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Spatial distribution of microbial biomass in relation to land-use in subtropical systems of north-east India

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Abstract: Spatial distribution of microbial biomass in relation to major land-use (grassland and agroecosystem) in subtropical region of north-east India was studied from April 2000 to March 2001. The mean microbial biomass C, N and P across the study sites at 0-10 cm soil depth ranged from 168 to 395, 20 to 68 and 8 to 30 µg g^{-1} soil $month^{-1}$ respectively. The microbial biomass exhibited seasonal peak in May and consistently decrease in both systems to the minimum in September and thereafter increase in later months. The microbial biomass in soil was characterized by a mean C:N:P ratio of 15:2:1 in grassland and 14:2:1 in agroecosystem. The microbial biomass was positively correlated to soil organic carbon, total nitrogen, phosphorous and soil pH. Higher soil microbial biomass was recorded in grassland than in agroecosystem indicating its decline on conversion of grassland into agroecosystem.

Resumen: Desde abril de 2000 hasta marzo de 2001 se estudió la distribución espacial de la biomasa microbiana en relación con los principales usos del suelo (pastizal y agroecosistema) en la región subtropical del nordeste de la India. Los valores promedio de C, N y P en la biomasa microbiana variaron de 168 a 395, de 20 a 68 y de 8 a 30 µg g^{-1} suelo mes^{-1}, respectivamente, en todos los sitios de estudio, a una profundidad del suelo de 0-10 cm. La biomasa microbiana mostró un pico estacional en mayo y consistentemente decreció en ambos sistemas hasta llegar a un mínimo en septiembre y a partir de allí incrementar en los meses posteriores. La biomasa microbiana en el suelo estuvo caracterizada por un cociente promedio de C:N:P igual a 15:2:1 en el pastizal y a 14:2:1 en el agroecosistema. La biomasa microbiana estuvo positivamente correlacionada con el carbono orgánico del suelo, el nitrógeno total, el fósforo y el pH del suelo. Se registraron valores más altos de biomasa microbiana del suelo en pastizal que en el agroecosistema, indicando así su declinación en la conversión de pastizal hacia agroecosistema.

Resumo: A distribuição espacial da biomassa microbiana em relação à principal forma de uso do solo (pastagem e agro-ecossistema) na região subtropical do nordeste da Índia foi estudada de Abril de 2000 a Março de 2001). A biomassa microbiana média C, N e P ao longo das estações estudadas na camada de solo entre os 0-10cm variou entre os 168 a 395, 20 a 68 e 8 a 30 µg g^{-1} de solo $mês^{-1}$, respectivamente. A biomassa microbiana exibiu um pico sazonal em Maio e desceu consistentemente em ambos os sistemas a um mínimo em Setembro a partir do qual cresceu nos últimos meses. A biomassa microbiana no solo era caracterizada por um valor médio do ratio C:N: de 15:2:1 na pastagem e 14:2:1 no agro-ecossistema. A biomassa microbiana estava positivamente correlacionada com o carbono orgânico, com o azoto total, o fósforo e o pH do solo. A pastagem registou um valor mais elevado da biomassa microbiana do solo do que o registado num agro-

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ecossistema indicando o seu declínio quando se passa de uma pastagem para um agro-ecossistema.

Key words: Agroecosystem, grassland, immobilization, microbial biomass, rice, seasonal changes.

Introduction

The soil microbial biomass is the labile pool of organic matter (Jenkinson & Ladd 1981) and act as both source and sink of plant nutrients (Singh *et al.* 1989). It plays a crucial role in nutrient cycling and its importance in soil fertility and nutrient concentration is well recognized. The studies on the measurement of microbial C, N and P in different natural and disturbed ecosystems have shown them to be important labile pools of C and mineral nutrients (Anderson & Domsch 1980; Smith & Paul 1990; Wardle 1992) from which nutrients are liberated after the death of the microorganisms. Changes in the microbial population in response to variations in soil conditions (moisture, organic C, nutrients, temperature, pH) have serious implications for nutrient cycling, with microorganisms acting as a source and sink of nutrient. Climatic seasonality has been reported to influence microbial populations (Diaz-Ravina *et al.* 1993) and soil microbial biomass (Granatstein *et al.* 1987; Lynch & Panting 1980) either directly by influencing microbial responses to soil changes or indirectly by influencing plant metabolism. Seasonal changes in microbial biomass in grassland and agroecosystem in tropical soils of India have been studied by Singh *et al.* (1989), Roy & Singh (1994). The present investigation was aimed to analyse seasonal changes in microbial biomass C, N and P at different soil depths in relation to land use changes from grassland to agroecosystem in northeast India.

