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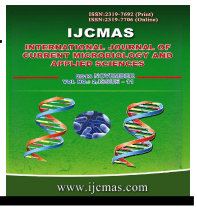

Original Research Article

Probing of microbial community structure, dehydrogenase and soil carbon in-relation to different land uses in soils of Ranichauri (Garhwal Himalayas)

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K e y w o r d s

Soil enzymes; Microbial biomass carbon; Organic carbon; Phospholipid ester-linked fatty acid; Different land use; Ranichauri.

A B S T R A C T

Indian Himalayas have a special place in mountain biodiversity. More and more lands in the Central Himalayan region were subjected to agriculture as a consequence of increasing food demand. Changing land use has significant impact on soil organic carbon, microbial biomass carbon and subsequently on soil microbial community composition. Soil biochemical indicators such as phospholipid ester-linked fatty acid (PLFA), microbial biomass carbon (MBC) and soil enzymes are highly sensitive to any change in microhabitat. We investigated six different land use system ranging from mixed undisturbed forest (> 100 years), Rice Wheat cropping system, Maize Wheat cropping system, Finger millet and Cabbage farm, and the uncultivable barren land for microbial biomass, organic carbon, soil enzyme dehydrogenase and Phospholipid fatty acid analysis. The soil organic carbon (SOC) and microbial biomass carbon (MBC) are significantly higher in forest than cultivated and barren lands. Microbial biomass carbon showed close relationship with soil phospholipids fatty acid profiles than organic carbon. Principal Component Analysis (PCA) drawn with respect to PLFA, MBC, OC and Soil dehydrogenase enzyme of six land use systems clearly segregates the agro ecosystems indicating the more or less similar type of microbial community composition. Fungal to Bacterial PLFA ratio were predominantly higher in forest systems. The soil functional fraction (PLFA) profiles with correlation with soil enzymes, microbial biomass carbon and organic carbon are effective to the study the impact of land use and management practices on soil microbial communities and apparently the soil health of a particular site.

Introduction

Soil is a biological habitat, which constitutes huge microbial gene pool (Blum et al. 2004) and one of the most complex biological communities on earth (Barrios, 2007). Due to increasing food demand, more and more lands were

subjected to agriculture in Indian Himalayas (Shah 1996, Ghosh and Dhyani 2005). The ecosystem conversion has a significant impact on the natural biodiversity of the soil. In addition, land intensification for agriculture leads to significant reduction of soil carbon fraction (Lal 2002; Steenwerth et al. 2005) and result in insightful changes in soil microbial diversity (Ding et al. 2013). Soil microbes are very useful to assess the impact of land use as they respond very swiftly to the change in their microhabitat. Soil microbial biomass carbon is viable and lively part of organic carbon present in soil, which quickly point out the land or soil quality degradation (Anderson et al. 1989; Powlson et al. 1987). Moreover, the active microbial fraction decomposes organic material and reflects the sum of all biological factors regulating the decomposition and transformation of the nutrients (Baath and Anderson, 2003).

Anthropogenic activities like agriculture and use of pesticides may predominantly affect soil biodiversity (Krik et al. 2004) and the unfortunate part is that we are unaware how the microbial diversity changes and influence the below ground biodiversity. Relationship between soil microbial diversity and function cannot be judged by the use of soil DNA and RNA extracted from soil alone, however it also require the high resolution technique to detect the viable microbial cell in the matrix (Nannipieri et al. 2003). Phospholipid relative abundance is the fine and easiest way to evaluate viable microbial activity (White, 1993; Tunlid and White, 1992) as phospholipids were not found in non-viable cells. Thus are efficient indicators of metabolic diversity of soil microbial communities (Yao et al. 2002; Steenwerth et al. 2002 Steenwerth et al. 2005).

From the soil, microbial metabolic diversity point of view the selected region of Ranichauri for this study is scientifically unexplored. In the present investigation we aim to study the impact of land use systems namely mixed forest (MF), Rice wheat cropping system (RW), Fingermillet (FM), Maize wheat cropping system, Cabbage (CB) and Barren land (BL) having similar type of soil in a particular transect on soil microbial communities (PLFA), soil dehydrogenase, microbial biomass carbon (MBC), organic carbon (OC).

Materials and Methods

Site description and sample processing

The study sites were from Tehri Garhwal districts located at 27^{o} 18' N, 86^{o} 00' E on the outer ranges of the mid-Himalaya of Uttarakhand state, India. The region has a sub-temperate to temperate climate characterized by moderate summer (May–June), extreme winter (Dec–Jan), general dryness, except during the southwest monsoon season (June–Sept), and the mean temperature ranges from 12.5^{o}C to 32^{o} C.

