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Comparative Study on Soil Microbial Biomass in Tarai and Hill Sal (*Shorea robusta* Gaertn.) Forests of Tropical Region in Eastern Nepal

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Abstract

A comparative study was conducted to investigate the effect of altitudinal variation and seasonality on soil microbial biomass carbon (MB-C), nitrogen (MB-N), and phosphorus (MB-P) betweenTarai Sal forest (TSF) and Hill Sal forest (HSF) of the tropical region in eastern Nepal. Soil microbial biomass was estimated by chloroform fumigation - extraction method in summer, rainy and winter seasons in the upper (0-15 cm) soil depth in both forests. Pre-conditioned soil samples were saturated with purified liquid chloroform, represented fumigated sample. Another set of soil samples without using chloroform, represented unfumigated samples and soil microbial biomass was estimated from these samples. MB-C, MB-N, and MB-P were higher by 66%, 31%, and 9%, respectively, in HSF than TSF. Distinct seasonality was observed in soil microbial biomass. It was maximum in summer and minimum in rainy season in both the forest stands. The value decreased from summer to rainy season by 46 to 67% in HSF and by 32 to 80% in TSF. Higher soil microbial biomass in the summer season may be due to its accumulation in soil when the plant growth and nutrient demand are minimal. Analysis of variance suggested that MB-C, MB-N, and MB-P were significantly different for both sites and seasons ($P < 0.001$). Soil organic carbon, TN, and TP were positively correlated with MB-C, MB-N, and MB-P in both the forests. In conclusion, the higher value of soil microbial biomass in HSF may be due to the higher concentration of soil organic matter and decreasing turnover rate of microbial biomass due to higher altitude. On the other hand, the lower value of microbial biomass at TSF may indicate its fast turnover rate due to lowland tropics to enhance the nutrient cycling process.

Keywords

Deciduous vegetation, microbial biomass, sal forest, seasonality, soil organic matter

Introduction

Soil microbial biomass is an active and living fraction of soil organic matter. It is a source and sink of available nutrients and plays a crucial role in nutrient exchange and conservation in forest ecosystems (Singh *et al*., 1989). Bacteria, fungi, actinomycetes, rotifers, and protozoa (up to $5\times10^3\mu m^3$ size) are the major soil organisms that constitute the soil microbial biomass (Jenkinson and Ladd 1981). Though, it represents a small portion of soil organic matter, it is an active part due to its rapid turnover rate and fast release of available nutrients into the soil. Thus, it contributes to the nutrient cycle process far greater than its size (Schnurer *et. al.,* 1985). The microbial activity also has a direct influence on ecosystem fertility and stability (Smith *et al*., 1993). Furthermore, microbial biomass acts as an essential ecological indicator that shows a quick response to any change in the ecosystem (Powlson and Jenkinson 1981). Microbial action is responsible for decomposition and mineralization of plant and animal residues in soil and thus helps to link the soil-plant nutrient cycling (Duffkova and Macurova 2011).

Variation in altitude is the main ecological factor that creates change in climatic conditions

and so also in soil properties by affecting physical, chemical, and biological processes (Hutchins *et al*., 1976). Similarly, it also influences plant distribution, morphology, physiology, and plant growth (Li pan *et al*., 2009). With a 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 microbes and their activities are also influenced by forest types, which mainly based on the quantity and quality of organic matter inputs. Besides this, seasonal variations in temperature, rainfall, and organic matter accumulation from litter fall also have a significant influence on soil microbial biomass (Chang *et al*., 2016).

Tarai Sal forests of Jalthal is a unique tropical forest of Nepal (Chaudhary *et al.,* 2015). It has dense vegetation representing the components of deciduous forest and harbors many tropical, sub-tropical, as well as endangered species. On the other hand, Hill Sal forest located in the sub-Himalayan tract (Shiwaliks) represents a fragile ecosystem with a weak landscape. This forest contains upper tropical deciduous and semi-deciduous vegetation. Nowadays, these forests are disturbed by overgrazing, firewood collection, litter collection, timber cutting, and forest fire due to human settlements near the vicinities. Hill Sal forest has also been subjected to many natural disturbances, including landslide due to high altitude, weak lithology, and steep slopes (Bhatt 1977). Anthropogenic disturbance in the forest results in significant alternation in the microbial community and its activities (Srivastava and Singh 1989) and also in soil-microbe-plant-nutrient cycling (Jordan 1985).

