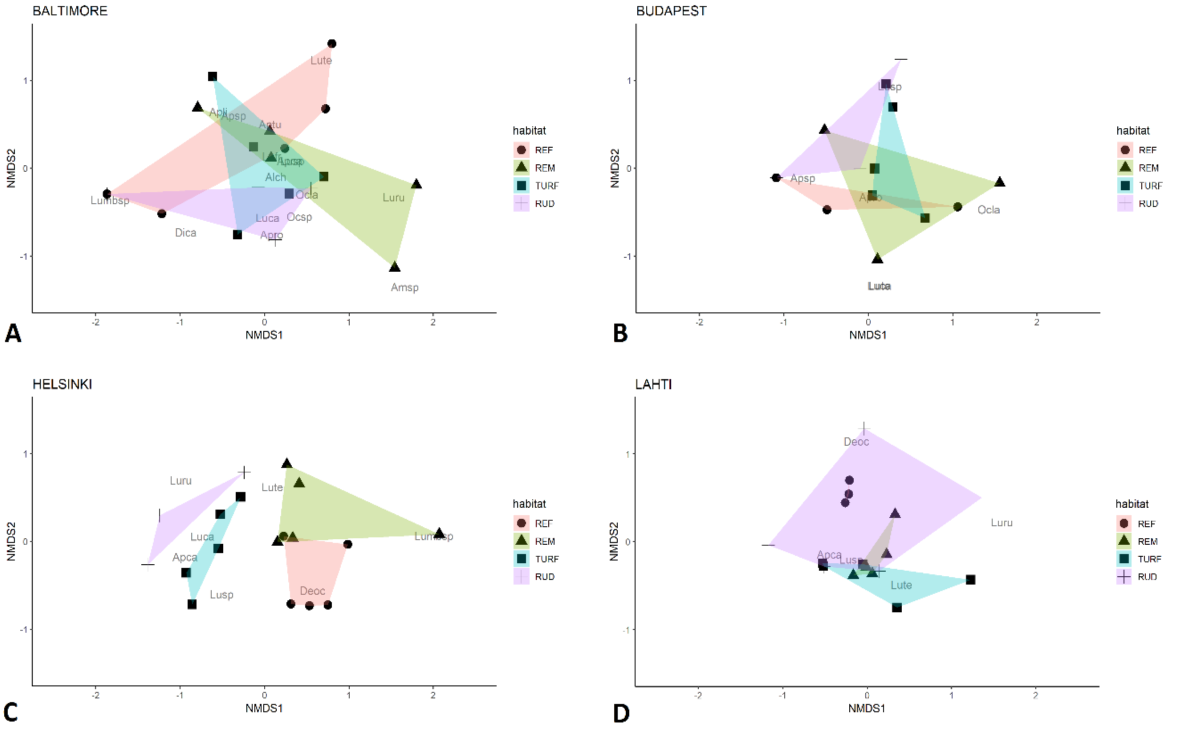

Figure 1: Nonmetric multidimensional scaling (NMDS) ordination plots based on Bray Curtis dissimilarities of earthworm communities by soil habitat types in four cities

REF: reference; REM: remnant; TURF: turfgrass; RUD: ruderal (Toth *et al.*, 2020)

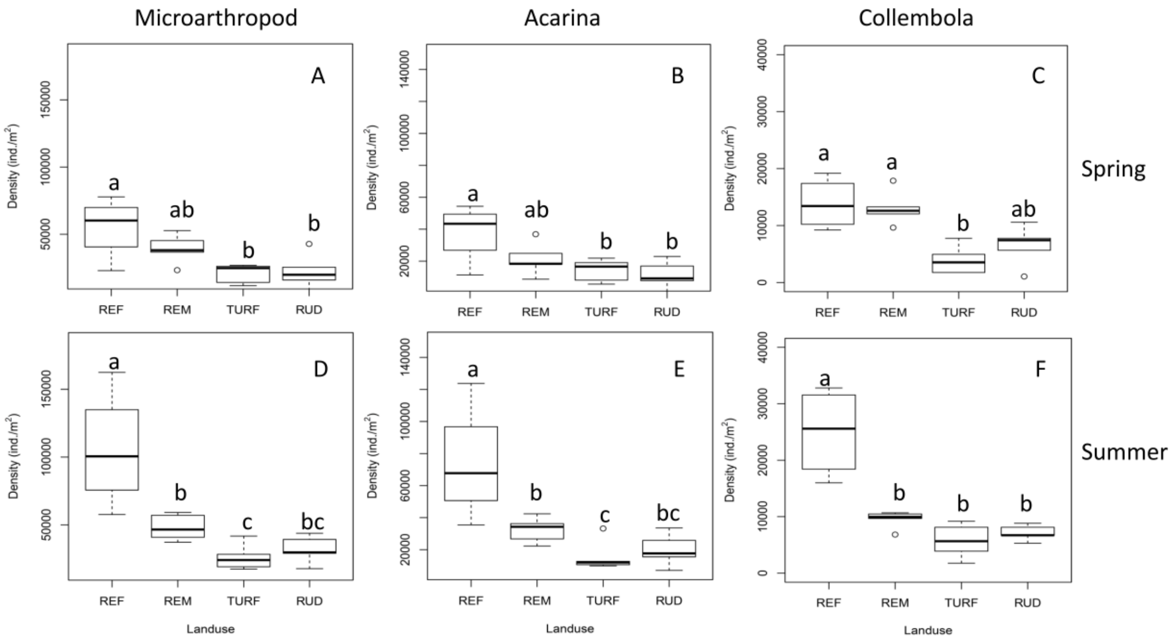

Figure 2: Abundances of microarthropod communities in four land use types in spring (A-C) and summer (D-F) in Baltimore

Different lowercase letters indicate significant differences among land use types (P < 0.05). (Huang, Yesilonis and Szlavecz, forthcoming)

A total of 78 microarthropod taxa were extracted with mites and springtails, accounting for 57.7 percent and 11.5 percent of the total richness, respectively. Soil organic carbon and bulk density had significant effects on mites (Acarina), while for springtails (Collembola), soil pH and soil moisture were significant factors.

The bacteria exhibited differentiation among cities and among land use categories. While some bacterial groups like Planctomycetes, which averaged 17 percent of bacterial sequences in Baltimore soils but made up less than 7 percent of sequences in each of the other four cities, exhibited noticeable geographic patterns (Figure3A), no single taxon alone explained the pattern of geography. Land use also imposed a filter on the bacterial community; within each city, communities in the reference and remnant sites were significantly different from turf and ruderal sites. Unlike the bacteria, turf and ruderal sites contained significantly fewer fungal ITS gene copy numbers than the reference and remnant sites. Among archaeal genera, PerMANOVA analysis shows no significant effect of city, but a significant effect of land use. Surprisingly, turf contained the most archaeal 16S rRNA gene copies; likewise, archaeal species richness was also higher in the turf sites.

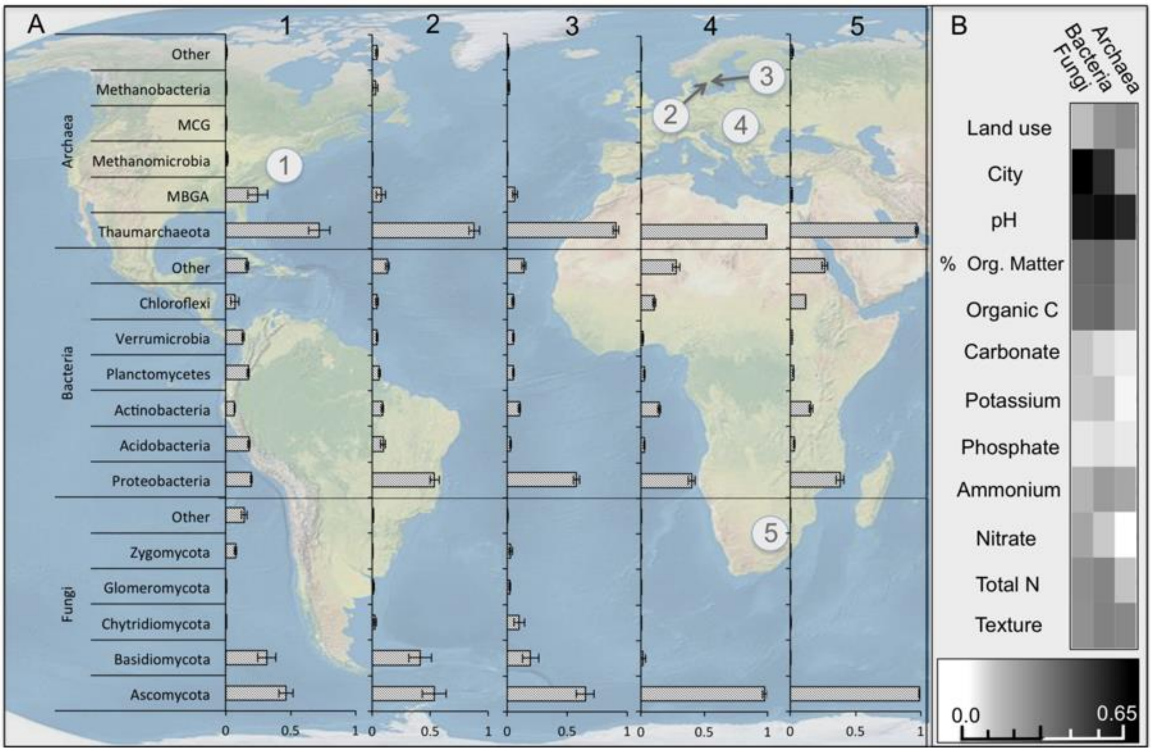

Figure 3: Panel A shows the geographic distribution of cities, and the mean (n=25) relative abundance of the most common microbial taxa averaged within each of the five cities

1) Baltimore, USA; 2) Helsinki, Finland; 3) Lahti, Finland; 4) Budapest, Hungary; 5) Potchefstroom, South Africa. Panel B shows a heat-map representing the R-squared values (0.0 – 0.65) of correlations between edaphic factors and community composition of each community (Epps Schmidt *et al.,* 2017).

Global bacterial and fungal community compositions were highly correlated with soil pH, percent organic matter, and organic C (Figure.3). Soil pH strongly correlated with Bray-Curtis distance in both bacteria and fungi, as has been previously reported. The archaeal community composition also correlated with pH but had lower correlation coefficients with percent organic matter and organic C (Figure 3B).

Discussion

Our regional comparisons have shown that although urbanization leads to changes in community composition across a wide range of taxonomic groups, each taxon has had specific response patterns to the different land use/soil habitat categories. Earthworm communities on different land uses were distinct in some cities but not in others (Figure1). Edaphic properties, such as pH and soil organic matter drove microbial and mesofauna communities but not earthworms. Soil disturbance resulted in lower taxon richness among microarthropods (Figure 2) and fungi, whereas archaea richness was actually higher on turf and ruderal sites.

Despite the clear differences in climate, soils and biogeography among the studied cities, the earthworm fauna showed remarkable similarity between the cities, but this similarity is a result of combination of different geographical and human history. Human facilitated dispersal, both at regional and local scales appears to be key in shaping urban earthworm assemblages.

Soil bacteria and fungi did not converge in cities, suggesting that human actions such as soil disturbance, alter microbial communities more than indirect effects such as increased temperature. Thus, distinct human processes define each of the high impact sites, and yet when grouped together they have lower variation than the reference and remnant sites across all three domains, suggesting that there is either a common set of organisms that is able to exploit these altered soil environments, or there is a consistent suppression of certain functional groups.

Conclusions

Urban soils should not be viewed as lifeless dirt; it is a complex medium sustaining a diverse belowground community. It is essential that we expand urban soil biodiversity for several reasons:

1) At present, most urban land conversion takes place in the developing world, where even the regional species pool is yet to be explored. It is therefore likely that species inventories will result in new species for science or new records for the region; at the same time local species extinction and colonization can be directly observed as a result of urbanization.
2) Land cover and the soil ecosystem are intimately linked, and, from a human perspective, soil biota harbors both beneficial and harmful species. Regardless, a diverse soil biota is essential to provide ecosystem services and maintain long-term soil health in the urban landscape.
3) Due to the close proximity to more than half of the global human population, urban soils have tremendous potential to inform the public about the importance of soil biodiversity and the ecosystem function.

Acknowledgements

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In vitro screening for highly effective strains of Azotobacter

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Abstract summary

This study aimed to screen strains of *Azotobacter* for *in vitro* nitrogen fixation, IAA production and phosphate solubilization. Completely Randomized Design was used with 90 *Azotobacter* isolates. High potential isolates were grown in Ashby's broth. Various *Azotobacter* isolates had significantly different N_2-fixation, IAA production and phosphate solubilization. Fixed-N was in the range of 4.0-6.5 mg/L. Four isolates had the highest nitrogen fixation, 6.5 mg/L. The PR-ND D-1 strain had the highest of IAA production, 44.87 µg/ml. Thirty-one isolates (33.3 percent) gave positive phosphate solubilization activity. Eight isolates are chosen as high-efficiency strains for further study.

Keywords: Azotobacter, nitrogen, IAA, phosphate solubilization, effective strains, local isolates, Thailand, plantations

Introduction, scope and main objectives

Many *Azotobacter* species benefit agriculture as the bacterial indigenous microbiome. *Azotobacter* can fix atmospheric nitrogen, increasing the availability of nitrogen in soil and plants (Bohlool, Ladha and Garrity, 1992). These microorganisms can also produce growth-regulating substance such as phytohormones (auxins, cytokinins, gibberellins) and vitamins which enhance plant growth. Such root microbiome increases phosphate availability through phosphate solubilization. Moreover, they produce antifungal substances that inhibit pathogenic fungi. Siderophores act as chelating agents for Fe^{+3}, suppressing plant pathogens through iron deprivation (Quispel, 1974; Holt *et al.*, 2000; Narula, 2000). According to Bashan (1998) and Mala (2003), highly effective strains of *Azotobacter* for biofertilizer production should have the following characteristics. The microbial strain should grow rapidly with large cells, have high ability for nitrogen fixation and IAA or plant hormone production. They should be pure isolates without contamination by plant pathogens with good potential for promoting plant growth. *Azotobacter* is an effective biofertilizer that can be applied in sustainable and organic agriculture in Thailand. The aim of this study was to isolate *Azotobacter* strains capable of high *in vitro* fixing nitrogen, IAA production and phosphate solubilization of ninety local isolates screening from soil rhizosphere of twenty-nine crops for further investigation.

Methodology

All *Azotobacter* isolates were screened for *in vitro* nitrogen fixation, indole acetic acid (IAA) production, phosphate solubilization and antifungal activity in a Completely Randomized Design (CRD) using 90 *Azotobacter* isolates with 2 replications.

In vitro* nitrogen fixation of *Azotobacter: A loopful of *Azotobacter* was transferred into a 125 ml Erlenmeyer flask containing of 50 ml of sterile nitrogen-free Ashby's broth, incubate at 28±2 °C at 90 rpm on a rotary shaker for a week. Then, the bacterial cultures were sterilized and nitrogen content was determined using a micro-Kjeldahl method (Sparks *et al.,* 1996).

In vitro* IAA production in *Azotobacter: Each bacterial strain was transferred into a 125 ml Erlenmeyer flask containing 25 ml of N-free Ashby's broth with 50 μg/L of tryptophan, and incubated at 28±2 °C at 150 rpm on a rotary shaker for 72 hrs. Five milliliters of cultures were transferred into the tubes and centrifuged at 3,000 rpm for 30 mins. After that, 3 ml of supernatant were taken and mixed with 2 ml of Salkowski's reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml of 35 percent perchloric acid ($HClO_4$)). After 30 mins, a pink color intensity was developed as IAA production. The absorbance of these samples was spectrophotometrically determined at 530 nm and the concentration of IAA revealed following Brick, Bostock and Silverstone *et al.* (1991).

In vitro* phosphate solubilization of *Azotobacter: *In vitro* phosphate solubilization was determined using tricalcium phosphate (Gaur, 1990). Bacteria were steaked on Pikovskaya's agar (10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g NH_4SO_4, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, trace of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract, 15 g agar/liter of distilled water), incubated for 7 days at 28±2°C. Then, clear zones on Pikovskaya's agar indicated a positive result. Data were analysed using Duncan's Multiple Range Test (DMRT).

Results

In vitro* nitrogen fixation by *Azotobacter Nitrogen fixation among *Azotobacter* isolates is presented in Table 1. Highly significant nitrogen fixation was seen. Fixed-N ranged from 4.0-6.5 mg/L. Four isolates showed 6.5 mg/L of fixed nitrogen. Other isolates showed lesser degrees of fixed-N, as depicted in Table 1.

In vitro* IAA production by *Azotobacter The IAA quantities produced by various *Azotobacter* isolates were statistically different (Table 1). IAA synthesis by these organisms ranged from 0 to 44.87 μg/ml.

In vitro* phosphate solubilization by *Azotobacter It was found that 31 of 90 isolates (34.44 percent) could dissolve tri-calcium phosphate resulting in a clear zone (positive result). The results for specific strains are presented in Table 1.

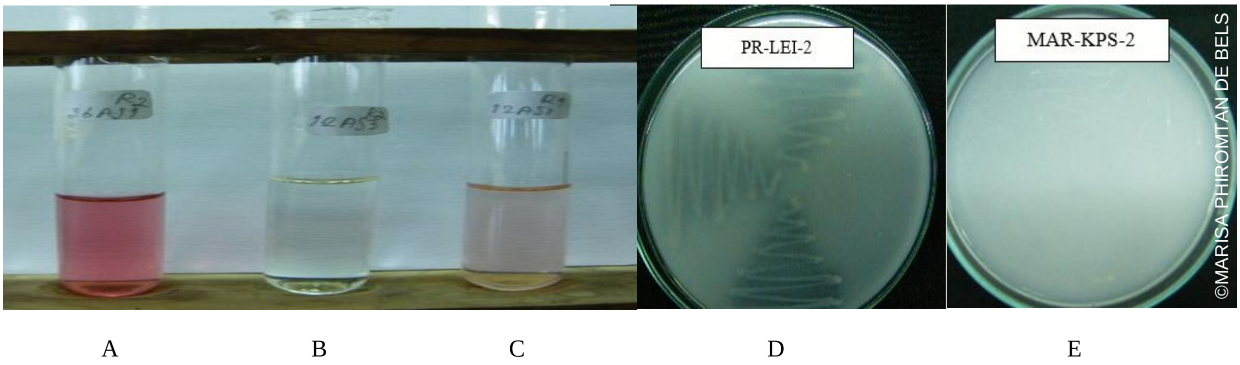

Figure 1: IAA production by *Azotobacter* cultured in Ashby's medium

Pink color (A and C) indicates IAA production, whereas (B) with no color is a negative result. D shows phosphate solubilization by *Azotobacter* (clear zone around the streak lines on Pikovskaya's medium, while E is a negative result.

Table 1: N-fixation, IAA and phosphate solubilization of *Azotobacter* spp. isolates

No.	Isolate No.	Fixed-N (mg/L)	IAA production (µg/ml)	Phosphate solubilizat
1	ROS-LRU-1	4.0d	2.72 y-A	+
2	GER-LRU-1	4.5cd	36.01 f-l	
3	GER-LRU-2	4.5cd	37.98 d-g	
4	MAR-KPS-1	4.0d	36.38 e-k	
5	MAR-KPS-2	4.0d	1.96 z-B	
6	MAR-KPS-3	4.0d	33.80 l-n	
7	JT-PR-1	4.5cd	0.60 AB	+
8	MAI-KPS-1	4.0d	38.59 c-e	+
9	PIN-NM-2	4.0d	0.58 AB	
10	PIN-NM-3	4.0d	0 B	
11	PIN-NM-5	5.0b-d	0 B	
12	GRA-KPS-1	4.5cd	0 B	
13	GRA-KPS-2	4.0d	0 B	
14	GRA-KPS-5	4.0d	0 B	
15	GRA-KPS-6	4.5cd	37.30 e-j	
16	SUG-KPS-1	5.0b-d	0.34 B	+
17	SUG-KPS-2	4.0d	2.38 z-B	
18	SUG-KPS-3	4.5cd	0 B	+
19	SUG-KPS-4	4.0d	0 B	+
20	SUG-KPS-5	5.0b-d	7.19 v	
21	SUG-KPS-6	5.5a-c	0.75 AB	
22	LIC-ML-1	5.5a-c	19.82 s	+
23	LIC-ML-2	5.0b-d	2.13 z-B	
24	LIC-ML-3	4.5cd	1.15 AB	
25	LIC-ML-4	4.5cd	35.67 g-l	
26	LIC-ML-5	4.5cd	5.97 vw	

No.	Isolate No.	Fixed-N (mg/L)	IAA production (µg/ml)	Phosphate solubilizat
27	LAN-NST-1	4.5cd	16.98 t	
28	BG-ML-1	5.0b-d	4.79 v-x	
29	BG-ML-2	5.5a-c	1.23 AB	
30	BG-ML-3	4.5cd	26.75 q	+
31	BAM-ML-1	4.0d	0 B	
32	BAM-ML-2	4.0d	0.61 AB	
33	TEA-ML-1	4.5cd	0 B	
34	TAM-NAD-1	6.5a	5.16 v-x	
35	TAM-KPS-2	5.5a-c	5.14 v-x	
36	TAM-KPS-3	5.0b-d	37.14 e-k	
37	PR-NDD-1	5.0b-d	44.865 a	+
38	PR-LEI-2	5.5a-c	0 B	+
39	PR-LEI-3	4.5cd	1.26 ab	+
40	BRO-PR-1	4.0d	0 B	
41	BRO-PR-2	4.5cd	36.29 f-k	+
42	BRO-PR-3	4.5cd	36.52 e-k	
43	BRO-PR-4	4.0d	35.30 i-m	
44	BRO-KPS-5	4.5cd	36.17 f-k	
45	BRO-KPS-6	4.5cd	41.65 b	
46	BRO-KPS-7	4.0d	40.695 b	+
47	BKL-LRU-2	4.0d	35.60 h-l	
48	BKL-LRU-3	4.5cd	1.17 AB	+
49	BKL-LRU-4	4.0d	35.78 g-l	
50	CK-NDD-1	6.5a	37.74 e-h	+
51	CK-KS-2	6.5a	35.61 h-l	
52	CK-KS-3	6.5a	36.08 f-k	
53	LET-NDD-1	6.0ab	30.64 o	
54	LET-NDD-2	5.5a-c	1.36 AB	
55	LET-NDD-3	5.0b-d	37.60 e-i	
56	CAN-NDD-1	5.0b-d	32.92 n	+
57	CAN-NDD-2	5.0b-d	34.85 k-n	+
58	CAN-ERW-3	4.0d	36.49 e-k	+
59	CAN-ERW-4	4.5cd	41.165 b	+
60	CAB-KPS-1	5.0b-d	1.13 AB	
61	CAB-KPS-2	5.0b-d	11.33 u	
62	CAB-KPS-3	4.5cd	0.63 AB	
63	CAB-KPS-5	4.5cd	1.87 z-B	
64	CHI-KK-1	4.5cd	39.92 b-d	+
65	CHI-KK-2	5.5a-c	33.23 mn	
66	CHI-KK-3	5.0b-d	0	+

No.	Isolate No.	Fixed-N (mg/L)	IAA production (µg/ml)	Phosphate solubilizat
67	CHI-KS-4	5.5a-c	1.09 AB	+
68	COR-NDD-1	4.5cd	2.92 y-A	+
69	COR-NDD-3	4.5cd	0 B	+
70	COR-NDD-4	5.0b-d	3.81 x-z	+
71	COR-NDD-5	4.5cd	0 B	
72	COR-ERW-6	4.5cd	40.225 bc	+
73	WC-KS-1	5.0b-d	30.28 o	+
74	SB-KS-1	4.0d	0 B	
75	SB-KS-2	4.5cd	26.48 q	
76	SB-KS-3	4.0d	35.46 h-l	
77	SB-KS-4	4.5cd	23.42 r	
78	IP-TMK-1	4.5cd	12.56 u	+
79	IP-TMK-2	4.0d	35.20 j-m	
80	IP-TMK-3	4.5cd	38.32 c-f	+
81	IP-TMK-4	4.0d	29.16 op	+
82	IP-TMK-6	4.5cd	28.23 op	
83	BM-TMK-1	4.0d	37.42 e-j	
84	BM-TMK-2	4.0d	2.88 y-A	
85	BM-TMK-3	4.5cd	37.14 e-k	
86	BM-TMK-4	4.0d	35.86 g-l	
87	BIT-TMK-1	4.5cd	11.64 u	+
88	BIT-KS-2	4.5cd	0.08 B	
89	CIT-TMK-1	4.0d	1.83 z-B	
90	CIT-TMK-2	4.0d	0 B	
		4.62	17.70	
		**	**	
		14.95	5.64	

Remark: Mean values in each column followed by the same letter are not significantly different at p≤.05 (a-z-AB)

Discussion

Azotobacter isolates showed various nitrogen fixation capacities depending on their species and plant origins as previously reported (Holt *et al.*, 2000; Narula, 2000; Ahmad *et al.*, 2008). Some strains showed high degrees of several beneficial effects in the laboratory. Of the isolates, 31.11 percent showed higher fixed-N than the mean, i.e., 13 isolates had N-fixing ability of 5.5-6.5 mgN/L. IAA production of all isolates was similar to nitrogen fixation depending on type of bacteria (Arshad and Frankenberger, 1993; Arshad and Frankenberger, 1997). Previous studies reported that 82.32 percent of *Azotobacter* spp. can synthesize auxins. Among IAA producing isolates, 5.55 percent synthesized more than 40 μg/ml, whereas most bacteria (34.44 percent) produced IAA in the range of 30-39 μg/ml, which is higher than reported by Ahmad, Ahmad and Khan (2008). Martinez-Teledo *et al.* (2000) found that *A. chrooccoccum, A. bejerinckii* and *A. vinelandii* produced phytohormones associated with plant root exudates having interactions between bacteria and maize root (Kumar, Amaresan and Bhagat *et al.*, 2011) similar to the findings of the current study. Various native isolates from the same plants and soil expressed different activities. The isolates from tree soils had larger clear zones around the colonies than those of other crops.

Conclusions

Ninety *Azotobacter* spp. isolates were demonstrated significant traits based on bacterial genetics, plant genotype and plant-microbe interactions. Eight selected isolates (8.89 percent) expressed higher in N_2-fixation, IAA production, and phosphate solubilization and fast-growing, which require more research in the future to explore their potential enhancing plant growth.

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Good agricultural practices help to restore sustainable biodiversity

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Abstract summary

At present time when climate change has negative effect on soil moisture content and can decrease signifitanly the productivity have good agricultural practises high importance via their direct influence on soil properties and biodiversity. Objectives of this study have been focused on assessment of good agricultural practises like minimum till, mulch, no-till and biofarm type of cultivation on soil. Our study shows positive effect of these practises on soil moisture content, biodiversity and soil structure stability. These findings can be used for further studies determining the ways of soil use in sustainable way.

Keywords: good agricultural practices, soil biodiversity, earthworms, microorganisms, soil enzymes, biofarm

Introduction, scope and main objectives

Soil as a complex system is an important part of environment. It acts as a reactor where many different processes between organic and inorganic phases occur. Soil biodiversity representing the variety of living organisms belowground is important soil health indicator. In general, soil biodiversity is directly influencing the main soil property, which is soil fertility. The higher the biodiversity, the better the soil fertility. Conservation and/or increasing soil fertility is extremely important because soil is a key element of the agroecosystem. Its biological activity, which is related to the processes occurring therein, can be greatly affected by anthropogenic interventions. Many biochemical reactions in this environment are dependent or influenced by the presence of soil enzymes (Šarapatka, 1996). The soil enzymatic activity reflects the activity of microorganisms, controls the release of plant nutrients and the growth of microorganisms (Kovácsová, 2011). Enzymes can be used as indicators of soil quality (García and Hernández, 2000; Liu, Lai and Helen, 2002; Mikanová, Friedlová and Šimon, 2009; Mijangos and Garbisu, 2010; Šíša, 1993). Soil sustainability can be evaluated using enzyme activities (Senwo *et al.*, 2007) and can give also direct information concerning soil biodiversity. Higher organisms like earthworms represent also soil biodiversity indicators. They react very sensitively to soil degradation or sanitation. Cultivation technologies which lead to the increase of soil microbial activity and occurrence of earthworms can be considered as good agricultural practices. Main reasons for cultivation technologies development can be divided to the economical, ecological and technological. Economic reasons evaluate mainly savings of work and energy, reducing workload, lowering staffing and higher machine performance. Ecological reasons are complex and they are focused on climate change mitigation, soil structure restoration and soil degradation prevention and/or sanation (Houšková *et al.*, 2019). They contribute directly to soil biodiversity conservation and/or improvement.

Methodology

Soil properties and biodiversity have been studied on two farms. One is biological farm Agrokruh (Figure 1) where soil cultivation is provided in spirals and individual fields have circle shape. Such cultivation is based on low soil surface disintegration without turning the soil layers and without heavy machinery and artificial fertilizers. It is realized by a rotating arm fixed in the middle of the field. Soil cultivating tool is mounted on a chain and is guided along the arm. It is possible to fix interchangeable tools that can serve as a spade, rotary tiller, and seeder or for drip irrigation. The circular fields are located side by side and one working arm can be easily moved to an adjacent field. On this farm vegetables are cultivated. Soil structure – stability of soil aggregates in water (according Baksajev) using was studied together with earthworms presence (number) and status (size and weight). Comparison was field cultivated in conventional way. Soil structure was evaluated as agronomically valuable soil structure (aggregates between 0.5 – 3mm, in percent) and as a coefficient of soil structure (*Cst*) according to the formula: $Cst = a/(b+c)$

where:

a – aggregates between 0,25–7mm

b - aggregates >7mm

c – aggregates <0,25mm

Second place is experimental farm Borovce (Figure 2) where different cultivation practises have been applied: conventional cultivation, minimum tillage, mulch and no-till with crop rotation: corn maize - winter wheat – spring barley – soya been. Influences of these types of cultivation on soil microbial activity and water content in soil profile have been studied. Soil microbial activity was expressed as production of CO_2 in soil and dehydrogenasis activity. Soil dehydrogenasis was measured according to the method described by Lenhard and determined colorimetrically on a T60 UV / Visable. Soil moisture was evaluated to the depth of 1 m of soil profile by gravimetric method.

Figure 1: Biofarm Agrokruh

Figure 2: Experimental farm, Borovce

Results

Biofarm Agrokruh

Coefficient of soil structure is higher in the soil from Agrokruh in the whole profile in comparison with soil with traditional cultivation. In varies according to the depth; from 1,8 for Agrokruh in the depth 0-20 cm, 2,3 in 20 – 40 cm to 5 in the depth 40-60 cm. For conventional cultivation it is: 1.6 in the depth 0-20 cm, 0,9 in 20-40 cm to the 1,7 in the depth of 40-60 cm. Amount of agronomically valuable structure was the highest on biofarm and varies from 42 percent in the depth 0-20 cm, 55 percent in 20-40 cm to 69 percent in the depth 40-60 cm. In comparison the traditional cultivation shows 40 percent in the depth of 0-20 cm, 31 percent in 20-40 cm and 47 percent in the depth of 40-60 cm. Amount of microaggregates (<0.25mm) shows that soil with traditional cultivation has the highest portion of such aggregates in the depth from 20 to 40 cm. All these results can be evaluated as lower aggregates stability which is direct effect of ploughing in comparison with the soil on biofarm which is cultivated without ploughing.

The earthworms density in soil monoliths from ecological farm recalculated per square meter shows that the amount of individuals was (average 2017- 2018 year) 249 – 246 - 7 for 5 years ecological farming, 3 years ecological farming and conventional farming respectively.

Concerning biomass, these results show significant positive effect of ecological farming on the amount of earthworms in comparison to conventional cultivation. The biomass was the highest in 3 years ecological farming comparing with 5 years. The biomass in conventional cultivation field was again significantly lower in comparison to the ecological fields.

Table 1: Earthworms biomass in soil on biofarm and conventional cultivation practice ($g.m^{-2}$ of soil), Agrokruh

Earthworms biomass in $g.m^{-2}$	2017 year	2018 year	average
ecological farming (5 years)	44,69	66,59	55,64
ecological farming (3 years)	37,34	102,68	70,01
conventional farming	10,61	0	5,305

Experimental farm Borovce

Microbial activity expressed as CO_2 productivity and dehydrogenase activity shows positive effect of soil saving technologies – good agricultural practices on its development (Table 2 and 3).

Table 2: CO_2 productivity in soil with different cultivation practice ($mg.100\ g^{-1}$ of soil), Borovce

average 2016 - 2018	Depth (cm)	Conventional cultivation	Minimum technology	Mulch technology	No-till
Spring sampling	0 - 10	3.32	5.44	7.36	8.55
	10- 30	3.67	2.59	2.81	4.21
	average	3.50	4.02	5.09	6.38
Autumn sampling	0 - 10	3.11	5.10	5.92	8.95
	10-30	3.35	2.97	3.49	4.40
	average	3.23	4.04	4.71	6.67

Table 3: Dehydrogenase activity (%) in soil, Borovce

Average 2016 - 2018	Depth (cm)	Conventional cultivation	Minimum technology	Mulch technology	No - till	**Average**
Spring sampling	0 - 10	69.6	96.9	107.0	126.5	**100.0**
	10-30	97.0	114.4	94.6	93.9	**100.0**
	average	83.3	105.7	100.8	110.2	**100.0**
Autumn sampling	0 - 10	77.5	100.3	102.2	120.1	**100.0**
	10-30	94.1	98.6	103.4	104.0	**100.0**

	average	85.8	99.4	102.8	112.1	**100.0**

Humus content increased in soils with soil saving treatment (Table 4) and confirmed their positive effect.

Table 4: Humus content (%) in soil, Borovce

Average 2016 - 2018	Depth (cm)	Conventional cultivation	Minimum technology	Mulch technology	No - till	**average**
Spring sampling	0 - 10	1.71	2.46	2.30	2.62	**2.27**
	10-30	1.74	1.85	1.81	1.76	**1.79**
	average	**1.72**	**2.15**	**2.06**	**2.19**	**2.03**
Autumn sampling	0 - 10	1.77	2.41	2.13	2.47	**2.19**
	10-30	1.77	1.86	1.81	1.74	**1.79**
	average	**1.77**	**2.13**	**1.97**	**2.10**	**1.99**

Increase of soil moisture content was the highest in mulch type of cultivation (Table 5).

Table 5: Effect of technology and depth on average soil moisture (%) in 2016-2018, Borovce

Technology	Depth (cm)								
	0,0 - 10	10 - 20	20 - 30	30 - 40	40 - 50	50 - 60	60 - 70	70 - 80	**average**
Conventional	16.57	16.11	16.05	15.71	15.44	15.18	14.80	14.29	**15.52**
Minimum	17.78	16.73	16.32	16.25	16.50	16.42	16.04	15.44	**16.44**
Mulch	17.93	16.80	16.95	17.17	16.86	16.36	15.80	15.19	**16.63**
No-till	17.53	16.95	16.63	16.50	16.17	15.73	15.26	14.66	**16.18**

Discussion

Cultivation of soil without ploughing can have several positive effects. It influences soil aggregates stability and biodiversity - reflected as development of rich earthworms population. All these influences are complex processes including soil physical, chemical and biological regimes.

Dehydrogenase activity (DHA) is influenced by the presence of readily degradable organic substances. It reflects the level of microbiological settlement of the soil as well as the supply of soil with organic matter. It is part of the metabolism of all microorganisms. It can be used as a general indicator of soil biological activity. DHA together with CO_2 production in soil shows positive effect of soil saving cultivation technologies.

Increase of stable humus content is also complex, time demanding process. Soil as CO_2 sink has important role in climate change mitigation.

Conclusions

Good agricultural practices represented by minimum tillage, much, no-till and biological farm type of cultivation have influence on soil properties restoration, improvement, and conservation. At present time when climate change has increasing effect, conservation of moisture in soil profile is very important and contributes directly to the sustainable use of natural resources. Soil biodiversity is crucial for soil properties improvement and good agricultural practices contribute directly or indirectly to the increase of soil biodiversity. Improvement of soil structure expressed as soil aggregates stability is direct evidence of good agricultural practices on soil sustainability.

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Earthworm abundance under dominant tree species affects soil aggregation and aggregate-associated C along a soil degradation gradient

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Abstract summary

Trees are known to play a critical role as ‘resource islands’ in agricultural landscapes, especially in soils most limited in nutrients and water. However, there is limited understanding of how tree attributes interact with soil biological activity that affects soil C storage. This study assessed the spatial influence of three dominant trees namely, *Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gilletii*, on soil aggregation and aggregate-associated C content along a soil degradation gradient resulting from continuous cultivation for 10, 16 and 62 years as affected by presence of soil macrofauna. Greater abundance of small macroaggregates and microaggregates were recorded in soils under the canopy of *Z. gilletii* with an average weight of 62.8 g and 9.4 g 100 g^{-1} soil compared to 53.9 g and 3.1 g 100 g^{-1} in soils under *C. megalocarpus* and 48.7 g and 3.9 g 100 g^{-1} of soils under *E. grandis*, respectively. These differences could be attributed to the high number of endogeic earthworm species, *Nematogenia lacuum* (Ocnerodrilidae) in soils under the canopy of *Z. gilletii* trees and reported decompacting activity and production of small faecal pellets that may have contributed to the fragmentation of large macroaggregates into small macroaggregates and microaggregates. The C content decreased by almost 40 percent in soils under longer duration of cultivation, with higher magnitude of differences associated with *Z. gilletii* trees. This study shows the significance of specific trees in shaping soil macrofauna communities influencing soil aggregation and soil C content which could have far-reaching implications for the long-term C storage in the soil and hence net contributions to climate change mitigation.

Keywords: Agroforestry, Nematogenia lacuum, Soil biodiversity, Spatial variation, Zanthoxylum gilletii

Introduction

Soil biota is a central constituent of any ecosystem, whether natural or managed, due to their role in regulating key soil functions such as organic matter decomposition, nutrient cycling and soil structure maintenance (Barrios, 2007). Soil macrofauna constitute an important component of soil biota given the significant impact of their activities on soil properties (Lavelle, 1997). Earthworms and termites, for example, have earned recognition as 'ecosystem engineers' due to their significant effects on soil structure and functions through their soil-feeding, nesting and burrowing habits (Jones, Lawton and Shachak, 1994). However, their activities could be affected by management practices largely through changes in organic inputs to soil which affect food availability, and through soil disturbance (e.g. tillage) which often kill the larger species (e.g. earthworms) or the structures they inhabit and interfere with their activities (Ayuke *et al.*, 2011; Mbau, Karanja and Ayuke, 2015). Furthermore, these management practices can also contribute to the spatial heterogeneity in soil properties which underlies the distribution of soil macrofauna. Consequently, soil macrofauna are usually not uniformly distributed within the soil in any given space and time, but rather, aggregated in 'hotspots' of carbon-rich areas such as the rhizosphere and organic detritus (Beare *et al.*, 1995; Kuzyakov and Blagodatskaya, 2015).

This study assessed spatial influence of three dominant trees (*Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gilletii*) on soil aggregation and aggregate-associated C content across three catchments that represent a soil degradation gradient resulting from different times since conversion from primary forest to agriculture. This provided a chronosequence experimental set-up where short/medium term effects of tree species and long-term effects of land-use change could be systematically studied.

Methodology

The study site is located in Kapchorwa, Nandi County in several farms along the Kakamega-Nandi Forest complex and the soils are classified as kaolinitic Acrisols. Detailed description of these catchments can be found in Recha *et al.* (2013) and Güereña *et al.* (2015). Selection of tree species of interest was conducted using participatory action research tools in the context of focus group discussions involving randomly selected farmers from all the three catchments (Barrios, Coutinho and Medeiros 2012). A ranked list of the most common trees within the area was created and the top three most abundant trees were selected, namely, *Croton megalocarpus, Eucalyptus grandis* and *Zanthoxylum gilletii*. Selection of trees to be sampled within the three catchments was based on dominance, distribution, tree attributes, and farm management practices.

Water-stable aggregates were determined using wet sieving method described by Elliott (1986). The soil samples were separated into four water-stable aggregate size classes: large macroaggregates (>2000 µm; "LM"), small macroaggregates (250-2000 µm; "SM"), microaggregates (53-250 µm; "m") and silt and clay sized aggregates (<53 µm; "s+c"). a portion of the small and large macroaggregates was dawn and combined into one sample (TM), to be used for further fractionation of the macroaggregates as described by Six *et al.* (2002). This second process resulted in three aggregates: coarse particulate organic matter and sand (>250 µm; "cPOM"), microaggregates-within-macroaggregates (53-250 µm; "mM") and silt and clay sized fraction within macroaggregates

(<53 µm; “s+cM”). The C content in all the aggregate fractions as well as whole soil (before fractionation process) was analysed.

Linear mixed-effect models were used to test the effects of duration of cultivation and tree species on soil aggregation and aggregate-associated C.

Results

Tree species had the greatest influence on soil aggregates weight and spatial distribution (Figure 1). The average weight of LM fraction was about 50 percent higher in soils under the canopy of *C. megalocarpus* and *E. grandis* trees than under *Z. gilletii* trees. In contrast, average weight of SM in soils under the canopy of *Z. gilletii* (62.8 g 100 g^{-1}) was about 15 percent and 30 percent higher than under *C. megalocarpus* and *E. grandis* trees, respectively. Similarly higher microaggregates weight was also observed in soils under *Z. gilletii* trees, with an average weight of 9.4 g 100 g^{-1} soil compared to 3.9 g 100 g^{-1} in soils under *E. grandis* and 3.1 g 100 g^{-1} in soils under *C. megalocarpus*. This was about 3 times higher microaggregates weight under the canopy of *Z. gilletii* than under the other two trees. Notably, the aggregate fractions increased with duration of cultivation.

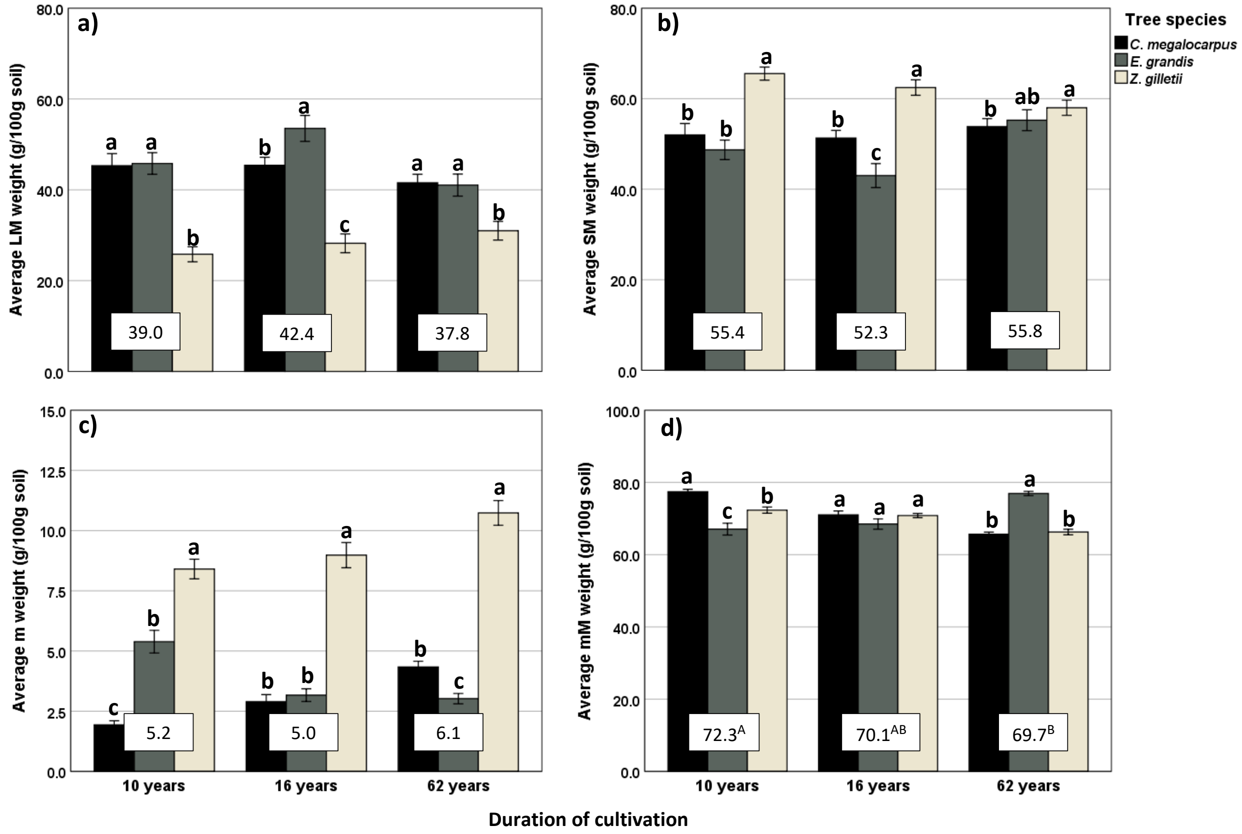

Figure 1: Soil aggregates weight distribution as affected by duration of cultivation and tree species (means and SE)

(a) LM = large macroaggregates (> 2000 µm), (b) SM = small macroaggregates (250-2000 µm), (c) m = microaggregates (53–250 µm), (d) mM = microaggregates-within-

macroaggregates (53–250 µm). Values in text boxes represent means of duration of cultivation. Bars indicate differences between the three tree species for a specific duration of cultivation.

Generally, C content of whole soil and soil aggregates fractions declined with increasing duration of cultivation (Figure 2). For instance, C content in TM was higher in soils after 10 years of cultivation (60.0 mg g-1) compared to 16 years (36.8 mg g-1) and 62 years (36.6 mg g-1), and this trend was similar to that of WS. Similarly, C content in microaggregates decreased with increasing duration of cultivation from 3.2 mg g-1 after 10 years to 1.9 mg and 2.5 mg g-1 after 16 and 62 years of cultivation, respectively. On average, this was close to 40 percent decline in C. Duration of cultivation also significantly influenced C content of mM, with C decreasing from 48.7 mg g-1 in soils after 10 years to 29.9 mg g-1 after 62 years of cultivation, which was more than 60 percent decline. Tree species influence on C content in was dependent on duration.

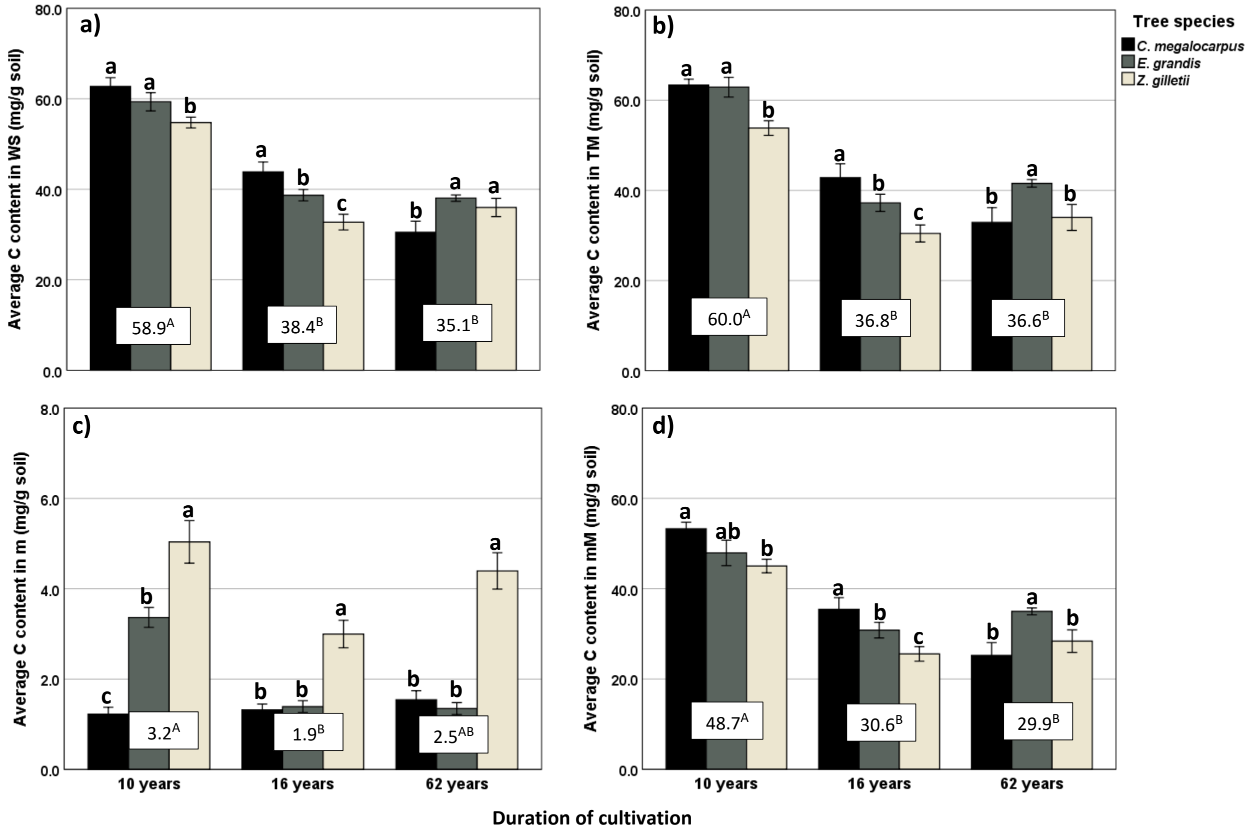

Figure 2: Distribution of whole soil and aggregate-associated C as affected by duration of cultivation and tree species (means and SE)

(a) WS = whole soil, (b) TM = total macroaggregates (>250 µm), (c) m = microaggregates (53–250 µm), (d) mM = microaggregates-within-macroaggregates (53–250 µm). Values in text boxes represent means of duration of cultivation. Bars indicate differences between tree species for a specific duration of cultivation.

Discussion

Conversion of forests to cultivated land negatively affects soil structure due to alteration in the amount and quality of litter input, litter decomposition rates and the processes of organic matter stabilization in soils. Such changes could be linked to increased tillage operations accompanied by removal of crop residues. Generally, decomposition rates under the trees can be enhanced due to the presence of higher quality organic matter and thus, the abundance of transient (e.g., microbially-derived polysaccharides) and temporary (e.g., fungal hyphae) organic binding agents which enhance soil macroaggregation. Besides litter and root inputs, trees intercept significant amount of incident solar radiation depending with a consequent reduction in soil temperature (Belsky *et al.,* 1989; Vandenbeldt and Williams 1992). This decreases the rate of evapotranspiration and hence a reduction of extreme moisture fluctuations which favours proliferation of soil biota. For instance, the number earthworms obtained in soils under the three tree species differed significantly, with higher abundance under Z. gilletii trees than C. megalocarpus and E. grandis reported in a previous study (Kamau *et al.,* 2017). Earthworms, especially endogeic species have been shown to play a critical role in initiating the process of soil aggregate formation (Zhang and Schrader, 1993; Shipitalo and Le Bayon, 2004). We could thus attribute the observed trends to the dominance of endogeic earthworm species, Nematogenia lacuum (Ocnerodrilidae). Previous studies have suggested mechanisms which may explain the observed trends. An early study by Shipitalo and Protz (1989), for instance, reported that pre-existing soil aggregates are disrupted during their passage through the earthworms' gut to form nuclei for new microaggregates. Blanchart *et al.* (1999), on the other hand, reported that smaller decompacting earthworm species such as Eudrilidae feed on casts from larger species to form smaller, delicate casts. Given that N. lacuum is a small earthworm species (40 to 55 mm long), we could infer that this species may have contributed to fragmentation of large macroaggregates into small macroaggregates and microaggregates in soils under the canopy of Z. gilletii, where high numbers of this earthworm species were found.

Fragmentation of large macroaggregates to small macroaggregates and microaggregates could cause a reduction in C content in the newly formed aggregates, which could explain the observed decrease in C content with increasing number of N. lacuum especially under the Z. gilletii tree. Freshly deposited casts are known to contain substantial amounts of partially digested organic matter, thus providing substrates for proliferation of microorganisms. Such increase in microbial population may increase C mineralisation rates and thus a reduction in overall C content over time. For instance, Alban and Berry (1994) reported an average decrease of about 0.6 Mg C ha-1 year-1 up to a depth of 0.5 m over a 14-year period after introduction of endogeic (Aporrectodea tuberculata) and epi-endogeic (Lumbricus rubellus) earthworm species in a deciduous forest. This demonstrates how dominance of a single macrofauna species, resulting from soil management decisions, could significantly affect soil C dynamics. However, as Brown, Barois and Lavelle (2000) noted, it is unlikely that these losses continue indefinitely, but rather until a new equilibrium is attained once the earthworm population stabilizes. Our results shows that the choice of tree species in an agroforestry system can significantly influence the amount of SOC either directly or indirectly through the mediation of soil biota, and this could have far-reaching implications for long-term soil C storage.

Conclusions

There was significant positive effect of specific tree species on soil macrofauna abundance, especially that of endogeic earthworm species Nematogenia lacuum. The decline in aggregate-associated C along the chronosequence could partly be stimulated by this earthworm species, demonstrating how dominance of a single macrofauna species, could significantly affect soil health. Thus, our study suggests that selection of tree species for an agroforestry system can be critical in shaping the soil aggregation process and soil C accrual which could have far-reaching implications for long-term C storage in the soil. More research is needed to guide the strategic management of tree attributes to positively influence soil biological activity underpinning soil C storage.

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Theme 3.

Soil biodiversity shaping the future of food systems

Keep soil alive, protect soil biodiversity

Soil biodiversity in action: ecological intensification of soil processes for agrosystem services in the tropics

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Abstract summary

With the development of agroecology, soil plays a particularly important role in the design of sustainable agricultural practices. The soils are the place of many processes operated by living organisms interacting with one another. Soil biodiversity performs various processes which determine the main aggregated functions and finally agrosystem services and sustainability. The understanding of these relationships is particularly important in a global context where biodiversity, including the soil one, is highly threatened. It becomes urgent to promote these ecological processes, to intensify them by appropriate practices considering the socio-economic constraints, and finally, to be able to measure them. Revisiting the ecological theories of the terrestrial ecosystem functioning is a need to improve our consideration of the soil in the agroecological transition. This theoretical approach is illustrated by four studies conducted in the tropics demonstrating the possibility to intensify the soil ecological processes and to solve agronomic dysfunctions. Finally, we highlight the need to define soil indicators based on ecological processes for an appropriate measurement of this intensification and we propose a methodological framework to optimize soil ecological functions for a sustainable supply of agrosystem services.

Keywords: Ecological intensification, agrosystem services, co-construction, indicators of functioning, soil biodiversity, sustainability

Introduction, scope and main objectives

The transition from intensive agriculture to more environmentally friendly agroecological practices that better reflect the expectations of sustainability and development has been widely documented. In these agroecological dynamics, soil occupies a very special place, in agreement with the numerous ecosystem services (plant production, pathogen regulation, carbon storage, climate regulation, etc.) it provides (Keesstra *et al.,* 2016). Many studies stress the need to promote soil biodiversity in cultivated systems, to assume their ecological complexity and to rely on ecological processes (Brussaard *et al.,* 2007). This ecological intensification is defined as an alternative approach to conventional one, with the objective to increase agrosystem services while minimizing negative impacts on the environment (Bommarco, Kleijn and Potts, 2013). In the current context of the sixth massive biodiversity extinction crisis, characterizing, understanding and optimizing soil biological functions within agrosystems is a urgent necessity (Dirzo *et al.,* 2014).

The ecologist E.P. Odum published in 1969 the strategy of terrestrial ecosystem development. In this paper, he described the development of ecosystem properties along ecological successions and highlighted the conflict between the 'maximum protection' and the 'maximum production'. Briefly, the pioneer stages quickly give way to the most productive, early, and transitory stages, consisting of fast-growing plant species, then to low-productivity mature stages composed of slow-growing species. During these successions, soil mineral fertility, organic matter content, the taxonomic and functional composition of edaphic biodiversity are also changing, the interactions between the organisms are accentuated and the closure of the biogeochemical cycles is reinforced. Similarly, the food web becomes more complex with the increased presence of heterotrophic organisms, the average size of organisms increases, the biochemical complexity of organic matter increases, the energetic fungal path becomes dominant and the carbon uptake efficiency increases (Morriën *et al.,* 2017). Ecological rules thus impose an opposition between a high transitory productivity and the dynamic stability of natural ecosystems. Maintaining highly productive terrestrial ecosystems means fighting against successional ecological mechanisms and requires a lot of energy. During the last decades, this energy was provided by tillage, mineral and organic fertilization, pesticides and herbicides. This is ecologically contrary to sustainability, and this justified the development of agroecology and the need for ecological intensification of soil processes (EISP). EISP aims to maximize agrosystem services provided by soil biodiversity while conferring better ecological resilience and sustainability (Yachi and Loreau, 1999).

Here we propose a methodology of soil ecological intensification based on ecological theories with the aim to deliver agrosystem services. Different results from past and ongoing projects in the tropics will be given to validate this approach.

Methodology

We propose a procedure for intensifying ecological soil processes to increase both productivity and sustainability (Figure 1):

(1) a local diagnosis of dysfunctions. This approach requires local action both in the diagnosis and understanding of soil ecological processes and in the deployment of appropriate tools. Indeed, the finalized outcome is contextual and does not tend towards a generality of the practice(s). Local site-specific knowledge is crucial.

(2) a detailed and integrative understanding of soil ecological processes involved in ecosystem functions and related to the dysfunction(s) diagnosed. As ecosystem functions, we refer here to the four aggregated functions proposed by Kibblewhite *et al.*, 2008,

i.e., carbon transformations, nutrient cycling, soil structure maintenance, biological population regulation. These functions aggregate different processes and are at the base of agrosystem services.

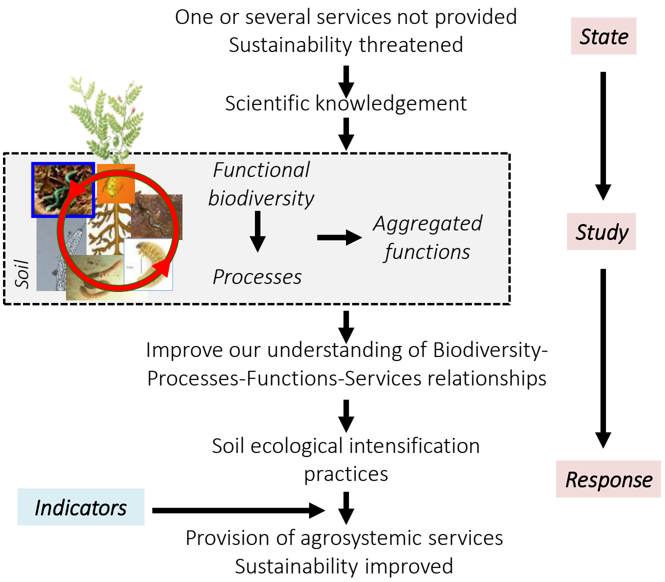

Figure 1: Diagram illustrating the methodological approach allowing to ensure the optimization of soil ecological functions for the sustainable provision of ecosystem services

(3) a co-construction of agricultural practices allowing EISP. The gain of scientific knowledge on the functioning of the soil crossed with the local socio-economic and agronomic constraints is exploited to co-construct innovative alternative agricultural practices. Soil ecological intensification practices are not exclusive of other agroecological practices and require advanced engineering combining several disciplines.

(4) finally, indicators of ecological soil intensification are deployed to measure the effect of practices on long-term ecological processes of the soil. There is a still a huge work to develop relevant indicators of soil processes and a great need of studies to quantify accurately chemical fluxes, to understand biodiversity-processes-functions-services relationships and their drivers, to understand interactions within ecological networks

Results

As results we present here successful case-studies of ecological intensification of soil processes from the tropics. As said above, a non-ecological intensified agricultural use of soils can be faced with one or different dysfunctions, e.g. low fertility, erosion susceptibility, loss of organic matter and/or biodiversity, etc. At some sites, scientists with users developed new, co-constructed, sustainable practices based on a deep knowledge of soil ecological processes.

1- A story of erosion, roots and organic matter. Vertisols from southern Martinique (West Indies) are very prone to erosion due to a high content in sodium. Maintaining high organic matter contents in these soils is the only way to reduce erosion. Scientific studies showed that grass roots such as in pastures have the ability to inject large amounts of carbon in soils and to control erosion (Blanchart *et al.,* 2004).

2- A story of pearl millet cultivars, rhizobacteria and soil aggregation. Soil structural formation is an important driver of soil fertility and depends on root exudation which contributes to soil carbon source and energy supply for microbes. Recent experiment in Senegal studied the plant genetic impact on root exudation and the factors promoting rhizosphere soil aggregation. Results showed that the amount of root-adhering soil per dry mass of root depends on cultivars (Ndour *et al.,* 2017).

3- A story of earthworms, silicon, rice and blast disease. Blast disease is responsible for important global rice production losses leading to high fungicide use. This disease is present in the Highlands of Madagascar and develops mainly after nitrogen fertilization. A recent experiment showed that disease can be controlled by earthworms and silicon (Blanchart *et al.,* 2020). Earthworms have the ability to increase Si availability, as well as P and N availability. Modulation of the expression of plant genes known to be responsive to stress has also been proposed as a mechanism.

4- A story of priming effect, nutrient cycling, organic management and bacteria. Fertilization management in the Highlands of Madagascar is mainly organic. Nevertheless, applications of organic matter can lead to a decrease in soil organic matter through the phenomenon of priming effect by nutrient mining. Recent studies have determined the drivers of this phenomenon, and the microbes involved in it (Razanamalala *et al.,* 2018).

Discussion

Above case-studies show that an ecological intensification of soil processes is possible in order to improve the provision of agrosystem services provided that (i) soil functions and dysfunctions are well described and (ii) innovative practices are co-constructed with users. All above case-studies resulted in propositions of innovative practices:

1- This led to alternative sustainable practices: superficial tillage to avoid organic matter mineralization, temporal rotation or spatial associations of market gardening crops with productive pastures.

2- This study highlighted the need to consider plant breeding to manage carbon content and soil physical characteristics.

3- The innovative practice was a replacement of excessive use of nitrogen fertilizers with agricultural practices involving the development of earthworm populations and coupled with micronutrient fertilization should be developed.

4- This led to the proposition that regular and frequent organic inputs with low C/N ratio (i.e. compost) would be preferable than a unique organic input with high C/N (i.e. residues) at the beginning of the cropping season to maintain carbon and nutrient stocks in soil.

Conclusions

In the context of erosion of global biodiversity, climate change, food security, it appears urgent (i) to improve our understanding of the soil functioning and the determinism of soil functions, (ii) to identify the agronomic levers that make possible to control biotic interactions, and (iii) to evaluate this ecological intensification. This problem-oriented approach proposes nature-based solutions and addresses the complex interactions between Sustainable Development Goals.

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Effect of free living nematodes and their associated microbial community on conservation biological control

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Abstract summary

Species of soil predatory mites feed on a diverse diet making them excellent candidates for conservation biological control programs. Free living nematodes (FLNs) are commonly found in soils and serve as prey for many soil predatory mites (SPMs). Some species must feed on FLN to lay eggs and others will need them to complete immature development. Surprisingly, as far as we know, FLNs have never been used as alternative prey to enhance the efficacy of SPM for conservation biological control. Here we present results of two case studies where we provisioned the FLN *Rhabditella axei* as complementary prey for predatory mites. In the first study, we used the SPM *Macrocheles embersoni* for housefly control and in the second, the SPM *Stratiolaelaps scimitus* for the control of the root knot nematode *Meloidogyne incognita*. In both systems, complementing the diet of predatory mites with FLNs resulted in higher predator abundance and better biological control, compared to the negative control and the release of predators without FLNs. Future research will focus on manipulations of soil management and evaluating soil amendments to enhance the abundance and diversity of FLNs and predatory mites in cropping systems for enhanced conservation biological control of soil pests.

Keywords: Laelapidae, Macrochelidae, Meloidogyne, Rhabditidae, Free living nematodes, Conservation biological control, Alternative food sources.

Introduction, scope and main objectives

While plant-parasitic nematodes receive most of the scientific attention, they are actually far less abundant in healthy natural soils than free-living nematodes (FLN) (Neher, 2010). FLN are high quality food due to essential nutrients such as omega 3 fatty acids (Menzel *et al.*, 2018). Interestingly, in several demographic studies on the potential of novel acarine biocontrol agents, FLN seemed to be more suitable as prey than the intended target pests. For example, the fecundity of the soil predatory mite (SPM) *Lasioseius floridensis* Berlese (Mesostigmata: Blattisociidae) was five-fold higher when fed the FLN *Rhabditella axei* (Cobbold) than when it was fed the broad mite *Polyphagotarsonemus latus* (Banks) (Britto *et al.,* 2012). Similarly, fecundities of the SPM *Cosmolaelaps jaboticabalensis* Moreira, Klompen and Moraes (Mesostigmata: Laelapidae) (Moreira *et al.,* 2015) and *Macrocheles embersoni* Azevedo, Berto and Castilho (Mesostigmata: Macrochelidae) (Azevedo *et al.,* 2018) were significantly higher when fed *R. axei* compared to their designated pests, the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) and the stable fly *Stomoxys calcitrans* (L.), respectively. For above ground systems, provisioning pollen as supplemental food to boost mite predators populations and maintain levels during low prey abundance periods, has been reported in numerous studies (for example Duso *et al.,* 2004, Warburg *et al.,* 2019). For below ground, mites belonging to Astigmatina have been used as alternative prey to conserve populations of the SPM *Macrocheles robustulus* (Berlese) to enhance the biological control of sciarid flies (Grosman *et al.*, 2011) and for *Cosmolaelaps* n. sp. for the control of the prepupae and pupae of the WFT (Muñoz Cárdenas, 2017). The use of specific astigmatine species as factitious prey is a very cost-effective solution for rearing and releasing predators for augmentative biocontrol in intensive cropping systems, but may not be suitable for soil application, as some species are not adapted to soils and others are pests. In contrast, as FLN evidently serve as SPM prey in nature, it seems only natural to use them as a supplementary food source in agricultural systems. Our general goal was to determine whether conservation biological control of soil pests by SPM could be enhanced by provisioning FLN. Here we present results of two case studies demonstrating the effects of *R. axei* on SPM biocontrol efficacy.

Methodology

In the first study (Azevedo *et al.,* 2019), two experimental setups differing temporally and spatially were conducted. The first, performed in small Petri dish arenas over 10 days, assessed *M. embersoni* fecundity and predation of L1 *Musca domestica* L., with or without supplementation of *R. axei*. The second, carried out in larger arenas over four weeks, was provisioned three times a week with *M. domestica* eggs and fresh larva diet, with or without nematode supplementation. The efficacy of fly immature predation was estimated by counting the adult flies that emerged over two weeks.

In the second study (Azevedo *et al.,* 2020), we conducted two experiments on dwarf tomato plants, with and without: 1) the root knot nematode *Meloidogyne incognita* (Kofoid & White), 2) the SPM *Stratiolaelaps scimitus* (Womersley), and 3) the FLN *R. axei* in its culture medium (FLNCM). To visualize the act of predation we performed high resolution imaging of S. scimitus feeding on *M. incognita*.

Results

In our first study, in the short-term small arena experiment, nematode supplementation reduced predation. Similarly, in the long-term experiment in plastic containers (Figure 1), more flies emerged in the nematode supplemented treatment during the 3rd week (the 1st week of fly emergence). However, in the 4th week, fly emergence dropped dramatically in the nematode supplemented treatment, whereas fly emergence continued to escalate in the treatment that received only fly eggs, and *M. embersoni* abundance was about a third of that in the nematode supplemented treatment.

In our second study, LT-SEM illustrated *M. incognita* held between SPM palps with the labrum, corniculi and extended chelicera ready to feed (Figure 2). As anticipated, gall abundance was lowest in the combined treatment of FLN and SPM but in contrast to the first study predator abundance was not high. Furthermore, similar reduction in gall abundance occurred when only SPM or only FLNCM was added to the soil mix. Finally, in the FLNCM treatment, foliar macronutrients N and K were significantly higher than the negative control.

Discussion

In both studies, in three experiments over a period 4-15 weeks, enhancing biodiversity by adding FLN with its associated microbial community added resilience to the system which translated to better pest reduction. Demonstrating once more that generalist predatory mites will be more effective as biocontrol agents when their habitat is more diverse in food and prey (Messelink *et al.*, 2010). In a review on the effects of plant amendments on plant parasitic nematode (PPN) control, Thoden *et al.* (2011) suggested that boosting bacterial populations and subsequent bacterial-feeding nematode numbers could stimulate crop growth, rendering plants less susceptible to PPN.

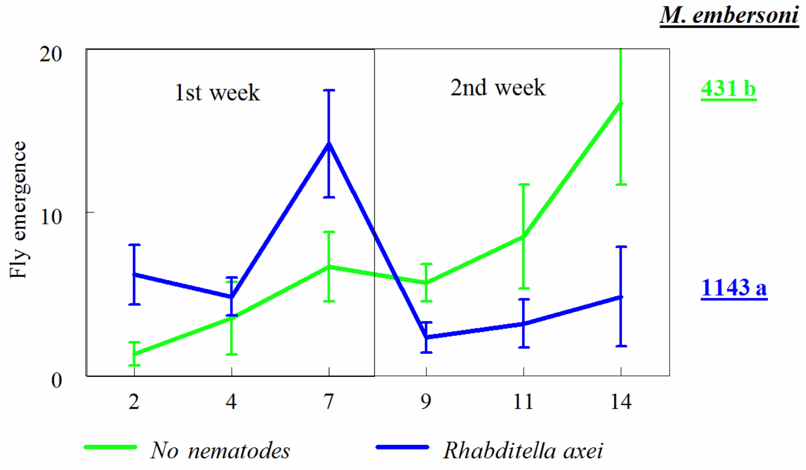

Figure 1: House flies collected in the first and second week of fly emergence, with and without provisioning of the free living nematode (FLN) Rhabditella axei

Numbers to the right of the figure are means of the soil predatory mite Macrocheles embersoni extracted at the end of the experiment with (in blue) and without (in green) FLN.

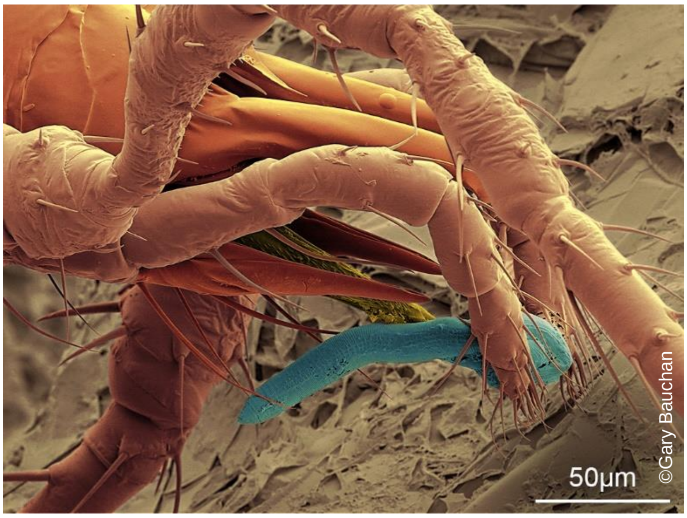

Figure 2: *Stratiolaelaps scimitus* with its palps (light brown), labrum (yellow), corniculi (red) and extended chelicera (orange) ready to feed on the J2 *Meloidogyne incognita* (turquoise)

Conclusions

Further studies are needed for the identification and conservation of FLN species that could be utilized for the conservation of soil predators. In outdoor crops, soil amendments have been utilized to enhance populations of naturally occurring FLN for nitrogen mineralization and disease management (Bulluck *et al.*, 2002; Thoden *et al.*, 2011; Rahman *et al.*, 2014; Ferris *et al.*, 2004). Similar soil manipulations could be used for identifying indigenous communities of FLNs and soil predatory mites in agricultural plots and their surrounding natural environments.

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Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield

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Abstract summary

Long-term adoption of intensive agriculture negatively affects soil fertility, microbiome and may compromise the quality and amount of crop production. To avoid these problems, application of organic materials has been proposed as a potential alternative strategy for soil management. Here, we compared the effects of conventional and organic soil management on crop yield, soil fertility, and microbiome structure through a 2-year long mesocosms experiment. Soil treatments were compared with conventional management practices with eight different organic treatments, in terms of organic matter quality and application frequency. Crop quality was assessed by measuring the NO_3^- content in leaves, whereas soil chemical and microbiological properties were evaluated to understand their effects on soil fertility. Changes in soil microbiota was assessed by high-throughput sequencing of bacterial and fungal rRNA gene markers. Application of organic amendments significantly improved crop yield. Application of synthetic fertilizer and fumigation induced soil acidification, increased soil salinity, and reduced soil microbiota diversity, activity and functionality, with negative effects on crop yield. In conclusion, this study indicates that long-term application of organic amendments effectively improved soil fertility and promoted the development of a beneficial soil microbiota capable of supporting high plant yield under intensive agricultural system.

Keywords: Organic amendment, Crop quality, Soil microbiota, Soil-borne pathogens, Soil sickness.

Introduction, scope and main objectives

Intensive agriculture is increasingly adopted for the cultivation of a wide range of agricultural species, in both open field and protected environment conditions (like greenhouse and plastic tunnel). However, the adoption of intensive agriculture systems for a long period, may lead to a deterioration of physical, chemical and biological quality of soil (Bonanomi *et al.*, 2011), which in turn may negatively affects crop yield and quality (Zoran, Nikolaos & Ljubomir, 2014). A possible solution for these problems is the application of organic amendments (Stockdale *et al.*, 2002). Many studies reported on the beneficial effects of organic amendments (like compost, green and animal manure, organic wastes and biochar) on soil properties, including the improvement of soil aggregation and available water holding capacity (Bronick and Lal, 2005), increase in soil organic matter (Li *et al.*, 2012), enhancement of microbial activity and biomass (Ros *et al.*, 2006), and plant protection from soilborne pathogens due to soil suppressiveness (Bonilla *et al.*, 2012). Most of the research focused their attention on the impact that single annual applications of organic materials have on crop productivity and/or soil properties (Diacono and Montemurro, 2011). On the contrary, only a few available studies considered the effects of frequent applications of organic amendments on soil processes and biological functions, including carbon (C) and nitrogen (N) mineralization (Mallory and Griffin, 2007), soil basal respiration (Nett *et al.*, 2012), enzymatic activities, soil fungistasis (Bonanomi *et al.*, 2017) and microbial biomass (Fließbach *et al.*, 2007). As such, the impacts on crop yield, soil fertility and soil microbial community of the type and application frequency of organic amendments have not yet been addressed. The main targets of the study were the following: (i) to assess the long-term (2 years) impact of organic amendment and conventional management on crop yield and soil chemistry; (ii) to explore how ordinary farming practices, or soils treated with different organic amendments influence the shifts in microbiota composition and functioning; (iii) to associate the changes in soil chemistry and microbiota composition, with the ecosystem functions i.e. crop yield and plan health.

Methodology

The mesocosms, consisting of plastic trays (32 liters), were set up in a greenhouse equipped with automatic temperature control. The soil was collected from a farm situated in Salerno (Southern Italy) in spring 2013. In the last ~10 years, the farm adopted an intensive farming system, characterized by rocket (Eruca sativa) monoculture in plastic tunnels (height ~4 m), intensive tillage (~6 rototilling per year), application of mineral fertilizers and soil disinfestation with fumigants, every three years. Four types of organic amendments, with different properties, were used: i. alfalfa straw (Medicago sativa), at the rate of 13 t ha-1 year-1; ii. Glucose, at the rate of 7 t ha-1 year-1; iii. compost manure, at the rate of 15 t ha-1 year-1; iv. wood biochar, at the rate of 30 t ha-1, applied once, at the start of the experiment.