The parent material of these soils consists of mica, schist, slates, sand stone, and calcium deficient granite and seynite rocks. Genetically these soils come under climatogenic podsolized grey-brown forest soils. All the systems were having acidic soil reaction except the soils of cultivated fields, which were slightly acidic. We selected six land use systems ranging from undisturbed forest (more than 100 years) rice-wheat, conventional maize wheat farm, finger millet and cabbage farm and the barren land from Ranichauri agricultural farm of College of Hill Agriculture and forestry, Uttrakhand, India

and nearby location. Three composite soil samples from each site of 0-20 cm depth were collected in July 2012. For making one composite sample, five soil cores were taken and mixed. Other workers (Patra et al. 2006) have also adopted pseudo-replication approach of sampling. The field moist soil samples were kept stored in refrigerator at temperature less than 4^{o}C for preserving the enzyme activities until the analysis were over. All chemical results are mean of triplicate analysis and expressed on oven dry basis. Soil moisture was determined after drying at 105°C for 24 h.

Soil dehydrogenase, microbial biomass carbon and organic carbon

Soil dehydrogenase activity was determined using the method of Klein et al 1985 by the mixture of 0.2 ml of 3% triphenyltetrazolium chloride (TTC) solution and 0.5 ml of 1% glucose to 1 gm soil sample. Samples were incubated at 28^{o} C for 24 hours and then 10 ml of methanol was added and again incubated at 28^{o} C for 8 hours. The pull out triphenyl formazan (TPF) was measured by absorbance at 485 nm.

MBC was determined using the chloroform fumigation method (Vance et al. 1987). 20g of sieved soil was weighed in six 100ml beakers. Three of these were extracted immediately with 75ml of 0.5M K_2SO_4.The other three were fumigated in a dessicator containing 25ml ethanol free chloroform and a few glass chips in a 50ml beaker and lined with wet filter paper (to maintain humidity). The dessicator was evacuated using suction until the $CHCl_3$ had boiled for 2 minutes. The fumigated dessicator was then placed in an incubator set at 25˚C in dark. After 24 hours, the beaker containing $CHCl_3$ was removed from the dessicator and residual $CHCl_3$ vapour in the soil was removed by repeated evacuation. The soil was then extracted with 75ml of 0.5M K_2SO_4 in a 250 ml flask. The flasks were shaken for 30 minutes on a shaker and the suspension was filtered using Whatman No. 42 filter paper. Organic C in the extract was measured using dichromate digestion. The difference between the carbon in the unfumigated and chloroform-fumigated samples was used to calculate the MBC with the correction factor related to the proportion of microbial biomass (Liu 2012).

The determination of soil organic carbon is based on the Walkley-Black chromic acid wet oxidation method (Allison, 1965). 1g soil was weighed in a 500ml Erlenmeyer flask to which 10ml of 0.1667M $K_2Cr_2O_7$ solution and 20ml of concentrated H_2SO_4 containing Ag_2SO_4 were added. The contents were mixed thoroughly and the flasks were allowed to stand for 30 minutes. The reaction mixture in the flasks was diluted by adding 200ml of deionised water and 10ml of concentrated H_3PO_4. 10ml of sodium fluoride solution and 2ml of diphenylamine indicator were then added. The unreduced dichromate was determined by back titration with 0.5M $FeSO_4$ solution till end point marked by change in colour from violet-blue to brilliant green was reached.

Soil Phospholipid Fatty Acid Analysis (PLFA)

Soil phospholipid fatty acid (PLFA) was extracted and measured using methods Buyer et al. 2010. Briefly, 5 g of lyophilized soil were extracted by Bligh-Dryer extraction and after evaporation the lipids were separated on solid phase

extraction coloumn. Then phospholipids were then eluted by 5 ml methanol. The extracted phospholipid were transesterified to fatty acid methyl ester and analyzed by Gas chromatograph Agilent Technologies, Wilmington, DE, USA) equipped with auto sampler and flame ionization detector and controlled with MIS Sherlock® (MIDI, Inc., Newark, DE, USA). The composition of soil microbial community was identified by microbial analysis software (Sherlock MIS 4.5 System, MIDI, USA) based on the spectrogram of specific PLFA. For each sample, individual PLFA values were expressed as a percentage of the total PLFAs (mole %) in the sample. Analysis was done in triplicates and the nomenclature of fatty acid was according to Feng et al. 2003.

Statistical Analysis

The concentrations of individual PLFA as calculated as the mole percentage of total PLFA were subjected to principal component analysis to determine the variation in PLFA signature with the impact of different land use studied. PCA was conducted using PAST software. Loading score of the sum of total PLFA signatures in mol percentage for each land use system were used to assess the relative importance of land use on microbial communities.

