*Article*

# Temporal Dynamics of Soil Microbial Biomass C, N and P along an Altitudinal Gradient in Kumaun Himalayan Belt of India

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**Abstract:** This study assessed the periodic fluctuations among microbial biomass Carbon (C), Nitrogen (N) and Phosphorus (P), and the consequences of variations in altitude and abiotic factors on the soil microbial biomass (C, N and P) in a temperate mixed-oak pine forest of Central Himalaya. This research was directed at three forest stands along an altitudinal gradient. Samples were collected in triplicates, seasonally from each selected site and microbial C, N and P were determined through the fumigation extraction method. Microbial biomass C, N and P decreased significantly ($P<0.01$, correlation coefficient -0.985, -0.963, -0.948, respectively) with increasing altitude, while the rainy season showed the highest values, and winter season revealed the least values. Microbial biomass C, N and P showed positive correlation with silt particles, water holding capacity, bulk density, porosity, soil moisture, organic C, total N and P, and negative correlations with sand particles and soil pH. The microbial biomass C showed strong associations with soil microbial N ($r=0.80$, $P<0.01$) and P ($r=0.89$, $P<0.01$) contents, while the soil microbial biomass N and P also showed strong positive correlation ($r=0.92$, $P< 0.01$). Soil microbial biomass was greatly influenced by the altitude and abiotic variables whereas, weakly by temporal variation. The microbial C: N ratio indicated that fertility of soil is influenced by the species assemblage. Our findings suggest that high microbial biomass and low C: N ratio during rainy season could be considered as a strategy to conserve nutrients by temperate mixed-oak pine forest ecosystem.

**Keywords:** abiotic variables; altitude; immobilization; mineralization; mixed oak-pine forest

## 1. Introduction

Soil microbes play an important role in the transformation of C and nutrients in the forest soils [1]. The transformation ability of microbial biomass helps in conversion of the complex organic matter present in soil in to inorganic compounds which can be reused by plants. As such, the biomass is considered both the source as well as sink of the nutrients (Carbon, Nitrogen, Phosphorus and Sulphur etc.) contained in the organic substance. In soils, the decomposition process of organic substances through microorganisms provides nutrient requirements to land plants [2-3]. It is the prime location for the majority of biological activities in soil and contains around 2-3% of total organic carbon, and can be considered as a labile pool of essential plant nutrients such as N, P and S, which are held in a form largely protected from loss due to leaching or fixation.

In the cycling of important nutrients including C, N and P, microbial actions are associated with the mineralization process of these nutrients [4]. In different soil processes like N mineralization and nutrient cycling, soil microbial biomass plays a vital part and acts as a sensitive bio-indicator to on-going climate change [5]. Microbial biomass can easily react to change the conditions of nutrients, moisture, temperature and the kind and quantity of organic matter in soils within a year [6]. These feature emphasize that the soil microbial

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biomass is an important indicator of changes in nutrient status [7-8] vegetation composition [9] and climatic conditions [10].

Forest composition affects the nutrient cycling, quality, amount and decomposition of litter, root growth, and soil properties due to the changes in microbial environment [11-15]. Tree diversity also influences on soil fertility and composition of microbial community, which ultimately affects the soil microbial biomass and microbial efficiency in nutrient consumption [16-18]. Moreover, the microbial biomass C to microbial biomass N ratio is an indicator of the organization and the state of microbial community. Subsequently, a greater microbial biomass C to microbial biomass N ratio specifies the high proportion of fungus in that microbial biomass, while a little value recommends that bacteria predominates in the microbial populations [19].

The activity of microorganisms in organic matter decomposition determines the mineralization and immobilization of nutrients which affects availability of nutrients in soil and plant growth [20]. Besides, the availability of nutrients like N and P in space and time strongly influences plant communities and ecosystem functioning [21-24].

We conducted a two- year field study in a mixed oak-pine temperate forest in Central Himalaya, India. The major objectives of the study were: (i). to analyse the effect of altitude on soil microbial biomass C, N and P (ii) to evaluate seasonal variation in microbial biomass C, N and P. (iii) To determine the relationships of soil microbial biomass C, N and P with soil physico-chemical properties. In the present study, we tested the following hypothesis (i) soil microbial biomass would differ with changing altitude and (ii) the soil microbial biomass would also differ seasonally as well as annually.

