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Cattle grazing influences soil microbial biomass in sub-tropical grassland ecosystems at Nambol, Manipur, northeast India

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Abstract: We assessed the effects of grazing on the soil microbial biomass C, N and P in the soils of non-grazed (NG), moderately grazed (MG) and heavily grazed (HG) sub-tropical grasslands in the Imphal valley of Manipur, northeast India, from January to December 2007. Mean soil microbial C (µg C g^{-1} soil) ranged from 258.5 to 347.8 in 0 - 10 cm soil depth and 87.6 to 146.1 in 10 - 20 cm soil depth across all sites. Soil microbial N (µg N g^{-1} soil) fluctuated between 38.3 to 45.4 soil in 0 - 10 cm soil depth and 11.7 to 16.2 in 10 - 20 cm soil depth across all sites. Mean soil microbial P (µg P g^{-1} soil) varied from 19.0 to 22.6 in 0 - 10 cm soil depth and from 5.8 to 7.6 in 10 - 20 cm soil depth across all sites. The soil microbial biomass C, N and P were highest in the MG site, followed by the NG and HG sites. Soil microbial biomass was strongly influenced by season and by soil organic C, total N and total P in the grassland soils, as evidenced by positive, significant correlations with these soil properties. Our results indicate that moderate grazing enhances soil microbial biomass C, N and P. Moderate grazing may benefit sub-tropical grasslands in northeast India by influencing nutrient dynamics and could be prescribed for the management of these grasslands.

Resumen: Evaluamos los efectosdel pastoreo en el C, el N y el P de la biomasa microbiana del suelo en suelos de pastizales subtropicales no pastoreados (NP), con pastoreo moderado (PM) y con pastoreo intenso (PI) en el vallede Imphalde Manipur, nordeste de la India, de enero a diciembre de 2007. En promedio el C microbiano del suelo (µg C g^{-1} suelo) varióentre sitios de 258.5 a 347.8 en la profundidad del suelo de 0 - 10 cm,y de 87.6 a 146.1 en la profundidad de 10 - 20 cm de profundidad.El N microbiano del suelo (µg N g^{-1} suelo) fluctuóentre sitios de 38.3 a 45.4 en la profundidad de 0 - 10 cm y de 11.7 a 16.2 en la profundidad de 10 - 20 cm. En promedio el P microbiano del suelo (µg P g^{-1} suelo) varióentre todos los sitios de 19.0 a 22.6 en la profundidad de 0 - 10 cm, y de 5.8 a 7.6 en la profundidad de 10 - 20 cm. Los valores de C, N y P en la biomasa microbiana del suelo tuvieron sus máximosen el sitio PM, seguidos por los sitios NPyPI. La biomasa microbiana del suelo estuvo influenciada fuertemente por la estacióny por los contenidos totales de C orgánico, N total y P total en los suelos de los pastizales, como lo ponen en evidencia las correlaciones positivas y significativas con estas propiedades edáficas. Nuestros resultados indicanqueel pastoreo moderadopromueveel C, el N y el P de la biomasa microbiana del suelo. El pastoreo moderado puede ser benéfico para los pastizales subtropicalesdel nordeste de la India gracias a su influencia en la dinámica de los nutrimentosy podría ser prescrito para el manejo de estos pastizales.

Resumo: Avaliaram-se os efeitos do pastoreio sobre os valores do C, N e P na biomassa microbiana em solos não pastados (NG), moderadamente pastados (MG) e fortemente pastados (HG) em pastagens subtropicais no vale Imphal de Manipur, no nordeste da Índia, de janeiro a dezembro de 2007. O C microbiano do solo (mg C g^{-1} solo) variou de 258,5 a 347,8 na camada de 0 - 10 cm de profundidade, de 87,6 - 146,1 na camada de 10 - 20 cm de profundidade, em todos os

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locais. O N microbiano do solo (mg N g^{-1} solo) oscilou entre os 38,3 - 45,4 na camadade 0 - 10 cm de profundidade,de 11,7 - 16,2 nos 10 - 20 cm de profundidade, em todos os locais. A média do P microbiano do solo (mg P g^{-1} solo) variou entre os 19,0-22,6 na camada dos 0 - 10 cm de profundidade e os 5,8 - 7,6 nos 10 - 20 cm de profundidade, em todos os locais. Os valores de C, N e P da biomassa microbiana do solo foram maiores no local da MG, seguidos pelos locais NG e HG. A biomassa microbiana do solo foi fortemente influenciada pela estação e pelos valores de C, N total e P total na matéria orgânicanos solos da pastagem, como evidenciado pelas correlações significativas, positivas, com aquelesparâmetros do solo. Os nossos resultados indicam que o pastoreio moderado aumenta os teores do C, N e Pna biomassa microbiana do solo. O pastoreio moderado pode beneficiar as pastagens subtropicais no nordeste da Índia, influenciando a dinâmica dos nutrientes e pode ser prescrito para a gestão desses campos.