Difference between mean values of soil dehydrogenase, microbial biomass carbon and organic carbon of different land use system three year were evaluated by a two-way analysis of variance, component of variation with Fisher's LSD tests as post-hoc tests and Pearson correlation analysis using the software SPSS 16.0 for test of significance. Unless otherwise stated, the level of significance referred to in the results is $P < 0.05$.

Results and Discussion

Soil organic carbon and microbial biomass carbon affected by different land use systems

Soil organic carbon and microbial biomass carbon are significantly affected by the land use and the management practices (Table 1). Microbial biomass C ranged from 619.63 to 156.75 mg kg^{-1} at surface soil (0-20cm) from forest to barren land. However, organic carbon content in six different land use system followed the order: Mixed forest (1.8) > Rice wheat cropping system (1.47) >Fingermillet cropping system (1.41) >Maize wheat cropping system (1.32) >Cabbage Farm (0.9) > Barren land (0.44).

The organic and microbial biomass carbon content was 309 % and 294 % higher in the forest soil in comparison to the barren land respectively. The microbial biomass carbon strongly correlated with total PLFA (R^2=0.75) than organic carbon (R^2= 0.70) while correlating microbial biomass carbon and organic carbon with total PLFA mole fractions of different land use system on XY graph (Fig 1). Makova et al. 2011 and Justin et al. 2013 found that variation in soil use significantly influenced microbial biomass carbon and soil organic carbon. Soil microbial biomass carbon values relays with the reported MBC values by Vance et al. 1987.

Microbial biomass carbon and organic carbon are strongly correlated (Table 2) in agreement with Sharma et al. 2004. Land use have the significant impact on soil microbial biomass carbon like the previous finding by Kara et al. 2007 and quantity and quality of organic carbon by Guo et al. 2013.

Table.1 Impact of Land use system on dehydrogenase (DHA-µg TPF g^{-1} soil h^{-1}), microbial biomass carbon (MBC- mg kg^{-1}), organic carbon (OC- %) and Total PLFA (mol %) 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 (Dun can's Multiple Range Test) for separation of means.

Land-use	**DHA**	**MBC**	**OC**	**PLFA**
At Depth 0-20cm				
Mixed forest	9.62^{a}	619.63^{a}	1.8^{a}	150.77^{a}
Rice Wheat	7.71^{b}	537.38^{b}	1.47^{b}	85.2^{b}
Finger Millet	6.12^{d}	409.28^{d}	1.41^{b}	80.82^{b}
Maize Wheat	5.84^{e}	463.51^{c}	1.32^{b}	60.31^{c}
Cabbage	7.26^{c}	327.42^{e}	0.9^{c}	76.88^{bc}
Barren	2.1^{f}	156.75^{f}	0.44^{d}	31.05^{d}

Table.2 Pearson's correlation coefficients among dehydrogenase, organic carbon and microbial biomass carbon, and total PLFA fraction from different land use systems of Ranichauri (Garhwal Himalayas)

Pearson's Correlation	MBC	OC	PLFA
DHA	$.889^{*}$	$.853^{*}$	$.910^{*}$
MBC		$.974^{**}$	$.837^{*}$
OC			$.838^{*}$

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

The findings of this study are in consent with Fang et al. 2011 that the decrease in soil nutrient with artificial disturbance reduces the microbial biomass carbon and affect other ecological processes. Within the agro ecosystem, the organic carbon shows 63% variation, however these variation are consequences of the management practices as if amendment of the organic fertilizer, and moreover SOC alone does not adequately reflect change in soil quality (Franzluebbers et al. 1995), however the microbial biomass carbon, a viable fraction when correlated may reveal the soil quality scenario.

This study result shows the impact of soil organic carbon has a significant impact on phospholipid fatty acid content of soil. For instance in agro system cabbage farm having comparatively less organic carbon subsequently having lesser microbial biomass carbon and total PLFA content in agreement with Potthoff et al. 2006 and Bailey et al. (2002).

Soil metabolic activity revealed by dehydrogenase enzyme activity

The dehydrogenase activity in surface was lower in the barren land (2.1 µg TPF g^{-1} soil h^{-1}) than forest soil (9.62 µg TPF g^{-1} soil h^{-1}) and cultivated soils; moreover, the cultivated soils (6.12-7.71 µg TPF g^{-1} soil h^{-1}) contain comparatively less DHA activity than forest soils for the similar type of soil (Table 1). Soil microbial viabilities are reflected through soil enzymes, which act swiftly to the environmental change and land use (Nannipieri et al. 2002; Garcia et al. 2000). In this study soil dehydrogenase activity is significantly affected by land use and significantly correlated with organic carbon in agreement with Skowron et al. 2010 and Leiros et al. 2000. Similar result were observed in the other parallel areas of North western Himalayas proposing the higher fertility index in the forest soil and reduced fertility in agro system followed by waste land by Pal et al. 2013.