Materials and methods

Study sites and soils

The study sites are located within the campus of Manipur University at 24° 72´N latitude and 93° 85´E longitude at an altitude of 782 m above sea level. The climate of the area is monsoonic with three distinct seasons in a year i.e. moist summer (March to May), rainy (June to October), and winter (November to February). The mean minimum air temperature varied from 3.2°C (January) to 21°C (July) and mean maximum temperature ranged from 24.3°C (January) to 32.7°C (May). The annual rainfall was 1587.9 mm and about 68% of the total annual rainfall occurs during rainy season. The parts of grassland areas have been converted into agroecosystem for last four years. The dominant grass species in the grassland ecosystem are *Imperata cylindrica* Beauv., *Leersia hexandra* Sw., *Hemarthria compressa* (Linn F.) R. Br. In agroecosystem, *Oryza sativa* L. (rice) is grown during the cropping period (June to October) and thereafter it was fallow land occupied by the weeds. No chemical fertilizers and manure was used during the cropping period of rice.

Sampling procedure and soil analysis

Soil samples were collected at different soil layers of 0-10 cm, 10-20 cm, 20-30 cm by using a steel corer (6.5 cm diameter) from five different plots of each study site at monthly intervals from April 2000 to March 2001. Individual core from each depth were placed in polythene bags. Three composite samples were made from each of the three depth segments. The field moist soils were sieved through a 2 mm sieve to remove stone, coarse, roots and other plant debris and were stored at room temperature for 24 hours. The half of the sub samples were air dried and analysed soil pH (1:5 soil water solution) by glass electrode pH meter (Systronic) and soil moisture content by gravimetric method. The soil organic carbon and total nitrogen was measured by acid dichromate calorimetry procedure and acid digestion micro- Kjeldahl method (Anderson & Ingram 1993). Available phosphorous was estimated by ammonium molybdate stannous chloride method following the method given by Sparling *et al.* (1985).

Analysis of microbial biomass

For the analysis of microbial C and N the field moist soil subsamples (equivalent to 50 g oven dry weight) were fumigated with ethanol free chloroform for 24 hours in the dark using vacuum desiccator. After removing the fumigant the soil were extracted with 200 ml of 0.5 M K_2SO_4 for 5 days in the dark. The resulting soil suspension was filtered through a Whatman No.42 filter paper. The filtered soil extracts of both fumigated and unfumigated samples were analysed for organic C using acid dichromate digestion method (Vance *et al.* 1987). Microbial biomass N in K_2SO_4 soil extract was determined by acid digestion Kjeldahl procedure (Brookes *et al.* 1985). The microbial biomass C (MBC) was then estimated from the equation MBC = 2.64 EC (Vance *et al.* 1987), where EC is the difference between C extracted from fumigated and unfumigated treatments. The soil microbial biomass N (MBN) was estimated from the equation MBN = 1.46 EN (Brookes *et al.* 1985), where EN is the difference between N extracted from fumigated and unfumigated samples.

For measuring microbial P, the moist soil samples (equivalent to 50 g dry weight) were fumigated with alcohol free chloroform in vacuum dessicator for 24 hours in dark and extracted with 200 ml of 0.5 M $NaHCO_3$ buffered at 8.5 for 5 days. The inorganic P in filtered extracts was estimated by the ammonium-molybdate stannous chloride method (Sparling *et al.* 1985). The microbial biomass P (MBP) was estimated from the equation. MBP = 2.5 EP (Brookes *et al.* 1982), where EP is the difference between P extracted from fumigated and unfumigated samples.

All the analyses were done in triplicate and results are expressed on an oven dry soil (105°C, for 24 h) basis.

