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Relationship Between Soil Microbial Biomass and Fine Roots in Tarai and Hill Sal (*Shorea robusta* Gaertn.) Forests of Eastern Nepal

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ABSTRACT

Relationship between soil microbial biomass and fine roots were studied in Tarai Sal forest (TSF) and Hill Sal forest (HSF) of eastern Nepal. Physico- chemical properties of soil were determined in upper (0-15cm) and lower (15-30cm) depth in the both forests. Soil microbial biomass (0-15cm depth) and fine roots (0-30cm depth) was estimated in summer, rainy and winter seasons in both forests. Soil organic carbon (SOC), total nitrogen (TN) and total phosphorus (TP) were higher in HSF than TSF whereas the values decreased depth wise in both the forests. Microbial biomass carbon (MB-C), nitrogen (MB-N) and phosphorus (MB-P) were higher by 66%, 31% and 9% respectively in HSF than TSF. Annual mean fine root biomass was significantly higher in HSF than TSF. Distinct seasonality was observed in soil microbial biomass and fine root in both forests. Soil microbial biomass is the main source of nutrients for the plant which showed opposite relationships with fine roots. Soil microbial biomass showed maximum value during summer season might be due to its accumulation when plant growth and nutrient demand are minimum. On the other hand, fine root showed maximum value during rainy season because of fast turnover of micro-organisms and optimum utilization of nutrients by the plants when growth and development remains at the peak. In conclusion, fine root mortality transfer considerable amount of organic matter and nutrients in forest soils which may enhance the level of soil microbial biomass.

Key Words: Fine Root Biomass; Relationship; Soil Microbial Biomass; Seasonality; Sal Forests.

INTRODUCTION

Sal (*Shorea robusta* Gaertn.) forest is significant vegetation in tropical region (Dobremez 1976). Now day forests are disturbed by over grazing, firewood collection, litter collection, timber cutting and forest fire due to human settlement near the vicinities. Thus, forest resource including soil characters is in danger due to adverse abiotic and biotic stresses (Bawa and Dayanandan, 1998). Disturbance in the forest results into significant fluctuation in microbial biomass and its activities (Srivastava and Singh 1989) and also in soil-microbes- plant- nutrient cycling (Jordan 1985).

Soil microbial biomass is the living microbial component of the soil organic matter, and mainly consists of bacteria, fungi, actinomycetes, rotifers and protozoan with a size smaller than $5\times10^3\mu m$ (Jenkinson and Ladd 1981). Though, it represents a small portion of soil organic matter but is an active part due to its rapid turnover rate and fast release of available nutrients to the plants. Thus, they contribute to nutrient cycle process far greater than its size (Schnurer et al. 1985). With rise in altitude, soil temperature decreases which in turn leads to a reduction in microbiological activity. Therefore, carbon has longer retention time in the soil and nitrogen levels are higher due to limited mineralization (Prichard et al. 2000). Soil microbial biomass and its activities are also influenced by forest types and quantity and quality of organic matter inputs. Besides this, seasonal variation in temperature as well as organic matter accumulation from litter fall also has great influence on soil microbial biomass (Chang et al. 2016).

