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Influence of Land Use on Soil Microbial Communities of Sub Himalayas of India: Insights from Phospholipid Fatty Acid Profiles, Ribosomal Intergenic Spacer Profiles, Soil Enzymes, and Carbon Pools.

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Research Article

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Influence of Land Use on Soil Microbial Communities of Sub Himalayas of India: Insights from Phospholipid Fatty Acid Profiles, Ribosomal Intergenic Spacer Profiles, Soil Enzymes, and Carbon Pools.

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Abstract

Land use and management practices have a significant impact on soil microbial populations and community composition, thereby influencing ecosystem processes. Soil microbial and biochemical indicators serve as highly sensitive tools for assessing the effects of land use systems. In this study, we investigated soils under ten different land use systems in the Central Himalayas, India, including natural forests dominated by oak (*Quercus incana*), deodar (*Cedrus deodara*), or pine (*Pinus roxburghii*) trees, orchards dominated by apple trees, and crop-based systems in uplands and valleys. We examined phospholipid ester-linked fatty acid (PLFA) profiles, soil enzymes, Ribosomal Intergenic Spacer Analysis (RISA) profiles, microbial biomass carbon and organic carbon as effected by land use. The results revealed that forest soils had significantly higher levels of soil enzymes compared to agricultural soils. Microbial biomass carbon and organic carbon showed a close relationship with the PLFA profiles across different land uses. Gram-positive bacteria (15:0 iso, 16:1 iso G, 16:1 iso H), gram-negative bacteria (10:0 2OH, 12:0 3OH, 17:0 cyc, 19:0cyc 8c, 18:1ω7c11), fungi (18:1ω9c), and arbuscular mycorrhizal fungi (AMF) (16:1) exhibited higher relative abundance in forest systems, whereas gram-positive PLFA markers (C15:0 anteiso, C17:0 iso, C17:0 anteiso) were more prominent in agro-ecosystems. Principal component analysis (PCA) of the PLFA profiles demonstrated that the microbial communities in deodar forest soils were compositionally distinct from other forest soils, while the cultivated soils were grouped together and exhibited higher similarity, except for the organic farming soil. The correlation between PLFA profiles and soil enzymes, microbial biomass carbon, and organic carbon provides insights into the impact of different land use and management practices on soil microbial health and, consequently, soil health.

Keywords: Soil enzymes, Microbial biomass carbon, Organic carbon, Phospholipid ester-linked fatty acid, Different land use, Kumaon Himalayas

Introduction

Land use practices have a profound influence on the diversity and functioning of microbial communities (**Liu et al 2022**). Microbes play a crucial role in various biochemical processes and exhibit organised community structures with defined and controlled functions (**Kolter and Greenbreg, 2006**). They are highly sensitive to changes in physical, chemical, and biological factors, thereby impacting the overall structure and function of microbial communities associated with specific land use and management practices. The intensification of land use often leads to the loss of microbial biodiversity and subsequent soil degradation (**Carrasco-Espinosa et al 2022**). In the Indian Himalayas, the increasing demand for food has resulted in the conversion of more land for agricultural purposes (**Ghosh and Dyani, 2005**).

Undisturbed forest ecosystems typically receive continuous litter fall, and soil microorganisms play a vital role in nutrient recycling processes (**Giweta 2020**). Previous studies have shown significant impacts of plant litter (**Chen and Stark, 2000**) and vegetation types (**Wang et al 2022**) on soil microbial composition in forest ecosystems. However, in agricultural systems, the microbial composition is not only influenced by the plant type but also by the application of organic and inorganic fertilizers (**Li et al 2017; Lazcano et al., 2012**). Although inorganic fertilizers have a positive impact on crop yields, their application can alter soil properties (**Zhong et al., 2007**) and soil quality (**Gregorich et al., 1994**), thereby potentially affecting microbial habitats and biodiversity. Therefore, understanding the impact of land use and management practices on microbial composition requires elucidating changes in microbial communities.

Phospholipid fatty acids (PLFAs) serve as efficient biomarkers that provide specific fingerprints of microbial communities (**Quideau et al 2016**). Similarly, soil enzymes are early indicators of biological changes (**Lee et al 2020**) and respond rapidly to environmental signals. Hence, measuring soil enzyme activities is valuable for understanding soil quality (**Karaca et al 2010**). Microbial biomass carbon, although comprising only 1-3% of soil organic matter (**McGonigle and Turner 2017**), represents the most active component involved in biogeochemical processes. It can serve as an effective indicator of the impact of management practices on microbial populations (**Hargreaves et al 2003**). In addition, another highly efficient and culture-independent method for analyzing the structure of microbial communities in environmental samples is Automated Ribosomal Intergenic Spacer Analysis (ARISA). ARISA, a PCR-based technique developed by

Fisher and Triplett (1999), has proven to be an effective approach for investigating microbial community dynamics at a community level.

The main objective of this study was to assess the impact of various factors (such as vegetation, altitude, and management practices) on different land use systems concerning microbial community composition, soil enzymes, microbial biomass carbon, and microbial abundance. We investigated the effects of ten different land use systems, including four distinct natural forests, three long-term inorganic fertilizer-based systems, one organically maintained agroecosystem, one orchard, and two conventional agricultural farms, on microbial community composition, soil enzymes, microbial biomas**s,** organic carbon and microbial abundance and relationship between the parameters.

Materials and methods

The study was conducted in the Almora region of Uttarakhand, India, encompassing ten different land use systems. Four systems (organic farming, soybean-wheat, maize-wheat, fodder crops) were located at the experimental farm, Hawalbagh, at an altitude of 1250 meters above mean sea level (amsl), belonging to the Vivekananda Institute of Hill Agriculture, Almora. The upland rice system was located in Someshwar Valley, approximately 25 km away from the farm. The forest land use systems included mixed forest (1800 mts amsl), undisturbed oak (2400 mts amsl) and pine (1800 mts amsl) forests of Binsar Wildlife Sanctuary (29°37' N and 79°20' E), and deodar forest in Jageshwar (29.65°N 79.58°E).

The soils in the study area consisted of mica, schist, slates, sandstone, and calcium-deficient granite and seynite rocks (**Singh et al., 2015**). These soils are classified as climatogenic podsolized grey-brown forest soils. The soil pH was acidic in most systems, except for the slightly acidic soils in cultivated fields. The climate in the region is sub-temperate, characterized by moderate summers (May-June), extreme winters (December-January), and overall dryness, except during the southwest monsoon season (June-September).

Three composite soil samples were collected from each site at two depths: 0-15 cm and 15-30 cm. Each composite sample was created by mixing five soil cores. Pseudo-replication sampling was adopted, following the approach of other researchers (**Patra et al., 2006**). The field moist soil samples were stored in a refrigerator at a temperature below 4°C to preserve the enzyme activities until analysis. All chemical results were the mean of triplicate analyses and expressed on an oven dry basis. Soil moisture was determined after drying the samples at 105°C for 24 hours.