To assess the impact of soil treatments on crop quality, nitrate (NO3-) content in rocket leaves was determined.

Soil chemical properties were assessed at the end of the experiment (i.e. after 360 days of cultivation). Briefly, soil pH, electrical conductivity (EC), organic matter (OM) and organic carbon (OC) content, total nitrogen (TN), total carbonates (TC) active carbonates (AC), cation exchange capacity (CEC), available phosphate (P2O5) and exchangeable bases (Ca2+, Mg2+, K+, Na+) were estimated. Soil nitrate (N-NO3-) and ammonium (N-NH4+) concentrations were assayed as well.

Total microbial activity was evaluated by using the fluorescein diacetate (FDA) analysis. The diversity and composition of soil microbial communities were analysed by Illumina high-throughput sequencing.

Results

Cumulated crop biomass was significantly higher ($p < 0.05$) in mesocosms amended with a single application of alfalfa and glucose, followed by soil treatments with a single application of manure, and frequent applications of organic materials. The lowest yield was observed in mesocosms subjected to soil fumigation and applications of synthetic fertilizers (Figure 1A).

The Δ in cumulated crop yield between the first and the second experimental year was reported in Figure 1B. Mesocosms treated with organic materials revealed a variable Δ in crop biomass, ranging from - 30.2 percent for the frequent applications of manure, to +31.7 percent for soil with char, and frequent applications of alfalfa and glucose. It is noteworthy, that mesocosms treated with conventional management showed a remarkable negative Δ ($p < 0.05$) in crop biomass between the two experimental years, corresponding to −66.5 percent for mineral and −77.1 percent for fumigated + mineral treatments (Figure 1B).

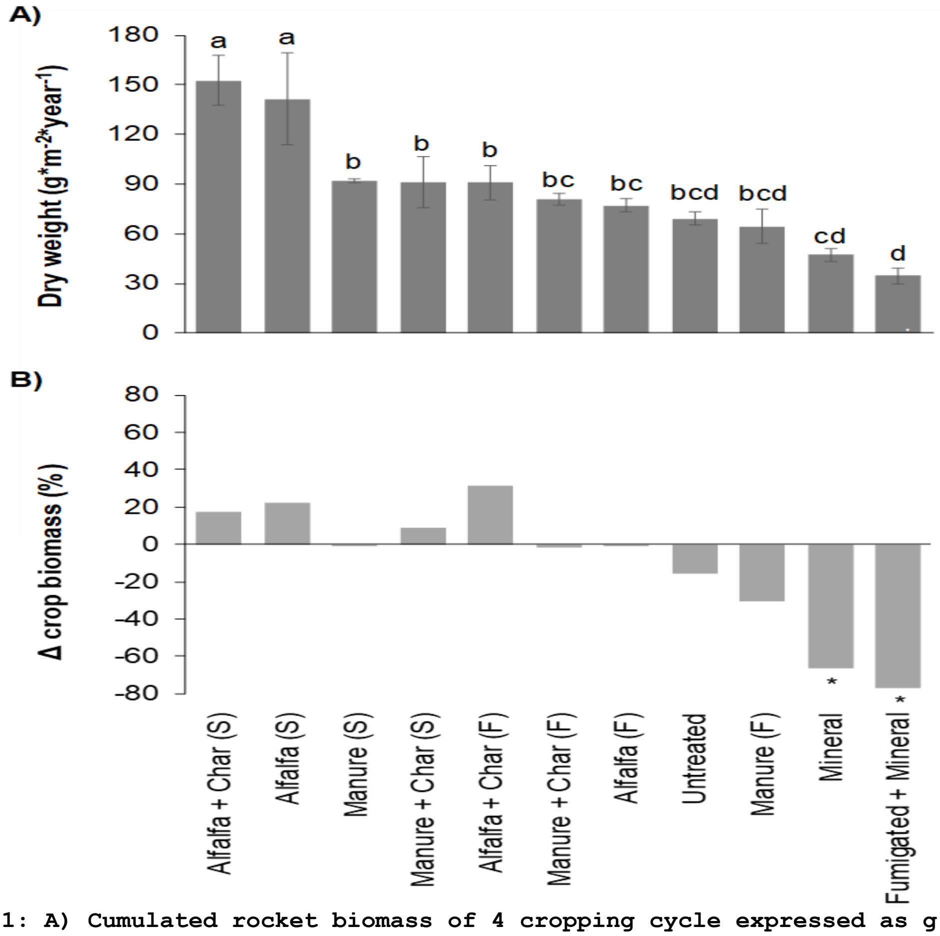

Figure 1: A) Cumulated rocket biomass of 4 cropping cycle expressed as g of dry mass per m^{-2} $year^{-1}$ and B) Δ of cumulated crop biomass between the first and the second year of soil conditioning

A) Data refer to mean of three replicates ± standard deviation. Different letters indicate statistically significant differences among treatments at $p = 0.05$ (Duncan test). B) Asterisk indicate statistically significant differences within each treatment at $p = 0.05$ (LSD test). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively

Discussion

After two years of soil conditioning, crop yield, plant health and leaf quality, as well as soil chemical properties and microbiota were significantly improved by the organic amendment. On the contrary, the use of mineral fertilizer, alone, or in combination with soil fumigation, had an overall negative impact on the functions of the soil ecosystem. Soil organic matter, by interacting with both the biotic and abiotic soil components, play a fundamental role in the conservation and restoration of soil fertility (Diacono and Montemurro, 2011). Several authors compared the effects of synthetic fertilizers and organic amendments, on soil properties and crop production (Goldstein *et al.,* 2004). Although these effects depend largely on the type of organic amendment, plant species, agricultural practices and environmental conditions, including soil type and growing conditions (Ahmad *et al.,* 2016), the application of organic amendments emerged as a necessary practice, to compensate for the amount of organic C lost through respiration and crop residues removal in the agro-ecosystems. In agreement with several authors, the shortage of organic matter input (into the soil) and the excessive use of synthetic fertilizers to support plant growth, negatively affect soil organic C stock (Li *et al.,* 2012; Ros *et al.,* 2006; Bonanomi *et al.,* 2011). In our study, the higher organic C content in soil treated with organic amendments, could be attributed to the input of organic materials, while the difference in soil organic C content between single and repeated applications, are probably due to the different application forms, i.e., powdered organic matter for single application, compared to liquid extract for the weekly application. Application of organic matter is essential to support soil microbial communities, which in turn influence organic matter decomposition, nutrient cycling, soil aggregation, plant growth and suppression of soilborne pathogens (Bonilla *et al.,* 2012; Sarker *et al.,* 2018). Generally, enzymatic activity, as well as microbial functional diversity, was found to be higher in soils with organic, rather than conventional management (Fließbach *et al.,* 2007). In agreement with these findings, we observed that the increase of soil EC and a reduction in soil pH, organic C and C/N ratio negatively affected the total microbial activity (FDA) and community-level physiological profile (AWCD) of soils treated with synthetic fertilizers.

Conclusions

Our study indicates that application of organic materials could be successfully introduced into the intensive agricultural systems to reduce the negative effects caused by the excessive use of synthetic fertilizers and soil fumigants. However, to identify the best strategy, further studies on the type, application rates and frequency of these organic amendments will be necessary, that will also take into account the relationship between different soil types and specific cultivated species.

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Phosphate solubilizing bacteria and arbuscular mycorrhizal fungi differentially benefit barley and enhance phosphate contents

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Abstract summary

The plant growth and their ability to take up phosphorus (P) from the soil can be modulated by beneficial soil microbes. Therefore, the aim of this study was to isolate and characterize new putative Phosphate Solubilizing Bacteria (PSB) to use as inoculums for plant growth and increasing the bioavailability of P in soil. To this aim, sixteen bacteria isolated from oats rhizosphere soils, were screened for their putative P-solubilization. The isolate MS1B15 showed the highest P solubilization activity 245.6±11.82 mg/l, and was identified by 16s rRNA gene sequencing as *Streptomyces albospinus*. In addition, it demonstrates its multiple plant growth promotion (PGP) traits including siderophores, Indol-Acetic Acid (IAA), Amino-Cyclopropane-1-Carboxylate Deaminase (ACCD), nitrogen fixation as well as antimicrobial activity against plant pathogenic fungi. Under greenhouse conditions, *Streptomyces albospinus* significantly increased fresh and dry biomass of barley plants, P contents in all plant tissues as well as the availability of P and nitrogen on soil, compared to Arbuscular Mycorrhizal Fungi (AMF) inoculation and uninoculated control. Overall results showed that the selected strain possesses high P-solubilization activity and therefore, it could represent potential candidate as biofertilizer in sustainable agricultural systems leading to increase plant growth, as well as P uptake.

Keywords: Phosphate solubilizing bacteria, Arbuscular Mycorrhizal Fungi, biofertilizer, Plant growth, P-uptake.

Introduction, scope and main objectives

Phosphorus is one of the major plant nutrients limiting plant growth. For this reason, most of the farmers regularly use chemical phosphate fertilizers which get incorporated into the soil by formation of complex non-bioavailable compounds, where phosphorus is wasted (Urrutia *et al.*, 2014).This mechanism generally causes a slow release of P, with highly accumulated P and thus not available for crops(Roy, 2017).The use of Phosphate solubilizing Bacteria (PSB) as biofertilizers is a sustainable and inexpensive biotechnological strategy (Zaidi *et al.*, 2009). Taking this into account the objectives of this original approach was i) to isolate and characterize new putative PSB, ii) to be evaluated in *vivo* on barley plant comparing to the biofertilizer Arbuscular Mycorrhizal Fungi (AMF) which act as a good biostimulant in nutrition and quality of horticultural crops as well as their productivity (Rouphael *et al.,* 2015) under greenhouse conditions.

Methodology

Screening of P-solubilizing bacteria

Sixteen isolates were isolated from oat rhizosphere to be screened *in vitro* for P-solubilizing activity on Pikovskaya's medium agar. The diameter of the clear halo zone surrounding the bacterial colony indicates the solubilization of Phosphorus. Phosphate solubilization index (PSI) was calculated using the formula of (Qureshi *et al.,* 2012). The strain that has the highest PSI was selected to quantify the concentration of solubilized P by molybdenum blue method on PVK broth, and it was identified by the sequencing of the 16S rRNA gene.

In addition, The selected isolate was evaluated for its putative PGP activities including siderophores, IAA, ACCD, nitrogen fixation and its antimicrobial against *Botrytis* sp. *Fusarium* sp. *Aspergillus* sp. and *oomycetes*, as described by (Fiorentino *et al.,* 2018; Viscardi *et al.,* 2016).

Plant inoculation

Greenhouse assay was conducted in an unheated polyethylene greenhouse. The soil used in this experiment was a Sandy clay soil, with a pH of 7.36, EC of 0.6 mS.cm-1, organic matter of 2.32g/kg, total N at 0.13g/kg, carbonates at 1.35g/kg, NO3-N and NH4-N at 39.39 and 4.48 mg kg-1, respectively, and olsen-P at 119.91 mg kg-1. Barley seeds were sterilized. The experimental design was as follows: treatment 1(BNOI): uninoculated seeds, treatment 2(BM): seeds treated with PSB and treatment 3(BG): seeds inoculated with AMF mixture of *Rhizoglomus irregulare* BEG72 *Funneliformis mosseae*. Three replicates per treatment were performed, leading to total 9 pots at plant density of 4 plants per pot. AMF treatment received 5 g pot−1 of the AMF inoculum while inoculation with PSB was performed two months after planting by fertigation 3.33 g of PSB suspension (5.8 × 106 CFU /g) in 100 ml of sterile distilled water in each pot. At the end of the experiment, Shoots and ears heights were measured, all plant tissues were weighed to obtain the fresh weight and were dried for 72 h for dry biomass determination. A sub-sample of the dried plants tissues was collected to determine the available phosphorus. A new physic-chemical characterization of soil was performed to evaluate the effect of the inoculums on P-content and total Nitrogen. Soil samples and roots were collected to obtain prokaryotic as well as eukaryotic profiles using Denaturing gradient gel electrophoresis (DGGE) analysis of rhizo-soil samples as described by (Ventorino *et al.,* 2016).

Results

The results indicated that 31.3 percent solubilized phosphate. Maximum PSI was observed by strain MS1B15 (PSI > 1.75). In addition, MS1B15 showed positive phosphate solubilizing efficiency under liquid assay in concentration of 245.6±11.82 mg/l. It exhibited 97 percent identity to *Streptomyces albospinus*. Interestingly, it showed positive PGP (AAC, IAA and siderophores) and antimicrobial activity against five from nine plants pathogenic fungi selected. Unluckily, showed a negative nitrogen fixation activity because did not contain *nifH* genes (Table3).

In the plants assay, the results showed a significant increase in most of the variables measured in this study when the seeds were inoculated with PSB compared to AMF inoculation and control, (Figure 1, Table 2). Statistically, DGGE prokaryotic profile permitted the classification of one cluster associated with the inoculums applied, while eukaryotic DGGE demonstrated a diverse profile not consistently associated with the inoculums applied in the sub-cluster formed. Both DGGE profiles showed that prokaryotic as well as eukaryotic populations are affected by the applied inoculums, as the results indicated that there was no high similarity between treatments (Figure2).

Table 1: Plant growth-promoting and antimicrobial attributes of *streptomyces albospinus*.

Strain	P-solubilization mg/l	Siderophores production †	Nitrogen Fixation	AAC deaminase	IAA production (mg/l)	Antimicrobial activity
MS1B15	245.6±11.82	4.09±1.10	-	+	6.34±0.33	+Against *Fusaruim* (FRAG, 3) +against *Botrytis* +against *Phytophthora* (PG2, P69)

Values represent the means ± SE of three replicates; † production siderophores unit (PSU); + positive activity, - negative activity

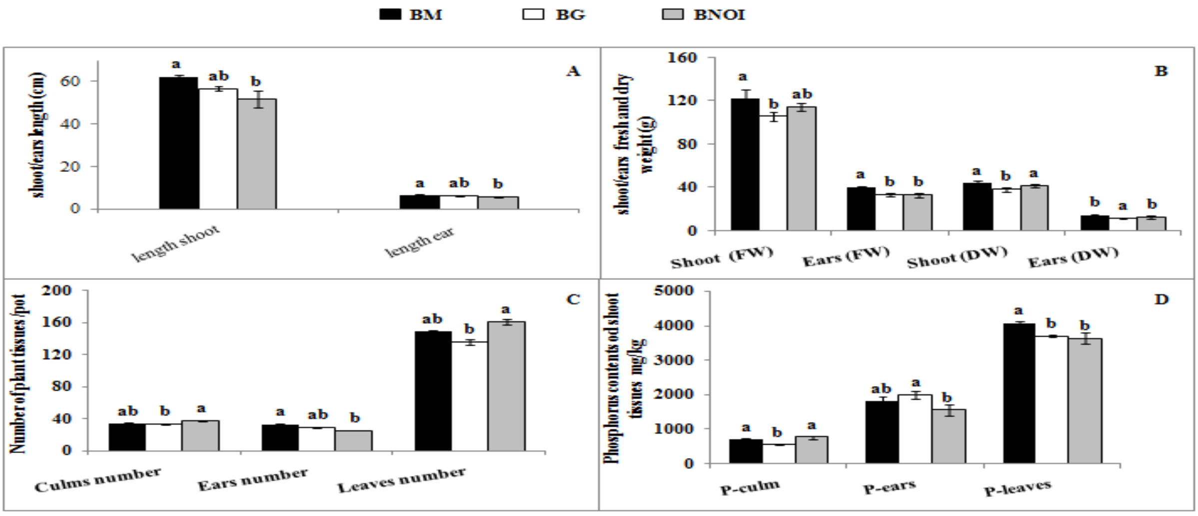

Figure 114: Effect of inoculums on shoot and ears length (A), fresh weight (FW) and dry weight (DW) (B), number of plants tissus/pot (C), and Phosphorus contents (D) of barley plant

Table 2: Soil properties at the end of the experiment

Treatment	**PSB**	**AMF**	**Control**	**ANOVA**
CE (ms/cm)	0.59±0.0	0.58±0.0	0.71±0.0	ns
pH	6.76±0.0	6.68±0.0	6.71±0.0	ns
No3-n (g/kg)	11.14±0.86	10.60±1.05	7.84±1.04	ns
Nh3-n (g/kg)	35.94±1.72	36.26±1.03	35.56±2.40	ns
Organic matter(g/kg)	2.99±0.06	3.00±0.13	2.70±0.13	ns
Total carbon (g/kg)	1.74±0.04	1.74±0.08	1.56±0.08	ns
Total nitrogen g/kg	0.17±0.0 **(a)**	0.15±0.0 **(b)**	0.15±0.0 **(b)**	*****
P2o5 mg/kg	125.09±2.82 **(a)**	99.80±1.77 **(b)**	110.28±4.28 **(ab)**	******

Values (mean± standard error).*, **: P< 0.05 and 0.01, respectively; n.s., not significant.

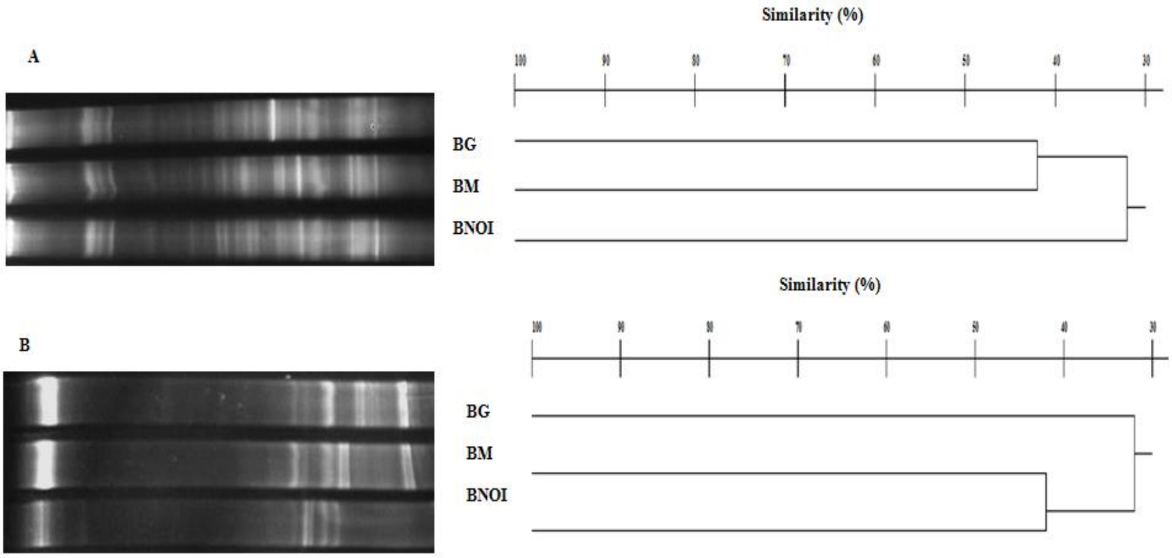

Figure 2: DGGE profiles and dendrogram showing the degree of similarity (%) of the PCR-DGGE profiles of the prokaryotes (A) and eukaryotes (B) in Barley rhizo-soil samples

Discussion

S. albispinus showed a high P- solubilization activity that exceeds the level of the other genera, including *Bacillus* and *Pseudomonas* as reported previously by (Habil-Addas *et al.,* 2017; Tiwari *et al.,* 2018.). Another interesting feature of this strain exerted antimicrobial activity against plant pathogenic fungi. Inoculation with our selected PSB possesses multiple PGP traits, was effective to benefit Barley growth compared to the uninoculated control and to inoculation with AMF which act as a good biostimulant in nutrition and quality of horticultural crops (Rouphael *et al.,* 2015).

In consistent to our findings (El-Tarabily, 2008; Gopalakrishnan *et al.,* 2014; Sadeghi *et al.,* 2012) reported the positive growth promoting attributes of tomato, rice, wheat towards inoculation with *Streptomyces* spp.

In our study, the P content of the inoculated plants were found to be significantly higher than in uninoculated plants; this makes us believe that PSB and AMF are responsible for P mobilization in the plants in treatments 2 and 3. However, the low similarity of rhizo-soil between treatments because of the increase of microorganisms number on rhizo-soil, due to an antifungal activity by PSB. The mechanism of PGP by this strain appears to include phosphorus mobilization, antifungal activity and increase Barley growth.

Conclusions

Overall results showed that the selected strain combines multiple PGP traits and antimicrobial activity. These characteristics have been found effective in positively increasing the growth of Barley plants and P contents. Therefore, this isolate could represent potential candidate as biofertilizer in sustainable agricultural systems leading to increase plant growth, as well as P uptake.

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Metabolic activity of *Pseudomonas* sp isolated from agricultural soils

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Abstract summary

The study of the biodiversity and metabolic properties of the microbiota present in soils has allowed us to apply alternatives in areas where it is sought to recover eroded soils or implement improvements in crop processes. The objective of the present work was to evaluate the metabolic processes in bacterial isolates in conventional and agroecological agricultural soils. Samples were carried out in plots with conventional and agroecological management of the state of Puebla and Tlaxcala, Mexico. Performing isolation and identification with selective and chromogenic media. From the bacterial isolates, metabolic activity was evaluated in CAM medium (starch, sucrose, dextrose), DNase activity, biofilm formation and its ability to degrade pesticides (malation, carbofuran, esteron). In conventional and agroecological systems, *Pseudomonas* sp was isolated and identified, highlighting the greater metabolic activity and DNase activity in the conventional system, in this system 60 percent of the isolates were biofilm-forming. With respect to pesticide degradation, it was observed that bacterial isolates were more active against ester with 48 percent degradation, malation with 46 percent and carbofuran with 35 percent. Bacterial isolates of the conventional culture system are more metabolically active.

Keywords: Soil, Pseudomonas spp, isolations, metabolic biodiversity, conventional and agroecological plots.

Introduction

Due to their characteristics, microorganisms actively participate in various functions and offer ecological services in soils, highlighting the maintenance of primary production and soil recovery. Among the microorganisms that contribute significantly to ecological soil services are bacteria, which have the ability to adapt to a wide variety of biotic conditions (Marcano, 2008). Bacterial metabolism confers the ability to use a large number of compounds as a source of carbon and energy, facilitating their survival in unfavourable environments and facilitating the transfer of compounds that are not available to plants (Bakhshandeh *et al.*, 2015). The objective of the present work was to evaluate the metabolic processes in bacterial isolates in conventional and agroecological agricultural soils.

Samples were carried out in plots with conventional and agroecological management of the state of Puebla and Tlaxcala, Mexico.

Methodology

Conventional and agro-ecological soil sampling was performed in the areas of Puebla and Tlaxcala, Mexico. Each sampling was 2 kg of soil and was carried out according to NOM-021-SEMARNAT-2000 in which it is indicated that the agricultural land take must be 0-15 cm deep. One gram of each soil sample was weighed and resuspended in 10 mL of nutrient broth, incubating at room temperature and stirring at 40 revolutions per minute/24 hours. 10 µL were reseeded in nutrient agar and incubated at 30ºC/24 hours, the colonies being selected with greater viability. The identification of each isolation was carried out with the "Nissui" EC™ Compact Dry chromogenic medium. The metabolic activity test was carried out with the CAM medium using as a source of carbon dextrose, sucrose and starch. To evaluate the DNase activity, the DNase Test medium was used, revealing the test with the addition of 1N HCl. For the quantification of biofilm, the nutrient broth growth of each of the isolates was carried out by adjusting the inoculum to 1X10^{6} CFU/mL. The different samples were placed in 96-well microplates, stained with violet crystal and following the technique described by Stepanovic *et al.* (2004) to obtain the cut-off point in the biofilm formation. To determine the degradation of pesticides (esteron, malation and carbofuran) by bacterial isolates, a silty soil (control) was used, of which 300 grams were weighed and divided into three 100 gram fractions, to each fraction was added a pesticide in a solution of 100 mL and a concentration of 100 ppm. Each sample was then interacted with 1 mL of the bacterial inoculum, allowing them to interact for 17 days. The UV-Vis spectra were determined in a spectrophotometer (Lamba 20), with the absorbance observed in the maximum of each spectrum the concentrations were calculated using the calibration curve of each pesticide to compare its degradation in the absence and presence of each bacterial isolation.

Results

Based on the isolates obtained from both types of soils (conventional and agroecological), the identification was carried out in the Compac Dry "Nissui" EC™ chromogenic medium, identifying *Pseudomonas* spp. For the evaluation of metabolic activity, the isolates of the agroecological system showed positive test in 20 percent and 40 percent in dextrose and sucrose, respectively, and in the conventional system the isolates were positive to sucrose and dextrose in 44 percent and 50 percent, respectively. DNase activity only in the conventional system a 20 percent positive test is reported. The data on the determination of biofilm formation of the isolates are presented in Table 1.

Table 1: Determination of biofilm production in the isolates of *Pseudomonas* spp

Samples	Conventional system	Agroecological system
1	Not producing	Moderate producer
2	Weak producer	Not producing
3	Weak producer	Not producing
4	Not producing	Weak producer
5	Weak producer	Not producing

The quantification of pesticide degradation by UV-Vis spectrum showed the following data: when comparing the degradation of the ester with and without inoculation of the isolates of *Pseudomonas* spp., A greater degradation was observed in the first seven days, on day three the sample with bacterial inoculation showed a 26.28 percent greater degradation difference compared to the test where the bacterial inoculation was not applied. Malation with bacterial inoculation showed the greatest degradation in the first nine days and for carbofuran it was observed that on days seven and fifteen without bacterial inoculation there was greater degradation.

Discussion

The differences between the metabolic activity tests are related to the type of agricultural management that each system presents. The isolates of the conventional system presented different activity due to the use of pesticides, which modifies their metabolic activity (Griffiths *et al.*, 2000; Gravina *et al.*, 2017). Pesticides can have an inhibitory effect on bacteria, causing alterations in biomass formation and metabolic activity, and as a result of these interactions bacteria develop the ability to form biofilms, favoring their ability to survive and adaptability (Dong *et al.*, 2011; Bjarnsholt *et al.*, 2013).

The isolates showed the highest degradation against the ester, followed by malation and carbofuran, the efficient degradation is related to the ease that bacteria have to metabolize chlorinated organic compounds (Koranda *et al.*, 2014).

Conclusion

The isolates can be considered together with other agroecological practices t degrade or decrease the concentration of pesticides in some soils affected by these compounds, taking advantage of the biodiversity of soil microorganisms.

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Influence of soil type on the biodegradation of pesticides by rhizobacteria: case of Glyphosate and paraquat

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Abstract summary

The present work aimed to evaluate soil type influence on pesticide degradation using bacteria strains. We assessed the basal respiration of four rhizobia (*Pantoea agglomerans, Rhizobium nepotum, Rhizobium radiobacter,* and *Rhizobium tibeticum*) inoculated in four different soils (Isohumic, Fersialitic, Vertisols, and Calcimagnesic) in presence of two herbicides (glyphosate and paraquat). The results showed a clear relationship between soil types, pesticide adsorption, and biodegradation. A significant difference was observed between the soils studied ($P < 0.05$). Furthermore, the production was the highest in Isohumic soil, whereas it was low in the Fersialitic soil, which is the richest in sand compared to Vertisols, which is the richest in clay. This investigation confirms that the soil parameters manage pesticide availability to microorganisms for degradation

Keywords: Soil parameters, Biodegradation, Respiration basal, Carbon dioxide, Pesticide

Introduction, scope and main objectives

The soil is an essential recycling centre for life maintenance. The human being thus always endeavoured to settle where the grounds were the best. Thereby Soil health is essential for healthy food production. It provides all necessary elements for the growth and development of plants essential such as nutrients, water, oxygen and support to the roots.

The United Nations Organization for Food and Agriculture Organization (FAO) has defined pesticides as any substance, or a mixture of substances of chemical or biological ingredients that are meant for repelling, destroying or controlling any pest, or regulating plant growth (FAO and WHO, 2014). These substances play a capital role in decreasing crop losses owing to pest infestation. However, excessive pesticide usage has various drawbacks and negative effects on the environment especially on the soil who is the first element that comes into contact with its substances (Maldani *et al.,* 2017).

Glyphosate (N- (phosphonomethyl) glycine is a broad-spectrum systemic herbicide that blocks an enzyme that the plant needs to make amino acids and proteins. Paraquat is a very effective and commercially important herbicide and widely used to control broadleaf weeds worldwide. Paraquat is a rapid, non-selective compound, destroys green plant tissue by interaction and translocation with plants (Yu, Cairns and Powles, 2006).

In the soil, pesticides degradation is done chemically, or biologically by microorganisms (Verma *et al.,* 2014). Likewise, pesticide biodegradation is influenced by different parameters like organic matter content, clay content, soil texture, pH and pesticide structure.

The aim of this study was to evaluate the influence of soil type on the degradation of two herbicides: glyphosate and paraquat by bacteria using the basic soil respiration method.

Methodology

Four types soils were sampled from the 0–20 cm layer: Vertisols (Latitude : 33°, 52′, 8″ N Longitude : 5°, 36′, 4″ W) ; Fersialitic (Latitude : 33°, 50′, 8″ N Longitude : 5°, 32′, 6″ W) ; Calcimagnesic (Latitude : 33°, 50′, 8″ N Longitude : 5°, 28′, 3″ W) ; Isohumic (Latitude : 33°, 51′, 6″ N Longitude : 5°, 28′, 0″ W) in Meknes province, Fez-Meknes region, Morocco. The soils were sterilized by autoclaving for 1 hour at 121 °C. Then 1.8 g/l of glyphosate or 1 g/l of paraquat was added to 100 g of each soil type and inoculated by four rhizobacteria: *Pantoea agglomerans, Rhizobium nepotum, Rhizobium radiobacter,* and *Rhizobium tibeticum*. The glyphosate and paraquat biodegradation in the different soil was evaluated by the carbon dioxide evolution method described by Freijer and Bouten (1991). The carbon dioxide amount released was measured at 2, 4, 8, 16, 24 and 32 days after glyphosate or paraquat application. All soils are bringing at 60 percent of maximum water holding capacity (WHC). Data were examined by ANOVA and post-hoc test (Tukey’s test at $p < 0.05$) was performed for multiple comparisons.

Results

Physicochemical analysis data of the soils are represented in the (Table 1). A significant difference ($p< 0.05$) was found between the soils concerning texture, exchangeable cations, and organic matter content.

Table 1: Physico-chemical characteristics of the studied soils

Soils	**Texture (%) (Pipette Robinson)**			**Exchangeable cations (meq 100g^{-1})**					**Organic matter (%)**
	Clay	Silt	Sand	Ca^{2+}	Mg^{2+}	Na^{2+}	K^{+}	CEC	
Vertisols	43.35^{a}	23.67^{a}	27.13^{a}	5.1^{ac}	2^{a}	0.52^{a}	0.96^{a}	49.13^{a}	5.8^{a}

Calcimagnesic	55.73^{b}	29.68^{b}	12.27^{b}	44.75^{b}	2.8^{b}	1.43^{b}	0.6^{b}	53.47^{b}	4^{bc}
Fersialitic	6.95^{c}	5.45^{c}	85.06^{c}	5.4^{a}	0.53^{c}	0.7^{c}	0.32^{b}	3.95^{c}	4.5^{c}
Isohumic	30.57^{d}	46.34^{d}	20.03^{d}	4.18^{c}	4.65^{d}	1.09^{d}	2.48^{c}	31.73^{d}	3.25^{b}

Values are the mean of n = 3. Values superscripted by different letters are significantly (Tukey's test, $P < 0.05$).

The effect of glyphosate and paraquat on microbial activity was measured by CO_2 production in four soil samples during 32 days of incubation.

Statistically, the data obtained of our experiment show a significant difference ($p < 0.05$) (Figure 1 and 2). For the four soils tested, total respiration increased progressively during the incubation period. Isohumic soil confirmed higher CO_2 production compared to other soil types under glyphosate and paraquat applications. For all strains tested, the respiration rate was significantly influenced by pesticide and soil type.

To approve the effect of soil and pesticide type on the biodegradation, each strain was studied separately, the soil effect is obviously clear in all soils. The CO_2 production in glyphosate treatment was the highest in all soils; this can be explained through the use of pesticide as a source of nutrients by the bacteria after degradation. Moreover, Comparing the microbial activity of the four strains in each studied soil, it is found that in the Isohumic soil the production of CO_2 was the highest, whereas it was low in the Fersialitic soil who is the richest in the sand, and the Vertisols which is rich in clay.

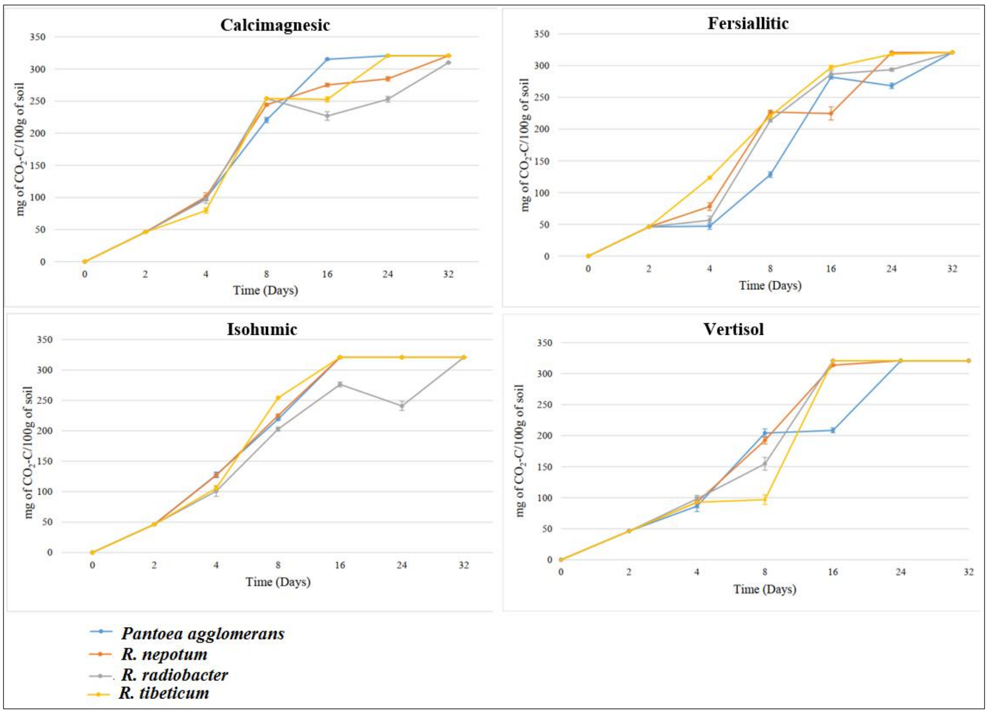

Figure 1: Carbon dioxide evolution from four soil types with glyphosate inoculeted with *Pantoea agglomerans*, *R. nepotum*, *R. radiobacter*, and R. *tibeticum*

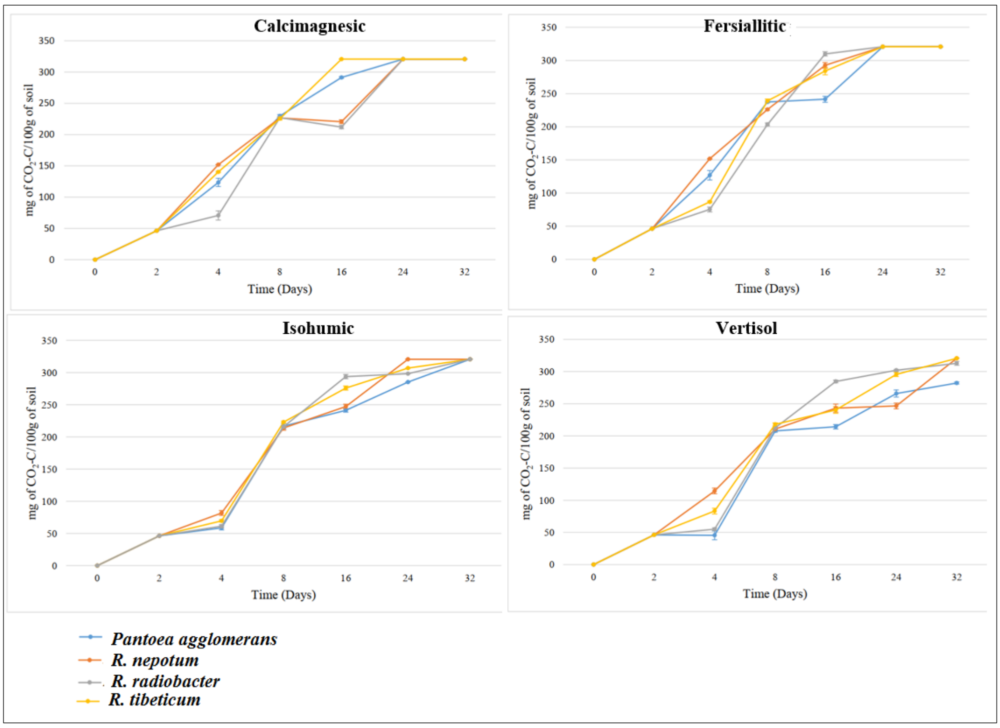

Figure 2: Carbon dioxide evolution from four soil types with paraquat inoculeted with *Pantoea agglomerans*, *R. nepotum*, *R. radiobacter*, and *R. tibeticum*

Discussion

The soil has the capacity to absorb various contaminants and pollutants, like pesticides, thus it functions as a pollution absorber. Consequently, it may contaminate the food chain, therefore threatens human health.

Our data indicate that the application of glyphosate and paraquat stimulated soil microbial activity and suggested that glyphosate and paraquat was the direct source of increased microbial activity this finding has been proved by Araújo, Monteiro and Abarkeli (2003) in their studies, they have shown that the soils with high microbial activity promote the rapid glyphosate mineralization as well.

The pesticide behaviour in the soil is depending on the soil composition. Amondham et al (2006) demonstrate in their studies on the uptake and absorption of paraquat in tropical soils suggests that paraquat is strongly adsorbed by soil particles, particularly in clay soils. Furthermore, Veiga *et al.* (2001) have shown that glyphosate is degraded very rapidly in soil surface (0-20) which is rich in clay and organic matter content than in the deep (20-35), which is poor in clay.

Moreover, when soil is rich in organic matter content, and poor in nutrients elements enhance glyphosate sorption, and facilitate its degradation, reducing the risk of pesticides despite its adsorption on soil components.

Conclusions

The above-mentioned data confirm that soil parameters such as organic matter content, clay content, soil texture influence the biodegradation of glyphosate and paraquat.

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Perennial energy crops and organic fertilization increase soil biological activity and soil fertility on sandy substrate

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Abstract summary

The extensive cultivation of perennial energy crops in combination with organic fertilization are discussed as a more sustainable alternative to the intensive cultivation of maize mono cropping for energy purposes on light and sandy soils, especially in the east of Germany. Perennial cropping systems leave the soil undisturbed for years and the continuous amendment of the soil with organic matter via digestate fertilization increases the carbon content of the substrate. Both factors bare a high potential benefit for soil biodiversity. Additionally, the overall biodiversity benefits from the fact that perennial energy crops, in contrast to annual maize cropping, grow without need for chemical pest control and produce flowers with nectar and pollen for pollinating organisms.

We present results from a cropping system for the perennial energy crop *Sida hermaphrodita* on sandy substrate. Fertilization is applied via biogas digestate. We compared this fertilization to a standard mineral fertilization as well as an unfertilized control treatment.

We show that within three years of digestate application, the system was able to increase soil carbon mass fraction and soil respiration in the sandy substrate. The related increase in soil fertility resulted in significant yield advantage (30 percent) of digestate over mineral fertilization.

Keywords: soil fertility, microbial activity, carbon mass fraction, soil respiration, organic fertilization, perennial energy crop, alternative cropping system, Sida hermaphrodita

Introduction, scope and main objectives

The intense and monotonous cultivation of maize for the production of biogas on light and sandy soils causes serious environmental problems as well as land use conflicts (FAO, 2009). First, the use of pesticides decreases the biodiversity in such agroecosystems, above and below-ground. Second, the intense and continuous mechanical cultivation of such substrates negatively affects soil organisms, the soil carbon content and overall soil fertility. Plus, maize cultivation leaves great parts of the soil uncovered during the main rain season, causing soil erosion and a loss of habitat for soil organisms, especially in the top soil.

Perennial energy crops are discussed as a more sustainable alternative to annual maize cultivation for energy purposes. After the establishment of the plants, the cropping system renders the soil uncultivated for up to 20 years, allowing the formation of a robust soil structure and the establishment of a diverse community of soil organisms. Additionally, the flowering nature of some perennial energy crops (e.g. *Silphium perfoliatum* and *Sida hermaphrodita*) and their cultivation without need for chemical pest control bare additional benefits for both, above- and belowground biodiversity.

Organic fertilization provides crops with all plant nutrients and at the same time amends the soil with organic matter. Thus, soil organisms are adequately supplied with a carbon and energy source. Plus, organic fertilizers, like biogas digestate, the remaining residue after anaerobic fermentation of plant material in a biogas fermenter, close the nutrient loop when applied on the fields of the plant biomass origin.

In our study, we examined an alternative cropping system based on the perennial energy crop *Sida hermaphrodita* cultivated on light and sandy substrate. Fertilization was applied via biogas digestate. Within the three-year experiment we aimed to increase the soil carbon content, microbial activity and increase the overall fertility of the substrate. *Sida hermaphrodita* is an herbaceous perennial that can be harvested yearly and regrows in the following season (Jablonowski *et al.*, 2016). In Germany it flowers from May until late October, providing constant supply for pollinating organisms. Its deep reaching and extensive root system potentially stimulates soil organisms within the rhizosphere and supports the formation of a soil structure in the sandy substrate and therefore potentially protects soil from erosion.

Methodology

Figure 115: Experimental Setup of 250 L mesocosms in a completely randomized design

An outdoor mesocosms experiment in 250-liter containers, filled with a sandy substrate was established at IBG-2: Plant Sciences, Research Center Jülich, in May 2014. *Sida hermaphrodita* seedlings were planted and grown for three full growing seasons with an annual harvest of above ground biomass in each year. Plants received either no fertilization or fertilization via mineral NPK (nitrogen, phosphorus, potassium) or biogas digestate, equivalent to a supply of 160 kg N ha^{-1}. Substrate samples (0-30 cm) were taken in 2014 and 2016 and analysed for pH (Hannah Instruments pH 209-meter using 0.01 M $CaCl_2$ solution) and carbon content (elemental analysis in VarioELcube). Complementary, a laboratory experiment was set up to monitor soil respiration in relation to different doses of digestate amendment (0, 20 and 40 g kg^{-1}) using a Respicond VIII system (Nogren Innovations AB). Statistical analysis was performed with analysis of variance (ANOVA) in R 3.0.3 (The R Foundation).

Results

Within the three-year experiment, the carbon mass fraction of the sandy substrate was strongly increased when *Sida hermaphrodita* was fertilized organically using biogas digestate. Mineral fertilization and the unfertilized control only resulted in a slight increase of the carbon mass fraction (Table 1). Both fertilizations decreased the pH to a slightly acidic environment, while the substrate of the unfertilized control remained slightly basic.

Table 1: The carbon mass fraction of the sandy substrate is clearly increased by digestate fertilization

	carbon mass fraction		**pH**
	2014	**2016**	**2016**
Control	0±0	0.1±0	7.4±0.1
Digestate	0.3±0.1	0.7±0.5	6.9±0.1
NPK	0.1±0	0.2±0	6.9±0.1

The pH is decreased to a more microbial friendly environment within three years (Nabel *et al.* 2017); NPK fertilizer.

Amendment of the sandy substrate with increasing doses of biogas digestate resulted in a linearly increased soil respiration rate (Figure 2).

With a dry matter yield of 9 t ha^{-1} in the third year of growth *Sida hermaphrodita* fertilized with digestate resulted in the highest biomass yield. Compared to the unfertilized control, fertilization with digestate increased biomass yield on average by a factor of six. The yield advantage of digestate over mineral fertilization got more and more pronounced over the three-year time period of the experiment. In the third year the yield of digestate fertilized plants was on average one third higher than for minerally fertilized plants (Figure 2).

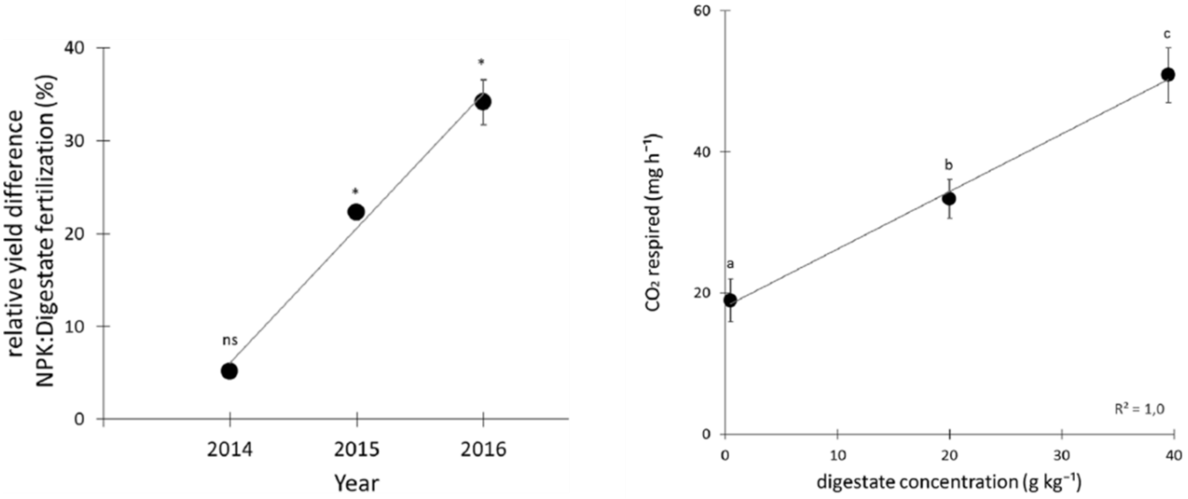

Figure 1 and 2: Increased digestate amendment of the sandy substrate results in increased soil respiration

Within three years the relative yield of digestate fertilization over mineral NPK fertilization gets more and more pronounced (Nabel *et al. 2017*).

Discussion

The results for soil carbon content, pH and biomass yield indicate the potential of the combination of perennial cropping systems with organic fertilization for soil carbon accumulation and increased soil fertility. The increased carbon content of the substrate serves as carbon and energy source for soil organisms, as shown by the increased soil respiration. Alburquerque *et al.* (2012) found similar effects on soil respiration after the amendment with digestate and argued that digestates consist of two fractions of organic matter: The first fraction is easily degradable and triggers activity of soil organisms, whereas the second fraction is more resistant to microbial degradation, contributing to the increase of soil organic matter. Furthermore, they were able to positively correlate the increased activity of soil organisms and soil respiration with the formation of soil aggregates, resulting in a positive effect on the water holding capacity. In the long term, this leads to an increase of the substrate fertility, also explaining the steady increase of the yield advantage of digestate compared to mineral fertilization.

Conclusions

Cropping systems on light and sandy soils using perennial energy crops like *Sida hermaphrodita* in combination with organic fertilization via digestate are a more sustainable alternative to cropping systems based on annual crops like maize and mineral fertilization. The combination of soil rest and organic amendment triggers the activity of soil organisms and creates a supporting habitat for them. In return, soil organisms facilitate the build-up of soil aggregates and soil structure and thus increase and sustain the substrate fertility. In the long term the investment into the soil system will return substantial and save yields for farmers with strongly reduced ecological follow-up costs, compared to conventional cropping systems. In future, soil biodiversity should receive more attention in designing sustainable cropping systems that contribute to the conservation of the world's biodiversity and food security.

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Cultivation and grazing impact on extracellular enzyme activity in Alberta grasslands

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Abstract summary

Grasslands cover a quarter of the planet's terrestrial surface and constitute 70 percent of the world's agricultural land area. Grasslands provide clean water, facilitate effective nutrient cycling, and provide necessary habitat and forage for livestock and wildlife. Grazing is one of the most common uses of grasslands and may alter C and nutrient mineralisation. Therefore, understanding the impact of different grazing systems (i.e. continuous and rotational) on C and nutrient cycling, as well as past management practices (cultivation), climate and soil properties, is of significant interest. This study examined the role of grazing systems on soil biogeochemical cycling by measuring extracellular enzyme activity (EEA), which is an indicator of soil biological activity.

Keywords: grassland, extracellular enzyme, grazing system, nutrient mineralisation

Methodology

Study sites

Study ranches (N = 24) were located in pairs at 12 sites within the Mixedgrass, Foothills Fescue, Aspen Parkland, and Boreal transition subregions of Alberta. Soil sampling was done three times in 2018: June, July, and September, representing spring, mid-summer and fall, respectively.

Most ranches within a ranch pair had the same cultivation history, with two exceptions. In total, there were sixteen cultivated and eight non-cultivated ranches. Within a pair, ranches were located relatively close to each other (< 5 km) and had similar soil properties (texture, pH), slope position, and elevation. A detailed survey of each ranch operator was conducted to obtain the information necessary to assess differences in soil EEA. Key metrics include whether the area had been previously cultivated, the number of paddocks, average length of a grazing period, and the minimum length of the rest period during the early to mid-growing season. These data were used to compute a rest to graze ratio (RGR) that indicates the number of days of rest per day of active grazing during the growing season. Finally, information on the number of cattle, size of area grazed, and the entry and exit dates of cattle were used to compute an average stocking rate (SR) of cattle for the grazed area.

Soil analyses

To determine soil pH, a soil:water solution (10 g:50 mL) was tested using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) (Robertson *et al.,* 1999). Soil moisture content was analysed by comparing weights before and after oven drying (105 °C). For determining soil available nitrogen (AN), 10 g of soil was mixed with 0.5M K_2SO_4 solution in a ratio of 1:5 (air-dried equivalent soil sample weight:K_2SO_4 solution). To determine nitrate (NO_3^-), soil extracts (from 10 g soil + 50 mL 0.5M K_2SO_4 solution) were mixed with vanadium chloride (Miranda, Espey and Wink, 2001) and to determine ammonium (NH_4^+), soil extracts were mixed with phenol and hypochlorite at pH 11.2 (Keeney and Nelson, 1982) and analysed on a spectrophotometer (Genesys 10 UV-Vis, Thermo Spectronic). Available N (AN) was represented by sum of NO_3^- and NH_4^+. The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by ethanol-free chloroform fumigation (Brookes *et al.,* 1985). To assess EEA of xylosidase (Xylo), cellobiosidase (Cello), β-glucosidase (BAG), N-acetyl-glucosidase (NAG), and phosphatase (Phos), a standard fluorometric method was used with 96-well microplates described by Sinsabaugh *et al.* (2003) with acetate buffer solution (pH 5.0), except for urease. Colorimetric method (Sinsabaugh *et al.,* 2000) was chosen to determine urease activity.

Data analysis

Data were analysed using a linear mixed-effect model (glme), with response variables being the individual EEA values, as well as the total of all EEAs (geomean) responsible for C cycling (specifically Cello, Xylo and BAG). A model selection approach (Anderson *et al.*, 2000) was used to compare models for their ability to explain each EEA response. All statistical analyses were conducted with R studio version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results and discussion

Results showed that a lengthy rest to grazing period ratio (mainly present in AMP ranches) increased β-glucosidase activity, while high cattle stocking increased urease activity. In contrast, soils previously cultivated had lower xylosidase and phosphatase activities, suggesting a legacy effect of past cultivation. The main factors regulating enzyme activities were available soil N and climatic aridity. Overall, grazing practices appear to be capable of altering C and nutrient cycling in these temperate grasslands. This study highlights the importance of grassland management in influencing biological activity.

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Transformation of the soil microbial community of the ordinary chernozem in irrigation

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Abstract summary

The results of research the transformation of the species and numerical composition of the microbial community of ordinary chernozem irrigated by unsalted (0.61 g/dm^3) and saline (2.9-3.2 g/dm^3) waters are presented. The objects of research are located in the Steppe zone of Ukraine (Donetsk region). The parameters of microbiological parameters of the studied soils are determined. The results of a comparative assessment of the soil microbial community in stationary objects and in soil-analogous on the parameters of microorganisms number, structure and its functioning are presented. Long-term irrigation by saline water led to degradation changes in the composition of microbial community (medium degree) and biological degradation index was 35 percent. The research results indicate the need to include microbiological indicators in the system of ecological and ecology-agroameliorative monitoring of irrigated soils. They should also be considered when evaluating soil ecosystem services.

Keywords: chernozem ordinary, microbiological indicators, soil microbial community, irrigation, unsalted water, saline water

Introduction, scope and main objectives

Soil is one of nature’s most complex ecosystems and one of the most diverse habitats on earth: it contains a myriad of different organisms, which interact and contribute to the global cycles that make all life possible. Soils contain a quarter of the biological diversity of our planet. Soil organisms act as the primary agents of nutrient cycling, regulating the dynamics of soil organic matter, soil carbon sequestration, modifying soil physical structure and water regimes and enhancing plant health. These services are essential to the functioning of natural ecosystems and constitute an important resource for the sustainable management of agricultural systems (FAO, 2015).

Soil biodiversity plays an important role in the functioning of ecosystems and their provision of services - providing, regulating, supporting. Microorganisms are very sensitive to changes of environmental factors, therefore, the number of microflora and the activity of microbiological processes can be diagnostic indicators of estimation of soil properties transformation. They are not only an important component for the functioning of natural ecosystems, but also provide sustainable management of agrolandscapes. The literature review showed soil fertility largely depends on the state of microbial community and biological activity. Therefore, biological indicators must be taken into account in monitoring irrigated soils, determining the degree of soil properties changes for the development of measures to increase the biological resistance of soils to loads.

Irrigation, as one of the powerful anthropogenic factors, leads to the restructuring of the entire soil ecosystem, changes the number and ratio of different groups of microflora, the nature and orientation of biochemical processes (Naydyonova and Baliuk, 2014; Frenk, Hadar and Minz, 2018). Changes in microbiological community can be both positive and negative depending on the quality of irrigation water, the duration and intensity of irrigation, type of soil, crop rotation.

The aim of the research was to determine the change in the microbial community of chernozem ordinary in irrigation by water of various quality (unsalted and saline water).

Methodology

The research was conducted in Steppe zone of Ukraine, where most of the irrigated land is located. The object of research was the irrigated agrolandscapes of Donetsk region (Ukraine) - Slavyansk and Maryinka stationary experimental sites (Figure 1).

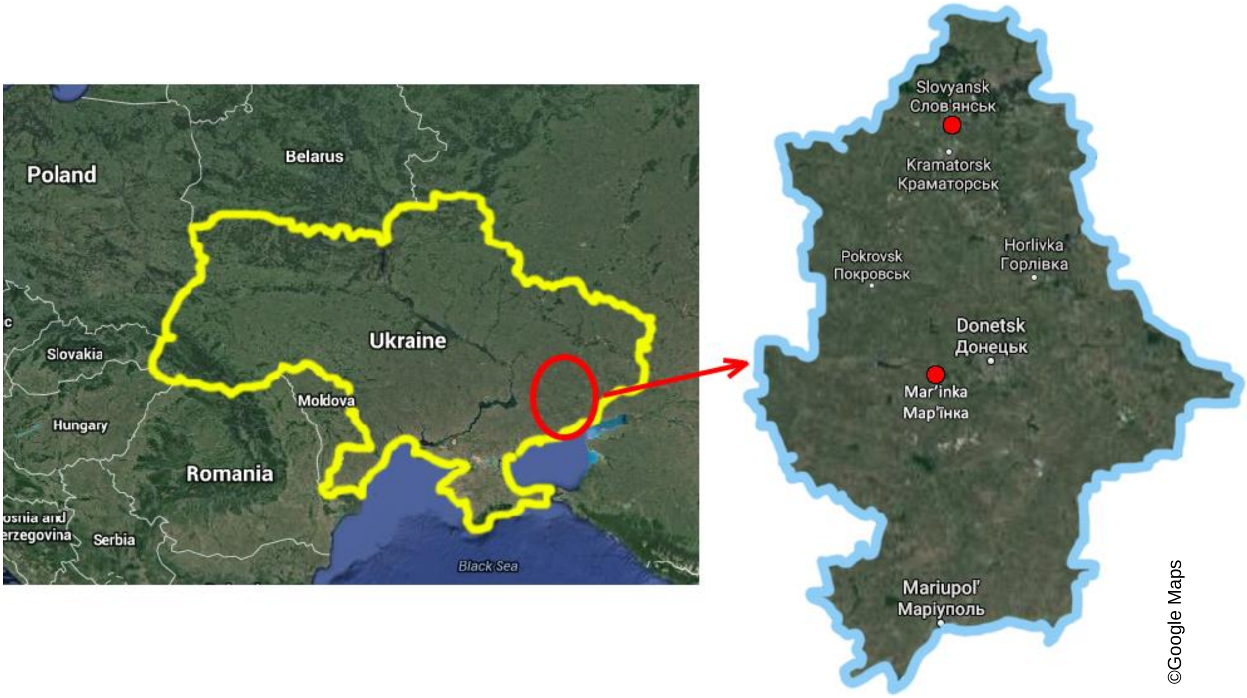

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Figure 1: The points of sampling at the stationary experimental sites, modified by the authors

In the Slavyansk stationary experimental site, unsalted water is used for irrigation (mineralization 0.61-0.63 g/dm^3). It classified as suitable for irrigation on agronomic and ecological criteria's. In the Maryinka stationary experimental site, the soils are long irrigated by saline waters (mineralization - 2.9-3.2 g/dm^3). They are estimated as unsuitable for irrigation, but given the lack of unsalted waters in this region, they are used for irrigation.

Irrigated lands a long time (45-50 years) have been used in irrigated agriculture. Soil samples for microbiological research were taken from a layer of 0-25 cm of Slavic and Maryinsky stationary experimental sites. The object-comparison were similar non-irrigated soils. On the territory of each stationary experimental site we laid 3-5 points on which soil samples were taken.

We determined number of soil microorganisms belonging to basic ecological-trophic, taxonomical and physiological groups by methods, generally accepted in soil microbiology (Zvyahintsev *et al.,* 1980; National Standard of Ukraine, 2016), namely by sowing dilutions of soil suspensions on dense nutrient media. The number of organic nitrogen assimilating bacteria was counted on the meat-peptone agar (MPA), mineral nitrogen utilizing bacteria and actinomycetes were grown on starch-ammonia agar (SAA), fungi on Rihter’s medium, oligotrophic microorganisms were cultured on starvation agar (SA). Sowing of the suspension was plated in fourfold replication from dilution 1:10^4. Summary biological index (SBI) and biological degradation index (BDI) were calculated by the method of relative values by Azzi (1959).

The main methods were field, analytical, and statistical. For soil quality estimation we also used the level of provision of ecosystem services (supply services, support services (providing a living environment for plants and microorganisms, ensuring biodiversity). 10 indicators were used, including the state of microbial community.

Results

Microbiological research showed that in the soil of the Slavyansk stationary experimental site (table 1), in conditions of long irrigation by unsalted water, the number of organic nitrogen assimilating bacteria changed insignificantly compared to non-irrigated soil (at the level of the least significant difference (LSD_{05}). Differences between the total number of microorganisms assimilating mineral nitrogen in the irrigated soil of this stationary experimental site and non-irrigated soil have not been established. However, due to irrigation, the number of bacteria increased (on 83 percent), and actinomycetes, vice versa, decreased (on 38 percent). The number of oligotrophs decreased on 35 percent, eutrophs - on 8 percent, mushrooms - on 48 percent.

Table 1: The number of microorganisms of the main ecological, functional, trophic and taxonomic groups in the soil of Slavyansk and Maryinka stationary experimental sites

Sampling points, stationary experiment	**Nitrogen assimilating microorganisms, mln. CFU/g**		**Actinomycetes, mln. CFU/g**	**Fungi, th. CFU/g**	**Oligotrophs, mln. CFU/g**	**Eutrophs, mln. CFU/g**	**Indices**	
	organic	**mineral**					**SBI, %**	**BDI, %**

Slavyansk, without irrigation	11,04	20,46	13,97	18,72	25,06	31,52	100,0	0
Irrigation by unsalted water	8,45	20,59	8,71	9,68	16,28	29,05	87,7	-12,3
Maryinka, without irrigation	12,24	19,98	6,21	41,60	22,88	32,26	100,0	0
Irrigation by saline water	6,62	11,11	3,94	79,86	16,94	17,81	64,6	-35
LSD$_{05}$	2,55	3,02	1,01	5,13	1,92	3,01	-	-

Under the influence of irrigation and the changes that occurred, the mineralization index in the soil increased, which indicates a more intensive course of mineralization processes. The coefficient of microbial transformation of soil organic matter and the summary total biological index were lower in irrigated soil. They were 11.9 and 87.7 respectively. This is a confirmation of the lower level of functional activity of microbial community. In the samples of the irrigated soils of the Slavyansk stationary experimental site, the total number of microorganisms decreased on 12 percent. The degradation degree of irrigated soils was estimated as low, and BDI was 12.3 percent.

At the Maryinka stationary experimental site, research was conducted in the vegetable-fodder crop rotation in long irrigation by saline water (for 20 and 50 years). The number of all groups of microorganisms (except fungi) in irrigated soil for 20 years was significantly lower than in non-irrigated: organotrophic bacteria - 23 percent, microorganisms assimilating mineral nitrogen - 20 percent, actinomycetes - 37 percent, oligotrophs - 16 percent, eutrophs - 21 percent.

With an increase the irrigation period (to 50 years), changes in microbial community were more significant. This is evidenced by a decrease in the number of mineral and organic nitrogen assimilating bacteria. The oligotrophic index increased from 0.71 to 0.95. This indicates about deterioration in the nutrient regime of irrigated soils.

SBI in irrigation by saline water decreased to 65 percent. This indicates about a low level of functional activity of microbial community (Naydyonova and Vorotyntseva, 2015). Long irrigation of chernozem ordinary causes a moderate degradation degree (BDI = 35 percent) and toxicosis is due to the accumulation of fungal toxins in the soil. A decrease in the intensity of changes is associated with a decrease of anthropogenic load on the soil and decrease the irrigation norms.

Discussion

The research results showed that the microbial community and the degree of their transformation in the studied soils depends on the quality of irrigation water, the irrigation duration and crop rotation. The quality of irrigation water influences upon directivity of soil processes and evolution of soils, often bringing to development epy soil degradation processes - salinization, alkalinization, changes in the qualitative and quantitative composition of microbial community and other of degradation processes.

In irrigation the chernozem ordinary by unsalted water, significant changes in the number of microorganisms assimilating organic and mineral nitrogen were not detected. An increase the mineralization index indicates a more intensive development of mineralization processes. The direction of changes in the microbial community of the chernozem ordinary of the Slavyansk stationary experimental sites was determined not only by irrigation, but also by crop rotation and fertilizer systems. The soil degradation degree of irrigated by unsalted water was rated as low.

At the Maryinka stationary experimental site, irrigation by saline water contributed to a decrease of the number of soil microflora and the number of microorganisms of the main ecology-functional groups. The decrease of the number of microorganisms in the irrigated ordinary chernozem increased with increasing time of irrigation. In the long-irrigated soil, we found an increase in the number of fungi due to the narrowing of their generic composition with a quantitative predominance Penicillium і Aspergillus.

Microbiological indicators are important indicators for assessment of the ecology-agroameliorative condition of the irrigated soil. Therefore, we have included them in the list of mandatory indicators. We also used them for evaluation of the ecosystem services of the studied soils.

The soils of the Slavyansk stationary experimental site (irrigated and non-irrigated) and non-irrigated soil of the Maryinka stationary experimental site were characterized by a high level of ecosystem services - 8.1-8.8 points. The irrigated soil of the Maryinka stationary experimental site was characterized by middle level - 5.6 points. One of the limiting factors is the low microbiological activity of the soil.

The results of estimation of the ecosystem services level provided by chernozem ordinary are the basis for making managerial decisions on their rational using and elaboration the complex differentiated, adapted measures for melioration and improvement of their condition.

Conclusions

Microorganisms are very sensitive to changes of ecological factors, therefore, the number and composition of microflora can be diagnostic indicators for estimation the transformation of soil properties. High information of the microbiological indicators necessitates their inclusion in the system of indicators of ecological and ecology-agroameliorative monitoring of irrigated soils.

Irrigation by saline water leads to the transformation of the soil microbial community. The soil characterized the middle level of degradation (BDI=35 percent). Therefore, it is necessary to apply measures to improvement of irrigation water quality and soil properties.

Soil biodiversity is one of the important indicators of soil quality and determines the level of provision of ecosystem services.

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Utilization of soil microbial diversity for crop production in Sri Lanka

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Abstract summary

Although microorganisms represent a very small fraction in soil, they play vital roles in the maintenance of soil health and provision of nutrients to plants in a sustainable manner. In these studies eubacterial, cyanobacterial and fungal taxa were isolated from different soils regularly used for short term crop cultivation. The isolates included heterotrophic eubacteria, symbiotic rhizobia from legume root nodules, photoautotrophic cyanobacteria and free living and rhizospheric fungi. Screened and selected isolates were lab and field tested for their ability to associate intimately with targeted crop plants, form efficient symbiotic root nodules with legume crops and the best candidates were used to prepare solid and liquid biofertilizer inoculants. Two main groups of biofertilizers: rhizobial biofertilizers and biofilm-biofertilizers were prepared and tested in farmers' fields in several different locations. Performances of the inoculants were always tested in comparison to mineral fertilizer levels recommended to the different crops. Results showed that rhizobial inoculants can replace the application of N-fertilizer (urea) completely and biofilm-biofertilizers can replace at least 50 percent of all three N, P & K fertilizers without any reduction and sometimes with increase in crop yields.

Keywords: biofertilizers, rhizobiology, biofilm-fertilizer, minimizing chemical fertilizer, eco-friendly agriculture.

Introduction, scope and main objectives

Application of chemical fertilizer though capable of producing high yields in short term crops, the yields level off with time and continuous use of chemicals becomes ecologically detrimental in the long run. It has been demonstrated that chemical fertilizer additions result in the depletion of soil microorganisms which maintain soil health (Seneviratne *et al.,* 2011) and increasing crop yields on such 'dead soils' is a tremendous task. Moreover, nutrient absorption from chemical fertilizers is highly inefficient and 60 to 70 percent of the added nutrients are not taken up by crop plants. The residual chemicals are liable to all types of losses resulting in environmental pollution. Losses of highly labile nitrogen fertilizers have been shown to be largely responsible for environmentally related health problems. Such consequences led to the decision to reduce chemical N-fertilizer use by at least 20 percent by the year 2030 as a sustainable development goal.

In Sri Lanka excessive application of chemical fertilizers had been suspected as a major contributory factor responsible for chronic kidney disease of uncertain etiology (Jayatilake *et al.,* 2013) and cancer and 'blue baby syndrome' particularly in Northern Sri Lanka (Jeyakumaran, 2013). Environmental pollution especially from P-fertilizer losses have led to toxigenic cyanobacterial bloom formation in freshwater reservoirs of Sri Lanka (Kulasooriya, 2017).

The present studies were therefore undertaken to develop biofertilizers that could minimize the application of chemical fertilizers in crop production.

Methodology

Rhizobial biofertilizer

Isolation and purification of rhizobia followed procedures described by Somasegaran and Hoben (1994). Initial isolations from root nodules of legumnous crops were done on Congo Red Yeast Mannitol Agar to detect contaminants that appear pink on this selective medium. Both split (for large nodules) and crush (for small nodules) methods were used. After several successive sub-culturing isolated pure cultures were maintained on Yeast Mannitol Agar (YMA). These isolates were authenticated using the original host plants and screened in a greenhouse for effective nodulation and plant growth. The best strains were then semi-mass cultured in 20L aspirator bottles under sterile conditions in YM broth. Once a culture reached a density around 10^8 cells/ml 15ml of the broth culture diluted to 150ml with sterile water was injected into 250g of autoclaved, modified coir dust packeted in a polypropylene bag (Seneviratne, Van Holm and Ekanayake, 1999). These bags were incubated in the dark, under room temperature (25°C to 33°C) for a week to mature. Such carrier-based inoculants are supplied for use by the farmers. Agronomic scale field trials were conducted with soybean (*Glycine max*), mung bean (*Vigna radiata*), vegetable bean (*Phaseolus vulgaris*), groundnut (*Arachys hypogea*) and the forage legume clover (*Trifolium repens*) to compare the yields obtained with the recommended levels of urea with those obtained with inoculation in the absence of urea.

Biofilm-biofertilizers are multi-microbial inoculants (bacteria and fungi) growing in a common matrix. Preparation of biofilm-biofertilizers followed the procedure described in Seneviratne *et al* (2011). Studies with biofilm-biofertilizer were designed to compare plant and endophytic microbial parameters under farmers’ practices of chemical fertilizer application with biofilm-biofertilizer inoculation using rice (*Oryza sativa* L.) as the test crop. Field trials were conducted in 37 representative farmers’ fields with diverse soil types spreading over thousands of hectares in Ampara (n=3), Kurunegala (n=14), Hambantota (n=8) and Polonnaruwa (n=12) districts. Two consecutive, uniformly managed paddy fields were used to apply the treatments separately. The treatments were (i) biofilm-biofertilizer practice (1000 ml of biofilm-biofertilizer with 225 kg NPK/ha) and (ii) Farmers' practices (425 kg NPK/ha). Paddy was broadcasted and irrigation water was managed separately in the two fields, without mixing from surrounding fields. The two consecutive treatment plots were taken as a block design in each site. Thirty-seven field locations acted as replicates.

Four random rice hills with rhizosphere soil were uprooted carefully at 50 percent flowering stage from each paddy field. Soil was carefully removed, and the root system was washed without damaging it. Roots and shoots were separated, and oven dried at 65°C for constant weight, and then root dry weight (RDW) and shoot dry weight (SDW) were recorded. Seed samples were collected from four random hills at physiological maturity stage. Yield was analysed by performing five 1 m x 1m crop cuts in each plot and thousand grain weight (TGW) was measured.

Endophytic diazotrophs (ED) and non-diazotrophs (END) in plant leaves were enumerated by culturing them at 10^{-6} dilution in combined carbon medium (CCM) (Rennie, 1981) and modified CCM medium (CCM + NH_4NO_3), respectively. Colony counts were taken after 48 hours.

Means and correlations of all the variables under biofilm-biofertilizer practice and farmers' CF practice were calculated. T-test was done for mean comparison after confirmation of normal distribution of data using normality test. All data were analysed statistically using Minitab 17 version.

Results

In all the trials with legume crops the yields obtained with inoculation were either equal or marginally above those obtained with urea application. A fertilizer N-response curve obtained with soybean showed that the effect of inoculation superseded that of the highest level of urea application (Figure 1).

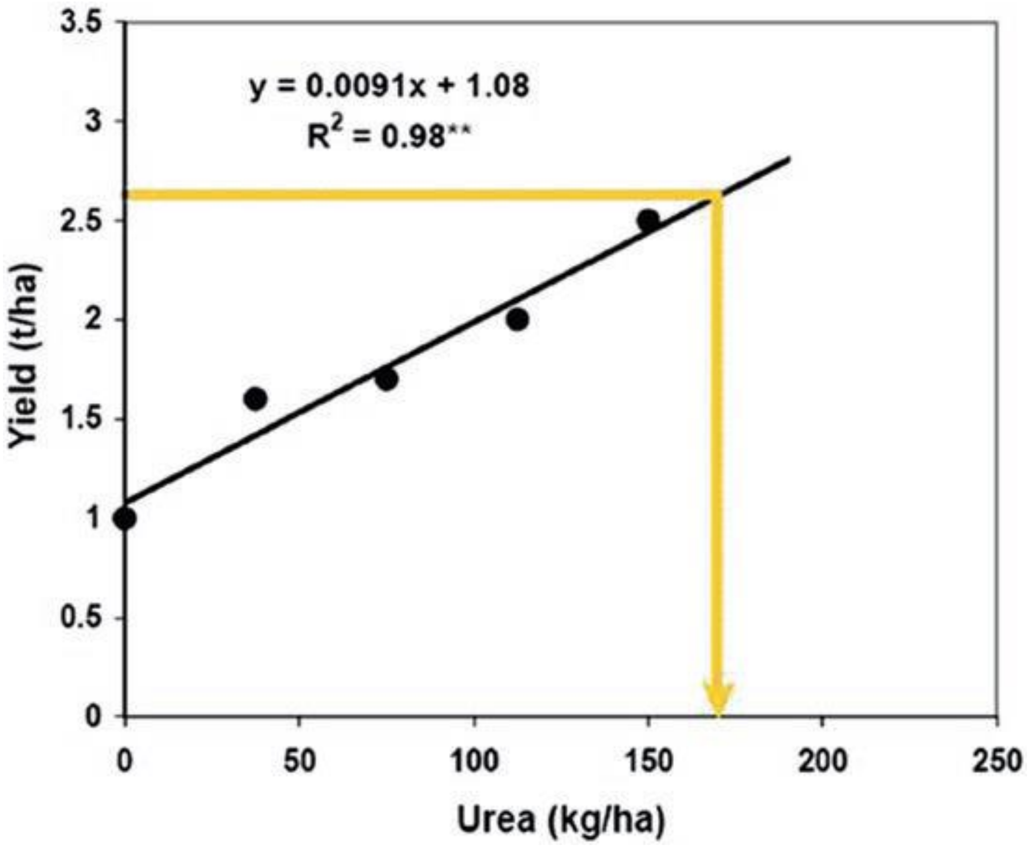

Figure 1: Yield response of soybean to different levels of urea fertilizer

The yellow arrow indicates the yield obtained with inoculation which marginally superseded that obtained with the highest level of urea. (Reproduced from Kulasooriya *et al.,* 2017)

A similar yield response curve was also obtained with vegetable bean (*Phaseolus vulgaris*). In this experiment measurement of weed biomass under the different treatments showed a 60 percent reduction in weed growth under inoculation in comparison to the full N-fertilizer application. This is understandable because nitrogen fixed by root nodules is largely available to the host legume while broadcast urea is freely available to all plants including the more aggressive weeds.

Rice grain yield increased by 24 percent (Table 1) with the biofilm-biofertilizer practice over farmers' CF alone practice (P = 0.002) due to improved grain filling as reflected from increased TGW (P = 0.063). Enhanced plant growth with the biofilm-biofertilizer application may have contributed to this, as shown by increased SDW (P = 0.000) and RDW (P = 0.017). Increased END more than ED with the biofilm-biofertilizer practice over farmers' CF alone practice (P = 0.082) seem to be responsible for this process as END's role in plant growth is well known (Hardoim, van Overbeek and van Elsas, 2008). Generally, EDs' role is to fuel communities of ENDs by supplying biologically fixed N_2 for their growth and functions.

Table 1: Plant and endophytic microbial parameters of the biofilm-biofertilizer practice and farmers' CF practice of rice cultivations

Parameter	Biofilm-biofertilizer practice (n = 37)	Farmers' CF alone practice (n = 37)	Difference*
Grain yield (kg/ha)	5860 ± 243	4733 ± 173	1127 (0.002)
Thousand grain weight (g)	20.92 ± 0.65	17.73 ± 0.72	3.19 (0.063)
Shoot dry weight (g/m^2)	3293 ± 245	2150 ± 135	1143 (0.000)
Root dry weight (g/m^2)	1130 ± 100	733 ± 128	397 (0.017)
Endophytic diazotrophs (colony count per plate)	191 ± 38	106 ± 30	85 (0.121)
Endophytic non-diazotrophs (colony count per plate)	56 ± 5	43 ± 5	13 (0.082)

Mean ± SE values in each column. *Values within parentheses are probability levels at which differences are significant. Plant samples were collected at 50% flowering whereas yield parameters were obtained at physiological maturity.