Sufficient information is not available on the status of soil microbial biomass in the forests having a distinct topographic variation. In the present work, an attempt has been made to evaluate the effect of the altitudinal change on the status of soil microbial biomass in Sal forests located in Tarai and Hill regions of the tropical belt in eastern Nepal. The work was designed to answer the following questions (i) What is the status of soil organic carbon, total nitrogen, and total phosphorus in these forests differing in altitude? (ii) How do soil microbial biomass carbon, nitrogen, and phosphorus vary in these forests? (iii) How does soil microbial biomass correlate with soil organic carbon, total nitrogen, and phosphorus and (iv) How seasonality effect on soil microbial biomass along the altitudinal gradient.

Methodology

Study area

The study was carried out in the Sal forests located in the Tarai and Hill regions of eastern Nepal. Sal forest of the Tarai region is addressed as Tarai Sal forest (TSF), which is situated at Jalthal near Kechana (Extreme low land of Nepal) of the Jhapa district. It occupies an area of 6300 ha, and altitudinal variation ranges from 62 to 129 m msl. The studied TSF is situated in between 87° 55' and 88° 03' E and 26° 27' and 26° 32' N (Fig. 1). Sal forest of Hill region is addressed as Hill Sal forest (HSF), which is located at Kiteni of Kolbung, Ilam district. This forest lies at the sub-Himalayan tract (Shiwaliks), where the altitude ranges from 500 to 850 m msl. The studied HSF is situated in between 88° 02' and 88° 04' E longitude and 26° 44' and 26° 47'N latitude.

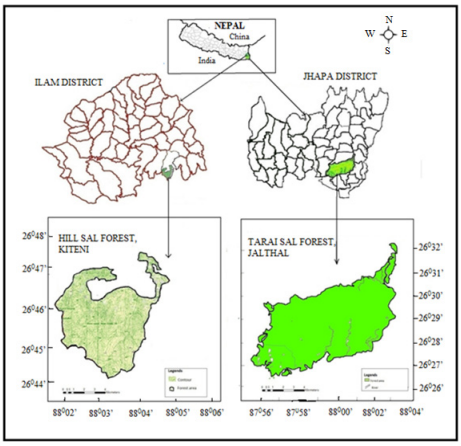


Fig. 1. Location of study area of Hill Sal forest at Kiteni, Ilam district, and Tarai Sal forest at Jalthal, Jhapa district in eastern Nepal.

Climate

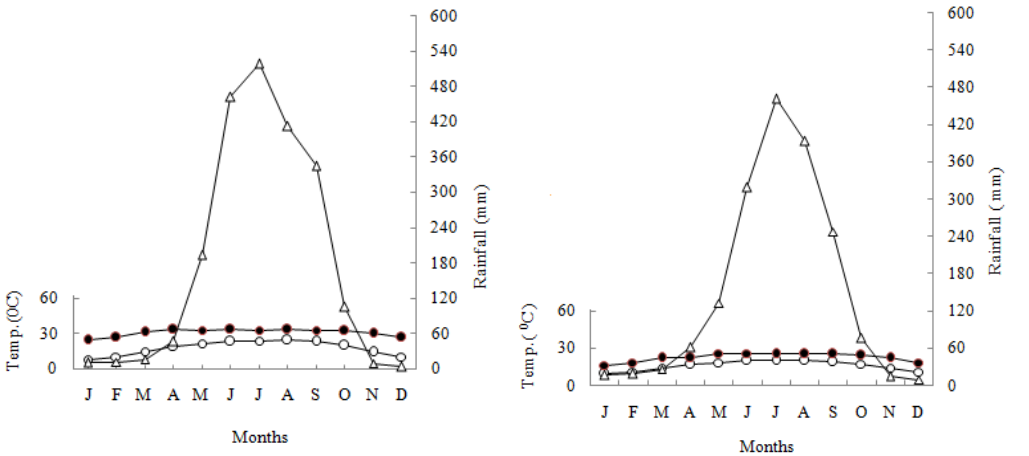
The climate of the study area is tropical monsoon type. Based on the data about the period 2001-2014, the mean monthly minimum temperature

ranged from 10.05^{0}C to 23.99^{0}C, and maximum temperature ranged from 23.92^{0}C to 33.45^{0}C in the TSF (Fig. 2a). Likewise, the mean monthly minimum temperature of HSF ranged from 9.36^{0}C to 19.88^{0}C, while the maximum temperature ranged from 16.45^{0}C to 25.91^{0}C (Fig. 2b). The average annual rainfall of TSF was 2130.4 mm, and HSF was 1776.07 mm (Fig. 2a & 2b). Data on temperature and precipitation were recorded at Kankai Irrigation Base Camp Observatory, Gaida, Jhapa (90 m msl), and Ilam Base Camp Observatory, Ilam Bazar, Ilam (1200 m msl).