For dehydrogenase activity was determined using the method as suggested by **Klein et al 1971**; and **Meena and Rao 2021**. Soil samples were mixed with a mixture of 0.2 ml of 3% triphenyltetrazolium chloride (TTC) solution and 0.5 ml of 1% glucose. The samples were incubated at 28°C for 24 hours, followed by the addition of 10 ml of methanol and incubation at 28°C for another 8 hours. The resulting triphenyl formazan (TPF) was measured by absorbance at 485 nm. Acid and alkaline phosphatase activities were determined using colorimetric measurements of p-nitrophenol (pNP) released when soil was incubated with pNP phosphate buffer pH 5 and pNP phosphate buffer pH 9 solutions, respectively (Tabatabai and Bremner, 1969). Nitrate reductase activity was determined using the method of **Mandal et al., 2007.**

MBC was determined using the chloroform fumigation method (**Vance et al., 1987**). The difference between carbon content in the unfumigated and chloroform-fumigated samples was used to calculate MBC, considering the correction factor related to the proportion of microbial biomass (**Liu et al., 2012**). Soil organic carbon was determined using the Walkley-Black chromic acid wet oxidation method (**Allison, 1965**).

PLFA analysis was performed using the methods described by **Buyer et al. (2010)**. A total of 5 g of lyophilized soil was subjected to Bligh-Dyer extraction, followed by separation on a solid-phase extraction column. The phospholipids were then eluted with methanol, and the extracted phospholipids were transesterified to fatty acid methyl esters. Gas chromatography (Agilent Technologies, Wilmington, DE, USA) equipped with an autosampler and a flame ionization detector was used for analysis. The composition of the soil microbial community was identified using microbial analysis software (Sherlock MIS 4.5 System, MIDI, USA) based on the spectrogram of specific PLFAs. Individual PLFA values were expressed as a percentage of the total PLFAs (mole %) in the sample. Triplicate analyses were performed, and the nomenclature of fatty acids followed the classification by **Feng et al. (2003)**.

The soil ribosomal intergenic spacer analysis (RISA) was conducted following the methodology described by **Ranjard et al. (2001)**. Total genomic DNA was extracted from 0.5 g of sieved soil using the PowerSoil DNA Isolation Kit (MO-BIO) according to the manufacturer's recommendations. The intergenic spacers between the small- and large-subunit rRNA genes were amplified using the primers S-D-Bact-1522-b-S-20 (eubacterial rRNA small subunit, 5'-TGCGGCTGGATCCCCTCCTT-3') and L-D-Bact-132-a-A-18 (eubacterial rRNA large subunit, 5'-CCGGGTTTCCCCATTCGG-3').. The PCR reaction mixtures (50 µl) contained 5 µl of a 10×

dilution buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 15 mM MgCl2, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.5% Tween 20 (vol/vol), 0.5% Nonidet P40 (vol/vol), 50% glycerol (vol/vol)), 1 µg T4 gene 32 protein (New England Biolabs), 0.5 µM of each primer, 200 µM of each dNTP, 2.0 U of PR polymerase (Genei), and 100 ng of purified soil DNA. The amplification was carried out in a Bio-Rad C1000 thermal cycler, starting with a hot start at 94°C for 3 minutes, followed by 25 cycles consisting of 94°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension of incomplete products for 5 minutes at 72°C. For gel electrophoresis, the volume of PCR reactions loaded on the gel was adjusted to obtain similar intensities per profile. To increase sensitivity, the forward primer D-Bact-1522-b-S-20 (eubacterial rRNA small subunit, 5'-TGCGGCTGGATCCCCTCCTT-3') was labeled with FAM dye at the 5' end. The PCR products were separated using standard ABI 310 denaturing electrophoresis conditions for 1 hour each, with the POP-4 polymer. The resulting data were analyzed using the GeneScan 3.1 software program (Perkin-Elmer).

Principal Component Analysis (PCA) was conducted using PAST software to determine the variation in PLFA signatures and RISA profiles based on band presence (peak) and intensity (height or area of peak)among different land use systems. The loading score of the sum of total PLFA signatures in mole percentage for each land use system was used to assess the relative importance of land use.

Results

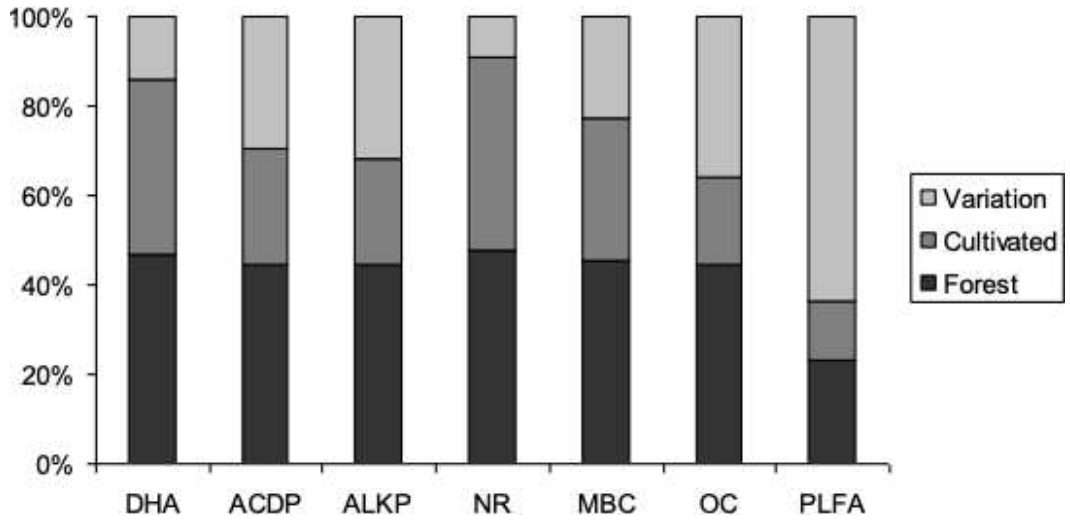
The results of the study showed significant variations in soil enzymes among the different land use systems (**Table 1**). The forest ecosystems (mixed forest, deodar forest, oak forest, and pine forest) exhibited higher soil enzyme activities compared to the agro ecosystems (maize-wheat cropping system, soybean-wheat cropping system, organic farm, and rice cropping system). Land use system accounted for the highest proportion of the total variation in soil alkaline phosphatase (32%) and acid phosphatase (30%), while nitrate reductase explained only 9% of the variation (**Fig 1**). The variability within the three-year period and replication of forest and cultivated ecosystems ranged from 9% to 32% of the total variation. Soil dehydrogenase activity showed a 14% variation when comparing forest to cultivated lands. However, the fodder cropping system and apple plantation orchards accounted for the highest proportion of the total variability in different land use systems, with 42.6% for dehydrogenase, 51.1% for acid phosphatase, 53.2% for alkaline phosphatase, and

Table 1: Impact of Land use system on dehydrogenase (DHA-µg TPF g soil h), acid phosphatase (AcP- µg PNP g^{-1} soil h-1), alkaline Phosphatase (AlkP- µg PNP g^{-1} soil h^{-1}), nitrate reductase (NR-mg kg^{-1}), microbial biomass carbon (MBC- mg kg^{-1}) and organic carbon (OC- %) at different depths under different land use systems in Central Himalayan region. The mean followed by different letters are significantly different at $p<0.05$, according to DMRT (Duncan!s Multiple Range Test) for separation of means.