Statistical analysis

Significant difference between the means of different months were examined by using ANOVA. Karl Pearson's coefficient of correlation was used for examining relationship between microbial biomass and soil properties.

Table 1. Physico-chemical characteristics of soil at 0-30 cm soil depth in grassland and agroecosystem at Imphal.

	Study sites	
	Grassland	Agroecosystem
Soil pH	5.74 (4.90-6.45)	6.16 (5.64-6.72)
Soil moisture (%)	43.56 (32.85-61.32)	43.79 (30.34-63.12)
Organic C (%)	1.05 (0.79-1.52)	0.95 (0.65-1.48)
Total N (%)	0.13 (0.06-0.23)	0.12 (0.06-0.20)
Available P (%)	0.017 (0.013-0.020)	0.016 (0.013-0.019)

*Values cited in the table are the annual mean and ranges of the value are given in the parenthesis.

Results and discussion

Soil physico-chemical characteristics

The soil of the two study sites is alluvial blackish brown in colour and clayey loam soil and moderately acidic in nature ranging from 4.90 to 6.72 (Table 1). The soil moisture content was obviously maximum during the rainy season ranging from 30.34 to 63.12% across the study sites and soil depths. The lower pH value in grassland soils than agroecosystem may be due to the higher nitrification of NH_4^+ and subsequent production of H^+ ions thus increase the soil acidity. The maximum concentration of organic C, total N and available phosphorous occurred during the summer season. The levels of soil microbial biomass remained low during the rainy season. The lower value of soil organic C, total N and P during the rainy season might be due to higher uptake of soil nutrients by the actively growing vegetation, whereas during the winter and summer season the uptake of soil nutrients by the plant is low coupled with additions of dry part of plant vegetation in soils during these seasons. Roy & Singh (1994) suggested that massive plant uptake of available nutrient during the rainy season supports active plant growth as compared to dry period. During the dry period available nutrients tends to

Table 2. Seasonal variation in soil microbial biomass C, N and P (μg g^{-1} soil) at different soil layers at grassland and agroecosystem at Imphal. Values are mean ± SE. MBC = Microbial biomass carbon, MBN = Microbial biomass nitrogen, MBP= Microbial biomass phosphorous.

Sample	Grassland			Agroecosystem		
	MBC	MBN	MBP	MBC	MBN	MBP
0-10 cm						
Summer	326±19.80	48±5.84	21±2.53	284±8.72	41±4.45	20±2.23
Rainy	255±12.48	31±2.79	14±1.69	216±12.58	26±1.94	12±1.69
Winter	310±14.05	37±1.45	19±1.34	262±11.19	29±1.51	15±1.58
10-20 cm						
Summer	93±5.94	17±1.69	8±0.53	69±2.82	15±0.98	7±0.38
Rainy	60±5.02	10±1.29	4±0.68	51±4.56	8±1.30	3±0.68
Winter	81±1.30	13±1.49	5±0.82	66±1.28	10±0.91	4±0.64
20-30 cm						
Summer	38±4.14	6±0.67	3±0.39	35±4.08	6±0.54	3±0.81
Rainy	32±2.19	4±0.55	2±0.27	23±2.63	3±0.65	2±0.30
Winter	36±0.63	5±0.09	2±0.21	31±1.22	4±0.57	2±0.29

Table 3. Nutrient ratio in microbial biomass and microbial C, N and P as proportion of soil organic C, total N and P in grassland and agroecosystem at Imphal.

	Microbial C:N ratio	Microbial C:P ratio	Microbial N: P ratio	Mic C / Organic C (%)	Mic N / total N (%)	Mic P / Soil P (%)
Grassland	6.84±0.22 (5.59-8.25)	14.8±0.67 (9.46-8.89)	2.18±0.04 (1.91-2.51)	2.11±0.12 (1.64-2.88)	2.44±0.10 (2.01-3.17)	10.15±0.43 (7..53-13.48)
Cropland	6.97±0.33 (4.66-9.83)	15.34±0.71 (9.46-8.92)	2.13±0.06 (1.67-2.45)	2.02±0.11 (1.62-2.85)	2.46±0.08 (2.03-2.98)	9.57±0.41 (7.13-13.26)

*Values cited in the table are the annual mean and monthly ranges are given in the parenthesis.

accumulate in soils owing to low plant uptake. The soil organic C, total N and P were comparatively higher in grassland soil than that of the agroecosystem. This may be due to harvest of major component of above ground parts for domestic purpose in latter ecosystem. In this system plant roots provide the major source of organic matter input in soil. Whereas, in grasslands major component of above ground biomass retain in the soil in addition to below ground parts.