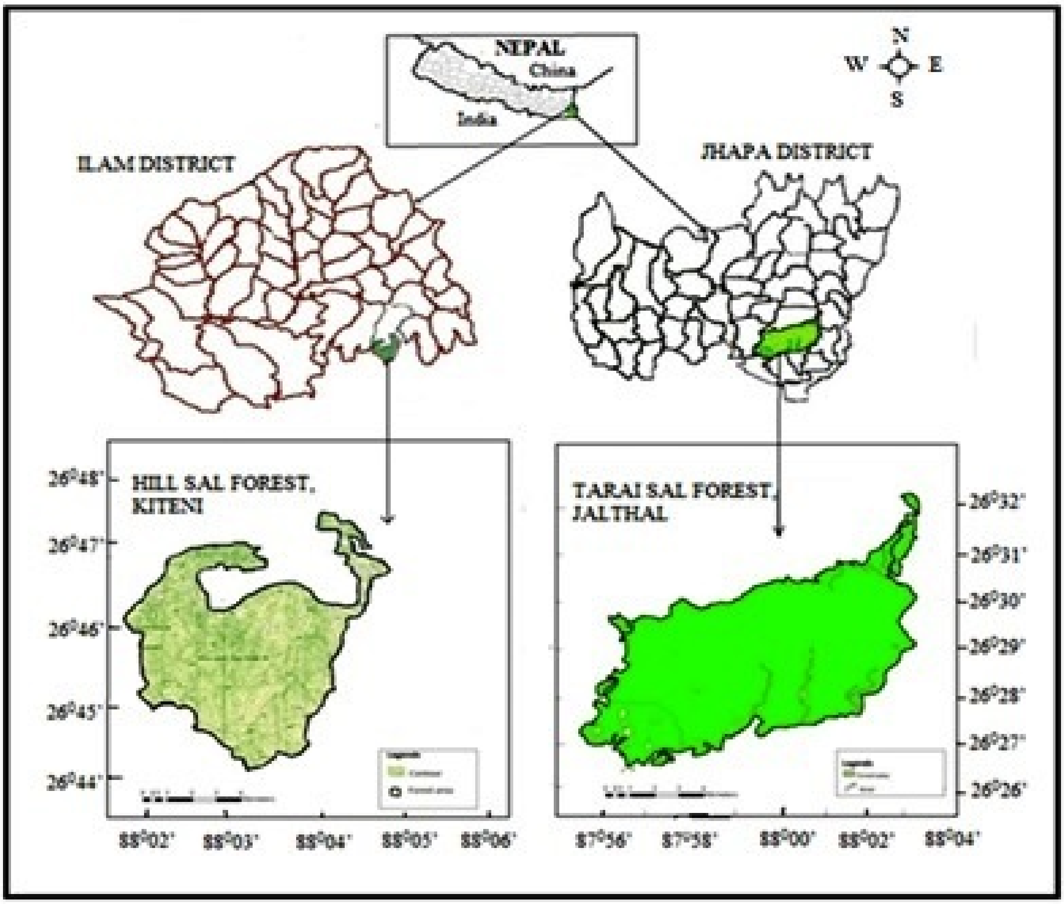


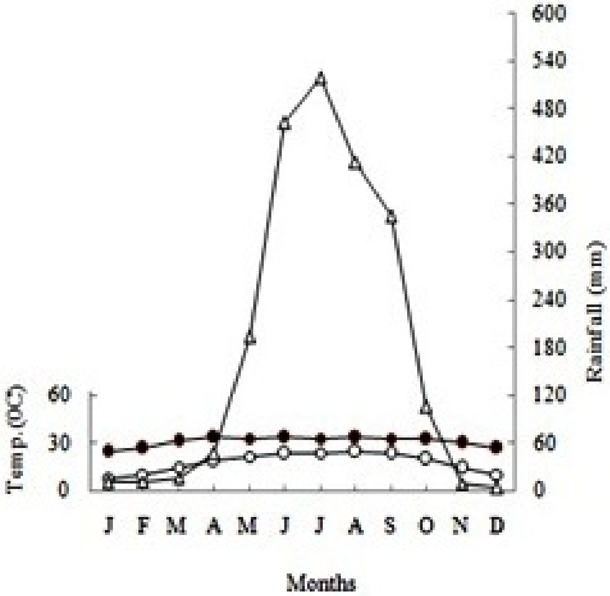
Figure 1. Location of study area of Hill Sal forest lies at Kiteni, Ilam districts and Tarai Sal forest lies at Jalthal, Jhapa district in eastern Nepal.

Plant species composition, genetic properties and seasonality are the determining factors for the growth and development of the fine roots (Barbuhiya et al. 2012). Fine roots the important below ground nutrient source, are responsible for water and nutrient uptake and cycling (Gordon and Jackson 2000). In addition, fine root mortality is also main source of forest soil nutrients which contributes 18 to 58% of total nitrogen (Vogt et al. 1986). Soil chemical properties have strong influence on fine root biomass. In general, soil nutrients show opposite relationships with fine root biomass (Maycock and Condon 2000). No sufficient information is available on the status of soil microbial biomass and its relationship with fine root in the Sal forests having distinct topographic variation. The work was designed to answer the questions as: (i) What is the status of soil organic carbon, total nitrogen and total phosphorus in Sal forests? (ii) What is the status of microbial biomass and fine roots in Sal forests differ in altitude? (iii) How does seasonality effect on soil microbial biomass and fine roots along the altitudinal gradient. (iv) How does soil microbial biomass relate with fine roots?

STUDY AREA

The study was carried out in the Sal forests located in Tarai and Hill regions of eastern Nepal (Figure 1). Sal forest of tarai region is addressed as Tarai Sal forest which is located at Jalthal Village Development Committee (VDC) near Kechana (extreme low land of Nepal) of Jhapa district in eastern Nepal. The forest floor is uneven and altitudinal variation ranges from 62 to 129 m altitude. TSF is situated between 87° 55’ and 88° 03’ E and 26° 27’ and 26° 32’ N. Sal forest of hill region is addressed as Hill Sal forest is located at Kiteni of Kolbung VDC, Ilam district. This forest lies at sub Himalayan tract (Shiwaliks) where the altitude ranges from 500 to 850 m altitude. HSF is situated between 88° 02’ and 88° 04’ E and 26° 44’ and 26° 47’ N.

