EVALUATION OF SOIL MICROBIAL BIOMASS CARBON AND NITROGEN DISTRIBUTION UNDER DIFFERENT AGRO-CLIMATIC CODITIONS

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ABSTRACT: Soil microbial biomass (SMB) is a chief marker of changes that may occur in soil fertility. It represents an important source and sink of nutrients and systematize the cycling of nutrients. The objective of the study was to determine soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) and their correlation with selected physical and chemical properties. Selected soils with different land uses (cultivated, forest and grassland) and climatic conditions were Eutrudepts and Hapludolls. The forest soils of Eutrudepts and Hapludolls showed the highest amount of SMBC that was 265.01 mg / Kg in Eutrudepts. In Eutrudepts, SMBN was higher (48.713 mg / Kg) in forest soils while in Hapludolls statistically non-significant difference was observed among land uses. The SMBC and SMBN were positively correlated with soil organic matter (OM) (0.81 and 0.73), total N (0.62 and 0.59) and extractable K (0.51 and 0. 52) at Hapludolls. The same trend was observed at Eutrudepts. The SMBN and SMBC were lowest (30.48 and 136.18 mg /Kg) in cultivated land while, Hapludolls had higher SMBC (213.49 mg /Kg) than Eutrudepts (205.79 mg /Kg) and lower SMBN (40.18 mg /Kg) than Eutrudepts (42.45 mg /Kg) .

Key words: Soil microbial biomass, Nitrogen and Carbon, Climatic Conditions, Soil properties.

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INTRODUCTION

There is a need to identify early indicators of soil fertility to ensure global food security. Soil microbial biomass (SMB) involved in majority of processes may give integrated measure of soil health (Sharma *et al*., 2011). The changes in SMB distribution indicate early sign of soil fertility or an early soil degradation warning (Trivedi *et al*., 2015). The SMBC comprises 1 to 5% of total organic carbon (Gil-Sotres *et al*., 2005; Nsabimana *et al*., 2004). The SMBC responds readily to soil environment due to its high turnover rate. The SMB acts in a sink; immobilization as well as mineralization of essential nutrients (Fosu *et al*., 2007) and get influenced by environmental changes such as moisture and temperature (Lodhi *et al*., 2009). Soil management practices and use of land has affected the SMB distribution (Singh *et al*., 2014). Grass and cultivated lands develop different characteristics by influencing soil chemical, biological and physical properties. The grasslands have higher soil organic carbon (SOC) and microbial biomass with higher variations within the soil profile as compared to cultivated soils (Caldero´n *et al*., 2000). Higher carbon storage with greater plant diversity is a direct function of the soil microbial community (Lange *et al*., 2015).

Many studies have reported seasonal variability of SMB with higher SMB, both in dry and wet seasons (Montaño *et al*., 2007; Devi and Yadava, 2006). Soil microbial populations fluctuate with changes in soil moisture, SOC, nutrients, temperature and pH which ultimately affect nutrient cycling (Li and Chen, 2004). Microbial biomass is also affected by several ecological factors, such as plant community composition (Bezemer, 2006; Mummey *et al*., 2002). The impact of both natural and agricultural practices on the SMB in the soil has been studied extensively (Venkateswarlu and Srinnivasa Rao, 2014).

The microbial biomass is also affected by plant cover type, soil's cropping patterns, organic, inorganic amendments and soil types (Velmourougane *et al*., 2013). The variation in SMBC distribution in different soil types is controlled by physical and chemical properties of the soil (Manna *et al*., 2008). Oyedele *et al*. (2015) reproted that in same ecological conditions the SMBC and SMBN were correlated to the physcio-chemical properties of soil and due to this relationship SMB is an important soil quality indicator, especially with respect to land use type.

Climatic changes influence SMB (Roy and Singh, 1994) both directly by soil changes and indirectly by affecting plant metabolism. Soil microbial biomass not only involved in nutrients transformation but conservation of ecosystems, both in tropical and temperate climates (Kujur and Patel, 2012; Yang *et al*., 2006). Soil organic matter (OM) yield can be affected by any kind of change in SMB activities or populations. Thus, the ecosystem stability and fertility could be directly influenced by activities of soil microbial community (Smith *et al.,* 1993). Fluctuations in the extent of SMB during the growing season are well thought-out as an important factor which controls the turnover of soil carbon and nitrogen. By regulating nutrient availability, SMB interacts with ecosystem productivity and determines soil carbon sequestration.