Seasonal changes in microbial biomass

The microbial C, N and P varied significantly ($P < 0.01$) across different seasons in both the study sites. The microbial biomass C, N and P at 0 to 10 cm soil depth across the sites ranged from 168 to 395, 20 to 68 and 8 to 30 μg g^{-1} soil respectively in different months throughout the year. Microbial biomass C, N and P attained the peak value in the month of May and decreased consistently to the minimum in September coinciding with the peak growth in both ecosystems (Fig. 1a-f). The plant residue inputs, soil water and soil organic matter influence the amount and temporal changes in microbial biomass. Wardle (1992) has indicated that seasonal variation in temperature and soil water have marked effect on soil microbial biomass. In present study, the highest value of microbial biomass C, N and P were recorded during summer season followed by winter and rainy season. Comparatively lower value of microbial biomass during the rainy season could be due to high turnover rate of microorganism and peak growth of vegetation during the wet periods, whereas during the dry periods of winter and summer seasons plant residues continue to decompose and products are immobilized in the microbial biomass in grassland and agroeco-system. Thus, in this study, there exist a reciprocal relationship between the plant growth rates and soil microbial biomass in both the ecosystems. The result of present study is in conformity of the reports of Singh *et al.* (1991) that due to strong demand for inorganic N and P by the higher plants during the rainy season there is a decrease in the nutrient pool during this season. Thus, it shows that moderate temperature and high organic nutrients promote the microbial activity in summer season. The summer time maxima of microbial biomass in tropical grassland and agroecosystem soil of India have been reported by Singh *et al.* (1989), Srivastava & Singh (1991) and Roy & Singh (1994).

In the present study, soil microbial biomass C, N and P were recorded to be higher in upper layer (0-10 cm depth) of the soil and contributed 72%, 67%, 68% and 73%, 66%, 68% of microbial biomass to total annual microbial biomass in grassland and agroecosystem respectively. The soil microbial biomass decreases with the increase of soil depth in both the ecosystems. The decline in lower layers may be due to low availability of soil organic matter, which shows that microbial biomass is sensitive to change in quality and quantity of soil regions. Several workers have also reported the higher value of microbial biomass in upper soil layer (Carter & Rannie 1982; Franzleubber *et al.* 1994; Woods 1989). Thus, the microbial biomass can be used as useful parameter to elucidate the changes in organic matter in different soil layers.

Nutrient concentration and ratio in microbial biomass

In the present study the microbial C, N and P contributed 2.11%, 2.44% and 10.15% to total soil organic C, total N and P in grassland and 2.02%, 2.46% and 9.57% of microbial C, N and P to total soil organic C, N and P in agroecosystem respectively. The proportion of microbial C and P to total soil organic C and P is found to be higher in grassland than that of agroecosystem, whereas proportion of microbial