Land Use	DHA	DHA	Acd-P	Acd-P	Alk-P	Alk-P	NR	NR	MBC	MBC	OC	OC
Depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
MF	15.3a	9.34d	896.02a	614a	253.81 b	177.4 a	1.6a	0.66cd	918.1b	778.4 a	3.2b	2.6a
DF	12.9b	9.74a	642b	343.6b	293.8a	147.3 b	1.2b	0.72c	1063.5a	822.8 a	3.4a	1.7b
OF	11.8c	8.07b	530.74c	262.1c	182.8c	181.2 a	1.74b	1.02b	765.89c	427.6 b	2.7c	1.6b
PF	6.7e	3.98e	272.63e	99.9h	164.5c d	84.59 c	0.79c	0.42ef	617e	303.6 c	1.3e	0.43e
FC	3.5f	2.8f	218.22f	123.9g h	85.65f	46.18f	0.49.d	0.44ef	473.59f	217.6 d	0.71g	0.39e
AP	2.9f	2.65f	332.5d.	145.1fg	48.13g	41.72f	0.79c	0.27f	234.1g	162.3 e	0.89fg	0.37e
MW	11.1c	3f	354.19 d	163.9f	142.31 d	78cd	1.65a	0.5de	624.99e	322.7 c	0.92f	0.75d
SW	9.5d	4.89d	328.39 d	228.5d	139.19 d	61.8e	0.82c	0.65cd	470.35f	277.2 c	0.89fg	0.69d
OR	11.3c	7.65bc	399.96c	247.6c d	157.3c d	81.7c d	0.72c	1.23a	696.1d	384.4 b	1.6d.	1.09c
UR	7.1e	3.48e	279.32e	254.5c	111.81e	88.3c	1.62a	0.98.4 b	594.39e	301.3 e	1.3e	0.32e

(MF-Mixed Forest, DF-Deodar Forest, OF- Oak Forests, PF-Pine Forest, FD- Fodder cropping system, AP- Apple Plantation, MW-Maize wheat cropping system, SW- Soybean cropping system, OR- Organic Farm, UR- Rice cropping system)

Figure 1: Total percent variation observed in soil dehydrogenase, acid phosphatase, alkaline phosphatase, nitrate reductase, microbial biomass carbon, organic carbon and PLFA explained by land use system.



42.9% for nitrate reductase activity, as these systems were severely affected by the land use system and exhibited lower soil enzyme activity. The organic farm showed better dehydrogenase activity compared to other cultivated ecosystems. All the enzymes studied showed significant positive correlations with each other, except for nitrate reductase activity (**Table 2**).

Table 2: Pearson's correlation coefficients among soil dehydrogenase, acid phosphatase, alkaline phosphatase, nitrate reductase, microbial biomass carbon, organic carbon and PLFA from different land use systems of Central Himalayan region.

	DHA	ACP	ALP	NR	MBC	OC
ACP	0.811**					
ALP	0.860**	0.809**				
NR	0.599	0.506	0.409			
MBC	0.846**	0.757*	0.960**	0.487		
OC	0.769**	0.897**	0.897**	0.500	0.891**	
PLFA	0.739*	0.680*	0.906**	0.438	0.907**	0.896**

**Correlation is significant at $P < 0.01$

*Correlation is significant at $P < 0.05$

When assessing the forest ecosystems in terms of soil enzymes, the order of activity was as follows: mixed forest > deodar forest > oak forest > pine forest. In contrast, for the cultivated eco

systems, the order was organic farm > maize-wheat > soybean-wheat > upland rice > apple plantation > fodder. Soil enzyme activity was 37% higher in the surface soil (0-15 cm) compared to the subsurface soil (15-30 cm).Soil organic carbon was significantly higher in mixed forest, deodar forest, and oak forest compared to other ecosystem soils, while it was lower in the fodder cropping system. In the surface soil, organic carbon was 60% higher in forest ecosystems (mixed forest, deodar forest, oak forest, and pine forest) compared to other cultivated ecosystems (fodder cropping system, apple plantation, maize-wheat, soybean-wheat, upland rice). When considering both soil depths together, forest soils accounted for 40% of the variation, while cultivated lands accounted for

34%. Organic farm exhibited an average of 42% higher organic carbon compared to other cultivated lands.

Similarly, microbial biomass carbon varied significantly among the different land use systems. In the surface soil, microbial biomass carbon was 38% higher in forest ecosystems compared to other cultivated ecosystems. Land use system in the surface soil explained the highest proportion of the total variation in microbial biomass carbon (23%) and organic carbon (36%). The dominant forest ecosystems (mixed forest, deodar forest, oak forest) exhibited the highest microbial biomass carbon. Microbial biomass carbon and organic carbon were highly correlated with soil enzymes and the phospholipid fatty acid (PLFA) fraction of different land use systems at the surface soil (**Table 3**).

The box plot analysis, considering soil enzymes, microbial and organic carbon, and PLFA profiles, effectively separates the forest systems from the cultivated systems, suggesting a potential decline in microbial abundance and diversity across the different land use systems **(Figure 2).** Soil dehydrogenase, acid phosphatase, alkaline phosphatase, microbial biomass carbon, and organic carbon were significantly and positively correlated with PC1 in the PCA analysis. PC1 explained 78.8% of the variance in the data, while PC2 explained 9.7%. The PCA plot (**Fig 3**) showed that mixed forest, oak forest, and deodar forest clearly segregated to the right, while other cultivated ecosystems were located on the left. Organic farm exhibited similarity with forest soils compared to other cultivated ecosystems.