Microbial Community Structure signified by Phospholipid Fatty Acid Analysis

PLFA analysis identified 65 different fatty acids and twenty of them were consistently present in the samples were used for data analysis (Table 3). Fatty acid analyzed having carbon chain C14 to C18 comprising branched saturated gram positive, mono saturated gram negative, cyclopropane gram negative, polyunsaturated fungi and methyl-branched actinomycetes fatty acids marker.

Bacteria were the major microbial group in the six different land use systems, accounting 48 % of total microbial soils ranges from 2.1 – 9.62 µg TPF g^{-1} soil h^{-1} and it was significantly affected ($P < 0.05$) by changing land use. For example, the DHA activity community, followed by fungi with comprising 24.2 and then by actinomycetes with 7.4%. The gram-positive, gram-negative and fungal populations were significantly higher in forest soils (Table 4). The mean fungal to bacterial PLFA ratio (the sum of mole percent for 15:0 anteiso,15:0 iso, 16:0 iso, 17:0 anteiso, 17:0 iso, 17:1 w8c, 18:1 w5c, 18:1 w7c 17:0 cyc to the sum of 18:1 ω9c, 18:2 ω6,9c and 18:3 ω6c) is majorly affected by agriculture as the values attaining nearer to the barren land quality.

In other reported studies by Potthoff *et al*., (2006) and Guggenberger *et al*. ,(1999), the fungal diversity are widely influenced by the management practices similar to our findings that land use systems has significant impact on the fungal communities ranging from 25.7-43.1 in agro ecosystems (Table 3). Mulder and Elser 2009 shows that fungal to bacterial ratio were significant higher in nutrient available sites rather than in neglected field. Their result may be interpret here, as the fungal to bacterial ratio in our study are much lower in cultivated systems as compared to the forest where soil receives continuous litter and have better nutrient status. Principal component analysis identified the fatty acids responsible for the variation in PLFA profiles (Figure 2). The PLFA gram positive bacterial marker 15:0 anteiso (2.4), 15:0 iso (10), 16:0 iso (1.6) were predominant in PC 1, however gram positive 17:0 anteiso (0.33), 17:0 iso (0.7) comprises PC 2. The PLFA gram-negative bacteria marker 18:1 ω7c (7.2) were major element in PC 1 and 19:0 cyc (1.2) were in PC 2 (Table 4).

Table.3 Mean relative abundance (mol PLFA-C %) of different land use systems of Ranichauri (Garhwal Himalayas). The mean followed by different letters are significantly different at p<0.05, according to DMRT (Dun can's Multiple Range Test) for separation of means.

Community	**PLFA**	**MF**	**RW**	**FM**	**MW**	**CB**	**BL**
Actinomycetes	10 Me 18:0	2.7b	nd	3.5a	1.1c	2.1b	0
	10 Me 17:0	0.37c	nd	0.89a	0.56b	1a	0.22d
	10 Me 16:0	7.59a	3.5c	3.9d	2.8b	5.8b	2.1d
Fungi	18:1 w9c	45.7a	8.7b	8.9b	8.2b	5.6c	2.4d
	18:2 w6,9	1.3c	6.2a	1.9b	nd	nd	1.8b
	18:3 w6c (6,9,12)	1e	4.6a	3.6b	3.2c	2.5d	nd
AM Fungi	16:1 w5c	2.1a	1.1b	0.91c	0.35d	1.1b	0.11d
Branched Gram Positive Bacteria	14:0 iso	2.2a	nd	1.5b	0.3d	1.1c	0.12de
	15:0 anteiso	8.8a	6.5b	5.4c	4.3d	5.1c	1e
	15:0 iso	15.5a	8.9b	6.1d	7.1c	6.2d	1.3e
	16:0 iso	9.2a	4.2c	5.8b	2.1e	4.6b	2.6e
	17:0 anteiso	2.4b	2.4b	3.1b	2.5b	2.3b	6a
	17:0 iso	5.2a	3.5c	4.2b	2.1e	4.5b	2.6e
Monosaturated Gram Negative Bacteria	17:1 w8c	0.51de	0.74c	1.5b	0.3e	0.88d	1.42a
	18:1 w5c	2.4a	1.1cd	1.29d	1.4b	2.3a	1.2cd
	18:1 w7c	14.2a	7.4b	5.9c	2.1d	5.6c	1.6d
Cyclo-Propyl Gram Negative Bacteria	17:0 cyc	5.1a	2.66b	2.4bc	1e	2.3d	nd
	19:0 cyc	3.9c	5.6a	1.9e	2.5d	4.2b	1.5f
Non Specific	16:00	15.7a	12.9a	14a	13.2a	15a	2.7b
	18:00	4.9b	5.8a	4.2c	5.2ab	4.7bc	2.08d
Total Mole Fraction (PLFA)		150.77	85.2	80.82	60.31	76.88	31.05