The climate of the study area is tropical monsoon type. Based on the data pertain to the period 2001- 2014, the mean monthly minimum temperature of TSF ranged from 10ºC to 24ºC and maximum temperature ranged from 23.9ºC to 33.4ºC (Figure 2a). Likewise, the mean monthly minimum temperature of HSF ranged between 9.4ºC and 19.9ºC and maximum temperature between 16.4ºC and 25.9ºC (Figure 2b). The average annual rainfall of TSF was 2130.4 mm and HSF was 1776.07 mm. Both Tarai Sal forest and Hill Sal forest (tropical moist forest according to the life zone classification of Holdridge *et al*., 1971) are dominated by *Shorea robusta* Gaertn. Some of the main associates eg. *Lagerstroemia parviflora* Roxb., *Dillenia pentagyna* Roxb., and *Schima wallichii* D.C Korth are common in both forests. However, TSF is peculiar in containing *Artocarpus chamsala* Roxb and some high altitude species like *Castanopsis indica* (Roxb.) Miq, *Michelia champaca* L. and *Madhuca longifolia* (Koenig) Mac. Soil of Tarai Sal forest is sandy loam Mollisols which has dark topsoil while soil of Hill Sal forest very shallow and contains much gravel, stones and rock fragment is a sandy loam Entisols (Jackson 1994).



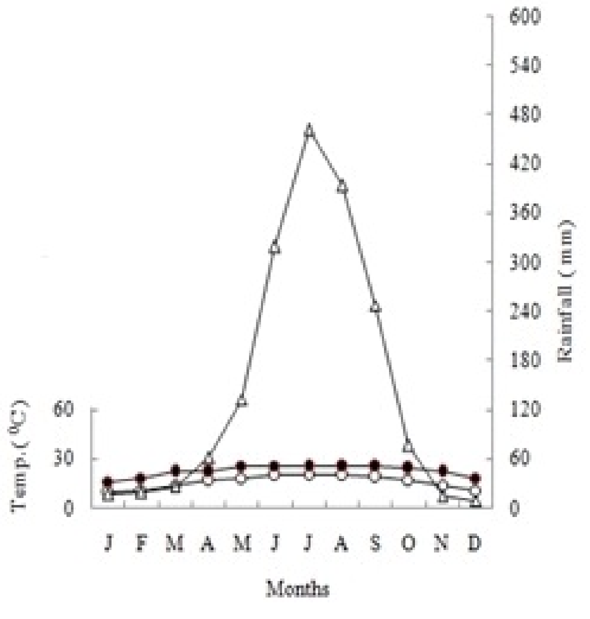


Figure 2.Ombrothermic diagram of the climate in (2a) Tarai Sal forest (above) and (2b) Hill Sal forest regions (below) of Nepal. The temperature (○ mean mimimum and ● mean maximum) and rainfall (∆) data are for the period 2001-2014.

METHODS

Soil Sampling and Analysis

Soil samples were collected in two soil depth (0-15cm and 15-30cm depths) from thirty locations in each forest site from randomly selected blocks. At each location the soil was collected from three pits, composited and pooled as one replicate. Soil sampling was carried out in May, July and January (2012 and 2013) representing the summer, rainy and winter season, respectively. Physico-chemical properties were estimated in both depths while soil microbial biomass carbon, nitrogen and phosphorus were estimated from (0-15cm) depth for each season.

Air dried soil samples were sieved through a 2mm mesh screen and used for further analysis. Soil pH was measured by using a glass electrode (1:5, soil: water). Water holding capacity (WHC) was estimated by perforated circular brass box method (Piper 1966). Bulk density (BD) was determined by inserting metallic tubes of known internal volume in soil and there after estimating dry weight of a unit volume of soil (Brady and Well 2013). Soil organic carbon was analyzed by using potassium dichromate and digestion of soil samples with H_2SO_4 and titration with ferrous sulphate (Walkley and Black 1934). Total nitrogen was estimated by micro-Kjeldahl method (Jackson 1958). Total phosphorus was determined colorimetrically by ammonium molybdate-stannous chloride blue color method after digesting the soil in tri-acid mixture of $HClO_4$, HNO_3 and H_2SO_4 in the ratio of 1:5:1 (Jackson 1958).