Soil sampling and analysis

Each forest stands of TSF and HSF was demarcated in the outer buffer area and inner core area. The inner core area considered as the sampling area was divided into 100 compartments. As per the partial random sampling, 30 compartments were selected randomly. Within each compartment, 20m × 20m permanent plot was fixed randomly for sampling. Soil samples were collected in two soil depths (0-15cm and 15-30cm) from thirty randomly selected plots in each forest site. At each plot, the soil was collected from three pits (10cm × 10cm × 15cm), mixed and pooled as one replicate. Such type of thirty replicates was taken for soil analysis. 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. Soil microbial biomass carbon, nitrogen, and phosphorus were determined in the upper depth (0-15cm) for each season.

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



(2a) (2b)

Fig. 2. Ombrothermic representation of the climate inTarai Sal forest (2a) and Hill Sal forest (2b) regions of Nepal. The temperature (-○-; mean monthly minimum and -●-; mean monthly maximum) and -△-; rainfall data pertain to the period 2001-2014(Source: Department of Hydrology and Meteorology, Government of Nepal)

Determination of soil microbial biomass

Soil microbial biomass was estimated by the chloroform fumigation extraction method. Microbial biomass carbon (MB-C) 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 sulfate. Microbial biomass carbon was then estimated from the equation: MB-C= $2.64E_c$ where E_c is the difference between carbon calculated from fumigated and unfumigated soils, both expressed as C µg g^{-1} dry soil (Vance *et al*., 1987). Soil microbial biomass nitrogen (MB-N) 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 derived from that of fumigated soil extract; the difference in the amount 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 (MB-P) was estimated in the $NaHCO_3$ extracts of fumigated and unfumigated soils using the ammonium molybdate stannous chloride method. Microbial biomass phosphorus 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).

Statistical analysis

The data obtained from the analysis of soil samples were subjected to two-way 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 by using SPSS 20 statistics software. Correlation analysis was done in between soil organic carbon, total nitrogen, total phosphorus, potassium, microbial biomass carbon, microbial biomass nitrogen, and microbial biomass phosphorus from upper soil depth (0-15cm) of TSF and HSF.

Results

Soil physico-chemical properties of TSF and HSF *viz*. texture, moisture, BD, WHC, pH, SOC, TN, TP, MB-C, MB-N, and MB-P are presented in Table 1 and Table 2. Both forests had sandy loam type of soil texture. The proportion of sand showed a marked increase with soil depth in both forests, but silt increased with depth only in TSF. Clay decreased along with the soil depth in TSF. Soil moisture of TSF was higher than HSF, and in both forest types, it was higher in the rainy season than winter and summer seasons. The pH value was slightly higher in HSF than TSF. However, the value increased in lower soil depth in both forest stands. The water holding capacity was marginally higher in TSF than HSF. It decreased with soil depth in both forests. Bulk density was higher in TSF than HSF and increased with soil depth. In the upper soil depth, soil organic carbon was higher in HSF (2.09%) than TSF (1.6%), and it decreased depth-wise. Total nitrogen was also higher in HSF (0.173%) than in TSF (0.129%), while total phosphorus was the same in both forest stands. The potassium, an extractable soil nutrient, also showed a higher value in HSF (312.13 µg g^{-1}) than TSF (238.47 µg g^{-1}). The value of SOC, TN, TP, and K decreased depth-wise in both forest stands (Table 1).

ANOVA suggested that the variation in MB-C, MB-N and MB-P were significantly different for sites (for MB-C, $F_{1,174}$ = 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 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).Adistinctseasonal variation was also observed in the content 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 (Fig. 3). The annual mean value of MB-C, MB-N, and MB-P showed an increasing trend along with the increasing altitude. Hill Sal forest showed 66%,31%, and 9% higher value of MB-C, MB-N, and MB-P over TSF (Table 2), respectively. Microbial biomass carbon, nitrogen, and phosphorus reduced by 46% to 67% from

summers 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 were higher in HSF than TSF (Table 2). Based on the annual mean value, MB-C, MB-N, and MB-P as a fraction of SOC, TN and TP were comparable between HSF and TSF except MB-C as a fraction of SOC which was higher in HSF (Table 3). Microbial biomass carbon, nitrogen, and phosphorus were positively and significantly correlated with SOC, TN and TP in both forests stands (Table 4 and 5).