For a given PLFA, values having different alphabet indicate significant mean difference within the series (rows) of PLFA biomarkers with respect to land use system observed (P <0.05 by LSD test, n=3). *nd= Not Detected.*

Table.4 Mole percentages (%) of soil microbes (Gram positive bacteria, Gram Negative bacteria, Fungi and Fungal to bacterial ratio) under different land use system. Mixed forest, Rice Wheat cropping system (RW), Finger millet cropping system (FM), Maize Wheat cropping system (MW), Cabbage farm (CB) and Barren Land (BL). The mean followed by different letters are significantly different at $p<0.05$, according to DMRT (Dun can's Multiple Range Test) for separation of means.

Land-use	**Gm+ve**	**Gm-ve**	**Fungi**	**Fun/Bac**
Mixed Forest	43.3^{a}	26.11^{a}	69.41^{a}	0.72^{a}
Rice Wheat	25.5^{b}	17.6^{b}	43.1^{b}	0.48^{b}
Fingermillet	26.1^{b}	12.92^{d}	39.02^{c}	0.39^{c}
Maize wheat	18.4^{d}	7.3^{e}	25.7^{d}	0.46^{b}
Cabbage	23.8^{c}	15.28^{c}	39.08^{c}	0.24^{d}
Barren Land	13.92^{e}	5.72^{f}	19.64^{e}	0.22^{d}

Table.5 PLFAs receiving scores on the first two principle components from soils of different land use systems of Ranichauri (Garhwal Himalayas)

PLFA Marker	**PC I**	**PC 2**
10 Me 18:0	-6.2491	-1.7714
10 Me 17:0	-9.2584	-2.6403
10 Me 16:0	0.1821	0.62997
18:1 w9c	37.994	-9.2751
18:2 w6,9	-7.0628	-0.81414
18:3 w6c (6,9,12)	-6.1774	1.9836
16:1 w5c	-7.4146	-2.8548
14.0 iso	-7.5158	-3.2453
15:0 anteiso	2.452	2.112
15:0 iso	10.032	2.3468
16:0 iso	1.8627	0.081918
17:0 anteiso	-5.5086	0.33967
17:0 iso	-2.2876	0.79221
17:1 w8c	-8.868	-2.2476
18:1 w5c	-6.6184	-1.7411
18:1 w7c	7.2479	-0.28114
17:0 cyc	-3.7252	-2.1025
19:0 cyc	-3.5121	1.2003
16:00	15.75	14.211
18:00	-1.3226	3.2758

Figure 1: 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, (Replication n=3 were averaged)