Discussion

Results obtained with all the legume food crops tested in farmers' fields in different locations in Sri Lanka have confirmed that rhizobial inoculants have the capacity to completely replace urea application without any reduction in crop yields and sometimes with marginal increases. Currently our project annually supplies rhizobial inoculants to about 10,000 acres of legume crop cultivations and the demand for inoculants by farmers show an increasing trend.

Application of biofilm-biofertilizer increases ENDs among other things, which contribute to hormone production, bio-control of pathogens etc., thus supporting the increase of plant growth and yield. Under large scale rice cultivations in farmers' fields, biofilm-biofertilizer can replace at least 50 percent of all three N, P & K fertilizers without any reduction of the harvest and sometimes with increase in crop yields. This biofertilizer practice has now been applied to thousands of acres of the rice cultivation in the country. Limited field trials conducted with biofilm-biofertilizer have also shown that at least a 50 percent reduction of N, P & K chemical fertilizer application to corn, several vegetable and fruit crops as well as plantation crops like tea can be achieved without any reduction in crop yields.

Conclusions

Application of biofertilizer inoculants has a tremendous potential to enhance eco-friendly agriculture. This is bound to receive a boost as the new Government of Sri Lanka had decided to embark upon an ambitious program from this year to minimize the application of chemical fertilizer in crop cultivation by adopting alternative non-chemical inputs.

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Bacterial community in melon/cowpea intercropping systems with reduced nitrogen application

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Abstract summary

The bacterial community and abundance of N-cycling microbial groups impact of different melon/cowpea intercropping systems under reduced nitrogen application was compared against usual monocrop system (melon or cowpea). We found that the bacterial communities in the melon/cowpea intercropping induced a synergetic interaction which modified the structure of microbial communities compared to monocropping. Furthermore, increase of different species identified as growth promoting bacteria were also observed and the N-cycling was improved.

Keywords: 16SrRNA; nirK; AmoA; melon/cowpea.

Introduction, scope and main objectives

Agricultural management practices and cropping systems can evidently influence crop yield, soil physicochemical characteristics, and soil microbial activity and composition (Singh *et al.,* 2016). Intercropping systems consist of simultaneous growth of two or more crop species on the same area and at the same time (Brooker *et al.,* 2014). It can effectively improve the mobilization and uptake of nitrogen, phosphorous, potassium, and micronutrients through interspecific interactions in rhizosphere (Zuo and Zhang, 2008). Most recent research has focalized on the potential of intercropping in sustainable productions and in particular by using grain legumes like cowpea (*Vigna unguiculata L. Walp)* adapted to stressful environments, associated with high temperatures, drought or low fertility, that can improve legume nitrogen uptake by enhanced nodulation of legumes (Li *et al.,* 2007).

Soil microorganisms play an important role in soil biogeochemical processes, decomposition of organic matter and suppression of soil-borne pathogens (Li *et al.,* 2012). Their composition and diversity are imperative to maintain the plant biodiversity, soil health and productivity (Mangan *et al.,* 2010). The changes in soil microbial communities may be both a cause and reflection of the better performance in the intercropping systems.

The purpose of this study was to explore the responses of microbial communities to different combinations of intercropping (melon (*Cucumis melo*)/cowpea (*Vigna unguiculata*)), with decrease of external fertilizer inputs in a Mediterranean semiarid farm during one year. Deeping on the outcomes, new strategies to improve agricultural development in a sustainable way could be proposed.

Methodology

This study was carried out in Cartagena, SE Spain, with semiarid Mediterranean climate. The field experiment was designed as a randomized block with three replications, and each plot had 150 m^2. We compared a melon monocrop (M) and different melon-cowpea intercropping systems in summer 2018 (one-year time): Mixed intercropping (MC1) and row intercropping 1:1 (melon:cowpea) (MC2), row intercropping 2:1 (melon:cowpea) (MC3). A cowpea monocrop was established at the density of 5 plants per square meter, with a spacing of 100 cm between rows and 20 cm between plants, the traditional pattern of this crop (C).

Melon seedlings were planted in a density of 0.4 plants m^{-2}, with a spacing of 200 cm between rows and 120 cm between plants in all plots (monocrop and intercropped systems). Cowpea seeds were sown between two rows of melon in the row intercropped systems, spacing 100 cm between melon and cowpea rows. Under row intercropped systems, cowpea was separated 20 cm plants in the same row. Density of cowpea plants was 2.5 and 1.5 per square meter in the row 1:1 and row 2:1 systems, respectively. In the mixed system, cowpea was sown in all melon rows between two melon plants, and so in a density of 0.4 plants m^{-2}, with a spacing of 200 cm between rows and 120 cm between plants. Density of melon was the same in the different treatments, but the density of cowpea changed depending on the crop system. All crops were drip irrigated and grown under organic management. The fertilizer rate was reduced by 30 percent in the intercropping compared to melon monocropping. Melon and cowpea were simultaneous harvested from 31 July to 10 August. Harvest was carried out manually as traditionally performed in the area to avoid damages in the melon fruits, since they lie on the soil.

Soil DNA from each treatment was extracted using the DNeasy Power Soil Kit (Qiagen). Ion 16STM Metagenomics kit from Thermo Fisher Scientific® was used to analyses bacterial community using the Ion Torrent™ semiconducter sequencing workflow. Two pair of primers from 16S ribosomal DNA regions (V2-4-8 and V3-6, 7-9) was used. The amoA, nirK and narG gene of the nitrogen cycle was investigated by qPCR in all treatments based on the ISO standard 17601 (ISO, 2016). The Shannon diversity index (H) and Inverse of Simpson index (1-D) was calculated using the Vegan package (Oksanen *et al.,* 2019) respectively in R.

Results

In intercropping systems, melon yield increased by 34-70 percent and marketable yield by 40-80 percent compared to melon monocrop. The best intercropping system for this parameter was mixed (MC1) and the row 2:1 intercropping (MC3), with values of 26,272 kg ha^{-1} and 24,759 kg ha^{-1} respectively. PCoA analysis based on OTU level indicated a significant difference between intercropping systems and monocropping (PERMANOVA F = 2.311; P = 0.014) (Figure 1). No significant differences on bacterial diversity represented by the Shannon index were observed (Figure 1).

The predominant phyla of bacterial community were Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes and Gemmatimonadetes, which occupied more than 90 percent of the total sequences. The relative abundance of different genera showed significant differences between intercropping systems and monocrop (PERMANOVA analysis F=3.851; P=0.002). Higher abundance of Proteobacteria in intercropping systems was observed. Compared to monocrop, intercropping systems had higher *Sphingomonas*, *Pseudomonas* and *Skermanella* and lower *Solirubrobacter, Rubrobacter* and *Amoniphilus* (Figure 2 A). The abundance of marker genes for nitrification (amoA) and denitrification (nirk, narG) did not show significant differences between treatments, although there is a decreasing trend in intercropping systems (Figure 2B).

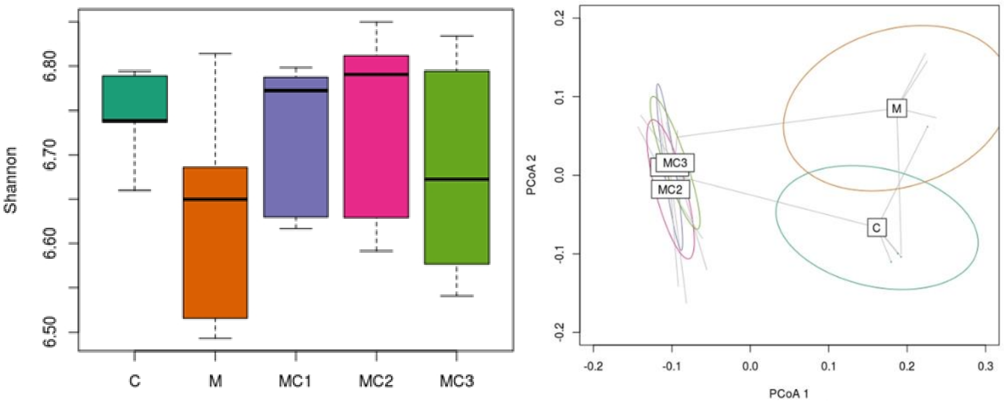

Figure 1: Shannon diversity index (left) and PCoA of the bacterial community of the different cropping systems (right)

Melon monocrop (M); Cowpea monocrop (C); Mixed intercropping (MC1); Intercropping 1:1 (melon:cowpea) (MC2); Intercropping 2:1 (melon:cowpea) (MC3).

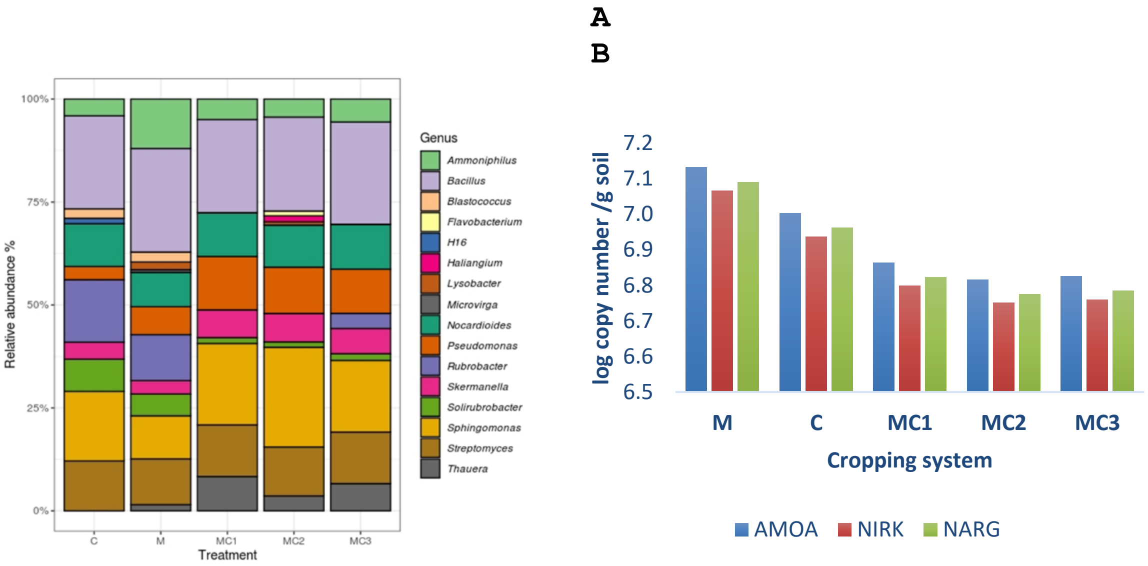

Figure 2: Relative abundance (>1%) of bacterial genus (A) and abundance of N-genes markers of cultivation systems

Melon monocrop (M); Cowpea monocrop (C); Mixed intercropping (MC1); Intercropping 1:1 (melon:cowpea) (MC2); Intercropping 2:1 (melon:cowpea) (MC3).

Discussion

In this study, the increase in crop yield with melon and cowpea intercropping systems was confirmed. Interspecific competition for nutrients resources and belowground interactions is well known to affect crop growth and grain yield in intercropping systems (Corre-Hellou, Fustec and Crozat, 2006). In addition, cowpea crop has a very active rhizosphere, with intense rhizodeposition that activates microbial populations, with the capacity of mobilizing soil nutrients (Sánchez-Navarro *et al.*, 2019).

The Shannon index of bacterial community was similar in all cultivation systems, similar to the results of Paungfoo-Lonhienne *et al.* (2017) using legumes in rotations. The overall microbial community composition significantly differed between monocrop and intercropping systems. This was in agreement with the previous work by Alvey *et al.* (2003), who also demonstrated that legume crop rotation has a substantial effect on the structure and diversity of soil microbial community. Dominant phyla found in this study was in line with previous studies investigating plant-associated bacterial and fungal communities (Bulgarelli *et al.,* 2013) and higher abundance of Proteobacterias in intercropping systems can be related to higher AOB in these soils. Also, in intercropping systems, higher members of the genera *Pseudomonas* and *Sphingomonas*, which are well-known as plant growth-promoting bacteria and/or for the production of secondary metabolites (Lodewyckx *et al.,* 2002). Moreover, effective use of nitrogen is considered the most important factor with respect to the advantages of intercropping. Abundance of genes related to N-cycling, in nitrification (amoA) and denitrification (nirk, NarG) genes, indicated no differences between monocrop and intercropping systems, but trending to be lower in the last; this fact ould indicate the effectiveness of legume (cowpea) in the intercropping compared to monocropping, that it could fix nitrogen, reducing the functional genes of soil microbial communities to N uptake.

Conclusions

Intercropping (melon/cowpea) can contribute to multiple agroecosystem services by increased yield, reduce the use of chemical fertilizer and change bacterial community to increase soil quality, increasing plant growth-promoting bacteria and the nitrogen efficiency use in the agroecosystem.

Acknowledgements

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Soil biodiversity and sustainable vineyards: Hints from the analysis of microarthropod communities

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Abstract summary

The aim of this study, started in 2011, was to evaluate the long-term effects of organic management on soil quality in a commercial vineyard situated in the district of Verdicchio of Matelica (Marche, Italy). Assessment of soil quality was performed by using microarthropods as indicators. In the selected vineyard, three study plots selected within three vineyards differing in the length of their organic management histories, were chosen. Namely, in the vineyards denominated as V92, V98 and V09, organic management was started in 1992 (19 years under organic management), 1998 (13 years) and 2009 (2 years) respectively. Soil samples were taken once a month from March to October 2011 for a total of 8 sampling events for each study plot. The measured biological parameters for microarthropods were: abundances, QBS-ar index, number of biological and euedaphic forms and diversity indices (Shannon, Margalef, evenness). Results show that plots with a longer organic management history, were associated with more stable/resilient microarthropods communities, thus supporting the hypothesis that organic management contributes to soil quality improvement at least in vineyards belonging to this particular pedoclimatic area.

Keywords: Bioindicators, QBS-ar, Agro-ecosystem, Microarthropods, Farming practices, Viticulture, Organic management, Soil health

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Introduction

Soil fauna is one of the most important factors influencing soil functionality, modifying its chemistry and structure. Faunal roles include pre-decomposition of litter, cycling of nutrients, modification of soil structure, but it also takes part in the control of populations of microorganisms, fungi and pests. Species richness and diversity of soil organisms influence soil functionality, thus empowering its competence in providing pivotal ecosystem services (Barrios, 2007). In the last years we assisted to an increase of the interest in this topic, and in the application of biological indicators to monitor soil health in both natural and agro-ecosystems. Among the various available indices to estimate soil biological quality, the QBS-ar index (Parisi *et al.*, 2005) is based on the analysis of the microarthropods communities. This index is based on the concept of biological and euedaphic (well-adapted to soil) forms, attributing EMI (Eco-Morphological Index) scores, which goes from 1 to 20, in proportion to the level of adaptation of the identified biological forms to soil habitat. Accordingly, euedaphic forms receive the highest score, while the less adapted forms receive lower scores. In final, QBS-ar value is calculated by adding up EMI scores of all the biological forms (euedaphic and non-) identified in the samples according to Parisi et al (2005).

In this study, started in March 2011, the QBS-ar Index was used to assess biological soil quality in 3 study plots, located in a commercial vineyard in the district of Verdicchio of Matelica (Marche, Italy), that were organically managed since 1992 (19 years under organic management), 1998 (13 years) and 2009 (2 years) (hereafter named as V92, V98 and V09, respectively). Further, vineyards have undergone to alternate management of the tractor-rows on either side of the vine row with tillage and non-tillage annual cover crop species (clover, common vetch, oats) (Figure 1a-e)

Methodology

Sampling design and QBS-ar Index

Sampling was performed following the method proposed by Parisi *et al.* (2005) once a month from March 2011 to September, except for the month of August. Two additional samplings were done in June and September, fora total of 8 sampling events.

As shown in Figure 1, the study plots were located at the centre of each selected vineyard (Figure 1a). Further, in each plot were identified two adjacent sampling units, each one represented by the disturbed (tillage) and un-disturbed (no-tillage) grassed inter-rows (Figure 1b). For the purpose of the QBS-ar analysis, a sampling unit is composed by three soil cores (replica) (10X10X10 cm),at about 2 m distance from each other, in both disturbed (tillage) (*ifd*) and not-disturbed (no-tillage) (*ifu*) grassed inter-rows.(Figure 1b-e).

Samples were collected using a corer of 10 cm diameter per 10 cm depth (ca. 785.40 cm^3), immediately transported in the laboratory and placed in a Berlese-Tullgren apparatus for the extraction of microarthropods. Collection and storage of extracted arthropods was done in a solution of 70° alcohol (2 parts) with glycerol (1 part). Identification of microarthropods was accomplished following the scheme of Parisi *et al.* (2005). Obtained data were used to calculate QBS-ar index, abundances of individuals (*N*) and richness values, taking into account the number of biological forms (BF) and the number of euedaphic forms (EF). During sampling the main chemical-physical parameters (pH, moisture, temperature) were also measured using a soil pH meter (PH-212; Lutron, London, U.K.), a moisture meter (PMS-714 Lutron), and a thermometer (HI-98501; Hanna Instruments, Milan, Italy), respectively. Soil texture analyses and further chemical analyses were performed at the laboratory of Agrochemistry of ASSAM (Jesi, Italy).

Statistical analysis

The following software were used for the statistical and multivariate analysis: PAST v.2.17c package (Hammer *et al.*, 2001) and BioEstat v.5.3 (Ayres *et al.*, 2007). Data used for statistical analyses were number of BF, number of EF and absolute abundances (*N*). Diversity indices were calculated at the level of order and family. In particular, index of *Shannon-Wiener*(*H'*), *Margalef index* (*d*) and equitability (*J*) were determined. The presence of significative differences between different sample sets was investigated using the non-parametric *Kruskal-Wallis* test.

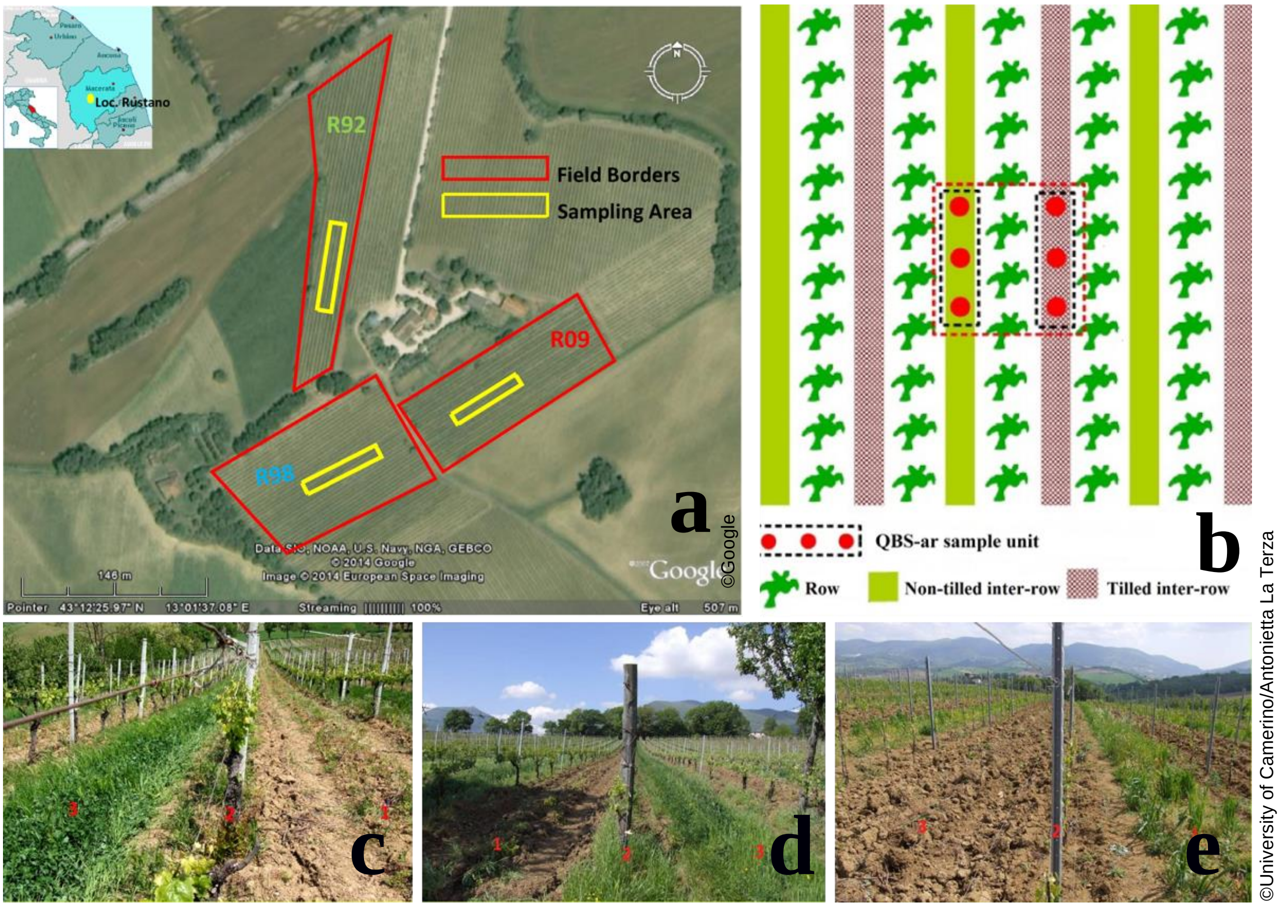

Figure 116: Google maps view of the three vineyards and study plots/areas (a); scheme of sampling design (b); representative images ofthe three study plots V92 (c), V98 (d), V09 (e), showing the disturbed (tillage) (*ifd*) and not-disturbed (no-tillage) (*ifu*) grassed inter-rows

Results

As shown in Table 1, the comparison of microarthropod communities in the disturbed (tillage) (*ifd*) and undisturbed (no-tillage) (*ifu*) inter-rows of the three plots, based on the richness of the biological forms (BF), abundances and diversity indices (H', d, J) highlight some differences between the *ifd* and *ifu* inter-rows. In all the three analysed study plots, and irrespective of the time under organic management, the abundances were higher in the undisturbed, no-tilled (*ifu)* inter-rows than those in the disturbed, tilled *(ifd*) inter-rows. However, no significant differences among the tilled (*ifd*) and no-tilled (*ifu*) inter-rows for H', d, J values were detected for the three plots. On the other hand, the comparison of microarthropod communities in the disturbed (tillage) (*ifd*) and undisturbed (no-tillage) (*ifu*) inter-rows of the three vineyards, based on the values of QBS-*ar* and abundances of FB and EF (Biological and Euedaphic forms), show and solely for the oldest vineyard V92, no significant differences (Table 2). On the contrary, significant differences (with the exception of the EF value in V98) between the disturbed (tillage) (*ifd*) and undisturbed (no-tillage) (*ifu*) inter-rows were detected for the V98 & V09 vineyards.

Table 1: Biological Forms (BF) richness (S), Abundances (N) and Diversity Indices (H, d, J) in the three study plotsV92, V98 and V09 for the disturbed (tillage) (*ifd*) and undisturbed (no-tillage) (*ifu*) inter-rows

	V92-ifd	V92-ifu	V98-ifd	V98-ifu	V09-ifd	V09-ifu
BF (S)	21	21	19	21	19	22
Abundance (N)	547	1147	733	1648	589	2291
Shannon (H)	2.349	2.46	2.287	1.448	1.982	1.906
Margalef (d)	3.172	2.839	2.728	2.7	2.822	2.714
Equitability (J)	0.7715	0.8081	0.7768	0.8042	0.6732	0.6165

Table 2: Kruskal-Wallis test: comparison between the disturbed (tillage) (*ifd*) and undisturbed, grassed (no-tillage) (*ifu*) inter-rows in the three study plots V92, V98 and V09

	V92	V98	V09
QBS-ar	0.6737 n.s.	0.0311	0.0269
BF	0.0691 n.s.	0.0067	0.0567
EF	0.4132 n.s.	0.3594 n.s.	0.0170

Discussion

Results obtained from QBS-ar calculation and statistical analyses highlighted differences in soil-dwelling microarthropods communities collected from the three vineyards (V92, V98 and V09), that differ in the length of their organic management histories. Results obtained from V92, the oldest vineyard, show no significant differences between tilled and no-tilled inter-rows relatively to QBS-ar, BF and EF values (Table 2). According to Elliot and Lynch (1994), the higher stability of the microarthropod communities in the vineyard V92 (but also, in part in V98) could be due, to a higher soil resilience, probably reached during the long-term organic management (19 years). Similar results were also obtained, by La Terza *et al.* (2015) using, in this case, ciliated protists as bioindicators. Indeed, analyses performed using ciliates revealed a higher stability of their communities in the oldest vineyards (V92 and V98)with respect to those present in the youngest vineyard (V09), thus being in agreement with the microarthropods analyses carried at the same locations.

Conclusions

Results of this study show the benefits of organic management on soil biodiversity and soil health. The microarthropods communities experience less fluctuations (are more stable) in the oldest vineyard V92. As reported by Elliott and Lynch (1994), this effect may be due to the greater resilience of the soils of the vineyards V92 possibly achieved during the long term organic management. Only in the oldest vineyard (V92) and irrespective of the type of disturbance applied, the microarthropods communities remain stable/resilient in both the disturbed (tilled) and undisturbed (no-tilled) inter-rows than in the other vineyards. The data also show that the time required to achieve this result (increased stability of biological communities) is quite long and it commence to be appreciable not before than at least 15-16 years of organic managements.

In final, this study helps in evaluating the long-term effects of common organic vineyard floor management practices as well as, in raising awareness among stakeholders and policy makers on the importance of soil biodiversity in preserving soil health and assisting them to select, promote and stimulate adequate sustainable farming practices.

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From soil to table: agroforestry systems as an alternative to regenerative agriculture

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Abstract summary

Soil quality is directly related to the diversity of life and availability of nutrients, and how these interactions happen, so that we have rich soil. The health of the plant depends on the health of the soil and the diversity of species, and just as it does below the ground, above it, there is also an intelligent, complex and perfect logic, which from the observation and practice associated with the study, allows us to understand these logics and manage the systems in order to harvest food, water and life, generating energy and leaving the environment more abundant and healthier.

Agroforestry systems are an alternative for agricultural production, where *Agro* refers to the act of farming the land and, *forest* refers to a system based on the principles of the forest.

Agroforestry systems can occur in different regions and climates, as long as it respects and follows the logic of the forest, which has general and specific characteristics, according to its region being planned and executed according to its principles.

Our practical experience has shown that the Agroforestry Systems contribute to the recovery of the soil, in the processes of decompression, erosion, humidity, increased diversity of meso and macrofauna, color, texture and increased production quality.

Keywords: agroforestry, syntropy, soils, regenerative agriculture, family farming, food security.

Introduction, scope and main objectives

This work aims to present the agroforestry system as an agricultural technique that contributes to the recovery of soil life, in levels of organic matter, macro and micro life, pH, base saturation, and levels of AL + 3, taking as an example my practical work in Brazil, where I produce vegetables, fruits, mushrooms, yerba mate and medicinal herbs as main products and sell them at an organic market weekly. Another objective is to present agroforestry as a possible and applicable technique in different regions of the world, producing food and improving soil quality, quality of life for families and the ecosystem as a whole.

The agroforestry system is the methodology used to practice agriculture based on the principles of the forest, which is a complex and balanced environment with the capacity to generate energy instead of just consuming it, presenting itself as an alternative system to the conventional agricultural system, which in turn degrades and destabilizes the soil and the entire ecosystem and teaches us how we, human beings inhabitants of this planet, through our management can optimize the recovery of the environment, as Ernst Götsch teaches us, In nature, the recovery of degraded soils can take a long time, and their abbreviation is one of the goals of restoration projects (Götsch, 1996).

If we let the environment manifest itself without human intervention, we will observe a sequence of facts that deal with forestry principles. The first species to be born will be the herbs and grasses that in agroforestry we call the *placenta,* which, like the human body, creates conditions for other plants to grow, heal, cover and protect the soil, producing organic matter, are more resilient plants and, grow on poorer soils. Then, with better living conditions in the soil, fast-growing shrubs and trees arrive, with deeper and larger roots that will work the land more intensively, seek water and nutrients from the depths, making it available on the surface; they are trees that produce more water when transpiring, produce more seeds and fruits, recovering fauna and flora and supplying all organic matter that feeds the soil, contributing to, subsequently, germinating the most mature trees that need understory conditions and more fertile soils to be there, in all stages of succession there is always a great diversity of plants.

According to Primavesi (2002), the diversification of life in the soil is linked to the amount of organic matter available. In a forested environment we will never see soil without organic matter on its surface, this organic matter feeds micro-life which in turn works to make the soil more porous and permeable, allowing water to return to the water tables, passing through the entire root system, in addition to the thermal balance provided by the vegetation cover.

By introducing a wide variety of plants into the system, we also balance the life of the soil as each plant interacts differently with the nutrients and life there. According to Primavesi (2002) in her book: Ecological soil management "In mixed and interspersed cultures, rooting can be perfect, if the plant species match. Different species can take root in the same space in the soil, as they have different nutritional requirements and different root excretions, which can even be absorbed directly by the other species, thus contributing to their better development.

Methodology

For the implantation of an agroforestry system, it is important to have clear which is the objective of the farmer and which are the initial conditions of the environment in question, respecting some basic principles of the forest, therefore the methodology will be divided into three phases: presentation of the basic principles of the agroforestry system as agricultural technique used, description of the area before handling, presentation of the implantation of the system and description of the handling.

Basic agroforestry principles:

- Natural Succession: Dynamic process that refers to the time and function of each plant in the system, where one plant creates conditions for the others that follow.

- Stratification: complementary to the principle of succession, it is the space that plants occupy in time, according to the need for sun and nutrients for their development, they are the "forest floors", because agriculture is the art of harvesting the sun.

- Pruning: consisting of cutting species or parts of them that have already fulfilled their roles in the system. Then they become fertilizers or can perform different functions, it is about the regeneration of the area that stimulates the growth of new and pruned sequential species, within the forest succession.

- Soil cover: In summary, the essence of the agroforestry system is the cover of the soil, which is the organic matter in the process of decomposition. The cover acts on the thermal and water balance of the soil, protects from direct sunlight and ensures greater water retention. Its decomposition provides nutrients and oxygen, creating structures necessary for microorganisms and micro fauna to develop and optimize the system, called soil flocks.

This work was done based on a system for the production of vegetables, fruits and yerba mate. After analysing the soil, with clay characteristics, a pH of 5.6 was found, with a low level of micronutrients, as well as an average percentage of Al + 3 (aluminum) 26 percent, organic matter level at 20 percent and a saturation low base - V percent of 31 percent. Based on these data, management and initial fertilization were defined.

For the tree lines, banana (Musa acuminata) and eucalyptus (Eucalyptus grandis and Eucalyptus globulus) were chosen as species that supply organic matter, mate herb (Ilex paraguariensis), avocado (Persea americana), lemon tree (Citrus aurantifolia and Citrus bigaradia), and pecan (Carya illinoinensis) as commercial species, in addition to several native trees. In the first planting, the trees were planted together: arugula, lettuce, broccoli, corn, yams and sweet potatoes.

As for the vegetable lines, several consortiums were made with more than 40 (forty) varieties to be marketed, respecting their production and harvest times and spaces, based on production cycles for these lines from 90 (ninety) to 150 (cent and fifty) days.

Once the species are defined and the soil is analysed, implantation and management begin.

Table 1: Atividade, MÊS and DIA

ATIVIDADE	MÊS	DIA
Land preparation with subsoiler and ploughing harrow.	1	1
Fertilization with rock powder (200g / m2), sheep manure (1kg / m2), shell limestone (350g / m2) and vegetable ash (600g / m2)	1	2

Incorporation of fertilizers into the soil with Tratorito.	1	2
Delimitation of space with divisions of lines of trees and lines of vegetables.	1	3
Coverage with organic matter from all lines, trees and vegetables.	1	4
Coverage with organic matter from all lines, trees and vegetables.	1	6 a 10
Planting 18 (eighteen) rows of vegetables.	1	11 a 24
First harvest of vegetables and medicinal herbs.	2	26
Second crop of vegetables and medicinal herbs	3	18
Third harvest of vegetables and medicinal herbs.	4	20
First pruning of bananas and eucalyptus. All organic matter chopped with a machete and arranged in the ground as a cover. Each tree line provided organic matter for its own line.	4	25
Fourth crop of vegetables	5	10
Second fertilization of the vegetable lines: mutton manure (750g / m2), shell limestone (100g / m2) and vegetable ash (300g / m2).	5	11
Incorporation of fertilization and preparation of vegetable lines again, using Tratorito.	5	12
coverage of vegetable lines and planting.	5	13

Second pruning of bananas and eucalyptus. Each tree line provided organic matter for its own line and a line of vegetables.	8	15
Third pruning of bananas and eucalyptus. Each tree line provided organic matter for its own line, plus two lines of vegetables.	12	20

Figure 1: Area in the implementation of the agroforestry system - own source, photo taken at

Figure 2: Area 6 months after the implementation of the agroforestry system, with vegetables between the lines and trees in the

Figures 3 and 4: Soil covered with banana pruning - own source, photo taken at the farm

Figure 5, 6 and 7: line of newly planted tree, with trees in the middle, such as Melaleuca alternifolia, Monteverdia truncate, Ilex paraguariensis and vegetables such as broccoli and corn - own source, photo taken at the farm

Figures 8 and 9: more photos of the agroforestry system of vegetables with trees and medicinal herbs- own source, photo taken at the farm

Figure 10: soil without agroforestry management and soil cover - own source, photo taken at the farm

Figures 11 and 12: soil with agroforestry management, typical in organic matter, micro and macro life - own source, photo taken at the farm

Results

One year after planting and subsequent management, the following advances were noted:

pH of 6.3 (increase of 0.7), Al+3 9 percent (reduction of 17 percent), level of organic matter in 45 percent (increase of 25 percent) and a base saturation - V percent of 52 percent (increase of 21 percent).

In addition to all the improvement reported above, the colour, smell, micro and macro life structure of the soil improved considerably, the moisture retention and soil porosity also improved.

Discussion

The management of the tree lines, in addition to providing organic matter for the soil, also opened space for the entry of light and contributed to the development of plants both in the tree lines such as lemon and avocado, as well as for vegetables and medicinal herbs.

An increase in the presence of birds, insects and other animals was found in a balanced quantity, positively interfering in the system.

The abundant agrobiodiversity of the agroforestry system has brought greater food and nutritional security for the family, improved quality of work, increased diversity in the food sold at the market and personal satisfaction for the family.

Conclusions

It is concluded that agroforestry systems, as they respect and reproduce the natural logic of the forest, can be applied in any biome, as long as it respects the principles of implantation and management, in addition to showing an alternative of agricultural production combined with the recovery of degraded soils, increasing the diversity of cultivars, in addition to presenting itself as a technique that uses inputs at decreasing levels, greater quantity in the implantation of the area, due to the reality of the soil conditions used by conventional crops previously, which over time, tends to decrease, and management tends to go from an intensive practice to an extensive reality with less intervention and work.

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What dead wood microbiota contributes to soil biodiversity?

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Abstract summary

We investigated the interaction between fungal communities of soil and dead wood substrates, quantified the rate of N_2 fixation in decaying logs and studied the potential drivers of the N_2-fixation activity. One third of the forest fungi inhabit both soil and dead wood substrate and one third is unique to the forest soil. Mycorrhizal species colonising dead wood in the late decay stages actively transfer N and C between soil and host plants. Asymbiotic N_2-fixation takes place in decaying wood and N_2 fixing methanotrophs are present in all wood decay phases. Interaction between soil and wood-inhabiting fungi and transfer of carbon and nutrients by fungi has importance to wood decay process and soil fungal community composition.

Keywords: fungi, functioning of microbiota, forest, methanotroph, N_2-fixation, nifH

Introduction, scope and main objectives

Fungal communities inhabiting forest soil and dead wood interact during the wood decay. Soil contact affects moisture and nitrogen content of dead wood via hyphae of diverse fungal community. However, the composition of shared fungal community is not widely studied and importance of decaying wood to composition and functioning of soil microbiota is not well known. Aim of this review is to compile and enhance the knowledge on the interaction between fungal communities of soil and dead wood substrates.

Methodology

In the studies reviewed for this paper we applied molecular species identification and stable isotope tracking to both soil and decaying wood in an unmanaged boreal Norway spruce-dominated stand (Mäkipää *et al.,* 2017; Mäkipää *et al.,* 2018; Rinne-Garmston *et al.,* 2019). Wood and soil samples were analysed for δ^{13}C and δ^{15}N by combustion under excess oxygen in an elemental analyser connected in continuous-flow mode to an isotope ratio mass spectrometer (IRMS). We used 6–18 mg of a sample for the analysis, depending on the sample's N percentage.

DNA extractions were performed with the commercial kit (PowerSoil DNA Isolation) following the standard protocol with slight modifications described in Mäkipää *et al.* (2017). DNA samples were amplified with 454-fusion primers targeting the fungal rDNA ITS2 region (Rajala *et al.*, 2015). Sequencing of the tagged amplicons was performed using a Genome Sequencer FLX Titanium XL+ System. The raw sequence data are archived at NCBI BioProject ID PRJNA341814.

Results

Altogether we observed 1990 operational taxonomic units (OTUs), out of which more than 600 were shared by both substrates and 589 were found to exclusively inhabit wood (Table 1). Most of the OTUs were rare and 30 most abundant OTUs yielded 50 percent of all sequences. On average the soil was more species-rich than the decaying wood, but the species richness in dead wood increased monotonically along the decay gradient, reaching the same species richness and community composition as soil in the late stages. Decaying logs at all decay stages locally influenced the fungal communities of soil, some fungal species occurring in soil only under decaying wood. Soil inhabiting *Piloderma sphaerosporum* and *Tylospora sp.* mycorrhizal species were highly abundant also in decayed wood.

Stable isotope analyses suggest that mycorrhizal species colonising dead wood in the late decay stages actively transfer nitrogen and carbon between soil and host plants. In addition to nutrient transfer, microbial communities in decaying wood affect forest soil nitrogen supply. Dead wood is initially a nitrogen (N) poor substrate, where the N content increases with decay, partly due to biological N_2 fixation. We used *nifH* genes as indicator of N_2 fixing capacity of microbiota. The number of *nifH* copies (g^{-1} dry matter) was higher at the later decay stages, but no correlation between the copy number and the in vitro N_2 fixation rate was found. All recovered *nifH* sequences were assigned to the order *Rhizobiales*, and therein mostly (60 percent) to methane oxidizing genera.

Table 1: Fungal species found exclusively in soil, exclusively in dead wood, and in both substrate types

Unique to soil **(793)**	Both in soil and wood (**608**)	Unique to wood (**589**)
Piloderma sp.	*Piloderma sphaerosporum*	*Fomitopsis pinicola*
Russula sp.	*Heterobasidium parviporum*	*Antrodia serialis*
Russula vesca	*Tylospora* sp.	*Phellopilus nigrolimitatus*
Tretomyces lutescens	*Gymnomyces monosporous*	*Phellopilus nigrolimitatus*
Amanita sp.	*Connopus acervatus*	*Phellinus viticola*
Pseudotomentella sp.	*Mortierella humilis*	*Phellopilus nigrolimitatus*
Cortinarius caperatus	Auriculariales sp.	*Exidia* sp.
Cortinarius rubellus	*Piloderma fallax*	*Atractiellales* sp.
Rhodocollybia sp.	Agaricales sp.	*Phellinis ferrugineovelutinus*

Cortinarius traganus	*Russula decolorans*	*Coniophora puteana*
Trechisporales sp.	*Hyphodontia pallidula*	*Basidioascus*
Pseudotomentella humicola	*Basidiodendron caesiocinereum*	*Pseudeurotiaceae* sp.
Cortinarius sp.	*Avachytrium platense*	*Sistotrema brinkmannii*
Piloderma sphaerosporum	*Russula emetical*	*Hyphoderma* sp.
Mortierella sp.	*Aphanobasidium pseudotsugae*	*Stereum sanguinolentum*

The 15 most abundant species are listed, and the numbers in brackets indicate the total number of species found. (Source: Mäkipää *et al.*, 2017)

Discussion

Soil- and wood-inhabiting fungal communities interact at all decay phases of wood that has important implications in fungal community dynamics and thus nutrient transportation. Mycorrhizal species colonising dead wood in the late decay stages actively transfer nitrogen and carbon between soil and host plants, which has importance to wood decay process. Furthermore, the presence of decaying logs has a positive influence on soil inhabiting mycorrhizal fungi.

Our studies confirmed that methanotrophs with potential N_2 fixing capacity (*nifH* gene) are present in all the wood decay phases. As methanotrophs are members of the *nifH* community in dead wood, where decomposition is driven by fungi, we suggest a hypothesis for further research: Decomposer fungi that are capable of producing CH_4 (Lenhart *et al.,* 2012) feed methane-oxidizing bacteria to fix atmospheric N_2. If this is the case, these fungi achieve a competitive advantage compared to other decomposers since N is the most important nutrient limiting the ecosystem processes.

Conclusions

We conclude that decaying wood has an important contribution to the overall fungal diversity of forest soils because a large fraction of all species was found to exclusively inhabit wood. Thus, wood specialised species are lost from managed forests where decaying wood is absent. We have shown interaction between soil and wood-inhabiting fungi and we suggest that transfer of carbon and nutrients by fungi has importance to wood decay process. Future challenge is to resolve the implications of nutrient transportation from soil to wood for the activity of wood-inhabiting fungal communities and the overall nutrient fluxes in forest ecosystems.

Acknowledgements

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Symbiotic relationships and effectiveness of soybean rhizobia in soils of the Nigerian savanna

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Abstract summary

Greenhouse experiment was carried out at National Cereal Research Institute Badeggi, Nigeria in 2013. The treatments were N sources (0 kg N ha^{-1}, 100 kg N ha^{-1}, USDA110 traded as Biofix, 532c traded as Legumefix) and 30 sites across the Nigeria Savanna fitted to Completely Randomized Design with three replications. Results revealed that population density of the indigenous rhizobia was highest in the southern Guinea and lowest in the northern Guinea. Similarly, symbiotic effectiveness of indigenous rhizobia was highest in the southern Guinea and lowest in the northern Guinea. Consequently, 50 percent and 20 percent of the locations responded to inoculation with Biofix and Legumefix respectively in the southern Guinea, while the percentage of locations that responded to these inoculants were 60 percent respectively in the northern Guinea , 50 percent and 30 percent in the sudan savanna. It is therefore recommended that the use of these elite inoculants (Biofix and Legumefix) be restricted to Northern guinea savanna while the potentials of indigenous rhizobia native to southern Guinea and sudan savanna be explored in local production of rhizobium inoculants.

Keywords: Effectiveness, Inoculation, Population, Rhizobia, Soybean, Symbiotic

Introduction, scope and main objectives

Introduction

Rhizobia can associate naturally with compatible host, or they can be introduced as exotic strains through inoculation. Inoculation however requires skills and plant response to inoculation may be limited by environmental stress and genetic incompatibility between plant and rhizobium. Scharf and Wiebold, (2003) reported that the numbers of indigenous Bradyrhizobia as well as the soil available nitrogen have been cited as some of the main factors that have a significant effect on symbiotic relationships. To avoid the need for inoculation, soybean breeders at the International Institute of Tropical Agriculture (IITA) Nigeria, developed new soybean genotypes for Africa. These genotypes known as Tropical Glycine Cross (TGx), nodulate with Bradyrhizobium spp populations indigenous to African soils (Mpepereki *et al.*, 2000) and have been tested in a number of African countries (Mpepereki *et al.*, 2000) including Nigeria (Osunde *et al.*, 2003). Much still needs to be done to ensure optimum productivity by the TGx by quantification of the nitrogen fixed by rhizobia, and in this way assess the symbiotic performance of the association of the TGx and the indigenous rhizobia in the soil.

Scope

This work is focused on ecological aspects that limit response of TGx soybean varieties to inoculation. It is also an inquiry into the population of rhizobia strains nodulating promiscuous soybeans and whether the population confers any significant symbiotic advantage to the plant.

Main objectives

The main objective of this study is to evaluate the symbiotic relationships and effectiveness of rhizobia strains that nodulate promiscuous soybeans in soils of the Nigerian savanna.

The specific objectives are to:

i. estimate the population and symbiotic effectiveness of indigenous rhizobia in soils of the Nigerian savanna in relation to agro-ecology.

ii. establish the relationship(s)between the symbiotic effectiveness of indigenous rhizobia population and the magnitude of response to inoculation in each soil.

Methodology

Soil Sampling and Analysis

Soils were sampled from 10 auger points per plot at the depth of 0-20cm using sterilized auger and bulked according to sites to form a composite. Thereafter, 5g of subsamples were sieved through 0.5mm and refrigerated for Most Probable Number of indigenous rhizobia count according to the methods of Woomer, Singleton and Bohlool (1988) while 125g of sub sample were air-dried, crushed and sieved with a 2mm screen in preparation for physicochemical properties using standard methods outlined by Van Reeuwijk (ed., 2002).

Most Probable Number Count (MPN) of Indigenous Rhizobia

Treatment and Experimental Design

The experiment was a factorial arrangement fitted to a Completely Randomized Design (CRD). Seeds of TGx 1448-2E were inoculated with tenfold serial dilution (10^{-1} – 10^{-6}) of each soil sample and replicated three times.

Planting and Crop Management

Coarse sand was severally washed with water and immediately sundried and autoclaved at 121^0C for 20 minutes. Thereafter, 2 kg was transferred to each 25 polybags per site followed by the watering of polybags to field capacity with sandsman nutrient solution. Seeds of TGX144 8-2E were surface-sterilized with 3 percent sodium hypochlorate for 5 mins and thoroughly rinsed with sterile distilled water before planting of four seeds per polypot and then thinning to 2 seedlings per polypot at one week after planting and before treatment application. Plants were constantly watered at a day interval with sandsman nutrient solution. The strength of the solution was quartered after 3 weeks, and the plants harvested at 7 weeks after planting (WAP).

Need-to-Inoculate Experiment

Soils sampled from thirty sites on farmer's field, collected in pots, were arranged in a greenhouse located at National Cereal Research Institute, Badeggi, Niger State, Nigeria in the month of June 2013.

Treatment and Experimental Design

Treatments are 4 N sources as follows: (0kg N ha^{-1}, 100 kg N ha^{-1}, Biofix, Legumefix) and 30 sites across the Nigeria Savanna; 10 sites each from southern Guinea (Agwanga, Lapai, Garatu, Maikunkele, Keffi, Gwada, Paiko, Bwari, Sabon Gida and Kampani Mailamba) northern Guinea (Kagara, Tegina, Shika, Maigana, Soba, Tasha Ango, Rigachikun, Birnin Yaro, Birnin Gwari and Tasha Iyali) and sudan savanna (Bebeji, Karaye, Madobi, Tofa, Kura, Karefi, Chiromawa, Rano, Amawa and Bagwai) fitted to Completely Randomized Design with 3 replicates.

Planting, Crop Management and Harvesting

Four kilogrammes of soil were transferred to polypots before watering to field capacity with deionized water, application of 60 kg P ha^{-1} as 1270 mg SSP and planting of surface-sterilized TGX 1448-2E seeds at the rate of four seeds pot^{-1}. At one week after planting, soybean plants were thinned to 2 seedlings pot^{-1} prior to Basal applications as follows: 60 Kg K ha $^{-1}$ applied as 116 mg KCl, 5 Kg Mg ha $^{-1}$ applied as 85.28 mg $MgSO_4$, 10 Kg Zn ha $^{-1}$ applied as 79.2 mg $ZnSO_4$, 0.1 Kg Mo ha $^{-1}$ applied as 3.12 mg $(NH_4)_6 Mo_7O_{24}.H_2O$, 0.1 Kg B ha $^{-1}$ applied as 13.104 mg $Na_2B_4O_{7.5} H_2O$. Soybean variety TGx1448-2E was obtained from International Institute of Tropical Agriculture substation, Kano.

Harvesting and Data Collection

At harvest (7 WAP), fresh and dry weights of shoots were measured before and after oven-drying at 65^0C for 3 days while Percentage symbiotic effectiveness (%SE) was derived as follows:

$$\% \text{ SE } = \frac{d-n}{d+n} \text{ X } 100 \quad \text{...eqn 1}$$

Where d-n and d+n are the dry weight of plants without N and plants with N respectively. Percentage Inoculation response (IR) was also derived as follows:

$$\% \quad IR = \frac{d+i-(d-n)}{d-n} \times 100 \quad \text{..eqn 2}$$

where d+i and d-n are the dry weight of inoculated plants and plants without N respectively.

Statistical Analysis

Data generated from symbiotic effectiveness of indigenous rhizobia and response to inoculation with Legumefix and Biofix were analysed using simple analytical tool that generated bar charts with error bars.

Results

Table 1: Indigenous rhizobia counts (MPN) and Total N of soils of the Southern Guinea Savanna

Soil characteristic	Akwanga	Lapai	Garatu	Maikunkele	Keffi	Gwada	Paiko	Bwari	Sabon Gida	Kampani Mailamba
MPN count trapped by TGx1448-2E	1.6 x 10^5	1.1 x 10^6	1.1 x 10^6	1.1 x 10^6	9 x 10^2	3.6 x 10^5	1.1 x 10^6	1.1 x 10^6	1.1 x 10^6	1.1 x 10^6
Soil Total N (g Kg-1)	0.25	0.14	0.17	0.03	0.22	0.25	0.13	0.10	0.24	0.17

Table 2: Indigenous rhizobia counts (MPN) and Total N of soils of the Northern Guinea Savanna

Soil characteristic	Kagara	Tegina	Shika	Maigana	Soba	Tasha Ango	Rigachikun	Birnin Yaro	Birnin Gwari	Tasha Iyali
MPN count trapped by TGx1448-2E	1.1 x 10^5	1.1 x 10^5	2.1 x 10^5	1.1 x 10^6	2.1 x 10^3	3.6 x 10^5	2.1 x 10^3	1.1 x 10^6	1.1 x 10^6	3.6 x 10^2
Soil Total N (g Kg-1)	0.18	0.24	0.18	0.22	0.27	0.20	0.28	0.17	0.18	0.15

Table 3: Indigenous rhizobia counts (MPN) and Total N of soils of the Sudan Savanna

Soil characteristic	Bebeji	Karaye	Madobi	Tofa	Kura	Karefi	Chiromawa	Rano	Amawa	Bagwai
MPN count trapped by TGx1448-2E	1.1 x 10^6	1.1 x 10^6	2.1 x 10^2	1.1 x 10^6	2.1 x 10^2	9.3 x 10^2	1.1 x 10^5	1.1 x 10^6	1.1 x 10^5	1.6 x 10^5
Soil Total N (g Kg-1)	0.29	0.17	0.25	0.14	0.20	0.29	0.21	0.21	0.18	0.28

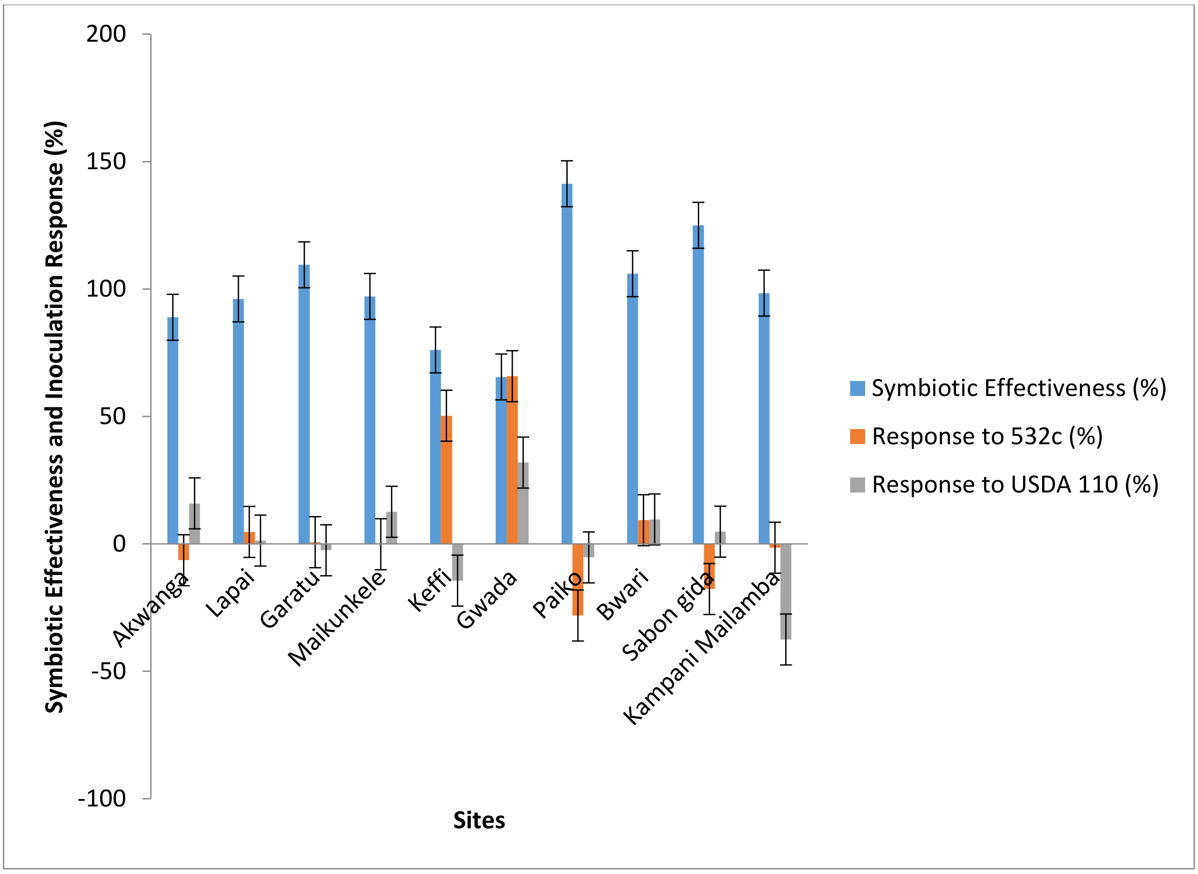

Figure. 1: Symbiotic effectiveness and inoculation Response of Soybean across Sites in the Southern Guinea savanna

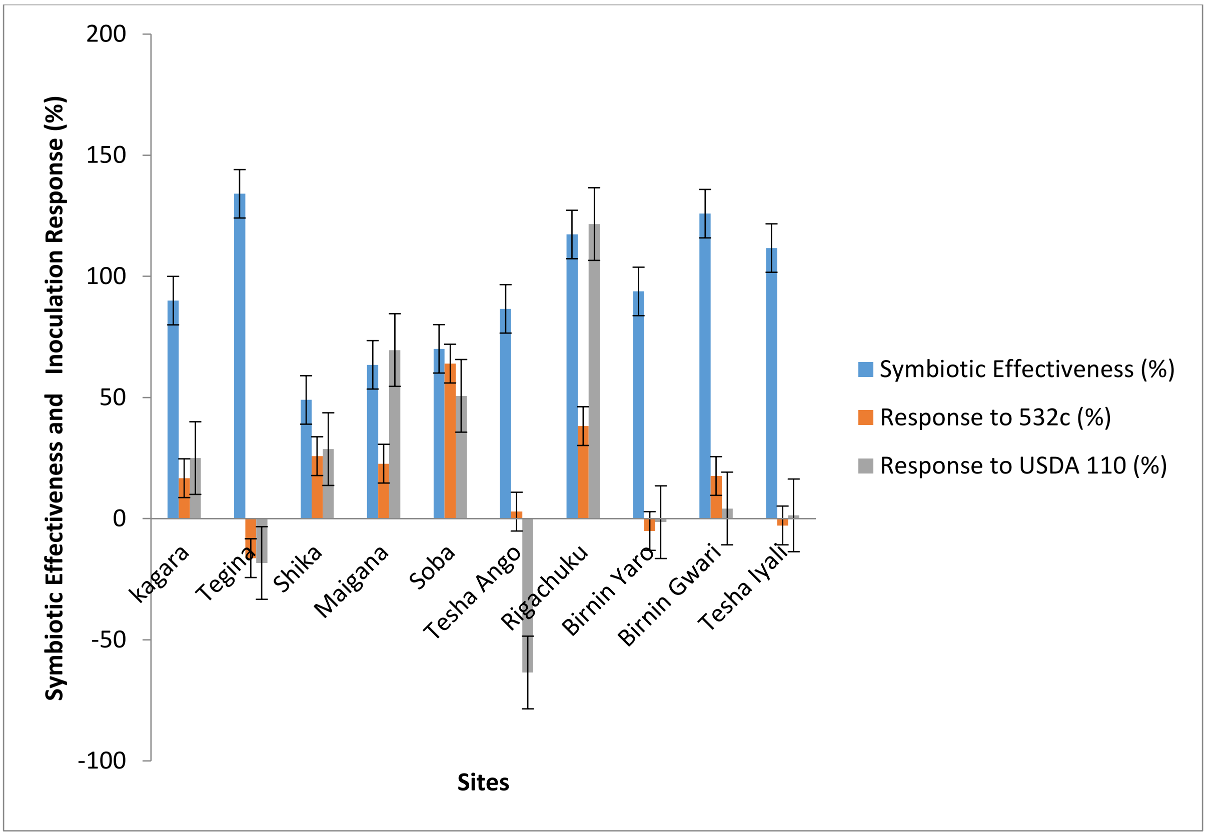

Figure 2: Symbiotic effectiveness and inoculation response of soybean across sites in the northern Guinea savanna

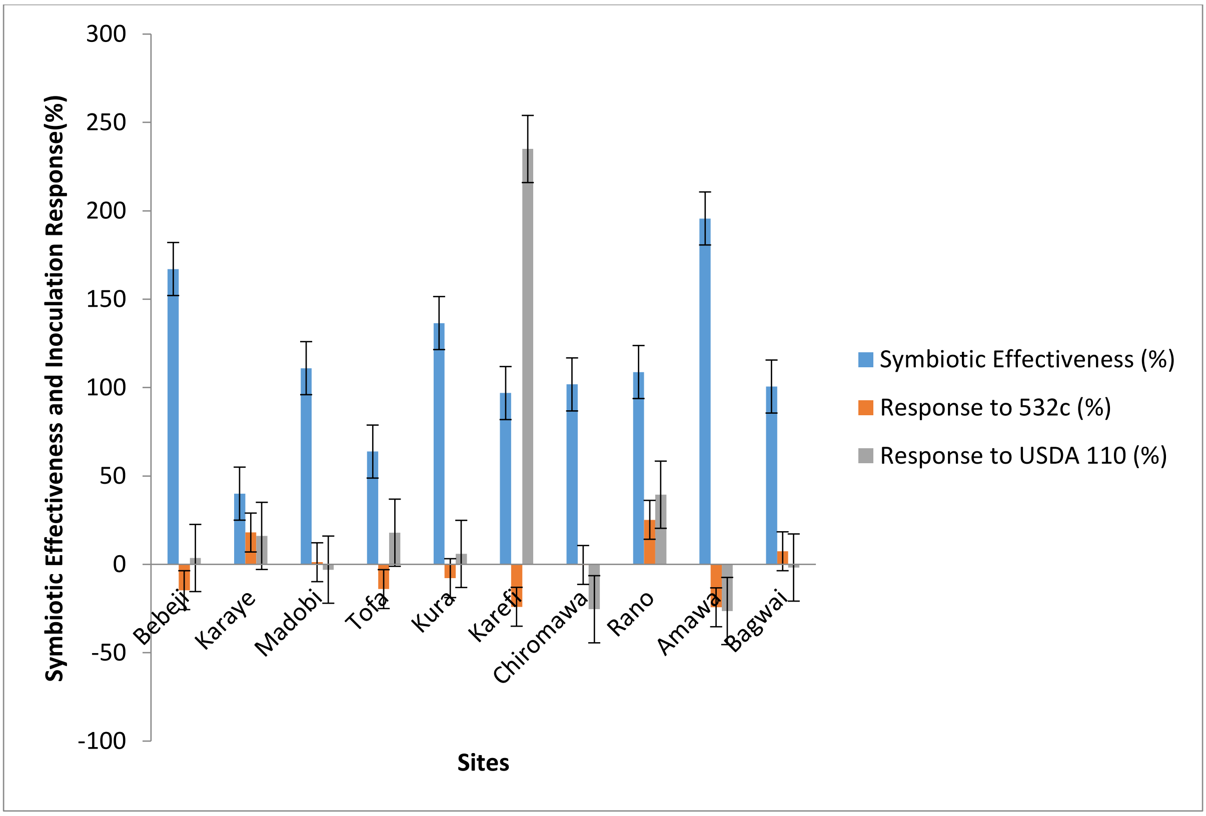

Figure 3: Symbiotic effectiveness and inoculation response of soybean across sites in the Sudan savanna

Discussion

In the southern Guinea savanna, indigenous rhizobial count averaged 822,090 cells g^{-1} (Table 1) and Symbiotic effectiveness of indigenous rhizobia ranged from 69 percent - 123 percent (Figure 1). This high population densities and symbiotic effectiveness of indigenous rhizobia may explain why 50 percent of the soils responded positively to USDA110 (Biofix inoculant) while 20 percent responded positively to 532c (Legumefix inoculant) (Figure 1).

In the Northern Guinea Savanna, results obtained showed that soybeans grown on 60 percent of the locations responded to USDA110 (Biofix) and 532c (Legumefix inoculant) respectively (Figure 2). The higher response to inoculant use in the Northern Guinea compared with the Southern Guinea might be a reflection of differences in soil nitrogen availability (Gentili and Huss-Daniel, 2003). Plants grown on soils obtained from Shika, Kagara, Soba, Maigana and Rigachiku responded positively to inoculation probably because averagely high soil N (Table 2) reduced the competitiveness of their indigenous rhizobia. Sites like Tegina, Birnin Gwari and Birnin Yaro with indigenous rhizobia populations that are competitive as suggested by the negative response to inoculation of soybean plants, had medium soil N averagely (Table 2). Result obtained in the Northern Guinea has demonstrated that lower population densities of indigenous rhizobia recorded higher symbiotic effectiveness and consequently lower response to inoculation with USDA110(Biofix) and 532c (Legumefix). For example, Symbiotic effectiveness of 121 percent and population of indigenous rhizobia of 1.1×10^6 cells g^{-1} obtained at Birnin Gwari were halved and doubled respectively at Shika, Maigana and Soba. Consequently, response to inoculation with USDA110 (Biofix) and 532c (Legumefix) were positive at these sites (Figure 2) even though negative at Birnin Gwari (Figure 2).

In the Sudan Savanna, indigenous rhizobial populations (Table 3) and their symbiotic effectiveness (Figure 3) were averagely higher than those obtained in the Northern Guinea Savanna (Table 2 and Figure 2). Consequently, 50 percent of the locations responded to Biofix while 30 percent responded to Legumefix (Figure 3). Amawa, Bebeji and Chiromawa soils responded negatively to both Legumefix and Biofix (Figure 3) and may not benefit from inoculation with these elite strains. Low to negative response to inoculation with Biofix and Legumefix in this Agroecology may be due to high levels of soil nitrogen (Gentili and Huss-Daniel, 2003) in addition to high population densities and high symbiotic effectiveness of indigenous rhizobia populations. With the exception of Amawa, Tofa and Karaye soils, total nitrogen of soils obtained from Sudan Agroecology were high (Table 3).

Conclusions and recommendations

In conclusion, 50 percent and 20 percent of the locations responded to inoculation with Biofix and Legumefix respectively in the southern Guinea (Figure 2), while the percentage of locations that responded to these inoculants were 60 percent respectively in the northern Guinea (Figure 2), 50 percent and 30 percent in the sudan savanna (Figure 3). It is therefore recommended that the use of these elite inoculants (Biofix and Legumefix) be restricted to Northern guinea savanna while the potentials of indigenous rhizobia native to southern Guinea and sudan savanna be explored in local production of rhizobium inoculants.

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Diversity and distribution of mycorrhizal arbuscular fungi associated to Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) in Benin

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Abstract summary

The Arbuscular Mycorrhizal Fungi (AMF) are microorganism group which enable to the plant to uptake soils nutrients for the growth. This study assessed the distribution and diversity of mycorrhizal fungi associated to Bambara groundnut in Benin. A survey has been done through 20 villages chosen on the basis of Bambara groundnut yield, cropping area affected to this specie and its production across five agro-ecological zones. Data collected are spore's diversity and root's infection levels. The results revealed that the cotton zone of northern Benin has recorded the highest density. Fourteen (14) morphotypes divided into five genus (*Glomus, Gigaspora, Acaulospora, Scutellospora* and *Diversispora*) were identified in the different studied zones with a dominance of *Glomus* genus in all AEZ. It will permit the production of mycorrhizal inoculum in order to increase the productivity of the Bambara groundnut in Benin.

Keywords: Arbuscular Mycorrhizal Fungi, diversity, soil fertility, Bambara groundnut, Benin.

Introduction, scope and main objectives

The food insecurity areas in worldwide are most concentrated under tropics especially in Africa in the south of Sahara. Indeed, Arbuscular mycorrhizal fungi enable the plant to get soils nutrients which are few steady in the soil like phosphorus, nitrogen, and most soil micronutrients (Saïdou *et al.*, 2012; Haougui *et al.*, 2013). In Benin, much research has been done to assess AMF diversity associated with different crops. For instance, Houngnandan *et al.* (2009) have focused on evaluating the indigenous Glomus species diversity in the clear forest for *Isoberlina doka* (Craib et Stapf) at Wari-Maro in the centre of Benin. Tchabi *et al.*, (2008) and Balogoun *et al.* (2015) have also studied the diversity of endomycorrhizal fungi associated to the cotton and cashew tree respectively. This different research has permitted the identification of some species of Glomeromyceta associated to many crops in Benin. To our knowledge, no research in West Africa and especially in Benin has so far been focused on AMF associated to Bambara groundnut in order to evaluate species and strain diversity of AMF associated to the crop.

So, the principal research question that this study will answer is:

- What are the distribution and diversity of arbuscular and vesicular mycorrhizal fungi associated with *Vigna subterranea* in different agro-ecological zones in Benin?
- The aim of this work was to apprehend Arbuscular Mycorrhizal Fungi community present in *Vigna subterranea* rhizosphere in different agro-ecological zones in Benin.

Methodology

Study areas

Statistical data on Bambara groundnut production in Benin also showed that it is cultivated from the first agro-ecological zone to the sixth agro ecological (Gbaguidi *et al.,* 2016) zone respectively: the Far North Benin (AEZ 1), Cotton region of North Benin (AEZ 2), Food crop region of South Borgou (AEZ 3), West zone of Atacora (AEZ 4), Cotton region of the centre (AEZ 5) and bar lands (AEZ 6).

At field

According to Bambara groundnut sowing date in the different areas and to his flowering date, the prospection phase has been done in September to be sure to find Bambara groundnut at flowering phase. Two regions have been selected per agro-ecological area. The best cultivation regions have been retained for making sampling. Each sampling place were georeferenced with GPS.

Per region, two composite samples have been considered. Indeed, in order to obtain composite sample, four soil sampling have been done at four different locations with a custom-made corer at 20 cm in depth.

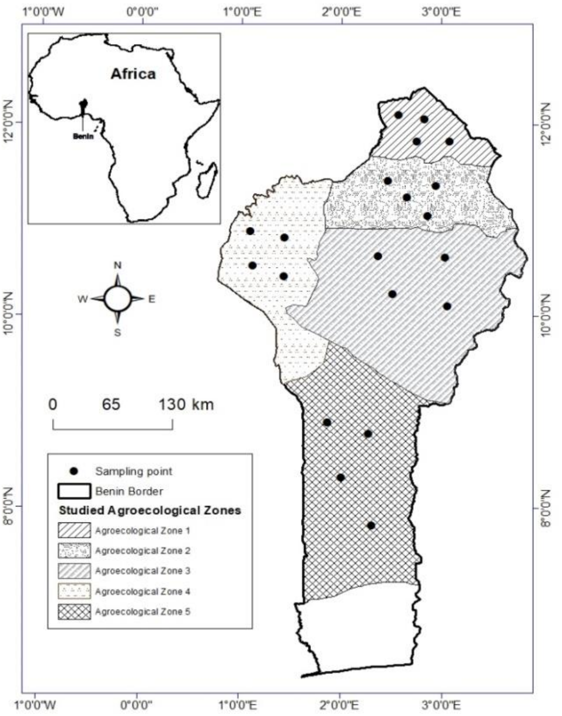

Figure 1: Benin geographic map with different sampling places

Spore extraction, counting and morphological identification

AM spores were extracted using the wet sieving and centrifugation method of Gerdemann and Nicolson (1963). After this process suspended spores were collected and count under binocluar loupe by using gridded petri dish (5cm of diameter) for making easier the counting. It is based on morphological criteria (colour, presence or absence of suspensor bulb and hyphae, etc) some AMF morphotypes have been identified, counted and grouped in genus by using of identification and description key of International Collection of Vesicular and Arbuscular Mycorrhizal fungi (INVAM, http//www.invam.caf.wdu.edu).

Assessment of mycorrhization

The earlier collected roots of bambara groundnut were used for determine mycorrhization frequency and intensity by Phillips and Hayman method (1970). The estimation of root's mycorrhizal infection was performed according to intersection method (Trouvelot, Kough and Gianinazzi-Pearson, 1986).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to assess the effects of agro-ecological zone (AEZ) on AMF spore diversity parameters and root's infection levels. When significant differences ($P < 0.05$) were found, a post hoc comparisons of means between AEZ were made using Student-Newman-Keuls' test. This ANOVA was performed with non-transformed data after ensuring conformity of these data with

ANOVA assumptions. Additionally, Pearson correlation test were performed between all of studied parameters in order to appreciate relationship between them. All statistical analysis were carried out using SAS software version 9.2.

Results

AMF diversity

In Bambara rhizophere in this study, fourteen mycorrhizal fungi morphotypes have been identified according to morphological criteria (colour, presence or absence of suspensor bulb and hyphae, etc.). After identification of these morphotypes with INVAM identification key, it was appeared that they belonged to five genus (*Glomus, Gigaspora, Acaulospora, Scutellospora and Diversispora*) which belong to four (04) different orders (Glomeraceae, Acaulosporaceae, Diversisporaceae and Gigasporaceae) and two (02) families (Diversisporales and Glomerales) in Glomeromycetes phylum. Relative abundance of these genus globally showed that *Glomus* are the most abundant genus which were present in all prospected AEZs and occupy about mean of 66.82 percent. Other genus were also represented in all prospected AEZs and scored 18.46 percent, 8.25 percent, 4.89 percent and 1.58 percent respectively for *Gigaspora, Scutellospora, Acaulospora* and *Diversispora* genus (Figure 2).

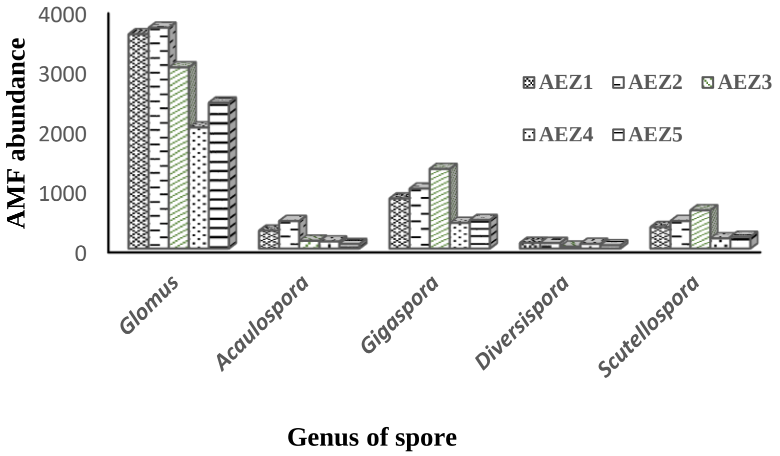

Figure 2: Relative AMF genus abundance of all prospected zone in Benin

Assessment of natural mycorrhization level

As it can be seen on the Figure 3, there was significant ($p<0.0001$) difference between the studied AEZs basing on the mycorrhization frequency. Highest frequency (45.625 percent) was registered in AEZ 1 which was different than remaining AEZs. Remains zones were not statistically different and the lowest rate of mycorrhization frequency was recorded in AEZ 4 (17.25 percent).

In the case of mycorrhization intensity, it appeared that AEZ 3 has registered the highest value (8.06 percent) and the lowest rate has been recorded in AEZ 4 (2.39 percent). All of AEZs were also significantly different ($p<0.0001$) according to mycorrhization intensity.

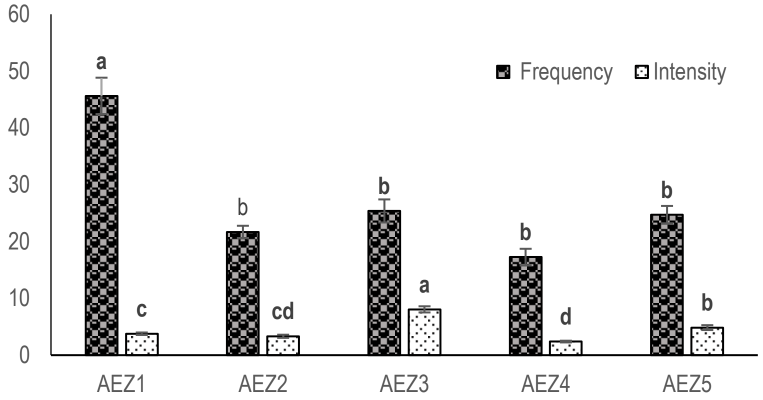

Figure 3: Mycorrhization frequency and intensity according to AEZ

Discussion

AMF diversity

Results from the present study showed that the spore diversity of fungi varies relatively little from one AEZ to another with a dominance of the *Glomus* genera in all zones. Indeed, this predominance of *Glomus* was reported in AMF morphotypes in various tropical soils (Tchabi *et al.*, 2008; Houngnandan *et al.*, 2009) and in agricultural soils from temperate zone (Oehl *et al.*, 2003). Abundance of this genera in different agro-ecosystems allow to consider it as be most adaptable and available in some environments (Brito, Goss and De Carvalho, 2012). Diversity analysis have also shown that AEZ 1 and AEZ 4 were characterized by genera *Glomus* and *Diversispora* and AEZ 3 by *Gigaspora* and *Scutellospora.* This means that in each zone, it exists a relationship between genera which characterize them. This will permit to determine which type of inoculum will be appropriate in each zone.

Mycorrhization frequency and intensity

Mycorrhization frequencies and intensities have widely varied between zones. The area which has recorded the highest mycorrhization frequency did not have a good infection level and also haven't recorded highest levels of soil chemical parameters. At the same time, the area with the highest levels of spore density and different soil chemical parameters recorded low mycorrhization frequency and intensity. This could stipulate that low levels of certain soil parameters (Nitrogen, Carbon, Phosphorus and pH) would favour the infection capacity of Bambara groundnut roots by the fungal species which were present. Indeed, these results are conformed with previous works of Houngnandan *et al.* (2009) who showed that, for example, plots with low Phosphorus levels were those with high frequencies of mycorrhization. Also, Liu *et al.* (2012) have shown that high levels of Nitrogen and Phosphorus have reduced Glomeromycetes populations in the soil. Moreover, there is a very weak correlation between the density, the frequency, and the intensity of mycorrhization. Living spores of AMF present in the soil may not function as propagules, they may be quiescent (inactive because soil conditions are unsuitable) or have an innate period of dormancy-mechanisms which may help them survive in periods of adverse soil conditions (Brundrett, 2009). In addition, came soils parameters which would diversely influence the establishment of the symbiosis between the mycorrhizal fungi and Bambara groundnut roots.