253 **Table 3**: Mean relative abundance (mol PLFA-C %) of different land use systems of Central Himalayas
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Microbes	PLFA	MF	DF	OF	PF	FD	AP	MW	SW	OR	UR
Actinomycetes	C17:0 10-	1.07^{b}	0.81^{b}	1.12	nd	0.16^{c}	0.31	1.59^{a}	1.14	nd	nd
	C18:0 10-	3.32^{b}	3.46	2.44	3.28	0.53^{d}	0.34	2.55^{c}	1.84	15^{a}	2.6^{c}
Fungi	C18:1w9c	20.2	63.8^{a}	22^{b}	11.2^{e}	7.55^{f}	10.8	7.82^{f}	7.36^{f}	17.4	6.95^{f}
	C16:1 w5c	1.34^{d}	1.54	2.41	1.44	0.17^{f}	1.41	2.76^{a}	0.82^{e}	2.02	1.65^{d}
Gram-positive bacteria	C12:0iso	0.24^{b}	0.15^{c}	1.42	nd	nd	nd	nd	nd	nd	nd
	C15:0 iso	9.1^{a}	3.92^{f}	8.46	4.94	0.46^{i}	3.26	5.94^{c}	2.28	4.5^{de}	4.51^{e}
	C15:0	3.37^{b}	2.63^{c}	2.32	2.54^{c}	0.25^{e}	1.49	2.4^{c}	2.35^{c}	2.62	5.5^{a}
	C16:1iso G	0.61^{b}	nd	4.15	nd	nd	nd	nd	nd	nd	nd
	C16:1iso H	nd	nd	0.73	nd	nd	nd	nd	nd	nd	nd
	C16:0 iso	3.73^{c}	3.16^{e}	8.03	3.46	0.54^{h}	1.83	3.53^{d}	2.49^{f}	3.37	4.18^{b}
	C16:1 w7c	nd	5.07^{a}	nd	nd	nd	nd	nd	nd	nd	0.45^{b}
	C16:1 w9c	nd	7.76^{a}	nd	nd	0.16^{c}	nd	0.38^{b}	0.27	0.43	nd
	C17:0 iso	2.03^{c}	1.53^{e}	1.72	1.99^{c}	0.35^{e}	nd	2.7^{b}	1.8^{de}	2.13	3.19^{a}
	C17:0	2.16^{c}	1.7^{d}	1.35	2.18^{c}	0.30^{f}	nd	2.32^{b}	1.41^{e}	1.76	3.23^{a}
Gram-negative bacteria	C10:0 2OH	0.25^{c}	2.14^{a}	nd	nd	0.14^{d}	0.15	nd	nd	nd	0.92^{b}
	C10:0 3 OH	0.13^{c}	0.12^{c}	0.24	nd	nd	0.29	nd	nd	nd	nd
	C12:0 2OH	nd	nd	nd	nd	nd	0.29	nd	nd	nd	nd
	C12:0 3OH	0.16^{b}	nd	3.47	nd	nd	nd	nd	nd	nd	nd
	C17:0 cyclo	1.44^{d}	3.26^{a}	1.85	1.49	0.21^{f}	0.86	2.4^{b}	0.85^{e}	1.49	1.33^{d}
	C19:0cyclo	5.42^{c}	10.8^{a}	5.44	9.15	0.36^{e}	0.23	nd	1.72	nd	0.44^{e}
	C18:1 w7c	nd	8.81^{a}	nd	nd	nd	nd	1.15^{b}	nd	0.86	0.51^{d}
Non-Specific	C10:00	nd.	nd.	0.13	nd.	nd.	nd.	nd.	nd.	nd.	nd.
	C12:00	3.3^{b}	1.99^{cd}	6.75	2.25	0.5g	0.7f	1.6de	1.3ef	0.64	nd.
	C17:0	4.5^{a}	1.21^{c}	2.09	1.27	0.14	nd.	1.08c	0.8c	1.03	0.93c
	C18:0	7.52^{b}	4.47	4.7d	3.99	3.82	nd.	5.84c	10.6	5.6c	7.25b
Total Mole Fraction		69.9	128.	80.8	49.2	15.6	21.9	44.1	37	58.9	46.6

255 MF-Mixed Forest, DF-Deodar Forest, OF- Oak Forests, PF-Pine Forest, FD- Fodder cropping system, AP- Apple Plantation, MW-
256 Maize wheat cropping system, SW- Soybean cropping system, OR- Organic Farm, UR- Rice cropping system.

Figure 2: Box-plot representation of A) Dehydrogenase, B) Acid phosphatase, C) Alkaline phosphatase, D) PLFA, E) Microbial biomass carbon and F) Organic carbon and PLFA in soils of different land use systems from central Himalaya, India. Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The lines within the box indicate the median. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Average values with different letter indicate significant differences between forest and cultivated ecosystem ($P < 0.05$).

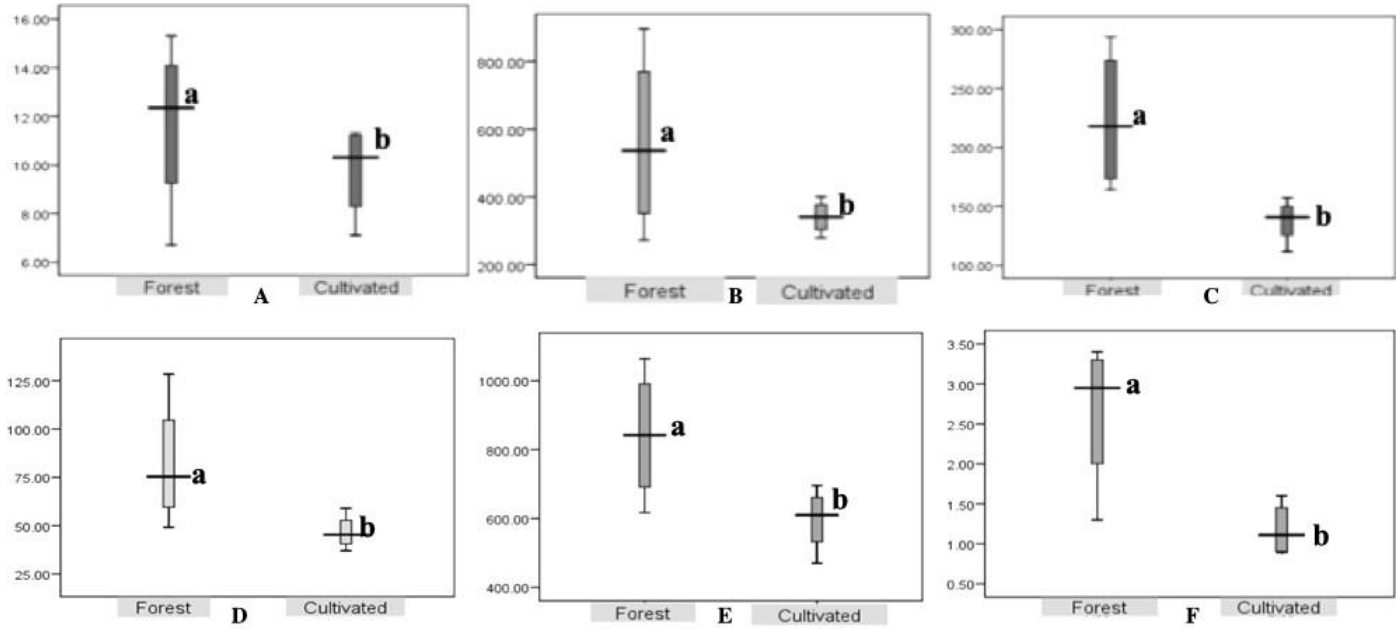
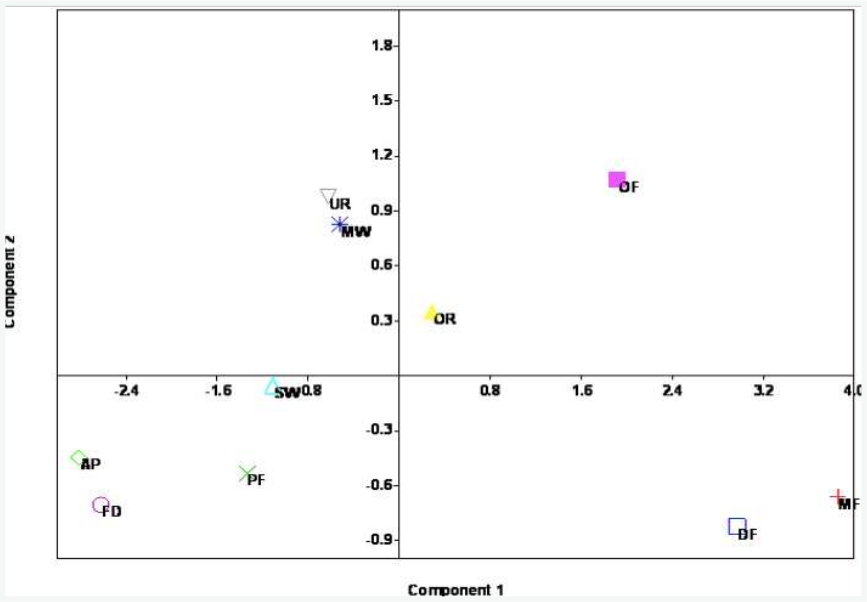