Determination of Soil Microbial Biomass

Soil microbial biomass (SMB) was estimated by the chloroform fumigation extraction method. Microbial biomass carbon was determined in the K_2SO_4 soil extract of fumigated and unfumigated soil samples by dichromate oxidation in a reflux system and titration with ferrous ammonium sulphate. MB-C was then estimated from the equation: MB-C = $2.64E_c$ where E_c is the difference between carbon estimated from fumigated and unfumigated soils, both expressed as C µg g^{-1} dry soil (Vance et al. 1987). Soil microbial biomass nitrogen was determined in the same soil extract of fumigated and unfumigated soil samples using the Kjeldahl digestion method. The MB-N value obtained for the unfumigated soil extract was subtracted from the value obtained from that of fumigated soil extract; the difference in the value of total nitrogen thus estimated was divided by a K_N value of 0.54 assuming that 54% of the MB-N was extracted in K_2SO_4 by chloroform treatment (Brookes et al. 1985). Soil microbial biomass phosphorus was estimated in the $NaHCO_3$ extracts of fumigated and unfumigated soils using the ammonium molybdate stannous chloride method. MB-P was calculated by dividing the value obtained as inorganic phosphorus (P) by a K_P value of 0.4 ($NaHCO_3$ inorganic P in fumigated subtracted from that of unfumigated), assuming that 40% of P in the soil microbial biomass is released as inorganic P by chloroform treatment. A correction was made for P fixation during the $NaHCO_3$ extraction by measuring the recovery of exogenously added inorganic P as KH_2PO_4 (equivalent to 20 µg P g^{-1} soil) as suggested by Brookes et al. (1982).

Determination of Fine Root Biomass

Fine root biomass (FRB) was determined from thirty soil monoliths (10cm x 10cm x 30cm depth) taken out from each forest (TSF and HSF) in summer, rainy and winter season during 2012 and 2013. Soil monoliths were washed over a sieve with fine jet of water to retrieve the fine roots which were oven dried at 80^o C. Fine root size <2mm and 2-5mm in 0-15cm and 15-30cm soil depth were separated and estimated separately. Summer, rainy and winter season values were averaged to obtain a mean annual FRB.

Statistical Analysis

The data obtained from the analysis of soil samples were subjected to two ways ANOVA to test the level of significance and least significant difference (LSD) by applying post hoc to distinguish the differences in soil chemical properties between two Sal forests located in different topography by using SPSS statistic 20 software. ANOVA was also used to test the level of significance of differences in FRB due to stand type and size class. Regression analysis was also done to find out the relationship between soil microbial biomass and fine roots.

RESULTS

Soil physico-chemical properties of TSF and HSF were analyzed and results are presented in Table 1. Both forests had sandy loam type of soil texture. Soil moisture of TSF was higher than HSF. The water holding capacity was slightly higher in TSF (59.6%) than HSF (58.41%). It decreased with soil depth in both forests. Bulk density was higher in TSF than HSF and increased with soil depth. The pH value was slightly higher in HSF than TSF however, the value increased in lower soil depth in both forest stands. In the upper soil layer soil organic carbon was higher in HSF (2.09%) than TSF (1.60%). Similarly, total nitrogen was also higher in HSF (0.173%) than in TSF (0.129%) while total phosphorus was more or less same in both forest stands. The potassium also showed higher value in HSF (312.13 µg g^{-1}) than TSF (238.47 µg g^{-1}). The value of SOC, TN, TP and potassium decreased in lower depth (15-30cm) in both forests. The C: N ratio of Tarai and Hill Sal forest was more or less similar.

Annual mean value of MB-C, MB-N and MB-P showed increasing trend along the increasing altitude. Hill Sal forest showed 66%, 31% and 9% higher value of MB-C, MB-N and MB-P over TSF respectively (Table 2). A distinct seasonal variation was also observed in the levels of MB-C, MB-N and MB-P in both the sites. Minimum values were obtained in the rainy season and maximum in the summer season (Figure 3). Microbial biomass carbon, nitrogen and phosphorus reduced by 46% to 67% from summer to rainy in HSF. The rate of reduction from summer to rainy was even higher (32 to 80 %) in TSF. The ratios MB-C: MB-N, MB-C: MB- P and MB- N: MB-P was higher in HSF than TSF (Table 2). ANOVA suggested that the variation in MB-C, MB-N and MB- P were significantly different for sites (for MB-C, $F_{1, 74} = 136$, $P< 0.001$; MB-N, $F_{1, 174} = 39$, $P < 0.001$; MB-P, $F_{1,174} = 86$, $P <0.001$) as well as for the

Table 1. Soil characteristics of Tarai Sal forest and Hill Sal forest of eastern Nepal. Values are means ±1 SE.