Conclusions

The present study has shown that there is an important spore density of AMF associated with Bambara groundnut and that differs significantly from one AEZ to another. Furthermore, it appeared that AEZ 1 and AEZ 4 were characterized by *Glomus* and *Diversispora* genera, but *Gigaspora* and *Scutellospora* characterize AEZ 3 are. So, it would be appropriate that further studies would be carried out in order to confirm these results. Also, achievement of molecular characterization will allow most accuracy in specie identification. This will enable an evaluation of effectiveness and efficiency of different collected species in order to develop some ecological technologies in Bambara groundnut fertilization based on AMF.

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Microbial biodiversity in the field is related to fruit and vegetable health and linked to postharvest quality

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Abstract summary

According to FAO statistics, 45 percent of all fresh produce is lost worldwide and the losses are especially high during postharvest storage. The study of microbial diversity is crucial to understand reduced postharvest quality and to improve fruit and vegetable quality. We studied this for field-grown sugar beet (*Beta vulgaris* L.) and apple (*Malus domestica* Borkh.) by a metabarcoding approach, targeting bacteria and fungi. The microbiome and its dynamics were accessed as well as microbial transmission from the field into the storage phase was investigated. Interestingly, there was a broad overlap between sugar beets affected by root rot in the field and those in storage. Bioinformatics predictions indicated a transmission of the field microbiomes into storage. Postharvest quality loss was accompanied by a loss in microbial diversity as well as the replacement of fungal health indicators (*Plectosphaerella* and *Vishniacozyma)*, with potential pathogens and degraders (*Penicillium*, *Candida, Fusarium* sp. and *Lactobacillus*) in rotting beets. Similar results were shown for apples. Managing practise on the field highly influences the apple microbiome diversity, which was shown to be important for postharvest quality of the fruits. The overall findings link microbial biodiversity and field health to postharvest quality loss and propose new microbiome-driven management strategies.

Keywords: Beta vulgaris, Malus domestica, storage rot, microbiome, health indicators

Introduction, scope and main objectives

Soil biodiversity is correlated with nutrient cycling and ecosystem health (Wagg *et al.*, 2014). This also directly translates to plant performance and health (Berendsen, Pieterse and Bakker, 2012; Berg, 2009; Philippot *et al.*, 2013). Microbes not only colonize soil and plant roots, they also colonize other plant tissues and are therefore an integrated part of the plant lifecycle (Sánchez-Cañizares *et al.*, 2017). Recently they were discussed to also play a key role in postharvest quality of fruits and vegetables (Droby and Wisniewski, 2018).

This study focuses on linking the field microbial diversity with postharvest fruit quality and health. Therefore, the sugar beet and apple microbial communities were studied in the field as well as after harvest. Bioinformatic predictions were applied to link between the field and postharvest storage microbiomes and implications for future managing practices were addressed.

Methodology

Apples from organic and conventional field sites, apples from storage facilities as well as sugar beets, taken from multiple locations in Austria and Germany, from field sites and storage piles, were processed in this study. DNA extraction was performed using the FastDNA spin kit for soil (MB Biomedicals, USA). The DNA extracts were used for 16S rRNA gene and internal transcribed spacer (ITS) amplicon amplifications. The primer pair 515f (GTGYCAGCMGCCGCGGTAA) and 926r (CCGYCAATTYMTTTRAGTTT) targeting the complete hypervariable region 4 of the 16S rRNA gene and the ITS1 region primer pair ITS1f (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) were used. Barcoded amplicons were sequenced on an Illumina MiSeq (2 × 250bp) machine. Data analysis was done with the QIIME2 pipeline according to the developer tutorials. More details to the methodology are provided by Kusstatscher *et al.* (2019a) and Kusstatscher *et al.* (2019b) as well as Wassermann, Kusstatscher and Berg (2019) and Wassermann, Müller and Berg (2019).

Results

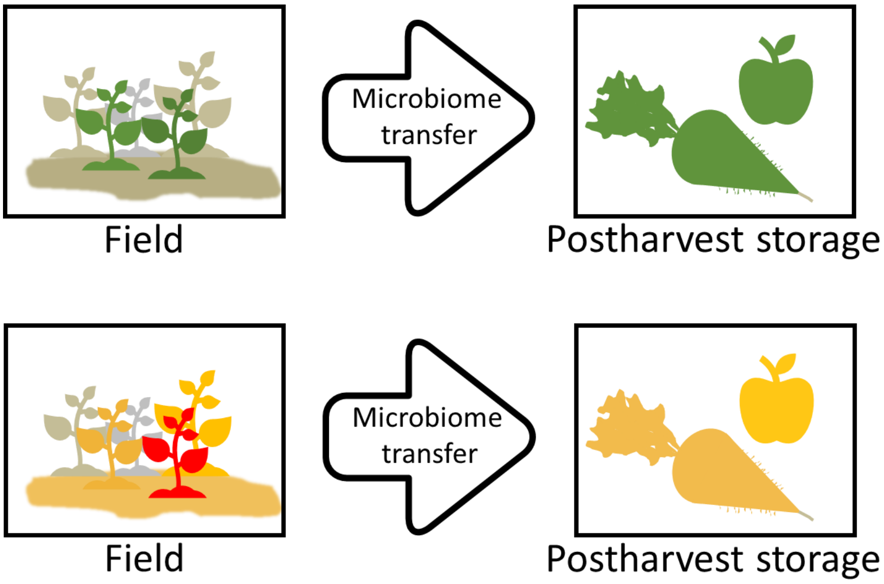

Figure 18: A high proportion of bacterial and fungal taxa are transferred from the field into storage

Healthy microbial communities on the field (green) as well as diseased ones (red, orange) can be linked to high or poor postharvest quality.

Comparing field managing practice in apple a significantly lower microbial diversity can be found for conventional field-grown apples (mean shannon index 4.2 compared to 5.6). Additionally, changed microbial communities colonizing different fruit tissues were observed. Especially the fruit pulp and the seed microbiome were changed. Apple storage trials revealed a link between fruit microbiome diversity and fruit postharvest quality. Postharvest disease of apple induced by *Penicillium expansum* or *Neofabraea alba* highly decreased the fruit microbiome diversity.

Similar results were observed for sugar beet. Postharvest disease led to a significant loss in diversity (average shannon index 4.5 compared to 5.5 (bacterial community) and 3.5 compared to 4.5 (fungal community)). Geographic location (cultivation soil) as well as health status of the beets were shown to be the two most influential factors for microbiome differences between the samples. Taxa which indicate low postharvest quality can be defined from compositional changes of sugar beet samples. Especially *Lactobacillus*, *Leuconostoc*, *Candida* and *Penicillium* were found correlated to postharvest disease.

Using specific bioinformatic tools, microbiome origins were tracked in different sample groups. A high transmission (60-80 percent) of field microbes into the postharvest period was observed. This way the health of the field microbiome impacts the postharvest quality (Figure. 1).

Discussion

In these studies, detailed insights into the bacterial as well as fungal community related to health and postharvest quality were obtained. Moreover, the impact of the field biodiversity on the postharvest storability was shown. Field managing practice impacts the soil and plant microbiome (Berg and Smalla, 2009; Lupatini *et al.*, 2017). The plant microbiome plays a key role in plant health and disease defense (Berg *et al.*, 2014; Berg *et al.*, 2017). However, the plant-colonizing microbes are also crucial for postharvest fruit and vegetable stability (Droby and Wisniewski, 2018). Our results show the strong link between field biodiversity, plant health and postharvest quality. Microbial signatures are directly transferred from the field to the postharvest storage. We could not only show a connection between diversity and fruit quality but also identify microbial signatures for both apple and sugar beets indicating postharvest disease. These findings increase our knowledge and understanding of the great importance of microbes associated to our food products and open the field for innovative new strategies to manage field and postharvest microbes.

Conclusions

Changes in field microbial diversity not only impact nutrient cycles but also highly influence plant performance and health. Moreover plant-associated microbes accompany plants throughout their life cycle and also into the postharvest phase. The microbial transfer from the field into the storage as well as the great importance of a diverse microbiome for postharvest quality was shown for apples and sugar beet. Future managing practices should be evaluated on their impact on the microbiome to guaranty a healthy environment and healthy fresh produce.

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Ecological intensification of Mediterranean vineyards: effects on soil conservation and economic viability

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Abstract summary

Three Mediterranean vineyards were studied for effects of management on soil physical and chemical properties and functional biodiversity. The vineyards were managed intensively (IN), under organic agriculture strategies (OR) and under ecological intensification (EI) respectively. Soils under intensive management were poor in nutrient, organic matter and biota and showed signs of physical deterioration. Under organic management or ecological intensification, soil organic matter content was higher than the average in the region. Biomass of bacteria, fungi, amoeba and flagellates was far higher in the organic and ecologically intensified than in the intensive vineyards, and ecological intensification was of particular benefit for soil protists. Conversion from intensive management to ecological intensification yielded environmental, productive and economic benefits after four years of transformation efforts.

Keywords: Ecological intensification, Mediterranean soils, functional biodiversity

Introduction, scope and main objectives

Fertility is the ability of soil to provide a suitable environment for plant growth and food production. During the past century, driven by the need to feed a rising population, the intensification of the agriculture, resulted in significant growth in food production and hunger reduction (Evenson and Gollin, 2003). However, agricultural landscapes were optimized for production in detriment of non-provisioning ecosystem services (Seppelt *et al.*, 2016) to such an extent that, in 2008, agriculture contributed 19 percent–29 percent of global anthropogenic green-house gas emissions (Vermeulen, Campbell and Ingram, 2012). Thorough studies forecast a 100–110 percent increase in crop demand from 2005 to 2050 which, under a business-as-usual scenario, will increase land clearing by 1 billion ha, will rise agricultural GHG emissions to 3Gt y^{-1} of CO_2-C equivalents, and will have major effects on species extinction, water quality and loss ecosystem services provision (Tilman *et al.*, 2011). Since 40 percent of the Earth's terrestrial area is already appropriated for agriculture, increasing food production must be achieved from existing agricultural land while reducing environmental impacts and conserving soil and water quality (Godfray *et al.*, 2010). This goal can be met by eco-intensification strategies that include improving the efficiency of inputs (water, fertilizers and energy) and managing correctly the carbon and nitrogen cycles is soils (Lal, 2020). Biodiversity cooperates to productivity in agricultural systems and there is evidence of significant contribution of belowground communities to farm yield through enhanced nutrient availability, prevention of pests and diseases, sequestering carbon and improved soil structure and water holding capacity (Wall, Nielsen and Six, 2015).

Here, we present a successful case of ecological intensification in a Mediterranean vineyard, in comparison with two neighbouring vineyards managed under organic and conventional intensive strategies.

Our aim is to show that there is no contradiction in the simultaneous pursuit of improving soil quality and biodiversity and maintaining production and economic gain.

Methodology

We worked in three farms in Marzà (Girona, Spain; 42°18′29″N, 3°04′14″E). The farms are contiguous and share geological substrate, soil type (Haplic Regosols, eutric) and Mediterranean climate (mean annual T: 15.2 °C; annual rainfall: 703 mm; summer drought) but differ in management strategies. One farm is intensively managed (IN) including tillage three times yearly, glyphosate and pesticides application and mineral fertilization. A second farm has been organically managed (OR) since 2007. At that time, ploughing ceased. Currently, the spontaneous grass cover is mowed twice yearly, and the residuals decompose on the site. Plague control is made with Bordeaux broth. The third farm was intensively managed until 2015, when an "ecological intensification" strategy (EI) was adopted. Ploughing was suppressed and cover crops were stablished. Currently, the soil is decompressed yearly by injection of air, and humic acids and microorganisms (Cho, 2018) are provided. Manure is applied as fertilizer. Fungi attacks are controlled with copper and sulphur, and harmful insects with pheromones (Figure1).

In the spring of 2019, we stablished four sampling areas at each farm, 10 m away of each other on a N-S transect with the aim of covering soil heterogeneity. At each area, we buried two pairs of tea bags to study soil ability for mineralization following the Tea Bag method (Keuskamp *et al.,* 2013) and sampled soil (four samples per area) for physical and chemical properties, respiration, functional biodiversity and energy and matter fluxes through trophic webs. The samples were taken in the transects defined by the tree rows avoiding sampling in the interrow spaces.

Figure1: general views of the vineyards

EI: vineyard under ecological intensification; OR: organic vineyard; IN: vineyard intensively managed.

Results

Table 1 shows some preliminary results of our probes. Management deeply affected soil quality. The soil of the intensively manged (IN) vineyards was very low in organic matter, nitrogen, available P, Ca, Na, K and Mg, with values significantly lower than in the EI/OR vineyards. Soil physical structure was also worse in IN than in EI and OR as indicated by higher bulk density and lower capacity for water retention. The IN vineyards also were biologically poor, with low biomass attributable to microbes and all groups of protists.

The OR and EI vineyards were comparable in terms of physical quality, but electrical conductivity and pH were significantly higher in EI than in OR. There were also significant differences in Ca content between both vineyards that cannot be attributable to calcium carbonates, whose concentration was negligible in the soil of the three farms.

Table 1: Soil properties in vineyards managed under ecological intensification (EI), organic (OR) and conventional intensive (IN) strategies

		EI	OR	IN	Model (p)	EI/IN (p)	EI/OR (p)	IN/OR (p)
E. conductivity	dS m^{-1}	0.146 ± 0.02	0.081 ± 0.06	0.066 ± 0.01	0.013	0.011	0.069	ns
pH (1:2.5 H2O)	-	7.1 ± 0.09	5.9 ± 0.08	5.9 ± 0.25	0.002	0.003	0.004	ns
Water content	percent	1.3± 0.08	1.1 ± 0.1	0.8 ± 0.0	0.002	0.002	0.117	0.047
Bulk Density	g cm^3	1.36 ± 0.1	1.15 ± 0.02	1.64 ± 0.06	0.044	ns	ns	0.037
SOM	%	2.09 ± 0.27	2.39 ± 0.25	0.86 ± 0.15	0.001	0.002	ns	0.001
N (Kjeldahl)	%	0.121 ± 0.017	0.121 ± 0.009	0.056 ± 0.006	0.001	0.002	ns	0.001
P (Olsen)	mg kg^{-1}	39.07 ± 11.09	19.85 ± 3.81	7.27 ± 0.75	0.003	0.02	0.192	0.036
Ca	mg kg^{-1}	1 498.5 ± 174.9	904.7± 162.1	739.5 ± 86.4	0.016	0.016	0.06	ns
Na	mg kg^{-1}	42.5 ± 2.5	39 ± 2.1	30.5 ± 1.9	0.0008	0.008	ns	0.037
K	mg kg^{-1}	251.2 ± 27.6	202.7 ± 18.7	69.5 ± 4.4	<0.0001	0.0001	ns	<0.0001
Mg	mg kg^{-1}	228.2 ± 30.8	167.5 ± 24.5	130.5 ± 14.9	0.074	ns	ns	ns
K (tea bags)	-	0.011 ± 0.001	0.114 ± 0.03	0.01 ± 0.00	ns	ns	ns	ns
S (tea bags)	-	0.235 ± 0.04	0.274 ± 0.08	0.211 ± 0.01	0.002	0.001	ns	ns
Plant roots	g m^{-2}	1.32± 0.3	2.37± 0.2	0.49 ± 0.18	0.02	ns	ns	0.018
Microbial biomass	g m^{-2}	20.4± 2.9	28.3 ± 3.8	14.5 ± 3.2	0.005	ns	ns	0.004
Bacteria	g m^{-2}	7.3± 1.945	11.6 ± 2.6	6.2 ± 2.6	0.049	ns	ns	0.038
Fungi (living)	g m^{-2}	13.9 ± 2.4	16.6 ± 2.6	8.3 ± 1.1	0.068	ns	ns	0.055
Flagellates	g m^{-2}	0.02 ± 0.004	0.002 ± 0.00	0.001 ± 0.00	<0.001	<0.001	<0.001	ns
Amoeba	g m^{-2}	0.905 ± 0.392	0.113 ± 0.054	0.030 ± 0.014	0.004	0.004	0.038	ns
Ciliate	g m^{-2}	0.03 ± 0.02	0.0005 ± 0.00	0.001 ± 0.00	ns	ns	ns	ns
Total protists	g m^{-2}	0.9 ± 0.4	0.12± 0.05	0.03 ± 0.01	<0.001	<0.001	<0.001	ns
Fungi/ bacteria	-	6.7 ± 3.3	7.1 ± 5.2	9.4 ± 4.2	ns	ns	ns	ns

Probability values (p) for effects of management on the measured properties and for (EI/IN, EI/OR, IN/OR) comparisons.

The OR and EI vineyards significantly differed in the abundance of flagellates and amoeba, that were higher in EI. The soils of the OR and EI vineyards were rich in organic matter by comparison with the Mediterranean standards. In fields, the stabilization factor (S) obtained from the Tea Bag method was higher than in IN, although management had no effect on the decomposition rate (k).

The ongoing long-term soil incubation (Figure 2) confirms the significant difference in C content between the IN and the OR and EI vineyards, and suggests greater labile to recalcitrant carbon ratio in EI compared to OR.

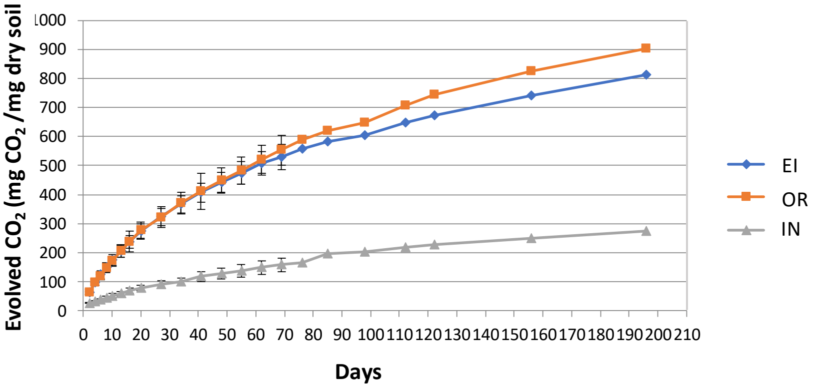

Figure 2: CO_2 evolved from soils of the EI, OR and IN vineyards in lab incubations under constant conditions

(T: 25°C, 40% WHC). Incubation still in course.

Discussion

It is well known that tillage (Beare *et al.*, 1994) and intensive use of agrochemicals (pesticides, herbicides) and mineral amendments (Thiele-Bruhn *et al.*, 2012) have caused the deterioration of agricultural soils and undermined their capacity to provide environmental services and even to sustain food production. Under the current uncertain climate scenarios, there is an exacerbate debate about the potentially negative effects of ecological intensification of agriculture on food production and farmers' economies (Kleijn *et al.*, 2019).

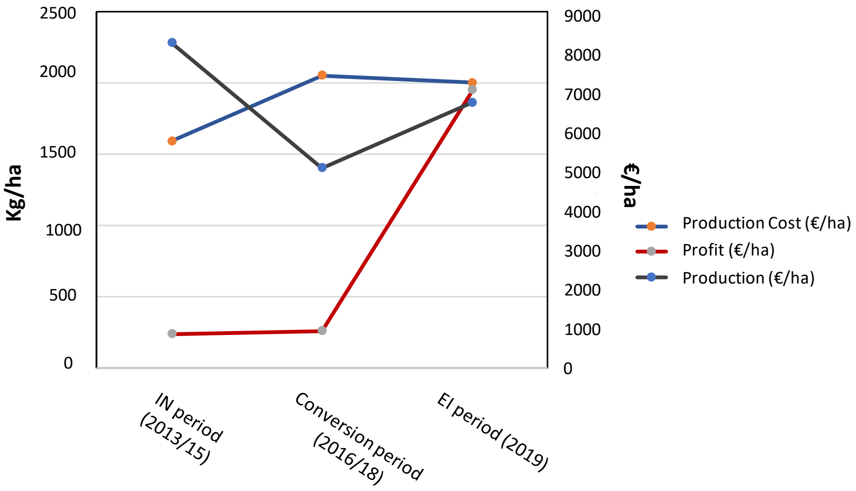

Figure 3: Evolution of yield and economic profit in the EI vineyard during the transition from intensive management to ecologically intensive management

Data from F. Font presentation to the 2019 EU Agricultural Outlook (Can Font Orgànic, 2019)

Our data show that ecological intensification of the agriculture can reverse soil organic matter and nutrient depletion in as little as four years and that the biological machinery and soil functional diversity responsible of the soil metabolism responds positively to this kind of management. In our study cases, the adoption of organic strategies together with the use of light machinery, improved microbial inoculums and intelligent water management has also resulted in net gains in production and economic profit three after three years of conversion in relation with the initial situation of intensive management (Figure3).

Conclusions

Under Mediterranean conditions, transforming vineyard management from intensive to ecological intensification strategies can be achieved in four years. In this short time, bacterial and fungal abundance and the biomass of several functional groups of soil protist significatively increase and soil nutrient and organic matter content rise to levels higher than the average in this geographic area. Conversion requires of temporal economic support, but soon agricultural production and economic profit equal or exceed the pre-conversion levels.

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The interdependence of soil function and soil biological diversity: lessons from an 8-year cover crop study in semi-arid Montana, USA

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Abstract summary

Measuring soil biological diversity is a significant task, but even more challenging is interpreting what that diversity means in terms of ecological function. Compounding the challenge of a highly diverse system is the reality that most of our methods of studying the soil involve a major disruption to habitats. I present the results of a study of soil processes in a semi-arid wheat system, where, because of limited growing season moisture, alternate year fallow has been practiced for decades. We designed an experiment to assess the effects of cover crops grown during the fallow period, comprised of four different plant functional groups (nitrogen-fixers, tap rooted plants, fibrous rooted plants, and Brassicaceae), on soil enzyme activity, mycorrhizal colonization, microbial biomass, and nitrogen cycling, along with effects on the subsequent year's wheat yield. The increase in soil biological activity was measurable after 2 and 4 years, and less evident after 8 years. Our results underscore the difficulty of interpreting soils processes when using just the plant community as the mechanistic driver. While ecological function can inform the relevance of soil biodiversity, soil biota drive process functions, and studies like ours will be difficult to interpret without knowledge of soil community composition.

Keywords: soil enzymes, nitrogen cycling, semiarid, wheat, soil function

Introduction, scope and main objectives

Nutrient cycling and accompanying organic matter decomposition is one set of ecological functions that supports aboveground communities. Linking soil biodiversity to soil functions is crucial for understanding the implications of soil biodiversity on food production and land management. As challenging as it is to address the high level of biodiversity in soil systems, soil function is also not straight forward. We provide an example in a study on the effects of cover crop mixtures (CCMs) on soil properties in a semi-arid system.

Dryland wheat farming in the northern Great Plains (USA) occurs in climates with barely enough precipitation to grow wheat profitably. Summer fallow has been widely practiced for decades as a way to store part of one year's precipitation for the next year's crop. While effective as a way to increase crop yield, the fallow year impacts soil quality as a result of reduced organic matter inputs and increased erodibility (Campbell *et al.,* 2000; Acosta-Martinez, Mikha and Vigil, 2007). We measured the effects of cover crop mixtures relative to fallow or a single-species pea cover crop on soil biological, chemical and physical parameters, along with yield and protein content of the subsequent year's wheat crop. We monitored four sites for two cropping cycles (four years), and tracked two of those sites for an additional two cropping cycles. With this study, we asked whether the presence of a cover crop could change soil biological parameters 9 months after termination of cover crops, when the cash crop (spring wheat) is seeded, compared to summer fallow; and whether the functional group composition of the cover crop affects soil biological parameters.

Methodology

Four sites were selected across Montana (USA), with a history of a minimum of 3 years of no-till wheat-fallow cultivation. Crop rotations were staggered, with two sites planted in 2012, and two sites in 2013. Cover crops were selected from four functional groups, each group represented by two species: N-fixing plants; tap-rooted plants, for their effects on soil compaction and water infiltration; fibrous-rooted plants, for their capacity to add C to the soil; and Brassicaceae, because of their unique biochemistry. Ten cover crop treatments and a fallow control were randomly assigned to plots (8 x 12m) in a split plot design including four blocks and three N fertilizer rates (0, 67, and 135 kg/ha). Cover crop treatments included a summer fallow (SF) control; legume green manure consisting of spring pea only (PEA), an eight-species/four-functional group cover crop mixture (FULL), four single-functional group treatments—N-fixers (NF), taproots (T), fibrous roots (FR) and Brassicaceae (BR)—and four mixtures that excluded one functional group—minus N-fixers (MNF), minus taproot (MT), minus fibrous roots (MFR), and minus Brassicaceae (MB).

In the spring of the year following CCM treatments, and prior to planting wheat, soils were sampled to a depth of 10 cm. Activity of five soil enzymes was measured in the lab (β-1,4,-glucosidase (active on cellulose), β-1,4,-N-acetyl glucosaminidase (active on chitin), arylsulfatase, and acid and alkaline phosphatases (Dick, 2011; Parham and Deng, 2000). Potentially mineralizable nitrogen (PMN) was calculated from those same soils as the difference between plant available N at time zero and after a 14-day anaerobic lab incubation (Keeney & Nelson, 1983). Microbial biomass was estimated by measuring substrate-induced respiration (Fierer, 2003). At wheat anthesis (2013-2015, 2018), single plants were harvested to quantify mycorrhizal colonization (McGonigle *et al.,* 1990).

Results

Following the first rotation, soil enzymatic activity of β-glucosidase, β-glucosaminidase, arylsulfatase, and acid and alkaline phosphatases showed few differences between the FULL, PEA, and summer fallow treatment soils, but many enzymes showed positive correlation with total aboveground cover crop biomass produced the previous year at two site-years. Geometric means of enzyme activity were positively correlated to cover crop biomass from the previous year at one site-year after one rotation (r = 0.74) and at another site-year after two rotations (r = 0.38). After two rotations at three of four sites, enzyme activity was 1.2 to 1.3 times greater and microbial biomass was 1.3 to 1.4 times greater where a cover crop was present the previous year at two of four sites. After four rotations, there were no differences in soil enzyme activity across any of the treatments at both sites monitored. For measures of nitrogen cycling, PMN was higher after the first and second cover crop cycles in either FULL or PEA than in summer fallow treatments in four of eight site years. After four cover crop cycles, PMN was higher in soils with either the FULL or PEA than compared to fallow, but did not differ between PEA or FULL treatments. When comparing single functional group treatments, there were no differences in enzyme activity, microbial biomass, or PMN. Mycorrhizal colonization of wheat roots after one rotation increased by 15 percent following either FULL or PEA treatments compared to summer fallow at one site, but among all site-years, results were inconsistent.

Discussion

Plants provide both habitat and nutrients to belowground communities, in a symbiotic exchange: soil communities break down organic matter and recycle nutrients to support plants, and plants provide photosynthetically-fixed carbon to support the soil community (Wardle and Bardgett, 2010). Our results after the first four years of this study suggested that soil response to cover crops relative to fallow was cumulative, and that total biomass of the cover crop was in fact more important than plant functional group identity. However, after eight years there were fewer differences in soil biological processes between treatments. This is especially unexpected because we measured an increase in soil organic carbon and total nitrogen of 10-15 percent in FULL and PEA relative to the fallow treatment. That soil enzyme activity was consistent between fallow and any of the cover crop treatments after 8 years of crop rotations suggests that we are missing part of the story.

One possibility is that soil processes respond to multiple preceding years of treatment, and we are analysing the data to determine whether cover crop biomass + previous year’s wheat biomass is better correlated to soil enzyme activity. Another possibility is that unusual precipitation events affected our results. Or, perhaps most logically, the cover crop treatments affected the composition and function of the soil community, which altered soil function in ways that are not predictable without including an assessment of biotic communities.

Our measurements of biological activity were taken in soils collected just prior to seeding spring wheat, as a way to assess whether the cash crop benefits from the cover crop treatments. In general, the presence of N-fixers in the cover crop mix increased wheat yield and grain protein, especially at the 0 N fertilizer rate. At the two higher rates of N fertilizer, yield either increased or decreased with the presence of N-fixers in the mix at one site, while grain protein consistently increased with N fertilization.

While we did not measure soil biological diversity in this study, instead focusing on soil processes in cover crop treatments, our results underscore the importance of considering the complexity of soil biological communities. Given both the diversity and richness, many studies focus on one or a few subgroups of soil biota. But functional processes are the result of a large number of direct and indirect interactions characteristic of soil food webs (Nielsen, Wall and Six, 2015). Integration of both functional and structural analysis of soil communities will be necessary to understand belowground systems.

Conclusions

The complexity of soil communities and the extent of both direct and indirect soil food web interactions suggests that soil processes respond to treatments in complex and not always predictable ways. Moving forward with linking soil biodiversity and soil processes will require systems-level thinking about taxa and functional guilds and their interactions within soil communities and with aboveground systems.

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Response of soil biological indicators after 37 years of wheat production management practices in a semi-arid climate

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Abstract summary

Agricultural management practices have substantial impacts on soil conditions and can influence soil microbial populations. Intensive soil cultivation practices particularly lead to soil organic matter (SOM) degradation, which also influences soil microbial populations. Conservation agriculture (CA) can restore SOM losses and improve the soil's biological status.

Soil samples were collected from a long-term wheat production management trial from two soil layers (0-5 and 5-10 cm) to examine the effect on SOM fractions, soil enzyme activities and microbial biomass. The applied field treatments were no-tillage (NT), stubble mulch (SM) and conventional tillage (CT), in combination with chemical weeding and non-burning of residues.

No-tillage and SM had the greatest influence on total C and N, dehydrogenase activity, ß-glucosidase activity and total microbial biomass than CT. Substantially higher SOM fractions were observed in 0-5 cm compared to 5-10 cm soil layers. Furthermore, a strong relationship existed between the measured biological indicators and SOM fractions, especially in the 0-5 cm soil layer.

These results demonstrate that conservation tillage practices have higher SOM content and microbial biomass than conventionally ploughed soils. Even though production management practices play a critical role in the response of soil parameters, it is important to consider site-specific conditions like climate and other soil characteristics.

Keywords: microbial biomass, soil biological status, soil quality, tillage, wheat

Introduction, scope and main objectives

Soil organic matter plays a vital role in biological, physical and chemical functioning of soils. In addition, SOM is a source of energy for soil microorganisms, and their biological activity depends on this. Soil microorganisms are involved with soil aggregation and stability in soil structure, ultimately protecting soils from erosion. These soil microorganisms also play a critical role in improving cation exchange and soil pH buffering capacity, and their role in association with SOM is therefore important.

Agricultural management practices, like tillage can alter soil conditions (temperature, water content, porosity), which influences the decomposition rate of SOM. Understanding the link between soil organisms and ecosystem functioning and the impact of agricultural practices is necessary to reduce negative impacts, and to ensure effective utilization of the benefits that soil biological activity holds for sustainable and productive agriculture. This study posed a unique opportunity to investigate the relationship between SOM and soil biological indicators in a semi-arid environment.

Methodology

Site description

A trial was established in 1979 near Bethlehem (28°09’S, 28°17’E; 1.680 m above sea level) in South Africa, to investigate the effects of selected wheat management practices on soil properties. The mean annual rainfall in this area is 743 mm, mean annual class-A pan evaporation is 1 815 mm, with a mean annual aridity index of 0.41. This Plinthosol has three diagnostic soil horizons: orthic A (0-300 mm), yellow-brown apedal B1 (300-650 mm), soft plinthic B2 (> 650 mm), containing 18, 23, and 36 percent clay, respectively.

Experimental design and treatments

The experimental design was a complete randomized block with three blocks. The sampling design was restricted to three tillage methods [no-tillage (NT), stubble mulch (SM) and ploughing (CT)] that coincided with chemical weeding and non-burning of residues and two depths (0-5 and 5-10 cm). Plots were annually cropped with winter wheat (*Triticum aestivum* L.), with no rotation involved.

In the ploughed treatments, wheat straw was incorporated by a two-way offset disc to 150 mm depth, followed by ploughing to a depth of 250 mm. The stubble mulched treatments were cut V-blade and then ripped with a plough, to the same depth and time as the ploughed treatments. The no-tilled treatments were not ripped or ploughed. Weeds were controlled by spraying non-selective herbicides (Roundup alternated with Paraquat).

Soil sampling, preparation and analyses

Soil samples were taken during May 2016 at 0-5 and 5-10 cm depth intervals (Kotzé and du Preez, 2008). Five samples were randomly taken from each plot and combined into one composite sample. Microbial samples were kept cool (approximately 4°C) before sieving (<2 mm), whereas soil used for other analyses was dried and sieved as soon as possible.

- **Total C and N:** The total C and N were analysed by dry combustion with a TruSpec Leco CN analyser. (Nelson and Sommers, 1996).

- **Enzyme activities:** Assays for the β-glucosidase (adaptation of Dick, Thomas and Halvorson, 1996) and dehydrogenase enzymes (Von Mersi and Schinner, 1991).
- **Microbial biomass:** A sensitive and rapid method of Schnürer and Rosswall (1982), modified by Adam and Duncan (2001) was used for the measurement of microbial biomass using FDA.
- **Data analyses:** All parameters were subjected to a two-way ANOVA using IBM SPSS (version 24) to determine the influence of tillage, sampling depth and their interactions. Means were compared with Tukey's post hoc test. All data were tested for normality and homogeneity using Shapiro-Wilk and Levene's test before carrying out the ANOVA at 95 percent confidence interval.

Results

The 0-5 and 5-10 cm soil layers displayed elevated total C and N, dehydrogenase and ß-glucosidase activity and total microbial biomass in the NT and SM plots (Table 1).

Table 1: Measured parameters within the three tillage practices (NT, SM and CT), over two soil layers (0-5 and 5-10 cm)

Tillage practice	Soil layer (cm)	Total C (%)	Total N (%)	Dehydrogenase (INF mg/kg/2h)	ß-glucosidase (ρ -nitrophenol mg/kg/h)	Total microbial biomass (µg FDA/g)
NT	**0-5**	0.8363	0.0798	106	8636	40
	5-10	0.7983	0.0732	104	7409	33
SM	**0-5**	0.7325	0.0787	57	4267	16
	5-10	0.7152	0.0668	46	5728	45
CT	**0-5**	0.7246	0.0702	56	3414	21
	5-10	0.7087	0.0707	45	4557	11

Correlations among the measured parameters were tested at 95 and 99 percent significance levels. Total N correlated with dehydrogenase and ß-glucosidase, while dehydrogenase correlated with ß-glucosidase ($p < 0.01$).

The first principal component (PC1) explained 26 percent and the second principal component (PC2) 21 percent of the total variance in the measured parameters (Figure 1a and b). The PC1 plot in Fig 1a had a clear negative loading score and the PC2 plot a clear positive loading score for all measured parameters. Figure 1b illustrates clear groupings found for tillage practices and soil layers sampled. The

PCA plot shows that the topsoil layers of the CA plots (NT and SM) are grouped together with negative loading scores on PC1, while the deeper soil layers sampled are grouped together with the CT plots on the positive loading scores. For the PC2 plot, both soil layers of CT plots, together with the deeper soil layer for NT plots had negative loading scores.

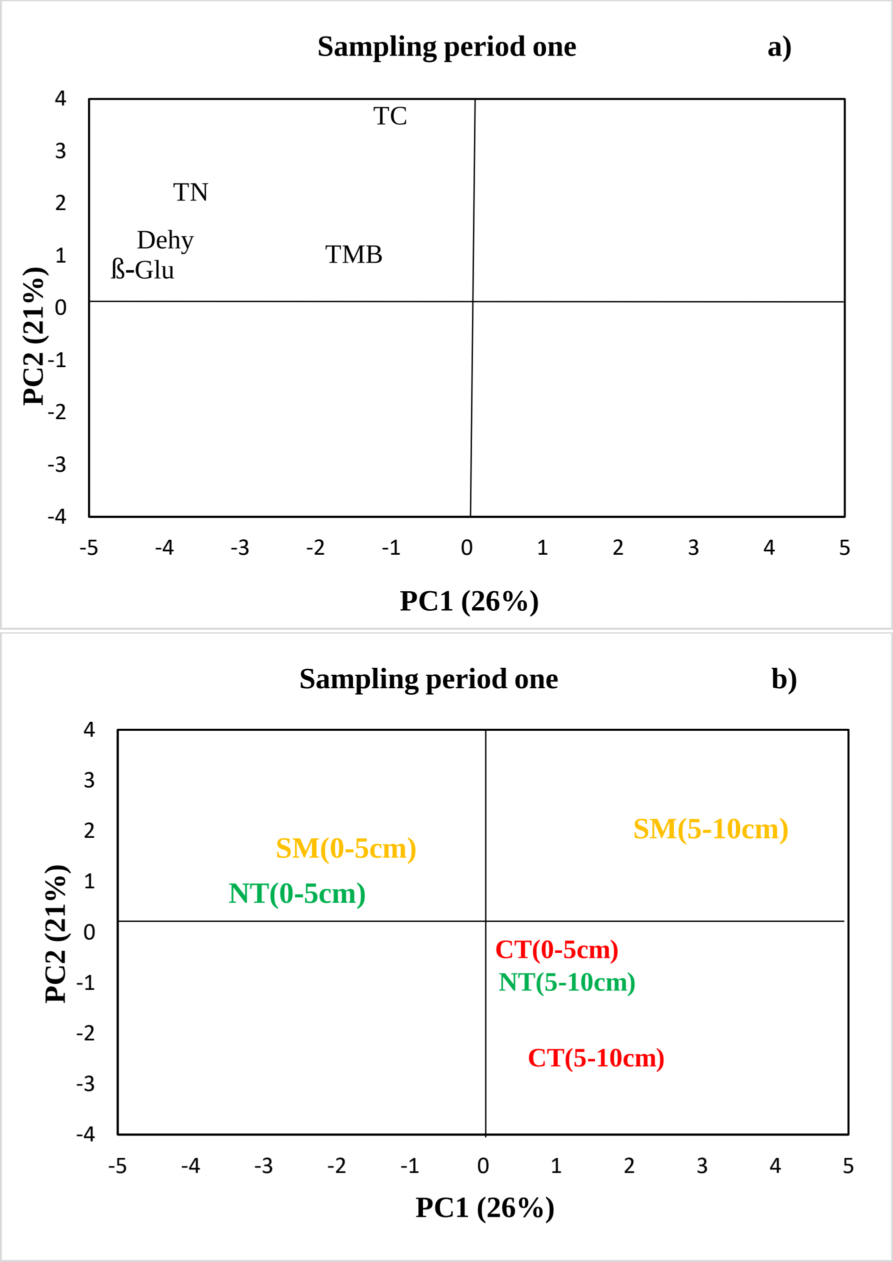

Figure 1: PCA separating all measured soil biological parameters.

PC1 and PC2 together explained 47% of the total variance. Abbreviations are a) TC: total C; TN: total N; Dehy: dehydrogenase; ß-Glu: ß-glucosidase; TMB: total microbial biomass. b) PCA separating the different tillage practices [NT (no-tillage) indicated in green, SM (stubble-mulch) in yellow, CT (plough) in red] over two soil layers (0-5 and 5-10 cm).

Discussion

The results revealed that most of the measured soil parameters were elevated in the CA tillage practices in the topsoil layer compared to CT practices in the second soil layer. Minimum tillage practices result in organic matter stratification and accumulation on or near the soil surface (0-5 cm), thus conserving C and N. Franzluebbers *et* *al.* (2001) and Gattinger *et al.* (2011) found stratification and accumulation of SOM to be beneficial to microbial populations, erosion control, water infiltration and soil nutrient conservation.

Microbial biomass in the soil is an essential part of SOM and consists of microorganisms that feed on C. They produce enzymes that are representatives of the C and N cycles and of microbial biomass (Aon and Colaneri, 2001), and explains the correlations found in this study between the measured parameters. Total N was strongly correlated with dehydrogenase and ß-glucosidase, and indicate that soil enzyme activities are quicker to respond to changes in production management practices and land use, compared to total C and N (Bergstorm *et al.*,1998).

The PCA showed that an increase in soil enzyme activities was associated with C in soils under CA practices, supported by numerous studies reporting lower soil enzyme activities in cultivated fields (Saviozzi *et al.,* 2001). Tillage destroys mycelia that typically produce these enzymes (Baldrian, 2014), and breaks down soil aggregates serving as a habitat for soil microorganisms. (Mummey *et al.,* 2006; Clayton, 2012).

This study shows that increased amounts of SOM leads to advantages like resistance to erosion, better water infiltration, suppressed soil borne diseases and a nutrient sink for plants (Gajda, Czyż and Dexter, 2016). Additionally, SOM affects the main processes that occur in soil, such as microbial activities, nutrient release, erosion protection and the promotion of biological activities, and ultimately the sustainability of many agroecosystems (Vázquez *et al.,* 2016).

Conclusions

The CA practices (NT and SM) supported the high expectation that these practices are associated with the abundance of various microbial populations. This is because CA practices retain higher organic C contents than the CT practices, which promotes a healthy soil ecosystem. Soil microorganisms can be used as sensitive predictors of soil quality, however these are often the least measured parameters in soil.

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Long term impact of soil fauna conservation practices on the introduced mesofauna and soil chemical properties in rain fed agro ecosystem

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Abstract summary

A lot of emphases have been made in alfisols to commercialize agriculture through agricultural practices. However, the crops are not responding to practices due to loss of below-ground biodiversity and resulted in progressive deterioration of agricultural productivity. The aim of this study was to evaluate the effect of fertilizer and farmyard manure at different doses along with introduced mesofauna rich soil on the survival of soil mesofauna richness and abundance in soybean ecosystem. The present study was carried out in the fixed plot for 15 years. This study demonstrated that: 1) Long term addition of 20t of FYM ha^{-1} and introduced native mesofauna rich soil enhances the mesofauna abundance, MBC, SOC, available nitrogen, phosphorus and potassium content in the soil. These enhanced the grain yield significantly. 2) FYM with lower dose of fertilizer also supports the establishment of soil mesofauna. 3) Addition of partially decomposed FYM or glyricidia leaves enhanced the abundance of mesofauna than well decomposed FYM. 4) Long term addition of 5t of FYM ha^{-1} alone documented higher mesofauna abundance and sustainability compared to recommended fertilizer alone.

Keywords: Introduced soil mesofauna, Rainfed agro-ecosystem, Mulching, Gliricidia, FYM.

Introduction

Alfisols are low in nutrient retention capacity, highly erodible with high infiltration rate. A lot of emphases have been made in this soil to commercialize agriculture through intensive agricultural practices. However, the crops are not responding to these practices due to loss of below-ground biodiversity and resulted in progressive deterioration of sustainable agricultural productivity. Recently, the importance of soil biodiversity is frequently discussed in relevance to the ecosystem functioning (Wardle, 2002). It holds true in the soil ecosystem; increased below-ground biodiversity gives rise to increasing crop productivity. Earlier, Keesing and Wratten (1998) highlighted the importance of indigenous faunal components in ecological restoration in agricultural landscapes for better sustainable function. The quantitative and qualitative composition of soil fauna reflects the quality of the soil. With this background, the present study considers the value of soil organic matter in agro-ecosystem for increasing the abundance and diversity of mesofauna. First, we compared the abundance and diversity of mesofauna in soybean agro ecosystem to test the hypothesis that higher soil organic matter content increases soil mesofauna abundance within different doses of farmyard manure (FYM) and inorganic fertilizer. Next, we compared the addition of native mesofauna rich soil to test whether the introduced native fauna increases the faunal abundance and diversity and sustainability in the already degraded agro-ecosystem. The long-term investigation was initiated in the year 2001 to know the impacts of biodiversity conservation practices on the native introduced soil fauna and sustainability under rain-fed condition.

Material and methods

The investigation was carried out at the Zonal Agricultural Research Station, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India during *Kharif* season under rainfed conditions. The site is located at an altitude of 929m above MSL. The soil is classified as oxic Haplustalf. The soil is lateritic derived from granite gneiss under subtropical semi-arid climate. The topsoil pH is 5.03 with the textural class of sandy-loam. Bi-modal distribution of rainfall accounting 928mm. This was the long-term experiment initiated during *Kharif* 2001 and continued till 2015. The experiment was started with the initial soil chemical properties of organic carbon (0.34 percent), available nitrogen (168.72 kg/ha), available phosphorus (11.69 kg/ha), available potassium (120.50 kg/ha) and pH (5.92). The experiment was laid out in a randomized complete block design with the following treatments and replicated thrice.

The treatments were,

1. Recommended fertilizers (25: 60: 25 Kg NPK ha^{-1}) + Recommended FYM (10 t ha^{-1}) + Phorate 10G @ 1kg *a.i.* ha^{-1} + Pre-emergent application of Lasso 50 EC @ 2.5 l ha^{-1} + seed treatment with Carbendazim 50WP @ 2g/kg.
2. 12.5t FYM ha^{-1}+ 75 percent of rec. fertilizer
3. 15t FYM ha^{-1}+ 50 percent of rec. fertilizer
4. 17.5t FYM ha^{-1}+ 25 percent of rec. fertilizer
5. 20t FYM ha^{-1}
5. 10t FYM ha^{-1}
6. 10t FYM ha^{-1} (partially decomposed)
7. 10t FYM ha^{-1}+ mulching (*Gliricidia* 2 t ha^{-1})
8. Rec. fertilizer alone
9. 5t FYM ha^{-1}

(Note: N, P, K- Nitrogen, Phosphorous, Potassium. FYM- Farm yard manure)

FYM was applied to the respective plots about one week before sowing. Chemical fertilizers and soil insecticide were applied at the time of sowing in furrows. Seed treatment with fungicides was done before sowing. Herbicide was applied to the soil as a pre-emergent application. Treatments were maintained in the respective plots year after year. The soybean variety Hardee was sown after treating with Rhizobium culture with a spacing of 30x10cm in 6x3.6m subplot during *Kharif* season under rainfed condition. Need-based plant protection measures (NSKE and Ha-NPV) were taken to manage foliar insect pest population. Two hand weedings' and one inter-cultivation operation were carried out. One kg of soil (0-10cm) of natural grassland was spread in each plot as a source of native soil fauna (mesofauna) from 2005 *Kharif* onwards.

Extraction of soil mesofauna

The soil samples (400g each) were collected from each plot at fortnightly interval using the circular core sampler (12cm diameter) to a depth of 10cm during the cropping season. The mesofauna was extracted from the soil samples using Rothamsted modified Mcfadyen high gradient funnel apparatus. The mesofauna were collected in a vial containing 70 percent ethyl alcohol after 48 hours. Collected fauna was sorted out into different taxonomic groups under stereo binocular microscope.

Soil samples were collected at 45 days after germination to a depth of 15cm for soil nutrient analysis. Collected samples were dried under shade and crushed into powder. These were sieved through 2mm sieve. Samples were used to estimate soil organic carbon (Walkley and Black, 1934), available nitrogen (Subbaiah and Asija, 1949), available phosphorus, available potassium (Jackson, 1973) and microbial biomass C (Carter, 1991) by using standard methods. Seed yield was recorded after threshing and winnowing the pods from the net plot area and expressed in quintals per hectare.

Statistical analysis

The total number of individuals of mesofauna, which appeared in each treatment was recorded and the mean of each season were considered for further analysis. The data were statistically analysed by adopting the analysis of variance. To test for the effect of the experimental factors (treatment and sampling period) in the variables analysed, a two-way ANOVA with interaction term was performed using SPSS statistics 23.0 software.

Results

The abundance of soil mesofauna

Introduction of mesofauna rich soil in 20t of FYM ha^{-1} applied treatment significantly enhanced the mesofauna abundance than the rest of the treatments. This was followed by 17.5t of FYM ha^{-1} +25 percent of recommended fertilizer treatment. Introduced faunal survival was significantly least in the recommended fertilizer alone treatment compared to the rest of the FYM based treatments. Even five tonnes of FYM ha^{-1} applied plot supported higher mesofauna. The buildup of mesofauna abundance was less in all the treatments untill the introduction of mesofauna rich soil (Figure 1).

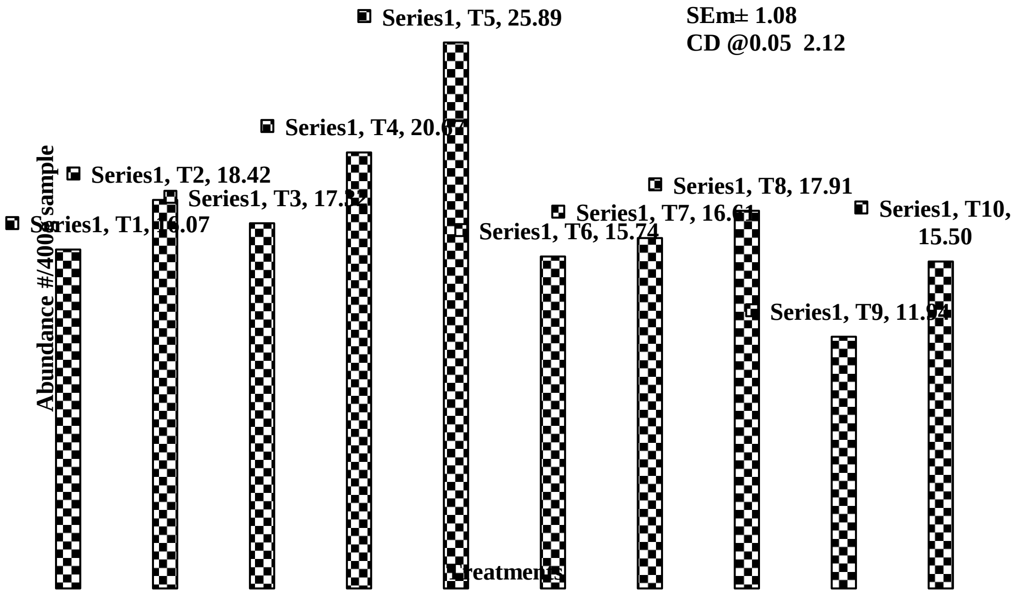


Figure 1. The abundance of mesofauna in different treatments

Microbial biomass carbon (MBC)

MBC content in 20t of FYM ha^{-1} applied treatment was 99.13 percent higher than recommended fertilizer alone treatment. This was followed by treatment 4. All the FYM based treatments supported higher MBC compared to the fertilizer alone treatment (Figure 2).

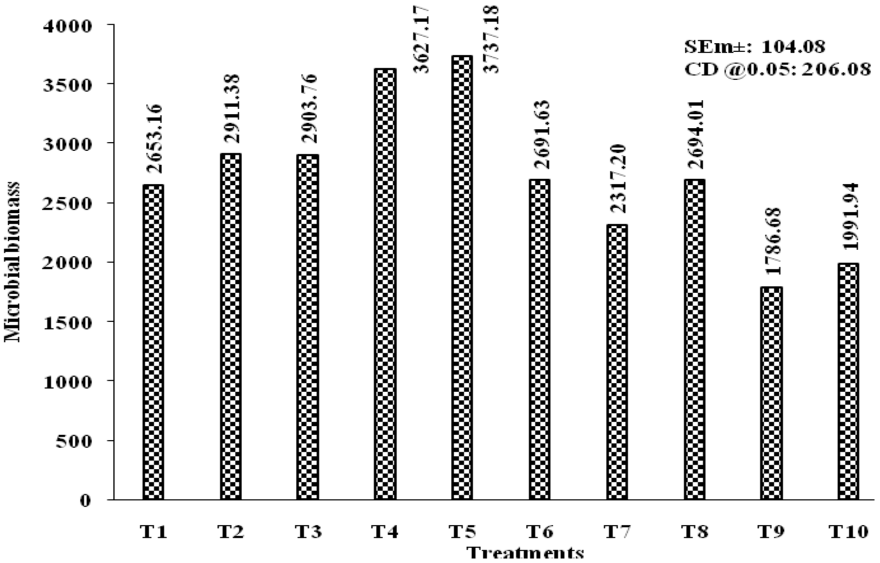


Figure 2: Microbial biomass carbon (µg/g soil) content in different treatments

Soil organic carbon (SOC)

Significantly higher SOC buildup was observed in treatment 5. This was followed by treatment 4. The latter treatment was on par in SOC with FYM +mulched and partially decomposed FYM treatments. Significantly least SOC was noticed in fertilizer alone treatment (Figure 3).

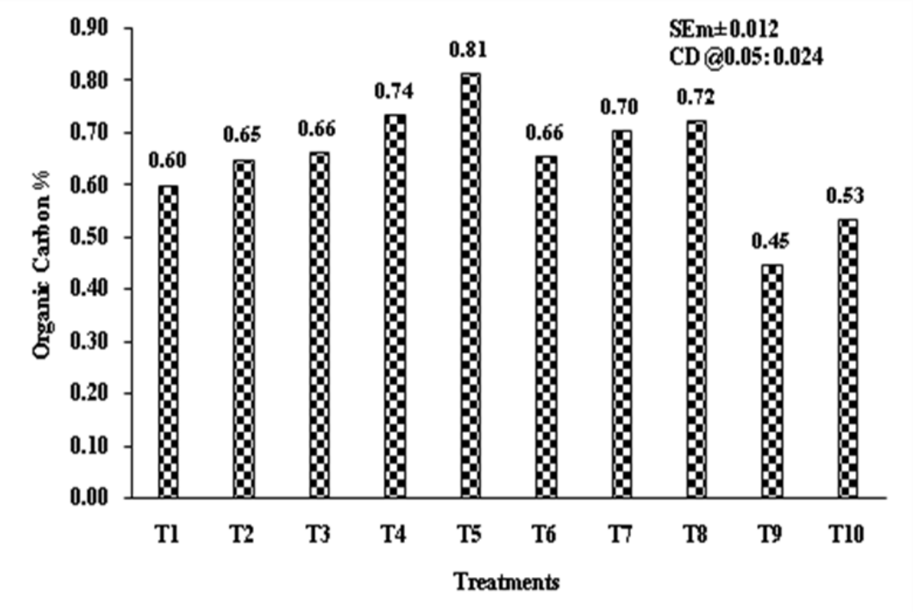


Figure 3: Soil organic carbon content in different treatments

Available nitrogen

Available nitrogen content in the treatment 5 was 26.63 percent higher than fertilizer alone treatment. The latter treatment documented significantly lower available nitrogen than the rest of the treatments (Figure 4).

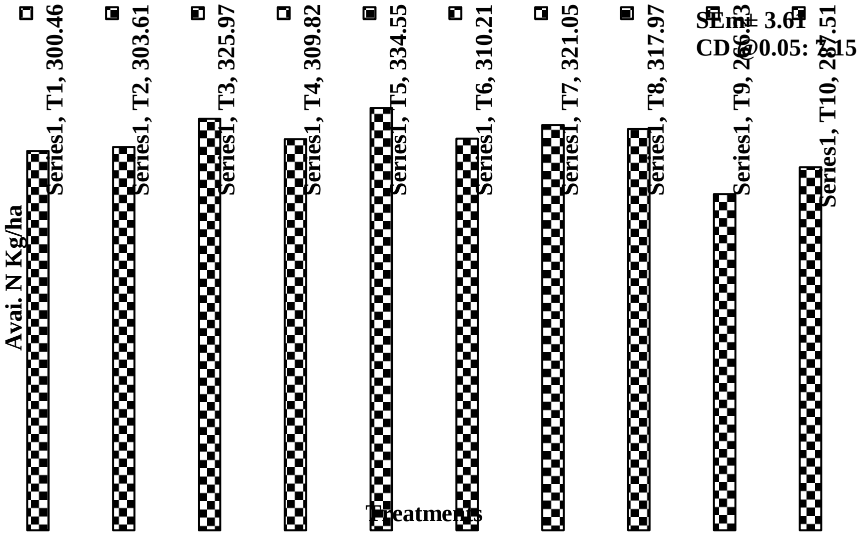


Figure 4: Available nitrogen content in different treatments

Available phosphorus

Available phosphorus content in treatment 5 was 35.95 percent higher over fertilizer alone treatment. This was followed by treatment 4 and 8. Even 5 and 10t of FYM ha^{-1} applied treatments were superior over fertilizer (Figure 5).

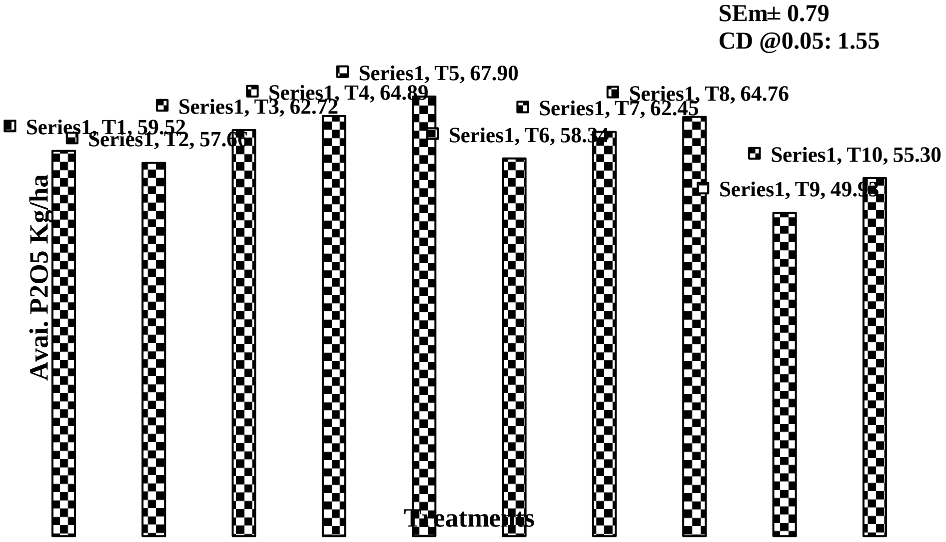


Figure 5: Available phosphorus content in different treatments

Available potassium

Available potassium content in treatment 5 was 38.84 percent higher over treatment 9. This was followed by treatment 4 and 3. Remaining treatments possessed 5.38 to 23.66 percent higher available potassium content over recommended fertilizer treatment (Figure 6).

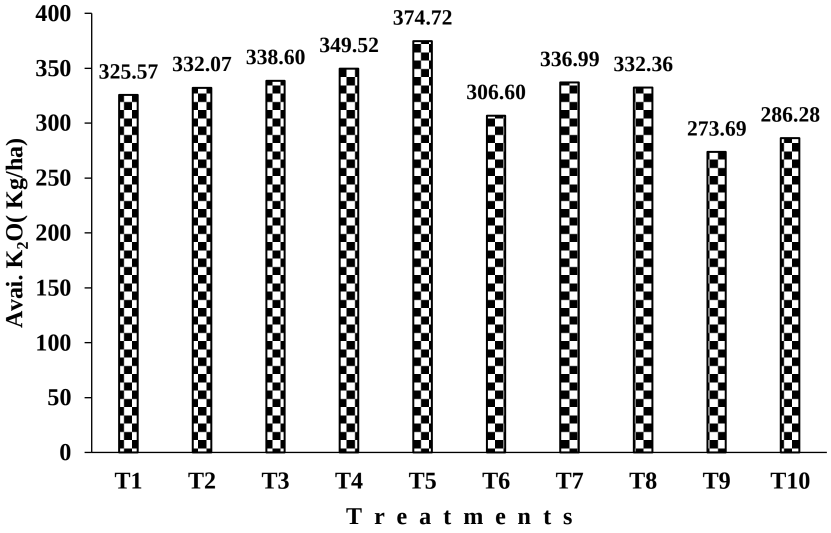
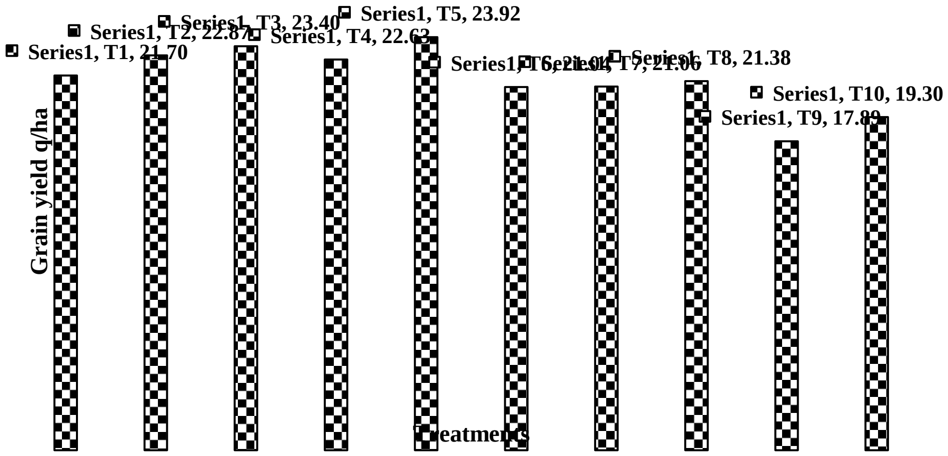


Figure 6: Available potassium content in different treatments (SEm±6.004, CD@0.05:11.77)

Soybean grain yield

Soybean grain yield was 33.46 percent higher in the treatment 5 and was on par with treatment 3. Latter treatment exhibited on par in grain yield with treatment 4 and 2 (Figure 7).



SEm± 1.83
CD @0.05: NS

Figure 7: Grain yield in different treatments

Discussion

The present investigation has demonstrated the benefits of the introduction of native soil mesofauna in the degraded agro-ecosystem for enhancing abundance and diversity. Higher mesofauna abundance was observed in the treatments after the introduction of native mesofauna rich soil. Earlier to this, these treatments supported only existing mesofauna in a lower number. The introduced mesofauna took almost two years to exhibit variation in abundance among the treatments. A Slight increase in abundance of mesofauna was noticed even in recommended fertilizer alone also. Higher abundance of mesofauna (Collembola, cryptostigmatids, mesostigmatids and other invertebrates) was observed in higher doses of FYM with the lower dose of fertilizer and FYM alone treatments. Higher abundance of mesofauna may be due to enhancement in food availability (organic matter and microbial biomass), optimum soil moisture and temperature, crop shade, regular addition of senescent leaves, less disturbance and settlement of soil particles due to rainfall after inter-cultivation at 25days after germination. Further, increased quantity of FYM and mesofauna abundance resulted in increased MBC, SOC, available nitrogen, phosphorus and potassium content in the soil. Similar to these observations, Gupta, Anil and Narawal (1988), Ben *et al.* (2007) and Ayuke *et al.* (2011) also reported the increase in soil biota and fertility by adding organic amendments.

Conclusion

Introduction of native mesofauna rich soil to all the FYM based treatments survived well with variation in abundance of mesofauna compared to the recommended fertilizer alone. Application of 5 to 10t of FYM ha^{-1} (well or partially decomposed or with glyricidia) harboured higher mesofauna than fertilizer alone treatment. Further, these treatments recorded higher MBC, SOC, available nitrogen, phosphorus, potassium content and soybean grain yield.

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GLOBAL SYMPOSIUM ON SOIL BIODIVERSITY | FAO HQ | Rome, Italy, 19-22 April 2021

Prospection, identification and antagonistic activity of native strains of beneficial microorganisms for the biological control of rice pathogens in Panama

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Abstract summary

The prospection and characterization of antagonistic microorganisms against pathogens in the rhizosphere, phyllosphere and espermosphere in rice plants in Panama was developed. For this, samples were taken from these three sections of a variety of rice (*Oryza sativa*), in a plot under the modality of organic agriculture. Fungi and bacteria were isolated determining differences in the total of isolates for the variables phenological phases (vegetative, flowering, maturation), sections of the rhizosphere (soil section, root section, soil + root interphase), sections of the phyllosphere (base, center, apex) and seeds (espermosphere). The antagonistic capacity of bacteria and fungi was evaluated against the phytopathogenic *bacterium Burkholderia glumae* and the fungus *Pyricularia oryzae*. The highest inhibitory capacity against *B. glumae* was found in strains of the genus *Bacillus*, as well as *Lactobacillus plantarum*, *Brevibacillus bortelensis*, *Aeromonas sp*. The fungal strains that showed antagonistic activity against *P. oryzae*, correspond to *Aspergillus flavus*, *Aspergillus niger*, Aspergillus sp, Penicillium sp, Trichoderma sp and Fusarium sp.

Keywords: rhizosphere, phyllosphere, espermosphere, bacteria, fungi, antagonistic

Introduction

Panama is the largest consumer of rice in Central America (68 kg / person) (FAO, 2018), being one of the essential foods in the diet of the population. Consequently, its production is of great importance at a social, political, economic and mainly in relation to food security in the country (Brathwaite, 2009). In rice, diseases of microbial origin are one of the factors that affect the obtaining of low yields. These diseases can be caused by various microorganisms such as fungi, bacteria and viruses (Acebo *et al.*, 2011). The bacterial blight of the rice panicle, caused by *B.glumae* is one of them, acquiring great importance in recent years. Another pathogen of high incidence in rice cultivars is the fungus *P. oryzae* considered one of the most important phytopathogens in this crop. Both were considered in our investigation, due to the fact of causing a decrease in the quality of the grain and low yields, which translates into economic losses, affecting the production of rice.

The rhizosphere, due to its environment rich in energy and nutrients, hosts large populations of most of the groups of microorganisms in the soil. The microbial flora found in this can play an antagonistic action against phytopathogens (Jaramillo, 2002).

The phyllosphere is considered as adverse for the colonization of organisms, as there is no source of nutrients there. The microbial communities of this space are diverse and include microorganisms that can be found as epiphytes on the surface of the plant or endophytes within the tissues of the plant. The espermosphere is a zone of influence of the seed in germination that produces stimulating exudates or inhibitors of the activity and / or growth of the autochthonous populations of the soil (Lindow and Brandl, 2003).

The objective of this investigation was to determine the presence of antagonistic microorganisms against the main rice pathogens in Panama and their distribution in the different sections (rhizosphere, phyllosphere and spermosphere) and in the phenological stages of the plant. According to our hypothesis, the presence of these microorganisms in the different sections of the plant, as well as in the phenological stages is fundamental to counteract the attack of phytopathogenic microorganisms.

Methodology

The samples were collected in the Republic of Panama, near the UTM coordinates (Datum WGS84) 591361.06 m E; 943492.23 m N. In the field, soil samples were taken for physicochemical analysis (Villarreal and Name, 1996). The sampling was carried out in a 10 m2 plot with rice variety Gab 8 during three phenological stages (vegetative, reproductive and maturative). The sampling site was selected for bioprospecting microorganisms because it was not subject to the effects of pesticides.

Rhizosphere. Isolation of rhizospheric bacteria

From each of the collected plants the rhizospheric zone was separated, dividing it into three sections, the exorhizosphere or rhizospheric soil (S), the root that involves both the endorhizosphere and rhizoplane (R) and a mixture of rhizospheric soil with roots (S + R). 30 g of the sample were weighed from each section and homogenized in 100 ml of peptonated water (Benitez *et al.*, 2007).

Rhizosphere. Isolation of rhizospheric fungi

Similar to the rhizospheric bacteria, serial dilutions were made and 0.7 ml was added from the 30 g sample taken from each of the sections of the rhizosphere of each dilution in potato dextrose agar (PDA) (Alpha Biosciences ®) (Benitez *et al.*, 2007).

Phyllosphere. Phyllosphere. Isolation of epiphytic bacteria

The methodology developed for the isolation of epiphytic bacteria, was performed in the sections of the apex, middle and basal areas of the collected leaves (Benitez *et al.*, 2007).

Phyllosphere. Isolation of endophytic fungi

For the isolation of endophytic fungi, healthy leaves were selected and each section of the leaf was cut into 2x2 mm squares, sections of plant tissue were placed on potato dextrose agar (PDA), incubated at 28 ° C (Schulz *et al.*, 2002).

Espermosphere

For the isolation of bacteria and fungi, 100 grams of rice seeds were weighed to which 200 mL of sterile distilled water was added. Then they were placed in a Stomacher (400 circulator) and shaken for 10 minutes. With the decanted water serial dilutions of $1x10^{-1}$ to $1x10^{-7}$ were made and spread on trypticase soy agar and potato dextrose agar, subsequently incubated at 30 ° C for 24 h.

Antagonism tests

The antagonism tests consisted of dual confrontations between the autochthonous microorganisms isolated from the rhizosphere, phyllosphere and espermosphere against the phytopathogens *B. glumae* and

P. oryzae, in order to quantify the possible inhibition of the growth of the pathogenic microorganisms. The in vitro antagonism test was based on the methodology of Alvis, Perez and Cordero (2017) with minor modifications.

Identification of microorganisms

For the identification of bacteria with antagonistic capacity against *B.glumae*, we used the API biochemical identification system (API 20 E and one of API 50 CH) Biomereux®. The strains with inhibition halo ≥ 4mm in diameter were considered as a significant antagonist. The monosporic cultures were identified with the help of the taxonomic keys were identified (Watanabe, 2010).

Results

Soil samples taken for physicochemical analysis revealed a percentage of organic matter 1.04, a pH of 5.80 and a sand-silt-clay composition of 70-8-12 percent respectively). The content of organic matter is considered low according to the parameters of Villareal and Name, 1996 for this type of soil. Despite an acid pH, the reported aluminum content of 0.40 Cmol / Kg and saturation of 4.35 (values considered low, Villareal and Name, 1996) generates low toxicity and contributes to the fact that acidity favours the prevalence of bacteria of the genus Bacillus (Calvo and Zúñiga, 2010) and of the fungi of the genera *Aspergillus* and *Penicillium* (Kavanagh, 2005).

In the three sections (rhizosphere, phyllosphere and espermosphere), a total of 400 bacteria and 180 fungi were isolated, of which 25 bacteria had antagonist activity against *B. glumae* and 11 fungi against *P. oryzae*. The presence of halos of inhibition ≥ 4 mm in diameter were considered as significant antagonists for the pathogens evaluated. In the rhizosphere, 317 isolates of bacteria and 10 antagonists were obtained, for the philosophical 18 isolates and 10 antagonists, and in the espermosphere, 65 isolates and 5 antagonists were obtained (Figure 1).

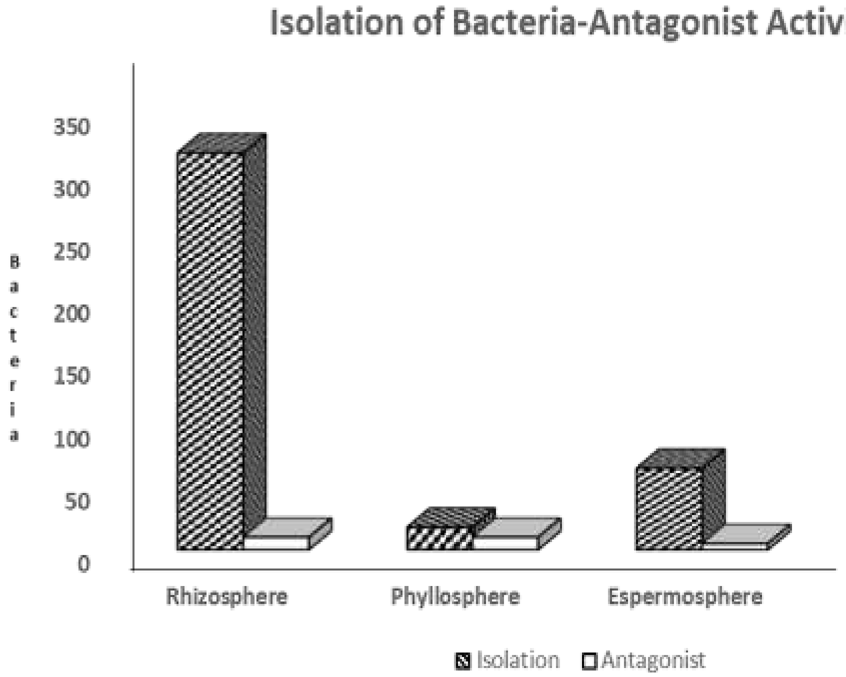

Figure 1: Isolation of bacteria and antagonistic activity against *B. glumae* in rhizosphere, phyllosphere and espermosphere in rice plants

In relation to the fungi, in the rhizosphere 112 were isolated and 6 had antagonistic activity, in the philosophical 68 isolations and 5 antagonists were obtained; no fungal isolates were obtained from the espermosphere (Figure 2).

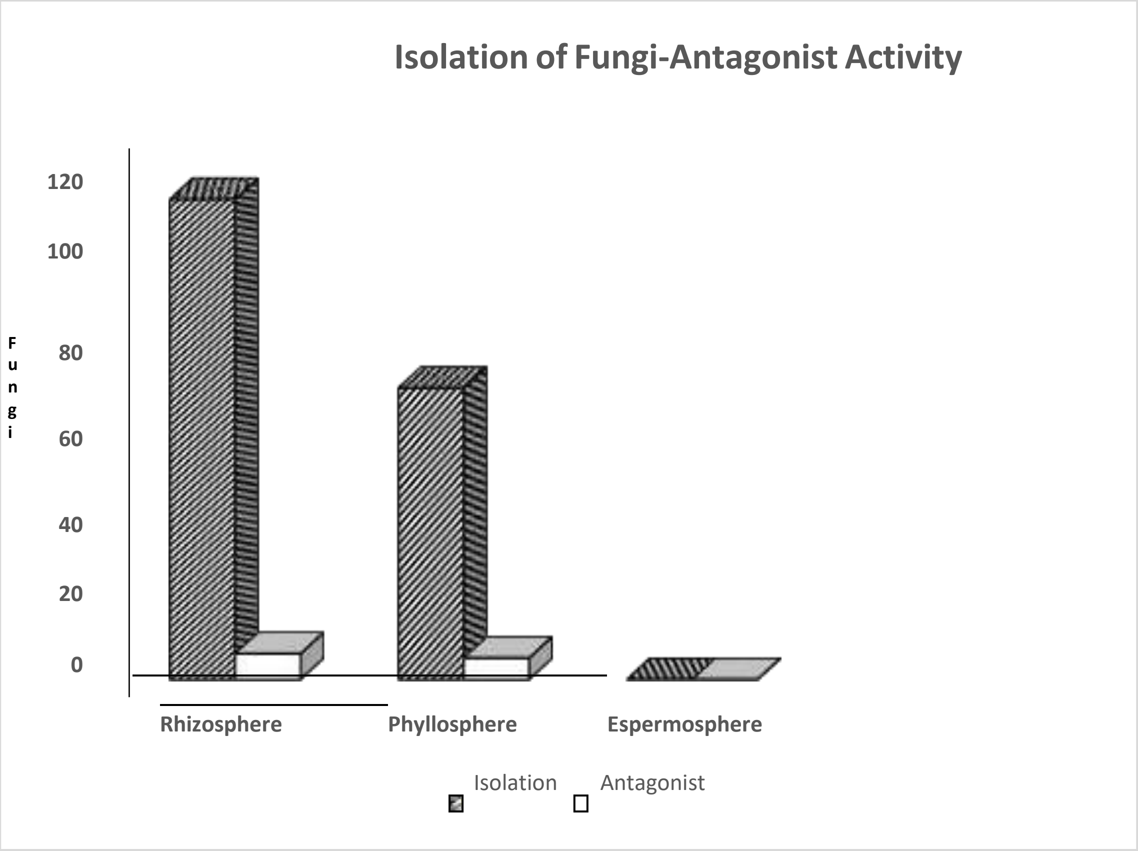

Figure 2: Isolation of fungi and antagonistic activity against *B. glumae* in rhizosphere, phyllosphere and espermosphere in rice plants

Discussion

In a conglomerate analysis, it was observed that there is a closer correlation between the bacteria of the espermosphere, the fungi of the phyllosphere and the fungi of the rhizosphere. However, there seems to be no correlation of these three sections with the rhizosphere bacteria and very weak correlation with the bacteria of the phyllosphere.

According to Jaramillo (2002), more bacteria were isolated in the rhizosphere, because this area is rich in nutrients and agrochemicals are not applied in the analysed soil, which increases its number, because the agricultural soils they are subject to continuous mechanization, to monoculture, to irrigation, to the application of agrochemicals and synthetic fertilizers, to compaction and to burning, they have a very low microbial flora that affects their fertility, which has an impact in obtaining possible antagonistic microorganisms against pathogens.