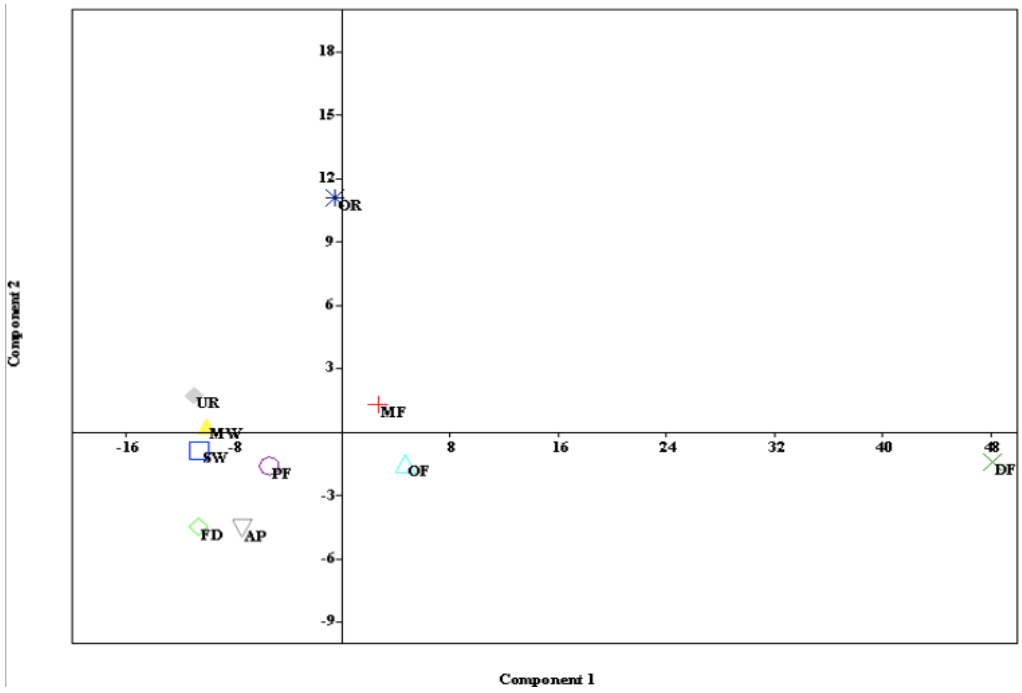
Figure 3: Ordination of ten different land use system soils sites in function of mean of three subsequent years in the space defined by the PC1 and PC2 axis of the PCA analysis carried out with soil enzymes. Component 1 and 2 represent 85 and 9.27 % of the variation in the data respectively.



MF-Mixed Forest, DF-Deodar Forest, OF- Oak Forests, PF-Pine Forest, FD- Fodder cropping system, AP- Apple Plantation, MW-Maize wheat cropping system, SW- Soybean cropping system, OR- Organic Farm, UR- Rice cropping system.

The analysis of the mole percentages of phospholipid fatty acid (PLFA) biomarkers showed that deodar forest had the highest mole fraction of PLFA, followed by oak forest and mixed forest ecosystems, while the lowest values were recorded for the fodder cropping system and apple plantation. Organic farm soil exhibited higher PLFA C fraction compared to other agro-ecosystems. Fungal biomarkers C18:1ω9c, C16:1ω5c, and universal marker C16:0 were relatively abundant in all land use systems. The PCA plot based on the mol% PLFA-C of microbial communities (**Fig 4**) showed distinct clusters for different land use systems. Cultivated ecosystem soils tended to be on the left of the plot, forming a major cluster, while forest ecosystems were on the right. Organic farming soil exhibited intermediate characteristics between cultivated and undisturbed forest ecosystems. Gram-positive PLFA markers C12:0 iso were found in forest ecosystems but not detected in cultivated ecosystems. Gram-positive (C15:0 iso, C16:1 iso G, C16:1 iso H) and gram-negative bacteria (C10:0 2OH, C12:0 3OH, C17:0 cyc, C19:0cyc 8c, C18:1ω7c11) were more abundant in forest systems, while gram-positive PLFA markers (C15:0 anteiso, C17:0 iso, C17:0 anteiso) were higher in agro-ecosystems.

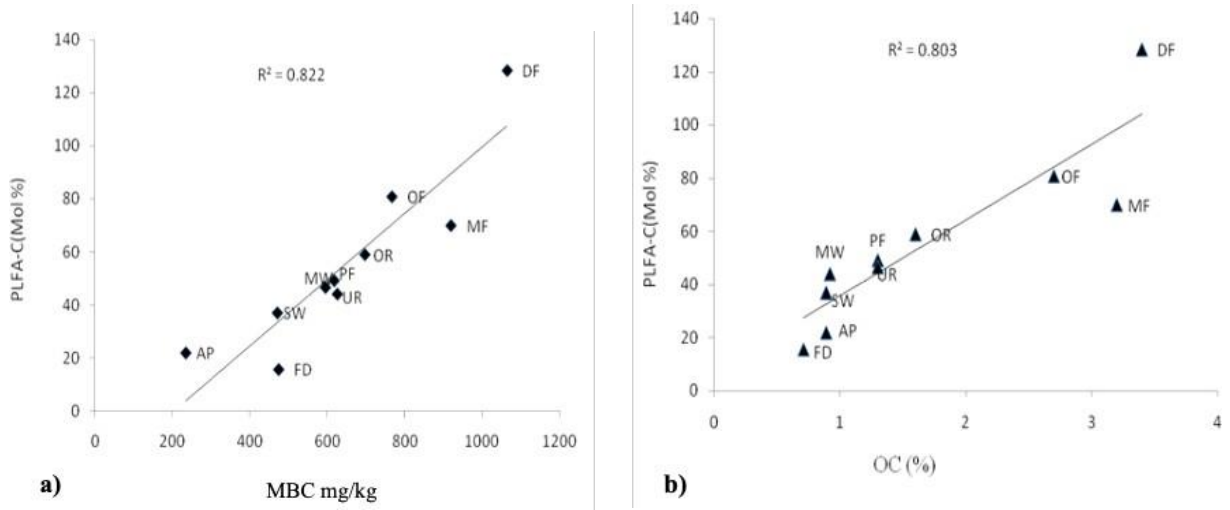
Figure 4: PCA ordination plot of the PLFA profiles for 10 different land use systems. Shorter distances between sites in the PCA ordination indicate high degree of similarity between systems and their respective PLFA profiles. Component 1 and 2 represent 31.2 and 24.2 % of the variation in the data respectively