Key words: Grazing intensity; northeast India; organic matter; soil microbial biomass; soil quality; sub-tropical grassland.

Introduction

The majority of Indian grasslands have developed following deforestation, abandonment of cultivated land, and burning. These grasslands are maintained at successional level (sub-climax stage) by repeated grazing and burning. There are several types of grassland ecosystems in north-eastern India, depending upon the original forest type, which range from tropical rainforest through sub-tropical and humid mountain forests to temperate forests (Champion & Seth 1968; Yadava 1990).

Soil microbial biomass plays a crucial role in nutrient cycling (Amador 2012; Hafich *et al.* 2012). It is a potential source of plant nutrients and may serve an indicator of soil fertility. The soil microbial biomass contributes 2 - 3 % of the total organic carbon in soil and is a relatively labile fraction of soil organic matter (Jenkinson & Ladd 1981). It is a key component of soil, since it defines the functional component of the soil micro-biota primarily responsible for decomposition, soil organic C turnover, and nutrient transformations (Dalal & Meyer 1987; Moussa *et al.* 2007; Smith & Paul 1990; Witter 1996). Soil microbial biomass does not respond uniformly to grazing by livestock or other large animals, and has been observed to increase or decrease in response to grazing of the plant community (Bardgett & Wardle 2003). Grazing intensity impacts soil biota through increased trampling, defoliation, and manure inputs (Bardgett *et al.* 1998; Kohler *et al.* 2005; Maharning *et al.* 2009), and affects both above and belowground processes in grassland ecosystems (Bardgett *et al.* 1998; Clegg 2006; Zhao *et al.* 2009). For example, Bardgett *et al.* (1997) reported increases in microbial biomass with increasing intensity of sheep grazing in hill grassland ecosystems.

Grasslands in the state of Manipur, which is situated in the northeastern part of India bordering Myanmar, are managed by local communities for cattle grazing and extraction of essential oils and thatching material. Although there are number of studies on the effect of grazing on soil microbial biomass dynamics in the different parts of the world (Bardgett *et al.* 2001; Li *et al.* 2005; Qi *et al.* 2010; Tracy & Frank 1998; Wang *et al.* 2006), there is limited information on the impact of grazing on the dynamics of soil microbial biomass in the grassland ecosystems in India (Singh *et al.* 1991; Singh & Yadava 2006; Singh *et al.* 2009; Srivastava 1992). Furthermore, there is no published record of the influence of grazing intensity on soil microbial biomass in grassland ecosystems in the eastern Himalaya.

The present study was undertaken to examine the effect of grazing intensity on (1) soil microbial biomass C, N and P in different months and seasons, and (2) and the interrelationships between abiotic factors and the microbial biomass in the grassland ecosystems of Manipur, northeast India, with the aim of improving management of these ecosystems. We hypothesized that excessive grazing would negatively affect microbial biomass C, N and P, which are the source of essential plant nutrients.

Materials and methods

Study area

Study sites were located at 24° 43' 03.2″ N latitude and at 93° 50′ 17.2″ E longitude at Nambol in the Bishnupur District of Manipur, northeast India, about 777 m above sea level. In this region the year can be divided into three seasons: summer (March to May), rainy (June to October), and winter (November to February). The mean minimum air temperature at the study area varied from 3.6 °C (January) to 22.2 °C (July) and mean maximum air temperature ranges from 20.8 °C (January) to 28.8 °C (August). The annual rainfall in the study area was 1522 mm. The temporal distribution of rainfall in the region is uneven; with three-fourths of annual rainfall occurring during the rainy season.

Three study sites were established in grasslands comprising a total area of 3.10 ha. Site I was a non-grazed (NG) grassland area that served as a control; Site II was a moderately grazed (MG) area, with a stocking rate of 12 - 15 cows ha^{-1} which were allowed to graze rotationally at one month intervals for 12 months; Site III was heavily grazed (HG) with a stocking rate of 12 - 15 cows ha^{-1} which were allowed to graze continuously throughout the year. Cows are mainly used for milk, but also for transportation and ploughing of agricultural fields. The cow breed is indigenous. The grassland at each site was dominated by *Imperata cylindrica*, *Cynodon dactylon*, and *Axonopus compressus*, and managed by the local population. The soil is clayey loam in texture, and soil colour varied from grayish brown (2.5Y, 5/2) in heavily grazed sites to dark grey (2.5Y, 4/1) in protected and moderately grazed grassland sites. Soil in all the sites was of same type and varied in the colour of the top soil.