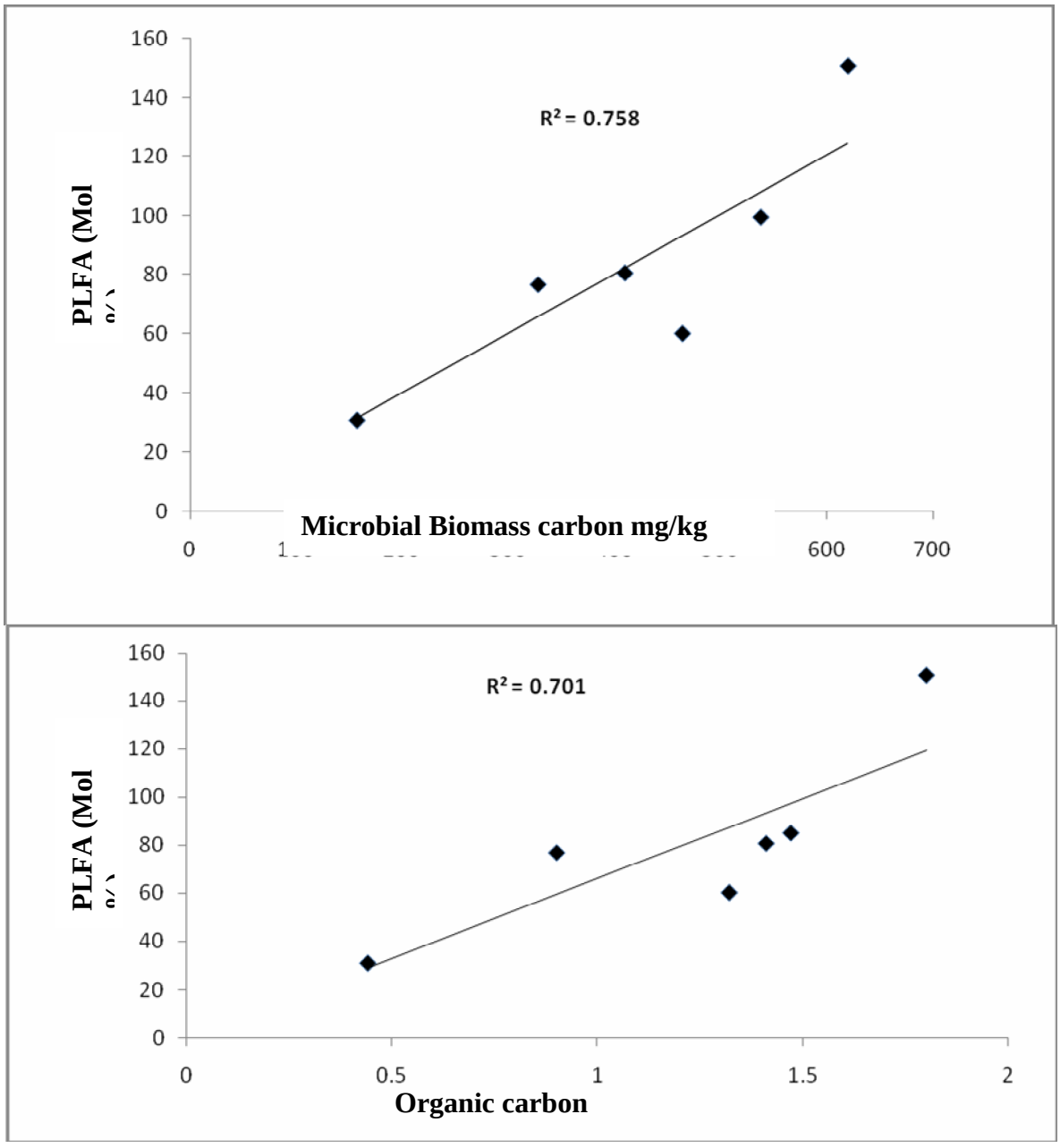
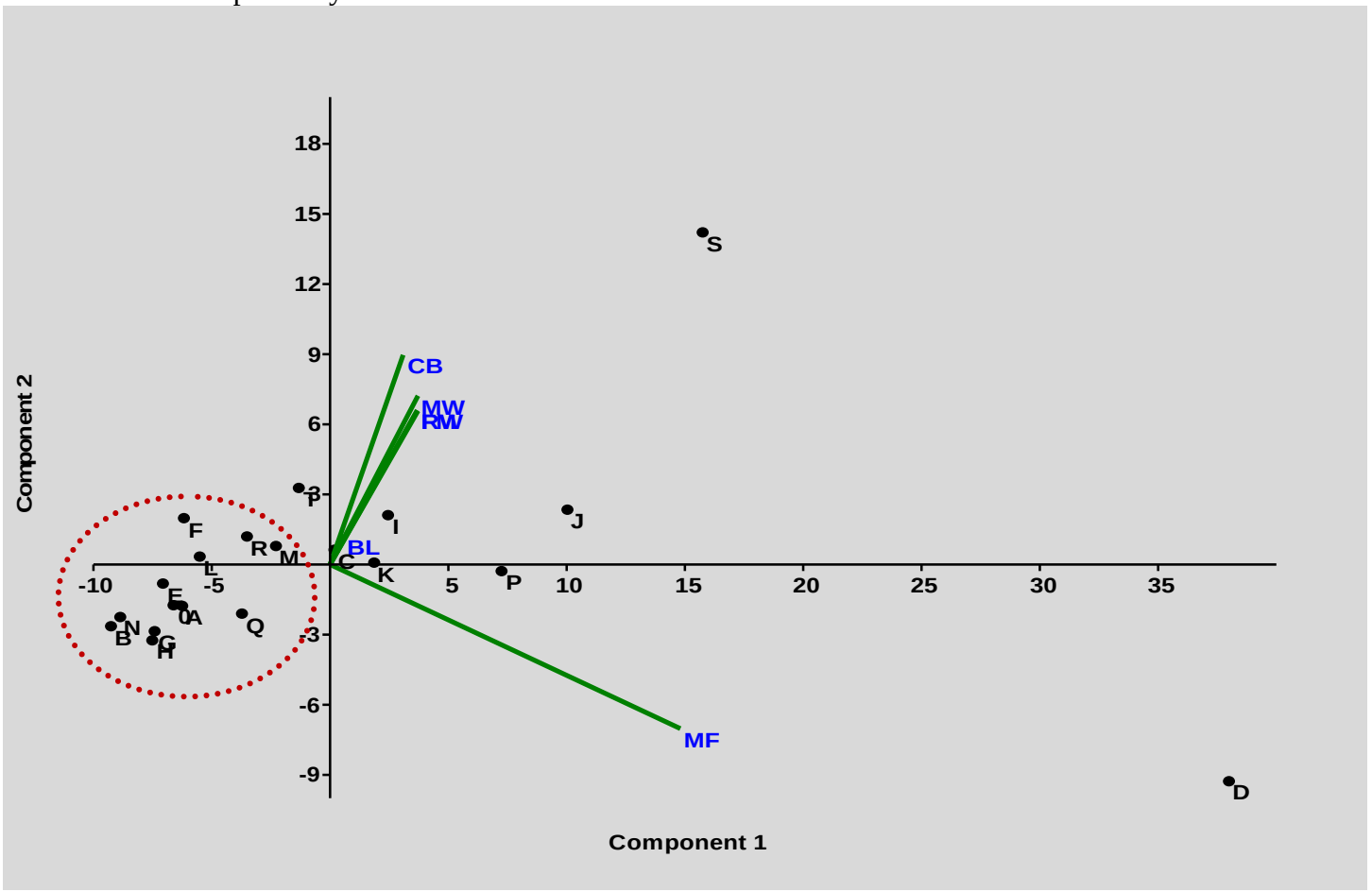