Soil properties	Tarai Sal Forest		Hill Sal Forest	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Soil texture (%)				
Sand	52.0 ± 1.7	57.66 ± 1.8	65.73 ± 0.7	68.13 ± 0.6
Silt	31.4 ± 1.6	33.93 ± 1.5	25.93 ± 0.8	21.8 ± 0.7
Clay	16.6 ± 0.9	8.46 ± 0.6	10.73 ± 0.6	11.0 ± 0.4
Soil moisture (%)				
Rainy	38.3 ± 0.1	40.13 ± 0.1	26.32 ± 0.2	28.35 ± 0.2
Winter	18.17 ± 0.2	20.53 ± 0.2	10.56± 0.1	12.13 ± 0.1
Summer	9.11 ± 0.9	10.96 ± 0.2	5.15 ± 0.2	7.21 ± 0.2
Soil temperature (^{0} C)	23.76 ± 0.06		20.57 ± 0.06	
Bulk density (g cm^{-3})	1.3 ± 0.004	1.44 ± 0.004	1.03 ± 0.007	1.13 ± 0.004
Porosity (%)	50	45	60	57
WHC (%)	59.6 ± 1.3	54.58 ± 1.2	58.41 ± 1.5	52.51 ± 1.4
pH	5.35 ± 0.03	5.59 ± 0.02	6.42 ± 0.03	6.58 ± 0.03
Soil organic carbon (%)	1.6 ± 0.09	0.908 ± 0.05	2.09 ± 0.12	1.53 ± 0.11
Organic matter (%)	2.76 ± 0.15	1.55 ± 0.08	3.6 ± 0.02	2.64 ± 0.18
Total nitrogen (%)	0.129 ± 0.003	0.106 ± 0.002	0.173 ± 0.005	0.124 ± 0.004
Total phosphorus (µg g^{-1})	619.95 ± .31	617.13 ± 0.19	669.53 ± 9.63	621.55 ± 0.38
Potassium (µg g^{-1})	238.47 ± 0.37	209.11 ± 0.18	312.13 ± 0.14	297.27 ± 0.15
C: N ratio	12.48	8.56	12.09	12.33

Table 2. Soil microbial biomass (MB-C, MB-N, MB-P) and their ratio in TSF and HSF of eastern Nepal. Values are mean of summer, rainy and winter seasons.

Forest	Soil Microbial Biomass (µg g^{-1})			MB-C: MB-N	MB-C: MB-P	MB-N: MB-P
	MB-C	MB-N	MB-P			
Tarai Sal forest	216.2 ± 11.8	29.67 ± 1.35	9.85 ± 0.04	7.28	21.94	3.01
Hill Sal forest	359.08 ± 13.94	38.72 ± 1.62	10.72 ± 0.08	9.27	33.49	3.61

seasons (for MB-C, $F_{2, 174} = 47$, $P< 0.001$; MB-N, $F_{2, 174} = 57$, $P < 0.001$; MB-P, $F_{2, 174} = 696$, $P <0.001$). The microbial biomass carbon, nitrogen and phosphorus were positively and significantly correlated with SOC, TN and TP in the forest stand (Table 3).

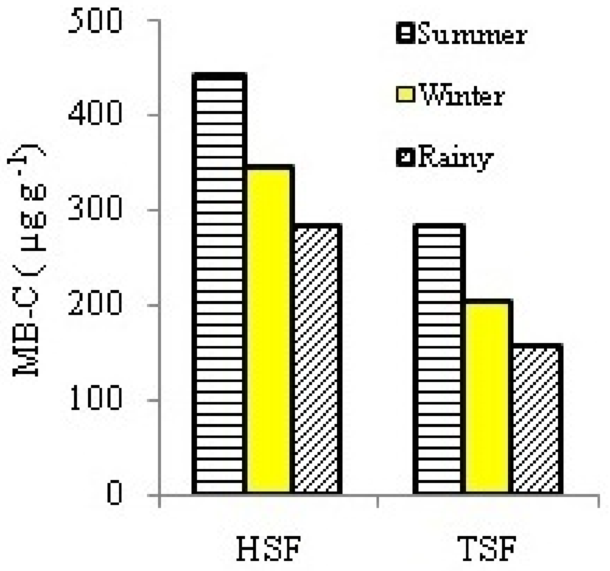
Annual mean fine root biomass of <5mm size in 0-30cm soil depth was higher in HSF (6.27 Mg ha^{-1}) than TSF (5.04 Mg ha^{-1}). The variation in annual FRB was significant. Annual fine root biomass of HSF was 21.14% more than TSF (Table 4). Similarly biomass of <2mm and 2-5mm size class were also significantly ($P < 0.001$) higher in HSF than TSF. In HSF biomass of <2 mm size class fine root was 22% higher and 2-5mm size

Table 3. Correlation between soil microbial biomass and other soil chemical properties in TSF of eastern Nepal.

	SOC	TN	TP	K	MB-C	MB-N
TN	0.640**					
TP	0.493**	0.683**				
K	-0.037	-0.213	-0.251			
MB-C	0.719**	0.668**	0.569**	-0.22		
MB-N	0.678**	0.761**	0.437*	-0.083	0.700**	
MB-P	0.448*	0.549**	0.562**	-0.155	0.536**	0.577**

Table 4. Annual fine root biomass (Mg ha^{-1}) in Tarai Sal forest and Hill Sal forest of eastern Nepal.