Sampling procedure and analysis of soil

Soil samples were collected at monthly intervals from January 2007 to December 2007 using a soil corer. The soil corer was 75 mm in diameter by 75 mm high. At each site, five samples were collected randomly from 0 - 10 cm and 10 - 20 cm soil depths and brought to the laboratory in polythene bags. Sites were not replicated, therefore caution is to be applied in interpretation of results due to pseudoreplication. The soil was sieved through a 2-mm-mesh sieve to remove stones, coarse roots and other plant debris, and stored at room temperature for 24 h prior to analysis. Half of the sample was air-dried and used for physicochemical analysis and the other half was used for the determination of soil microbial biomass.

Soil pH, bulk density, moisture, temperature, organic carbon, total N and available phosphorus were determined on a monthly basis. Soil pH was determined (1:5 water suspension) with a pH meter (Systronics-Eutech instruments, Cyberscan 510, Singapore). Soil moisture was determined gravimetrically, and soil temperature was measured using an electronic soil thermometer. Soil texture was analyzed by the pipette method (Gee & Bauder 1986). Soil organic C was determined using the methods given by Anderson & Ingram (1993), total soil N and total soil P was determined by FIAstar 5000 (Foss Tecator AB, Sweden).

Microbial biomass C, N and P was determined by the fumigation-extraction method (Anderson & Ingram 1993). The C content of the extracts was determined by the modified Walkley Black method and biomass C calculated using the equation of Vance *et al.* (1987):

Microbial C = $K_{EC} \times 2.64$

where, K_{EC} is the difference between C extracted from fumigated and unfumigated soil.

The N content of the extracts was determined by the micro-Kjeldahl method (Bremner & Mulvaney 1982) and calculated using the equation of Brookes *et al.* (1985):

Microbial N = $K_{EN} \times 1.46$

where, K_{EN} is the difference between N extracted from fumigated and unfumigated soils.

The P content of the extracts was determined using the ammonium molybdate stannous chloride method (Sparling *et al.* 1985) and calculated using the equation of Brookes *et al.* (1982).

Microbial P = $K_{EP} \times 2.5$

where, K_{EP} is the difference between P extracted from fumigated and unfumigated soils.

Student's t-test, linear regression and one way ANOVA were used to statistically analyze the data.

Results

Physiochemical characteristics of soil

The soil was clayey loam in texture and acidic in nature across the sites (Table 1). Soil pH was highest in the heavily grazed site and lowest in the moderately grazed site. Soil bulk density was slightly higher in the heavily grazed site but not significantly. Soil temperature ranged from 15.5 to 29.5 °C across the sites. The soil C : N ratio ranged

Table 1. Physico-chemical characteristics of soil in the study sites. Values are the mean of 12 months ± S.E. Ranges are given in parentheses.

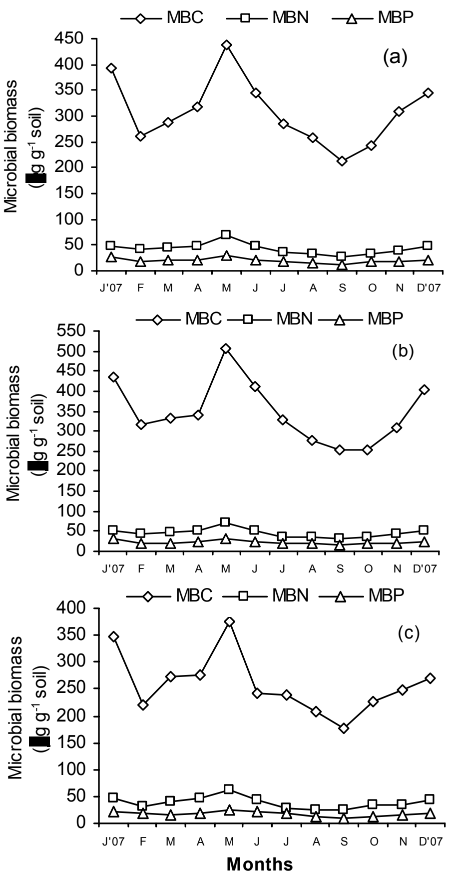
Soil characteristics	Grassland type		
	Non-grazed	Moderately grazed	Heavily grazed
Soil temperature (ºC)	23.2 ± 0.9	22.1 ± 2.0	24.8 ± 0.9
	(15.5 - 28.5)	(16.5 - 28.5)	(17.0 - 29.5)
Soil moisture (%)	34.9 ± 3.8	33.2 ± 3.7	31.7 ± 3.7
	(19.8 - 52.8)	(19.2 - 49.5)	(16.9 - 48.2)
Soil Texture:			
Sand (%)	14.0	12.0	14.4
Silt (%)	33.6	32.4	32.0
Clay (%)	52.4	55.6	53.6
Soil Bulk Density	1.2 ± 0.007	1.2 ± 0.007	1.2 ± 0.009
	(1.18 - 1.26)	(1.18 - 1.27)	(1.19 - 1.28)
Soil pH	5.89 ± 0.13	5.61 ± 0.10	6.01 ± 0.12
	(5.53 - 6.56)	(5.07 - 6.12)	(5.37- 6.72)
Soil Organic C (%)	1.28 ± 0.07	1.3 6 ± 0.07	0.99 ± 0.06
	(0.82 - 1.70)	(0.90 - 1.26)	(0.61 - 1.41)
Soil total N (%)	0.11 ± 0.01	0.13 ± 0.01	0.09 ± 1.12
	(0.07 - 0.18)	(0.09 - 0.21)	(0.05 - 0.16)
Soil total P (g g^{-1})	133.7 ± 3.1	146.9 ± 3.3	125.6 ± 2.7
	(116.0 - 153.3)	(128.0 - 169.0)	(111.0 - 144.5)
Soil C : N	11.05 ± 0.51	10.85 ± 0 .46	9.26 ± 0.93
	(8.53 - 14.21)	(8.48 - 14.3)	(8.0 - 13.3)