The SMB distribution varies with respect to climate, soil type and vegetation. Soil microbes mediates 80 to 90 % of the soil processes and reactions (Nannipieri and Badalucco, 2003). The statistics on SMB determination allows an estimate to be made of the SMB distribution under different agro-ecosystem. In this study, the concentrations of SMBC and SMBN, SOC, total N and the relationships between SMB distribution with physico-chemical properties in the 0 to 15 cm and 15 to 30 cm soil layers were compared under different agro-climatic conditions.

MATERIALS AND METHODS

Two sites with different climate, vegetation and soil type located at District Poonch, Azad Jammu and Kashmir, Pakistan inluding Eutrudepts and Hapludolls were selected. Physico-chemical properties of study sites i.e. Eutrudepts and Hapludolls were determined (Table-1).

Collection of samples: The soil from three land uses including forestland, grassland and cultivated land of Eutrudepts and Hapludolls were collected. Twenty-four composite samples were collected from upper (0-15 cm) and lower (15-30 cm) soil depths.

Soil analysis: Particle size percentage was measured by Bouyoucos hydrometer (Gee and Bauder, 1986). Soil pH was measured from 1:2 (soil: water) soil suspension (Mclean, 1982). Water contents by gravimetric method were determined (Hesse, 1971). Gravimetric water contents were converted into volumetric water contents by multiplying with bulk density. For the determination of soil total nitrogen the Kjeldahl method (Bremner and Mulvaney, 1982) was used. The available P and extractable K was determined by AB-DTPA method (Soltanpour and Workman, 1979). Soil OM and SOC were determined by potassium dichromate method (Nelson and Sommers, 1996). The Blake and Hartage (1986) method was used for soil bulk density determination.

Microbial biomass carbon and microbial biomass nitrogen: The SMBC and SMBN were measured by the fumigation-extraction method (Okalebo *et al*., 1993).

Fumigation: For fumigation 30 g of each fresh soil sample was weighed and vacuum was applied to the fumigated samples till the chloroform get boiled. The fumigated and non-fumigated samples were stored in dark for 72 hours at room temperature. After completion of duration, both non-fumigated and fumigated samples were opened and transferred to 250 ml flasks. Then 100 mL of 0.5 M K_2SO_4 solution was added and each sample was shaken for one hour. A clear extract was obtained by filtrating the suspension. The SMBN and SMBC were determined from same extract.

Soil microbial biomass nitrogen determination: The filtrate (50 mL) was allow for digestion and allow to digestion at 150 ºC to 380 ºC for 3 hrs. After digestion, volume was made and samples were distillated and analyzed for N, the process of distillation was same as for total N.

Soil microbial biomass carbon determination: For determination of SMBC, 8 mL of the filtrate was transferred to 100 mL digestion tube. The digestion was done for 30 minutes at 150ºC. Volume (250 mL) was made and then 2 to 3 drops of 1.10 phenalthroline indicator was added to each sample in flask and each sample was titrated with 0.2 N ferrous ammonium sulfate solutions until the colour changed from bluish green to reddish brown.

Statistical analysis: Statistical analysis of the results was done (Steel *et al*., 1997). The correlation between soil microbial biomass distribution and soil physico-chemical properties were constructed. Correlation coefficients (r) were used to measure association between different variables. The least significance difference (LSD) among means was measured at $P \leq 0.05$.

RESULTS

The physico-chemical properties of study site (Table-1) showed higher organic matter in forest soil and lower in cultivated. The total nitrogen (2 g Kg^{-1} in Eutrudepts and 3.6 g Kg^{-1} in Hapludolls) was higher in forest soils followed by grassland (1.6 g Kg^{-1} in Eutrudepts and 3 g Kg^{-1} in Hapludolls) and (1.4 g Kg^{-1} in Eutrudepts and 0.9 g Kg^{-1} in Hapludolls) cultivated. However, at both sites K was higher (190 mg/Kg in Eutrudepts and 186 mg/Kg in Hapludolls) grassland. The higher soil bulk density (1.23 g cm^{-3} in Eutrudepts and 1.25 g cm^{-3} in Hapludolls) was found in cultivated land and lower (1.03 g cm^{-3} in Eutrudepts and 1.01 g cm^{-3} in Hapludolls) in forest soils at both sites.