MF-Mixed Forest, DF-Deodar Forest, OF- Oak Forests, PF-Pine Forest, FD- Fodder cropping system, AP- Apple Plantation, MW- Maize wheat cropping system, SW- Soybean cropping system, OR- Organic Farm, UR- Rice cropping system

The PLFA biomarker for actinomycetes (C17:0 10methyl, C18:0 10methyl) and fungi (C18:1ω9c) exhibited varying mole percentages among the land use systems. Pearson's correlation analysis **(Table 2)** showed strong correlations of different land use systems with soil microbial biomass carbon (R2=0.90; P < 0.01) and organic carbon (R2=0.89; P < 0.01). At the surface soil, MBC (r2=0.88) and OC (r2=0.80) exhibited a strong correlation with PLFA microbial composition (**Figure 5**). Electropherograms of various land use profiles revealed peaks (bands) ranging from a 50-bp IGS to 1,200 bp (a 1,050-bp IGS), as determined by the GeneScan 1,000-bp ROX standard. Prior to automated sequencer analysis, the profile bands were assessed on a 1% agarose gel (Figure 7.1). Each profile exhibited a range of 189 to 265 bands. The electropherograms clearly demonstrated distinct differences in the number and intensity of bands, enabling easy differentiation of bacterial communities among the ten sites.

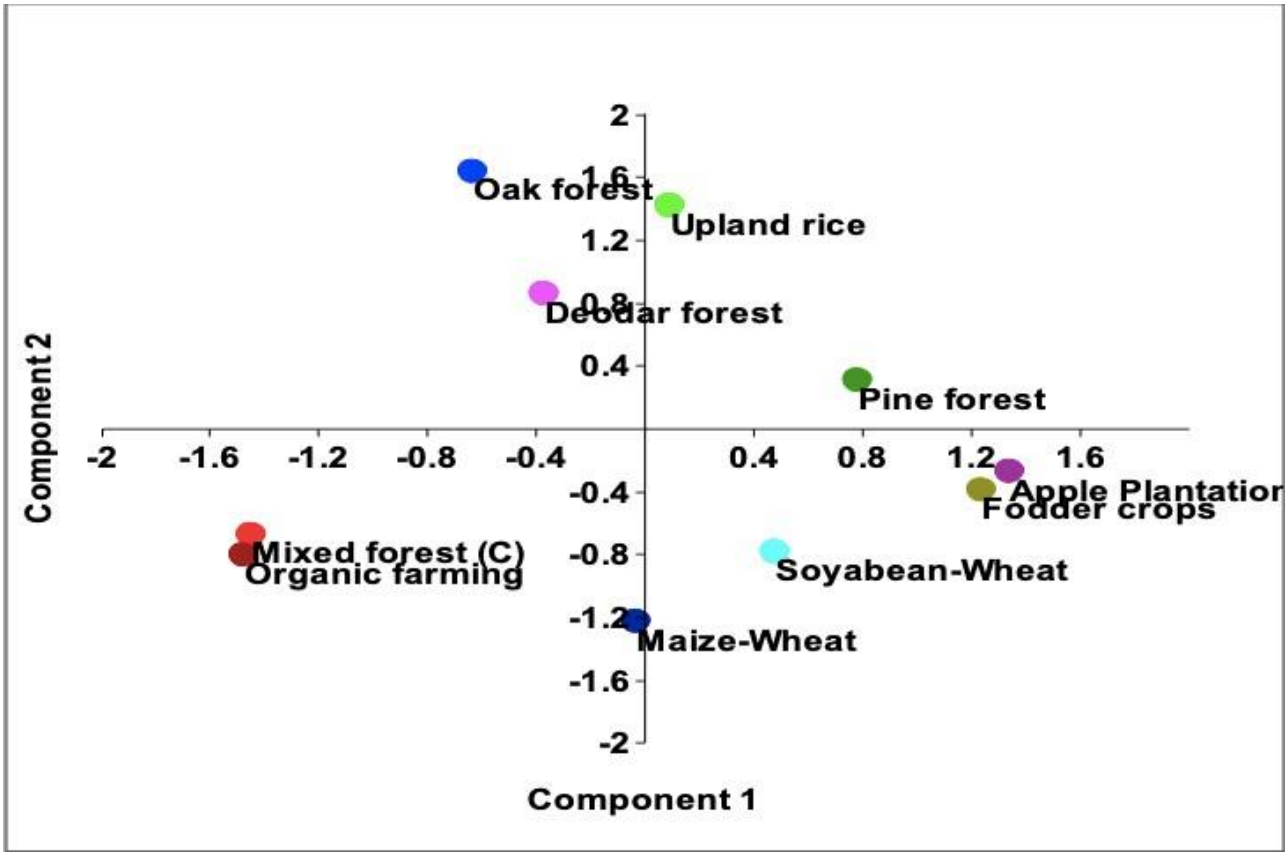
Figure 5: Impact of soil carbon fraction on soil microbial community composition (PLFA). (a) Correlation between Microbial Biomass C and PLFA (b) Correlation between soil organic carbon and PLFA mole fraction



The profiles exhibited variations in structure, characterized by the number and length distribution of major bands with the highest relative fluorescence intensity, which differed across soil types. Principal component analysis (PCA) was conducted on ARISA profiles to assess between-site variations. Bacterial-ARISA fingerprinting enabled the differentiation of each site. The differences observed between sites were more significant compared to the variations within independent repetitions of the same site. PCA analysis of RISA peaks, using the first two principal components (PCs), accounted for 68% and 12.8% of the total variance, respectively (**Figure 6**). Forest

ecosystems formed a distinct cluster, while organic farming exhibited close similarity with the mixed forest ecosystem. All other agro ecosystems clustered together.

Figure 6: Ordination of ten different land use system soils sites in function of mean of three subsequent years in the space defined by the PC1 and PC2 axis of the PCA analysis carried out with Automated Ribosomal Intergenic Spacer Analysis. Component 1 and 2 represent 68 and 12.6 % of the variation in the data respectively.



MF-Mixed Forest, DF-Deodar Forest, OF- Oak Forests, PF-Pine Forest, FD- Fodder cropping system, AP- Apple Plantation, MW- Maize wheat cropping system, SW- Soybean cropping system, OR- Organic Farm, UR- Rice cropping system

Discussion

The aim of this study was to investigate the impact of different land use systems on soil microbial community and composition. The researchers considered various land use systems, including forest systems (Mixed forest, Deodar forest, Oak forest, and Pine forest), well-maintained agricultural lands (Maize wheat cropping system, Soybean wheat cropping system, Organic farm, and Rice cropping system), and two long-term overlooked conventional ecosystems (Fodder crops and Apple plantation). The study focused on evaluating the range of variations within and between these land use systems by assessing soil enzymes, microbial biomass carbon, organic carbon, microbial abundance, and PLFA composition.