Meanwhile in the area of the espermosphere and phyllosphere less bacteria were obtained; This is based on the fact that the sperm is a zone of influence of the seed that produces exudates that can inhibit the activity and growth of the indigenous populations of the soil. Then when the plant develops the root system (rhizosphere) substances are produced that allow interactions to be maintained with the microbial population that originally colonized the seed when germinating). In the phylosphere, epiphytic bacteria develop in a very hostile environment, basically at the expense of the few nutrients exuded by the plant.

Conclusions

It is remarkable the omnipresence of the genus *Bacillus* in the whole rice plant, which reinforces its role like a promising biocontrol organism for root, foliar and postharvest diseases in rice. Additionally, strains of *Bacillus* sp. can induce systemic resistance resulting in an increase tolerance to harmful microbes. Although the importance of the genera *Penicillium*, *Aspergillus* and *Trichoderma* cannot be ignored, which depending on the environmental conditions and the agronomic management can represent an alternative to the use of agrochemicals.

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Biofertilizer applications in India: current status and future prospects

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Abstract summary

Soil harbours numerous microflora which participates in providing ecosystem services. Thus a soil rich in biodiversity will be most versatile in nature and can support numerous activities to provided ecosystem services. With only 2.5 percent of the land area India accounts for 7 percent-8 percent of the recorded species of the world. In agriculture the soil microbial diversity has traditionally been utilized for supplementing plant nutrient in the form of biofertilizers. Biofertilizers are the preparations containing living or latent form of microbes which affects the plant growth beneficially upon their application. *Rhizobium, Azotobacter, Azospirillum,* Phosphate Solubilizing Bacteria (PSB), Blue Green Algae (BGA) and Mycorrhiza have been used as biofertilizer in India for a long time and has been incorporated in Fertilizer Control Order (FCO) of India. Nitrogenous biofertilizers can fix 15-200kg of N/ha in a year. The government of India has been promoting the research and use of biofertilizers in the country and consequently there has been increase in production and use of biofertilizers. Use of biofertilizers and biopesticides are not only economic but are eco-friendly as well. Hence, biofertilizer application has to be seen not only in terms of economic viewpoint by nutrient supplementation to the plant but also other accrued ecological benefits received from their application and curtailment of chemical fertilizers. In this presentation, an overview of current scenario of biofertilizer application in India and future prospects and challenges are highlighted.

Keywords: Soil biodiversity, Biofertilizers, agriculture, India

Introduction

Increasing population and reducing fertile land due to diversion for non- agricultural purposes requires more productivity from existing arable land to meet the food demand. On the other hand, intensive cropping with use of high analysis fertilizers without adequate recycling of organic materials is contributing to yield stagnation and reduced factor productivity. The ill effect of overuse of agrochemicals and fertilizers to boost farm productivity has started surfacing, posing challenge on sustainable production and soil health maintenance. India is second largest consumer of chemical fertilizers in the world after China, with more than 26.5 million tonnes of fertilizer consumption (Sharma and Thaker 2011). Domestic demand of N and P fertilizers often exceed the production whereas entire potassic fertilizers requirement is met through imports due to unavailability of economically viable potash minerals. Despite large amounts of total

P and K is inherently present in the soil, only a small fraction is available for plant uptake. The contribution of inorganic P in most of the Indian soil ranges from 54 to 84 percent whereas the organic P may contribute to 16-46 percent (Sanyal *et al.,* 2015) in different states depending on the organic matter content, climate and other edaphic factors. The fixed or insoluble phosphorus compounds can be turned to soluble forms by potential soil micro-organisms. Similarly, despite the potassium constitutes about 2.5 percent of the lithosphere, the concentrations of soluble potassium in the soil are usually very low (Parmar and Sindhu, 2013) with more than 90–98 percent K reserves in soil system are in non-exchangeable forms (Sattar *et al.,* 2019). This necessitates finding alternative to improve the use efficiency of applied fertilizers as a major portion of P and K fertilizers are fixed in soil. Harnessing the potential of microbes in solubilizing the nutrient from the non-exchangeable pool and supply nutrient to the plants besides promoting the plant growth in sustainable way could be a possible approach. Thus, there has been emphasis on biological technologies like composting, legume BNF, biofertilizers, integrated nutrient management, biopesticides, etc. As population is increasing, increase in food production in India is obvious, and that will require additional fertilizer. Visualizing the economic burden and environmental cost of applying huge quantity of additional fertilizer, even if a small part of this increased demand if can be met from biological sources like biofertilizers, that will have large impact. Biofertilizers are known to not only improve yields and produce quality but also improve nutrient use efficiency. The use of cheap and eco-friendly inputs like biofertilizers is especially important for India where most of the farming will continue to be in the hands of small and marginal farmers (<2 hectares land). Biofertilizers unlocks the nutrients that are naturally abundant in soil or atmosphere and supply to the plants. Besides supplying the nutrients, they also enhance soil processes which may augment the availability of nutrients from soil by plants. Very often the efficient microorganism does not vigorously colonize in the rhizosphere thus their effect remains subtle. Therefore, artificially multiplied strains of efficient selected microbes are required to be applied to accelerate the microbial processes in soil.

Types of biofertilizers

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Microorganisms that are commonly used as bio-fertilizer includes; nitrogen fixers (N-fixer), potassium and phosphorus solubilizers, growth promoting rhizobacteria (PGPRs), endo and ectomycorrhizal fungi, cyanobacteria and other useful microscopic organisms (Table 1). The use of bio-fertilizers leads to improved nutrients and water uptake, plant growth and plant tolerance to abiotic and biotic factors (Ju *et al.*, 2018). These potential biological fertilizers would play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost-effective inputs for the farmers. Biofertilizer has potential to reduce chemical fertilizer as *Rhizobium* alone can substitute around 108.6-217.3 kg, Azolla 20-40 kg, *Azospirillum* 60 kg in maize and *Frankia* up to 195 kg of urea in agricultural fields. As estimate suggests in global agricultural systems rhizobial N-fixation contributes significant amounts of N ranging from 20 to 22 Tg N per year up to 40 Tg N per year (Herridge, Peoples and Boddey, 2008).

Table 1: Microbial groups involved in nutrient cycling in soil ecosystem

S.No.	Group	Micro-organism	Mechanism of action	Reference
1.	Diazotrophs	*Rhizobium*	Fix 50 – 100 Kg N/ha and yield increase of 10-70% over uninoculated control in legumes	(Singh *et al.*, 2013)
		Azospirillum (A. brasilense, A. lipoferum, A amazonense, A. halopraferens and A. irakense)	Yield increases by 43% in wheat, 44% in finger millet and 60% in barley and can substititute 15-20 kg N /ha as inorganic fertilizer	(Chapke *et al.*, 2018)
2.	Phospahte solubilizing microbes	*Bacillus* and *Pseudomonas*	Chelation via 2-ketogluconic acid, lactic, citric, gluconic and glyconic acids for phosphorous uptake by plants	(Dhandapani, 2011)
		Arbuscularmycorrhizal fungi	Mineralization via enzymes like phytases	(Kalayu, 2019).
3.	Iron chelator	Bacillus megaterium*and* *Azotobacter*vinelandii	10-35 % increase in grain yields over un-inoculated control	(Ahemad and Khan, 2010)
4.	Plant growth promoter (PGPR)	*Bacillus, Pseudomonas, Glomussp*	Phytohormones (IAA, ethylene, cytokines), nutrient acquisition via HCN, nitrogen fixation, P-solubilization and siderophoreproduction	(Yadav *et al.*, 2017)

Biofertilizer production in India

The total biofertilizer production in India increased from 88029.3 metric tonnes (MT) carrier based and 6240.926 KL liquid inoculums during 2015-16 to 109020.1 MT (solid carrier based) and 7526.3343 Kilo litres during 2016-17. The state wise carrier based highest production of biofertilizer was reported from Karnataka (31553.06 MT) followed by Tamil Nadu (27427.96 MT) whereas Gujarat was reported to produce highest number of liquid inoculums (2857.77 Kilo litres) amongst all the states during 2016-17 (Table. 2). Category wise in the year 2016-17, the liquid inoculums of PSB was highest produces biofertilizer. Despite increase in the trend of biofertilizer production, there is huge gap between capacity and actual production of biofertilizer in the country (Figure1).

Table 2. State wise biofertilizer production (KL) in India during 2016-17 (NCOF annual report 2016-17)

State	**Carrier (MT)**	**Liquid (KL)**

ANDHRA PRADESH	3375.91	365.24
ARUNACHAL PRADESH	119.7	0
ASSAM	1359.05	26
BIHAR	107	0
CHHATTISGARH	955.07	10.23
GOA	822	0
GUJARAT	3909.82	2857.77
HARYANA	2360.64	70.148
HIMACHAL PRADESH	3.28	194.7
JAMMU & KASHMIR	0	0
JHARKHAND	18.552	0
KARNATAKA	31553.06	993.443
KERALA	4993.87	59.6143
MADHYA PRADESH	5609.01	238.103
MAHARASHTRA	8323.62	398.33
MANIPUR	25	-
MEGHALAYA	-	-
MIZORAM	2.5	-
NAGALAND	51.45	-
ODISHA	516.28	31.79
PUNJAB	5533.77	210.177
RAJASTHAN	711	-
SIKKIM	16.25	-
TAMIL NADU	27427.96	875.292
TELENGANA	-	-
TRIPURA	1153.5	-
UTTARAKHAND	3720.68	461.19
UTTAR PRADESH	2835.79	696.9
WEST BENGAL	3195.18	26.21
DELHI	116.2	-
PONDICHERRY	203.97	11.197
Total	109020.1	7526.334

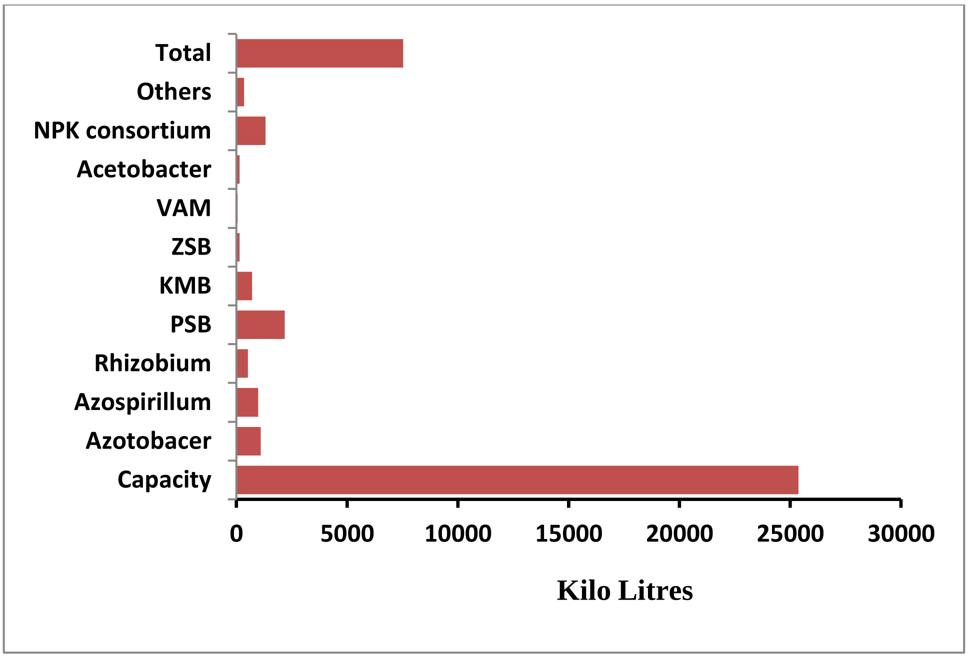

Figure 117: Category wise liquid based biofertilizer production (KL) in India during 2016-17

Government intervention in quality control of biofertilizers

Biofertilizers are included under Schedule-III Part-A of the FCO, 1985. Presently, *Rhizobium, Azotobacter, Azospirillum,* Phosphate Solubilizing Micro-organism (PSM), Potash Solubilizing Micro-organism, Zinc Solubilizing Micro-organism, and Mycorrhizal Bio-fertilizer, *Acetobacter*, consortia of microorganism are included. Organic fertilizers including city waste compost, vermi-compost, organic manure, de-oiled castor cake and Phosphate rich Organic manure (PROM) manure are notified in Schedule-IV Part-A of FCO, 1985. With the inclusion of biofertilizers and organic fertilizers under Fertilizer Control Order (FCO), a regulatory mechanism has been introduced in the country w.e.f. March 2006. National centre on organic farming (NCOF), Gaziabad and its seven regional centres have been declared as Notified Quality Control Analysis laboratories. Under the system samples are being drawn by the statutory authorities of State Agriculture Departments and submitted to NCOF or concerned RCOF for quality analysis.

Challenges and future prospect of biofertilizer in Indian agriculture

According to FAO there is a need to increase the agriculture production by 60 percent to feed about 9 billion populations in 2050. India is a tropical country and agricultural yield will be regional biased and largely depend upon crop type (Fitton *et al.*, 2019). India is projected to face substantial local yield reductions, particular for wheat and maize. Therefore, relying on microbes to restore agricultural yields along with improving soil fertility is a better option. It is also predicted that there will be inevitable global reduction in agricultural yields due to climate change (Sun *et al.*, 2019). For instance, there will be 10 percent reductions at 1.5ºC warming, ~15 percent between 1.5ºC to 2ºC and yield will be drastically reduce at 3ºC in case (IPCC). Considering the extent of biodiversity loss and climate change Indian agriculture can be reshaped through intensive use of biofertilizers along with chemical inputs. As evident from figure 1, there is wider gap between production capacity and actual current production of biofertilizers. Also, there is trend of increasing use and production of biofertilizers in India, indicating greater promise for utilization of indigenous microbial diversity for bioinoculant production and use.

Conclusion

This paper provided brief information about the significance of soil biodiversity and role of different microbial biofertilizer in agriculture. Due to intensive agriculture the gap between demand and supply of plant nutrients is prevalent in India. Supply of nutrients only through chemical fertilizers may not be viable in the context of its cost and environmental impact of chemical fertilizers. Improving soil biodiversity and use of biofertilizers would be a viable option in an integrated nutrient management option to increase agricultural productivity of small and marginal farmers. Strengthening the research on screening of potential bioinoculant, extensive field demonstration to visualize the impact of biofertilizers, focused research on improving shelf file of bioinoculant, mode of delivery, storage etc will add to further popularization of biofertilizers. Government policy has to be in place to encourage the industrial participation, and at the same time the quality control system has to be stricter to build up the confidence of users in biofertilizer.

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Enabling barley production in arid soils by only exploiting the indigenous microbial biodiversity

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Abstract summary

Mediterranean region is characterized by scarcity of natural resources such as water and productive soils. Agricultural practices, until now, have been based on the intensive use of fertilizers and water resources although limited. A knowledge-based bioaugmentation strategy that exploits the local soil biodiversity may provide microbial toolboxes to enhance target metabolic functions, in order to create optimal conditions for promoting specific crop growth under adverse conditions. This work is carried out under ERANETMED SUPREME project and presents the effect of bacterial bioaugmentation on plant growth under water stress. The bacterial formula JOR is composed by PGP autochthonous strains selected from an arid soil . The site is located in Al-Ghweir, an agricultural station for cereals cultivation, among which barley is the major crop. An exploratory pot experiment was set up to investigate the role of the microbial formula, in comparison with fertilisers, to support the growth of barley during the tillering phase. Results showed that under water stress only bacteria were able to sustain the vitality and the growth of barley. These results are confirmed by a second pot experiment replicated in Jordan, using the same microbial formula. The same bioaugmentation approach is applied in a two-year open-field trial now underway in the Al-Ghweir site.

Keywords: Soil functions, Biotechnology, Environmental sustainability, Resource efficiency.

Introduction, scope and main objectives

Mediterranean region is characterized by scarcity of natural resources such as water and productive soils. Agricultural practices, until now, have been based on the intensive use of fertilizers and of the limited water resources. As a result, local communities are experiencing soil impoverishment and water resources overexploitation. This work is part of the EU project "SUPREME" (Developing tools for sustainable food production in Mediterranean area using microbes) that aims at favouring the set-up of a sustainable agricultural production frame, addressing vulnerable communities living in semi-arid and arid areas in the Mediterranean region. The project objective is to combat impoverishment of soils and to reduce the use of water, fertilizers, and pesticides harnessing the microbiome potential to improve soil functions and promote plant growth. A knowledge-based bioaugmentation strategy that exploits the local soil biodiversity will provide microbial toolboxes to enhance target metabolic functions, in order to create optimal conditions for promoting specific crop growth under adverse conditions. This project addresses local communities distributed over 4 different Mediterranean countries (Italy, Jordan, Cyprus and Algeria) that have been increasingly challenged by water scarcity and by low agricultural productivity due to the scarce bio-geochemical functions of soils. Different soils and crops (tomatoes, legumes, such as fava beans and vetch, barely, wheat or high biomass leading grasses like sorghum, health crops and cereals) are considered in the test sites. The project's scope will be achieved through integration of state-of-the-art biotechnologies and leading-edge characterization, monitoring and modelling tools, accessed through an innovative, interactive web-based observation system. The assessment of soil characteristics, hydrological and climate conditions as well as type of crops will be the basis to measure the efficacy of the geo-biotechnology applied to improve crop production and to reduce negative effects of agricultural practices on the environment.

Microbial biodiversity at the soil level is a key issue to create optimal condition for crop growth. Microbes have already been proven to improve agriculture practice in controlled situations by reducing the need of water demand, fertilizers, and by improving crop resistance to pathogens.

Bacteria and fungi can effectively influence plant physiology, growth, defence mechanisms and nutrient uptake (van der Heijden *et al.*, 2008). Microorganisms associated with plant roots increase the absorption of nutrients, particularly phosphorus and nitrogen, enhancing the growth of crops and trees (Berg and Smalla, 2009; Gosling *et al.*, 2006). Inocula of bacteria can be used to improve soil functions and the resistance of plants to drought periods, and thus can contribute to reducing irrigation needs (Marasco *et al.*, 2012). Especially on arid and bare soils, bacterial inoculants can positively influence biogeochemical element cycles and formation of soil. Thus, they can help to reduce water, N and P requirements by augmenting the soil functions, and to reduce the effect of climate change on crop production.

The use of tailor-made consortia, functionally linking the indigenous microbial community with the chemical characteristics of the native soil and the needs of the target plant species, represents a successful strategy towards a rational selection of stable and reliable inocula (Sprocati *et al*, 2014a).The selection of indigenous strains can overcome the risk of introducing foreign microorganisms that could have a negative influence on the different components of soil biodiversity (Sprocati *et al*, 2014b).

In this work we present the set-up of a bacterial formula composed by some indigenous strains selected from the agricultural research station of Al-Ghweir in Jordan, used for cereals cultivation and especially for barley crop. The performance of the formula to support the growth of barley in conditions of reduced supply of water and fertilizers was tested in pot experiments prior to the field-scale step.

Methodology

Ten endemic botanical species were sampled with the soil in ten different points of the Jordan site. The plant and soil samples were collected in sterile bags and transported to the laboratory for further study. The rhizospheres of the ten samples were then mixed to create a composite sample and immediately processed for microbiological analysis.

Fifty grams of the rhizosphere sample were suspended in 500 ml of Sodium Pyrophosphate 0.1 percent W/V in a sterile flask and placed in an orbital shaker at 180 rpm for 90 minutes at room temperature. Biolog ECOPlates (Biolog Inc.,Hayward, CA, USA) were inoculated with the soil suspension (containing the dissolved bacterial community) in order to analyse the Community-Level Physiological Profile (CLPP).

The cultivable bacterial community was isolated spreading the soil suspension on three different solid agar media (TSA, MM and N-fix) in Petri dishes and incubating them at room temperature. The isolated strains were tested for plant growth promoting (PGP) traits. For strains identification, single colony DNA amplification was performed by polymerase chain reaction (PCR) with the Euroclone Gradient One thermocycler (Euroclone, Milano, Italy) using the universal Eubacteria primers 9bmf (5′- GAG TTT GAT YHT GGC TCA G -3′) and 1512r (5′- ACG GHT ACC TTG TTA CGA CTT -3′) to amplify the 16S rRNA gene (ca. 1500 bp).

The tailor-made microbial formula (JOR) was composed on the basis of the best-performing PGP traits, and was tested as bioaugmentation agent in a 20 days pot experiment with barley seeds and soil from the Jordanian experimental field. In the first pot experiment, the effects on the barley growth of the addition of the microbial formula JOR or a fertilizer (Diammonium phosphate) in optimal irrigation (100W) or water stress (25W =25 percent of the optimal) were compared.

The pot experiment was replicated in Jordan with the JOR formula (B), testing three irrigation levels: 100, 50, 25, under the same three conditions: just water W, W + DAP, W + B.

Results and discussion

The metabolic activity of the soil measured by CLPP showed high oxidation substrates kinetics as well as a high functional diversity index (94 percent, in terms of number of oxidized substrates), comparable to any agricultural soil.

Forty different colony morphotypes were isolated. The bacterial strains were distributed among the phyla of *Proteobacteria* (52.4 percent) *Actinobacteria* (38.1 percent), and *Firmicutes* (9.5 percent) (Figure 1).

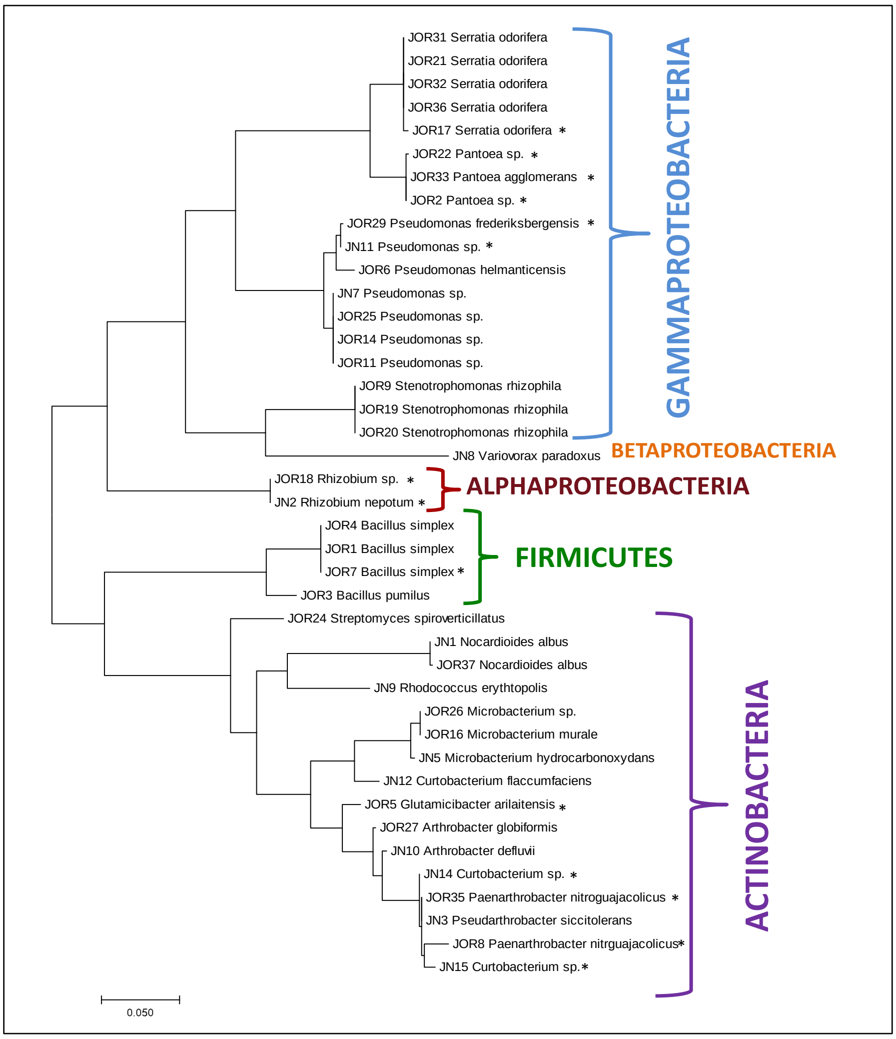

Figure 1: Neighbour-joining tree of 16S rDNA gene sequences showing the phylogenetic relationships of soil strains

The strains marked with * are those selected for JOR bacterial formula.

Some plant growth promoting (PGP) traits of the isolated strains were assessed: 88 percent were able to fix nitrogen, 29 percent to solubilize phosphates, 38 percent to produce siderophores and 16.7 percent to produce auxin. On the basis of these data, 16 of the best performing strains were selected to establish a bacterial formula that encompasses all the PGP functions and reflects the phylogenetic composition of the cultivable indigenous community.

The tailor-made bacterial formula (JOR) was tested as bioaugmentation agent in a 20 days pot experiment with barley seeds grown in soil comung from the Jordanian experimental field. Subsequently the formula was tested in a second experiment replicated in Jordan with Al-Gweir soil.

The effects of the treatments, either the microbial formula (B) or a fertilizer (DAP) in optimal irrigation (100W) and water stress (25W) conditions, were compared during the tillering phase of barley growth.

Both soil (CLPP) and plant parameters (aerial apparatus and roots system) were observed. The soil metabolic activity and the functional diversity were improved in the pots treated with bacteria, in both the conditions of optimal water supply and under water stress.

Under optimal water supply, the growth of plantlets (grams of fresh weight) was statistically equivalent (as induced from the t-test) with both bacteria and fertilizer and was greater than in the 100W control. Under 25W condition, only the presence of bacteria allowed plantlets to survive healthy, while the plantlets grown in the control and with fertilizer showed severe stress symptoms (Figure 2).

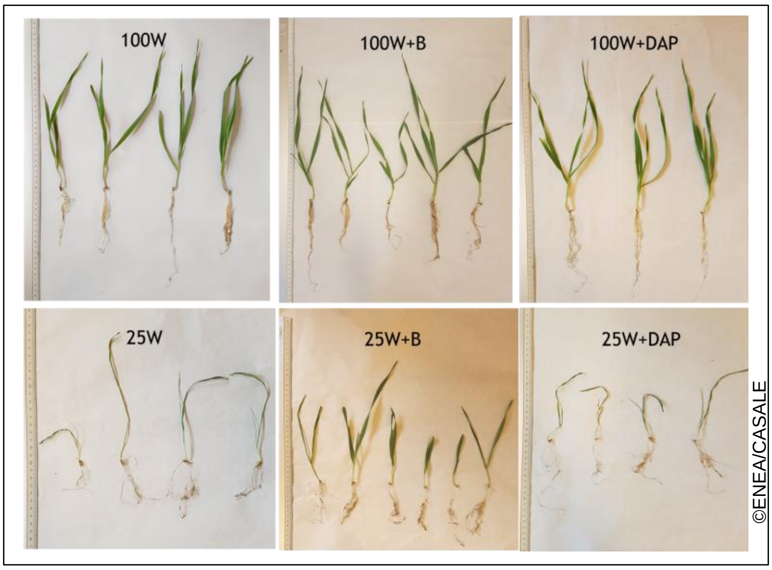

Figure 2: Exp 1-Comparison of the growth of plantlets in different condition: optimal irrigation (100W) and water stress (25W) with the addition of the microbial formula (B) or a fertilizer (DAP)

The root system was analysed by counting the number of adventitious and lateral roots and by measuring the length of the adventitious roots.

Anova and Student's t-test have highlighted as follows: in optimal water conditions the average number of adventitious roots was statistically equal for all treatments while under water stress the bioaugmentation improved the formation of adventitious and of lateral roots, compared to the other treatments; the bioaugmentation induced a different effect on the average length of adventitious roots compared to the other treatments, determining a shorter length in both 100W and 25W.

The second experiment confirmed previous results: under water stress (25W) only bacteria are able to support the growth of barley during the tillering phase; under optimal (W100) and intermediate (W50) water supply the microbial formula JOR can replace fertilizers (figure 3).

On this basis, a field trial for two harvest seasons was planned at the Al-Gweir site. The second year is currently underway.

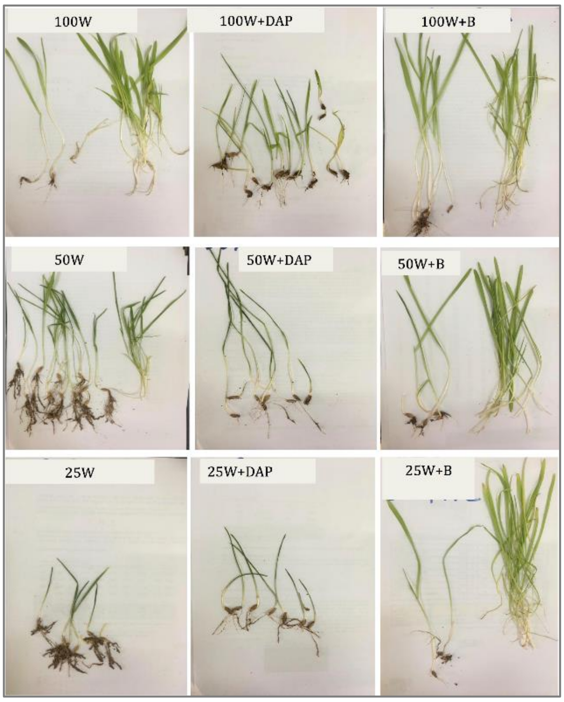

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Figure 3: Exp 2. comparison of the growth of barley plantlets in different conditions : optimal water (100W), intermediate irrigation (50W) and water stress (25W), with the addition of the microbial formula (B) or fertilizer (DAP)

Conclusions

1. At Al-Ghweir agricultural research station the soil biodiversity retains an intrinsic microbial potential suitable to be exploited, in order to support the cultivation of barley in semi-arid soil and under water stress.
2. In the presence of optimal water supply, no major differences in the results are observed between the treatments, highlighting that the selected microbial formula JOR can replace DAP fertilizer, commonly used in cereal crops.
3. The bioaugmentation with the native bacteria showed to be the only treatment that allowed barley plantlets to survive under water stress, as well as maintaining good physiological performances.
   The bioaugmentation with JOR microbial pool improved the metabolic activity and the functional diversity of soil.

Acknowledgements

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ZEOWINE ZEOlite and WINEry waste as innovative product for wine production

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Abstract summary

The ZEOWINE project is aimed at improving the soil protection and management, the plant health and the quality of production in the wine-growing sector, including organic and biodynamic productions.

Starting from the results of the application to agricultural soils of zeolites and compost in a separate way, the synergic and positive effects of these matrices will be demonstrated through the use of the innovative substrate ZEOWINE, resulting from the composting process of wastes of the wine productive chain and zeolite. In order to develop the composting protocol of pomace and steam with zeolite for the production of ZEOWINE, three productive composting cycles will be carrying out and the obtained compost will be applied to young and productive vineyards in different terroir.

In particular, the application of ZEOWINE in viticulture will be able to improve soil agronomic and biological properties in terms of:

- carbon sequestration;
- biodiversity;
- soil structure;
- water retention capacity;

In addition, the improvement of the physiological properties of the plants in terms of tolerance to abiotic stress and absorption of nutrients, will be demonstrated.

Finally, with the closure of the production cycle of waste material, the ZEOWINE product will increase the environmental sustainability and the competitiveness of the wine supply chain.

Keywords: zeolites, compost, soil biodiversity

Introduction, scope and main objectives

Due to the increasing pressure imposed to agricultural soils and to their consequent fertility decrease, the development of management strategies able to increase the quality and productivity of soils has become a common priority. In particular, Mediterranean vineyards are exposed to fertility decline due to continuous tillage to maintain bare soils, and to contamination due to repeated applications of copper, to fight against vine diseases such as mildew.

In particular, the loss of soil biodiversity, playing a key role in maintaining soil fertility and its health status, could irreversibly jeopardize the state of health of the soil and, therefore, crops. The ZEOWINE project proposes the adoption of the ZEOWINE product, derived from the composting of winery wastes and zeolite, in the vine supply chain management system to:

1. improve the quality of the vineyard soils, in terms of organic matter contents, biodiversity, water retention capacity, mineral resources availability and soil structure;
2. reduce the mobility of copper in the soil, the main anti-hygrostemic used mainly in biological and biodynamic viticulture, and therefore the risk of transferring the pollutant to other environmental compartments;
3. define and disseminate standards of intervention for the application of ZEOWINE in the various phases of the vine production cycle and in all production systems (conventional, organic and biodynamic), replicable also at European level;
4. increase the sustainability and competitiveness of the wine-growing sector, by implementing the efficiency of mineral and water nutrition and reducing energy consumption by closing down the production cycle of waste materials and ensuring higher yield stability and quality of grapes; product best suited to modern market demands;
5. improve consumer health protection: creating growth and development conditions suitable for crops in order to detect improvements in terms of endurance, healthiness and plant production.

Methodology

During the first year of the project, the waste material come from the wine supply chain of the 2018 harvest was mixed with the natural zeolite, clinoptilolite 80 percent with granulometry 1-2.5 mm, to form four different composting piles characterized as follows:

- One control pile: 5 tons of pomace and stems (10 m^3);
- Two piles ZEOWINE 1:10 w/w: 0.5 tons of zeolite (0.5 m^3) + 5 tons of pomace and stems (10 m^3);
- One pile ZEOWINE 1:2.5 w/w: 2 tons of zeolite (2 m^3) + 5 tons of pomace and stems (10 m^3).

Figure 1: zeolite (clinoptilolite) and winery waste piles set up

The composting piles were periodically turned (at least once a month) with a scraper to promote aeration. In order to maintain a moisture of about 50 percent an irrigation by sprinklers on top of each pile as needed was performed. Temperature and humidity were recorded every day until the end of the termophilic phase, successively every week. Three composite samples for each pile were collected at the start of composting, at the end of termophilic phase and at the end of composting process.

The samples were air-dried and sieved (2 mm) and stored at room temperature until physical, chemical, biochemical, toxicological and hydrological analyses.

In the spring 2019, the obtained ZEOWINE composts have been applied to two vineyards, a new (not in production yet, cultivar *Sanforte*) and a productive vineyard (cultivar *Sangiovese*) located in Tuscany (Italy), and incorporated by ploughing to a depth of 30 cm.

In each vineyard the following treatments were set up in triplicate (three sub-plots):

1. Commercial compost at the rate 20 t/ha;
2. Zeolite at the rate10 t/ha;
3. ZEOWINE at the rate 30 t/ha.

Immediately after treatments (Spring 2019) and after the grape harvest (six months from treatments application, September 2019), three composite soil samples were taken from each of the three sub-plots per each treatment, between rows at the 0–30 cm layer, in both the vineyards.

Methods for the analytical phase

Electrical conductivity (EC) and pH were measured in a 1/10 (w/v) aqueous solution using selective electrodes. Total organic carbon (TOC) and Total Nitrogen (TN) content were measured with LECO, U.S.A. RC-412 Multiphase Carbon and FP-528 Protein/Nitrogen Determinators, respectively.

Total phosphorus (TP) and potassium (TK) were extracted with nitric-perchloric acid digestion (HNO3:HClO4, 5:2) in microwave. Total phosphorus (TP) was measured using the method reported by Murphy and Riley (1962). Available potassium (Kav) was extracted with ammonium acetate (Helmke and Sparks, 1996). Total (TK) and available potassium (Kav) were determined by ICP-OES (Varian AX Liberty). $N\text{-}NH_4$ and $N\text{-}NO_3$ were determined in 1:10 (w/v) KCl extracts 0.5 M; $N\text{-}NH_4$ was detected with ion selective electrode (Seven- Multi, Mettler Toledo) and $N\text{-}NO_3$ was detected by Norman, Edberg and Stucki's method (1985). Sodium pyrophosphate (0.1M, pH 11) at 60 °C for 24h under shaking at 200 oscillation min^{-1} was used to extract Total Humic Carbon (THC). The THC extract was separated into humic (HA) and fulvic (FA) acids by addition of H_2SO_4; the extract was kept overnight at 4 °C, and then the flocculent (HA) and the supernatant (FA) were centrifuged. THC and FA were determined by the Yeomans and Bremner (1988) method, while HA were obtained by subtracting FA from THC.

Dehydrogenase activity was assayed using the method of Masciandaro *et al.* (2000). Cation-Exchange Capacity (CEC) of the soils was determined by Sumner and Miller (1996) method, using barium chloride (pH 8.1). Pyrolysis-gas chromatography (Py–GC) was used to evaluate soil organic matter quality. It is based on a rapid decomposition of organic matter under a controlled high flash of temperature, in an inert atmosphere of gaseous N2 carrier. The obtained pyrolytic fragments were separated and quantified by using the gas chromatographic technique (CDS Pyroprobe 190 coupled to a Carlo Erba 600 GC) (Macci *et al.*, 2012).

Results

Considering a single application of ZEOWINE at a dose of 30 t / ha, the project will demonstrate the effectiveness of the application of the product to demonstrative vines in improving the soil quality producing the following results in terms of:

- **Organic substance** - The organic carbon will go from 39 to 45-46.8 tons/ha; the total nitrogen will go from 3.6 at 3.9 tons/ha; humic carbon will drop from 0.78 to 1.90-2.13 tons/ha; total potassium will go from 7.5 to 8.4 tons/ha.
- **Biodiversity** – An increase in biodiversity in terms of enzymatic activities related to the nutrient cycles, microbial abundance and soil fauna. In particular, the QBS-ar index will go from 80 to 110; basal respiration from 5 to 10-15 mg CO_2-C/kg/day; the □-glucosidase activity from 250 to 350 mmol/kg/h; the phosphatase activity from 150 to 200 mmol/kg/h. These variations indicate an increase of about 25 percent in enzymatic activities.
- **Water retention capacity of soil** - For one ha of soil treated with ZEOWINE, an increase in the water reserve of 8.3 m^3 of water is expected.
- **Soil structure** - The average diameter of the water-stable aggregates of the vineyard soil will pass from 0.5 to 1-1.2 mm.
- **Reduction of bioavailable copper content** - Bioavailable copper will go from 15 to 10.5 mg Cu/kg.

The ZEOWINE project will have an impact on greenhouse gas emissions generated by specific agricultural activities. The phase of composting and application of the product itself will help to increase the ability of soil to fix and maintain the reserve of mineral elements in the soil, decreasing the need for fertilization, increasing the water retention capacity of the soil, and reducing the number of soil fertilization processes with consequent reduction of emissions linked to the use of agricultural machinery. They expected total reduction of greenhouse gas emissions is approximately 18.48 tons/ha of CO_2 per year.

Discussion

Zeowine proposes a solution, in the context of the circular economy, for the reuse of the green waste of the wine supply chain, the enhancement of their value thanks to the mixing with the zeolites and the simultaneous improvement of healthiness both for the soil environment and for the plant with its products. Zeowine represents both a product and a process that can be exported to every single agricultural activity.

Conclusions

The waste management system of the wine supply chain proposed by the ZEOWINE project represents an innovation in the composting process, with the closure of the production cycle of waste materials and their reuse as soil amendment. The wastes and zeolite based compost (ZEOWINE) represents an effective tool to reduce the dependence on mineral fertilizers in vineyard systems and to improve their agronomic and biological fertility.

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Perennial crops for sustainable soil management – Symbiotic fungi benefit from cultivation of a perennial cereal in Europe

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Abstract summary

Thinopyrum intermedium (intermediate wheatgrass) is used as a model for future perennial cereal grain production and the influence of agricultural perennialization was assessed in terms of the biomass of arbuscular mycorrhizal fungi (AMF) in soil. Cultivating a perennial cereal (*T. intermedium*) proved to promote higher biomass of AMF in comparison to conventional, but also organic, annual wheat production as well as in comparison to annual wheat and rye under no-tillage cultivation. Our results indicate improved conditions for mycorrhizal fungi in perennial cereal production stands, which may provide enhanced plant nutrient status of the crop and overall crop performance, due to larger hyphal networks and improved soil structure.

Keywords: Mycorrhizal fungi, soil fungi, soil ecology, perennial cereal grain crops, agriculture, soil ecosystem services

Introduction, scope and main objectives

Intensive agricultural practices are important drivers to the global loss of soil biodiversity and soil functions (Tsiafouli *et al.,* 2015). Thus, innovative management practices to improve soil biodiversity needs to be implemented in contemporary agriculture. *Thinopyrum intermedium* (intermediate wheatgrass) provide a perennial, multifunctional and multipurpose cereal crop (Duchene *et al.,* 2019). A shift from systems based on annual to perennial grain-crop production with permanent to semi-permanent plant cover, without the negative consequences of inverting soil tillage, is expected to benefit the delivery of soil ecosystem services (Crews *et al.,* 2016). The soil microbial community structure and biomass can be used as early indicators of the effects of changed cropping practices on the soil quality. In particular, the hyphal growth of fungal organism groups, i.e. saprotrophic (Barreiro *et al.,* 2018) and mycorrhizal fungi (Hydbom *et al.,* 2017), benefit from less disturbance, and extensive management in general (Barreiro *et al.,* 2019). This would fulfil part of the second Global Sustainable Development Goals (SDGs), calling for more resilient agricultural practices, enhancing both productivity and ecosystem quality. Increased soil biodiversity would also contribute to SDGs 12, 13 and 14 through improved efficiency in the management of natural resources, improved climate resilience and reduced nutrient loss. We determined the effect of agricultural perennialization of arable cropping systems by *T. intermedium* on the soil microbiome. We hypothesize that biomass of arbuscular mycorrhizal fungi (AMF) will be higher in the soil under a perennial cereal crop compared to under annual cereals.

Methodology

Mycorrhizal biomass indicators were analysed in two field experiments. One field experiment was carried out at Alnarp (55.65°N, 13.06°E) in southernmost Sweden (established 2016), and another one in Maubec (50.37°N, 4.48°E) in southeast France (established in 2017). The Swedish experiment included sole crop stands of *T. intermedium* under organic management, intercrops of *T. intermedium* and *Medicago sativa* (lucerne) under organic management, and *Tritium aestivum* (winter wheat) under conventional and organic management. The French experiment included conventional sole crop stands of *T. intermedium* in an annual grain crops rotation with *T. aestivum* (2017-2018), with direct sowing applied since 2007. Both experiments were replicated in four blocks for each treatment/crop. Soil sampling took place at the end of the growing season in 2018 using soil cores were four sub-samples made up one composite sample for each sampling depth and treatment plot. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) 16:1ω5 were used as indicators of AMF biomass (Olsson, 1999; Frostegård and Bååth, 1996; *Olsson et al.,* 1995). PLFA 16:1ω5 indicate both arbuscular mycorrhizal fungi (AMF) and Gram negative (G-) bacteria (Olsson, 1999; Frostegård and Bååth 1996; Olsson *et al.,* 1995), thus, the NLFA 16:1ω5 is used as an accompanying indicator of AMF due to its high correlation with AMF spore counts in soil (Sharma and Buyer 2015). PLFAs and NLFAs were extracted using the protocol presented by Frostegård, Bååth and Tunlid (1993) using $CHCl_3$:MeOH:citrate buffer (1:2:0.8 v/v/v). The lipids were separated using pre-packed silica columns subsequently rinsed with $CHCl_3$ (neutral lipids) and MeOH (phospholipids). The neutral and phospholipids were subjected to metanalysis after adding FAME 19:0 as internal standard, and the resulting fatty acid methyl esters quantified by gas chromatography with flame ionization detector (GC-17A, Shimadzu).

Results

In the Swedish experiment, the abundance of the one of the biomass indicators of AMF was higher in the perennial sole crop than in the three other crops at the 0-30 cm soil level (F_{AMF_N}= 13.48 (df=3) $p<0.001$) (Fig. 1:I). The biomass indicators of AMF (F_{AMF_P}=112.6 (df=1) $p<0.001$; F_{AMF_N}=38.54 (df=1) $p<0.001$) decreased from 0-30 cm to 30-60 cm depth. In the French experiment, the abundance of PLFA biomass indicator of AMF was higher in the perennial sole crop than in the annual crops at the level of 0-10 and 10-30 cm soil depth (F_{AMF_P}=4.441 (df=1) $p<0.05$) (Fig. 1:II). The biomass indicators of AMF were generally higher in the upper soil level than further down the soil profile (F_{AMF_P}= 121.1 (df=3) p<0.001; F_{AMF_N}=6.516 (df=3) p<0.01).

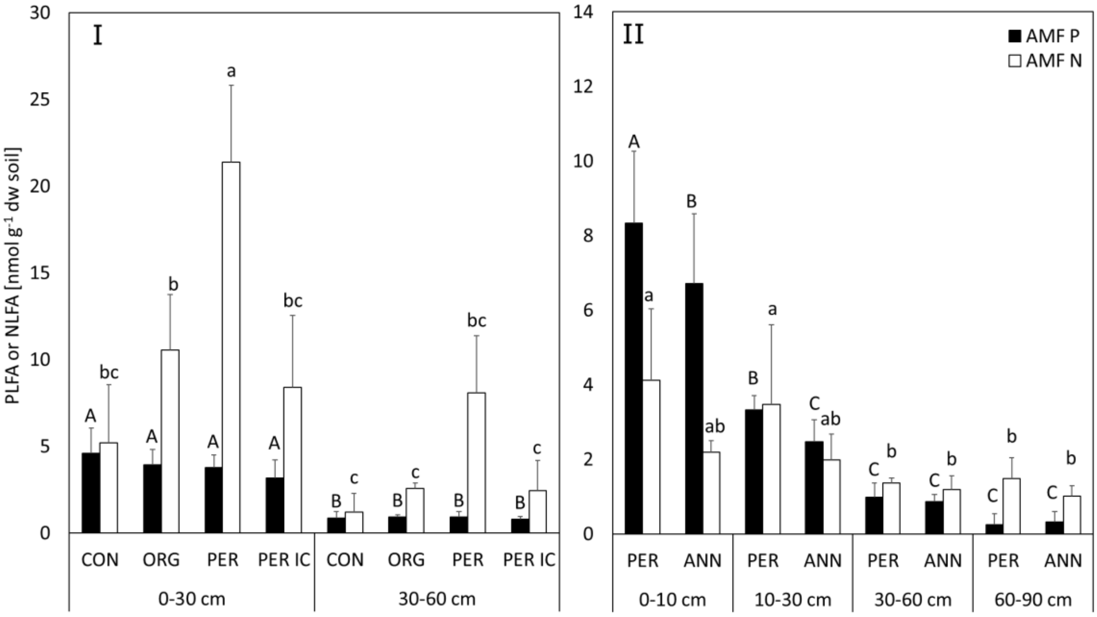

Figure 1: I) The abundance of neutral lipid and phospholipid fatty acid indicators (nmol g^{-1} dw soil) of arbuscular mycorrhizal fungi (AMF P and AMF N) in the conventional annual wheat (CON), organic annual wheat (ORG), organic perennial wheatgrass (PER), organic perennial wheatgrass and lucerne intercrop (PER IC) in the SITES AGROECOLOGICAL FIELD EXPERIMENT (SAFE) at Lönnstorp, SLU, Sweden. II) The abundance of AMF P and AMF N (nmol g^{-1} dw soil) in perennial wheatgrass (PER) and annual wheat (ANN) at Maubec, France.

Data are presented as means from each sampling depth (cm) and crop treatment with standard deviations. Different letters indicate differences at the significance level of p<0.05 (ANOVA with Tukey´s post hoc test using RStudio). Upper case letters are used for AMF P and lower case letters for AMF N.

Discussion

Our hypothesis was partly confirmed in the Swedish experiment through the higher abundance of the NLFA marker for AMF biomass. The high abundance, and clear response, of the NLFA marker reflect the larger abundance of AMF spore counts (Sharma and Buyer, 2015) in soil under the perennial crop. The lack of influence on the PLFA marker for AMF biomass may be due to the rather young age of the perennial stand. It has been shown that AMF colonization increases with increasing age of co-occurring plant (Grigera, Drijber and Wienhold, 2007), motivating further studies to evaluate the longer-term development of the soil-crop interactions. The French experiment confirms the expectation to increase abundance of AMF biomass in soil under the perennial crop. The rather low response of the markers for AMF biomass may result from the overall application of no-tillage practices in both the annual and perennial crop, combined with the recent introduction of the perennial (as in the Swedish experiment). Further questions to explore is the potential to increase the plant´s ability to acquire nutrients (Tran, Watts-Williams and Cavagnaro, 2019) and the potential of AMF to improve soil structure and related soil functions. Intercropping with legumes, to exploit biological N_2 fixation, is an important measure to contribute to sustainable development (Voisin *et al.*, 2014) through increased yield stability (Raseduzzaman and Jensen 2017) and the potential higher biomass N concentration and N accumulation in the intercropped partner (Bedoussac *et al.,* 2015). The occurrence of N_2 fixing bacteria and the higher levels of accessible soil N usually benefit the abundance of AMF. Our results indicate that the expected higher abundance of AMF under perennial crops is not realized when intercropped with lucerne, which could be explained the competitive interactions resulting in less *T. intermedium* roots for the AMF to colonize. Furthermore, the otherwise expected AMF colonization of lucerne roots may not have occurred and the interactions needs to be further investigated.

Conclusions

Agricultural perennialization will lead to healthier soil microbiomes, in this case proven by higher abundance of the obligate symbiotic soil mycorrhizal fungi. The outcome is in line with the global sustainability goals meaning that the implementation of improved, less interruptive agricultural practices should be promoted.

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Aromatic plants in vineyards: Mixed intercropping as a chance for promoting soil biodiversity and increasing economic revenues?

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Abstract summary

Diversification might be an important measure for climate change adaption and increased resilience of agricultural farms. The perennial growth of grapevines shows a high potential for crop diversification in the form of intercropping. Our study aims on evaluating intercropping grapevine with aromatic plants (Oregano and Thyme). Additional plant biomass may provide food and habitat for soil organisms, leading to beneficial effects. Otherwise, adverse effects of aromatic plants may occur, as they contain plant secondary metabolites, which have been reported to show anti-microbial properties. Therefore, we assess community structure, biomass and activity of microorganisms in vineyard soil affected by aromatic plants. Grapevine mono-crop serves as control. After two seasons of intercropping, there is a distinct decline of total microbial biomass and activity under aromatic plants. However, more specific microbial indices are increased by intercropping with aromatic plants. This differentiated reaction displays the complexity of the soil ecosystem and highlights the ability of specialized organisms to thrive on aromatic plant residues. Potentially, intercropping with aromatic plants creates unique habitats for specialized organisms resulting in beneficial impacts on soil biodiversity.

Keywords: Viticulture, Crop diversification, Intercropping, Aromatic plants, Plant secondary metabolites, AM fungi

Introduction, scope and main objectives

Wine is one of the most labour- and capital-intensive agricultural goods and therefore, its production comprises a high degree of specialization as well as intensive management. Moreover, many wine-growing areas play an important role for tourism and cultural heritage. Since grapevines profit from increased radiation on slopes and cope with shallow soil depth conditions, vineyards are frequently located in sloping landscapes all around the globe.

Common management practices encompass the removal of vegetation in the area underneath the grapevines by mechanical tillage or herbicide to prevent fungal diseases and competition on water, yet resulting in an increased risk of soil erosion and soil organic matter (SOM) depletion. Cover crops such as grasses and legumes have the potential to protect soil from erosion or SOM depletion; however, they do not benefit to the value chain and may compete with grapevines or increase pest pressure.

Alternatively, perennial aromatic plants such as *Origanum vulgare* and *Thymus vulgaris* appear to be appropriate intercrops for vineyards, since they are economically valuable (e.g. via extraction of essential oils), adapted to the specific pedoclimate, have a low water demand and a low growing habitus, ideal to cover soil for erosion control and weed suppression.

In addition, herbal plant litter fall, root growth and exudation may provide food and habitat for soil microorganisms and thus, potentially promote soil biodiversity and functionality. In contrast, plant secondary metabolites (PSM) such as thymol and carvacrol (both found in *Origanum vulgare* and *Thymus vulgaris*) have been reported to show anti-microbial activity. PSM might be released to the soil ecosystem and may exert adverse effects on soil microbial biomass and its activity. This could result in decreased soil biodiversity and hampered functionality. Hence, our main objective is a comprehensive evaluation of possible effects of intercropping grapevines with aromatic plants (as compared to grapevine monocrop with regular tillage) on soil microbiological properties. Our main objective is the interrelationship between microbial diversity, activity and soil functionality and we intend to answer the objectives:

i. Does the cultivation of aromatic plants alter soil microbial community structure?

ii. Does soil microbial biomass, its activity and subsequently carbon and nutrient cycles are affected?

iii. Do alterations of soil microbial properties cause further (long-term) changes of soil functionality?

Methodology

In May 2018, aromatic plants have been introduced by hand as seedlings (± 5 between two grapevine plants) to a commercial organically managed vineyard (0.3 ha). The vineyard is located in the Saar valley, Germany, on soil classified as Terric Anthrosol (WRB) and mainly developed from Devonian schist. These vineyard soils are typical for the area and can be specified as shallow and rocky with a pedoclimate dominated by hot and dry conditions. The field experiment is set-up as randomized block design with three blocks (Figure 1a), each consisting of treatment

a. Control (*Vitis vinifera* ‘Riesling’ monocrop with regular tillage),

b. Oregano (*Vitis vinifera* ‘Riesling’ intercropped with *Origanum vulgare*; Figure 1 b),

c. Thyme (*Vitis vinifera* ‘Riesling’ intercropped with *Thymus vulgaris*; Figure 1 c)

Figure 1: Experimental layout a) and photos of grapevines planted in slope oriented lines, undergrown by b) oregano and c) thyme plants

Photos taken in April 2019, nearly one year after planting of seedlings.

Soil samples are taken from each treatment twice a year, at the beginning and the end of each crop cycle, from 2018 until 2020. Analytical protocols are described in Álvaro Fuentes *et al.* (2019). Microbiological analysis comprises the determination of microbial biomass after fumigation extraction, basal respiration, enzymatic activities associated with carbon- and nutrient cycling as well as functional genes related to nitrogen cycling. Microbial community structure is assessed by metabarcoding of bacterial and fungal DNA and determination of lipid fatty acid fingerprints (PLFA/NLFA). Water contents of samples are adjusted to 40-50 percent of WHC_{max} prior to analysis.

Results

Since the field experiment is still in progress, only preliminary and selected results are presented. Due to severe drought after introducing aromatic plants in 2018, seedling establishment was poor and possible impacts on the plant-soil-microbe system were restricted. Thus, soil microbial parameters were not altered in 2018. However, as aromatic plants recovered and ultimately demonstrated vital growth in 2019, we observed a distinct trend showing decreased total microbial biomass and activity (as indicated by microbial biomass carbon and dehydrogenase activity; see Figure 2 a and b).

Simultaneously, aromatic plants tend to maintain or even increase levels of C16:1w5 (neutral lipid fatty acid - NLFA), a measure of arbuscular mycorrhiza fungi (AM fungi) biomass in soil, as well as activity patterns of particular enzymes such as N-acetylglucosaminidase, which is involved in chitin degradation (see Figure 2 c and d). Increased biomass of AM fungi is in accordance with microscopic observations of mycorrhizal structures within root tissue of aromatic plants shown in Figure 3.

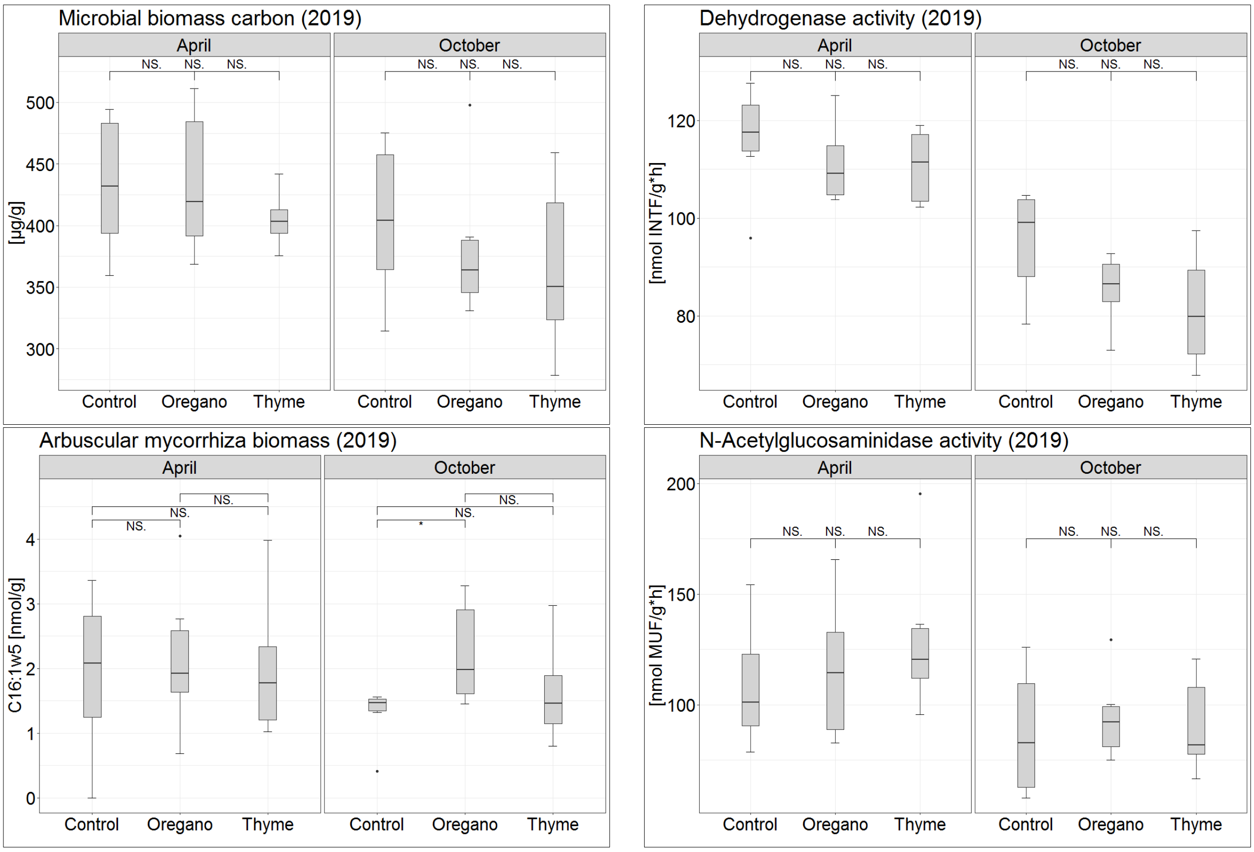

Figure 2: Effects of mono-cropping (control) or intercropping (oregano and thyme) underneath grapevine plants on a) the microbial biomass carbon, b) dehydrogenase activity, c) C16:1w5 content and d) N-acetylglucosaminidase activity in soil

Samples taken from vineyard in April and October 2019 in 5-10 cm depth.

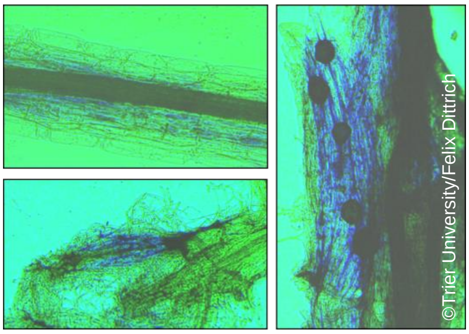

Figure 3: Microscopic images of AM fungi structures (blue) penetrating root tissue of aromatic plants after clearing and staining with an ink-vinegar-solution according to the method of Vierheilig *et al.* (1998)

Discussion

Soil functions and provision of ecosystem services crucially depend on a diverse and intact community of organisms. Therefore, results showing pronounced negative effects of aromatic plants as intercrops in viticulture, compared to mono-cropping, on total soil microbial biomass and activity underline the anti-microbial potential of aromatic plants and do, for the moment, not support their cultivation as intercrops in order to increase soil microbial biodiversity.

However, we also observed similar or increased levels of more specific soil microbial parameters under aromatic plants compared to mono-cropping, suggesting that some organism groups and their activity are not adversely affected or even promoted by aromatic plants. Especially the role of AM fungi on yield and quality of essential oils in aromatic plants reported by Khaosaad *et al.* (2006) highlight the potential advantages of intercropping, soil biodiversity and profitability. Overall, we expect a shift of the microbial community to occur after adaptations to food and habitat sources took place. Upcoming steps comprise metabarcoding of bacterial and fungal DNA, allowing deeper insights into the microbial community structure. A final evaluation of aromatic plants as intercrops will largely depend on the question if an adapted community restores and maintains long-term soil functionality.

Conclusions

We assume aromatic plants as suitable intercrops for viticulture, although selected results indicate also negative impacts on general soil microbial properties after two seasons of intercropping grapevine with aromatic plants. On the one hand, microbial adaption to agricultural management practices has been frequently reported. Moreover, the influence of aromatic plants on the vineyard soil ecosystem is spatially restricted but may create unique habitats for specialized organisms constituting an important advantage in terms of soil biodiversity conservation and promotion. Generally, aromatic plants as intercrops may provide further advantages such as erosion control, attraction of beneficial insects, weed suppression and decreased economic vulnerability against extreme weather events.

Acknowledgements

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Effects of conservative practices on soil ecosystem of Mediterranean high-density olive orchard

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Abstract summary

Edaphic arthropod communities can provide valuable information about the prevailing status of soil quality to improve the functionality and long-term sustainability of the soil. The present study aimed at evaluating the effect of plant and grass cover on the functional biodiversity and soil characteristics in a mature olive orchard (*Olea europaea* L. cv. Frantoio) in a Mediterranean environment. The orchard (Venturina, Italy) has been managed for ten years by two conservation soil practices: natural grass cover and minimum tillage. Soil samples were collected underneath the olive canopy and in the inter-row space. The abundance of Acari, Collembola and other arthropods and functional biodiversity were assessed; moreover, changes in crop yield components, soil structure and hydrological properties, concentration and storage of soil organic carbon pools were determined. Vegetal component, in the belowground ecosystem, impacts on the diversity and population abundance of soil arthropods; in fact, only in inter-row, soil organic carbon pools (total and humified) were negatively affected by minimum tillage, not under the canopy, without loss in fruit and oil yield.

Soil functionality was enhanced by plant and grass cover in terms of aeration, moisture and organic matter content and biodiversity was well-preserved.

Keywords: microarthropods, oribatids, soil management, feeding habits, ecosystem services

Introduction, scope and main objectives

Sustainability improves agricultural productivity to meet increasing demand (FAO, 2017). Furthermore, risk analysis of land degradation requires the monitoring of suitable indicators of soil quality (Bordoni *et al.*, 2019). These are commonly based on soil chemical, physical and biological properties that are directly related to soil ecosystem functions and highly responsive to soil disturbance, such as soil organic matter and its fractions, soil aggregate stability, soil porosity, soil biological activity and diversity (Vignozzi *et al.*, 2019).

The major role of soil biota in aggregate formation and stabilization is generally acknowledged even if direct empirical evidence to quantify the microarthropods' contribution is scarce (Culliney, 2013). Soil management considerably affects the soil structure's dynamics and the composition of microarthropod groups: edaphic species, mainly the no-burrowers, tend to colonize air-filled pore spaces and to set different community structures (Menta, 2012). Acari are among the most abundant groups of soil decomposers, inhabiting various organic substrates and using a wide range of food sources; together with Collembola, they affect ecosystem functioning by regulating the soil microbial activity by direct feeding on microorganisms, as well as by changing the soil structure through litter comminution, casting and other mechanisms (Maaß *et al.*, 2015).

This case was studied to analyse the effects of two inter-rows agronomical practices on physical, chemical and biological soil properties focusing on traditional agricultural techniques of tillage and permanent grass cover. Possible land use management acting as mitigation measures for olive orchard susceptibility could be implemented and supported to reduce currently high levels of soil erosion and run-off to watercourses, mainly in intensified-traditional and modern-intensive systems, by promoting changes in management practices.

Methodology

The experiment was carried out in a high-density olive orchard (*Olea europaea* L. cv. Frantoio) in coastal Tuscany region (43°01'N; 10°36'E - Venturina, Italy). The soil was a Typic Haploxeralf (sandy loam) under the sub-humid Mediterranean climate. Ten years after the beginning of the different management, soil samples using the core sampling method at 0.0-0.1 m were collected at two distances from the olive trunk: 1) underneath the canopy (UC) and 2) in the inter-row (IR) space (Figure 1).

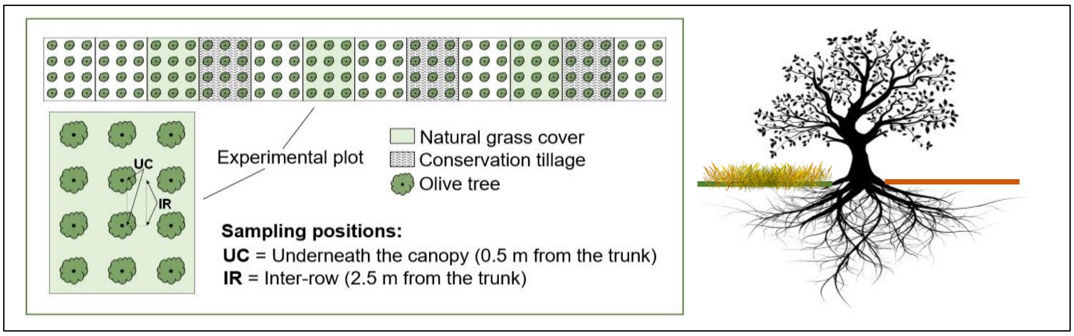

Figure 1: Layout of the experimental design; distribution of treatments across the different plots and sampling positions

A Principal Component Analysis (PCA) was performed to assess the relationships between soil physical and chemical properties and the abundance of microarthropods. For animal density analysis, all microarthropods were divided into three major groups: Acari, Collembola and "Other arthropods" including insects, myriapods, other arachnids and crustaceans. Functional roles of arthropods about microhabitat preference (soil-dwelling species –euedaphic; litter-dwelling species –hemiedaphic; surface-dwelling species –epedaphic) and/or mouthpart structure (macro- and microsaprophages, mycophagous, polyphagous, predators/parasitoids, herbivores/fluid feeders) (Vignozzi *et al.* 2019). According to the latest classification, the mites (Acari), can be divided into two superorders: Parasitiformes (Mesostigmata) and Acariformes (Trombidiformes Prostigmata and Sarcoptiformes- suborders Oribatida, Endeostigmata and Astigmata) (Krantz and Walter 2009).

Results

More than 13,500 arthropods were collected; the most representative groups were Acari (53.7 percent) and Collembola (35.9 percent). On the whole, mites were dominated by Oribatida (51.3 percent), followed by Prostigmata (26 percent), Mesostigmata (20.7 percent) and Astigmata (2.1 percent); Astigmata were occurring only in CT.

All soil characteristics analysed are shown in Table 1. The soil macroporosity did not change across the whole NC profile, while the highest frequency of fissures was observed in the CT-IR surface layer (78 percent of total macroporosity). The highest percentage of transmission pores, instead, was measured in the surface layer of CT-UC. Soil structure and hydrological properties were not affected by the distance from the plant, while water movement and retention increased where no-tillage was applied.

Table 1: Biological (Acari, Collembola and other arthropods densities), chemical (Total Organic Carbon, HC) and physical (MWD, AWC, AC, regular pores, total pores) properties (mean value±SD)

	Acari	Collembola	other Arthropds	TOC	HC	BD	MWD	AWC	AC	Regular pores	Total pores
NT-UC	304.2±120.1	357.2±384,3	90.0±38.6	1.8±0.3	0.4±0.1	1.2±0.2	5.7±0.3	18.2±1.7	16.4±6.0	1.7±0.4	8.3±3.5
NT-IR	293.0±146.8	228.3±151.4	89.8±75.0	1.9±0.3	0.4±0.2	1.4±0.2	6.6±0.2	16.7±0.4	13.3±8.1	1.5±0.3	7.1±1.3
CT-UC	489.8±294.4	154.2±87.7	39.3±21.7	1.8±0.4	0.4±0.2	1.2±0.1	5.3±0.9	15.7±0.5	26.7±1.8	0.9±0.4	27.0±10.7
CT-IR	127.0±67.0	70.3±57.6	16.3±8.0	1.4±0.1	0.2±0.1	1.4±0.1	1.7±0.3	14.2±0.1	28.9±2.3	1.0±0.2	25.8±3.0

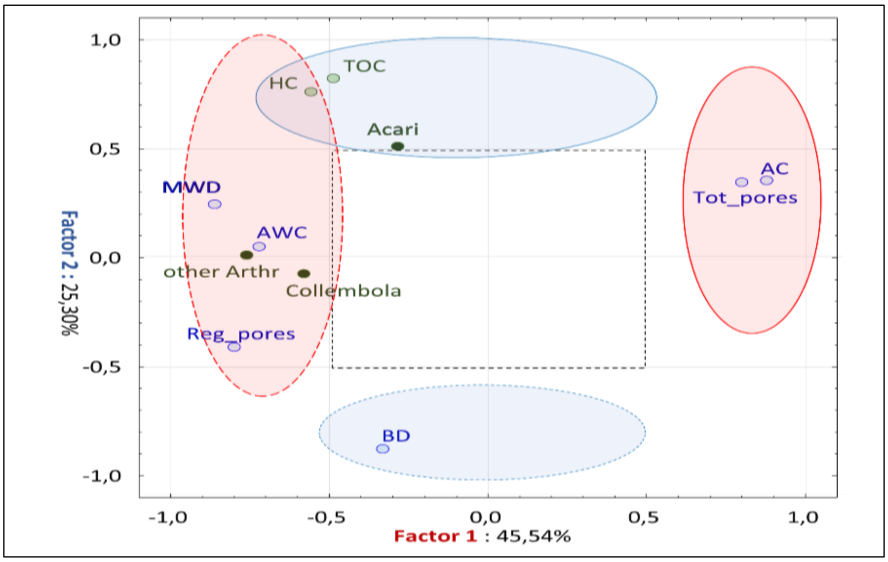

Figure 2: Principal component analysis (PCA) biplot of variables used to study responses of the main arthropod groups (green) to the environmental factors (blue) in different managed soil

The MWDwet was significantly affected by management; Collembola and the other Arthropods were positively related to NC soil structure characteristics. In CT-UC the water-stable aggregates (size class: 4.75-10 mm), high TOC and low BD values were related to a more abundant mite population.

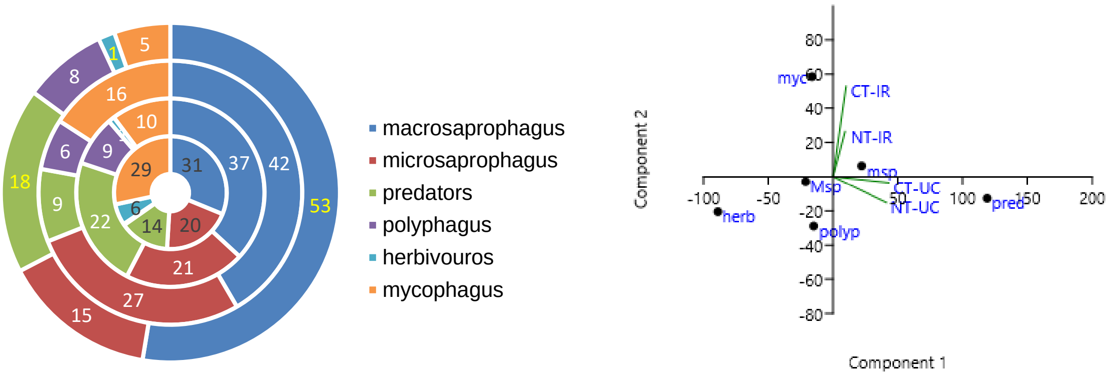

Figure 3: Functional biodiversity

a) community structure of soil arthropods (%) in each management in this order: Macro NT-UC (external circle), NT-IR, CT-UC, CT-IR (internal circle); b) relationship between abundance of soil mites and different soil microhabitats/management.

Concerning microarthropods, to provide an overview of soil functions, the relative frequencies of their feeding habits groups showed different composition between managements; especially macrosaprophages - mainly represented by Oribatids, epigeic forms of Collembola Enthomobriomorpha and Sminthuridae, Diplopoda Julidae, Coleoptera larvae - were abundant in NT-UC (Figure 3a). According to conclusions of Menta (2012), no vegetation cover and low soil moisture led to the dominance of some arthropod groups usually able to quickly thrive in stressed environments. Focusing on feeding habits of Acari, mycophagous and microsaprophages were abundant in inter-rows (CT-IR and NT-IR) (Figure 3b). Among the oribatid mites, the presence of Oppioidea and 'Lower Oribatida' (Paleosomata, Holosomata and Mixonomata) known as euedaphic fauna, was higher in NT.

Discussion

Permanent grass covering, compared to tillage-based management, was more effective - - in maintaining or improving elements of soil functionality. This practice did not affect a fruit and oil yield (Vignozzi *et al.*, 2019) while yield reduction can happen in young olive groves (Gucci *et al.*, 2012). Tillage reduced the microarthropod abundance, in particular Collembola, and eu-edaphic forms, which are the most sensitive groups to soil perturbation. Soil pore volume, moisture and air ventilation provide a suitable biotope to eudaphic mesofauna while epedaphic arthropods can tolerate desiccation better than hemi- and euedaphic forms (Lavelle *et al.*, 2006); these forms can survive when microhabitat conditions have been changed after tillage by wandering horizontally for select optimal conditions (Eisenbeis and Wichard, 1987). NT plots denote better soil conditions and the analysis showed that the relations between the abundance of each microarthropod main group, organic carbon fraction and soil structural condition (aeration and moisture) were dependent on the management.

A higher density of Acari was measured underneath the canopy independently on management; only the presence of predators correlated with the distribution of their prey. The olive plant provided a positive effect on the micro-environmental conditions and, then, on soil functional diversity in terms of more availability of resources and coenosis (Culliney, 2013).

Conclusions

Considering the importance of olive oil production in Italy and southern Europe and the scarcity of data on SOC content in olive groves, it is important to deep research concerning the role in C sink dynamics of these systems. Soil arthropod communities, highly affected by factors such as pH, aeration, organic matter composition, nutrient availability, vegetation, and soil structure, play a determining role as bioindicators of environmental quality and resilience of soil ecosystem. The use of feeding habits (Lavelle *et al.,* 2006) and higher-taxon levels as criteria in this evaluation allows both to save time/cost-consuming nature of species-level identification and to acquire information on environmental degradation (Culliney, 2013).

The most common soil management practice used in intensive modern olive production, in the Mediterranean region, is tillage, frequently applied to reduce competition of natural grass cover (Palese *et al.* 2014). However, if properly managed, the adoption of green cover deriving from weed flora, can represent a resource able to increase soil fertility and biodiversity (Gucci *et al.,* 2012). In vineyard, Bordoni *et al.* (2019) showed that tillage-induced perturbations by increasing risk in soil erosion and concomitantly decreasing C input to soil. By integrating evaluations of functional biodiversity and soil properties can represent a useful tool to assess the sustainability of the crop management adopted.

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Soil organic carbon and enzymatic activity under conservation agriculture in Moroccan dry land

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Abstract

Enhancing soil health is essential for sustainable agriculture under changing climate conditions. Therefore, soil organic carbon and biological activities research is gaining momentum in Dry land region. Our objective is to quantify effects of conservation agriculture (no tillage (NT) and conventional tillage (CT) on soil organic carbon stock (SOCs) in one the most productive soil in Central Morocco (Vertisol). Chemical analyses were used to determine how tillage affected various humic substances. Our results showed that, SOCs was significantly higher in NT compared to CT (10 percent more). A comparison of humic substances showed that humic acids and humin were significantly higher under NT compared to CT, but fulvic acid concentrations were significantly lower. Concerning the enzymatic activity, double strand DNA (ds DNA) and Alkaline phosphatase were significantly higher under NT soil compared to CT but a l.ow difference was observed in the other activities (Arylsulfatase and leucin-amminopeptidase) between the two treatments.