The soil biomass carbon (SMBC) of the forest, grass and cultivated land uses of Eutrudepts is given in Table-2. The highest SMBC (265.01 mg / Kg) in forest soils followed by grass (215.57 mg / Kg) and cultivated (136.79 mg / Kg) land use was found. Higher SMBC (23 %) was found in forest land compared to grassland (215.57 mg / Kg). However, higher SMBC (57.59 %) was observed in the grassland compared to cultivated land (136.79 mg / Kg). The higher SMBC of 246.45 mg /

Kg was at 0-15 cm and 165.14 mg / Kg at 15-30 cm. The SMBC of Hapludolls affected by land uses is presented in Table-3. The distribution of SMBC was found in the order of forest land (274.89 mg / Kg) > grassland (230.01 mg / Kg) > cultivated land (135.57 mg / Kg) use. Forest land had 20 % higher SMBC than grassland and 103 % higher compare to cultivated. In the same way, grassland had 69.66 % higher SMBC than cultivated land (135.57 mg / Kg). The upper soil depth 0-15 cm had higher SMBC (272.86 mg / Kg) than lower 15-30 cm depth (154.11 mg / Kg).

The SMBC of Eutrudepts and Hapludolls of different land uses (Table-4) illustrated the higher SMBC (269.95 m / Kg) in forest soils and the lowest (136.182 mg / Kg) in cultivated land use. Whereas, means of sites revealed higher ($p \leq 0.05$) SMBC of 213.490 mg / Kg of Hapludolls followed by 205.793 mg / Kg of SMBC of Eutrudepts. Hapludolls had 3.74 % higher SMBC than Eutrudepts (205.79 mg / Kg).

The SMBN under different land uses of Eutrudepts is given in Table-5. The SMBN (48.713 mg / Kg) was higher in forest

Table-1: Physico-chemical properties of Eutrudepts and Hapludolls under different land uses.

Parameters	Eutrudepts			Hapludolls		
	Land Uses			Land Uses		
	Forest	Grassland	Cultivated	Forest	Grassland	Cultivated
Soil organic matter (%)	2.48	1.74	1.33	4.03	2.27	1.54
Soil organic carbon(g Kg^{-1})	14.18	10.13	7.73	23.42	13.18	8.98
pH	6.73	7.10	6.69	6.70	7.22	7.07
Total Nitrogen (g Kg^{-1})	2	1.6	1.4	3.6	3	0.9
C:N	7.09	6.33	5.52	6.50	4.39	9.98
*Phosphorus (mg/Kg)	3.77	3.41	3.59	3.75	3.51	3.60
*Potassium (mg/Kg)	170	190	143	134	186	95
Bulk density (g cm^{-3})	1.03	1.13	1.23	1.01	1.09	1.25
Temperature ($^{\circ}C$)	20.88	24.82	24.40	13.60	16.47	16.55
Water contents (v/v %)	20.06	16.85	17.95	28.83	13.51	30.76

*Available Phosphorus; Extractable Potassium

Table-2: Soil microbial biomass carbon of soil under different land uses, humid, subtropical, temperate climate of Eutrudepts.

Depth	Land uses			Means
	Cultivated (mg / Kg)	Forest (mg / Kg)	Grassland (mg / Kg)	
0-15cm	162.30	311.53	265.50	246.45 [a]
15-30cm	111.28	165.65	165.65	165.14 [b]
Means	136.79 [b]	265.01 [a]	215.57 [ab]	
LSD				
Land use (L)		84.23		
Depth (D)		68.77		
D×L		NS		

Means followed by the different letter (s) are significantly different at ($p \leq 0.05$), NS= non-significant

Table-3: Soil microbial biomass carbon of soil under different land uses, humid, subtropical/temperate climate of Hapludolls.

Depth	Land uses			Means
	Cultivated (mg / Kg)	Forest (mg / Kg)	Grassland (mg / Kg)	
0-15cm	155.51	396.51	266.57	272.86 [a]
15-30cm	115.62	153.27	193.45	154.11 [b]
Means	135.57 [b]	274.89 [a]	230.01 [ab]	
LSD				
Land Used (L)		114.39		
Depth (D)		93.40		
D×L		Ns		

Means followed by the different letter (s) are significantly different at ($p \leq 0.05$), NS= non-significant

land, intermediate in grass (45.022 mg / Kg) and lower in cultivated (33.593 mg / Kg) land. The 8.19 % higher SMBN was in forest land than the grassland and 45 % higher compare to cultivated land. Similarly 34.62 % higher SMBN was in grassland compare to cultivated land. The statistically higher SMBN of 45.76 mg / Kg was at upper depth (0-15 cm) and 39.12 mg / Kg at lower (15-30 cm) soil depth.