The researchers found that natural undisturbed soils under confined vegetation displayed the greatest equilibrium among the soil properties, which served as a reference to compare soils affected by agriculture (**Gil-Sotres et al., 2005**). Among the forest systems, the Mixed forest from Chubatiya exhibited higher enzyme activities compared to other forest ecosystems (Deodar forest, Oak forest, and Pine forest). This could be attributed to the similar litter decomposition materials that layer the canopy soils in the latter forest systems, affecting the secretion of extracellular enzymes by the microbial population (**Ushio et al., 2008**).

Significant variations in soil enzyme data were observed among different land use systems over the course of three years. Similar variations have been reported in other studies investigating the impact of land use on soil enzymes (**Acosta-Martínez et al., 2007**; **Rudramurthy and Shilpashree, 2011**). The forest soils generally exhibited higher soil enzyme activities compared to agro ecosystems, which is consistent with previous research indicating the influence of low organic matter content and subsequently lower soil enzyme activities in agricultural soils (**Dou et al., 2007**). The results of this study also align with the findings of **García-Ruiz et al. (2008)** and **Lori et al. (2017)**, which showed that organic farms tend to exhibit higher soil microbial enzyme activities compared to other conventional field soils.

The proximity of the organic farm to the other two long-term fertilizer-used farms (Maize wheat and Soybean wheat) may explain the comparatively lower levels of soil enzymes observed. Other studies have reported that repeated use of fertilizer can restrict microbial growth and consequently decrease soil enzyme production (**Reid et al 2021**). The apple orchard soils studied in this research were contaminated due to long-term usage of fungicides and pesticides, resulting in significantly

lower enzyme activities compared to the other soils. Similar findings have been observed in studies by **Wang et al. (2009)** and **Bielińska and Pranagal (2007)**, indicating the impact of long-term use of fungicides on the soil microbial population. Similarly, anthropogenic influences on fodder crop soils led to reduced microbial enzyme activities.

All enzyme activities showed a reduction with increasing depth, consistent with findings from other research studies **(Baligar et al., 2005; Trasar-Cepeda et al., 2000**; **Baldrian, 2009**). Microbial biomass carbon was found to be more sensitive than soil total organic carbon, as it represents the fraction of soil microbes that is highly responsive (**Onwosi et al 2019)**. The higher microbial biomass carbon in forest soils compared to other cultivated soils could be attributed to the higher organic matter content, which is reflected in the status of microbial biomass carbon. Similarly, at greater depths, organic matter content decreases, resulting in a proportional decrease in microbial biomass carbon, as found in this study and in accordance with other research **(Fierer et al., 2003)**. The study demonstrated a clear impact of land use on soil microbial biomass carbon and organic carbon, as these two parameters were highly correlated with each other and with other enzymes, consistent with studies highlighting the strong relationship between microbial biomass carbon and organic carbon (**Lepcha and Devi 2020**).

When considering soil enzymes, microbial biomass carbon, and organic carbon fractions, the forest and cultivated systems clearly segregated from each other. Forest systems were positioned on the right side of the PCA plot, while cultivated ecosystems were on the left, and the organic farm tended to occupy a middle position, sharing some properties of both forest and cultivated systems.

PLFA fingerprints were used to provide information on the composition of the microbial community and represent its active potential. While individual fatty acids cannot represent specific species of the microbial community, when linked with other metabolically functional components such as soil enzymes and microbial biomass carbon, the data can be practical for evaluating the impacts of physical, chemical, and biological factors on microbial composition.

The impact of different land use systems on microbial community composition has been widely studied in various contexts, such as nitrogen-amended cropping systems, organic farming, comparison of inorganic and organically amended soils, and tillage and no-tillage cultivated soils. However, there is limited research comparing forest, agriculture, and orchard ecosystems affected by different factors such as altitude and management practices.

The diversity and abundance of gram-positive bacteria were found to be high in nitrogen-amended cropping systems, consistent with the results of **Denef et al. (2009)**. The study also showed increased abundance of gram-negative bacteria in forest soils compared to cultivated soils, which can be attributed to the higher organic matter content in forest soils, in agreement with other researchers (**Zhong et al., 2010**) who have highlighted the influence of organic matter content on the growth of gram-negative bacteria.

The fungal biomarker 18:1w9c, which is present in most fungal species, was significantly higher in forest soils and organic farming sites compared to other cultivated ecosystems. This indicates the relatively higher presence of several fungal groups in forest soils. Similar findings of lower fungal biomass in cultivated soils have been observed in studies by **Lazcano et al. (2012**), which explain this decrease by intensive agriculture, anthropogenic disturbance, and the complexity of nutrient inputs compared to undisturbed and organically maintained soils.

Ribosomal intergenic spacer analysis (RISA) was used to analyze the structure of microbial populations in soil (**Rincon-Florez et al 2013)** by comparing the profiles after polyacrylamide gel electrophoresis. At most, only a few tens of bands were detected, suggesting a probable underestimation of diversity due to difficulty in identifying weak bands and resolving contiguous bands. The automated version of RISA, the ARISA, is a rapid and precise technique that allows microbial communities to be investigated and compared easily; highlighting the taxonomic diversity, evident from the marked variability in ribosomal spacer length, in the prokaryote genomes **(Fisher and Triplett 1997)**.

In conclusion, this study demonstrates higher variations in microbial community composition, soil enzymes, soil organic carbon, and microbial biomass carbon among different land use systems. The cultivation and management practices employed in agricultural systems have a negative impact on these parameters, emphasizing the importance of adopting sustainable land management practices to preserve soil health and microbial diversity. PLFA profiles permits the better chance to understand microbial community under different land use systems and may insights the impact of agricultural chemical fertiliser on soil micro biota.

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Conflicts of interest. The authors declare no conflicts of interest.

References

Acosta-Martinez, V., Mikha, M.M., Vigil, M.F. (2007) Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat-fallow for the Central Great Plains. Appl. Soil. Ecol. 37, 41-52.

Baligar, V.C., Wright, R.J., Hern, J.L. (2005) Enzyme activities in soil influenced by levels of applied sulfur and phosphorus. Comm. Soil Sci. Plant Anal. 36, 1727-1735.

Baldrian, P. (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environment 55, 370-378.

Bielinska E.J., Pranagal J. (2007) Enzymatic activity of soil contaminated with Triazine herbicides. Pol. J. Envron Stud. 16 (2), 295.

Carrasco-Espinosa, K., Avitia, M., Barrón-Sandoval, A., Abbruzzini, T.F., Salazar Cabrera, U.I., Arroyo-Lambaer, D., Uscanga, A., Campo, J., Benítez, M., Wegier, A., Rosell, J.A. (2022) Land-Use Change and Management Intensification Is Associated with Shifts in Composition of Soil Microbial Communities and Their Functional Diversity in Coffee Agroecosystems. Microorganisms 10(9), 1763.

Chen, J., Stark. J. (2000) Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology and Biochemistry 32, 47–57.