## 2. Materials and Methods

### *2.1. Description of the Study Site*

The present study was conducted in the Kumaun Himalayan region of Uttarakhand, India (29°19′29°28′ N Latitude and 79°22′79°38′ E longitude). The three forest sites selected for this study were dominated by Chir pine (*Pinus roxburghii* Sarg.) and Banj oak (*Quercus leucotrichophora* A. Camus), which cover a 200 m vertical transition area having the elevations of about 1500 m (HB), 1600 m (HS) and 1700 m (HT), with the similar topographic and environmental factors, like aspect slope and forest composition. The dominant shrub species were *Hypericum cernuum* and *Berberis asiatica* at the HB; *Rumex hastatus* and *Pyracantha crenulata* at the HS; and *Rubus ellipticus* and *Berberis asiatica* at the HT.

During the first year of study, average monthly minimum temperature was observed between 4°C (January) and 17°C (June), while the average monthly maximum temperature was observed between 11°C (February) and 26°C (June). The total precipitation was recorded for about 2527 mm, and ranged between zero (November) to 842 mm (July) (Figure 1a). During the second year, the average monthly minimum temperature was observed between 5°C (January) and 16°C (May, June, July and August) while, average monthly maximum temperature was observed between 13°C (January and December) to 26°C (May and June). The total precipitation was recorded for about 1996 mm, and ranged from 2 mm (December) to 589 mm (July) (Figure 1b).

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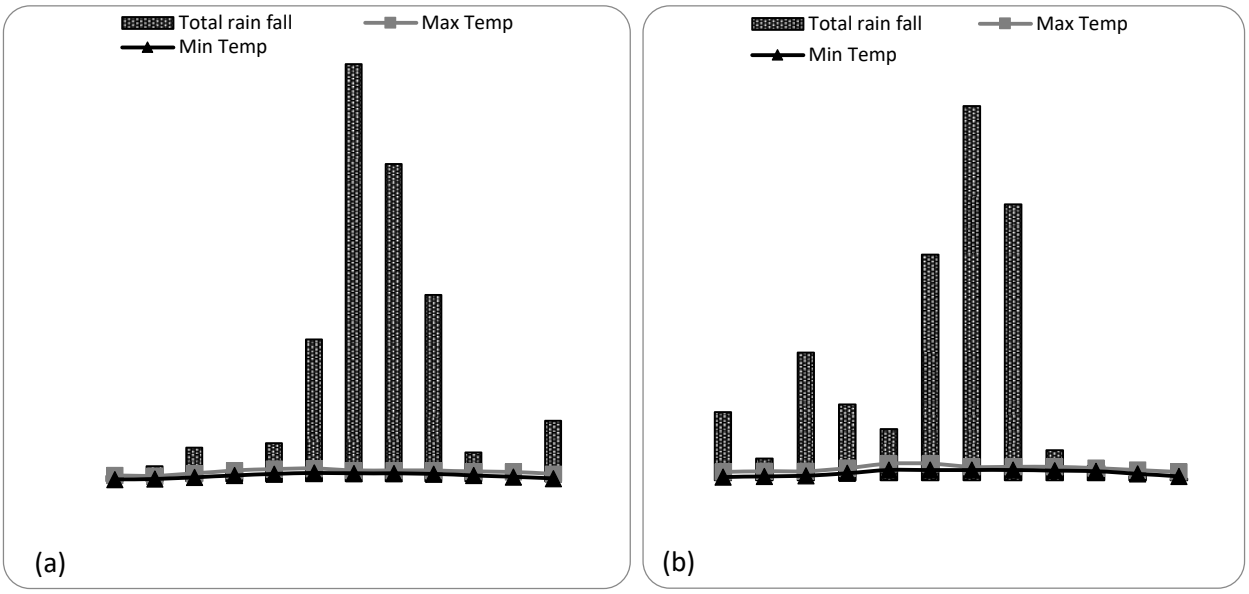


**Figure 1.** Meterological data (a) during January 2014 to December 2014 (first year) and (b) January 2015 to December 2015 (Second year). (Source: ARIES, Nainital).