Discussion

Variation in altitude within a short distance is a common phenomenon due to the widespread of mountains in the forest ecosystems of Southeast

Table 1. Soil characteristics of the Tarai Sal forest and Hill Sal forest of eastern Nepal. Values are means ±1 SE (n=30).

Soil properties	Tarai Sal forest		Hill Sal forest	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Soil texture (%)				
Sand	52 ± 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 ± 0.4
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

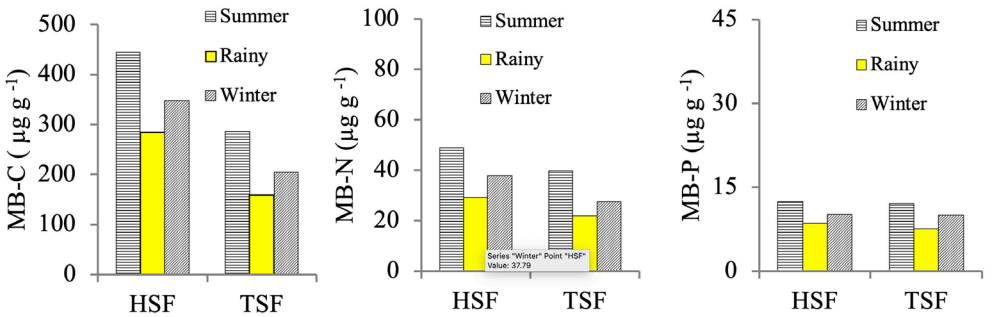


Fig. 3. Seasonal variation in MB-C, MB-N, and MB-P in Hill Sal forest and Tarai Sal forest of eastern Nepal.

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 (n=90).

Forests	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

Table 3. Soil microbial biomass (MB-C, MB-N, MB-P) as a percentage of SOC, TN, and TP in the Tarai Sal forest and Hill Sal forest of eastern Nepal.

Forests	Soil microbial biomass as % of		
	Soil organic carbon	Total nitrogen	Total phosphorus
Tarai Sal forest	1.3	2.3	1.5
Hill Sal forest	1.7	2.2	1.6

Table 4. 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**

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

able 5. Correlation between soil microbial biomass and other soil chemical properties in HSF of eastern Nepal.

	SOC	TN	TP	K	MB-C	MB-N
TN	0.734**					
TP	-0.038	-0.100				
K	0.671**	0.603**	-0.219			
MB-C	0.789**	0.579**	0.023	0.439*		
MB-N	0.565**	0.668**	0.036	0.219	0.705**	
MB-P	0.349	0.387*	0.053	0.072	0.373*	0.663**

T** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Asia. This variation can influence the microclimate, such as the temperature and moisture (Chang *et al*., 2016). Finally, it changes the soil's physical, chemical, biological properties, including soil microbial biomass. In the present study, the soil texture in both the forests was sandy loam. The values of soil moisture and water holding capacity were higher in the Tarai Sal forest which might be due to the presence of a relatively higher amount of clay, giving them the exceptional ability to adsorbed water and other substances (Brady and Weil 2013).

Moreover, TSF 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 might be enriched with available nutrients causing higher water holding capacity (Reth *et al.,* 2005). The bulk density in 0-15cm depth was higher in TSF than HSF and increased in lower depth in both Sal forests. The relatively lower value of bulk density in HSF might be due to the presence of higher organic matter accumulation in the soil than the TSF. Tarai Sal forest contained acidic soil than Hill Sal forest, and acidic nature decreased along with soil depth in both the forests.

Tarai Sal forest had a lower value of soil organic carbon and total nitrogen, which increased along with higher altitude. At the lower elevated landscape of TSF, higher soil moisture and temperature cause a fast turnover of litter and fine root due to which there may be less accumulation of organic matter. On the other hand, higher accumulation of soil organic carbon and total nitrogen at Hill Sal forest may be due to low soil temperature and moisture, causing reduced decomposition and slow turnover of organic matter. The same trend relating to altitude, temperature, and decomposition has also been reported by Griffiths *et al.,* 2009. Tsui *et al*., (2013) also found a positive relationship between elevation and soil organic matter.