These studies confirm that NT does have beneficial effects on the quantity and quality of SOC and the improvement of the soil enzymatic activity.

Keywords: Soil Organic Carbon, Conservation Agriculture, Enzymatic Activity, Morocco

Introduction, scope and main objectives

Management of soil organic matter (SOM) in arable lands has become increasingly important in many areas of the world in order to combat land degradation, increase food security and to enhance the mitigation/ the adaptation to climate change (Lal, 2006). In fact, soil carbon cycling and composition are essential components of comprehensive agricultural and ecological impacts and forecasting. Bot and Benites (2005) have considered SOM as keys to developing drought-resistant soils (i.e., water conservation, evaporation and erosion control, and soil water infiltration ease) and ensuring sustainable food production (crop productivity, fertilizer use efficiency, reduced pesticide use, and crop sustainable intensification).

In Morocco, previous investigations have shown that SOM content in most soils is low (<2 percent) and the decadal average (from 1987 to 1997) loss of the SOM due to intensive land use is about 30 percent.

The decline of SOM in cultivated soil of Morocco, due to the tillage intensification, decreased soil quality and increased the risk of soil degradation (Soudi *et al.,* 2003).

To deal with this situation, conservation agriculture has been recommended as an alternative strategy to invert the soil degradation spiral in many parts of the world. No-tillage systems, which consist of eliminating soil tillage and inversion, maintaining crop residue cover, and ensuring proper crop sequences, have been reported to improve SOM level and ensure carbon accumulation and sequestration in diverse soils from contrasted climate regimes (Kassam *et al.*, 2012; Mrabet *et al.*, 2012).

SOC is a continuum of substances including humic acids, fulvic acids, hymatomelanic acids, and humins. Humic and fulvic substances enhance plant growth directly through physiological and nutritional effects (Bot and Benites,2005), but also improve soil health through amelioration of soil’s physical, biological, and chemical properties. It is evident to account no-tillage systems as durable management practices; they have to improve and protect simultaneously soil organic matter and its active fraction. Also, soil enzymes play biochemical functions in the overall process of organic matter decomposition in the system; they are important in catalysing several reactions, necessary for the life processes of microorganisms in soils, the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, and nutrient cycling, providing an early indication of the history of a soil and its changes in agricultural management (Kandeler, Tscherko and Spiegel, 1999).

Despite the widespread interest in CA, the magnitude and effect by CA on soil enzymatic activity remains unquantified, especially in low-input systems of dry lands in North Africa. Consequently, the objectives of this study are:

(i) to investigate the long-term effect of CA on the SOC quantity and quality in semiarid Vertisol;

(ii) to quantify the effect of CA on the enzymatic activity in Vertisol.

Methodology

This study was conducted at the Merchouch plateau (33° 34 N, 6° 42 W, and 425 m elevation), in Morocco. Its Mediterranean climate is characterized by a mean annual rainfall of 450 mm. The experiment was established since autumn 2004. In the selected site, the trial consists of two tillage systems: no-tillage system (NT) and conventional tillage system (CT), performed on two adjacent plots of 200 m long and 100 m wide each. The CT plots were ploughed, according to farmers’ practice in the region, at 30 cm of depth with a “stubble plow” at the end of August each year. For seed- root bed preparation, a pass of a chisel, operated at about 15 cm depth, and two passes with a disc harrow at about 10 cm depth were needed during mid-September. The soil was not disturbed in the plots under NT which were maintained covered with flat and stubble residues at 30 percent levels. Wheat-lentil rotation was adopted and the crop management was similar in CT and NT treatments. Indeed, winter wheat was sown in mid-November at a 140 kg ha^{-1} seed rate. In mid-December, lentil was sown at seed rate of 40 kg ha−1. Before sowing, wheat and lentil received a complex fertilizer (14N-28P2 O5 -14K2 O), at a rate of 150 kg ha1 and 100 kg ha−1, respectively. In addition, wheat received 100 kg ha−1 of urea at the end of February. The control of weeds is based primarily on ploughing the soil in CT plot before sowing; while in the NT plot this control was achieved by chemical weeding.

Soil sampling and analysis

- **Soil organic carbon content:** In summer, after wheat harvest, disturbed and undisturbed soil samples were taken for determination of the SOC and soil bulk density, at the same time. The soil samples were collected at four depths (0–5, 5–10, 10–20, and 20–30 cm) in the NT and CT plots with three replicates per treatment. Immediately after sampling, the disturbed soil samples were dry sieved at 2 mm to remove plant debris. The SOC content was determined indirectly by oxidation of organic carbon following the classical method of Walkley and Black.
- **Humic substances:** To study the effect of tillage practices on humic substances, soil samples were collected randomly from the Vertisol at 0–20 cm depth in the NT and CT plots with three replicates per treatment in mid-season (March). Extraction of humic substances was done using the alkaline solvents method (1988).
- **Soil enzymatic properties:** Three soil enzymatic activities involved in soil Carbon, Nitrogen and Phosphor were analysed using leucine-amminopeptidase, alkaline phosphatase and arylsulfatase (Tabatabai, 1982). Double strand DNA (ds DNA) was extracted using the same procedure as for enzymes but using 0.12 M pH7.8Na3PO4 buffer as extractant.

Statistical analysis

The effects of tillage system on the SOCc, humic and enzymatic substances were tested in the different soil types using SPSS version 17. Analysis of variance (ANOVA) was used to determine significance of tillage effects in each soil type and t-test (Student’s t-test) was applied for comparing treatment means.

Results

Soil Organic Carbon (SOC)

Our results showed that SOC near the surface (0–10 cm) was significantly higher under NT compared to CT (Table 1). The reduction of SOC in conventionally tilled soil could be explained by the excessive removal of biomass after harvest. At 10–20 cm depth, SOC was significantly higher in NT than CT. However, in the deepest horizon (20–30 cm), no significant difference of SOC has been observed between the CT and NT systems. The same findings under similar semiarid Mediterranean conditions were reported by (Moreno *et al.*, 2006).

Table 1: Soil Organic Carbon content stock (Mg·ha^{-1}) under 12 years of adopting No tillage (NT) vs Conventional Tillage (CT) In Vertisol (Merchouch-Central Morocco)

Depth (cm)	**NT**	**CT**
0-5	5.39^{a}	4.14^{b}
5-10	5.83^{a}	4.62^{b}
10-20	11.32^{a}	10.77^{b}
20-30	9.34^{a}	9.26^{a}
Total (0-30 cm)	31.89^{a}	28.79^{b}

For the same soil and the same depth, treatments with the same letter are not significantly different; Student's *t*-test (*P* < 0.

Humic substances

According to Figure 3, the organic matter under NT was composed of significantly higher amounts of humic acids (HA) and humin (HU) and lower amounts of fulvic acids (FA) compared to CT in Vertisol. This is consistent with results obtained by Szajdak, Jezierski and Cabrera (2003). The relative decrease in FA under NT compared to CT was probably due to the humification process which was favored by residue management under NT and resulted in a significant increase of the most stable fraction (HA and HU).

According to Piccolo (1988), additional HA serve to bond particles together and can improve soil water content, and reduce soil erosion .In fact, in the studied Vertisol, positive effects of NT on aggregate stability and soil water content have been reported in a previous study by Moussadek *et al.* (2011) Moussadek *et al.* (2014).

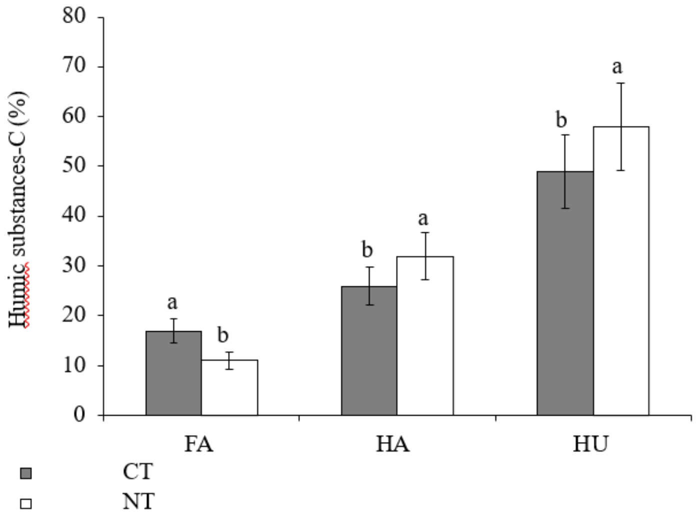

Figure 1: Humic (HA), fulvic (FA) acids and humine (HU) under the no tillage (NT) ann conventional tillage (CT)

Soil enzymatic activities

- Double strand DNA (ds DNA): After 12 years of adopting CA at Merchouch site. The figure below shows that under NT the ds DNA is higher under NT compared to CT.

- As presented in the figure below, Alkaline phosphatase was significantly higher (133 unite) under NT soil compared to CT (93 unite). Low difference was observed in the other activities (Arylsulfatase and leucin-amminopeptidase) between the two treatments.

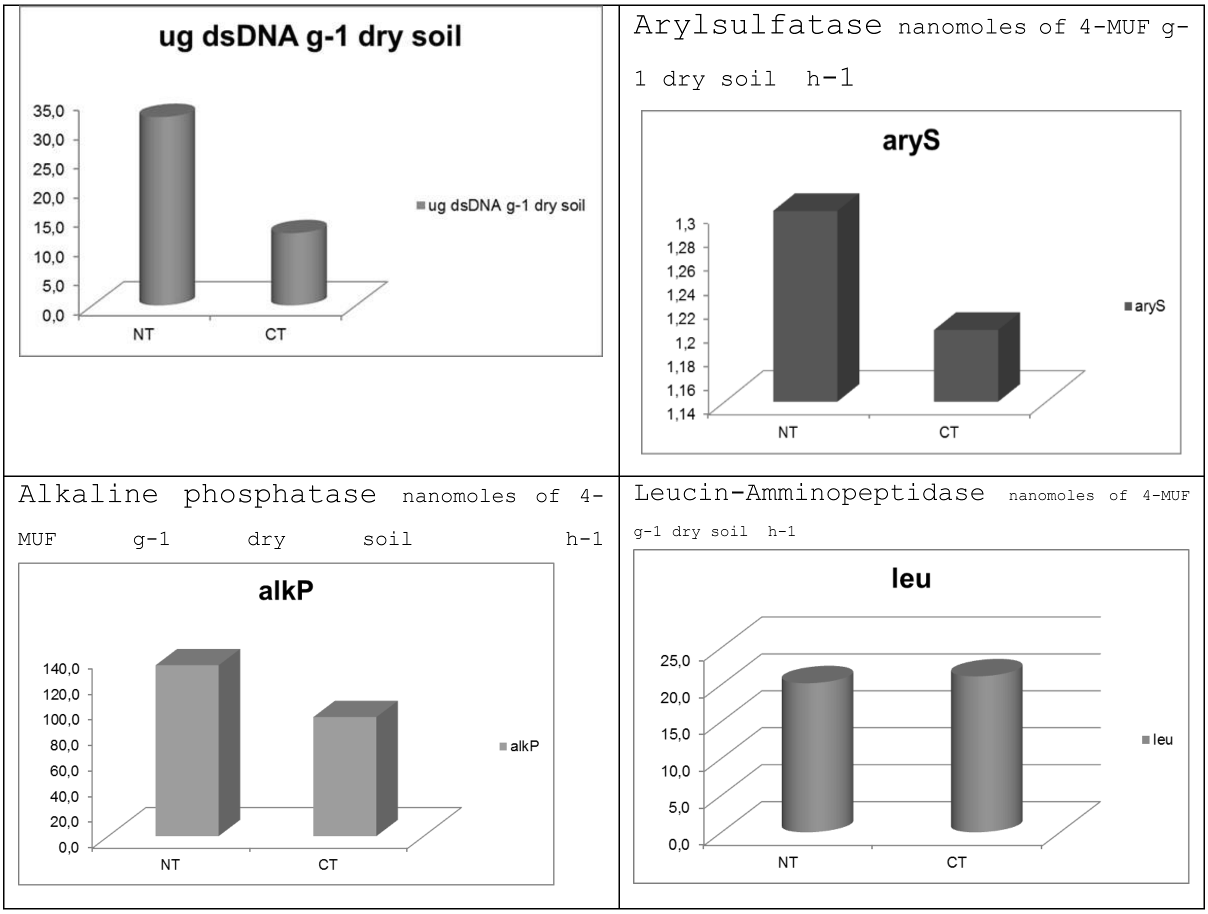

Figure 2: DNA and Enzymatic activity under NT and CT in Semi-arid Vertisol-Merchouch Morocco

Conclusions

This study attests of the effectiveness of the conservation agriculture for enhancing soil organic carbon quantity and quality. This study showed also a positive effect of adopting NT on soil biological enzymatic activities.

Acknowledgements

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Biodiversity in hay meadows: effects of intensive agriculture on ground-dwelling macro-invertebrates

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Abstract summary

Intensification of agriculture has led to a decrease of biodiversity in grasslands, leading also to lower epigeic invertebrate numbers in those habitats. Because vegetation structure changes due to the increasing number of cuttings and the high input of fertilizers, ground-dwelling invertebrates decrease. Our study sites were located in Barbian/Barbiano, South Tyrol (Italy) where we installed pitfall traps in intensively as well as in extensively managed meadows. The results show that there is a difference between the two agricultural habitats in species composition and in the abundance of certain groups.

Keywords: Intensive agriculture, grasslands, ground-dwelling macro-invertebrates

Introduction

Intensification of agriculture has increased in the last decades, leading to a decrease of biodiversity in the habitats concerned (Robinson and Sutherland, 2002). Invertebrates play an important role in ecosystem functioning of agricultural grasslands, for example they contribute to biocontrol or pollination and may serve as prey for other animals. Declines in invertebrate populations in response to more intensive grassland management are mainly caused by changes in the plant communities, which themselves react to increased mowing frequencies, grazing, higher fertilization and reseeding (Vickery *et al.,* 2001).

Grasslands, especially meadows and pastures, represent a large part of the province of South Tyrol. Here, animal husbandry plays an important role and is one reason for an increasing intensification of agriculture. To maintain a higher stocking density, a higher productivity of the meadows is required, therefore fertilizers like liquid manure or inorganic fertilizers are increasingly used to induce a higher number of cuttings.

The present study aimed at establishing how the community structure of ground-dwelling invertebrates is affected by management intensity. Specifically, we evaluated the impact of key management practices, namely the number of cuttings per year and the amount of fertilizers applied, on species composition and frequency.

Methodology

The study sites were located at elevations of 1100–1250 m a.s.l. in Barbian/Barbiano, South Tyrol (Italy). Of the six meadows studied, three were subjected to extensive and three to intensive agricultural practice. The former were mowed once a year, the latter fertilized up to five times a year and mowed three to five times a year. We installed each four pitfall traps per meadow for a period of four weeks in autumn 2018 (i.e. 29 days) and repeated the sampling in spring 2019 (i.e. 31 days). The pitfall traps were filled with a saturated saline solution as collection fluid and emptied weekly (i.e. four sampling dates).

All macro-invertebrates were identified at least to family level under a dissecting microscope; selected groups such as ground beetles (Carabidae), rove beetles (Staphylinidae), ants (Formicidae), grasshoppers (Orthoptera), spiders (Araneae)were identified to species level.

In addition, we conducted a vegetation survey to identify all plant species within a plot of 10 × 10 m per meadow and calculated the Ellenberg *et al.* (1992) indicator value for nitrogen. For a first comparison of the community from the two meadow types and both seasons, a non-metric multidimensional scaling (NMDS) was calculated using the package VEGAN in the statistical programming language R. (R Core Team, 2020; Oksanen *et al.,* 2019).

The community data was composed by the community matrix with the abundance of each species scaled to the days of exposure (total trap abundance divided by the day of exposure as individuals per sampling day, ind./day).

Species diversity was represented by Shannon-Wiener index (computed with function diversity in the package VEGAN; Oksanen *et al.,* 2019). For the statistical analysis of taxon activity density (individuals per day), richness and diversity, a linear mixed model (LMM, function *lmer()* in the package LME4; Bates *et al.,* 2015) was constructed. The season was modeled as a random factor to account for temporal autocorrelation and seasonality and the field ID was modeled also as a random factor to account for spatial autocorrelation between the pitfalls of each field.

Results

We found significant differences between species composition and treatments as well as species composition and seasons. The first axis of the NMDS plot (Fig. 2) separates well the two seasons, spring and autumn. The second axis depict well the management intensity, separating extensively used hay meadows from the intensively used ones. The PERMANOVA shows significant results for the two different treatments and for both seasons (Table 1). For groups for which we had species data (Lycosidae, Carabidae, Staphylinidae and Formicidae) we found variable patterns. Lycosidae showed a clear separation for treatment and season (Fig. 2A). Species composition differed significantly between treatments and seasons, respectively. Lycosidae and Carabidae showed a clear difference between the two seasons regarding their species composition (Fig 2, A, B). Lycosidae also showed a clearly different species composition regarding treatment.

Carabidae exhibited a similar pattern, but differences were not as clear as for the Lycosidae (Fig 2, B). Investigating the species community of the Staphylinidae (Fig. 2C), the first axis represented the treatment, indicating a separation where some species preferred either extensively or intensively used meadows. The differences between the two seasons were not as clear as for the other two groups (Lycosidae and Carabidae). Formicidae showed the most indistinct pattern of the four groups (Fig. 2D). There were no clear differences between treatments or seasons regarding their species composition. The PERMANOVA showed significant results for the treatment for all four groups. For the season only the results of the Formicidae were not significant (Tab. 1). Plant species richness was higher in the extensively used meadows as shown by the arrow pointing towards plots of this management type (Fig 2, A-D). We calculated the activity density for the four selected groups (Lycosidae, Carabidae, Staphylinidae and Formicidae) for both treatments and seasons, using the parameters individuals per day, their species richness and the Shannon-Wiener biodiversity index. Lycosidae showed significant differences between the two seasons for all three parameters; for the treatment the differences were not significant (Table 2). Lycosidae showed higher activity densities in spring, whereas Formicidae had a higher activity density in autumn. Staphylinidae had a significant higher species richness in intensively used meadows. For Lycosidae, Carabidae and Formicidae the differences between treatments were not significant for these three parameters. Carabidae had a higher species richness in autumn in the extensively used meadows, whereas the Formicidae showed higher species richness in the intensively used meadows, always regarding to autumn (Fig. 3).

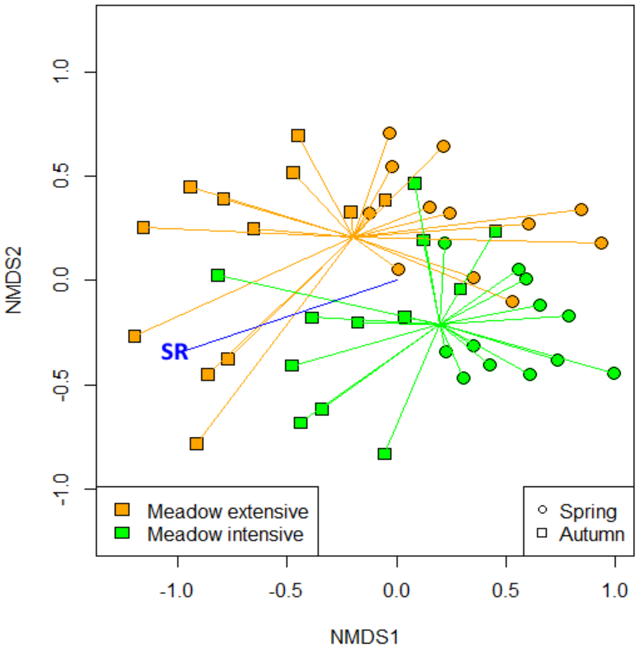

Figure 118: Non-metric multidimensional scaling (NMDS) of the full taxonomic community of ground-dwelling invertebrates by including the two treatments (intensive and extensive) and the two seasons (spring and autumn)

Each spot represents one pitfall trap. Spider web centers are the weighted centroids of each management type.

Table 14: PERMANOVA results table once for treatment and once for season for the different taxonomic groups (Full community, Lycosidae, Carabidae, Staphylinidae and Formicidae) using the method "bray"

Treatment				
	df	**residuals**	**F**	**p**
Full community	1	46	6.109	0.001 ***
Lycosidae	1	46	11.92	0.001 ***
Carabidae	1	36	3.707	0.002 **
Staphylinidae	1	34	4.676	0.001 ***
Formicidae	1	39	2.965	0.003 **
Season				
	df	**residuals**	**F**	**p**
Full community	1	46	11.621	0.001 ***
Lycosidae	1	46	11.263	0.001 ***
Carabidae	1	36	7.827	0.001 ***
Staphylinidae	1	34	2.730	0.004 **
Formicidae	1	39	1.124	0.275

Df: degrees of freedom; F: F value by permutation; p: p value based on 999 permutations. Significance levels: ** < 0.01 * < 0.001.**

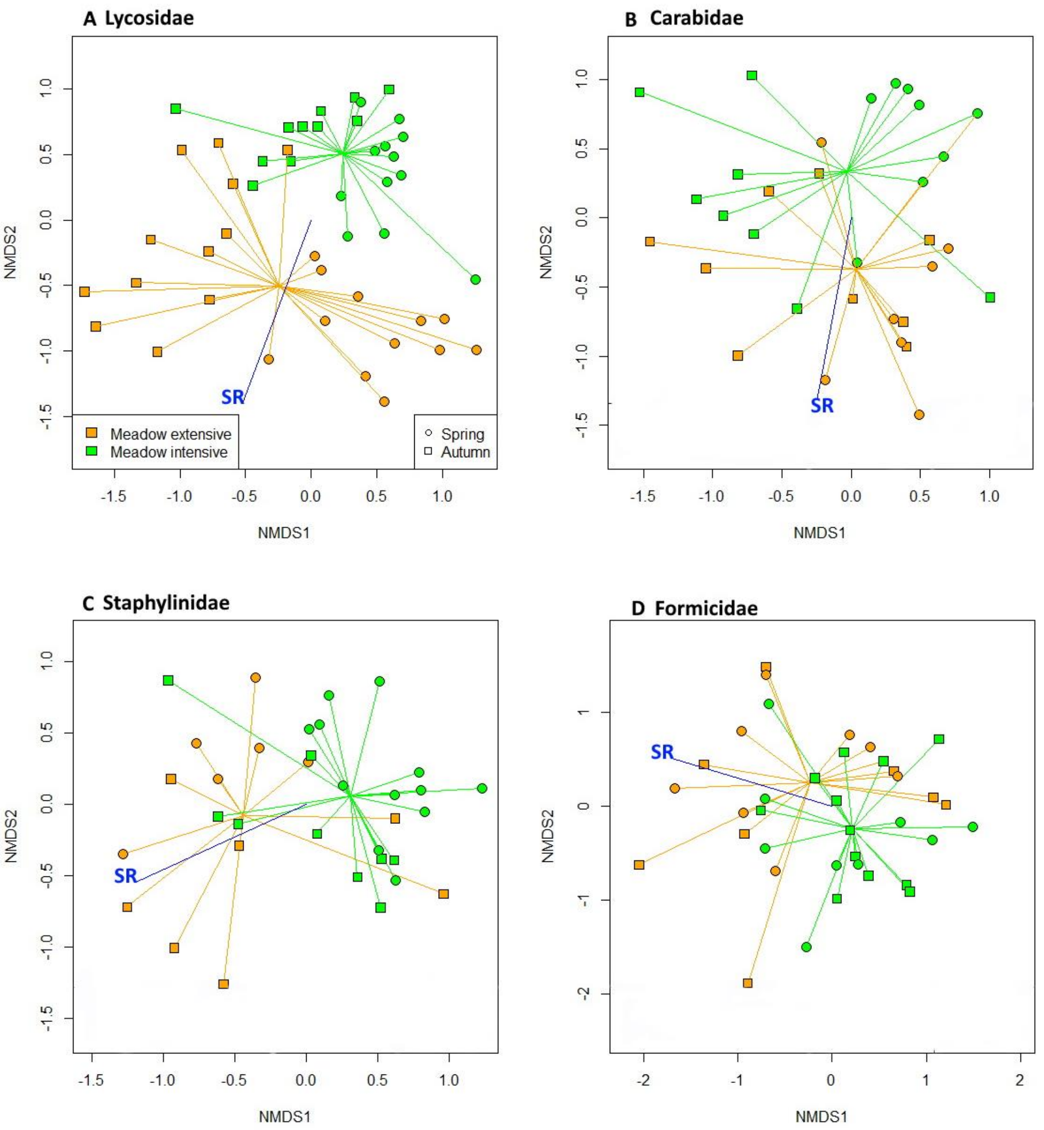

Figure 219: Non-metric multidimensional scaling (NMDS) of selected ground-dwelling invertebrates (Lycosidae, Carabidae, Staphylinidae and Formicidae) for the two treatments (intensive and extensive) and the two seasons (spring and autumn)

Each data point represents one pitfall trap. The spider web centers are the weighted centroids of each management type.

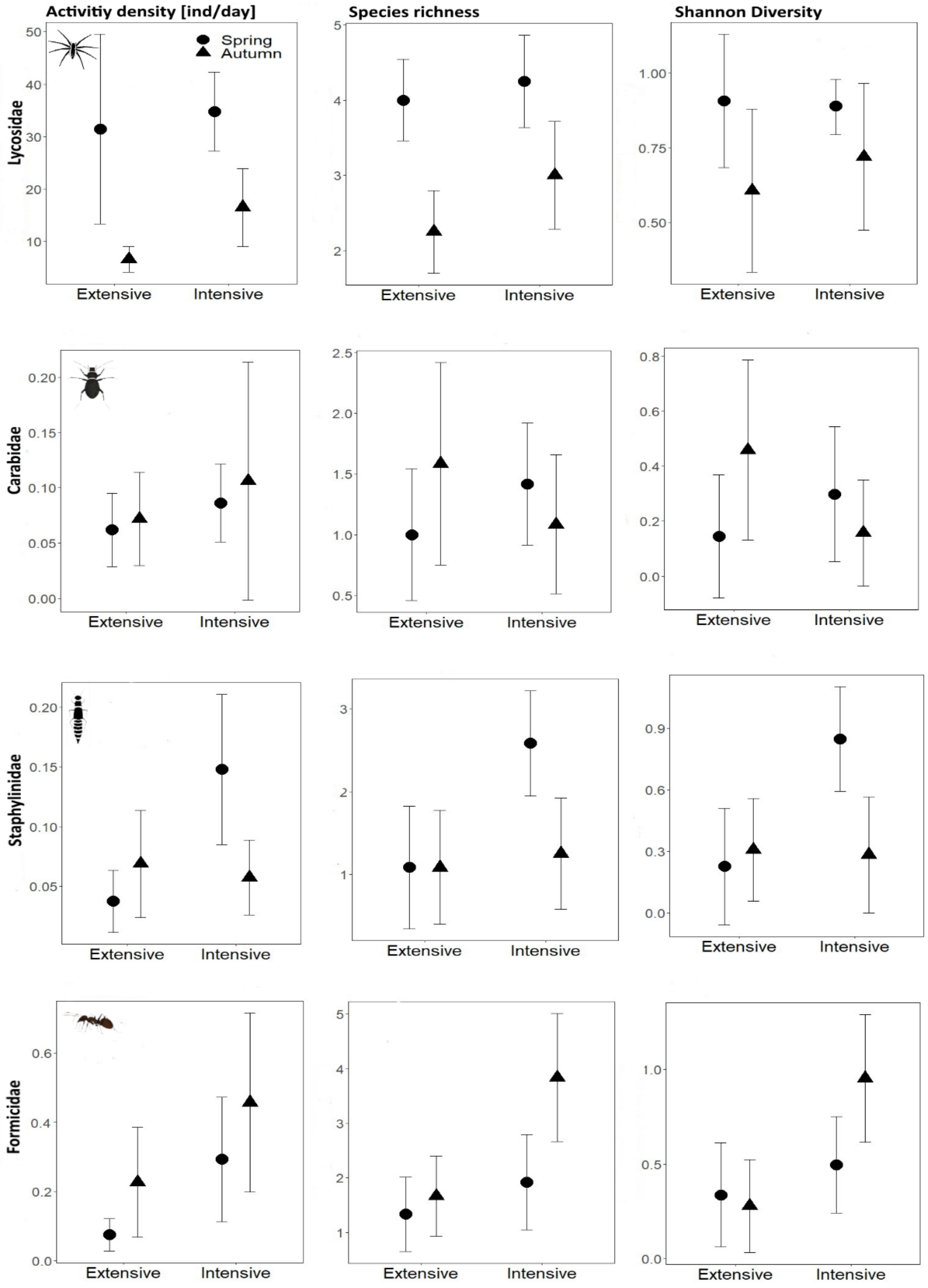

Figure 3: Boxplots of activity density in individuals per day, species richness and the Shannon biodiversity index on the four selected groups (Lycosidae, Carabidae, Staphylinidae and Formicidae) in the extensively and intensively used hay meadows and the two seasons (spring and autumn). n = 4

Table 2: Results of the linear mixed model once for treatment and once for season

Treatment			
	Activity density [ind./day]	**Species richness**	**Shannon Diversity**
Lycosidae	0.3714	0.3732	0.7628
Carabidae	0.2879	0.8821	0.5239
Staphylinidae	0.1014	0.0240*	0.0288*
Formicidae	0.1581	0.1346	0.1010
Season			
	Activity density [ind./day]	**Species richness**	**Shannon Diversity**
Lycosidae	0.0151*	0.0098**	0.0486*
Carabidae	0.5857	0.6561	0.4537
Staphylinidae	0.1553	0.0040*	0.0473*
Formicidae	0.0705	0.0396*	0.1156

It includes the activity density in individuals per sampling day, the species richness and the Shannon biodiversity index for the four selected groups (Lycosidae, Carabidae, Staphylinidae and Formicidae). n = 4
Significance levels: * < 0.05 ** < 0.01

Discussion

With this study we conducted one of few studies dealing with the effects of agricultural management intensity on ground-dwelling macro-invertebrates. We found significant differences in species composition between the two treatments (i.e. intensive and extensive management) and the two seasons of spring and autumn. This led to that intensification of agriculture has a strong impact on ground-dwelling invertebrates. Our results are in line with other studies, which showed that high mowing frequency, fertilization and grazing have strong impacts on ground-dwelling invertebrates. For example, Marini *et al.* (2009) observed reduced diversity of orthoptera and butterflies with high fertilization and high cutting frequency. Our results showed that Caelifera activity densities (expressed as ind./day) were much lower in intensively than in extensively used meadows in autumn. We found significant differences in species composition of the two investigated hay meadows in terms of season and treatment, but this not necessarily mean that the extensively used hay meadows had higher species richness or diversity. Differences regarding to the season can be explained by the life history of species for Lycosidae, Carabidae and Staphylinidae. The period of activity of spider and beetle population varies throughout the season (Thiele, 1977; Nentwig *et al.,* 2020). Formicidae did not show differences in seasonality, because colonies of ants are long living and therefore species were found throughout the year (Keller, 1998).

We did not find such clear differences between the two treatments and the activity density on the four selected taxa Lycosidae, Carabidae, Staphylinidae and Formicidae, but we found some species that had preferences for one of the two types of treatment.

Conclusions

We can confirm that intensively used hay meadows differ considerably from the extensively used meadows regarding to the ground-dwelling macro-invertebrates communities. Orthoptera were inhibited by the intensive management practices while spiders like Lycosidae were not as much affected by the intensive land use.

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Earthworms and microbial diversity under conventional and organic farms. Interaction with actual and inherited pesticides

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Abstract summary

Diverfarming is a H2020 European project who promotes soil quality conditions through plant diversification, within the framework of the project, we investigated what is the state of the art of different agricultural soils under organic and conventional management. Knowing that aboveground diversity will enhance belowground diversity, we aimed at assessing earthworms and soil microbial diversity as well the content of pesticide residues in soils of the long term diverfarming farms; we searched at identifying relationships between pesticide residues and earthworms or microbiome abundance/diversity, as well as pathogens or beneficial microorganisms. The study was carried out in 12 farms at Groningen, The Netherlands. The sampling took place before the application of agrochemicals in April 2018. Seventeen pesticides residues were found in the whole study, from where DDT, prosulforcab and AMPA (first metabolite of glyphosate) were detected in all soil samples. Microbial biomass and earthworm's abundance were inversely proportional to the number and concentration of pesticides, the highest bacterial diversity was obtained in those farms with high manure application, and the highest earthworm's diversity was observed in organic farms, which had less or no pathogen microorganisms, and they had also the highest content of beneficial microorganisms.

Keywords: earthworms and microorganisms diversity, actual and inherited pesticides

Introduction, scope and main objectives

Earthworms as indicators of soil quality (Bartz, Pasini and Brown, 2013) and promoters of several soil ecosystem services, (Blouin *et al.,* 2013) impeccably reflect what occurs aboveground. Soil microorganisms, responsible of different biogeochemical process interact as the earthworms with the actual and inherited soil conditions. Among inherited soil conditions are found the pesticides. The Diverfarming project is a H2020 project who enhances soil quality through plant diversification and rotation. Therefore, inside the framework of this project, we aimed at assessing earthworms and soil microbial diversity as well the content of pesticide residues in soils from long term diverfarming farms; we searched at identifying relationships between pesticide residues and earthworms or microbiome abundance/diversity, as well as pathogens or beneficial microorganisms.

Methodology

The study took place at Groningen, the Netherlands, where 12 farms (Figure 1, Table 1) were sampled in April 2018, before the application of agrochemicals. Soil samples, soil microorganisms and earthworms were collected following the protocols of the project. Soil physicochemical characteristics were assessed, and earthworms and soil microorganisms were quantified and identified according to the Diverfarming handbook. For soil pesticides characterization, soil samples were collected from the soil surface (0-5cm) (3 samples per farm) following the European soil sampling guidelines (Theocharopoulos *et al.,* 2001). Pesticides residues were determined by LC-MS/MS analysis following the methodologies proposed by Anastassiades *et al.* (2003), Mol *et al.* (2008) and Yang (2016). Statistical analysis (One-way anovas) was performed with normalized data in order to observer significant differences between conventional and organic farms in relation to the tested parameters.

Figure 1: Sampling area, Groningen, The Netherlands

Results

Organic farms had significantly the highest earthworm's diversity (Figure 2), and the highest content of beneficial microorganisms (Figure 3). The highest bacterial diversity was found where manure is highly applied. Seventeen pesticides residues were found in this study, where DDT, Prosulfocarb and AMPA (first metabolite of glyphosate) were present in all farms (Figure 4). An inverse relationship between soil microbial biomass and the number of pesticides in soils was also found. Earthworm's abundance decreased while the diversity of pesticides increased (Figure 4).

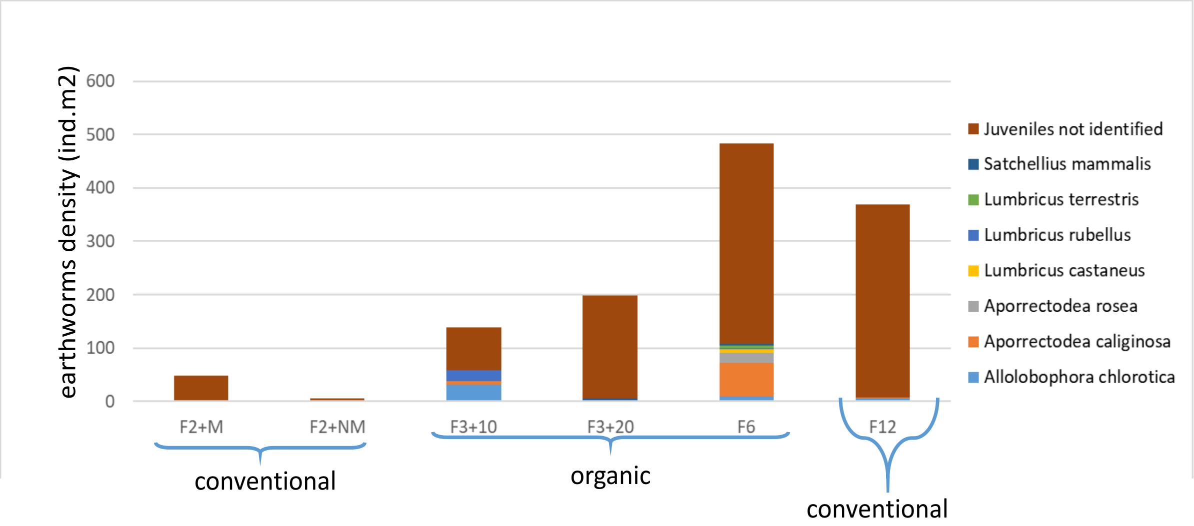

Figure 2: Earthworms abundance and diversity per farm

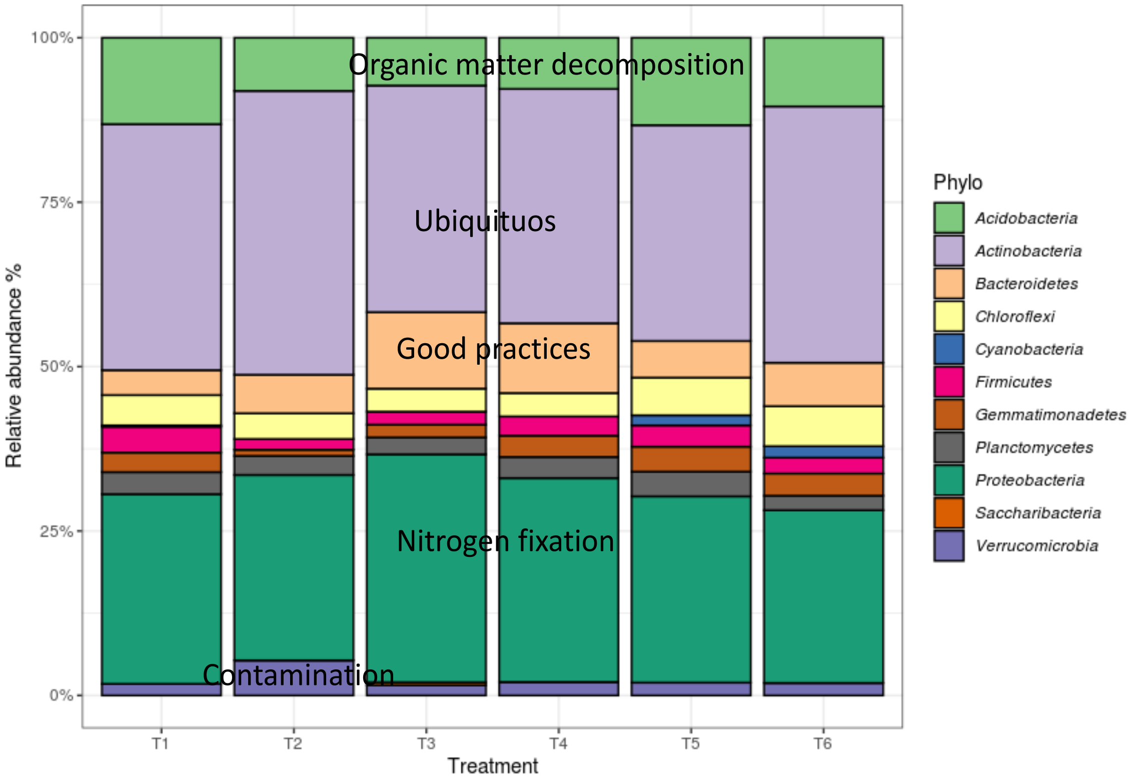

Figure 3: Bacteria diversity per farm

T3 & T4 are organic farms

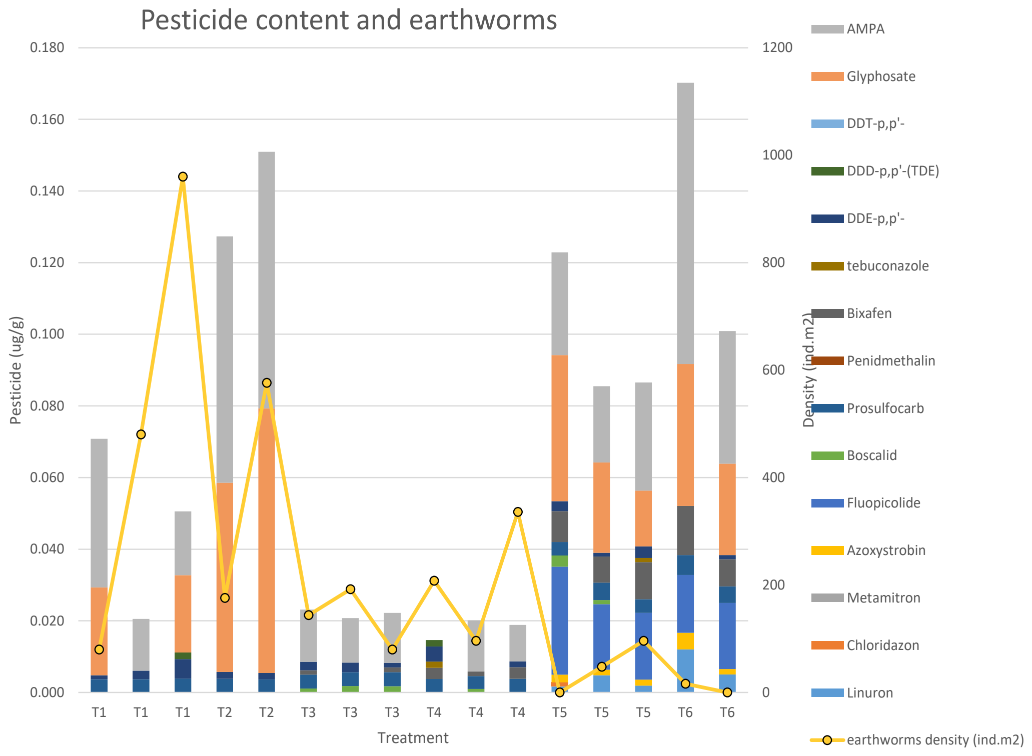

Figure 4: Pesticides residues and earthworms abundance among the studied farms T3 & T4 are organic farms.

Discussion

Soil invertebrates respond to a gradient of perturbation, when stress factors are present their biomass, abundance and diversity may decrease (Kammenga *et al.*, 2001). In this study we observed clearly how soil microorganisms and earthworms are influenced by the aboveground conditions. Organic farms practices in this study seemed to allow higher soil diversity than in the conventional farms, due to the aboveground diversity and the non-application of pesticides. Pesticides as glyphosate, prosulfocarb and DDT were found in almost all farms, even in the organic farms. More studies are required in under to understand the way of pesticides dissemination in this area.

Conclusions

Soil earthworms and microorganisms diversity are clearly influenced by actual and inherited pesticides.

Acknowledgements

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Soil biodiversity enhancement in European agroecosystems to promote their stability and resilience by external inputs reduction and crop performance increase – SoildiverAgro

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Abstract summary

SoildiverAgro is a project financed by H2020 programme focused on fostering synergies between crop production, biodiversity and the delivery of ecosystem services. The main objective of SoildiverAgro is the adoption of new management practices and cropping systems that enhance soil genetic and functional biodiversity to reduce the use of external inputs while increasing crop production and quality, the delivery of ecosystem services and the EU agricultural stability and resilience. SoildiverAgro outputs consider: 1) enhancement of soil biodiversity; 2) reduction of pest/diseases incidence; 3) increases in plant growth and development; 4) increases in crop yields, quality and value; 5) the reduction of inputs; 6) increases in soil fertility; 7) reductions of soil contamination; 8) reduction in GHG emissions; and 9) increases in soil C sequestration. To ensure rapid adoption of measures fostering soil biodiversity, improved methods and tools including for monitoring will be developed. The successful crop management practices will be also analysed from the environmental, economic and social perspectives. A key action for the project success is an adequate engagement of end-users, since farmers will not adopt cropping systems and management practices that enhance soil biodiversity unless the socio-economic profitability of an adequate soil biodiversity management is demonstrated to them.

Keywords: Functional diversity, Genetic diversity, microorganisms, macroorganisms, ecosystem services, farm resilience, crop health, inputs reduction

Introduction, scope and main objectives

The agricultural sector is essential for European food security, and it is present in many socio-economic EU´s frameworks. In fact, the European Common Agricultural Policy (CAP) is one of the most important European policies in terms of budget (39 percent in 2015). However, there are many challenges for Europe around agriculture that have not been adequately addressed yet. The most important is the livelihood of millions of farmers who face a high number of threats related with agricultural profitability and stability. Loss of soil biodiversity represents one key challenge and may adversely affect the functioning, stability, resilience and adaptability of agro-ecosystems and associated ecosystem services, both within and beyond the farm level. Therefore, the protection and enhancement of soil biodiversity should be an important objective for farmers. However, there is currently a lack of incentives (agronomic, economic and social) for farmers to safeguard biodiversity. With the long-term view of fostering the synergies between agricultural production, biodiversity and the delivery of ecosystem services of local, regional and global relevance, the SoildiverAgro consortium come together to develop and deploy innovative soil biodiversity research and crop management innovation. SoildiverAgro will contribute to the enhancement of soil biodiversity in Europe, one objective of the post- 2020 CAP as well as the delivery of a circular economy and development of a sustainable Europe future (both priorities of EU in the Commission Work Programme 2018). The main objective of SoildiverAgro is the adoption of new management practices and cropping systems that enhance soil genetic and functional biodiversity to reduce the use of external inputs while increasing crop production and quality, the delivery of ecosystem services and the EU agricultural stability and resilience.

Overall concept and methodology

The overall concept of SoildiverAgro is that to enhance soil biodiversity across European croplands, the benefits of soil micro- and macro-organisms for agricultural production should be better understood, applied and transferred to farmers. Farmers will not adopt cropping systems and management practices that enhance soil biodiversity unless the economic and environmental profitability of an adequate soil biodiversity management is demonstrated for agro-ecosystems, providing evidence that this approach can foster stability and resilience of European agroecosystems. To achieve this goal, it is essential that end-users and stakeholders are involved in all project phases to develop, hone and embrace crop management through effective plant-soil interactions and the use of soil organisms. Stakeholders` and end-users´ knowledge will be used in SoildiverAgro to define the experimental design by identification of farming threats, problems, needs and crop management alternatives.

The main hypothesis of the project is that an adequate soil biodiversity management implemented via different agronomic practices tailored for different pedoclimatic conditions under various cropping systems will contribute to enhance soil micro- and macro-organisms diversity, and thus, reducing current economic, social and environmental challenges. The conceptual design of SoildiverAgro starts with the identification of farmers’ threats and needs to tackle them, followed by the identification and development of management practices and cropping systems that can enhance soil micro- and macro-organisms genetic and functional diversity to promote beneficial synergistic plant-organisms interaction. This leads to improvement in crop health, growth and development, crop productivity, quality and value, and delivery of other ecosystem services (inputs reduction, increases in soil fertility, pollution reductions, greenhouse gas emissions reductions and increases in C sequestration).

SoildiverAgro provides a holistic approach to soil biodiversity management in European agricultural soils which can contribute to soil biodiversity increase together with economic benefits to farmers (cost reductions and increases in crop value). It also contributes to circular economy reducing agricultural inputs and raw materials importations, reusing by-product and contributing to lower GHG emissions. In the end, the main ambition of SoildiverAgro which provides novelty and innovation is to produce healthier food and feed with higher value by novel sustainable practices and cropping systems while increasing the stability, resilience and adaptability of the EU agricultural sector by effective plant-soil interactions and the use of soil organisms. SoildiverAgro also provides innovation by developing three new products based on soil microorganisms formulations to increase soil fertility and quality and crop production.

SoildiverAgro concept will be put into practice through the scheme in Figure 1. The left-hand shows the WP1 (Consortium coordination and project management) which affects to the other WPs, as well as WP8 (Communication, dissemination, exploitation and stakeholders engagement) shown in the right-hand. The other work packages were divided in three groups:

1) Identification of end-user needs, potential management practices and cropping systems, soil biodiversity status and innovative tools for soil biodiversity evaluation. This first group is composed by WP2 (Identification of main challenges in European agricultural cropping systems and data mining); WP3 (Soil biodiversity assessment in European cropping systems); and WP4 (Best tools for soil biodiversity evaluation).

2) Innovative management practices and cropping systems for soil biodiversity enhancement together with farm, social and environmental profitability. This groups need incomes from the previous group and is composed by WP5 (Impacts of soil biodiversity on crop production and other ecosystem services) and WP6 (Environmental and socioeconomic assessment of soil biodiversity management and conservation).

3) Finally, WP7 (Development of strategies and tools for sustainable crop management) need inputs from the other two groups.

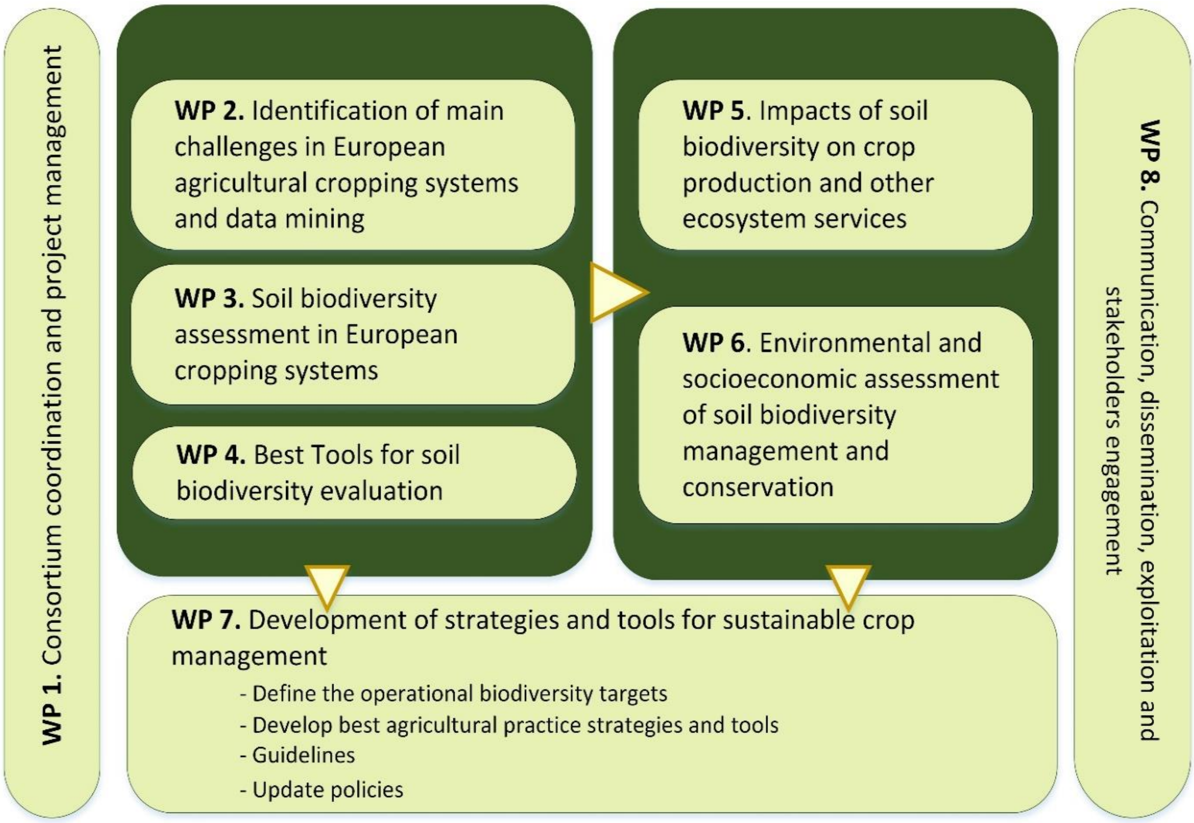

Figure 19: SoildiverAgro work packages structure

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Conservation of biodiversity through sustainable management of phytonematodes in plantain cultivated soils (*Musa* AAB SIMONDS)

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Abstract summary

In plantain plants *Dominico Hartón* was evaluated the effect of inoculation with arbuscular mycorrhizal fungi (AMF), plantain rachis compost leach (Lx) and worm compost (L), on the population of phytonematodes, the presence of AMF and the diversity of worms. We used a randomized full block experimental design under a split plot arrangement, analysing data using Poisson, Binomial Negative, Poisson Zero Inflated and Binomial Negative Zero Inflated models; finally selecting the best models through Pearson chi-square comparison, full log likelihood, AIC and SBC. The results obtained suggest that the response of nematode populations depends partly on the parasitic characteristics of the genera present and that the application of biofertilizers should be taken into account to regulate phytonematode populations in the crop and to conserve soil biodiversity.

Key words. Biofertilizers, diversity, worms

Introduction

In 2017, plantain cultivation in Colombia occupied 474,612 ha, of which 4,111,696 t were produced, with a yield of 9.89 t ha^{-1} (MADR, 2018), being of vital importance for the country's food security. Of the various pests that affect musaceous crops, phytoparasite nematodes as inhabitants of the rhizosphere cause serious problems of anchorage and considerable damage to the root system, which in turn serve as a gateway to other pathogens that seriously affect plant development and productivity. In order to propose alternative practices to the chemical management of this phytosanitary problem, which tend to the conservation and sustainable use of soil biodiversity, strategies have been proposed that involve the modification of the activity of microorganisms through the management of soil resources, the environment, the conditions of the plantations and the inoculation of antagonists (Cook and Baker, 1983). According to the previous considerations, the purpose of the present research was to evaluate the effect of inoculation of arbuscular mycorrhizal fungi (AMF), compost leaching from plantain rachis and worm compost, on nematode populations and beneficial organisms present in soils cultivated with plantain *Dominico Hartón* (*Musa* AAB SIMONDS).

Methods

Location and design of the experiment

The study was carried out in the municipality of La Tebaida, Quindío, Colombia. An experimental design of complete randomized blocks under a divided plots arrangement, with nine plants and three replications, was used. The application of AMF (M); earthworm compost (L); AMF plus earthworm compost (ML); the combination of the latter with plantain rachis leachate (MLx, LLx and MLLx) and the traditional management of the producer (P) evaluated as control, were evaluated during two productive cycles of *Dominico Hartón*.

Evaluated variables

Nematode populations were recorded in soil and roots by extraction (Varón and Castillo, 2001). Previous clarification and staining of roots (Vierheilig *et al.*, 1998), AMF colonization through the MYCOCALC program (Dodd, Clapp and Zhao, 2001) and bush identification using the scale proposed by Trouvelot *et al.* (1986). For the registration of the worm population, sampling was carried out according to the TSBF method - Tropical Soil Biology and Fertility (Anderson and Ingram, 1993), separating and identifying epigeous and/or endogenous organisms.

Statistical analysis

Considering that the variables evaluated were recorded through counts, the data were analysed using Poisson, Binomial Negative (BN), Poisson Inflated with Zeros (PIC) and Binomial Negative Inflated with Zeros (BNIC) models, selecting the best model by comparing Pearson's chi-square, full log likelihood, AIC and SBC. The parameters were considered significant when they presented a value of $P \leq 0,05$. For the statistical analysis, Proc Countreg of the Statistical Analysis System 9.4 (SAS) software was used.

Results

Both in soil and in roots the nematodes *Radopholus*, *Meloidogyne*, *Helicotylenchus* and *Pratylenchus,* were identified, which are part of the phytonematode complex of the plantain in the coffee zone of Colombia (Guzmán-Piedrahita and Castaño-Zapata, 2005); In addition, saprophytic nematodes, such as *Rhabditis, Plectus*, *Aphelenchus* y *Aphelenchoides,* were detected, which are classified as bacteriophages and fungivores (Yeates, 2007).

The comparison made allowed to select count models based on negative binomial distribution for *R. similis*, *Meloidogyne* sp., saprophytes, *Pratylenchus* sp., *Helicotylenchus* sp., total populations in soil and roots and percentages of colonization and shrubs of AMF. On the other hand, zero-inflated Poisson count models were identified for epigeous and endogenous worm populations.

The models obtained allowed us to appreciate a positive effect of cycle 1 (C1) for the amount of *R. similis* and saprophytic nematodes in soil; if compared with cycle 2 (C2) (**Eq. 1** and **Eq. 2**). A positive effect of L and M, their application together (ML) and their combinations with Lx (LLx and MLLx), was seen on *Meloidogyne* sp. in soil and roots in contrast to P; however, the application of MLLx in C1 had a negative effect with respect to C2 (**Eq. 3** and **Eq. 4**). On *Helicotylenchus* sp. and *Pratylenchus* sp., in roots there was a negative effect of M and MLx relative to P; in turn MLx in C1 has a positive effect on the populations of these two nematodes and saprophytes in roots (**Eq. 5**, **Eq. 6** and **Eq. 7**). Finally, in the total populations of phytonematodes in soil and roots a negative effect of LLx and MLx, respectively, was observed; and a positive effect of C1 and MLx in C1, with respect to C2 (**Eq. 8** and **Eq. 9**):

***R. similis* soil** = -3,008 + 2,727 ciclo_1 (**Ec. 1**)

Saprophyte soil= 4,471 - 1,270 ciclo_1 (**Ec. 2**)

***Meloidogyne* soil** = 2,602 + 2,159 L + 2,728 M (**Ec. 3**)

***Meloidogyne* root** = 5,168 + 1,838 L + 2,821 M + 1,820 ML + 1,492 LLx + 2,963 MLLx - 2.730 MLLx*ciclo_1 (**Ec. 4**)

***Helicotylenchus* root** = 8,359 - 1,060 M - 2,168 MLx + 2,268 MLx*ciclo_1 (**Ec. 5**)

***Pratylenchus* root** = 8,996 - 3,339 MLx + 4,161 MLx*Ciclo 1 (**Ec. 6**)

Saprophyte soil = 6,518 + 3,847 MLx*ciclo_1 (**Ec. 7**)

Total soil = 5,763 - 0,724 LLx (**Ec. 8**)

Total root = 9,643 – 2,205 MLx + 1,089 ciclo_1 + 2,923 MLx*ciclo_1 (**Ec. 9**)

A positive effect of ML on C1 was observed with respect to C2 (**Eq. 10** and **Eq. 11**) frequency and number of AMF bushes; whereas ML and C1 had a negative effect on the number of bushes compared to P and C2, respectively (**Eq. 11**). For the worm population C1 had a negative effect on epigeoid organisms compared with C2; for endogenous worms L, ML and their combinations with Lx (LLx, MLx and MLLx) had a positive effect relative to P; whereas C1, the application of MLLx and its interaction to it showed a negative effect (**Eq. 12** and **Eq. 13**).

Mycorrhizal Frequency = 3,354 + 1,276 ML*ciclo_1 (**Eq. 10**)

Mycorrhizal Bushes = 2,764 - 2,071 ML - 1,406 Ciclo_1 + 3,048 ML*ciclo_1 (**Eq. 11**)

Epiphygeal Worms = -22,904 - 1,389 ciclo_1 (**Eq. 12**)

Endogenous Worms = 4,507 + 0,385 L + 1,632 ML + 0,632 LLx + 0,162 MLx + 0,567 MLLx - 1,446 ciclo_1 - 2,325 ML*ciclo_1 - 1,954 MLLx*ciclo_1 (**Eq. 13**)

Discussion

The positive effects of treatments evaluated with respect to P indicate a greater probability of increasing the populations of phytonematodes; in this sense, the amount of *Meloidogyne* sp. in soil increases 9 and 15 times for L and M, respectively; as for its population in roots the treatments L, M, ML, LLx and MLLx increase their probability 6, 17, 6, 5 and 19 times, respectively. The opposite case occurs for the other phytonematodes in roots, since, with the application of M and Lx it increases the probabilities of reduction of the populations in 65 percent and 89 percent for *Helicotylenchus* sp. and *Pratylenchus* sp. respectively and 96 percent in MLx for *Pratylenchus*.

The positive effect of C1 on *R. similis* in soil (**Eq. 1**) indicates a reduction 15 times in the number of individuals in C2, which can be considered important from the phytosanitary point of view if we take into account that this nematode is considered one of the most harmful because due to its migratory habit, it penetrates the roots moving inside them destroying the cells until finishing its cycle (Agrios, 2005). For the population of saprophytes in soil, the effect of C1 (**Eq. 2**), indicates an increase of 72 percent in C2, being this positive since the identified genera are bacteriophages and fungivores (Yeates, 2007).

The negative effect of MLLx at C1 on *Meloidogyne* sp. in roots (**Eq. 3**) indicates a 93 percent increase of the nematode in C2; this may be due to the sedentary endoparasite habit it has and the abundance of roots from the effect of AMF inoculation. In the populations of *Helicotylenchus* sp. and *Pratylenchus* sp., the positive effect of MLx on C1 (**Eq. 5** and **Eq. 6**) can be translated as a 10 and 64-fold decrease in C2; respectively; which is important because these are within the plantain nematode complex.

Since a poly-specific population of nematodes lives in the rhizosphere, and the frequency and abundance of each species may change according to the agro-ecological conditions of each area (Araya, 2003); it is important to analyse the effect of factors on the total amount of phytonematodes. According to this, the negative effect of LLx on the total population in soil, is translated in a reduction of 52 percent with respect to P (**Eq. 8**); while in roots the negative effect of MLx and positive of C1 represents a 89 percent reduction with respect to the producer, and the positive effect of C1 and its interaction, represent a reduction of 3 and 19 times less in the population (**Eq. 9**).

According to the models analysed for AMF and the respective effects (**Eq. 10** and **Eq. 11**), the frequency and number of shrubs decreased 4 and 21 times at C2 with LM; however, the latter showed an increase of 76 percent at C2 and 87 percent with M (**Eq. 10**). Finally, epigeous and endogenous worm populations in C2 increased by 75 percent and 76 percent, respectively (**Eq. 12** and **Eq. 13**); for the latter, during C2 the application of ML and MLLx represented an increase of 90 percent and 86 percent, respectively (**Eq. 13**). This result is remarkable because epigeous worms as surface inhabitants of the soil feed on organic matter without ingesting soil, reproduce rapidly and adapt to conditions of soil variability; while endogenous worms have a low rate of reproduction, inhabit greater depths and tolerate the absence of food (Domínguez and Gomez-Brandon, 2010).

Conclusions

The results obtained in this research suggest that the application of biofertilizers, contributes to the reduction of phytonematode populations, while favouring the presence of saprophytic nematodes; however, this is largely influenced by the habits of each genus present in the rhizosphere. This confirms the importance of using sustainable soil management practices to achieve an appropriate balance of biological populations.

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Conversion of native grasslands to food and fibre systems impact soil fauna community in Brazilian Pampa

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Abstract

Soil biodiversity plays a crucial role on important ecosystem services such as biomass production, decomposition processes, pest control and climate regulation, but it is largely unknown in Brazilian natural ecosystems. To address this knowledge gap in the Pampa region, in this study we evaluate soil biodiversity in different land use types to assess the effect of native grassland conversion on soil fauna communities. We compared pairs of native grasslands with adjacent food and fiber systems (soybean and eucalyptus plantations) regarding soil fauna abundance, richness and composition. Our results show that native grassland conversion reduced soil fauna abundance and changed species composition. These findings indicate that changes in soil habitat caused by conversion of the native ecosystem affect soil fauna biodiversity. The consequences of these changes on ecosystem services, food, water and energy security will be further explored.

Keywords: land use conversion, soil biodiversity, Coleoptera, eucalyptus, soybeans

Introduction, scope and main objectives

The global demand for food, fiber and energy has been the main cause of human pressure on natural ecosystems (FAO, 2018), leading to increasing rates of conversion of native ecosystems to croplands and silviculture, which may affect biodiversity and the provision of ecosystem services (Foley *et al.*, 2005). In Brazil, conversion of natural vegetation has increased drastically in the past decades, particularly in non-forest ecosystems (Overbeck *et al.*, 2015), with expected consequences on ecosystem services (Metzger *et al.*, 2019). Native grasslands provide important ecosystem services at regional scale, such as carbon stocks, forage for livestock, erosion control, and habitat for biodiversity (Andrade *et al.*, 2015). However, at present, only about 35 percent of the original land cover of Pampa grasslands remain in southern Brazil (Oliveira *et al.*, 2017).

Soil and its biodiversity are essential compartments of ecosystems that need special attention to achieve a sustainable use of natural resources (Hatfield *et al.*, 2017). Soil properties influence soil functions (i.e. biomass production, carbon storage, water infiltration), affecting food and fibre production, water and nutrient regulation, biomass decomposition, and climate regulation (Adhikari and Hartemink, 2016). It is a consensus that soil biodiversity is crucial for most ecosystem processes, because soil biota regulates soil microbiota communities, nutrient uptake by plants, and nutrient distribution into the soil (Wagg *et al.*, 2014).

Thus, considering soil biodiversity importance and the scarcity of studies in Brazilian soils (i.e. Podgaiski *et al.*, 2013; Suleiman *et al.*, 2017; Winck *et al.*, 2017, Winck, Rigotti and Saccol de Sá, 2019; Boeno *et al.*, 2019), here we evaluate the effects of conversion of native grasslands to food and fibre systems (soybean and eucalyptus plantations) on soil communities across the Brazilian Pampa. We hypothesized that: (i) conversion to tree plantation (i.e. eucalyptus) will cause stronger impact in soil fauna, given the drastic changes from predominantly grassy to forest habitats; and (ii) impact of soybean cropland on soil fauna will be more related to community composition than species richness or abundance, since specific taxa such as those considered pests may occur abundantly in croplands.

Methodology

Sites description and experimental design

We collected data at nine sites across the Brazilian Pampa in the framework of the project NEXUS: "Scenarios of native vegetation conversion and the sustainability of agroecosystems in the Pampa biome" (Figure 1). Our sampling comprised 36 paired plots of converted sites (soybean, rice and eucalyptus) and native grassland traditionally used for free-range cattle production, totalling 72 sampling units distributed across the sampled region. Here we focus on two study cases about the effects of soybean and eucalyptus plantation on soil fauna communities.

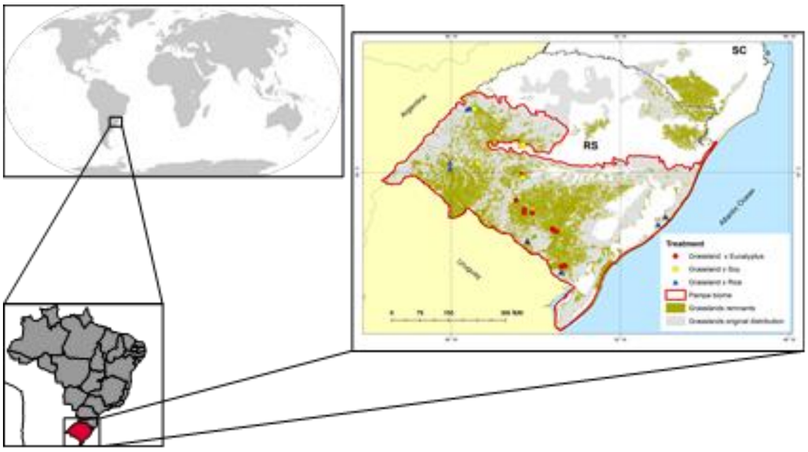

Figure 1: Location of the study sites in southern Brazil and distribution of the sampled sites in the Pampa biome

Case study 1 (eucalyptus vs. grassland)

We compared grassland paired with eucalyptus at 10 sites (Figure. 1) based on surface-dwelling soil fauna abundance and composition. The sampling was carried out from October 2018 to July 2019. At each site and land cover type we located a 250 m transect along an isocline, which was systematically subsampled at each 50 m using pitfall traps filled with alcohol 70 percent (50 mL centrifuge tubes; 3 traps per point), totalling 300 pitfall traps. The traps remained in place for 24 hours, after which organisms were counted and sorted into four high-taxa (Collembola, Formicidae, Coleoptera, Araneae).

Case study 2 (soybeans vs. grassland)

We compared grassland paired with soybean crops at nine sites (Figure. 1) based on surface-dwelling beetle (Insecta: Coleoptera) abundances, morphospecies richness and composition. Also, edge effects between habitats were explored. We sampled grasslands and soybean plantations during the soybean flowering stage (January – March 2019). At each site and land cover type, we established three 50 m transects located at 10, 125 and 250 m from the edge between habitats, where we installed at each 10 m pitfall traps filled with alcohol 70 percent (500 mL pots), totalling 270 traps. The traps sampled surface-dwelling beetle for 72 hours, after which the organisms were counted and sorted into morphospecies.

Data analysis

The effects of grassland conversion into eucalyptus and soybean plantations on soil fauna abundance, richness and composition were evaluated by uni- and multivariate analysis of variance with permutation test. The multivariate analysis was based on Bray-Curtis dissimilarities.

Results and discussion

Case study 1: We found higher abundance of soil fauna in grassland sites (2641 individuals) than in eucalyptus (843 individuals) being Formicidae and Collembola the most abundant groups in both land-uses. Total abundance was statistically different between grassland and eucalyptus ($P < 0.001$), except for Araneae ($P = 0.26$). These changes in abundance also reflected on significant differences in soil fauna composition (Figure 4A, $P > 0.001$).

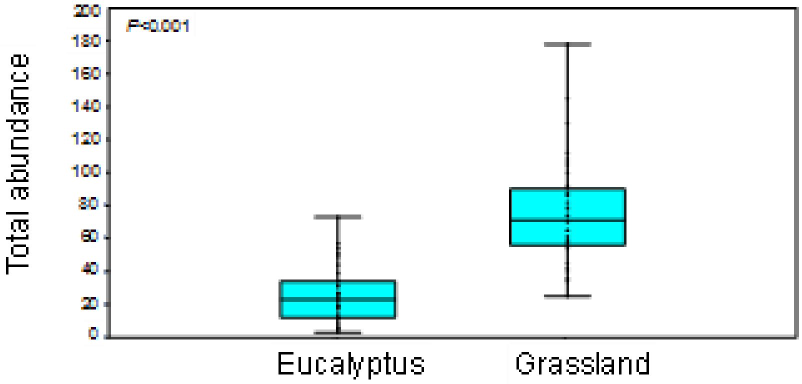

Figure 2: Mean abundance of soil fauna and standard deviation in 10 pairs of grassland and eucalyptus

Case study 2: We collected a total of 865 beetles, being 452 in grassland and 413 in soybean plantations and the most abundant beetle family was Nitidulidae (25 percent). We found 137 morphospecies, being the highest richness observed in Carabidae (21 morphospecies), Staphylinidae (18 morphospecies) and Curculionidae (14 morphospecies). Grasslands showed 61 exclusive morphospecies, while soybean plantations showed 35, and 41 were common to both land-uses (Figure 3A). (Figure 3A). Contrary to our expectation, we did not observe significant effects of grasslands conversion into soybean plantation for Coleoptera abundance ($P = 0.77$) and morphospecies richness ($P = 0.14$). Distance from edge also did not affect abundance ($P = 0.62$) and richness ($P = 0.19$) for both types if habitat (Figure 4B to E). However, as noted in the first study (Figure 4B), beetle community composition differed between grassland and soybean ($P < 0.001$).

Overall, the results confirmed our hypothesis that conversion of native ecosystems into cropland or silviculture reduces soil fauna abundance and changes species composition. However, we also expected to find higher richness of Coleoptera in grassland sites (Cramer and Willig, 2005). In another study in Pampa grasslands, using collembola as model group, Winck *et al.* (2017) found lower abundance of Collembola in eucalyptus, but higher richness and diversity than in grassland. The turnover we observed on fauna composition between habitats is likely related to changes in soil fauna habitat and food resource availability. Grassland plant communities present high species diversity (21 species per m^2 in average in our study sites), resulting in high habitat and resource heterogeneity and favouring soil fauna species coexistence. However, in soybean plantations, besides the homogenization of habitat and resources, management including fertilization, pesticides, soil tillage, and harvest, is much stronger than in natural grasslands grazed by cattle. These changes can negatively affect soil fauna communities and change their composition.

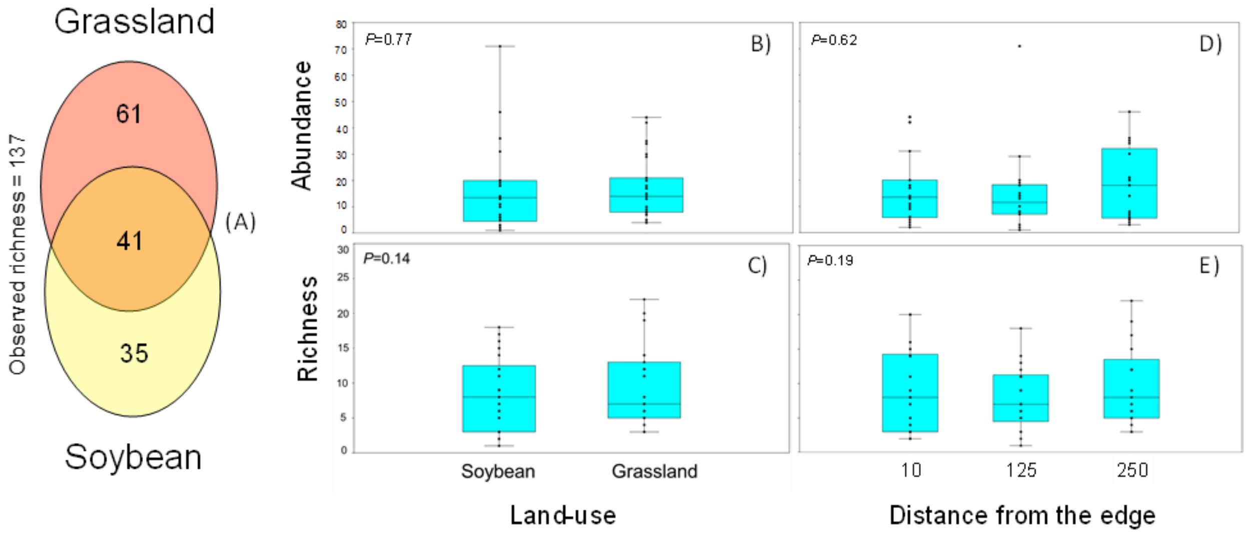

Figure 3: Venn diagram (A) of beetle richness and abundance (B and C) and richness (D and E) in soybean and grassland, and at each distance from edge

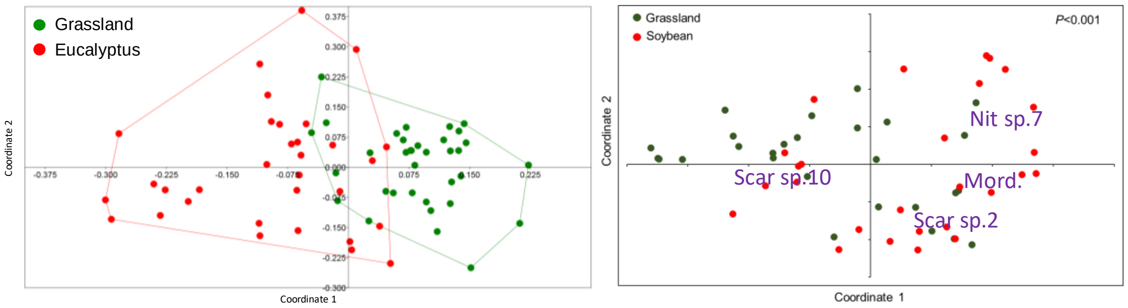

Figure 4: Principal coordinate analysis based on Bray-Curtis dissimilarities calculated using all soil fauna (A) and beetle morphospecies (B) abundances in Brazilian Pampa

Circles indicate sampling units

Conclusions

There is evidence that species richness of soil communities influence multiple ecosystem functions (Wagg *et al.,* 2014), but these effects may depend on species composition (Maestre *et al.,* 2012). Thus, we concluded that conversion of native grassland of Brazilian Pampa into food and fibre systems affects the composition and richness within soil fauna communities, and these changes might affect ecosystem services.