The study sites were geologically situated in the lesser Himalaya. According to Valdiya [25], these rocks are the compound mixture of alluvial, low grade transformed and igneous rocks which belong to Krol series in the lesser Himalaya. A sequence of limestones, grey and greenish-grey, siltstones, purple slates, and the upper part having huge dolomites follow the Blaini without perceptible break, and was named as Krol series by Medlicott [26]. The Blaini rock contains siltstones and conglomerates. Moreover, the Krol development involves majorly lime stones, slates and marl in the lower portion and dolomites on upper portion [25].

*2.2. Experimental design*

Soil was sampled from surface layer (0-15 cm) because microbial activities are majorly confined at this region [27-28]. Soil samples were collected randomly from each forest stand in rainy, winter and summer seasons in three replicates by digging soil monoliths (10 cm wide × 10 cm long × 15 cm deep). In order to randomize, the distance between soil samples, we kept the space between the soil samples at each site for at least 50 m. The collected soil samples were kept in dry ice box and were brought to the laboratory for the analysis of physical, chemical and biological properties. The soil texture was analyzed following Indian Standards [29], soil moisture by gravimetric method. Soil pH was determined through pH meter (1:5 water suspension). The soil bulk density (g $cm^{-3}$) was estimated by mass and volume. Estimation of pore space was done by using the bulk density as well as particle density. Soil organic C was estimated by Rapid titration method [30-31], total N by Micro Kjeldhal digestion technique [32] and total P by Spectrophotometer [31].

*2.3. Analyses of Soil microbial biomass*

The fresh soil samples were separated into two equal halves/sets; one set was immediately extracted (0.5 M $K_2SO_4$ for microbial C and N or 0.5 M $NaHCO_3$ for microbial P), and the other set was extracted after the fumigation by chloroform [33-34]. Subsequently, the soil microbial biomass C (SMBC) was estimated by reformed method of Walkley Black and calculated following Jenkinson and Ladd [35].

Microbial C = KEC X 0.45

The estimation of microbial biomass N was done through microkjeldahl method, and was calculated following Brookes et al. [34]:

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Microbial N = KEN X 0.54

The microbial biomass P was determined through the ammonium molybdate stannous chloride method, and was calculated following Brookes et al. [33].

Microbial P = KEP X0.40

Where, KEC, KEN and KEP are the changes between C, N and P extracted from fumigated and non-fumigated soils.

### *2.4. Data processing*

All variables were subjected to repeated measures ANOVA using 16.0 SPSS software package for Windows (SPSS Inc., IL, USA) and excel stat software.

## 3. Results

### *3.1. Soil characteristics*

Across the study sites, soil was sandy loam with 70 – 78 % sand, 10–13 % clay and 12–18 % silt. The soil moisture ranged from 11 to 22 %, soil pH, 5.6 – 6.1, soil organic carbon (SOC) 3.24–5.24 %, soil total nitrogen 0.17– 0.38 %, total phosphorus 0.041– 0.092 %, soil bulk density, 42– 65 g $cm^{-3}$, and C: N ratio was observed between 9.8 and 18.2 (Table 1).

**Table 1.** Characteristics of the studied forest stands. Values are site means (± SE, when provided).

| Parameters | Site | | |
|---|---|---|---|
| | Stand I (HB) | Stand II (HS) | Stand III ( HT) |
| | Soil | | |
| Sand (%) | 70±0.88 | 78±0.88 | 78±0.88 |
| Silt (%) | 18±0.88 | 12±0.88 | 12±0.58 |
| Clay (%) | 13±1.76 | 11±0.33 | 10±0.33 |
| Bulk density (g $cm^{-3}$) | 0.65±0.01 | 0.60±0.09 | 0.42±0.01 |
| WHC (%) | 45.65±0.43 | 42.76±0.61 | 41.47±0.69 |
| Moisture (%) | 22.48±0.37 | 14.70±0.19 | 11.36±0.35 |
| porosity | 75.35±0.33 | 77.23±3.28 | 84.28±0.33 |
| pH | 5.67±0.03 | 5.87±0.03 | 6.13±0.03 |
| Organic C (%) | 5.24±0.09 | 3.75±0.07 | 3.24±0.04 |
| Total N (%) | 0.38±0.01 | 0.38±0.01 | 0.17±0.01 |
| Total P (%) | 0.092±0.00 | 0.056±0.00 | 0.041±0.00 |
| C:N | 13.4 | 9.8 | 18.2 |
| | Vegetation | | |
| Tree Species richness | 05 | 03 | 02 |
| Tree density (stems $ha^{-1}$) | 670 | 590 | 570 |
| Basal area ($m^2$ $ha^{-1}$) | 32.39 | 27.73 | 27.20 |