Similarly, DFRS (2015) also showed that SOC stock in the forest was found to increase with increasing altitude. The total nitrogen of the Hill Sal forest was higher than the Tarai Sal forest. A similar trend was also reported by Li *et al*., (2013) in the Gongga mountain of western China. Lower temperature due to high altitude in the Hill Sal forest may cause the slow rate of mineralization and nitrogen turnover; thus, accumulation occurs at a high rate. Moreover, there is a longer residence time of nitrogen in the litter and a decrease in soil nitrogen losses at a higher altitude than lower altitude (Smith *et al*., 2002).

Soil microbial biomass is the primary source of nutrients for the plant (Singh *et al*., 1989). They regulate the decomposition of plant litter, increase the level of available nutrients and rate of N-mineralization. The status and activities of microbial biomass are generally influenced by altitude (Chang *et al*., 2016), vegetation types and quality as well as quantity of plant litter (Hackl *et al*., 2004; Jin *et al.,* 2010). In the present study, soil microbial biomass carbon, nitrogen, and phosphorus in the low elevated stand were lower than in high elevated, i.e., it was found higher in HSF. This finding might be attributed to the decreasing of microorganism turn over rate at high altitude. The relatively lower microbial turn over rate in Hill Sal forest 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 differences in altitudinal topography, which influences the environmental conditions and alters air temperature, atmospheric humidity, and soil moisture (Scowcroft *et al*., 2000). An increase in the size of microbial biomass is linked with a widening of MB-C: MB-N ratio from TSF to HSF. It suggests that the topographic variation alters both the size and composition of microbial biomass.

Furthermore, soil microbial biomass and its activities are dependent on the quality, quantity, and decomposing organic matter present in the forest floor (Barbhuiya *et al*., 2008). Hill Sal forest had a higher value of organic matter than the Tarai Sal forest, and so also soil microbial biomass carbon, nitrogen, and phosphorus were high. It is evident from the significant positive correlation of MB-C, MB-N, and MB-P with soil organic matter. Chang *et al*., (2016) and Chen *et al.,* (2005) also reported that the soil microbial biomass is highly correlated with soil organic matter. Relatively higher accumulation of litter and fine root favor the growth of microorganisms and the collection of carbon, nitrogen, and phosphorus in the microbial biomass. Microbial composition and activity also

depend upon nutrient availability of soil as there is a positive correlation between MB-C, MB-N, and MB-P with SOC, TN, and TP in both forests (Couteaux *et al*., 1998).

Soil microbial biomass carbon, nitrogen, and phosphorus showed distinct seasonal variation in both forest stands. The maximum value of soil microbial biomass during the summer season might be due to its accumulation when plant growth and nutrient demand are minimal. On the other hand, the value of soil microbial biomass was minimum during the rainy season because of the fast turn over of microorganisms and optimum utilization of nutrients by the plants when growth and development remain at the peak. A similar trend of seasonal variation was observed by Singh *et al.,* (2001) in a moist tropical Sal forest located at Panchakanyaplateau of Sunsari district, eastern Nepal. Arunachalam and Arunachalam (2000) in the humid subtropical forest of Northeast India, Singh *et al*., (2010) in the tropical forest of Vindhyan Plateau India and Tripathi and Singh (2012) in Indian tropical forest have also reported same trends regarding the effects of seasonality on soil microbial biomass. The low value of soil MB-C, MB-N, and MB-P during the rainy season might also be due to sudden change in environmental conditions (drying, rewetting, and soil temperature fluctuations) that cause the death of a large amount of the microbial biomass (Singh *et al*., 2010). The death of the microbes and release of microbial nutrients at the start of the rainy season supports the initiation of plant growth.

Conclusion

Hill Sal forest influences the environmental conditions due to having high altitude. As a result, may have an impact on accumulation, decomposition, and turnover of organic matter. It has a higher value of soil organic matter than the Tarai Sal forest, and so also soil microbial biomass carbon, nitrogen, and phosphorus are higher. Soil microbial biomass carbon, nitrogen, and phosphorus also showed distinct seasonal variation in both forest stands. It was maximum in summer season due to its accumulation in soil when the plant growth and nutrient demand are minimum, and minimum in the rainy season which may be due to high turnover of microbial biomass and proper utilization of converted nutrients in a situation when growth and development of the plants lie at the peak.

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