Size class	TSF			HSF		
	0-15 cm	15-30 cm	0-30cm	0-15 cm	15-30 cm	0-30cm
<2 mm	2.65±0.1	0.85±.06	3.5±0.08	3.39±0.12	1.09±0.15	4.48±0.14
2-5 mm	0.84±0.1	0.7±0.12	1.54±0.13	1.25±0.07	0.54±0.08	1.79±0.09
0-5 mm	3.49±0.14	1.55±0.14	5.04±0.12	4.64±0.15	1.63±0.11	6.27±0.16



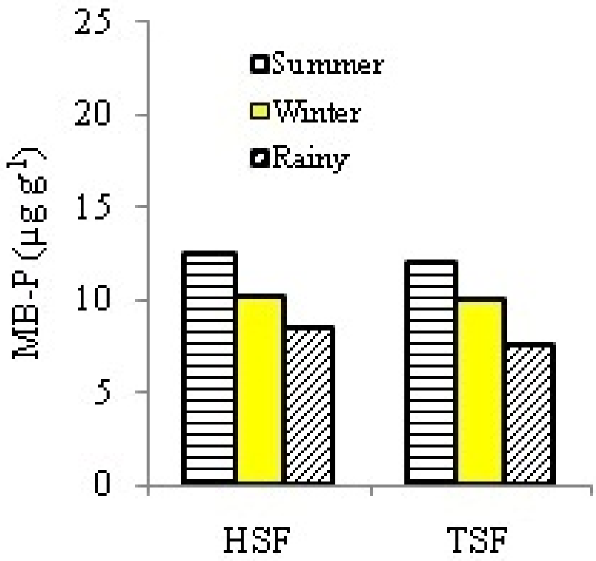
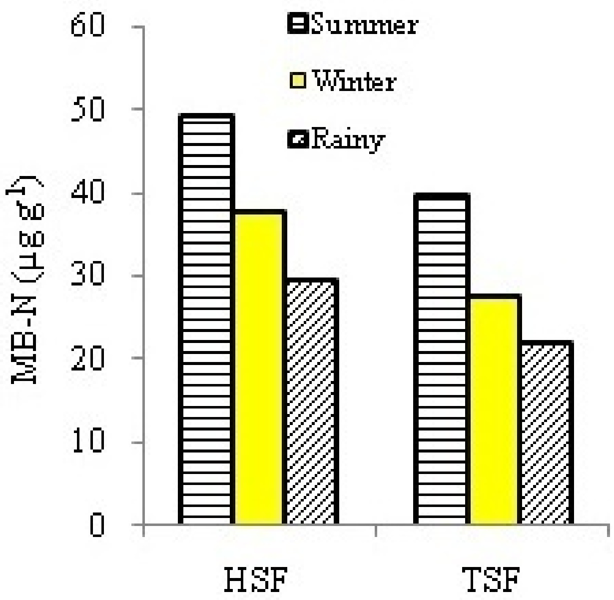


Figure 3. Seasonal variation in MB-C, MB- N and MB- P in Hill Sal forest and Tarai Sal forest of eastern Nepal



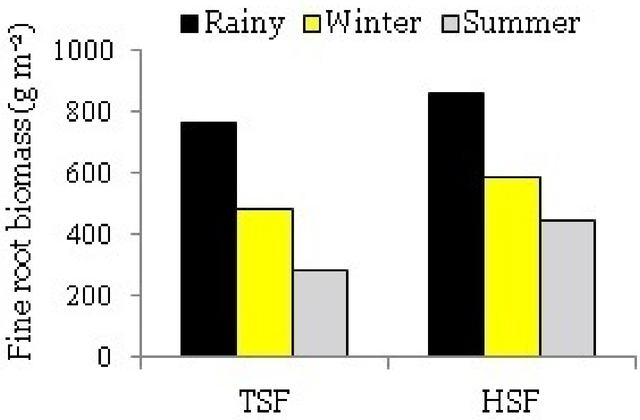


Figure 4. Seasonal variation in fine root biomass (Mg ha^{-1}) in Tarai Sal forest and Hill Sal forest of eastern Nepal.