Table 2. Seasonal changes in microbial C, N and P in the soils of non-grazed, moderately grazed, heavily grazed grasslands (µ g g^{-1} soil ± S.E.) at 0 - 10 cm soil depth. Values are means (n = 5) ± S.E.

Seasons	Non-grazed	Moderately grazed	Heavily grazed
Microbial C			
Summer	347.7 ± 45.9	393.8 ± 56.3	308.7 ± 33.1
Rainy	269.4 ± 22.1	305.0 ± 29.6	218.2 ±11.9
Winter	327.1 ± 27.8	366.9 ± 31.3	271.3 ±27.1
Mean of 12 months	309.2 ± 18.6	347.8 ± 22.2	258.5 ± 16.2
Microbial N			
Summer	53.0 ± 7.4	56.4 ± 7.0	49.6 ± 6.6
Rainy	35.1 ± 3.6	37.4 ± 8.1	31.1 ± 3.8
Winter	44.7 ± 1.9	48.0 ± 1.8	38.6 ± 3.6
Mean of 12 months	42.6 ± 3.1	45.4 ± 3.4	38.3 ± 3.2
Microbial P			
Summer	23.8 ± 3.7	25.8 ± 3.9	19.8 ± 3.1
Rainy	17.1 ± 1.7	19.8 ± 1.4	14.9 ± 2.3
Winter	21.1 ± 1.9	23.8 ± 2.7	18.2 ± 0.9
Mean of 12 months	20.1 ± 1.4	22.6 ± 1.5	17.2 ± 1.3

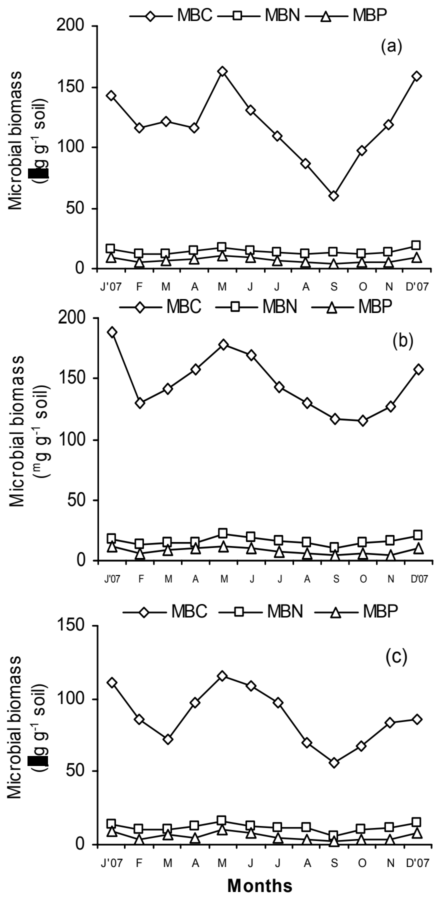
Table 3. Seasonal changes in microbial C, N and P in the soils of non- grazed, moderately grazed and heavily grazed grasslands (µg g^{-1} soil ± S.E.) at 10 - 20 cm soil depth. Values are means (n = 5) ± S.E.