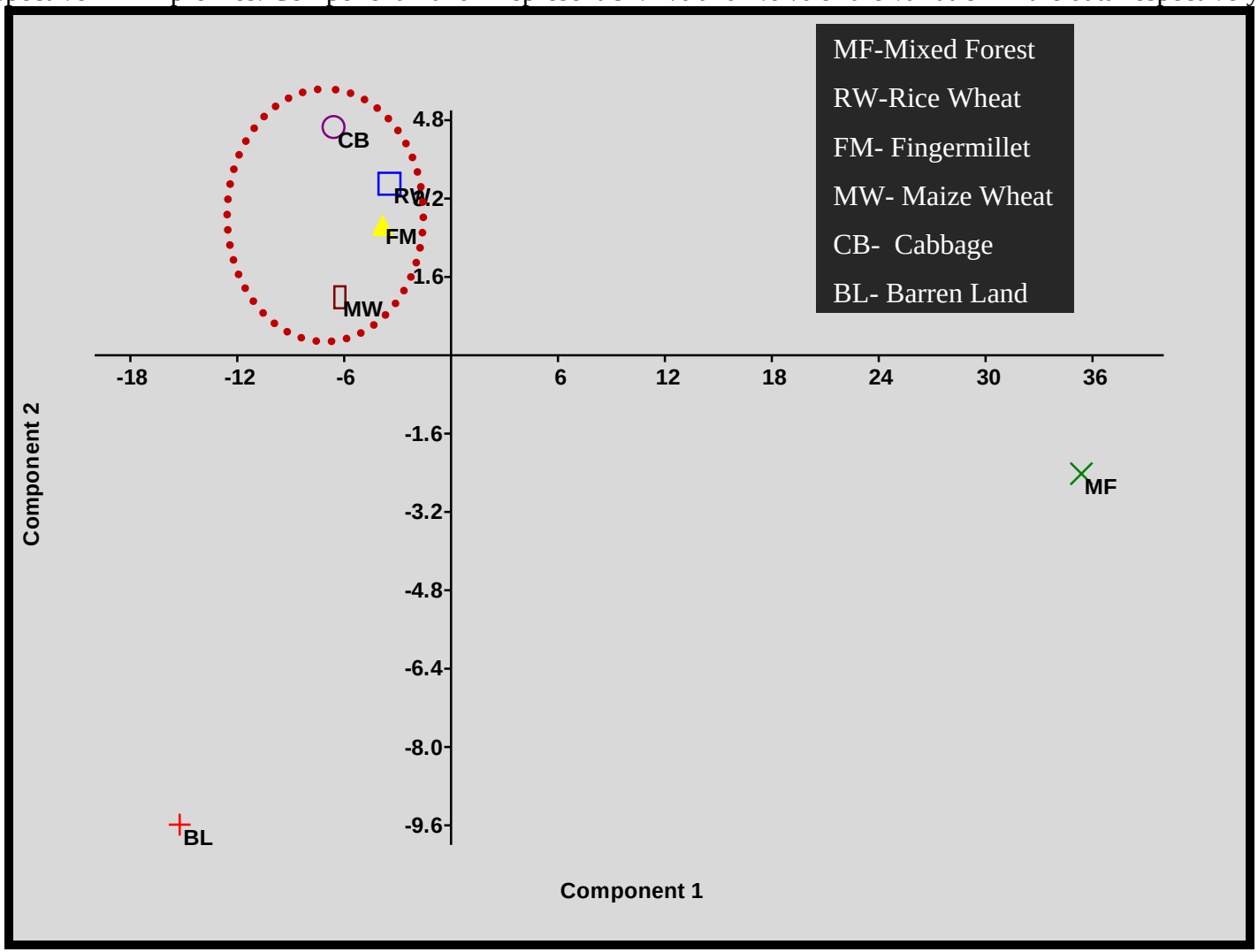