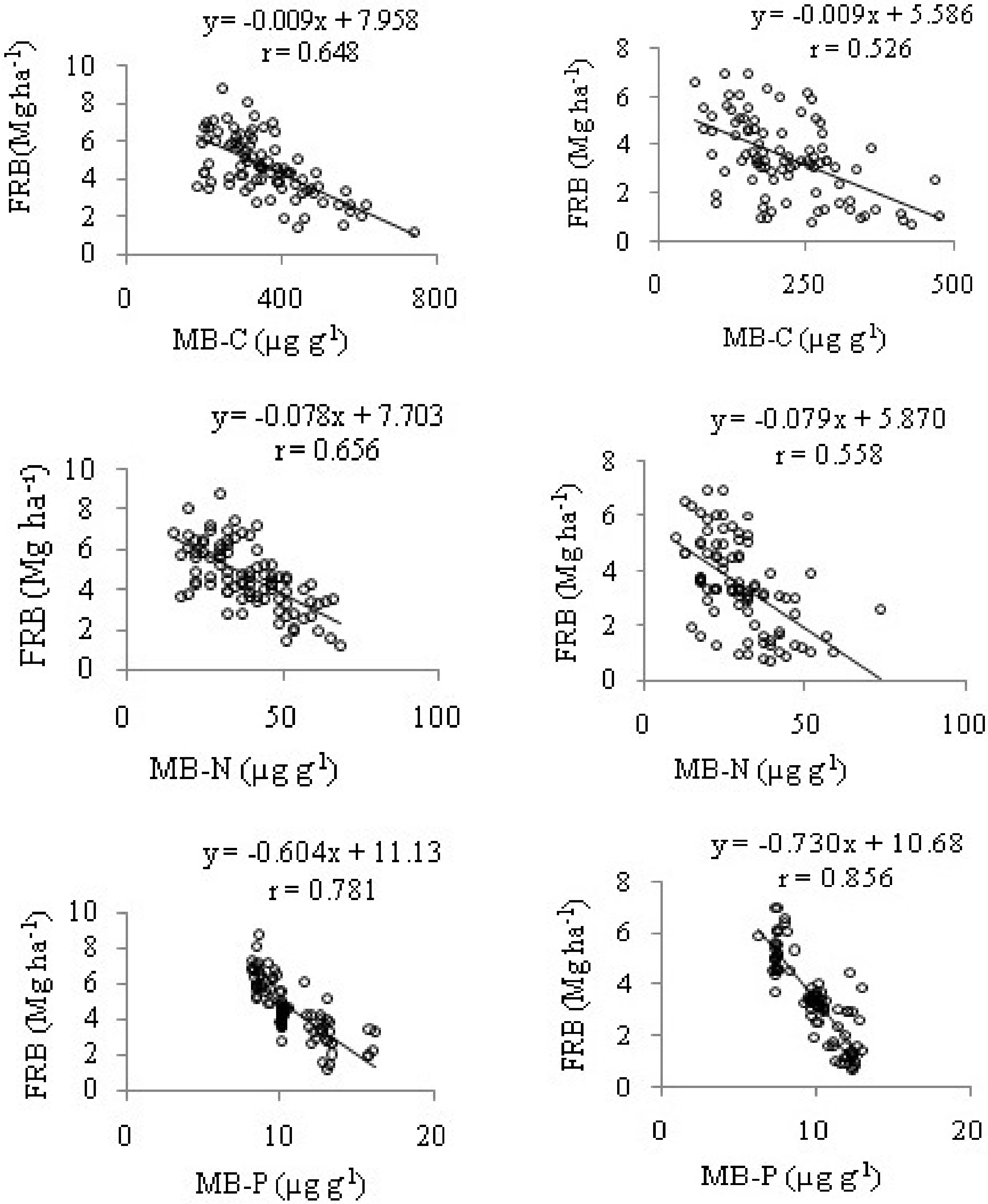


Figure 5. Regression analysis of fine root biomass (FRB) with soil microbial carbon (MB-C), nitrogen (MB-N) and phosphorus (MB-P) in upper soil layer of Tarai Sal forest and Hill Sal forest of eastern Nepal.($n = 90$, $P < 0.001$)

class was 14% higher than TSF. Biomass of < 2mm size class of HSF and TSF were also significantly ($P < 0.001$) higher than that of 2-5 mm size class.

Variations in the FRB between seasons were significant ($P <0.001$) in TSF and HSF. The maximum FRB was recorded during rainy season and minimum during the summer season (Figure 4). Fine root biomass in TSF decreased by 53.12% in summer and 26.41% in winter compared to that in rainy season while the trend was 44.52% and 27.61% in HSF. Significant ($P < 0.001$) seasonality was also observed in the biomass of different fine root size class in TSF and HSF. Regression analysis of FRB with MB-C ($r = 0.526$; $r = 0.648$), MB-N ($r = 0.558$; $r = 0.656$) and MB-P ($r = 0.856$; $r = 0.781$) in upper soil layer showed significant negative relationship in TSF and HSF respectively (Figure 5).

DISCUSSION

In forest ecosystem the nutrient succession depends on site, climate, topography, and biotic communities (Foster and Bhatti 2006). Amounts of organic matter in the soil are regulated by addition of plant residues in the form of litter and fine root and rate at which these various organic resources decompose (Batjes 1996). Addition of organic matter in the soil increases the level of soil microbial biomass and its activities like decomposition and mineralization which are the main process of nutrient input in soil.