### *3.2. Microbial C, N and P*

The microbial C, N and P were recorded highest at HB forest stand and least at HT forest stand. The microbial biomass C ranged between 730 μg $g^{-1}$ and 751 μg $g^{-1}$ at HB, 718 and 737 μg $g^{-1}$ at HS, 681 and 697 μg $g^{-1}$ at HT. The values of microbial biomass N were 111 to 143 μg $g^{-1}$ at HB, 93 to 111 μg $g^{-1}$ at HS, and 73 to 99 μg $g^{-1}$ at HT. While, the microbial biomass P was estimated between 53 and 72 μg $g^{-1}$at HB, 38 and 49 μg $g^{-1}$ at HS, 23 and 36 μg $g^{-1}$ at HT. (Table 2). Similar seasonal variations were recorded at all the stands with maximum values of microbial C, N and P during the season of rain and least values during cold (Table 2).

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**Table 2.** Microbial C, N and P in the soils of forest stand I, II and III (μgg$^{-1}$ soil ± S.E.).

| | Stand I (HB) | Stand II (HS) | Stand III (HT) |
|---|---|---|---|
| Microbial biomass carbon (MBC) | | | |
| Rainy | 751±2.58 | 737±1.02 | 697±0.55 |
| Winter | 730±0.86 | 718±1.32 | 681±1.81 |
| Summer | 738±1.39 | 725±1.18 | 685±2.01 |
| Annual mean | 739.67 | 726.67 | 687.67 |
| Microbial biomass nitrogen (MBN) | | | |
| Rainy | 143±8.54 | 111±4.43 | 99±2.14 |
| Winter | 111±1.78 | 93±1.41 | 73±1.38 |
| Summer | 120±1.45 | 103±1.42 | 89±1.07 |
| Annual mean | 124.67 | 102.33 | 87.00 |
| Microbial biomass phosphorus (MBP) | | | |
| Rainy | 72±3.03 | 49±3.69 | 36±1.86 |
| Winter | 53±1.57 | 38±1.53 | 23±1.55 |
| Summer | 65±1.79 | 45±1.91 | 26±1.73 |
| Annual mean | 63.33 | 44.00 | 28.33 |
| Microbial C:N | | | |
| Rainy | 5.25 | 6.63 | 7.04 |
| Winter | 6.57 | 7.72 | 9.33 |
| Summer | 6.15 | 7.03 | 7.69 |
| Annual mean | 6.16 | 7.23 | 8.19 |
| Microbial C:P | | | |
| Rainy | 10.66 | 14.31 | 19.71 |
| Winter | 13.81 | 18.41 | 35.89 |
| Summer | 11.42 | 16.43 | 27.56 |
| Annual mean | 11.96 | 16.38 | 27.72 |
| Microbial N:P | | | |
| Rainy | 1.98 | 2.26 | 2.75 |
| Winter | 2.09 | 2.45 | 3.17 |
| Summer | 1.85 | 2.29 | 3.42 |
| Annual mean | 1.97 | 2.33 | 3.11 |
| Microbial C/ organic C (%) | | | |
| Rainy | 1.44 | 1.95 | 2.13 |
| Winter | 1.76 | 2.16 | 2.25 |
| Summer | 1.45 | 2.07 | 2.22 |
| Microbial N/ total N (%) | | | |
| Rainy | 2.66 | 2.92 | 5.40 |
| Winter | 3.36 | 2.93 | 5.31 |
| Summer | 3.24 | 3.02 | 5.83 |
| Microbial P/ total P (%) | | | |
| Rainy | 7.20 | 8.16 | 9.00 |
| Winter | 7.57 | 9.50 | 11.50 |
| Summer | 9.28 | 11.25 | 8.66 |