Table-4: Soil microbial biomass carbon of soil under different land uses of Eutrudepts and Hapludolls (Data are combined over locations).

Sites	Land uses			Means
	Cultivated mg / Kg	**Forest mg / Kg**	**Grassland mg / Kg**	
Site 1(H)	135.57	274.89	230.01	213.49NS
Site 2(E)	136.79	265.01	215.58	205.79
Means	136.18 [c]	269.95 [a]	222.79 [b]	
LSD				
Land use (L)	42.89			
L×S	NS			

H= Hapludolls E= Eutrudepts
Means followed by the same letter (s) are not significantly different at ($p \leq 0.05$); NS = non significant

Table-5: Soil microbial biomass nitrogen of soil under different land uses, humid, subtropical, temperate climate of Eutrudepts.

Depth	Land uses			Means
	Cultivated (mg / Kg)	**Forest (mg / Kg)**	**Grassland (mg / Kg)**	
0-15cm	34.51	52.68	50.09	45.76
15-30cm	32.68	44.75	39.95	39.12
Means	33.59 b	48.71 a	45.02 ab	
LSD				
Land Used (L)		12.65		
Depth (D)		NS		
D×L		NS		

Means followed by the same letter (s) are not significantly different at ($p \leq 0.05$); NS = non-significant

The land uses effect on SMBN of Hapludolls (Table-6) showed that forest, grass and cultivated lands had SMBN of 49.52, 49.52 and 27.36 mg / Kg, respectively. The forest land has 13.44 % higher SMBN than grassland and 81 % higher than cultivated land. However, the 59.3 % higher SMBN was found in grassland as compared to cultivated soil.

Table-6: Soil microbial biomass nitrogen of soil under different land uses, humid, subtropical/temperate climate of Hapludolls

Depth	Land uses			Means
	Cultivated (mg / kg)	**Forest (mg / kg)**	**Grassland (mg / Kg)**	
0-15cm	29.34	66.97	47.37	47.90
15-30cm	25.37	32.06	39.92	32.45
Means	27.36	49.52	43.65	
LSD				
Land use (L)	NS			
Depth (D)	NS			
D×L	NS			

NS= non significant

The upper 0-15 cm soil depth had higher ($p \leq 0.05$) SMBN compared to lower 15-30 cm depth.

Soil microbial biomass carbon of land uses of Eutrudepts and Hapludolls is shown in Table-7. The significantly higher SMBN (49.12 mg /Kg) was observed in forest soils and the lowest (30.48 mg / Kg) in cultivated land use. Whereas, the means of sites revealed higher ($p \leq 0.05$) SMBN of 213.490 mg / Kg in Eutrudepts followed by 205.793 mg / Kg of SMBN in Hapludolls. Microbial biomass nitrogen was 5.64 % higher in Eutrudepts than Hapludolls.

Table-7: Soil microbial biomass nitrogen of soil under different land uses Eutrudepts and Hapludolls (Data are combined over locations).

Sites	Land uses			Means
	Cultivated (mg / Kg)	Forest (mg / Kg)	Grassland (mg / Kg)	
Site 1(H)	27.36	49.52	43.65	40.18
Site 2(E)	33.58	48.72	45.02	42.45
Means	30.48 ^{b}	49.12 ^{a}	44.34 ^{a}	
LSD				
Land use (L)		8.72		
L×S		NS		

H= Hapludolls E= Eutrudepts

Means of different land uses followed by the different letter (s) are significantly different at $p \leq 0.05$

The soil microbial biomass carbon and soil microbial biomass nitrogen (C:N) was higher in forest land among land uses at both sides. While the comparison of sites showed higher C:N at Hapludolls than Eutrudepts.

Table-8: Soil microbial biomass carbon and soil microbial biomass nitrogen ratio under different land uses of Eutrudepts and Hapludolls (Data are combined over locations)

Sites	Land uses			Means
	Cultivated	Forest	Grassland	
Site 1(H)	4.95	5.55	5.27	5.26
Site 2(E)	4.07	5.43	4.79	4.76
Means	4.51	5.49	5.03	

H= Hapludolls E= Eutrudepts

The Positive relationship between SMBC and TN at both Hapludolls and Eutrudepts were found (Table-9). The SMBN had also a positive correlation with TN of both Hapludolls and Eutrudepts. Negative correlation between SMBC and soil available phosphorus at both Hapludolls and Eutrudepts was observed in Table-9. While SMBN had a positive correlation with soil available phosphorus at Hapludolls and negative correlation of SMBN with soil available phosphorus was observed at Eutrudepts. A strong positive correlation between SMBC and soil K was observed at both Hapludolls and Eutrudepts. The similar positive trend was seen between SMBN and soil K at both Hapludolls and Eutrudepts.