Seasons	Non- grazed	Moderately grazed	Heavily grazed
Microbial C			
Summer	133.3 ± 15.1	159.1 ± 10.2	94.5 ± 12.6
Rainy	97.0 ± 11.7	134.9 ± 9.9	80.1 ± 6.5
Winter	134.0 ± 10.4	150.5 ± 6.9	91.5 ± 6.6
Mean of 12 months	118.4 ± 8.4	146.1 ± 6.8	87.6 ± 5.5
Microbial N			
Summer	14.5 ± 1.7	17.2 ± 2.4	13.1 ± 1.6
Rainy	12.9 ± 0.4	14.9 ± 1.6	10.1 ± 1.2
Winter	14.7 ± 1.7	17.0 ± 1.5	12.6 ± 1.2
Mean of 12 months	14.6 ± 0.8	16.2 ± 1.0	11.7 ± 0.8
Microbial P			
Summer	8.6 ± 1.2	10.4 ± 1.1	7.2 ± 1.5
Rainy	5.8 ± 8.4	7.0 ± 0.8	4.6 ± 0.8
Winter	7.3 ± 1.3	8.1± 1.4	6.1 ± 1.3
Mean of 12 months	7.0 ± 0.7	8.9 ± 0.7	5.8 ± 0.7



MBC = Microbial biomass C; MBN = Microbial biomass N and MBP = Microbial biomass P

Fig. 1. Monthly variations in microbial biomass C, N and P in (a) non-grazed (b) moderately grazed and (c) heavily grazed grassland sites at 0-10 cm soil depth. Values are means (n=5).



MBC = Microbial biomass C; MBN = Microbial biomass N and MBP = Microbial biomass P

Fig. 2. Monthly variations in microbial biomass C, N and P in (a) non-grazed (b) moderately grazed and (c) heavily grazed grassland sites at 10-20 cm soil depth. Values are means (n=5).

Table 4. Nutrient ratio in microbial biomass and microbial C, N and P as proportion of soil organic C, total N and total P in grassland ecosystem. Values are means (n = 12); range of values shown in parentheses.

Grassland type	Microbial C : N ratio	Microbial C : P ratio	Microbial N : P ratio	Microbial C/ Organic C (%)	Microbial N/ total N (%)	Microbial P/ Soil total P
Non- grazed	7.3 (6.1-8.2)	16.6 (13.8-18.6)	2.1 (1.8-2.4)	1.9 (1.3-2.5)	2.7 (2.4-3.0)	10.6 (10.2- 15.9)
Moderately grazed	7.7 (6.5-9.1)	15.4 (13.9-17.0)	2.0 (1.6-2.3)	1.9 (1.4-2.2)	2.5 (1.7-3.3)	13.5 (10.7-17.0)
Heavily grazed	6.9 (5.4-8.2)	15.4 (11.3-20.3)	2.2 (1.6-2.7)	1.9 (1.3-2.7)	2.3 (1.9-3.3)	10.0 (6.0-12.6)

Table 5a. Correlation between soil microbial biomass C, N and P and soil properties in non-grazed grassland ecosystem at 0 - 10 cm soil depth.

Variables	R	y	df	t	*P*	Variability as explained by regression (%)
Microbial C						
ST	-0.02	317.12-0.3746x	10	0.036	> 0.05	0.036
SM	-0.501	394.24-2.3785x	10	13.18	> 0.05	21.5
pH	0.846	-334.11+112.19x	10	16.41	< 0.01	71.5
SOC	0.785	76.492+137.6x	10	16.60	< 0.01	61.6
STN	0.921	78.04+1461.3x	10	16.62	< 0.01	84.7
STP	0.877	-478.34+4.5486x	10	8.72	< 0.01	76.9
Microbial N						
ST	0.06	38.104+0.1902x	10	0.31	> 0.05	0.31
SM	-0.621	60.17-0.4857x	10	1.03	< 0.05	33.6
pH	0.799	-57.381+17.471x	10	12.48	< 0.01	71.5
SOC	0.814	3.0296+23.522x	10	13.74	< 0.01	66.9
STN	0.924	4.535+241.92x	10	13.90	< 0.01	85.8
STP	0.947	-97.333+0.8095x	10	-110.80	< 0.01	88.3
Microbial P						
ST	-0.015	19.594+0.0222x	10	0.26	> 0.05	0.26
SM	-0.51	26.898-0.1876x	10	-3.46	> 0.05	21.1
pH	0.787	-26.234+8.0973x	10	10.82	< 0.01	61.9
SOC	0.785	2.1636+10.666x	10	13.60	< 0.01	61.6
STN	0.911	2.4746+112.05x	10	13.99	< 0.01	82.9
STP	0.903	-42.645+0.363x	10	-64.72	< 0.01	81.0

ST= Soil temperature; SM = Soil moisture; STN = Soil total nitrogen; SOC = Soil organic carbon; STP = Soil total phosphorus.

from 8.0 to 14.3 across all sites (Table 1). Soil organic C, soil total N and available P were highest in the MG site followed by NG and HG sites.