*3.3. Contribution of microbial biomass to the soil nutrient pool*

At HB forest site, contribution of microbial biomass C to the total SOC ranged between 1.44 and 1.76 %, and was highest during the winter season and minimum during the rainy season. The microbial biomass N and P added 2.66 to 3.36 %, and 7.20 to 9.28 % to the total N and total P, respectively. At HS forest site, the contribution of microbial biomass C to total organic C was 1.95–2.16%, microbial biomass N to total soil N was 2.92 –3.02 %, and microbial biomass P to total soil P was 8.16–11.25%. At HT forest stand, the

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percent contribution of microbial biomass C to total soil organic C was 2.13–2.25%, microbial biomass N and P to total N and total P was 5.31– 5.83% and 8.66–11.50 %, respectively. At all the three forest stands maximum contribution was estimated during summer season and minimum during winter season. Across the three forest stands, the microbial C: N and C: P ratios were observed between 5.69 and 9.47 and 10.66 to 35.89 (Table 2).

Moreover, ANOVA showed the substantial variance in microbial biomass at all the studied stands, i.e., MBC ($P < 0.05$), MBN ($P < 0.01$) and MBP ($P < 0.01$), and their annual variations were also significant ($P < 0.01$) (Table 3).

**Table 3.** ANOVA showing soil microbial properties in all studied forest stands (Position).

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Altitude | 0.087 | 2 | 0.043 | 0.059 | 0.943 |
| MBC | 710.291 | 2 | 355.145 | 0.575 | 0.571 |
| MBN | 6697.936 | 2 | 3348.968 | 21.677 | 0.000 |
| MBP | 6067.508 | 2 | 3033.754 | 48.487 | 0.000 |

## 4. Discussion

Our values of microbial C were similar to the ranges of 61–2000 mg $g^{-1}$ for different soils of temperate and tropical forest [36-37] and 978– 2088 mg $g^{-1}$ for sub-tropical forest [38]. Comparatively the microbial N also showed the similar trend with coniferous forest soils reported by Martikainen and Palojarvi [39] as 52–125 mg $g^{-1}$, and with the evergreen forest soils reported by Diaz-Ravina et al. [40] as 42–242 mg $g^{-1}$, but less than the soils of broad leaved deciduous forest [40] (132–240 mg $g^{-1}$). The microbial P value falls well within the informed range of 5.3–67.2 mg $g^{-1}$ for the soils of arable land, grassland and woodland [34], and 14–46 mg $g^{-1}$ for sub-tropical moist forest reported by Arunachalam and Arunachalam [38].

### *4.1. Seasonal variation in microbial biomass*

The species composition of the three forest stands were different and this has significant effect on soil microbial biomass, however the seasonal pattern of microbial biomass was common at all the three sites, indicating that seasonal pattern of soil microbial biomass was regulated by climatic factors. The microbial C, N and P were significantly higher during the rainy season ($P < 0.01$) and lower in winter season (Table 2). It might be due to greater rate of nutrient immobilization through the microbes from the decomposing litters as the rate of litter decomposition and microbial activities are at the peak during the rainy period. Various authors [41-44] reported that due to high humidity and temperature, growth of microorganisms increased during this season and contributed to the soil microbial biomass. In contrast, for the tropical dry deciduous forest, temperate pastures and savanna, Saratchandra et al. [45] and Singh et al. [2] reported maximum values of microbial biomass during summers, and for subtropical humid forest, Arunachalam and Arunachalam [38] reported maximum value during winters. This may be due to the differences in quality of litter and rainfall pattern in these forest types. Low values of microbial C, N and P during winter season in this study might be due to the low microbial activities and slow litter decomposition in dry and cool period. Diaz-Ravina et al. [46] reported that water scarcity limits the biomass of microbes more than temperature since lesser microbial biomass was recorded during the dry season, as compared to the rainy season.