In Tarai and Hill Sal forests soil texture was sandy loam. Values of soil moisture, water holding capacity and bulk density were higher in TSF and showed decreasing trend with increasing altitude. Tarai Sal forest showed higher soil temperature and moisture than HSF which may enhance the decomposition of litter and fine root turnover rate, as a result the soil may enrich with available nutrients causing higher water holding capacity (Reth et al. 2005). Soil moisture and bulk density increased while water holding capacity decreased depth wise in the both Sal forests. Soil of Tarai Sal forest was found acidic than Hill Sal forest and acidic nature decreased along soil depth in both the forests. Soil organic carbon increased along the increasing altitude however it decreased along the increasing soil depth. Total nitrogen and phosphorus of HSF was higher than TSF. Lower temperature together with high atmospheric humidity of HSF may cause slow rate of soil organic matter decomposition, mineralization and nitrogen turnover thus accumulation occurs at high rate.

In the present study MB-C, MB-N and MB-P in low elevated stand were lower than in high elevated i.e. it was found higher in HSF than TSF. This finding might be attributed to the decreasing of microorganism turnover rate at high altitude. Relatively lower microbial turnover rate in HSF is evident from the higher microbial C: N: P ratio in HSF (33:5:1) than the TSF (22:3:1). This variation is mainly due to difference in altitudinal topography which influences the environmental conditions and alters air temperature, atmospheric humidity and soil moisture (Scowcroft et al. 2000). Furthermore, soil microbial biomass and its activities are dependent on the quality, quantity and turnover of decomposing organic matter present in the forest floor (Barbhuiya et al. 2008). Hill Sal forest has higher value of organic matter than TSF and so also soil microbial biomass carbon, nitrogen and phosphorus are higher. This is evident from significant positive correlation of MB-C and MB-N with soil organic carbon. Chen et al. (2005) also concluded that soil microbial biomass depends on soil organic matter. Reduction in soil organic matter also causes the reduction in soil microbial biomass. Relatively dense vegetation greater accumu-lation of litter and fine root favor the growth of micro-organisms and accumulation of carbon, nitrogen and phosphorus in the microbial biomass. Soil microbial biomass carbon, nitrogen and phosphorus showed distinct seasonal variation in both forest stands. It was maximum in summer season, middle value in winter and minimum in rainy season. Low value of soil MB-C, MB-N and MB-P during rainy season might be due to sudden change in environmental condition that cause death of large amount of the microbial biomass.

Annual mean FRB (<5 mm) was significantly higher ($P <0.001$) in HSF than TSF. Hill Sal forest accumulated more organic matter due to lower decomposition rate. This results into higher organic form of nitrogen which could be related to high value of fine root biomass (Nasholm et al. 2009). A significant ($P < 0.001$) seasonality was observed in the FRB in both forest stands. Fine root biomass of both size classes was maximum in rainy season followed by winter and summer season in both forests. The maximum FRB of both root size classes during rainy season in both stands might be associated with the higher nutrient availability and soil moisture. Fine root biomass of TSF and HSF decrease in winter by 37% and 32% and summer by 63% and 48% respectively indicates a rapid turnover of fine roots (Pei et al. 2012).

Soil microbial biomass is the main source of nutrients for the plant (Singh et al. 1989). Vegetation types, quality and quantity of litter and amount of fine root nacromass all influences the status of soil microbial biomass and its activities (Jin et al. 2010). In general, soil nutrients show opposite relationships with fine root biomass (Maycock and Condon 2000). It is evident from a negative correlation of MB-C, MB-N and MB-P with amount of fine root biomass. Fine root mortality transfer considerable amount of organic matter and nutrients in forest soils which may enhance the level of soil microbial biomass (Jenkinson and Powlson 1976). In the Sal forest, soil microbial biomass showed maximum value during summer season might be due to its accumulation when plant growth and nutrient demand are minimum. However, fine root showed maximum value during rainy season because of fast turnover of micro-organisms and optimum utilization of nutrients by the plants when growth and development remain at the peak.

CONCLUSION

Hill Sal forest showed higher value of soil microbial biomass due to being at a higher altitude than TSF. Hill Sal forest has higher value of organic matter than TSF and so also soil microbial biomass carbon, nitrogen and phosphorus are higher. Annual mean fine root biomass was significantly higher in HSF than TSF. Hill Sal forest accumulated higher organic matter due to lower decomposition rate. This results into higher organic form of nitrogen which could be related to high value of fine root biomass. Soil microbial biomass and fine root showed distinct seasonal variation in both forest stands. Soil microbial biomass is the main source of nutrients for the plant which showed opposite relationships with fine roots. Soil microbial biomass showed maximum value during summer season might be due to its accumulation when plant growth and nutrient demand are minimum. However, fine root showed maximum value during rainy season because of fast turnover of micro-organisms and optimum utilization of nutrients by the plants when growth and development remains at the peak. In conclusion, fine root mortality transfer considerable amount of organic matter and nutrients in forest soils which may enhance the level of soil microbial biomass.

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