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Exploring the potential of three *Rhizobium* strains from Peruvian soils as biofertilizers for the common bean (*Phaseolus vulgaris*)

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Abstract summary

Common bean (*Phaseolus vulgaris*) is an important grain legume for human consumption as it has a high protein content. Rhizobia are nitrogen-fixing soil bacteria which stablish a mutualistic symbiosis with plant legumes like the common bean. These bacteria can be used instead of nitrogen chemical fertilizers. This study aimed at evaluating three rhizobial strains obtained from Peruvian soils as potential biofertilizers for *P. vulgaris*. The strains were identified as belonging to the species *Rhizobium tropici*, *Rhizobium etli* or *R. sophoriradicis* by sequence analysis of the *recA* and *glnII* genes. Besides its nitrogen fixation capability, the rhizobial strains displayed other characteristics like indoleacetic acid (IAA) production and/or di-calcium phosphate solubilization, abilities which may contribute to its plant growth promoting (PGP) potential. Bean plants inoculated with the rhizobial strains grew equally or better than plants provided solely with chemical nitrogen. Furthermore, a synergistic effect was observed when *Rhizobium etli* and *R. sophoriradicis* were combined as this treatment showed the highest pod weight.

Keywords: IAA, PGPR, SPAD, Rhizobium

Introduction

The common bean (*Phaseolus vulgaris*) is a plant legume grown worldwide for human consumption. In Peru, this legume is the most consumed and have the largest cultivated area (MINAG, 2014). Soil bacteria known as rhizobia have the ability to stablish a mutualistic symbiosis with legumes. Rhizobia induce the formation of specialized structures, called nodules, in the roots of legumes and within them they fix atmospheric nitrogen (N_2) into ammonium (NH_4^+) thanks to the bacterial nitrogenase enzyme. The NH_4^+ ion is assimilated by the plant which in return provides carbon sources to rhizobia. Soil nitrogen is a limiting growth element for plants and usually must be provided in the form of chemical fertilizers which are expensive and can have negative impacts on the environment. Rhizobia, on the other hand, can be used as inexpensive and ecologically-friendly fertilizers for legume production in agriculture.

P. vulgaris symbionts have been studied in many countries but little is known about rhizobia inhabiting soils of Peru which is a center of origin of this crop. We have previously isolated rhizobia from two bean growing areas in Peru and selected three strains based on a preliminary screen for their effects on bean growth until flowering. The objectives of this study were to determine the species affiliation of the selected strains as well as their *in vitro* plant growth promoting (PGP) activities and effects of bean growth as single or dual strain inoculants.

Methodology

Phylogenetic analysis

DNA was extracted from bacteria grown in YEM medium (Vincent, 1970) with the GeneJet Genomic DNA purification kit (Thermo Fisher Scientific) following the manufacturer instructions. Fragments of the *recA* and *glnII* genes were amplified as previously described (Aguilar, *et al.*, 2004; Lloret, *et al.*, 2007; López- López, *et al.*, 2010) and Sanger sequenced at Macrogen Inc (Seoul, South Korea). Edited sequences were aligned to those of rhizobial type strains retrieved from the GenBank database. A neighbor joining phylogeny with Tamura Nei distances was constructed with the concatenated alignment of *recA* and *glnII* using Mega v7 (Tamura, *et al.*, 2013).

in vitro plant growth promoting (PGP) activities

Production of indole compounds, such as the plant hormone indoleacetic acid (IAA), was determined in bacteria grown in YEM broth supplemented with L-tryptophan (1 g/l) for 48-72 h at 28 ° C until an OD_{600} of 0.8. A 500 ml aliquot was centrifuged at 12000 rpm for 8 min and 250 ul of the supernatant was mixed with 1 ml of Salkowsky reagent. Reaction proceeded in the dark for 30 min, then, measures were taken at 530 nm and compared to a standard curve (Zúñiga, 2012). For di-calcium phosphate solubilization assays, bacteria grown in YEM broth until an OD_{600} of 0.8 were inoculated in discs which were placed in plates of NBRIP medium (Nautiyal, 1999). Plates were incubated at 28 ° C for 15 d and the diameter of solubilization haloes were measured. Both tests were performed with 3 replicates per rhizobial strain.

Effects of the symbiosis on bean growth

Plants were grown in a vermiculite/sand (5:3) mixture. The substrate was sterilized two times at 121 °C and 15 psi. Seeds of *P. vulgaris* cv. Canario Centenario were surface disinfected by successive treatments in 70 percent alcohol and 3 percent sodium hypochlorite, then rinsed ten times with sterile distilled water and finally inoculated with 1 ml of bacterial cultures grown until an OD_{600} of 0.8. Inoculations were performed with one strain or a combination of two strains. A non-inoculated control was included. Seeds were placed in pots with 500 g of sterile substrate. The experiment was conducted in a growth chamber at 20-25 °C using a completely randomized experimental design with three replicates per treatment. Pots were watered with nitrogen-free Broughton & Dilworth solution (Broughton and Dilworth, 1971) except the control that received nitrogen fertilization. The indirect measurement of chlorophyll content (SPAD index) was measured during vegetative growth. At pod formation (56 d), dry weight of nodules shoots and pods was determined. Statistical analyses were performed with Minitab 17 using the Tukey's range test for mean comparisons at a significance level of 0.05.

Results

Species affiliation of native P. vulgaris rhizobia isolated from Peruvian soils

The three rhizobial strains showed fast growth and acid production in YEM medium, characteristics which indicated that they may belong to the *Rhizobium* genus. A phylogenetic analysis using the sequences of two house-keeping genes confirmed that they were indeed *Rhizobium* strains and revealed that each strain belonged to a different rhizobial species (Fig. 1). Strain 30C, 22C and A6 could be assigned to the species *Rhizobium tropici*, *Rhizobium etli* and *Rhizobium sophoriradicis*, respectively.

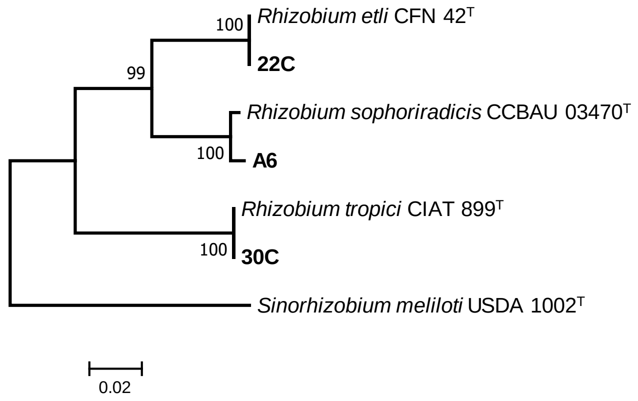

Figure 1: Phylogenetic relationships between Peruvian native rhizobia and related species based on concatenated *recA* and *glnII* gene sequences

Plant growth activities

IAA production, evaluated by the formation of indole compounds, was detected in the three rhizobial strains (Table 1). Strains 30C (*R. tropici*) and 22C (*R. etli*) produced approximate equal amounts which were slightly higher than that produced by strain A6 (*R. sophoriradicis*). Phosphate solubilization was observed only for strains 30C and 22C (Table 1).

Table 1: AIA production and di-calcium phosphate solubilization by three *Rhizobium* strains from Peruvian soils

Species	Strain	IAA concentration (µg/ml)	Solubilization index (mm)

Rhizobium tropici	30C	38,10	1,46
Rhizobium etli	22C	38,29	1,40
Rhizobium sophoriradicis	A6	36,22	0

Analysis of the inoculation effects of Rhizobium strains on bean plants

Variable responses were observed when bean plants were inoculated with the three native rhizobial strains (Table 2). All inoculated treatments showed equal or best responses in fresh and dry weight parameters than the control non-inoculated treatment which received chemical nitrogen. The *R. tropici* strain induced the highest shoot dry weight while he combination of *R. etli* and *R. sophoriradicis* gave the best results for fresh and dry pod weight.

Table 2: Bean response to rhizobia inoculation

Treatment	***Shoot dry weight (g)***		***Pod fresh weight (g)***		***Pod dry weight (g)***		***Nodule dry weight (g)***	
	Mean	***Groups***	***Mean***	***Groups***	***Mean***	***Groups***	***Mean***	***Groups***
R. tropici	3,603	A	6,04	B	1,357	C	0,2175	A
R. etli	2,350	B	9,10	AB	3,787	AB	0,1300	AB
R. sophoriradicis	2,720	AB	5,627	B	2,273	BC	0,1225	AB
R. tropici + R. etli	3,185	AB	10,63	AB	3,572	AB	0,1825	AB
R. tropici + R. sophoriradicis	2,965	AB	9,41	AB	3,337	AB	0,1750	AB
R. sophoriradicis + R. etli	2,6825	AB	13,540	A	3,902	A	0,0900	BC
Control N+	2,4875	AB	7,93	B	2,505	ABC	0	C

Means in the same column followed by the same letter did not significantly differ according to the Tukey test ($p<0.05$)

For the chlorophyll content measured as SPAD index, it was observed that the *R. etli* + *R. sophoriradicis* treatment had the best values at 36 and 56 d followed by the treatment with *R. etli* alone (Figure 2). Plants inoculated with the *R. tropici* strain reached their maximum values at 45 d. Finally, *R. sophoriradicis*-inoculated plants showed a similar behavior as the control plants.

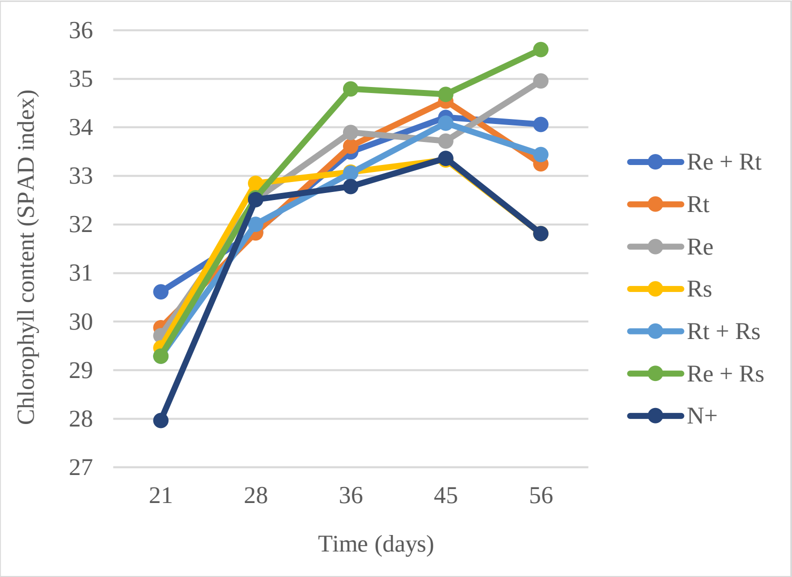

Figure 2: Chlorophyll content (SPAD index) of bean plants inoculated with rhizobial strains

Re, R. etli; Rt, R. tropici; Rs, R. sophoriradicis

Discussion

The results showed that three *Rhizobium* species able to stablish symbiosis with bean are present in Peruvian soils, none of these species has been described before in Peru. Besides its nitrogen fixation capability, the rhizobial strains analysed here displayed other characteristics like IAA production and/or phosphate solubilization, abilities which may contribute to its PGP potential. Bean growth parameters induced in symbiosis by the strains were not always correlated. The *R. tropici* strain stands out in shoot and nodule dry weight but not in pod weight. Chlorophyll content in plants inoculated with that strain increased until day 45 but then decreased, this may explain its better performance in shoot versus pod weight. On the other hand, the

R. etli + *R. sophoriradicis* treatment showed a good correspondence between a high pod weight and better and steady increase in chlorophyll content over time. However, that same strain combination did not significantly differed from most other treatments in dry nodule weight which suggest that strain effectiveness did not always correlate with nodule quantity.

Conclusions

- Three species of the bacterial genus *Rhizobium* able to nodulate the common bean were found in Peruvian soils.
- Although all strains were able to promote the growth of bean, a combination of the species *Rhizobium etli* and *R. sophoriradicis* stands out in chlorophyll content and pod yield.
- More studies are recommended to test the rhizobial strains under field conditions.

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Application of bacterial biostimulants in productive landscapes of *Allium cepa* L.

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Abstract summary

The bacterial strains belonging to the genera *Azospirillum, Burkholderia, Gluconacetobacter* and *Herbaspirillum* have been combined in consortium and inoculated in *Allium cepa* L. seeds before sowing. The seeds were sowed in different open fields and compared to control experimental conditions. The cultivations were followed until harvesting and plant growth and development parameters (*i.e* plant height, chlorophyll contents) and some properties on harvested products (*i.e.* crop yields, bulbs dry matter, total phenolic contents and antioxidant activities) were investigated. The influence of bacterial biostimulants treatment on microbial communities physiological (CLPP) and molecular profiles (PCR-DGGE) were also assessed. The results showed positive influence of bacterial application on plant germination and growth. The excellent ability to stimulate plant growth by the consortium was demonstrated in the productivity and quality of the crop. The results obtained so far underlined that the seed treatment with PGPR consortium is an effective and replicable methodology that improves yields in productive landscapes and food quality, whilst safeguarding soil biodiversity.

Keywords: Sustainable agriculture, PGPR, Bio-fertilization, soil biodiversity conservation

Introduction, scope and main objectives

Nowadays, productive landscapes are characterized by a massive use of synthetic chemical fertilizers. The application of these products entails significant environmental pollution, soils depletion and crop productivity declines (Savci, 2012). In Italy, biostimulants have been included in the Annex 6 of Law 75/2010 as "Products with specific action on plant". Biostimulants are defined as materials that added to another fertilizer or to the soil or plant, could favor or regulate the absorption of nutrients or correct certain physiological disorders (Toscano *et al.*, 2018). Among the biostimulants, plant growth promoting rhizobacteria (PGPR) represent a sustainable technique thanks to their ability to improve crops productivity (e.g. by hormones production) whilst safeguarding soil biodiversity. Moreover, these bacteria support plant nourishment by mobilizing soil essential elements that are wedged in complex structures (e.g. phosphorous, potassium) and prevent losses of these essential chemical elements (Alori, Glick and Babalola, 2017). Aim of this study was to evaluate the applicability of the selected bacterial biostimulant on the Fucino productive landscape, an Abruzzo region territory particularly suited for the cultivation of horticulture crops. Among the Fucino's production, two varieties of *Allium cepa* L. were selected and the main objectives of the study were to: (i) confirm on large scale the inoculum biostimulant capability already underlined on small scale; (ii) evaluate the effects on crop yields and (iii) on food products quality.

Methodology

The bacterial consortium was prepared as previously described by Botta *et al.* (2013). Onion seeds (var. Moondance and Meranto) were treated with PGPR consortium and dried overnight at room temperature. Controls were treated in the same way, utilizing only water. Sowings were performed by automatic seeder following a split-plot arranged experimental design. During the cultivations, plant height and chlorophylls (Arnon, 1949) were monitored and at harvest yields (q/ha) were quantified. Bulbs were extracted by microwave extraction (Mirzapour, Hamedi and Rahimipanah, 2010) and extracts were assayed for their total phenolic content (by Folin-Ciocâlteu reagent) and antioxidant activity (by DPPH, ABTS and FRAP assays) (Pellegrini *et al.*, 2018). The soil microbial communities were investigated in terms of physiological (CLPP) an molecular (PCR-DGGE) profiles, following the procedures and ecological indexes calculations as previously described in Moretti *et al.*, (2017) and Cacchio and Del Gallo (2019), respectively.

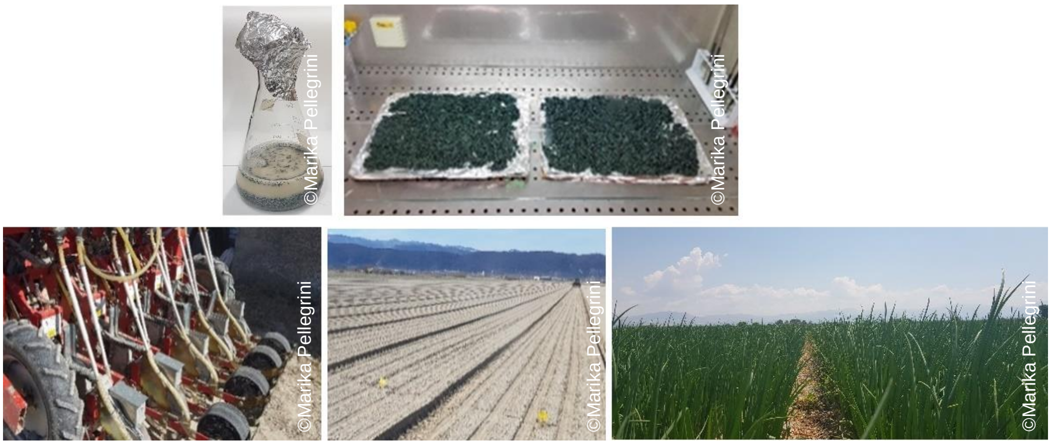

Figure 1: Onion seeds treatment and experimental fields sowing

Results

As shown in Figure 2, the treated experimental conditions (panels on the left) obtained highest plant growth and development parameters compared to the control one (panels on the right). The differences between the experimental conditions were underlined in terms of plant height (+ 18 percent on average, $p<0.001$) and total chlorophylls (+ 42 percent on average, $p<0.001$), crop yields (+ 13 percent on average, $p<0.01$) and bulbs dry matter (+ 3 percent, $p<0.05$). The differences between control and treated experimental conditions were also underlined in the bulb extracts in terms of total phenolic contents (+25 percent on average, $p<0.01$) and antioxidant activities (an average of +20 percent for the three antioxidant activity assays, $p<0.05$). For the same experimental condition (PGPR or control), no significant differences were found among the two varieties and the fields ($p>0.05$).

Figure 2: Seedling, plants and bulbs of *Allium cepa*

The PGPR experimental condition are in the panels on the left, while controls are in the right panels

The ecological indexes calculated on DGGE profiles, show that the soil microbial communities were not modified in terms of richness, but the community diversity and functionality were positively affected by the treatments. This positive contribution was also found in the ecological indexes calculated on CLPP profiles; for the treated experimental conditions a major utilization and diversity of the carbon sources utilized by soil microbial communities were detected.

Discussion

United Nations assessed that the global population would increase to more than 9.7 billion by 2050. This situation requires that food productions must increase; this growing demand must come with the utilization of sustainable and eco-friendly methodologies to counteract the increasing resource depletion and ecosystem degradation (Colla *et al.*, 2014; Rouphael and Colla, 2018). The bacterial biostimulants use in productive landscapes represent a valid tool to face this challenge. The results obtained in this study underlined this potential. The seed treatment with PGPR consortium, has positively influenced growth and development parameters in both plants and products. Several studies already demonstrated the PGPR biostimulants properties by direct and indirect mechanisms (Vejan *et* *al.*, 2016). The abilities that usually allowed an effective biostimulation are to ascribe to the synthesis of hormones like auxins, that stimulates plant development and have positive influence on pigments synthesis (Urana, Singh and Sharma, 2019). The capability of solubilizing nutrients such as P and K give a huge contribution to the crop; plants receive more nourishment for their growth than the control and have the possibility to thrive best, with consequent higher yields. The phenolic contents and the related antioxidant activity are other positive consequences of this biostimulants effects, that influence also plant secondary metabolism (Pagnani *et al.*, 2018).

Conclusions

The results so far obtained underlined that the seed treatment with PGPR consortium is an effective and replicable methodology that improves yields and quality of the products in productive landscapes of *A. cepa*. Further studies should be undertaken to assess the biostimulant ability on other vegetal species, in any case, the results so far obtained by scientific world suggest that increasing bacterial biodiversity could be a valid eco-friendly technique to increase food quality whilst safeguarding soil biodiversity.

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Minimal herbicide-based conservation tillage enhances soil macro fauna abundance and distribution in Uganda

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Abstract

Herbicide Based Conservation Tillage (HBCT) for weed management has been adopted widely by several farmers in Africa. However, the extent of its impact on soil biodiversity has not been well documented. A study was conducted to assess the impact of HBCT on soil macro fauna in Uganda. Banana and Coffee Fields of 40 m by 20 m that are ten days after glyphosate application, sixty days after application and control with no herbicide were studied. Sampling was done randomly along the transect in each experimental plot using quadrats. Soil monoliths were excavated up to 30 cm depth and termites, earthworms, millipedes and centipedes were sorted out within and between the given soil layers at varying depth of 0-10 cm, 10-20 cm and 20-30 cm. HBCT affected biodiversity with macro fauna abundance and distribution significantly affected ($p<0.05$). The abundance of earthworms, millipedes, centipedes and termites was found to be significantly lower in soil that had been sprayed 10 days back, with lower soil macro faunal distributions in the first soil layer compared to the second soil layer. This pattern was evident even after 60 days. Minimal use of HBCT is vital for enhancing soil biodiversity.

Keyword: Soil Macrofauna, Uganda, Herbicide based conservation, HBCT

Introduction

Use of pesticides and other agrochemicals has extensively increased worldwide to control weeds and pest incidences (Lee and Thierfelde, 2017). The increased global food security challenges that have come with the increasing global population and food demands has led to over reliance on agrochemicals. The use of agrochemicals as a conservation tillage practice, is believed to conserve SOM, control soil erosion; and is economically friendly approach to poor resource endowed farmers. However, organic matter is also believed to be a major factor in management that can affect earthworm abundance (Leroy *et al.*, 2008). The use of herbicides in thus a conservation tillage practice, but disruptions on SOM cycling or soil functioning can lead to loss of biota. Glyphosate is a widely used agrochemical in Uganda, and this is on assumption that its less toxic in nature to humans. It tends to have less likelihood of persisting in the environment as compared to other herbicides. There is massive adoption and use of the herbicide in low-input agricultural systems rather than use of mechanization. This can result in significant impact on soil biodiversity and soil health over years. Loss of soil biodiversity affects soil organic matter dynamics, soil physical properties, water and nutrient release due to toxic effects, making macrobiotic less active hence contributing to the weak biological functioning (Pulleman *et al.*, 2004; Ouédraogo, Mando and Stroosnijder, 2006). The decrease in the abundance, distribution and biodiversity of soil macro fauna can result in an ecological problem in agriculture. The impact of using herbicides in Uganda to control weeds has had limited studies, that link the chemicals to soil health development efforts. Glyphosate, which is widely used herbicide by farmers could be having long-term effects on soil macro fauna and soil health. Studies in Uganda have not considered the extent of the impact of herbicide-based tillage agriculture conservation practice and soil biodiversity (Mutema *et al.,* 2013; Holland, 2004). The study was conducted to assess the impact of herbicide-based conservation tillage (HBCT) on soil macro fauna variations in soils of Uganda.

Methodology

The study was conducted in two parishes Nyarubungo and Nyakikara in Rwampara district in southwestern Uganda. The physical landscape of the area is composed of fairly rolling and sharp hills and mountains, shallow valleys and flat land. The area is characterized under coffee-banana farming system with the major land use activities of crop and livestock farming. New farmers' fields have opened for production using glyphosate as a practice for weed control. The study was designed targeting new fields to be targeted for maize fields. The fields comprised of the treatment 1 – (Fields that had been sprayed in the last 10 days (T1) with glyphosate herbicides; Treatment 2 - fields spread 60 days back (T2) in order to determine the persistence of glyphosate herbicide in the soil over a period of time after the half-life of 30 days (Giesy, Dobson and Solomon, 2000), and Treatment 3 – the control with field that had not been sprayed by glyphosate herbicide before. Fields subjected to same treatment were studied and a total of three randomized locations in each treatment were sampled for soil macro fauna studies (termites, earthworms, millipedes and centipedes). Monolith sampling was used and according to the standard Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram, 1993). The sampling was done along a transect using quadrats measuring 65 cm by 65 cm and the inner quadrat measuring 25 cm by 25 cm x 30 cm. Soil monoliths were also excavated to 30 cm depth (n=3) per plot. Soil layers within the quadrants were evaluated at 0-10 cm, 10-20 and 20-30 cm depths. Termites, earthworm, centipedes and millipedes were sorted and count manually on plastic trays. Abundance (that represents the number of individuals per m^2 and biomass, that was calculated as g m^{-2}) were computed at 0-10 cm, 10-20 cm and 20-30 cm soil depth. Data was subjected to analysis of variance (ANOVA) using GENSTAT. Treatment differences were evaluated using Fisher's least significant difference at $P<0.05$).

Results

Effect of herbicide-based conservation tillage on soil macro fauna abundance and distribution

Overall, herbicide use in conservation tillage significantly affected ($p<0.05$) the abundance and biomass of earthworms, millipedes and termites, with notable decline in individuals (abundance/m^2) per unit area in the soils of south western Uganda. There was evidence of no termites after application in the first 10 days but the numbers tended to increase after 60 days (Table 1). There were generally decline in abundance of soil macro-fauna on application of herbicides in the first 10 days and 60 days (Figure 1-4)

Table 1: Soil Macro fauna abundance as affected by herbicide-based tillage in top soil layer (0-10cm)

Treatment with herbicides	Earthworm abundance/m^2	Earthworm biomass (g/m^2)	Millipede abundance/m^2	Millipede biomass (g/m^2)	Termites Abundance (m^2)	Termite Biomass (g//m^2)
T1 (10 DAHA)	14.9a	0.43a	0	0	0	0
T2 (60 DAHA)	24.6a	0.46a	2.1a	0.34b	1.61a	26.2a
T3 (control)	33.5b	1.97b	9.9b	1.7c	5.17b	64.2a
LSD 5%	13.92	1.276	4.83	0.24	2.712	47.37

T1, T2, T3 are treatments representing Days After Herbicide Application (DAHA) for 10 days, 60 days and no application as the control, respectively.

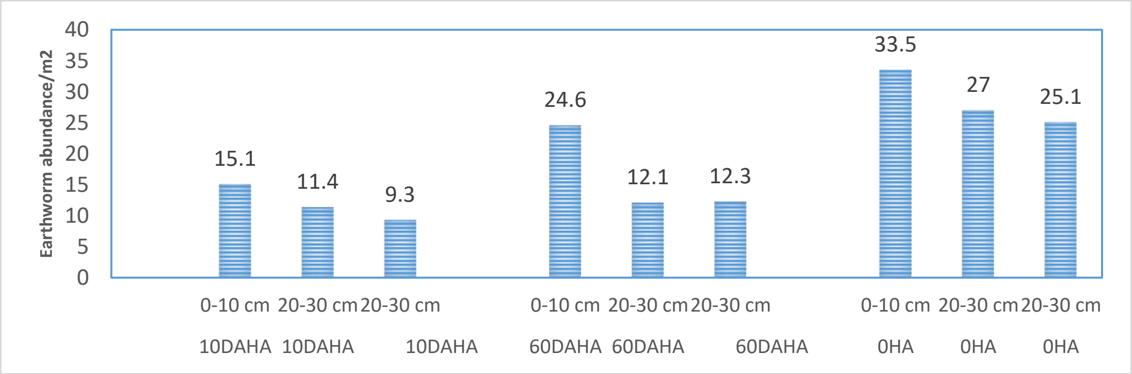

Figure 1: Earthworm abundance - Days After Herbicide Application (DAHA) for 10 days, 60 days and no application as the control

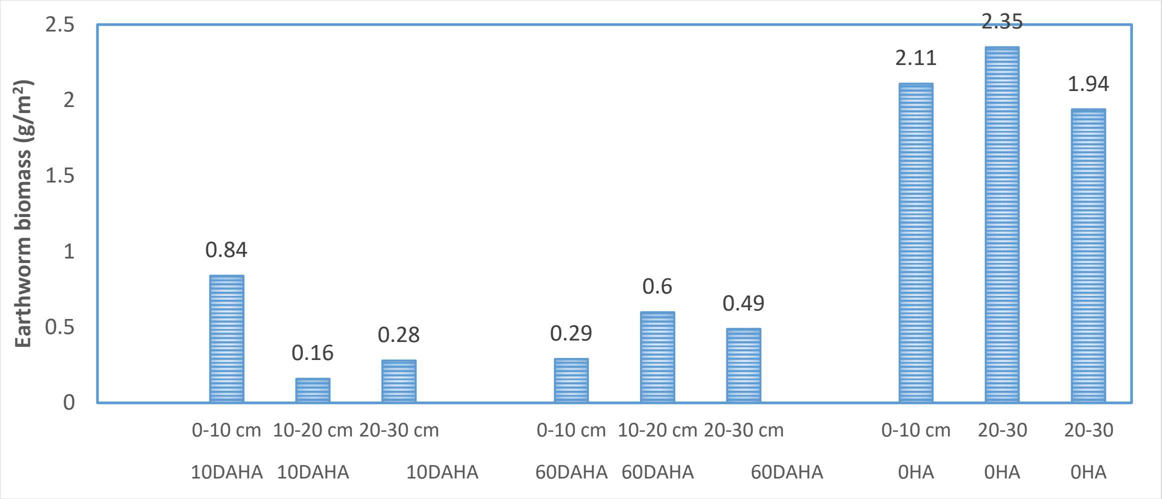

Figure 2: Earthworm Biomass - Days after Herbicide Application (DAHA) for 10 days, 60 days and no application as the control at various soil depth

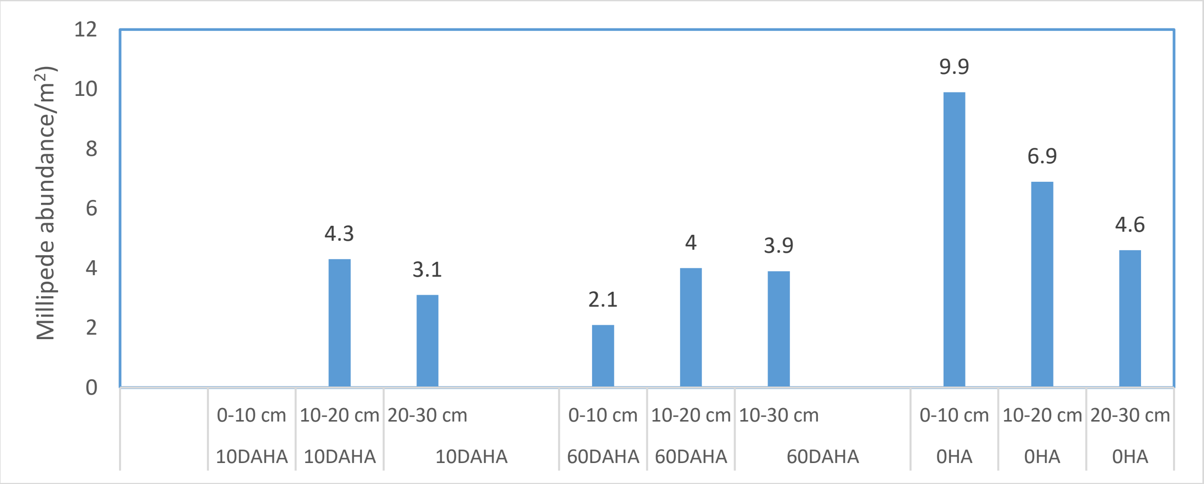

Figure 3: Millipede Abundance - Days After Herbicide Application (DAHA) for 10 days, 60 days and no application as the control at various soil depth

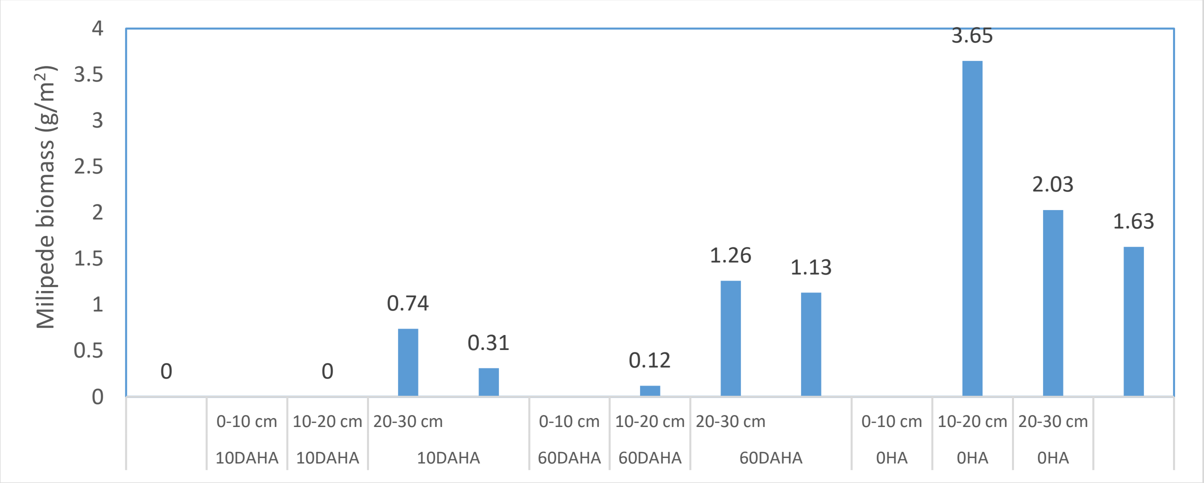

Figure 4: Millipede Abundance - Days After Herbicide Application (DAHA) for 10 days, 60 days and no application as the control at various soil depth

Discussion

The abundance of earthworms, millipedes, centipedes and termites were significantly lower in soils that had been sprayed with glyphosate herbicide, 10 days back while the soil that had not been spayed by the herbicide registered a higher abundance of soil macro fauna. The lower soil macro fauna in herbicide treated soil could be due to the losses that are caused by the herbicide through death and avoidance behaviour of the fauna. The study reaffirms that agricultural chemicals tend to induce an avoidance mechanism in soil macro fauna that renders them absent. For instance, earthworms are sensitive to the presence of chemicals in the soil due to the chemo receptors distributed on their body surface which are characteristically associated with their locomotors abilities, that renders them the chance to avoid contaminated areas where soil habitat function has been affected (Reinecke *et al.,* 2002). García-Pérez *et al.* (2016) demonstrated that earthworm biomass, development and reproduction can be severely affected by glyphosate at a range of concentrations. Our study affirms such observations with clear effects of toxicity impacting on soil biota. Similar patterns of the observations were observed with millipedes and termites. The total absence of termites or millipedes in 10 and 60 days of herbicide application further demonstrated how toxic these herbicides can cause to micro-biota. The impacts seem to affect life in the soil as a whole. In some cases, the effects in 60 days half-life of glyphosate were not so toxic as 10 days, which corresponds to an assertion that some herbicides in some in agricultural soils have a half-life of 30 to 32 days from the date of application. Therefore, after 60 days of application, the glyphosate could have dissipated from the soil causing the abundance of soil macro fauna to increase as observed in this study. The effect of the glyphosate herbicide was more severe in the immediate soil layers as they receive more of the excess chemical sprayed on the weeds. This explains the absence of soil macro fauna in the first top most soil layer of soil 10 days after herbicide application. Nevertheless, soil macro fauna like termites are more abundant in the first soil layer due to the availability of soil organic matter in the top soil layer that is feed to the macro fauna in soil.

Conclusions

Herbicides use in tropical soils for weeding and conservation tillage is a practice that is significantly disrupting the patterns of soil biodiversity. There was clear effect of herbicides use on soil biota abundance which is a key indicator of agrochemical impacts on soil. Further studies are needed to study the soil biological components in the soil over time as a result of continuous use of glyphosate herbicides. This will enable analysis of long-term effects of agricultural management practices on the soil macro-fauna, and help understand soil biodiversity.

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GLOBAL SYMPOSIUM ON SOIL BIODIVERSITY | FAO HQ | Rome, Italy, 19-22 April 2021

A new integrate land degradation assessment approach considering soil biodiversity, humus forms and vegetation

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Abstract summary

Any terrestrial environment is subject to several dynamics, whose quality levels are influenced by several factors (e.g., soil types, humus forms, fungi, vegetation, soil fauna, human activities). In literature, specific indicators (or indexes) were used to assess soil biological quality, soil types and humus forms.

In this conceptual poster, we will discuss the possibility to use a battery of multilevel indicators to overcome the traditionally neglected indicators of soil quality and try to assess the environmental quality and monitor land degradation, according to a holistic point of view. Soils influence land cover of any terrestrial habitat. Humus forms are soils’ living part and associate soil types to their biota. Fungi are the most neglected kingdom in environmental assessments, despite their crucial role, both in plant pathology and in soil organic matter and nutrients cycling. Soil micro-arthropods are the easiest component of soil fauna to study, and provide useful insights on the above- and below-ground environmental quality levels. A thorough analysis of these components might be the right scenario to grant a robust and easy-to-apply assessment of land conditions, providing useful hints both for a sustainable soil use and for land management.

Keywords: soil biodiversity, humus forms, soil, vegetation, land degradation neutrality

Introduction, scope and main objectives

To achieve an effective monitoring of land degradation, the ideal approach should be a holistic point of view that incorporates the biosphere, society, and the economy, as well as their driving processes (Chasek *et al.*, 2019). This holistic point of view requires a multi-scale approach from landscape level (i.e., vegetation, society, economics flows …) to a microscopic level (i.e., presence or absence of some biota species, organic carbon content …).

Soil connects the landscape level to the microscopic level, considering that soil is itself a living organism and a landscape unit (FAO, 2012).

Starting from the landscape level, the land cover and land use change (e.g., EEA, 2017) are the base layers for any spatial analysis useful to monitor the effects of their dynamics. The landscape patterns should afford to reckon the connectivity between the habitat and community levels.

Soils bond the biotic and abiotic elements of the landscape, and their evaluation should include at least the depth, the AWC, the soil texture and soil pH (FAO, 2008). These base parameters influence directly the plant community, the vigor of vegetation and the land cover (Tsegaye and Hill, 1998).

Humus, the living part of the soils, is the link between biosphere and geosphere. The main changes in the organic carbon from vegetation to stable organic carbon occur in the humus layers. To evaluate humus quality, it is important to record the presence or absence of O horizons and their type (Of or Oh), and an accurate characterization of the A horizon (OC content, pH, presence of enchytraeids) (Ponge 2003; Ponge 2013; Zanella *et al.* 2011; Jabiol *et al.* 2013).

Most humus literature is focused on enzyme activities, organic carbon cycling and other metabolic parameters, however research on humus forms distribution and relationships with the environment is incomplete.

The extant knowledge is limited also for other disciplines, namely the achievement of a complete check-list of the macromycetes, which still is a work in progress, as well as for the humus, where there is a detailed literature on its specific components, but a thorough assessment and knowledge of relationships with the other components of soil is still debated.

To deal with these shortfalls, in this poster we are proposing to use a battery of multilevel indicators, qualified to assess the environmental quality of terrestrial environments.

Methodology

In our first attempt to formulate a new methodology, we assembled the above mentioned core parameters to ideate a complex index which might be easy to use and easy to reproduce.

The vegetation is the visible biota of the landscape pattern, therefore it is fundamental in itself and as main driver of soil microbiology (Millard and Singh, 2010; Prober *et al.* 2015). Therefore, it should be evaluated in its quantity, quality and functionality. The knowledge of the species distributions, and their changes over the years, is an indispensable tool for the conservation and protection of biodiversity.

The knowledge of fungal biodiversity (particularly, macromycetes and myxomycetes) assumes a primary importance, as research carried out in various parts of Europe (Arnolds, 1991; Jakucs, 1988; Fellner and Peskova, 1995) revealed that forest degradation is related with a reduction of the macromycetic flora, in particular of the symbiotic (mycorrhizal) species. The community composition of each terrestrial ecosystem, either natural or recently planted through restoration or re-naturalization plans, is subject to a delicate balance. Monitoring mycological species allows to assess the ecosystem state and infer on its health or deterioration. Soil microarthropods influence the composition of soil microbiota, chewing, pruning and disseminating spores and hyphae (Moore, St. John and Coleman, 1985; Lussenhop, 1992; Kuťáková *et al.,* 2018). Themselves are sensitive, and that's why have been used in monitoring land quality, to several causes of disturbance, including heavy metals, pesticides, soil erosion and loss of organic matter (Parisi *et al.* 2005; Menta *et al.* 2018).

Under the persistence of perturbation conditions, that cause deterioration phenomena, plants progressively lose their ability to select the most efficient fungal symbionts, allowing their replacement with other species, more suitable for the changed environmental conditions. At the same time, the declining mycorrhizal community leaves the way to the most aggressive pathogenic fungi (primary parasites).

Thanks to the normal presence, even in stable ecosystems, of pioneer and non-specific ectomycorrhizal fungi, with a partially saprophytic value, a primary and rapid colonization of young natural root systems or plants resulting from environmental restoration, can be followed by a succession of other ectomycorrhizas. They gradually are replaced by ectomycorrhizas more specific and more suitable both to the changing needs of the plant, and to the modified pedological context that changes simultaneously and consequently to the aging of the vegetable consortium.

The studies conducted by Fellner and Soukup (1991) and Schlechte (1991) affirm that a percentage of mycorrhizal species (compared to the total of macromycetes) between 40 percent and 60 percent indicates a good state of health of the ecosystem investigated; percentages between 20 and 40 percent imply an acute disturbance phase, while values below 15-20 percent express a lethal disturbance phase.

Discussion and conclusions

The assessment of land degradation through a complex integration of soil, humus forms, vegetation, fungi and soil microarthropods is approached in an innovative and still unvalued manner. Although preliminary in our conclusions, this innovative approach would like to show that with simple and easy-to assess parameters and indicators it could be provided a holistic and cheap methodology to assess land degradation on a wide scale and with a good resolution.

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Effect of different inoculants in growth of *solanum tuberosum* and the control of *rhizoctonia solani*

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Abstract summary

Growth-promoting bacterial microorganisms (PGPR) and phytopathogenic antagonists part of soil biodiversity applied as biological fungicides is one of the solutions to improve agricultural productivity and recover soil biodiversity progressively, so the United Nations declared "International Year of Health Vegetable (AISV)" to 2020, motivating our research objective to apply isolated rhizosphere biocontrollers to control *Rhizoctonia solani*. At the greenhouse level, coinoculation tests were carried out with different bacteria in botanical seed of potato cultivation, in a completely randomized design of 14 treatments and 5 repetitions. At the field level, a trial with 2 potato varieties (*Unica* and *Canchan*), 5 treatments and 4 repetitions in a block design was installed. In the greenhouse there were significant differences in the parameters evaluated, with the strain 4BPP8 + Azo16M2 being the one that produced the greatest weight of small tubers and the strain 5ACPP5 + Azo16M2 which most reduced the damage in the plant (chancre), compared to the positive control. In the field, the emergency percentage was different according to the varieties of the crop used, but the different strains improved this parameter.

Keywords: Soil biodiversity, PGPR, phytopathogenic antagonists, Solanum tuberosum

Introduction, scope and main objectives

The soil is one of nature's most complex ecosystems: it contains countless organisms that interact and contribute to the global cycles that make life possible (FAO, 2015), many of these bacterial microorganisms are growth promoters (PGPR) and phytopathogens antagonists increasing their use as mixed inoculants, by combining mechanisms between different microorganisms. These mixed inoculants provide excellent results and show great potential to be used more and more by farmers *(*Hungria *et al.,* 2015*)*. In summary, some studies of mixed inoculants are those that combine microorganisms whose main processes are; phytohormones production, phosphate solubilization or biological control, and if these microorganisms cannot be combined into a single product, they are manufactured separately *(*Santos *et al.,* 2019*)* whose preventive application is not only one of the solutions for agricultural productivity sustainable but also to progressively recover degraded soil biodiversity *(*Pratush *et al.,* 2018*)*. Alternatively, biological approaches are considered the most effective, ecological and low-cost methods for remediation. And as this concern increases internationally, the United Nations has declared “International Year of Plant Health - AISV” to 2020 (FAO, 2020). The objective of this research was to evaluate co-inoculation of different isolated rhizosphere PGPRs with *Azotobacter* strain in the growth of plant and decrease of damage (caused by *R. solani*) under greenhouse conditions. In addition, in field conditions the effect of inoculation in the emergence of two varieties of *Solanum tuberosum* var. *Unica* and *Canchan* was evaluate.

Methodology

This test was carried out in a greenhouse with climatic conditions: $T°_{min.}$15°C – $T°_{max.}$21°C, HR_{min}58 percent - HR_{max} 80 percent. Botanical seeds (previously disinfected according to *Zúñiga, 2012*) of potato *S. tuberosum cultivate Yungay x Ccompis (*Osorio, 2009), were sown in sterile germination trays of 2.7 cm3 with sterile substrate (organic matter and sand in 1: 1 ratio), a pH 7.32, EC 1.27 (dS/m), 4.07 percent organic matter and free sand texture. At 30 days each seedling was transplanted into sterile pots of 1.5 L capacity. The experimental design was completely randomized with 14 treatments and 5 repetitions, inoculating a PGPR *Azotobacter* sp. (Azo16M2) of the LEMYB-UNALM strain bank in combination with each of the 11 strains; Chemical treatment (Benomyl), positive control (*R. solani)* and negative control (without inoculation) were considered. 1 mL of each bacterial strain [10^8 CFU / mL] was applied to each seedling, the first inoculation was performed in the transplant, and the re-inoculation at 20 and 30 days directly to the plant neck. Infection with the fungus *R. Solani* was performed at 25 and 40 days. Making measurements of plant height, chlorophyll, length of chancre, fresh and dry weight of the small tuber at 90 days.

Subsequently, a field trial was installed in the department of Junín. Its climatic conditions were: $T°_{min.}$7°C - $T°_{max.}$21°C, HR_{min} 58 percent - $HR_{max.}$66 percent and precipitation $(PP)_{min.}$91-$PP_{max.}$102 (mm/month). Prebasic seeds of two potato varieties, var. Unica and Canchan (Torres *et al.,* 2011). The soil characteristics were: pH 7.55, EC 0.25 (dS/m), organic matter 2.62 percent and clay loam texture. The experimental design was completely randomized blocks with factorial arrangement of 2 varieties, 5 treatments and 4 repetitions, inoculating the following strains 4BPP8, BPP4, MRPJ4 and 5ACPP5 (Verástegui *et al.,* 2019) each at a concentration of 10^8 CFU/mL plus a control treatment, inoculation and re-inoculation was performed at sowing and 15 days after the emergency to the plant neck and leaves *(*Contreras *et al., 2017*). The first emergency percentage data were evaluated. In both trials the data were processed in the STATGRAPHICS-Centurion program.

Results

At the greenhouse level, significant differences were observed between treatments. The coinoculated plants with the different bacteria presented better results than the control with pathogen and without pathogen (Figure1A). The 4BPP8–Azo16M2 interaction was the one that gave the highest plant height, highest chlorophyll content and highest small tuber weight, compared to controls (Table.1). It was also observed that all bacteria decreased the damage to the neck of the plant (smaller chancre size) compared to the pathogen control (Table .1). At the field level (Figure 1B), there were no differences in the emergency percentage of var. Unique among all treatments, however in var. Canchan the effect was variable according to the inoculated strains (Figure 2).

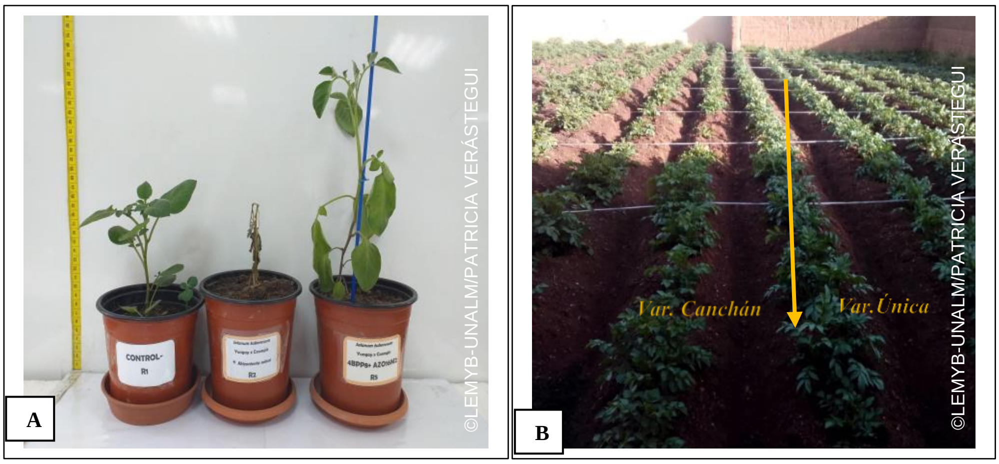

Figure 1: A) Effect of coinoculation on plant growth (control -, control +, and 4BPP8 + Azo16M2); B) Field of cultivation *S. tuberosum* var

***Unique* and *Canchan* in the Village Center of Vicso-Junín.**

Table 1: Effect of the best inoculants on growth and damage reduction in potato plants

Treatments	**Chlorophyll (nm)**		**Plant Height (cm)**		**Chancre (mm)**		**Small tuber Fresh Weight (g)**		**Small tuber Dry Weight (g)**	
4BPP8+AZO16M2	48.92*	a	23.26*	a	30.17	efg	0.71*	a	0.09*	a
BPP4+AZO16M2	43.54	ab	21.44	ab	37.10	def	0.61	abc	0.077	abc
5ACPP5+ AZO16M2	48.73	a	19.04	abc	28.67*	fg	0.68	ab	0.086	ab
Benomyl	42.16	ab	18.21	abc	18.79	g	0.25	abcde	0.032	abcde
Negative control	37.38	bc	14.10	cd	0.00	h	0.20	de	0.025	de
Positive Control	35.38	bc	15.20	cd	71.36	a	0.05	e	0.006	e

*** Means in the same column followed by the same letter did not significantly differ according to the LSD test ($p<0.05$).**

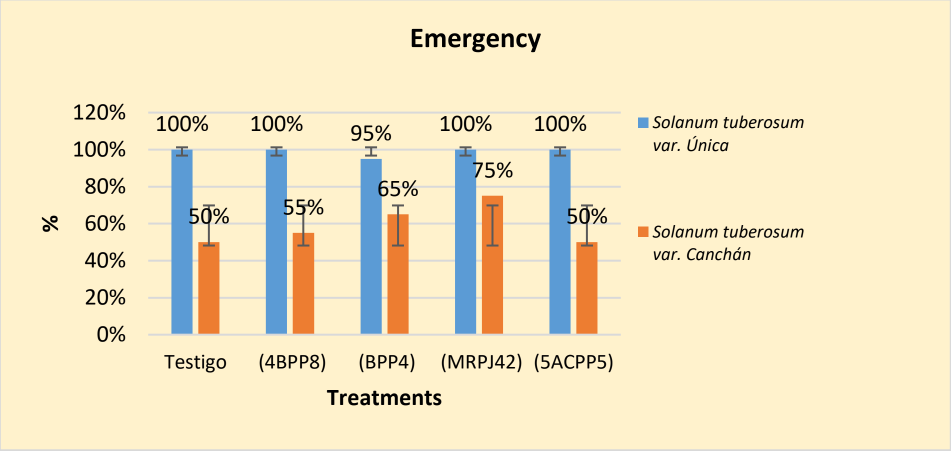

Figure 2: Effect of inoculation in the emergence of potato plants var. Unique and Canchan

Discussion

Under greenhouse conditions, the three inoculated treatments showed significant differences in tuber production, over control +, control - and chemical treatment with Benomyl, the same effect on plant height and chlorophyll index was also observed where the 4BPP8+Azo16M2 treatment had the highest values. Regarding the damage caused by *R. solani* in the plant, the 5ACPP5+Azo16M2 treatment reduced the size of the chancre from 71.36 mm (positive control) to 28.67 mm, the Benomyl product reduced it to 18.79 mm. Santos *et al.* (2019) mentions that if these microorganisms cannot be combined into a single product, they are grown separately, since not always a coinoculation can give better results, this would depend, possibly on some mechanism of antagonistic action or resistance induced according to *(*Oswald *et al., 2010)*.

In field conditions, the first result is 30 days after sowing where the emergency percentage in the *Unica variety* was 100 percent in all treatments except for the strain (BPP4) that was 95 percent; however, in the *Canchan variety*, 75 percent emergency was found when it was inoculated with the strain (MRPJ42) and 65 and 55 percent with the BPP4 and 4BPP8 strains respectively, surpassing the control that was only 25 percent (Figure 2). The specific effect of bacteria is dependent on each genotype of potato plants *(*Contreras-Liza *et al.*, 2019).

Conclusions

At the greenhouse level, the plants inoculated with the different strains had higher values in the growth parameters studied, including the dry weight of small tubers, also, the strains decreased the damage (chancre) in the plant caused by *R. solani*. At the field level, the strains improved the emergence of the plants depending on the variety.

It is important to carry out more field and farmer tests to validate these biotechnological tools, to be applied within a sustainable crop management, which would mean lower cost in chemical fertilizers and fungicides and improve the production of healthy and environmentally friendly foods.

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Rhizosphere ecological interactions for improved plant health and nutrition in sustainable agricultural production

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Abstract summary

Microbial-root associations are important for a plant's successful establishment in an environment as they support nutrient cycling, nutrient uptake and defense from abiotic and biotic stressors. Managing these interactions offers an opportunity for improving the efficiency and sustainability of agricultural production, reducing the inputs of agrochemicals. Strawberry cropping systems rely heavily on soil fumigants for the reduction of potentially devastating soil-borne pathogens. Restrictions in pesticide use have fostered robust breeding efforts aimed at improving strawberry cultivar tolerance to soil-borne fungal pathogens. Several cultivars exist that are tolerant to some of the major diseases, yet the resistance mechanisms are still unknown. We assessed the relationship between the structure of the rhizosphere prokaryotic community of strawberry cultivars and plant tolerance to two soil-borne pathogens. We characterized the prokaryote community from the bulk and rhizosphere soil of ten strawberry cultivars infected with and displaying varying degrees of tolerance to the two pathogens under field conditions. Differences between the prokaryotic structure of the strawberry rhizosphere and the adjacent soil showed a distinctive strawberry rhizosphere microbiome. In addition, significant differences in prokaryotic communities between cultivars were found, which were associated to the resistance to the soil borne pathogens and other aboveground plant traits such as biomass and nutrient uptake.

Keywords: sustainable agriculture, microbiome, prokaryotes, strawberry, soil-borne pathogens, Verticilium dahliae, Macrophomina phaseolina

Introduction, scope and main objectives

Because of its role in plant health, the rhizosphere is probably one of the most critical interfaces for plant productivity (Berendsen, Pieterse and Bakker, 2012); yet understanding the complexity of the rhizosphere microbiome, both from a taxonomic and ecological perspective, remains a challenge and the practical application of ecologically based agricultural management strategies is still not feasible (Sergaki *et al.*, 2018). Development of such practices is particularly important for crops that rely in large inputs of harmful agrochemicals. An example of such crops are strawberries (*Fragaria x ananassa*) a fruit crop highly appreciated throughout the world and therefore of major economic importance (Food and Agriculture Organization of the United Nations (FAO), 2018; Simpson, 2018), where high yields were traditionally achieved through heavy applications of pesticides to prevent the incidence of soil-borne diseases. Current restrictions in pesticide use have shift the interest of the industry towards integrated strategies combining cultural techniques with the use of resistant cultivars. To date, strawberry cultivars have been identified that are tolerant to soilborne pathogens like *Verticillium dahliae* and *Fusarium oxysporum* (Shaw *et al.*, 2010; Shaw, Gubler and Hansen, 1997). Tolerance against these soil-borne pathogens seems to be achieved through the expression of multiple genes, some of them involved in the production of defence substances in the roots with known antifungal activity such as catechin or caffeic acid, and the release of citric acid (Antanaviciute *et al.*, 2015; Besbes, Habegger and Schwab, 2019). Thus, resistance to soil borne pathogens in strawberries likely takes place through an interplay between the plant and soil microorganisms. Yet, to date no information is available on the possible role of the rhizosphere microbiome in strawberry plant health.

The objective of this study was to evaluate the relationships between the rhizosphere microbiome and strawberry plant traits such as resistance against soil-borne pathogens, plant growth and nutrition, under field conditions, relevant to commercial production.

Methodology

Two replicated field trials were established on the California Polytechnic State University campus in San Luis Obispo, California (USA) to evaluate 90 strawberry cultivars and elite selections for resistance to the soilborne fungal pathogens *Macrophomina phaseolina* and *Verticillium dahliae*. Within each field, beds measuring 1.6 m wide and 0.3 m high, were established. Beds were split into 90 randomly distributed plots (one per cultivar). Each plot had 20 plants of the same cultivar. A total of 4 beds (replicates) were established resulting in a total of 360 plots (90 cultivars x 4 replicates). Differences in pathogen history between the two field sites prompted distinct methods for ensuring host infection. While *V. dahliae* was naturally present in the soil in one trial, in the *M. phaseolina* trial the soil was chemically fumigated and then inoculated with the fungus. Bare-root strawberry seedlings were transplanted in Fall 2016 and grown under standard fertigation practices.

At harvest, we assessed plant mortality and we selected the 5 most resistant and 5 most susceptible cultivars within each field trial for further study. Plant, soil and rhizosphere samples were collected in July 2017. Four plants were randomly selected from each plot while avoiding dead or dying plants. Plants were consolidated by plot and fresh biomass was recorded in the field. Subsamples from each plot were then collected for the determination of aboveground plant biomass and leaf nutrient contents. Root samples were collected using 5 cm diameter cores centered around the strawberry crown to a depth of approximately 10 cm. A bulk soil sample (without the presence of roots) from each plot was also collected from the center of each strawberry bed. Rhizosphere soil was collected by dry sieving of the roots on sterilized 500µm sieves. Samples were lyophilized and stored at -80°C prior to DNA extraction.

To study prokaryote (bacteria and archaea) diversity in the soil and rhizosphere samples, DNA was first extracted using the DNeasy PowerSoil Kit (QIAGEN, Venlo, Netherlands). The V4 region of the 16S rRNA was subsequently sequenced using bacterial and archaeal primers using the Illumina MiSeq platform.

Leaf tissue N content was analysed through dry combustion using a vario Max CNS Macro Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Concentrations of phosphorus, potassium, calcium, magnesium, and sodium in leaf tissue were analysed on an Ultima 2 ICP-OES (Horiba Jobin Yvon S.A.S., Logjumeau, France).

For comparisons in the structure of the microbial community across samples, we generated resemblance matrices for the OTU tables using Bray-Curtis similarity of the standardized and square root transformed data. Interpretations of multivariate distances were conducted using NMDS plots generated from the resemblance matrices. ANOSIM was used to determine significance levels between groups. The taxa contributing to the dissimilarities between groups were assessed through SIMPER analysis at the phylum level. Furthermore, the differences in the relative abundance of a subset of well-known fungal antagonists in the soil and rhizosphere samples were evaluated using ANOVA. Distance-based redundancy analysis (db-RDA) was used to investigate relationships between the rhizosphere prokaryote community and other plant traits. All data was analysed in PRIMER v7 (Quest Research Limited, Auckland, NZ) and JMP Pro v14.0 statistical software (SAS Institute Cary, NC, USA).

Results

Analysis of the two field trials used in this study showed significant differences in their microbial community structure and diversity, in spite of being relatively close to each other and having the same soil type. Interestingly, the rhizosphere microbial community structure and diversity index of the strawberry plants across the two field trials was more similar to each other than to their respective bulk soils.

A closer look at the composition of the rhizosphere microbiome of the strawberry plants revealed a higher relative abundance of well-known beneficial microorganisms and fungal antagonists such as Rhizobium, Pseudomonads and Streptomyces as compared to bulk soils (Figure 1).

Comparisons among the 10 cultivars selected in each field trial, showed a significant effect of the plant cultivar in the structure of the rhizosphere microbiome. Distance-based redundancy analysis (db-RDA) showed significant correlations between the rhizosphere microbial community structure, and aboveground traits such as shoot biomass and leaf tissue nutrient content, although this depended on the trial. In the *M. phaseolina* trial, the rhizosphere prokaryote community was not explained by the leaf nutrient contents aboveground biomass or plant mortality. In the *V. dahliae* trial, changes in rhizosphere prokaryote community were explained by the leaf N contents in percentage, above ground dry mass, and leaf Ca:Mg ratio. Further analysis of the rhizosphere microbial community showed significant differences between strawberry cultivars with different levels of disease resistance.

Taxa found in higher abundance in the cultivars resistant to *V. dahliae* included, *Burkholderia*, *Sphingomonas and Novosphingonium* which have been found to suppress this fungal pathogen in previous studies (Inderbitzin *et al.*, 2018; Snelders *et al.*, 2020). Additionally, higher abundances of unclassified Acidobacteria (Gp6, Gp16 and Gp4) and Actinobacteria (*Arthrobacter, Nocardioides* and *Gaiella*) were found in resistant strawberry cultivars. In the *M. phaseolina* trial, cultivars with high resistance were characterized by greater abundances of *Pseudomonas*, actinobacteria (*Nonomuraea*, *Arthrobacter, Streptomyces, Nocardioides* and *Gaiella*), which are well-known to be fungal antagonists and plant growth promoters (Cha *et al.*, 2016; Inderbitzin *et al.*, 2018; Mendes, Garbeva and Raaijmakers, 2013). Additionally, the rhizosphere of resistant plants was also characterized by higher abundances of unclassified Acidobacteria (Gp6, Gp16 and Gp4).

Discussion

Modern strawberry cultivars show varying degrees of resistance to soil borne pathogens, which could be related to their capacity to recruit beneficial microorganisms to their rhizosphere. In this study we show that field-grown strawberry cultivars have a distinct microbial community in their rhizosphere that is more similar to the rhizosphere of other strawberry plants in different environments than to the bulk soil where they live. This indicates that the strawberry plants exert a strong selective pressure on the microbial community around their roots, and the possible existence of a core rhizosphere microbiome shared by all strawberry cultivars. This core rhizosphere microbial community shows a higher abundance of bacteria and archaea that are well known to promote plant growth or are antagonist to fungal pathogens, confirming that the strawberry plants have the capacity to recruit beneficial microorganisms from their environment. These differences in microbial community structure were associated to the capacity of the plants to take up nutrients and respond to biotic stress. The structure of the rhizosphere microbial community seemed to be plant-genotype dependent, as shown by the differences between cultivars. This means that the plant-driven selection of soil microorganisms could be a heritable trait that could be introduced into breeding programs.

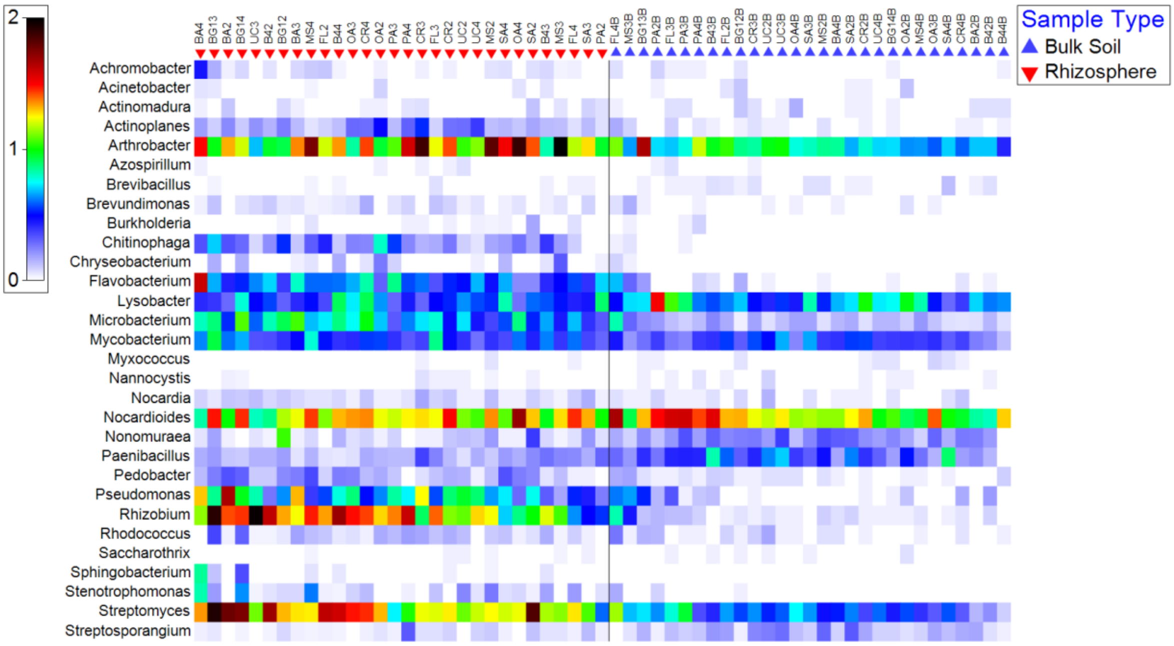

Figure 1: Heat map showing the relative abundance of fungal antagonist and other beneficial prokaryotes in the rhizosphere and bulk soil of the strawberry cultivars grown in the presence of the fungal pathogen *Verticilium dahliae*

Conclusions

Strawberry plants can recruit microorganisms to their rhizosphere from the pool of microbes available in the soil, increasing the relative abundance of beneficial microorganisms involved in nutrient uptake and resistance to soil-borne diseases. Selection of rhizosphere microorganisms is strongly associated to the different genotypes and therefore seems to be a genetic trait that could be selected through breeding; nevertheless, the microbiome selected by a specific cultivar depends largely on the environmental conditions and microbiome of the surrounding soil. Thus, this suggests that soil health may be important in the establishment of these beneficial plant-microbial interactions.

Acknowledgements

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Construction of a *nif*- mutant strain from a psychrotolerant soil bacterium to evaluate the contribution of the biological nitrogen fixation in crop plants

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Abstract summary

Pseudomonas syringae GR12-2 is a nitrogen-fixing bacterium that promotes growth of plants of agricultural interest, such as canola (*Brassica campestres*), presenting potential application as a biofertilizer. Nitrogen-fixing microbial associations are efficient and well characterized in legumes but are limited in non-leguminous plants. The combined capabilities of nitrogen fixation, root colonization, and plant growth-promotion may be useful for the development of an inoculant for temperate and cold regions to reduce input of nitrogen fertilizers on the agricultural crop. In this work, a mutant strain of GR12-2 was constructed with a precise marlerless deletion of the *nifHDK* genes encoding nitrogenase. The construction of mutants strains has been an important strategy to understand the function of genes in different organisms.

Keywords: biological nitrogen fixation, diazotrophs, genes nif, non-legumes, inoculant

Introduction, scope and main objectives

Nitrogen is an essential element for life, and low levels of nitrogen in soils reduces crop yields. Since the Green Revolution, massive amounts of synthetic nitrogen fertilizers have been produced from atmospheric nitrogen and natural gas, threatening the sustainability of global food production and degrading the environment. There is a need for alternative means of bringing nitrogen to crops, and taking greater advantage of biological nitrogen fixation seems a logical option (Pankievicz *et al.,* 2019). Diazotrophic bacteria have the ability to develop different types of root associations with different plant species. The best studied symbiotic interaction between diazotrophic bacteria and plants are those that involve legumes and nitrogen- fixing bacteria of *Rhizobium* genera (Oldroyd, 2013).

The associative and endophytic diazotrophic bacteria in non-legumes naturally colonize and contribute with fixed N to several economically important plant species, comprising a natural system to be better explored (Carvalho *et al.,* 2014).

The rhizobacterium *P. syringae* GR12-2, isolated from the roots of an Arctic grass, is a strong candidate for development as a soil inoculant to enhance crop yields (Lifshitz *et al.,* 1986). Inoculation of canola, tomato, and other agriculturally important plants with this strain results in substantial promotion of seedling root growth (St-Arnaud *et al.,* 1995); Hall *et al.,* 1996).

Some endophytic diazotrophs, such as *Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans,* and *Burkholderia silvatlantica*, can fix nitrogen in the vasculature and intercellular spaces of sugarcane stems (Lery *et al.,* 2011; Dong *et al.*, 1994). Diazotrophs, including *Herbaspirillum* species, living in mucilage released from the aerial roots of maize landraces from Sierra Mixe, Mexico, can provide up to 82 percent of the host nitrogen (Van Deynze *et al.,* 2018). There is no evidence, yet that nitrogen reduced by *P. syringae* GR12-2 in the rhizosphere of inoculated plants is transferred to host plants.

Our objective was to construct a mutant of *P. syringae* GR12-2 with a precise markerless deletion of *nifHDK* genes encoding the nitrogenase enzyme complex to evaluate the contribution and benefit of biological nitrogen fixation in crops of agricultural importance.

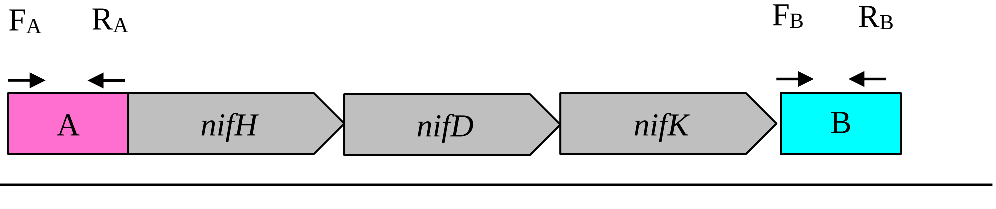
Methodology

Bacterial strains and growth conditions

All strains were routinely cultured in Luria–Bertani (LB) broth, at either 30 °C for *P. syringae* GR12-2 or 37 °C for *E. coli.*

Cloning and construction of expression and deletion vectors

Design primers to amplify regions flanking nif genes



Regions A (650 bp) and B (550 bp) flanking the *nifHDK* genes to be deleted were amplified separately using primers:

FA CTTCGTACTAGTAGGGAACGATATTGCCGCTC

RA CAGCGACGACAATAAAAGTCCTCGGTTTCGTTC

FB GACTTTTATTGTCGTCGCTGTCCGGTATGT

RB CTTCGTCTCGACGCAGCTGACGCTCGTTAATG

The reverse primer R_A for the A fragment and the forward primer F_B for the B fragment contained overlapping sequences of 20 nucleotides. That is, add 10 nucleotides (blue above) that are the reverse complement of the F_B primer (underlined sequence in the F_B primer) at the 5' end of the R_A primer, and 10 nucleotides (red above) that are the reverse complement of the R_A primer (underlined sequence in the R_A primer) at the 5' end of the F_B primer. Forward primer F_A has a recognition site for the restriction enzyme SpeI and reverse primer R_B has a recognition site XhoI. PCR was performed using a thermal cycler programmed to execute in the fellow cyling conditions: 98°C 5 min; 35 cycles of 98°C 30 s, appropriate °C 1 min, 72°C 1 min; 72 °C 10 min. To ligation reaction, the vector pJQ200SK with SpeI and XhoI were used to digest AB fragments.

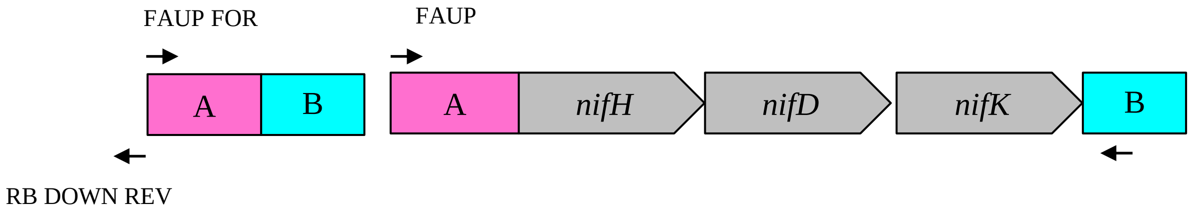
Generating the deletion mutant

Constructed deletion vector pJQ200SK-nifAB carries a gene for gentamicin resistance and *sacB* for counterselection on sucrose. The deletion vector was transformed into wild-type *P. syringae* GR12-2 by electroporation (1.8 kV pulse) and transformants that had integrated the vector into the chromosome by recombination were selected on LB agar containing gentamicin at 5 µg/mL. Isolated transformants were grown for 24 h in LB broth and then plated on LB agar containing 10 percent sucrose to select for loss of *nifHDK* by a second recombination event. Loss of the plasmid was confirmed by sensitivity to gentamicin, and deletion of the *nifHDK* genes was confirmed by PCR using primers UP and DOWN sequencing: CACTGGTGAAAGGGTGAGGT A and CCTTGGGCGAAATCTGATAG, respectively. To confirm the loss of nitrogen fixation ability can be used acetylene reduction assay (ARA) a sensitive and accurate method of assessing nitrogenase activity, via the indirect measure of reduction from acetylene to ethylene by nitrogenase.

Result

Integration of a vector pJQ200SK-nifAB at sequence B flanking the *nifHDK* genes in the *P. syringae* GR12-2 genome was confirmed by PCR using the external primers UP FOR / DOWN REV. The PCR reaction to confirm vector integration yielded products of 5.5 kb and 1.2 kb as expected.

Strategy used to confirm vector integration



10.0 kb

WT M.M C1 C2 C3 C4 C5 C6 C7 C8

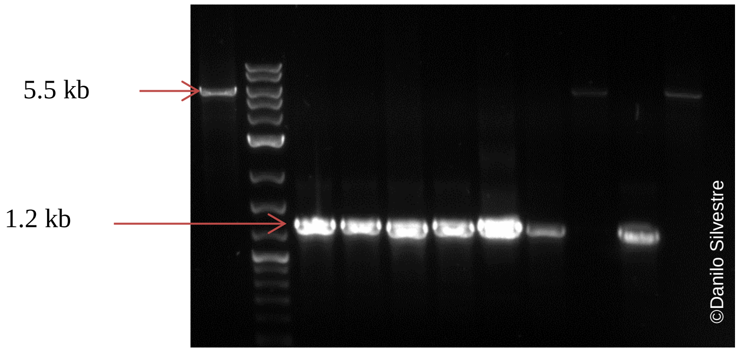
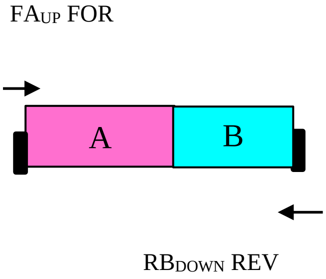


Figure 120: Electrophoresis profile at 0.8 % agarose gel in 1X TAE of the PCR product for confirmation of pJQ200DSK-nifAB integrants of *P. syringae* GR12-2. MM: molecular marker; WT (wild-type strain): transconjugant colonies of strain GR12-2 containing (1.2 kb) copies of the *nifHDK* gene

Primers used: FA$_{UP}$ FOR / RB$_{DOWN}$ REV.

Strategy used to confirm deletion mutants

Colonies capable of growing in medium containing 10 percent sucrose and which were sensitive to the antibiotic gentamicin, were subjected to PCR reaction using the primers FA$_{UP}$ FOR / RB$_{DOWN}$ REV to confirm the deletion of the target region of the gene.



Deletion of *nifHDK* by a second homologous recombination event was confirmed by the presence of a 1.2 kb PCR product (Figure 2).

Two colonies of the deletion mutant strain were verified by sequencing the amplification products. Confirmation of double mutants containing *nifHDK* deleted of *P. syringae* GR12-2 in the genoma.

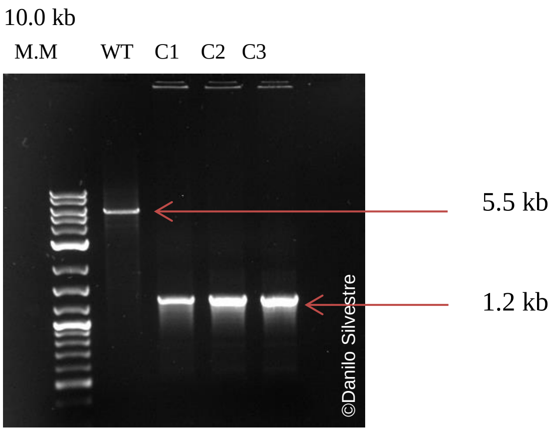


Figure 2: Electrophoresis profile at 0.8% agarose gel in 1X TAE from PCR product to confirm deletion of *nifHDK* in the *P. syringae* GR12-2 genome

MM: molecular marker; C1, C2 and C3: deletion mutants.