### *4.2. Effect of altitude*

Our hypothesis that the decrease in microbial biomass with increase in altitude in all the seasons in central Himalayan mixed oak-pine forest was partly demonstrated in this study. During all the sampling seasons, microbial C, N and P decreased with the increase

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in altitude (Table 2). Koch et al. [47] and Liu and Wang [48] also reported that microbial biomass decreased along the increasing altitude. According to Lipson et al. [1], at low altitude snow cover provides a protective effect during winter, while before the commencement of growing period snow melts and leaching of nutrients from high altitude provides rich substrate input to low altitude, which results in higher microbial activity. Higher soil moisture content (Table 1), low sunlight, greater diversity of plants [43] and better quality of litter at HB promotes the microbial growth. Patel et al. [49] also reported that microbial C, N and P were comparatively greater in foothill forest than the forests of higher altitude. However, Wardle [50] reported no consistent temporal pattern change of microbial biomass in the ecosystems of tropical and temperate regions. With increasing altitude, the ratios of microbial C, N and P to total soil organic C, total N and P increased. This specifies that the microbial biomass/nutrients (C, N and P) more frequently immobilized at high altitude i.e., HT stand.

*4.3. Microbial quotient*

The microbial C/N ratio (5.25-9.33) reported in this study is similar to the range reported by Martikainen and Palojarvi [39] for different forest soils (6–9) and by Fenn et al. [51] for chaparral soils (7–13). C/N ratio of fungi is often 10–12 and that of bacteria usually between 3 and 5. Since C/N ratios in this study are greater than 5, which indicates the dominance of fungal community. In this study, microbial biomass C/N ratio increased with increasing altitude (Table 2). Arunachalam and Pandey [52] stated that microbial C/N ratio is an ecosystem recovery indicator, the low values required the shorter time to build-up the microbial population. An increase in microbial biomass C/N ratio indicates the changes in the microbial community, with the governance of fungal population over the bacterial population; thus, it could be suggested that the soil at HT is fungi-dominated compared with HB and HS. It is possible that the restoration of soil at HT would take longer time than the soil at HB as apparent from the lower C/N ratio in the HB as HS and HT (Table2). The microbial C:P in this study (10.66- 35.89) lies under the range 10.6–35.9 reported by Brookes et al. [34], but less than and sub-tropical humid forest (33.2–98.5) reported by Arunachalam and Arunachalam [38] which might be due to greater microbial biomass P in the present forest stands. Microbial biomass C/P ratio and N/P ratio also showed the similar trend as described for C/N ratio (Table 2).

*4.4. Relationship between microbial biomass and abiotic variables*

The microbial biomass C, N and P revealed a substantial positive correlation with altitude, silt particles, water holding capacity, soil moisture, OC, total N and total P (Table 4). These findings indicate that the microbial biomass is strongly influenced by the abiotic variables. The microbial biomass C showed positive correlation with bulk density but adversely correlated with sand particles, soil porosity and soil pH. The microbial N and P exhibited a substantial adverse correlation with the sand particles and soil pH (Table 4).

**Table 4.** Correlation coefficients for the relationship of microbial biomass (C, N and P) with altitude and abiotic variables.

| | Altitude | Sand | Silt | Clay | WHC | BD | Mo | Po | pH | C | N | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MBC | 0.985** | -0.720* | 0.685* | 0.455 | 0.822** | 0.819** | 0.916** | -0.819** | -0.967** | 0.897** | 0.939** | 0.908** |
| MBN | 0.963** | -0.905** | 0.907** | 0.496 | 0.928** | 0.650 | 0.987** | -0.649 | -0.890** | 0.992** | 0.734* | 0.992** |
| MBP | 0.948** | -0.957** | 0.898** | 0.624 | 0.920** | 0.619 | 0.987** | -0.619 | -0.884** | 0.987** | 0.685* | 0.984** |

* Significant at (P< 0.05) and ** Significant at (P< 0.01)

The soil moisture content could be a better indicator of seasonal variations in soil microbial biomass C, N and P as indicated by significant positive correlation between the microbial biomass C, N or P and moisture content (Table 4). Similar reports related to

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significant positive correlation between soil moisture and soil microbial biomass in wet tropical deciduous forest of India have also been reported by Devi and Yadava [42]. In Central Himalayan region, the availability of soil moisture depends on rainfall. Any change in the rainfall pattern may have an impact on the soil microbial biomass dynamics, which, in turn would influence the C, N and P cycling in the region.

Soil microbial biomass C and total soil C, soil microbial biomass N and total soil N and soil microbial biomass P, and total soil P were strongly and linearly related (Table 4). This corroborates previous research showing strong correlation between microbial biomass and soil C and nutrient availability [50]. However, present study also indicated that microbial biomass C was strongly associated with the soil microbial N (0.80, $P<0.01$) and P (0.89, $P<0.01$) contents, suggesting that the stoichiometry of the soil microbial biomass is strictly maintained. These strong relationships between all elements also indicates that soil microbial biomass C depend on the soil N and P to maintain the required microbial element stoichiometry. Soil microbial biomass N and P also showed strong linear relationship (0.92, $P< 0.01$).