Denef, K., Roobroeck, D., Wadu, M., Lootens, P., Boeckx, P. (2009) Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology & Biochemistry 41, 144-153.

Dou, F., Wright, A.L., Hons F.M. (2007) Depth distribution of soil organic C and N after long-term soybean cropping in Texas. Soil & Tillage Research 94, 530-536.

Fierer, N., Schimel, J., Holden, P. (2003) Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

473 Fischer, M.M., Triplett, E. (1999) Automated approach for Ribosomal Intergenic Spacer Analysis of
474 microbial diversity and its application to freshwater bacterial communities. Applied and
475 Environmental Microbiology 65, 4630-4636.
476
477 García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A. (2008) Suitability of enzyme activities
478 for the monitoring of soil quality improvement in organic agricultural systems. Soil Biology and
479 Biochemistry 40, 2137-2145.
480
481 Ghosh, P., Dhyani, P.P. (2005) Nitrogen mineralization, nitrification and nitrifier population in a
482 protected grassland and rainfed agricultural soil. Tropical Ecology 42, 173-181.
483
484 Giweta, M. (2020). Role of litter production and its decomposition, and factors affecting the
485 processes in a tropical forest ecosystem: a review. Journal of Ecology and Environment, 44(1), 11.
486
487 Gil-Sotres, F., Trasar-Cepeda, C., Leiro´ s, M. C. (2005). Different approaches to evaluating soil
488 quality using biochemical properties. Soil Biology and Biochemistry, 37, 877-887.
489
490 Gregorich, E. G., Carter, M. R., Angers, D. A., Monreal, C. M., & Ellert B. H. (1994). Towards a
491 minimum data set to assess soil organic matter quality in agricultural soils. Canadian Journal of Soil
492 Science, 74, 367–385.
493
494 Hargreaves, P. R., Brookes, P. C., Ross, G. J. S., & Poulton, P. R. (2003). Evaluating soil microbial
495 biomass carbon as an indicator of long-term environmental change. Soil Biology and Biochemistry,
496 35(3), 401-407.
497
498 Karaca, A., Cetin, S. C., Turgay, O. C., & Kizilkaya, R. (2010). Soil Enzymes as Indication of Soil
499 Quality. In G. Shukla & A. Varma (Eds.), Soil Enzymology (pp. 127-141). Springer.
500
501 Klein, D. A., Loh, T. C., & Goulding, R. L. (1971). A rapid procedure to evaluate the
502 dehydrogenase activity of soils low in organic matter. Soil Biology and Biochemistry, 3(4), 385-
503 387.
504
505 Kolter, R., & Greenberg, E. P. (2006). Microbial sciences: The superficial life of microbes. Nature,
506 441, 300–302.

Lazcano, C., Gómez-Brandón, M., Revilla, P., & Domínguez, J. (2012). Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. Biology and Fertility of Soils, 48(7), 807-816.

Lepcha, N. T., & Devi, N. B. (2020). Effect of land use, season, and soil depth on soil microbial biomass carbon of Eastern Himalayas. Ecological Processes, 9, 23.

Lee, S. H., Kim, M. S., Kim, J. G., & Kim, S. O. (2020). Use of soil enzymes as indicators for contaminated soil monitoring and sustainable management. Sustainability, 12(19), 8209.

Li, F., Chen, L., Zhang, J., Yin, J., & Huang, S. (2017). Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. Frontiers in Microbiology, 8, 187.

Liu, G., Bai, Z., Cui, G., He, W., Kongling, Z., Ji, G., Gong, H., & Li, D. (2022). Effects of Land Use on the Soil Microbial Community in the Songnen Grassland of Northeast China. Frontiers in Microbiology, 13, 848.

Lori, M., Symnaczik, S., Mäder, P., De Deyn, G. and Gattinger, A. (2017). Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression.

Mandal, A., Patra, A. K., Singh, D., Swarup, A., Masto R. E. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology 98, 3585-3592.

McGonigle, T.P., Turner, W.G. (2017). Grasslands and croplands have different microbial biomass carbon levels per unit of soil organic carbon. Agriculture, 7(7), p.57.

Meena, A., Rao, K.S. (2021). Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region, India. Ecological Processes, 10(1), pp.1-12.

Onwosi, C.O., Odimba, J.N., Igbokwe, V.C., Nduka, F.O., Nwagu, T.N., Aneke, C.J., Eke, I.E. (2019). Principal component analysis reveals microbial biomass carbon as an effective bioindicator of health status of petroleum-polluted agricultural soil. Environmental Technology.

Patra, A.K., Chhonkar, P.K., Khan, M.A. (2006). Effect of green manure Sesbania sesban and nitrification inhibitor encapsulated calcium carbide (ECC) on soil mineral-N, enzyme activity and nitrifying organisms in a rice–wheat cropping system. European Journal of Soil Biology, 42, 173-180.

Quideau, S.A., McIntosh, A.C., Norris, C.E., Lloret, E., Swallow, M.J., Hannam, K. (2016). Extraction and analysis of microbial phospholipid fatty acids in soils. JoVE (Journal of Visualized Experiments), (114), p.e54360.

Ranjard, L., Poly, F., Lata, J.C., Mougel, C., Thioulouse, J., Nazaret, S. (2001). Characterization of bacterial and fungal soil communities by Automated Ribosomal Intergenic Spacer Analysis fingerprints: biological and methodological variability. Appl. Environ. Microbiol. 67, 4479-4487.

Reid, T.E., Kavamura, V.N., Abadie, M., Torres-Ballesteros, A., Pawlett, M., Clark, I.M., Harris, J., Mauchline, T.H. (2021). Inorganic chemical fertilizer application to wheat reduces the abundance of putative plant growth-promoting rhizobacteria. Frontiers in Microbiology, p.458.

Rincon-Florez, V.A., Carvalhais, L.C., Schenk, P.M. (2013). Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity, 5(3), pp.581-612.

Singh, R.D., Patra, A.K., Bhattacharya, R., Ghosh, B.N. (2015). Sustainable crop production and soil health in the north-western Himalayan region. Soil Biodiversity: Inventory, Functions and, p.163.

Trasar-Cepeda, C., Leiros, M.C., Gil-Sotres, F. (2000). Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, NW Spain): specific parameters. Soil Biology & Biochemistry 32, 747-755.

Ushio, M., Wagai, R., Balser, T.C., Kitayama, K. (2008). Variations in the soil microbial community composition of a tropical montane forest ecosystem: Does tree species matter? Soil Biology and Biochemistry 40, 2699-2702.

Wang, Q.Y., Zhou, D.M., Cang, L. (2009). Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biology and Biochemistry 41 (7), 1504-1509.

577 Wang, S., Mori, T., Zou, S., Zheng, H., Heděnec, P., Zhu, Y., Wang, W., Li, A., Liu, N., Jian, S., Liu,
578 Z. (2022). Changes in vegetation types affect soil microbial communities in tropical islands of
579 southern China. Global Ecology and Conservation, 37, p.e02162.
580
581 Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., Shen, W. (2010). The effects of
582 mineral fertilizer and organic manure on soil microbial community and diversity. Plant and Soil
583 326, 511-522.