Discussion

A study showed that *A. diazotrophicus* wild-type strains actively fixed N_2 inside sugarcane plants, whereas the Nif- mutants did not. In N-deficient conditions, sugarcane plants inoculated with *A. diazotrophicus* PAl5 generally grew better and had a higher total N content 60 days after planting than did plants inoculated with mutante MAd3A or uninoculated plants. These results indicate that the transfer of fixed N from *A. diazotrophicus* to sugarcane might be a significant mechanism for plant growth promotionin this association (Sevilla *et al.,* 2001)

Mutants in bacterial hormone biosynthesis and production have been described, and their use is helping to better understand the role of phytohormones during association. In *Azospirillum* sp., IAA (indole-3-acetic acid) might be synthesized by at least two biosynthetic pathways; therefore, mutants that completely lack IAA production could not be generated (Steenhoudt and Vanderleyden, 2000).

Transcriptome analysis of *A. brasilense* mutant in the *ipdC* gene, that encodes an indole-3-pyruvate decarboxylase involved in IAA biosynthesis, revealed broad transcriptional changes in the mutant, suggesting that IAA production can have a role on bacterial physiology and that it can possibly act as an important signalling molecule in this association (Van Puyvelde *et al.,* 2011).

With this construction of this *nif*-mutants will be possible to study whether there is a contribution from biological nitrogen fixation and N transfer in non-legumes plant during association, in addition to evaluate the nitrogen metabolism in the hosted plant.

Conclusions

Improving and understanding nitrogen fixation in non-leguminous crops has been a dream of the agronomic community for more than a century. The global challenges that our world is facing make the realization of this dream urgent. Fortunately, natural diversity holds solutions that the scientific community overlooked possibly because of the intense focus on legume nodules. Technological developments such as the advent of next-generation sequencing, gene editing, and synthetic biology allow the dissection and manipulation of plants and microbes at an unprecedented scale. We are confident that combining the prospecting of plant and bacterial natural diversity with genetic engineering will deliver solutions in the short and long terms and will help to feed the world in a more sustainable manner.

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Effect of different species of mycorrhizal fungi on growth and physiological characteristics of sorghum (*Sorghum bicolor* L.) in cadmium contaminated soil

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Abstract summary

Among the different micro-organism which could change the soil biodiversity, arbuscular mycorrhizal fungi (AMF) have a key role. These fungi not only affect the growth of plants by symbiosis, but also could improve the soil properties. In order to study the effects of AMF species on growth and physiological traits of sorghum an experiment was conducted in controlled condition of greenhouse. Experimental treatments were two levels of cadmium (0 and 40 mg.kg $^{-1}$ soil) and four levels of AMF application (F0: without fungi, F1: application of 20 g *Glomus mosseae*, F2: application of 20 g *Glomus intraradices*, F3: application of 10 g *Glomus mosseae*+10 g *Glomus intraradices*). Soil contamination with cadmium negatively affected all of the traits of sorghum plant. The lowest amount of shoot and root dry weight measured in Cd contaminated soil. Application of both species of mycorrhiza improved the produced biomass, however simultaneous application of two mycorrhiza fungi species had more positive effect rather than mono-inoculation and these results were more pronounced under cadmium stress conditions. AMF application both in the presence and absence of cadmium significantly increased the phosphorus uptake. Different AMF treatments also has positive effect on photosynthetic pigments and relative water content of leaf.

Keywords: fungi species, Glomus, soil microorganism, symbiosis

Introduction, scope and main objectives

The positive effects of soil-born arbuscular mycorrhizal fungi (AMF) on different aspects of plants growth are well documented. The symbiosis of plant roots with different fungi and especially AMFs could improve the plant growth and yield under the different environmental stresses such as drought, salinity and soil pollution (Rostami and Rostami, 2019). The key role of AMF is to improve uptake and translocation of water and different plant nutrients such as phosphorus and nitrogen (Smith and Read, 2008). In addition to above mentioned benefits, AMF can increase plant tolerance to both biotic and abiotic stresses (Augé, Toler and Saxton., 2015).

AMF are among the most abundant micro-organisms in the rhizosphere which are able to start symbiosis relation with the roots of about 90 percent of terrestrial plant species (Ahanger *et al.,* 2014), however considering the species diversity, the AMF has low species diversity and about 240 species of these fungi have been recorded (Krüger *et al.,* 2012). The high concentration of different heavy metals in the soil not only reduces crop growth (Rostami, Karamian and Joulaei., 2015), but also has negative effects on human health (Rostami *et al.,* 2020). Therefor the aim of current experiment was to study the effect of mono-inoculation and co-inoculation of two AMF species on growth and physiological traits of sorghum in the cadmium contaminated soil.

Methodology

In order to study the effect of two AMF species on sorghum plant under cadmium stress, the current factorial experiment was conducted based on completely randomized design with three replications in controlled condition of greenhouse. Experimental treatments were two levels of cadmium (0 and 40 mg.kg $^{-1}$ soil) and four levels of AMF application (**F0**: without fungi, **F1**: application of 20 g Glomus mosseae, **F2**: application of 20 g Glomus intraradices, **F3**: application of 10 g Glomus mosseae+10 g Glomus intraradices). The soil was collected from a filed in Malayer city, Iran. After air drying, the soil was passed through a 4 mm sieve and in order to delete the native microorganisms, soil was sterilized for one hour at 120 °C. The pots were filled with 2 Kg soil and 10 seeds were planted in each pot. After emerging, the weak seedlings were discarded and only 5 plants were kept in each pot. In order to avoid drought stress the pots were irrigated daily with adequate amounts of water. During the experiment using the destructive sampling method physiological traits of plant was determined. Chlorophyll pigments and catalase activity were measured using Lichtenthaler (1987) and Aebi (1984) methods. In order to measure produced biomass, all parts of plants in each pot were harvested 80 days after emergence. The obtained data were analysed using Minitab 16 software and in order to compare the mean values at $P < 0.05$ level, Duncan’s Multiple Range Test (DMRT) was used.

Results

Soil contamination with cadmium negatively affected all of the studied traits of sorghum plant. The lowest amount of shoots and roots dry weight measured in Cd contaminated soil. Application of both species of mycorrhiza improved the produced biomass; however simultaneous application of two mycorrhiza fungi species had more positive effect than mono-inoculation. In uncontaminated soil even without application of AMF, the biomass of produced root significantly was higher than all of the Cd contaminated treatments. Soil contamination with cadmium resulted in decreasing the relative water content of leaf. Application AMF improved relative water content in both of cadmium treatments. In the presence and absence of cadmium application of AMF significantly increased amount of phosphorus in leaf.

Regarding the catalase activity, in cadmium free treatment there was no significant different among the AMF treatments, but in cadmium polluted treatments in the absence of AMF (F0) catalase activity significantly was higher than other treatments. In cadmium free treatments there was no significant different between the treatments regarding the photosynthetic pigments but, in cadmium polluted treatments application of AMFs improved both chlorophyll a and chlorophyll b, however the highest effect was related to simultaneous application of AMF species (F1+F2).

Table 1: Interaction of cadmium and arbuscular mycorrhizal fungi on different traits of sorghum

Treatments		**Shoot dry weight**	**Root dry weight**	**Relative water content**	**Leaf phosphorus content**	**Catalase activity**	**Chlorophyll a**	**Chlorophyll b**
Cadmium	**AMF**	g. pot^{-1}	g. pot^{-1}	%	%	U mg^{-1}	mg. g $^{-1}$ FW	mg. g $^{-1}$ FW
40 mg.kg^{-1}	**F0**	4.77 c	1.76 e	71.1 d	0.225 d	0.52 a	1.002 d	0.51 c
	F1	5.32 c	2.13 de	75.3 cd	0.283 bc	0.30 c	1.11 cd	0.56 abc
	F2	5.24 c	2.17 cd	77 c	0.312 bc	0.36 b	1.07 cd	0.54 bc
	F1+F2	6.41 b	2.55 bc	79.5 bc	0.322 b	0.28 c	1.16 bc	0.59 ab
0 mg.kg^{-1}	**F0**	6.58 ab	2.80 ab	79.7 bc	0.274 c	0.11 d	1.29 a	0.61 a
	F1	7.06 ab	3.07 a	83.9 ab	0.424 a	0.11 d	1.26 ab	0.58 ab
	F2	7.02 ab	2.99 a	85.5 a	0.413 a	0.13 d	1.34 a	0.59 ab
	F1+F2	7.09 a	3.11 a	84.6 ab	0.422 a	0.12 d	1.33 a	0.61 a
CV (%)		8.3	6.4	5.9	4.1	1.83	3.2	2.7

Discussion

Cadmium stress negatively affected the growth parameters of sorghum in this study. These results are in accordance with previous findings (Pirdosti, Movahedi and Rostami, 2018; Nourbakhsh Rezaei *et al.*, 2019). The results of current study showed the positive effect of *Glomus mosseae* and *Glomus intraradices* on growth and physiological traits of sorghum which are in agreement with other studies. Based on our results co-inoculation of two species of AMF resulted in highest positive effects on plant growth and physiological characteristics, however the observed effects of co-inoculation (i.e. F3 treatment) significantly was higher under cadmium stress condition. Results of the previous study also showed that when the plant was under environmental stress, inoculation with mixture of AMF species belonging to different families was more efficient than mono-inoculation (Crossay *et al.*, 2019).

It seems that co-existence of different species of AMF under cadmium stress condition by increasing the root growth and consequently improving nutrient and water uptake enhanced shoot growth. In the current study, considering the plant growth, there was not any different between two species of AMF. Results of Bharti *et al.* (2013) also showed the greater similarity and effectiveness of *Glomus mosseae* and *Glomus intraradices* in improving productivity and biochemical traits of mint (*Mentha arvensis*). However, our results are in contrast with the previous studies which found that effect of AMF inoculant species on the same host species is different (van der Heijden *et al.*, 1998).

Conclusions

Soil contamination with cadmium had negative effects on all of the studied traits. It seems that cadmium by affecting different physiological traits negatively changed the produced biomass of root and shoot. Application of AMF improved the different studied characteristics of sorghum however the relative effect of AMF in contaminated soil was higher. Simultaneous application of both species of mycorrhiza significantly affected all of the traits whereas, under cadmium stress condition mono-inoculation with *Glomus mosseae* or *Glomus intraradices* had less positive effect on plant growth, therefor in heavy metal contaminated soils, application of different species of AMF to enhance the plant growth and performance is recommendable.

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Perceptions on soil macrofauna in the agricultural field

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Abstract summary

In this study we have evaluated social perceptions on soil macrofauna among farmers and other stakeholders working in an agricultural context in Brazil. During the National No-Tillage Meetings of 2008 and 2018, we have interviewed a total of 171 people (87 in 2008 and 84 in 2018), where 35 percent were farmers (33 percent in 2008, and 36 percent in 2018). A questionnaire with 12 questions was used to determine the profile of interviewees, their soil management practices and perceptions regarding soil macrofauna. From a list of nine soil organisms only earthworms, spiders and centipedes, generally, were not considered pests. When asked about pest incidence, 61-73 percent of the interviewees noticed an increase, mostly related to the excessive use of pesticides (25-33 percent) and monocultures (38-55 percent) for both years. When asked about soil health indicators, more than 80 percent mentioned the presence of a large number of taxa, even including some that were considered pests earlier. The results showed a significant increase in pest incidence after 10 years, together with a decreasing trend in prevalence of good practices. This is profoundly worrisome and highlights the urgent need to foster capacity building and to stimulate more effort in dissemination of information about the importance and function of soil biodiversity, and their vast benefits to society.

Keywords: Bioindicators, soil quality, soil macrofauna

Introduction, scope and main objectives

Soil is the habitat for several organism and holds one quarter of the world biodiversity, considered by many to function as a living organism (Harshberger, 1911, Decaëns *et al.*, 2006). The soil fauna can be divided into well-defined groups according to body size: microfauna, mesofauna and macrofauna, each providing a unique contribute to soil functioning, in particular to the food web (Swift, Heal and Anderson, 1979, Lavelle, 1996). The activity of these organisms is tightly associated with the set of ecosystem services provided, not only by directly impacting nutrient cycling, organic matter break-down, the soil structure and water retention, but also their unique role on soil trophic webs (Lavelle, 1997). The feedbacks between soil management and the functioning of soil biota are profoundly important to promote appropriate conservation measure and stimulate a sustainable agriculture (Wolters, 2001).

Many organisms of the soil fauna are bioindicators of the environment quality and their presence/absence is directly related with how the environment and the soil are managed by man (Bianchi, Aquino and Almeida 2007; Santos *et al.,* 2019). Farmers and who work directly with soil have considered some organisms as beneficial to soil and know hey assist in the agriculture productivity (Schiedeck *et al.,* 2009). Considering these associations several studies have been focusing on understanding how human action affects soil fauna and whether this information, in particular related to preservation and conservation, is disseminated to the entire society (Pulleman *et al.,* 2005; Lima *et al.,* 2016). Moreover, in assessments that aim people’s perception on soil fauna, it is important to emphasize that the main idea is not evaluate people’s knowledge, but to understand their points of view in relation to the subject (Bruyn and Abbey, 2003).

The aim of this study was evaluated the social perceptions concerning soil macrofauna among farmers and other stakeholders working in an agricultural context mainly in Brazil.

Methodology

A questionnaire composed by a set of 12 questions as used (including professional activity, geographical and educational background, but also size of explored area). We have included questions about the management practices (major crops produced, livestock, soil management and crop waste residues – straw), soil macrofauna perceptions (organisms considered pests, trends in pest incidence, causes of the pest increase, but also focused on which organisms are considered beneficial to the soil and which management practices suggest increase in soil biological activity) and how the people assess the health of the soil. The questionnaire had multiple-choice questions, and most of them with an open-ended question to complement the answers and the interviewees could choose more than one answer.

The first survey was conducted in 2008 during the 11th National Meeting of No-Tillage on the Straw, held in Londrina, Paraná, Brazil, and the second was conducted in 2018 at the 16th National Meeting of No-Tillage on the Straw, held in Sorriso, Mato Grosso, Brazil. The questionnaire was included in the meeting bag and participants were free to answer.

Results

In 2008, the National No-Tillage on the Straw Meeting had 600 participants and 87 answered the questionnaire, and in 2018 had 570 participants and 84 answered, about 20 percent of the participants in both events, totalling 171 answered questionnaires.

Table 1: Profile of the interviewees

Question		**Responses (%)**	
		2008	**2018**
		n=87	**n=84**
Education	Agronomist	61	50
	Other formation	11	13

	Agricultural technician	7	20
	Collage	11	14
	High School	7	8
	Middle School	2	1
Profession[1]	Farmer	33	31
	Researcher	14	11
	Professor	2	8
	Autonomous/Consultant	4	11
	Extensionist	9	0
	Technical Assistance	20	14
	Other	18	24
Country of origin	Brazil	93	100
	Paraguay	7	0
Region of origin in Brazil[2]	North	1	10
	Northeast	0	4
	Midwest	20	78
	Southeast	19	8
	South	60	0
Size of the management area[3]	< 20 ha	13	8
	21 to 50 ha	9	0
	51 to 100 ha	5	4
	101 to 500 ha	28	10
	501 to 1000 ha	6	10
	1001 to 2000 ha	3	7
	> 2001 ha	36	61

[1] Various interviewees answered more than one option; [2] n=78 for both years; n=64 in 2008 and n=71 in 2018.

In 2008, 33 percent of the interviewees were farmers, 20 percent technical assistance. In 2008, 36 percent of the interviewees managed an area larger than 2001 ha and 21 percent areas 101 to 500 ha. In 2018, farmers were the larger proportion of interviewees (31 percent). Regarding the size of the exploration area, 51 percent had managed areas larger than 2000 ha (Table 1).

From the list of nine organisms (Oligochaeta – earthworms, Hemiptera – stinkbugs, Formicidae – ants, Diplopoda – centipedes, Araneae – spiders, Isoptera – termites, Coleoptera – beetles, Chilopoda – millipedes and Gastropoda – slugs), only earthworms, spiders and centipedes were, generally not considered pests (Table 2). When asked if they observed an increase in pest incidence, 61 percent of the interviewees noticed an increase in 2008 and 73 percent in 2018. This increase was related mostly to the excessive use of pesticides (25 percent) and monocultures (38 percent) for both years, though the number of people relating these practices increased in 2018 (31 percent and 52 percent, respectively). When asked what kind of management was used for pest control, the chemical, mechanical and fallow practices were the most cited in both years, but the fallow decreased almost the half and other options, like biological and alternative managements and the Integrated Pest Management (IMP) increased from 0 to 3 percent in 2008 to 17 percent to 32 percent in 2018 (Table 2).

Table 2: Responses about organisms considered to be pests, their control and management practices

Question	Responses (%)

		2008	2018
		n=87	n=84
Organisms considered pests	Oligochaeta	1	2
	Hemiptera	52	62
	Formicidae	41	33
	Diplopoda	33	14
	Araneae	2	7
	Isoptera	55	38
	Coleoptera	62	43
	Chilopoda	6	8
	Gastropoda	37	38
	Others	17	20
Increase in plagues/pests	Sim	61	73
	No	39	27
Reason for the increase of plagues/pests	Pesticides	25	31
	Monocultures	38	52
	Pest/plague resistance	21	13
	Other	16	3
Management used for pest control	Chemical[1]	40	49
	Biological[2]	0	17
	Mechanical[3]	33	39
	Alternatives[4]	3	17
	IPM[5]	3	32
	Fallow	94	48
	Nothing	3	4

[1] Use of traditional pesticides; [2] Use of viruses, bacteria, parasites, etc.; [3] plowing, harrowing, etc.; [4] Homeopathy, herbal medicine, etc.; [5] Integrated pest management.

Most respondents considered earthworms (93-100 percent) and spiders (45-64 percent), to be beneficial animals. The management practices considered to enhance soil biodiversity were mainly green manures, crop rotation, integrated pest management and the use of no-tillage (all >65 percent in 2008), although the number of responses including these practices decreased slightly 10 years later. In 2008 <40 percent of the respondents considered that maintaining native vegetation fragments was important to improve soil biodiversity, but in 2018 only 29 percent considered this option. When asked about soil health indicators, >80 percent mentioned the presence of many organisms (although most animals had been considered by many respondents to be pests earlier), while roughly half mentioned the presence of increased number of earthworms and soil aggregation.

Table 3: Responses on soil macrofauna as beneficial organisms, good management practices and soil health

Question		**Responses (%)**	
		2008	**2018**
		n=87	**n=84**
Organisms considered beneficial	Oligochaeta	93	100
	Hemiptera	7	7
	Formicidae	22	23
	Diplopoda	18	23
	Araneae	64	45
	Isoptera	10	15
	Coleoptera	26	18

	Chilopoda	17	13
	Gastropoda	5	10
	Others	7	5
Management to favor soil biodiversity	Green manure	90	77
	Crop rotation	91	89
	Native forest fragments	40	29
	IPM	15	65
	Terracing	22	19
	Subsoilong[1]	5	6
	No-Tillage System	90	82
	Minimum tillage	11	17
	Other	3	0
Destination of the straw on soil	Left on the soil	93	88
	Burned	2	0
	Incorporated in soil	7	11
	Animal feeding	6	7
	Silage	1	4
	Other	3	1
How assess soil health	Many organisms	80	85
	Many earthworms	51	45
	Soil color	20	37
	Texture	15	45
	Soil aggregation	49	46
	Plants as indicator	21	0
	Other	22	0

[1] Mechanical practice using equipment (subsoiler or rippers) to break up soil compacted layers (30 to 50 cm depth).

Discussion

The social perception, especially from farmers and other stakeholders working in agriculture, concerning the functions, importance and benefits promoted by soil macrofauna, is still lacking. Generally, farmers recognize that organisms are capable of modify soil structure, the dynamic of organic matter and nutrients and balance of the food web, but few are aware about how these activities can assist water infiltration, aeration, improve soil fertility and plant growth, reflecting directly the soil health.

A review was conducted by Pauli *et al.* (2016) on the studies performed and the knowledge of farmers regarding the use of soil macrofauna. Across continents, the authors observed that most of the studies are focused mainly in one taxonomic group and this inclination happens according to the location and the importance or how strong negative impacts were observed (Pauli *et al.*, 2016).

In Brazil, throughout the different regions, is possible to observe some knowledge regarding the benefits of the macrofauna for the soil, mostly for earthworms, as several farmers emphasize that a soil with earthworms is a healthy soil with better fertility and helping with crop production (Schiedeck *et al.*, 2009; Van Groenigen *et al.*, 2014; Schiavon *et al.*, 2015). In some regions, where the dissemination of information is more difficult and most farmers use agriculture for subsistence, there is almost nothing regarding the knowledge about soil macrofauna or how to classify a healthy soil (Lima *et al.,* 2016).

Talking directly to farmers in situ and enquiring about their actions towards a sudden increased amount of organisms (insects, bugs, millipedes, crickets, etc.) in their crop fields, they spontaneously answered: "I apply pesticides!" (personal observation M.L.C. Bartz). There is a major misunderstanding concerning the function and importance of the soil biodiversity that embodies the perceptions of farmers, technical workers and other professionals linked to agriculture. We suggest that these patterns are associated with market and consumer perceptions, especially shaped by the ones that sell products for farming, and that are not well prepared to work with a biodiverse environment. Moreover, in Brazil and probably elsewhere, there is a profound gap between the academic community with those that directly work in agriculture.

Conclusions

In this study, the fact that most soil macrofauna were not perceived as beneficial and that the number of responses showed an increase in pest, together a decreasing trend in the application of good practices after 10 years is worrisome, and highlights the need to foster capacity building and to stimulate dissemination of evidence regarding the importance and function of soil biodiversity to society.

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Evaluation of PGP bacteria against *rhizoctonia solani* in *solanum tuberosum* plants under greenhouse conditions

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Abstract summary

The soil harbours growth promoting microorganisms, in recent years interest in its use has increased, because they have beneficial effects on plants and are biocontrol agents of phytopathogenic fungi. These microorganisms can reduce the use of chemical fungicides, promoting their use without negative impact on the environment, promoting the objective of this research that was to evaluate PGPR bacteria against *Rhizoctonia solani*, isolated from the rhizosphere and phylosphere of *Solanum tuberosum* from five regions of Peru such as *Bacillus* spp, *Pseudomona* spp and *Actinomycetes* spp, the bacteria were inoculated into sexual potato seeds using the completely randomized design with 14 treatments and 5 repetitions in greenhouse conditions. It turns out that in the production of small tuber there were significant differences, the strain 4BPP8 being better; in the plant stem the strain BPP4 further reduced the chancre caused by *R. solani*. These strains could be used at the field level to control plant pathogens and reduce the risk of contamination in soils by not using chemical fungicides. Further studies are recommended so that this biotechnology can be used in agriculture within a sustainable management.

Keywords: Soil, inoculation, antagonistic strains, Solanum tuberosum

Introduction, scope and main objectives

The soil is an essential resource and a vital part of the natural environment in which most of the world's food is produced (FAO, 2015), where the great diversity of microorganisms that are part of the soil are the invisible engines of fertility, since The growth of plants depends on a set of functions performed by the same microorganisms that inhabit the roots, because when secreting polysaccharides they adhere to soil particles avoiding erosion while regulating the hormonal balance of plants helping them cope with abiotic stressors and protecting them against a variety of insect pests, parasites and other pathogens. (Saleem, Hu and Jousset, 2019).

That is why at present, the use of bacterial soil microorganisms arises as new alternatives, taking advantage of their ability to synthesize bioactive compounds that have beneficial effects on plants, having among the main microorganisms with this activity *Bacillus* spp., Pseudomonas spp., *Actinomycetes* spp., etc. (Barahona, 2012; Quiñones-Aguilar, Evangelista-Martínez and Rincón-Enríquez, 2016; Méndez-Úbeda, Flores and Páramo, 2017). Thus, the specific advantages offered by these bacterial groups are to promote the optimal growth and development of plants and their antagonistic capacity against to phytopathogenic fungi (Martínez, 2019). Driving the objective of this research was to evaluate the inoculation of 11 antagonistic strains against *R. solani* in sexual seeds of potato *cultivate Yungay x Ccompis* using the completely randomized design with 14 treatments and 5 repetitions in greenhouse conditions.

Methodology

Bacterial strains of rhizosphere and phylosphere of *S. tuberosum* were isolated from different regions of Peru (Puno, Cajamarca, Huancavelica, Huánuco and Lima). Table 1 shows sampling areas, coordinates and soil characteristics. The test was carried out in greenhouse conditions of the LEMYB-UNALM, with the 10 isolated antagonist strains, the BAC15MB strain of (Calvo, *et al.* 2010), chemical fungicide (Benomyl), negative control (Control -) and *R. solani* (Control +).

Table 1: Characteristics of the rhizosphere and phylosphere sampling regions of *S. tuberosum*

N°	**Department**	**Province**	**Type of sample**	**Altitude (msnm)**	**Latitude**	**Length**	**pH**	**Texture**	**Structure**	**Strain**
1	Huancavelica	Acobamba	Phylosphere	4185	12°40'37,2''	74°41'1.69''	4.8	Clay loam	Granular	BPP4
2	Lima	Cañete	Phylosphere	70	-13084815	-76354898	7	Loamy	Granular subangular	4BPP6
3	Lima	Cañete	Phylosphere	113	-12.974502	-76.403824	6.8	Clay loam	Granular subangular	4BPP8
4	Cajamarca	Cajamarca	Rhizosphere	3642	7°2'2.36''	78°19'46.21''	4	Loamy	Migajosa	3PSPP8
5	Huancavelica	Tayacaja	Phylosphere	3949	12°14'30.78''	75°3'31.67''	4.7	Loamy	Granular	ACPP02
6	Huancavelica	Acobamba	Phylosphere	4185	12°40'37.2'	74°41'1.69''	4.8	Clay loam	Granular	ACPP03
7	Huánuco	Pachitea	Phylosphere	3155	9°59'42.71''	76°1'27.12''	4.8	Clay loam	Granular	2ACPP04
8	Huánuco	Yarowilca	Rhizosphere	3657	9°53'19.23''	76°29'55.02''	5.7	Clay loam	Granular	2ACPP08
9	Puno	Azángaro	Rhizosphere	4224	14°39'0.4''	70°9'30.04''	4.6	Loamy	Granular	5ACPP5
10	Puno	Azángaro	Rhizosphere	4224	14°39'0.4''	70°9'30.04''	5.6	Loamy	Granular	5ACPP6

The experimental design was completely randomized of 14 treatments with 5 repetitions. Potato sexual seeds *cultivate Yungay x Ccompis* obtained by the Agricultural Crops Research Center (CICA-Huancayo) were used. The climatic conditions of temperature varied between 15°C and 21°C, the environmental relative humidity between 58 percent and 80 percent.

The seeds were sown at 0.5 cm deep in 2.7 cm3 germination trays, after 25 to 30 days an emergency seedling was obtained with 4 true leaves (Figure 1A), transplanting 3 seedlings per bag 3 L volume. The different strains were immediately inoculated with 1 ml [$1x10^8$ CFU/mL] directly to the neck of the seedling, the control + inoculated with wheat grains infected with mycelia of *R. solani* (Figure 1B). At 30 days after the transplant the treatment strains were reinoculated and 7 days later it was inoculated with wheat grains infected with mycelium of *R. solani* directly to the neck of the plant. The following parameters were evaluating at harvest (42-45 days): fresh and dry weight of the small tuber, size of chancre, height of plant, index of chlorophyll and the data were processed with the STATGRAPHICS Centurion program.

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Figure 1: A) Seedlings in germination trays, B) Seedlings inoculated with *R. solani*, C) Damage (chancre) in the plant neck with *R. solani* D) Effect of strain 4BPP8 on the number of tubers compared to control + and control -

Results

Table 2 shows three treatments inoculated with different PGPR strains that had better results in the plant, compared to the positive and negative controls, showing the effects on small tubers production (fresh and dry weight), chancre size, height plant, chlorophyll index, finding significant differences between treatments.

Table 2: Effect of three PGPR strains on the growth of potato plants, under greenhouse conditions

Treatments	Fresh tuber weight (g)		Dry tuber weight (g)		Chancre (mm)		Plant height (cm)		Chlorophyll (nm)	
4BPP8	11.906	a	1.5081	a	39.93	cd	28.9	a	33.68	bc
BPP4	8.716	b	1.1040	b	19.90	ab	29.6	a	35.94	abc
5ACPP5	8.212	bc	1.0402	bc	25.10	ab	22.88	bcd	35.3	abc
BENOMYL	7.624	bcd	0.9657	bcd	16.26	a	24.56	bc	41.22	abc
CONTROL+	4.898	fgh	0.6204	fgh	55.50	efgh	19.64	cde	37.5	abc
CONTROL -	5.878	cdefgh	0.7445	cdefgh	0	-	23.32	bcd	45.08	a

*** The means were compared using the LSD test p <0.05.**

Discussion

The three strains had better fresh and dry weights of small tubers than the positive and negative control, highlighting strain 4BPP8 with 11.9 g of fresh weight and 1.5 g of dry weight of small tubers (Figure 1D). different bacteria have great stability to be a good biofertilizer and biofungicide for that; it is very important to know this diversity of the non-pathogenic microorganism-plant relationship in the rhizosphere. (Calvo and Zúñiga, 2010). In chancre size (Figure 1C), the BPP4 strain was the one that most reduced this damage from 55.5 to 19.9 mm, which does not differ significantly from the chemical fungicide Benomyl (16.26 mm), the other two strains also reduced the damage such as 5ACPP5 at 25 mm and the 4BPP8 at 39 mm. In agreement with Sagredo-Beltran*, et al.* (2018) and Yamunarani, Sundaram and Pandiyan (2019)'s work, bacterial isolates with pathogen inhibitory activity that establish friendly interactions with host plants of the same crops could offer more possibilities of controlling phytopathogens. Finally, only the first two strains shown in Table 1 were significantly higher than the positive and negative controls, in plant height; as mentioned in Liza, Laura and Dávila (2019), the specific effect of inoculation depends on the genotype of *S. tuberosum*.

Conclusions

This research demonstrates that the PGPs strains studied may potentially be applied for control *R. solani* compared to other commercial options (chemical fertilizers or fungicides). In addition to improving plant growth, they could restore soils damaged by pesticides and chemical fungicides. Field level studies are recommended using biotechnologically the resources of microbial diversity to improve food quality with sustainable agricultural management.

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Soil biodiversity management for food security

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Abstract summary

A significant proportion of Life on Earth is hosted in soils, where soil organisms co-exist in a hierarchical food web and interact in complex ways. Soil communities are key for soil processes and functioning, and influence ecosystem services. Knowledge on conservation and sustainable use of soil biodiversity is therefore crucial to improve food security. We present here some results of our recent review examining the role of soil biodiversity for ecosystem functioning and services that are essential to sustain food security (El Mujtar *et al.*, 2019). We also discuss the scientific evidence for the potential of soil biodiversity management to improve food security, aiming to contribute to and encourage the research agenda on emerging approaches for soil biodiversity management.

Keywords: Soil communities, soil biodiversity management, rhizosphere manipulation, agro-ecosystems

Introduction, scope and main objectives

Living soils are heterogeneous environments in which soil biota evolve. Soil organisms inhabit soils interacting in complex ways and over different soil compartments. These communities play essential roles in soil processes and impact ecosystem functioning. Living soils are therefore indispensable to sustain plant and animal/human life.

In the current context of increased food demand, concerns about human food security are high on the sustainable development agenda. Four interrelated pillars have been traditionally considered as the base of food security: "availability", "access", "utilization including nutrition & safety", and "stability"; while a fifth dimension "agency" has been proposed recently. Negative consequences of global change, inadequate management practices and high dependency on external inputs are important threats to the different pillars of food security. In the last decade the sustainable use of soil biodiversity (SBD) has been postulated as key to reinvent agricultural systems that are more resource-efficient, resilient and adaptive.

We present here a summary of the results of our review "*Role and management of soil biodiversity for food security and nutrition; where* *do we stand?”* (El Mujtar *et al.,* 2019). The main goals of the review were: (i) to synthesize current information on SBD and on their role on soil processes and ecosystem functioning and services, and (ii) to discuss the potential to improve food security through SBD management.

Methodology

Briefly, available literature on SBD was reviewed based on a systematic literature retrieval using the Scopus database and four search strategies (Table 1). Each article from the search output list obtained by following strategies 2, 3 and 4 was then assessed for relevance to the scientific research topics (by reading the abstract) and selected if it focused on them. Articles from all search strategies were classified according with research topics (Figure 1).

Table 1: Search strategies for Scopus systematic literature retrieval (modified from El Mujtar *et al.*, 2019)

Search strategy	Search terms [a]	Period
1	**Soil (T) AND biota OR microb* OR fauna OR “food webs” OR communities OR diversity OF enzyme (T,A,K)**	**unrestricted**
2	**“soil biodiversity” (T)**	**unrestricted**
3	**Productivity OR yield (T) AND bacteria OR fungi OR nematode OR protoz* OR mite* OR springtails OR *worm OR protist OR *arthropods OR rhizob* OR mycorrhiza * AND soil (T,A,K)**	**restricted 2012-2017**
	Productivity OR yield (T) AND microb* or micro* or *fauna AND NOT microalga* AND soil(T,A,K)	
4	**soil biodiversity AND wheat (T,A,K)**	**unrestricted**
	soil biodiversity AND corn (T,A,K)	
	soil biodiversity AND rice (T,A,K)	
	soil biodiversity AND tomato (T,A,K)	
	soil biodiversity AND banana(T,A,K)	
	soil biodiversity AND potato (T,A,K)	
	soil biodiversity AND chickpea (T,A,K)	
	soil biodiversity AND common bean (T,A,K)	
	soil biodiversity AND cowpea (T,A,K)	

[A] Search on T=title, A=abstract, K=key.

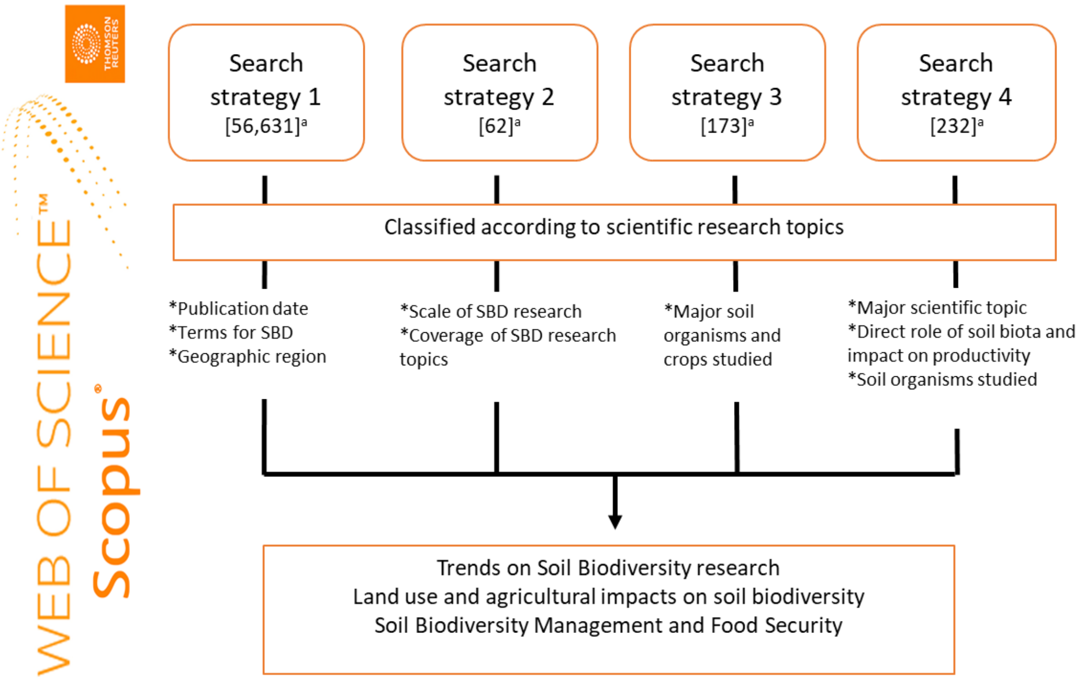


Figure 1: Summary of the research literature retrieval from Scopus database, indicating search strategy, classification criteria and scientific topics

^{a} Number of retrieved articles. modified from El Mujtar *et al.* (2019).

Results and discussion

Soil biodiversity, soil processes and ecosystem services

Soil biodiversity (Figure 2) comprises a huge number of organisms which can be generally classified in three functional groups: chemical engineers (CE), biological regulators (BR) and ecosystem engineers (EE). These organisms have specific spheres of influence accounting for the spatial and temporal variation in soil habitats, such as the rhizosphere (1) and the aggregatusphere (2). Soil environmental conditions modelling these groups are also specific in zones exposed to light (3), in aggregate surfaces and the water films between aggregates (4), inside aggregates (5) and within depth (6: soil profile). Several drivers (Figure 2) influence diversity and activity of soil organisms. These drivers can act directly on soil biodiversity (e.g. use of synthetic or organic fertilizers) or indirectly, since management, land use and climate change also induce or revert soil degradation. Global change impacts on SBD are mostly negative, and affect not just taxonomic diversity and species abundances, but also functional diversity, community composition, organisms' ratio (e.g. fungal/bacterial ratio) and their interactions. However, positive responses of soil organisms to agricultural management have been reported for organic farming (e.g. greater microbial biomass and enzyme activities, increased richness, better network interactions). Soil ecosystems services (Figure 2) depend on diversity and specific functions of CE, BR, and EE (Figure 3) and on biotic factors (aboveground diversity) and above/belowground interactions. Moreover, each functional group have main and minor roles in soil processes influencing differently soil ecosystem services (Figure 3).

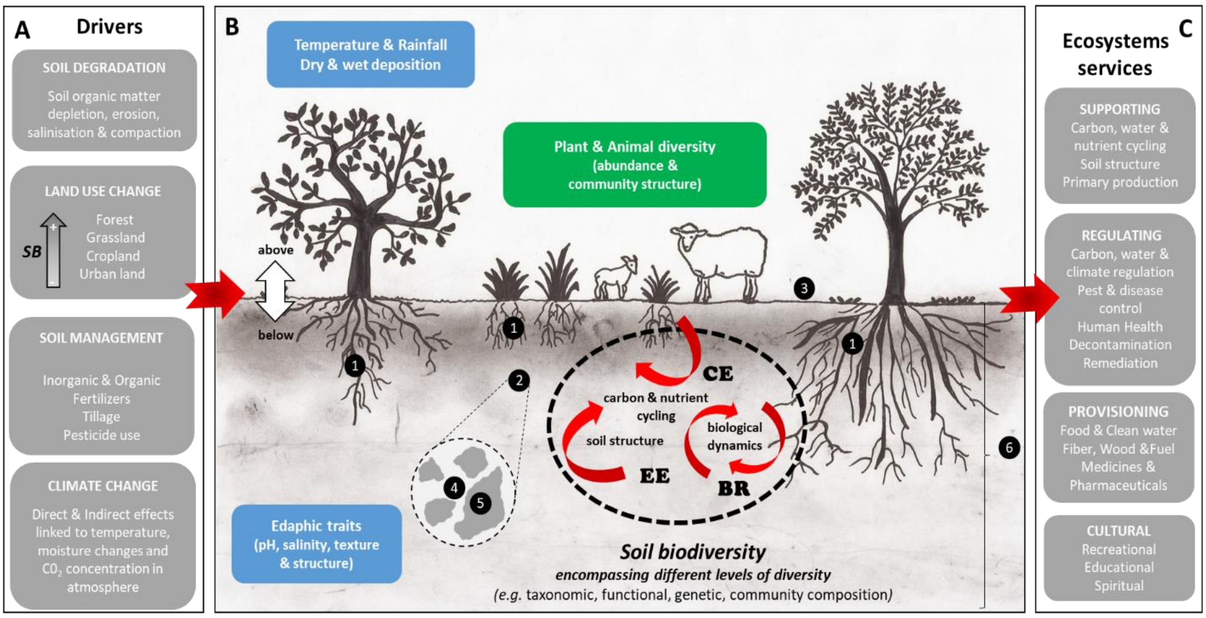


Figure 2: Soil biodiversity overview (from El Mujtar *et al.* 2019)

see text for abbreviations and number references.

Since 1920, scientific publications referring to SBD and the terms used, had an exponential increase (Figure 4) indicating a growing understanding of the complexity of SBD (*search strategy 1*). However, most studies (87 percent) were performed in the northern hemisphere, with barely 4 percent coming from Africa or South America. According to our review, up-to-date, soil biodiversity research: (i) is mostly focused either at the local or continental level, with a few articles referring to national or global levels, (ii) is mostly dealing with the effect of different disturbances on soil biota (50 percent) and on the relationship between SBD and soil functioning (38 percent), and (iii) has a knowledge gap on SBD in relation to agricultural productivity and food security (*search strategy 2*). Moreover, bacteria and fungi studies on agricultural productivity outnumber by far those on other soil organism groups (*search strategy 3*).

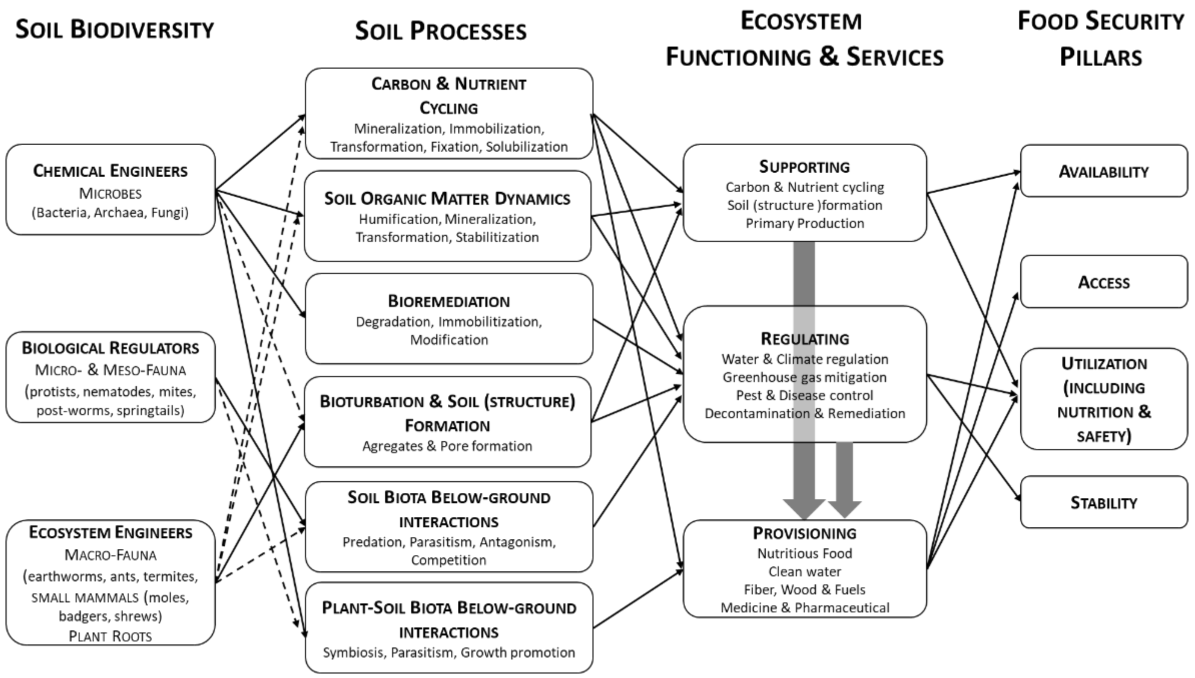
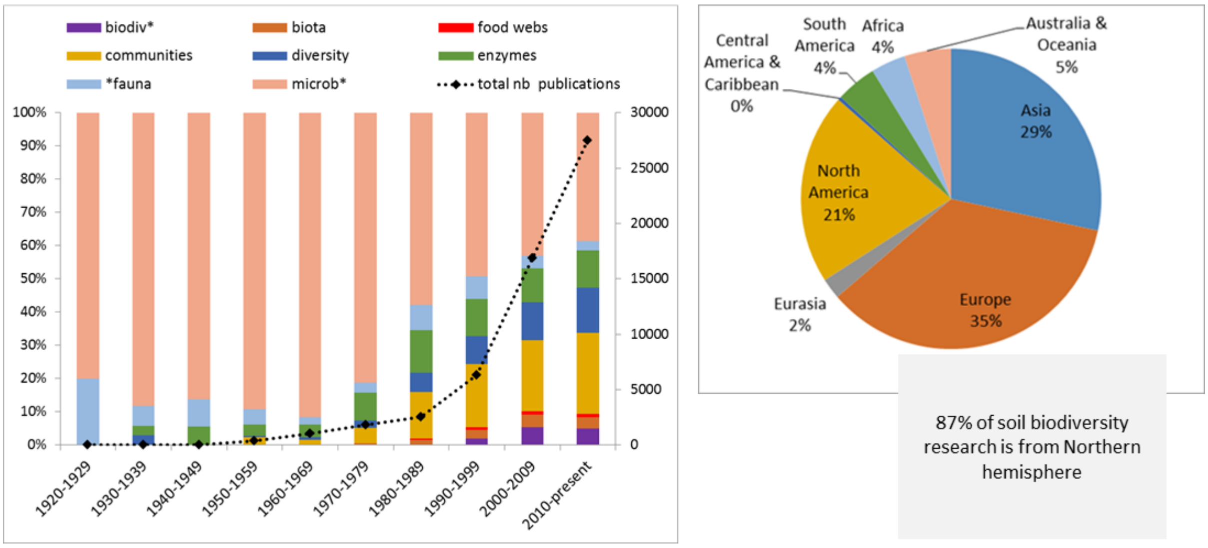


Figure 3: Relationships between soil biodiversity and food security pillars through soil processes and ecosystem functioning and services

Black arrows and black dashed arrows indicate, respectively, main and minor roles of functional groups on soil processes. Gray arrows indicate the relationships among supporting, regulating and provisioning ecosystem services. From El Mujtar *et al.* (2019).



87% of soil biodiversity research is from Northern hemisphere

Figure 4: Soil biodiversity research since 1920s, including number of publications and geographic distribution of research (modified from El Mujtar *et al.* 2019)

Research on SBD in the major crops (*search strategy 4*) shows that: (i) 50 percent of the studies are focused on the impact of land use and soil management, (ii) 25 percent on the impact of soil type, environmental conditions and plant genotype, and (iii) only 10 percent on the impact of plant trait (including growth stage/tissue). Regarding soil organisms, around 50 percent of total research was focused on bacteria, while soil fauna and fungi represented 22 percent and 17 percent respectively. This overview also shows that crop type and management practices affect SBD, and indicates that certain technologies (e.g. organic management, reduced tillage) can positively impact soil biodiversity.

These results globally evidence that SBD contributes through soil processes to ecosystem functioning and services. Although some SBD knowledge gaps remain, they also indicate that it could be possible to develop agricultural systems favouring the sustainable utilization of SBD while improving food production.

Soil biodiversity and food security

Soil biodiversity has direct and indirect impacts on agricultural productivity and plant response to stress, and therefore contributes to food availability and stability. Direct impacts occur through plant-soil interactions (Figure 3), particularly in the rhizosphere. These impacts are mostly due to bacteria, which represent 90-95 percent of the rhizosphere population. Plant growth promotion and biocontrol are the main mechanisms underlining direct impacts of SBD. Indirect impacts occur via soil processes in which soil biota are involved (Figure 3), influencing plant growth and resistance to climatic events. Soil biota also regulates fluxes of greenhouse gases, and could therefore be a key player for mitigation and adaptation to climate change. *Search strategy 4* revealed that only 15 percent of research focused on the impacts of SBD on plant productivity, but also shown that manipulation of rhizosphere community is increasingly considered as an alternative to improve food production or replace/reduce agrochemical inputs.

Different groups of bacteria and fungi inhabiting the rhizosphere, in particular arbuscular mycorrhizal fungi, have direct and indirect impact on net plant nutrient uptake and up-regulate the biosynthesis of secondary metabolites or enhance the activity of antioxidant enzymes. Arbuscular mycorrhizal fungi also mitigate contamination of food products with heavy metal, wastewater or pesticides. All these interactions have a role on plant growth and resistance to biotic and abiotic stress, improving not only agricultural productivity but also the nutritional value and safety of edible plant tissues. Rhizhosphere microbes therefore influence food availability, stability and utilization. Moreover, indirectly, biodiverse soils enhance the capacity of farmers to reduce their dependence on external inputs, thereby increasing their autonomy, which contributes to reinforcing the “agency” aspect of food security.

Rhizosphere microbes make therefore important contribution to almost all pillars of food security through their role on soil processes and ecosystem functioning and services. This knowledge can contribute to the development of well-informed approaches for soil biodiversity management.

Emerging approaches for soil biodiversity management

Despite some knowledge gaps it has been recently claimed that enough is known to develop sustainable agriculture through soil biodiversity management. The effects of soil biota and plant-soil interactions on plant productivity and food security is an active field of research, in particular the role of rhizosphere microbes in plant growth promotion and biocontrol. Our review revealed two main approaches to the application of SBD knowledge in agricultural practice, both of them focused on rhizosphere manipulation.

The *reductionist approach* is based on knowledge of SBD and soil-plant interactions and considers SBD as a pool of candidate organisms or genes to be tapped through biotechnology. Although tapping candidate organisms could be easy in practical terms, the approach is crop specific and mostly focused on chemical engineer’s impact on soil processes. Yet significant challenges lie ahead to up-scale results on specific pairwise interactions from the laboratory to complex communities in heterogeneous field conditions. Moreover, several concerns have been reported regarding long-term environmental consequences of several strategies for this approach (e.g. change on network interactions, increase of antibiotic resistance).

The *holistic approach* consists of managing soil ecosystems and their diversity in favour of multifunctionality, resilience and soil adaptive capacity against environmental changes, improving agricultural productivity, resource use efficiency and production stability through niche differentiation, functional complementarity and functional redundancy.

Although both approaches are not necessarily mutually exclusive, the second one emphasizes understanding soil communities-soil process relations through the analysis of their complexity. In practical terms, the holistic approach implies working more closely with producers to increase understanding and knowledge sharing about soil communities and soil biological processes through farmer-researcher networks or farmer-to-farmer training, thereby building capacity and autonomy among producers - a possible fifth pillar of food security.

Conclusions

The maintenance of high levels of functional diversity in the soil is closely related to the management of functional diversity above-ground and highly susceptible to changes in the soil environment; both are the direct or indirect consequence of land use and management decisions. For these reasons, soil biodiversity has to be understood, assessed and managed holistically, and research on specific traits should be informed and designed considering a multifunctional perspective and the potential trade-offs and synergies among the various soil and land functions.

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Arbuscular mycorrhizal fungi mitigate drought impacts and improve nutrient status of *Commiphora myrrha* seedlings

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Abstract summary

In dry ecosystems, tree and shrub seedling establishment, growth and survival are limited by access to moisture and nutrients. Arbuscular mycorrhizal fungi (AMF) increase seedling establishment and survival by enhancing nutrient and moisture acquisition. The mycorrhizal symbiosis operates in natural and agro-ecosystems by enhancing plant performance, while reducing the need for fertilizers. We executed a fully-factorial greenhouse experiment to determine the interactive effect of arbuscular mycorrhiza (AM, with and without), water deficit (four levels), and soil fertility (topsoil and subsoil) on the biomass, growth, nutrient availability, and root colonization of seedlings of *Commiphora myrrha*, a tree species that dominates large areas of dry forest in the Horn of Africa. AM increased the biomass of *C. myrrha* seedlings and the allocation of biomass to shoots of the seedlings. Mycorrhizal seedlings also had higher nutrient concentrations. Seedlings grew better in more nutrient-rich topsoil than in subsoil. Mycorrhizal colonization of roots of *C. myrrha* seedlings were higher with decreased moisture and higher in topsoil than in subsoil. The increased performance of mycorrhizal *C. myrrha* indicates that mycorrhization is a major component of the adaptive strategy of seedlings of this species, similar to other species in these dryland deciduous ecosystems.

Keywords: arbuscular mycorrhiza, dry deciduous woodland, water deficit, soil fertility

Introduction, scope and main objectives

In the dry tropics, soil conditions are not always optimal for seedling establishment, as they are generally low in moisture, nutrients, and microbial populations (Muthukumar and Udaiyan, 2006) so seedling establishment and natural regeneration are poor (Vieira and Scariot, 2006). AMF often improve tree seedling establishment, resulting in enhanced potential for restoration (Wubet *et al.*, 2003). Glasshouse experiments on the effects of drought, induced by withholding water (Gindaba *et al.*, 2005), on soils of differing fertility, may provide insights into how species respond to the interactive effects of water shortage and different nutrient availability. AMF increase drought tolerance and that these plants therefore can maintain higher growth rates (Bati *et al.,* 2015).

The effect of AMF on *C. myrrha* seedlings is poorly known. A better understanding of a species' mechanisms to withstand drought at the seedling stage would enable an appropriate method for regenerating valuable species such as *C. myrrha* in marginal arid lands. Therefore, a greenhouse experiment was conducted to evaluate the effect of AMF inoculation on soils of differing fertility and with different simulated drought levels on the growth of *C. myrrha* seedlings. We grew *C. myrrha* seedlings under greenhouse conditions in order to address the following research questions:

1. Do water availability (drought), AMF, and soil fertility affect the growth and biomass allocation of *C. myrrha* seedlings?
2. Do water availability (drought) and soil fertility affect the level of AM colonization of roots of *C. myrrha* seedlings?

The objective of the work was to analyse the growth and biomass response of *C. myrrha* seedlings to different levels of water deficit, mycorrhizal inoculation, and soil fertility. We quantified the effects of AM colonization, soil fertility, and moisture deficit on the growth and biomass of *C. myrrha* seedlings. We assessed the AMF colonization levels of *C. myrrha* seedlings under different levels of soil fertility and moisture. We hypothesized that: (1) mycorrhizal seedlings would grow more rapidly and achieve higher nutrient concentrations than non-mycorrhizal seedlings; (2) the beneficial effect of AMF would be stronger under conditions of drought or low nutrient availability; and, (3) AMF colonization of roots would increase with higher soil fertility and increased drought.

Methodology

A greenhouse experiment with *C. myrrha* seedlings was conducted in northern Ethiopia at Mekelle University's greenhouse, located at 13°29′N and 39°28′E. The mean daily temperature of the greenhouse was 27 °C during the day and 22 °C during the night, with a mean daily average relative humidity of 62 percent for the study period.

Seeds of *C. myrrha* were obtained from the Central Ethiopia Environment and Forest Research Center (CEE-FRC) in Addis Ababa, Ethiopia, and were treated prior to germination. Inoculum was collected during the dry season from the rhizosphere of *C. myrrha* trees. The fungal inoculum added to the experimental seedlings consisted of a mixture of soil, spores, and root fragments derived from both the rhizosphere and the roots of precolonized *Sorghum bicolor* plants. About 50 g of fungal inoculum was added near the roots of each seedling at the centre of the pot. We used a three-factorial experimental design for our study. The factors were arbuscular mycorrhiza (inoculated – AM+ and not inoculated – AM-), four water levels (field capacity = 100, 75 percent of field capacity = 75, 50 percent of field capacity = 50, 25 percent of field capacity = 25), and two soil fertility levels (topsoil and subsoil). The topsoil was excavated in the upper 15 cm depth and subsoil below 15 cm depth. The amount of water to compensate for daily loss was estimated from measurements of pot weight. The mass of water required daily was then supplied to each treatment. The treatment units were arranged on the greenhouse bench in a completely randomized block design. There were 6 replications, so in all there were 96 pots.

We determined plant size, mass, and growth rates as seedling traits. Leaf surface area was measured using an AM 100 Leaf area meter. Harvested seedlings were divided into roots, stems, and leaves, and their dry mass was determined after oven drying the samples at 80 °C until constant weight was achieved. Total root length was estimated using the grid line intersect method.The mineral status of the plants was obtained by performing a shoot and root tissue elemental analysis.

Mycorrhizal colonization was assessed for the presence or absence of arbuscules, vesicles, and hyphae, using the grid line intersection method (Giovannetti and Mosse, 1980). Mycorrhizal responsiveness (MR) and drought response index (DRI) were determined in accordance with (Osonubi *et al.*, 1991). Mycorrhizal dependency was expressed as the ratio of total dry weight of the mycorrhizal plant and non-mycorrhizal plant. The drought response index was calculated as the ratio of total dry weight of the mycorrhizal plant under conditions of water deficit to the mycorrhizal plant under well-watered conditions.

Results

AM increased the biomass of *C. myrrha* seedlings and the allocation of biomass to shoots of the seedlings.

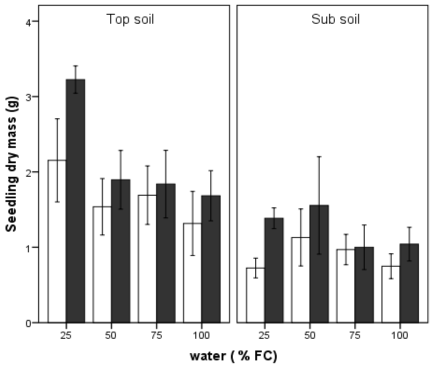

Figure 1: Effect of arbuscular mycorrhiza – inoculated (black bars) and not inoculated (white bars), soil fertility (topsoil and subsoil), and water availability (% field capacity, FC) on *C. myrrha* seedling biomass

Mycorrhizal seedlings also had higher nutrient concentrations. Seedlings grew better in more nutrient-rich topsoil than in subsoil.

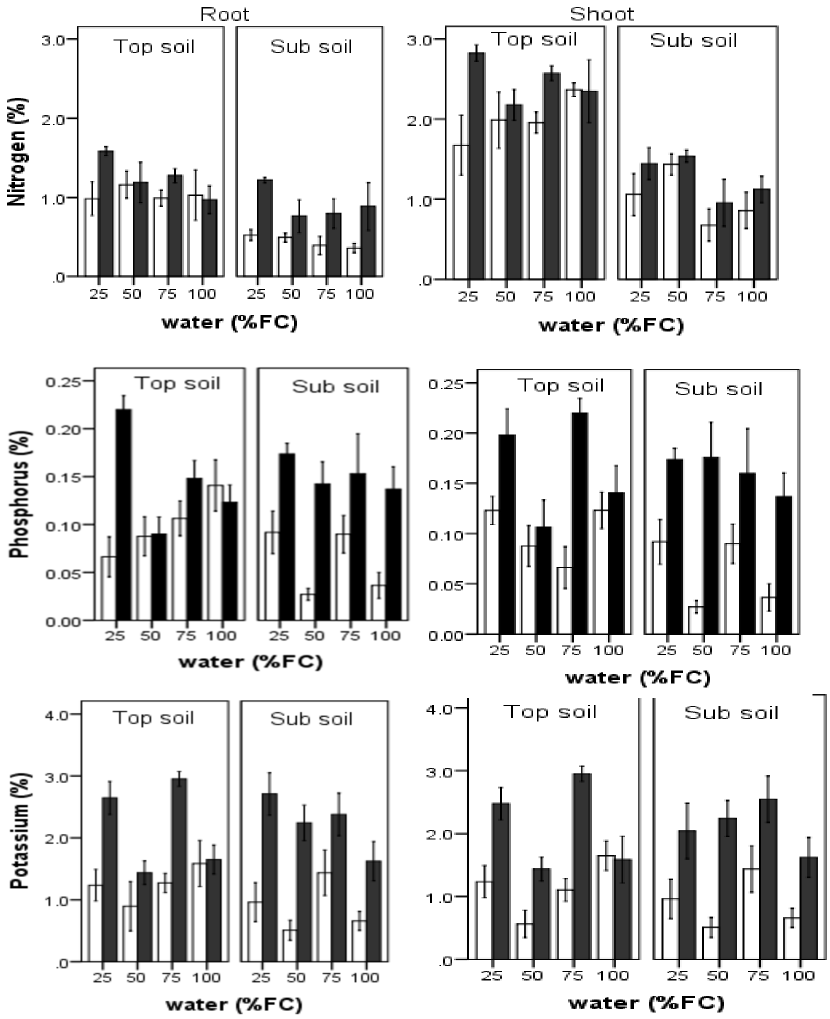

Figure 2: Effect of water level (25% field capacity (FC), 50% FC, 75% FC, and 100%FC), mycorrhizal inoculation (inoculated - black bars and not inoculated - white bars), and soil depth (topsoil and subsoil) on shoot and root N, P, and K concentration of *C. myrrha* seedlings.

Mycorrhizal colonization of roots of *C. myrrha* seedlings were higher with decreased moisture and higher in topsoil than in subsoil.

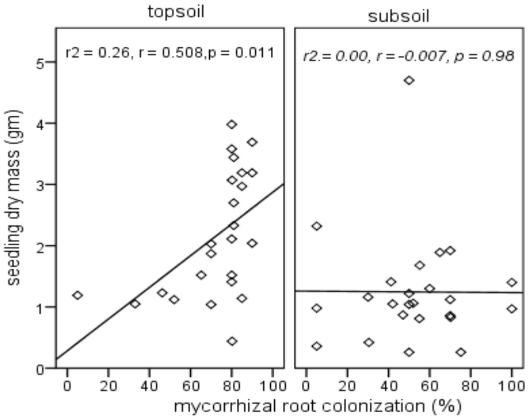

Figure 3: Relationship of mycorrhizal root colonization and dry mass of *C. myrrha* seedlings and soil depth (topsoil and subsoil)

Discussion

The hypothesis that mycorrhizal seedlings grew more rapidly than non-mycorrhizal seedlings is supported by the results of our study and concurs with studies on other tree species (Birhane *et al.,* 2012). The increase in biomass of the mycorrhizal *C. myrrha* seedlings might be because the mycorrhiza enhance the rooting system thus, in turn, allowing more effective nutrient acquisition (Xie *et al.,* 2014). Similarly, mycorrhizal seedlings had longer fine roots than non-mycorrhizal inoculated seedlings. Based on the results of a study of temperate species (Guo *et al.,* 2008), a large part of the fine root length can be associated with absorptive and mycorrhizal anatomical traits. Therefore, the symbiotic association enables increased host growth rates during drought by affecting nutrient acquisition (Augé, 2001).

The *C. myrrha* seedlings grown under conditions of water deficit all grew better than those grown at field capacity. This was most likely due to the greater adaptation and better mycorrhizal symbiosis of the species in dryland environments. Studies of tree species other than *C. myrrha* found that the dry biomass of seedlings increased with increasing water level (Birhane *et al.,* 2012).

Under conditions of water deficit, seedlings inoculated with AMF had substantially higher nutrient concentrations than did non-mycorrhizal seedlings. Therefore, we conclude that mycorrhizal fungi inoculation improved the N, P, and K concentration in *C. myrrha* roots and shoots. Our results also support previous findings that AMF are potentially more important to the growth of plants under drought conditions than under surplus soil moisture (Augé, 2001).

Conclusions

C. myrrha seedlings were responsive to AMF. Seedlings raised for restoration activities in drylands should be inoculated with AM fungi in the nursery prior to out planting in order to increase growth in the field.

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Microbial resources for Mozambican agriculture: use of arbuscular mycorrhizal fungi as a sustainable alternative to chemical input in cotton production

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Abstract

Mozambique is a sub-Saharan country subsisted by familiar agriculture in which the use of pesticides and chemical fertilizers are common. Local farmers however poorly understand the harmful effect of chemical inputs, either for themselves or soil biodiversity. For decades cotton have been one of the major cash crops levying around 80 percent of the national investment. Constraints to cotton cultivation were greatly presence of insects and weeds and soil nutrients deficit. Arbuscular mycorrhizal fungi (AMF) arisen representing a viable and self-renewable alternative to agrochemicals along with benefits for the environment. AMF are obligate root fungal symbionts that facilitate plant growth, by capturing nutrients in depleted soils like the Mozambican one's, fact that makes them a possible solution to limit chemical input. In the present work two cotton varieties (CA 324 and ALBARSZ 9314) were pot cultivated with previously tested commercial inocula in order to evaluate whether these fungi could be able to infect these particular varieties. It was verified that AMF infected cotton roots of both varieties, being the commercial inoculum from MycAgro Lab the one that showed best infection/typical structures presence ratio. Secondarily we are studying soil native fungal communities present in cotton cultivated field in Nampula province (Mozambique).

Keywords: arbuscular mycorrhizal fungi, cotton, soil fungal communities, biofertilizers

Introduction, scope and main objectives

Cotton (*Gossypium* spp.) is a shrub with soft fibre that grow on a protective case – boll (Figure 1), brought to African continent in the 19th century. In Mozambique cotton detain 97 percent of the national investment and in central and northern regions it's one of the main sources of income for rural families (Tombez and Bokusheva, 2011). With decades of civil war, the country's agricultural sector fell drastically, and cotton cultivation was not an exemption, having also been characterized by environmental harmful due to use of agrochemicals and low productivity due to presence of insects, weeds and low fertility soils.

Figure 1: Cotton plants

a) grown in green house, b) grown in open field (Nampula – Mozambique) showing boll (B), c) cotton cultivated field in Nampula province (Mozambique)

Since a long time, the endophytic fungi that form arbuscular mycorrhizae (AM) have gained notoriety representing an eco-friendly alternative to agrochemicals. AM fungi can symbiotically interact with plant roots facilitating plant growth by capturing nutrients in depleted soils and, by this, becoming a sustainable alternative to cotton fertilization and protection against diseases (Quilambo, 2003). Advances in molecular biology and biotechnology have resulted in the development of powerful tools and techniques that have assisted the evolution in crop improvement. AMF influence on cotton cultivation is scarcely reported (Eskandari *et al.*, 2017). In 2017 a project entitled "Microbial Resources for Agriculture: Arbuscular Mycorrhizal Fungi in Cotton and Their Potential Use as Biofertilizer (MYCOTTON)", between DCB/FC (UEM) and IPSP (CNR) and DBIOS (UNITO) has been funded by FIAM (Fundo Para Investigação Aplicada e Multisectorial) aiming to isolate and molecularly characterize AMF to formulate a cotton-tailored biofertilizer inoculum. The main objective of MYCOTTON Project (2017-2020) is to develop functionally and efficient AMF based inocula for cotton biofertilization. In this context, the aims of this work are: 1) to test two cotton varieties, commonly used in Mozambique, in order to verify their ability to be mycorrhized; 2) to extract and amplify AMF DNA from soil and roots, sampled in cotton cultivated field, in Nampula province (Mozambique).

Methodology

Biological materials

Two cotton varieties, ALBARSZ 9314 and CA 324, were pre-germinated in Petri dishes and the obtained seedlings were then transferred into pots filled with a sterilized substrate. Each pot from treated group were inoculated with three different commercial inocula (MycAgro Lab, Micosat F Radinet, Micosat F SEMI) containing AMF (Figure 2) previously tested by Caser *et al.* (2019), while a set of plants non-inoculated were used as control. All the plants were maintained several months in greenhouse.

Figure 2: Display of pots in greenhouse

A - inocula in pots containing sterilized substrate, B – treated pots with different inocula + control pot

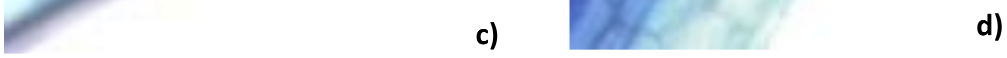
AMF Evaluation

During the vegetative phase of both control and treated cotton plants groups, roots were harvested, rid of topsoil, cleaned and stained with 0.1 percent (w/v) cotton blue in 80 percent lactic acid overnight, then distained 3 times with lactic acid for 18 h, cut into 1-cm-long segments and placed on microscope slides for the morphological analysis. Approximately 25 fragments were observed under light microscope for each sample. Fungal colonization was determined and calculated as described by Trouvelot, Kough and Gianinazzi-Pearson (1986).

For the preparation of the roots for further observations in light and transmission electron microscopy we randomly selected fragments of cotton mycorrhized roots. Root segments were excised under a stereomicroscope and quickly fixed in 2.5 percent glutaraldehyde in 0.1 M cacodilate buffer (pH 7.2) for 2 hours at room temperature and overnight at 4 °C. The samples were then post-fixed in 1 percent OsO4 in the same buffer and dehydrated in an ascending series of ethanol to 100 percent, incubated in two changes of absolute acetone and infiltrated in Epon-Araldite resin. The resin was polymerized for 24 h at 60 °C. Semi-thin (1 micron) sections were then stained with 1 percent toluidine blue and ultra-thin (70 nm) sections were counter-stained with uranyl acetate and lead citrate and used for electron microscopy analyses under a transmission electron microscope.

Molecular analysis

In order to optimize DNA extraction from soils and roots, collected in cotton cultivated field in Nampula province (Mozambique), two kits were tested: DNeasy Power Soil kit (QIAGEN) for soil and DNeasy Plant kit (QIAGEN) for eukaryotic DNA extractions. PCR amplification was done by means of specific primers targeting fungi. The resulting PCR products were visualized on 1.5 percent agarose gel.



Results

Greenhouse cotton roots morphological observation and mycorrhizal evaluation

Two cotton varieties treated with three different inocula (MycAgro Lab, Mycosat F Radinet and Mycosat F SEMI) were tested in pot. AMF successfully infected cotton roots of both varieties and, as expected, not inoculated plants were not mycorrhized. The colonization was seen after 45 days of pot cultivation in greenhouse, showing highly AMF presence in some root fragments, detected by the presence of arbuscules, vesicles and inter-intracellular hyphae (Figure 3). MycAgro Lab inoculum was the best inoculum in terms of ability to colonize, as treated plants were able to form all AMF typical structures mentioned before.

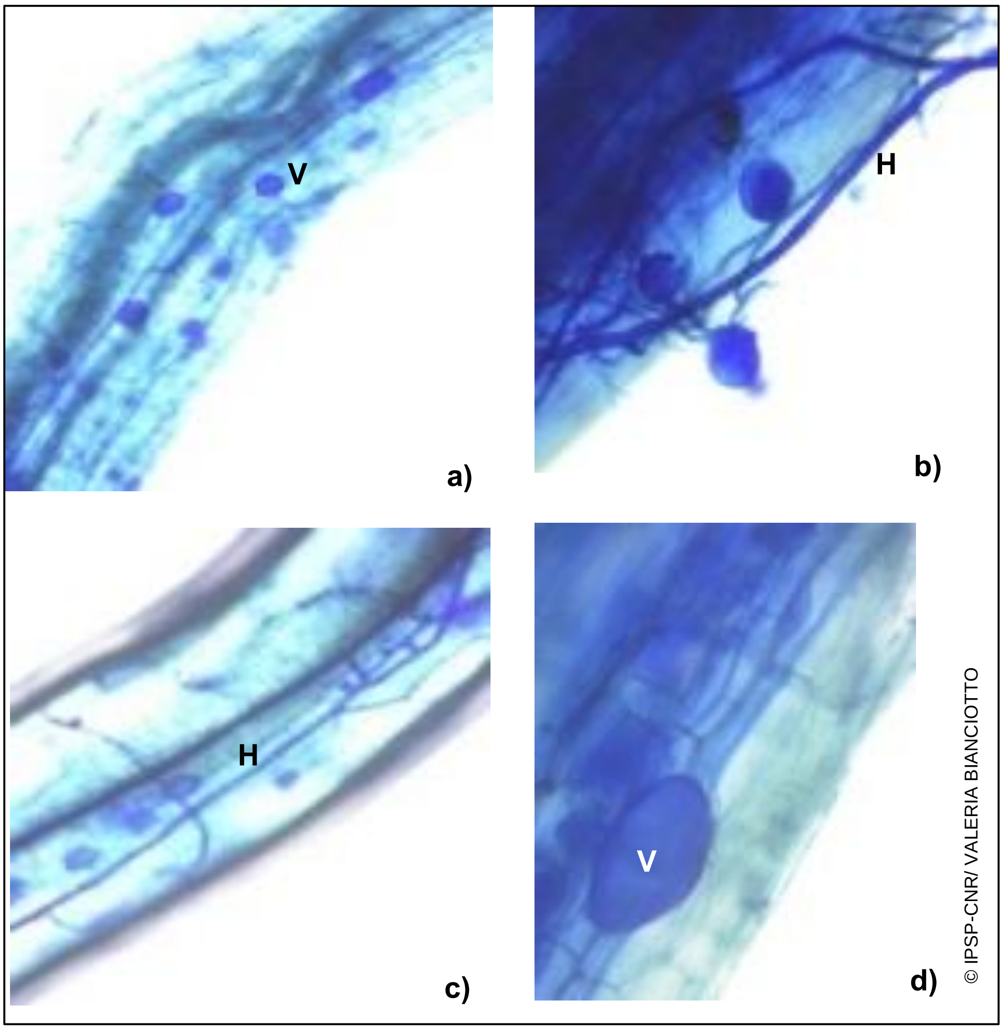


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Figure 3: Optical microscope images showing cotton roots cortical cells

In the pictures above (a-d) are presented cotton roots colonized by MycAgro Lab inoculum showing hyphae (H), vesicles (V), on ALBARSZ 9314 variety.

Under light and transmission electron microscopes by observing semithin and thin sections hyphopodia were observed on epidermal cells. The root epidermis and the first layer of root cortical parenchyma were free of colonization while the deeper layer of parenchyma (near central cylinder) was all colonized by typical AMF structures. At this time of pot cultivation very few mycorrhizal structures were detected in roots treated with Mycosat F Radinet and Mycosat F SEMI inocula.

Open field soil and roots DNA extraction and amplification

Using DNeasy Power Soil kit (QIAGEN) and DNeasy Plant kit (QIAGEN) for soil and cotton roots DNA isolation, we were able to successful extract template DNAs for amplification and subsequent NGS. Previously extracted DNAs resulted amplifiable by fungal specific primers.

The characterization of fungal communities associated to soil and cotton roots samples, from cultivated field in Nampula province (Mozambique), will be carried out with paired-end Illumina MiSeq data, cover the full ITS2, a suitable target for environmental fungal community assessment. In the meanwhile trap cultures, with autochthonous soils, will be set up in greenhouse experiments in order to isolate and characterize "local adapted" AMF inocula that will be tested as tailored-biofertilizer for cotton.

Discussion

This study presents, for the first time, experimental evidence for directly inoculation of two cotton varieties cultivated in Mozambique. The AMF are soil microorganisms able to establish a mutualistic interaction with most land plants (Berruti *et al.*, 2016), thus as expected al three used inocula were able to spread into the root systems of almost all infected plants after 45 days in greenhouse. Mycorrhizal fungi from all three commercial inocula were able to infect cotton, MycAgro commercial inocula however, showed the best performance confirming that an inocula with more than one AM fungi readily colonized plant roots fact that was stated previously by Bianciotto *et al.* (2016). All three inocula differently infected cotton roots (data not shown). This fact can be explained because each inoculum (AMF isolate) may act differently both ecologically and functionally, depending on the plant species (Janousˇkova *et al.*, 2017).

Commercial DNA extraction kits are widely available and have become favourable because they are often faster than traditional extraction methods (Mahmoudi, Slater and Fultrorpe, 2011). It is noteworthy that successful DNA extractions, characterized by high quantity and integrity of dsDNA and low quantity of contaminants that absorb at 230 and 280 nm, are the first important step for a future reliable characterization of microbial communities associated to soil and plant samples and Next-Generation Sequencing (NGS) analyses.

Conclusions

AMF commercial inocula can infect cotton plants and can be a great alternative in cotton cultivation by reducing the use of agrochemicals. Inocula with more than one AM fungi tend to readily infect cotton plant roots as previously described for many other plant species. For this reason, all the research efforts to select and improve local AMF production and use are welcome.

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