*4.5. Multivariate analyses (PCA) of microbial properties of soil*

Principal component analysis (PCA) was carried out to differentiate the forest types with physico-chemical and biological properties of soil (sand, silt, clay, WHC, soil microbial biomass). The multivariate analysis indicated that F1 (active sites with 38.64% variation) and F2 (active variables with 31.24% variation ) components exhibited the maximum variations with physico-chemical and biological properties of soil (Figure 2), and their cumulative variability was about 70% (Table 4).

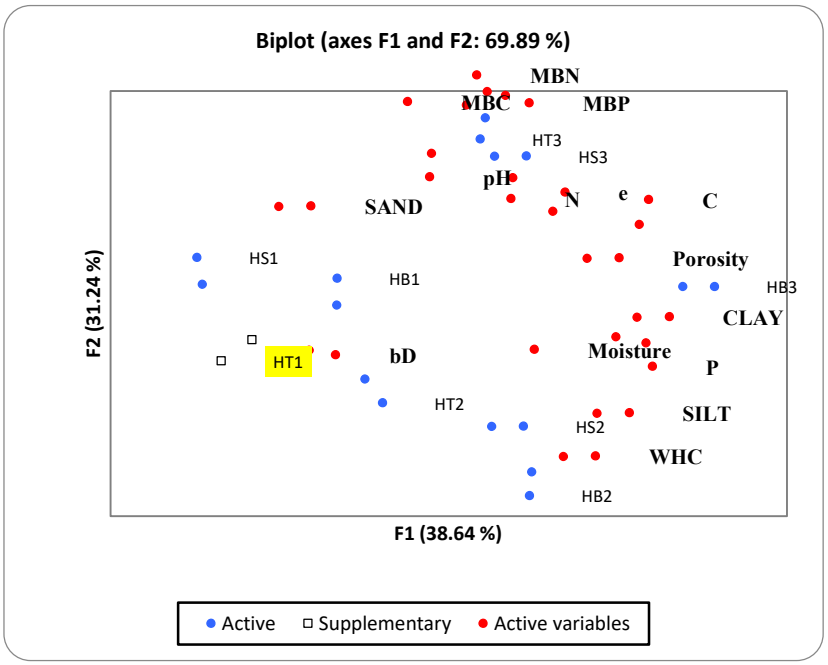


**Figure 2.** Principal component analysis (PCA) of physical, chemical and microbial parameter of soil in different stands of mixed oak-pine forest. PCA axis 1(38.64%) and 2 (18.66%) represent for first and second coordinates of sites, respectively (bD= Bulk density, WHC= Water holding capacity, e= Void ratio, C= Carbon, N= Nitrogen, P= Phosphorus, MBC= Microbial biomass carbon, MBN= Microbial biomass nitrogen, MBP= Microbial biomass phosphorus, HT1= Hill top 1, HT2= Hill top 2, HT3= Hill top 3, HS1= Hill slope 1, HS2= Hill slope 2, HS3= Hill slope 3, HB1= Hill base 1, HB 2= Hill base 2, HB3= Hill base 3).

## 5. Conclusion

This study concluded that soil microbial biomass showed a poor seasonality with a minimum value during winter season and maximum during the rainy season. The abiotic

factors highly affected the soil microbial biomass. The high value of microbial biomass carbon, nitrogen and phosphorus in the hill base stand indicated that this region had a higher amount of microorganism which sustains better soil quality. As compared to other forest stands, hill base showed higher soil moisture content, plant diversity, better litter quality and less exposure to sunlight. This site is also enriched with substrate input through rain water, snowmelt and leaching of nutrients from hill top which favored the high microbial activity. Moreover, the microbial C:N ratio indicates that soil fertility is highly influenced by the species composition of the forest stands.

**Author Contributions:** VM performed the experiments and analyzed the data. KB wrote and revised the manuscript. SSB guided the research and modified the manuscript. RKC provided editorial advice and finally revised the paper

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