Microbial biomass C, N and P

Soil microbial biomass C, N and P were highest in the summer months, followed by the winter and rainy seasons in all the study sites (Tables 2 & 3). Soil microbial biomass was highest in the moderately grazed site, followed by non-grazed and heavily grazed sites (Figs. 1 & 2). The soil microbial C accounted for 1.87 % to 1.92 % to total soil organic C, whereas soil microbial N contributed 2.3 % to 2.7 % to total soil N, and microbial P contributed from 10.0 % to 13.5 % to soil P across the sites (Table 4). The contribution of microbial biomass to organic C, total N and soil P varied seasonally across the sites, but did not differ

Table 5b. Correlations between soil microbial biomass C, N and P and soil properties in moderately grazed grassland ecosystems at 0 - 10 cm soil depth.

Variables	R	y	df	t	*P*	Variability as explained by regression (%)
Microbial C						
ST	0.36	326.40+0.87062x	10	15.61	> 0.05	0.135
SM	-0.43	437-2.5808x	10	12.99	> 0.05	19.08
pH	0.75	396.59-136.9x	10	15.46	< 0.01	56.49
SOC	0.79	15.775+196.72x	10	15.61	< 0.01	62.75
STN	0.92	56.374+1673.4x	10	15.64	< 0.01	86.22
STP	0.84	576.43-5.1875x	10	8.81	< 0.01	71.74
Microbial N						
ST	0.011	42.324+0.1268x	10	-3.39	> 0.05	0.012
SM	-0.65	63.894-0.534x	10	1.77	< 0.05	42.9
pH	0.68	-47.02+17.004x	10	13.13	< 0.05	47.08
SOC	0.82	-7.9116+28.865x	10	14.26	< 0.01	68.28
STN	0.89	5.83+227.38x	10	14.51	< 0.01	80.06
STP	0.84	-101.49+0.8247x	10	110.38	< 0.01	89.71
Microbial P						
ST	-0.006	22.38+0.0103x	10	6.35	> 0.05	0.144
SM	-0.42	28.477-0.1691x	10	4.21	> 0.05	28.09
pH	0.69	-23.148+8.4194x	10	12.24	< 0.05	52.99
SOC	0.81	-2.2756+13.476x	10	14.69	< 0.01	46.63
STN	0.87	4.2466+105.56x	10	15.226	< 0.01	71.91
STP	0.79	-35.222+0.3247x	10	59.52	< 0.01	90.07

ST = Soil temperature; SM=Soil moisture; STN=Soil total nitrogen; SOC=Soil organic carbon; STP =Soil total phosphorus.

under different grazing intensities except for the contribution of microbial biomass P to total P, which was highest in MG sites and lowest in HG sites (Table 4). Microbial C : N, C : P and N : P ratios ranged from 5.4 to 9.1, 11.3 to 20.3 and 1.6 to 2.7, respectively in NG, MG and HG sites (Table 4). The relative variability in microbial biomass due to pH, organic C, total N and P was recorded to be maximum in NG followed by MG and HG grass-land sites (Tables 5a, b, c).

One-way analysis of variance (ANOVA) indicated significant monthly differences in microbial biomass C, N and P ($P < 0.01$) in all the three sites at 0 - 10 cm, and in microbial biomass C and N ($P < 0.01$) at 10 - 20 cm over 12 months. In addition, the results of the ANOVA show there were significant differences in microbial biomass C, N and P among the three study sites.

The soil microbial biomass C, N and P was positively correlated to soil pH for all the sites except for soil microbial biomass C in the HG site. Soil microbial biomass N had a significant negative relationship with soil moisture in NG and MG sites, whereas soil microbial biomass C had a significant negative relationship with soil moisture in the HG site. Soil microbial biomass C was significantly correlated with soil organic C in the NG and MG grasslands. Soil microbial biomass N and P at 0 - 10 cm soil depth were also significantly correlated with total N and soil total P, respectively, in all the sites (Tables 5a, b, c).