DISCUSSION

Compare to agricultural soils, the carbon content was found significantly higher in the forest and pasture soils. It is attributed to higher organic matter and OM increases biological activity, however, these biochemical activities get changed when natural land use such as forests are replaced by cultivated land (Cardelli *et al.*, 2012). Oyedele *et al*. (2015) reported low contents of SMBC in cultivated land as against grazing land. Due to high dense forests at Hapludolls site there was more OM content. Similarly, the higher amount of SMBC in Hapludolls could be due to favorable climatic conditions. Climatic conditions have a great effect on SMBC (Murrieta *et al.*, 2007). Velmourougane *et al*. (2013) studied the effect of different soil types on SMBC and reported higher SMBC in Halic Haplusterts and the lowest in Gypsic Haplusterts.

Land use influences soil microbial processes and structure of microbial communities (GarcíaOrenes *et al*., 2013; Blagodatskaya *et al*., 2011). The soils with high OM soils have a significantly higher microbial biomass, larger fractions of mineralizable C and N (Reganold *et al*., 2010). Microbial biomass nitrogen was found significantly higher in the forest than agricultural soil. Previous studies (Singh *et al*., 2010 and Singh *et al*., 2009, 2007) also found the higher SMBN in forest soils as compared to farm manure soils.

Overall comparison of both sites showed higher SMBN in Eutrudepts, the higher content of SMBN at Eutrudepts could be the climatic conditions as the temperature of Eutrudepts was relatively higher than the Hapludolls and that temperature was favorable for decomposition and mineralization process so more microbial nitrogen was observed in Eutrudepts. As the temperature increases, soil ammonification and nitrogen mineralization also get increases. The N mineralization is also benefited with medium soil moisture. The temperature has pronounced influence on nitrogen mineralization as with temperature increase from 12 ºC to 36 ºC mineralization also get increases (Chen *et al*., 2009).

Haripal and Sahoo, 2014 showed the ratio of MBC:MBN is 4.14-11.7 and this is close to our results (4.07-5.55) and close to the findings of Moore *et al*. (2000). Campbell *et al*. (1991) reported that higher SMBC:SMBN is due to fungal dominant SMB and lower SMBC:SMBN is due to bacterial dominated biomass. Ravindran and Yang, 2015 reported the SMBC: SMBN ratio of spruce, hemlock, and grassland soils were 5.2-6.5, 4.8-6.6, and 4.1-5.6, respectively, showing the dominancy of bacteria.

The strong positive correlations between SMBC and OM, SMBN and OM at both Hapludolls and Eutrudepts is attributed to the higher activity of microbial biomass (Wright *et al*., 2005). The more organic matter means more SMB because soil OM is an energy source for microorganisms. The SMBC and SMBN accumulation increases total organic carbon contents of soil (Bingbing *et al*., 2010; Arunachalam and Pandey, 2003). A significant positive correlation among SMBC, SMBN and TN has also been studied by Shah *et al*. (2010). The microbial activity increases due to supply of nutrients (C, N) as energy source, it increase the N mineralization rate (Frey *et al*., 2003). The nutrient P affects SMB activity (Wright and Reddy, 2001) and negative correlation shows lower P contents due to higher microbial activity (Oyedele *et al*., 2015). In table-9, it is illustrated that negative correlation between SMB distribution and soil bulk density at both Hapludolls site and Eutrudepts. This negative correlation between SMB distribution and soil bulk density might be due to the higher OM matter and due to more OM higher porosity of soil which in turn has decreased the bulk density of soil. The higher OM of the soil increases the microbial activity of the soil that alleviates soil aggregates which in turn have the beneficial influence on nutrient dynamics, and soil porosity (Haynes and Naidu, 1998).

Conclusion: Forest soils showed higher microbial biomass nitrogen and carbon in both types of soil as compared to the cultivated and grassland soils. Hapludolls have higher SMBC and lower SMBN. The SMBC and SMBN concentrations were positively correlated with OM, TN and K.

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