Figure.2 PCA ordination biplot of the individual PLFA profiles for 6 different land use systems. Shorter distances between sites in the PCA ordination indicate high degree of similarity between systems and their respective PLFA profiles. Component 1 and 2 represent 83.49 % and 12.6 % of the variation in the data respectively



A =10 Me 18:0 **B**=10 Me 17:0 **C** =10 Me 16:0 **D**= 18:1 w9c **E** =18:2 w6,9 **F** =18:3 w6c (6,9,12) **G**= 16:1 w5c **H** =14:0 iso **I** =15:0 anteiso **J** =15:0 iso **K** =16:0 iso **L** =17:0 anteiso **M**= 17:0 iso **N**= 17:1 w8c **O** =18:1 w5c **P** =18:1 w7c **Q** =17:0 cyc **R**=19:0 cyc **S**= 16:00 **T**= 18:00

Figure.3 PCA ordination of the PLFA profiles, Dehydrogenase enzyme activity, microbial biomass carbon and organic carbon for 6 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 87.4 % and 7.6 % of the variation in the data respectively.



The fungal marker 18:1 ω9c (37.9) were present in PC 1 and 18:3 ω6c (6, 9, 12) (1.9) were segregated to PC 2 (Table 5). Zhao et al. 2013 suggested that different land uses have indirect effect on soil microbial community functional diversity through the changing soil nutrient availability. Our study supports this agreement as our findings clearly shows the forest having higher microbial dehydrogenase activity proportionally higher microbial population due to continuous decomposition and has much better microbial diversity profile than the cultivated systems and we can discriminate the diversity loss in the agro ecosystem.

The ecological interpretation of the community structure is tedious as the individual fatty acid cannot represent specific species of the microbial community, however if linked with the other metabolically functional part as soil enzymes and microbial biomass carbon the data may be practical to evaluate the impacts of physical, chemical and biological factors on microbial composition. Fig. 3 shows the plots of the Principal Component Analysis based on the mol % PLFA-C of the microbial communities, soil dehydrogenase, microbial biomass carbon and organic carbon with respect to different land use systems. The first principal component axis (PC1) explained 87.4% of the variance in the data while the second principal component axis (PC2) explained only 7.6%. The principal component analysis of PLFA profiles of six different land use systems were sketched, the cultivated ecosystem soils (maize wheat, rice wheat, finger millet and cabbage) were tend to be on the top left of the PCA plot and segregated in a major cluster. However, the barren land segregated on the left bottom with a large-scale difference with right hand segregated forest ecosystem soil. The forest soil are high in organic matter content as a consequence the significantly diverse and higher microbial contents were observed in our study, similarly Moeskops et al. 2010 observed that the organically managed farms contains significantly higher phospholipid fatty acid marker than the conventional farm. The total PLFA contents of different land use systems are significantly ($P < 0.05$) correlated with soil dehydrogenase activity.

It was conclude that land use has the significant impact on the soil dehydrogenase, organic carbon, microbial biomass carbon and microbial community structure in studied soil samples of Ranichauri, Indian Himalayas. Phospholipid fatty acid biomarkers shown to be an effective indicator to evaluate the soil quality and can easily interpreted with other viable factors like soil enzymes, microbial biomass carbon etc for the conclusive remarks. By taking the forest soil as positive reference and barren land as negative reference of soil quality, the microbial diversity loss may be evaluated in the similar type of soils. Our study as far as we researched is the first attempt to understand the viable soil microbial community structure or microbial diversity in soils of Ranichauri, Garhwal Himalayas

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