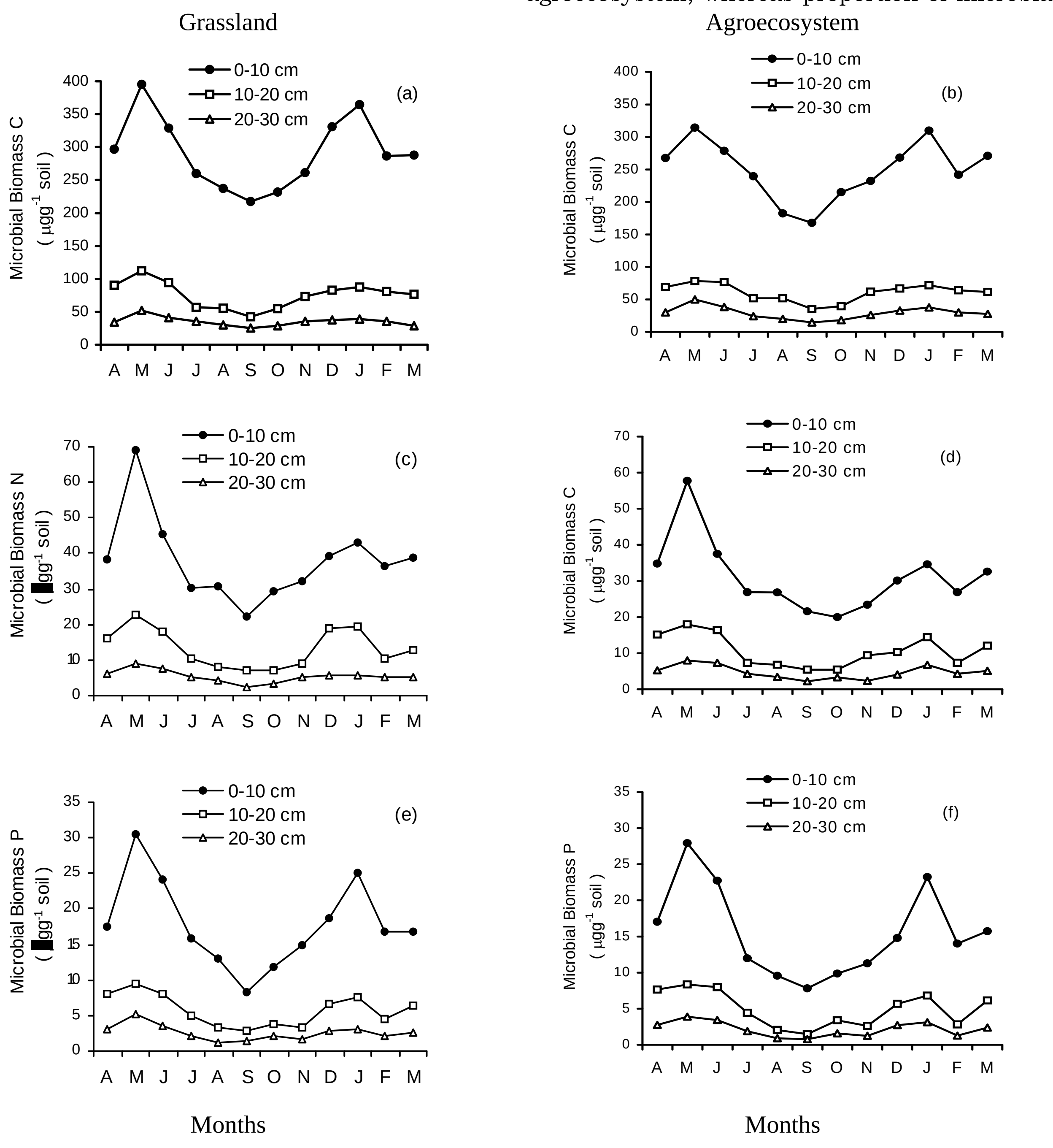

Fig. 1. Seasonal variation in soil microbial biomass carbon (a & b), nitrogen (c & d) and phosphorous (e & f) at different soil depths in grassland and agroecosystem respectively.

N to soil N is more or less similar in both sites. It indicates that soils are rich in C and P in grassland than that of agroecosystem. The C, N and P concentration in microbial biomass in present study falls within the range reported by earlier workers (Brookes *et al.* 1984; Kaur *et al.* 1998; Srivastava & Singh 1989). The maximum value of nutrient ratio of microbial C : N and C : P fluctuates between rainy (grassland) and winter (agroecosystem) season and minimum value in summer season in both the study sites. There is a minor difference in microbial C : N, C : P and N : P ratio between the soils of grassland and agroecosystem suggesting that both the study sites reached an identical stage of microbial biomass turnover and mineralisation of organic matter. The nutrient ratio of microbial biomass of the present study falls within the range reported by earlier workers (Brookes *et al.* 1985; Srivastava & Singh 1989; Singh & Singh 1995) in grassland and cropland soils of different climatic regions of the world. The microbial C:N ratio is often used to describe the structure and the state of the microbial community. Jenkinson & Ladd (1981) reported that fungi and bacteria have considerably different C:N ratio i.e. ratio of the fungal hyphae is often 10-12 and that of bacteria usually between 3-5. A high microbial C:N ratio indicates that the microbial biomass contains a higher proportion of fungi, whereas low value suggested that bacteria predominate in the microbial population (Campbell *et al.* 1991). In the present study, the mean microbial biomass C : N ratio is higher than 3-5 indicating that fungal population predominates in both the sites though agroecosystem exhibits higher fungal population in comparison to grassland ecosystem. Thus, it shows that soils are more fertile in grassland than that of agroecosystem.