Discussion

Maximum values of soil organic C, total N and total P in the MG site may be due to the presence of a large standing pool of organic matter and a higher rate of decomposition of plant litter through trampling by cattle. The low total soil organic C and N in the HG sites may be due to reduction in

Table 5c. Correlation between soil microbial biomass C, N and P and soil properties in heavily grazed grassland ecosystems at 0 - 10 cm soil depth.

Variables	R	y	df	t	*P*	Variability as explained by regression (%)
Microbial C						
ST	-0.07	285.73-1.0658x	10	-14.32	> 0.05	0.42
SM	-0.58	339.8-2.47x	10	12.102	> 0.05	33.64
pH	0.56	-217.15+80.525x	10	15.67	> 0.05	32.19
SOC	0.56	115.67+103.94x	10	15.94	> 0.05	31.25
STN	0.80	110.59+1069.6x	10	15.98	< 0.01	65.41
STP	0.86	-409.73+4.0645x	10	7.09	< 0.05	75.44
Microbial N						
ST	0.04	35.114+0.1241x	10	-0.10	> 0.05	0.14
SM	-0.53	52.994-0.4473x	10	0.88	> 0.05	28.09
pH	0.73	82.569+20.457x	10	10.37	< 0.01	52.99
SOC	0.68	3.7277+25.134x	10	11.13	< 0.05	46.63
STN	0.85	7.5539+222.11x	10	11.94	< 0.01	71.91
STP	0.95	-106.28+0.8793x	10	-115.9	< 0.01	90.07
Microbial P						
ST	0.09	13.85+0.7317x	10	11.02	> 0.05	0.99
SM	-0.39	21.69-0.1365x	10	-3.50	> 0.05	15.97
pH	0.60	-23.672+6.92x	10	9.182	< 0.05	37.02
SOC	0.57	5.3934+8.5945x	10	12.68	< 0.05	33.29
STN	0.92	3.6241+98.196x	10	13.27	< 0.01	85.74
STP	0.85	-35.625+0.3213x	10	-60.32	< 0.01	73.46

ST= Soil temperature; SM=Soil moisture; STN=Soil total nitrogen; SOC=Soil organic carbon; STP =Soil total phosphorus.

aboveground biomass owing to excessive grazing by the cattle. Similar findings were also reported by Bardgett *et al.* (2001) in a sub-montane ecosystem and Gao *et al.* (2008) in a grassland in Inner Mongolia. Soil C : N ratios were influenced by grazing pressure i.e. the highest ratios in the NG site and the lowest ratios in HG site were observed. Bardgett *et al.* (2001) also reported the highest C : N ratio in ungrazed areas and lowest in heavily grazed areas for a sub-montane grassland ecosystem. The highest soil pH in heavily grazed site indicates that acidity in the soil decrease with grazing intensity in this study.

The distribution of microbial biomass within the soil profile depends on several biological and environmental factors, including quality and quantity of organic matter, temperature, soil moisture, pH and texture. Soil microbial C, N, P were highest in MG grassland, followed by NG and HG grasslands. Our results support our initial hypothesis that heavy grazing has a negative effect on the microbial biomass. However, moderate grazing enhances the soil microbial biomass in the grassland ecosystem. Cattle grazing at moderate levels may increase plant litter inputs to the soil, perhaps by trampling, and higher inputs of organic matter through feces, resulting in an increase in microbial biomass. Several researchers have reported that moderate grazing increases root exudation that may enhance microbial biomass in the field (Holland *et al.* 1996; Mawdsley & Bardgett 1997; Singh *et al.* 1991). The low values of microbial biomass in the HG site may be influenced by a lower rate of supply of organic matter and other nutrients owing to reduction in biomass by cattle. Holt (1997) has also reported that reduction in microbial biomass in the heavy grazed treatment may have been influenced by lower rate of organic matter input as a result of reduction in herbage biomass. Patches of bare soil due to heavy grazing result in loss of soil organic matter through wind erosion (Hoffman *et al.* 2008; Schneidera *et al.* 2008), which adversely affects the structure and processes of the plant community (Tong *et al.* 2004), and ultimately results in decline in the microbial biomass. Contrary to the present findings, Wang *et al.* (2006) reported for a sub tropical grassland in the south of central Florida, that microbial biomass C and N increases with patchy heavy grazing by cattle.

Soil microbial C, N and P were significantly higher during the summer, followed by the winter and rainy seasons. Higher values in the summer may be due to faster decomposition of plant residues and immobilization of products in the microbial biomass, whereas lower values of microbial biomass in rainy season are likely due to high demand of nutrients from the soil for the peak growth of vegetation during the time. Similar patterns in the temporal distribution of microbial biomass were reported in tropical dry deciduous forests, savannas in India and temperate grasslands in China, i.e. peak values in summer and lowest in the rainy season (Chen *et al.* 1995; Saratchandra *et al.* 1984; Singh *et al.* 1989).