Relation between the microbial biomass and soil properties

Highly significant correlationship ($P < 0.01$) between microbial biomass C, N and P and soil organic C, total N and P was recorded, which shows that microbial biomass is highly influenced by soil organic nutrient in both the ecosystems. Relatively higher correlationship existed between microbial biomass C, N and P and soil organic C, N and P in grassland ($r =$ 0.957, 0.946, 0.959; $P < 0.01$) than agroecosystem ($r =$ 0.845, 0.941, 0.956; $P <$ 0.01) respectively, which indicates higher organic content in grassland soil than that of agroecosystem. Positive relationship between microbial C, N and P and soil organic C, total N and P in grassland and agroecosystem have been reported by Singh & Singh (1995), Ghoshal & Singh (1994) and Moore *et al.* (2000). The soil pH was also significantly ($P < 0.05$) related to soil microbial biomass in both the study sites indicating an important effect of soil pH on microbial biomass. The soil microbial biomass C, N and P were negatively related to soil moisture within the annual cycle in contrast to results obtained from other arid ecosystems (Schnurer *et al.* 1986; West *et al.* 1987). The negative relationship between microbial biomass and soil moisture content apparently due to the saturation of the soil especially during the rainy season (both the study sites were in water logging condition), results in low value of soil microbial biomass. Lower value of microbial biomass during rainy season could be due to anaerobic environment of soil, which favours only the growth of anaerobic microorganisms. The result of the present study is in accordance with the results reported by Ross (1987) and Srivastava (1992). The inverse relationship between soil water and microbial C, N and P within annual cycle at each sites indicated a pulsed turnover of microbial nutrient in these soils. Raghubanshi *et al.* (1990), Srivastava (1992) suggested that pulse release of microbial nutrients at the beginning of the rainy season not only support the initiation of plant growth, but also helps to shorten the act of nutrient immobilization phase of litter decomposition.

In comparison of the study sites, grassland ecosystem exhibited a higher value of microbial biomass productivity than agroecosystem. In present study, there were decline of 16, 19 and 14% of microbial biomass C, N and P on conversion of these grassland to agroecosystem. Haas *et al.* (1957) compared organic C and total N concentration in cropland soils with that of natural grassland in Great Plains of North Dakota and reported 41 and 31% loss in C and N respectively due to cultivation. The decrease in microbial biomass on conversion of natural grass-land into cropland has also been reported by several workers (Dalal & Mayer 1987; Gupta & Germida 1988; Srivastava & Singh 1989). It may be due to high organic matter generated in grass-lands in comparison to agroecosystem, whereas in agroecosystem most of the plant produce is harvested for human and animal consumption and only a small fraction of organic matter is returned in the soil. The organic matter being important parameter for regulating microbial biomass status, the grassland soil comparatively being higher in organic matter content exhibited higher microbial C, N and P than agroecosystem.

In conclusion, concentration of soil microbial biomass showed seasonality, exhibiting maximum during summer and minimum in rainy season in subtropical systems of northeast India. It further indicates that microbial biomass decline with increase of soil depth and more than 60% of the total microbial biomass is contributed by upper layer of the soil (0-10 cm). The study also shows the decline of 16% of microbial biomass on conversion of grassland to agroecosystem in subtropical region. However, further study is necessary to assess the loss of soil fertility on conversion of grassland to agroecosystem on long term basis for sustainable crop productivity.

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