The soil microbial biomass C in the present study falls within the ranges (109 - 390 μg C g^{-1}) in an oil palm plantation in west Malaysia (Haron *et al.* 1997) and in the Buck Island Ranch soil (210 to 781 μg g^{-1}) in south Florida, USA (Wang *et al.* 2006), and is comparable to the range (365 - 468 μg g^{-1}) in non-grazed and grazed sites of steppes in Inner Mongolia (Qi *et al.* 2010). The soil microbial N values fall within the range (37 - 136 μg g^{-1}) in a Buck Island Ranch soil in south Florida (Wang *et al.* 2006) and is comparable to soil (17 - 39 μg g^{-1}) of dry land rice paddy soil with and without plant residue treatments (Singh *et al.* 1999). The microbial P in our study was close to the range 5.3 - 67.2 μg g^{-1} for arable land, grassland and woodland soil of temperate and sub-tropical systems (Brookes *et al.* 1984; Singh & Yadava 2006).

The proportion of microbial biomass C and N to organic C and total N was more or less similar in all the three sites, whereas the proportion of microbial P to total P was higher in MG grassland, but the difference was not statistically significant. The latter indicates that microbial biomass P immobilized a greater proportion of P in MG sites. The proportions of microbial biomass C to organic C in the present study are comparable to those (0.3 % to 3.9 %) reported for savanna (Singh *et al.* 1991a), pastures (Iyyemperumal *et al.* 2007; Saratchandra *et al.* 1984). The contribution of microbial biomass N to total N falls well within the reported range (2.0 - 6.0 %) for agricultural soils (Brookes *et al.* 1985). The contribution of microbial P to total P falls more or less within the range (7.5 - 13.5 %) found in sub-tropical grassland soils (Singh & Yadava 2006). There was very little difference in microbial C : N, C : P and N : P ratio among the soils of NG, MG and HG grasslands, suggesting that all the study sites are at a similar stage of microbial biomass turnover. The microbial C : N ratio is used to describe the structure and state of the microbial community. Fungi and bacteria have considerably different C : N ratios (Jenkinson & Ladd 1981). The ratio of fungal hyphae ranges from 10 - 12, whereas for bacteria it ranges from 3 - 5. In this study, microbial biomass C : N is higher than 3 - 5, indicating the dominance of fungi in these acidic soils.

Significant positive correlations of microbial biomass with soil C, total N and total P in all the three study sites show that microbial biomass is strongly influenced by soil organic C and nutrients at all three sites. A similar positive relationship has also been reported in other grasslands and agroecosystems (Ghoshal & Singh 1994; Moore *et al.* 2000; Singh & Singh 1995). Concentrations of total N and organic C in the soils have been shown to have a direct effect on the soil microbial biomass N (Billore *et al.* 1995). Significant positive correlations of microbial biomass with soil organic C and total N suggest that soil organic C content and total N are the important factors determining the population size of the soil microbial community (Qi *et al.* 2010). By contrast, soil moisture negatively influenced soil microbial biomass N in NG and MG sites. This may be due to high turnover rate of microorganisms and high uptake of nutrients during the wet periods for growth of vegetation. Previous studies have reported soil moisture to negatively influence soil microbial biomass in both dry tropical forest and savanna ecosystems of India (Singh *et al.* 1989; Srivastava 1992). In New Zealand, soils under tussock grassland and introduced pasture displayed similar trends with soil moisture (Ross 1987). In two semi-arid soils in California, the response of microbial biomass to rapid change in soil moisture potential was examined. Results showed that increasing soil moisture could significantly decrease microbial biomass C (Keift *et al.* 1987). Soil microbial biomass C, N and P were also influenced by soil pH in all the three sites as evident from significant positive relation between them. Thereby it shows that increase in the pH value enhances the microbial activity in sub tropical soils.

Conclusions

Our results show that, in a sub-tropical grassland in northeast India, moderate grazing enhances the soil microbial biomass C, N and P. The soil microbial biomass is highly influenced by pH, organic C, total N and total P in the grassland soils under different grazing intensities. The results of our study indicate that there is strong seasonality in dynamics of soil microbial biomass, which exhibits maximum values in summer, followed by lower values in winter and rainy seasons. Our data suggest that moderate grazing is an appropriate grazing strategy in these sub-tropical grassland ecosystems. Additional, long-term studies on carbon and nutrient transformations in these ecosystems are necessary to develop a better understanding of the effects of grazing intensity on nutrient cycling in the grassland ecosystems of Northeast India.

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