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Response of microbial biomass to grazing in an alpine meadow along an elevation gradient on the Tibetan Plateau

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ABSTRACT

Although grazing is a common land use type, few studies are available about the response of soil microbial biomass to grazing especially above 4300 m on the Tibetan Plateau. Therefore, three fenced enclosures were made at three alpine meadow sites along an elevation gradient (4313 m, winter pasture; 4513 m and 4693 m, summer pasture) in July 2008. Soil samples inside and outside the fenced enclosures were gathered in July, August and September 2011. Microbial biomass C (MBC) and N (MBN) were determined using the chloroform fumigation–extraction method. Grazing marginally declined MBC by 21.60%, 4.83% and 5.36% across sampling dates at elevation 4313 m, 4513 m and 4693 m, respectively. Grazing significantly declined MBN by 39.58% and 18.88% across sampling dates at elevation 4313 m and 4693 m, respectively, whereas it slightly declined MBN by 1.50% at elevation 4513 m. Microbial biomass at elevation 4693 m was significantly higher in comparison with elevation 4513 m and 4313 m, whereas soil temperature at elevation 4693 m was 2.3 °C and 2.8 °C lower than that at elevation 4513 m and 4313 m, respectively. Our findings suggest that MBN may respond more rapidly to grazing than MBC and that climate warming and grazing may decline microbial biomass for the alpine meadow.

As living fraction of labile soil organic matter, soil microbial biomass plays an important role in the transformation of nutrients for plant growth [1]. Microbial biomass C (MBC) generally comprises 1–4% of soil organic C (SOC) [2–4], but its turnover represents an obvious contribution to C cycle [5].

Responses of microbial biomass to grazing are different among different ecosystems with respect to vegetation types and soil characteristics [6]. Grazing-induced significant increase in microbial biomass has been observed in hill grassland [4], humid grassland [7] and temperate salt marsh [8]. Olsen et al. [8] ascribed it to higher root turnover and root exudation under grazed conditions in comparison with ungrazed conditions. In contrast, significantly negative effect of grazing on microbial biomass has also been reported in various terrestrial ecosystems, such as semiarid savanna [9], semi-arid sagebrush steppe [10] and alpine swamp meadow [11]. However, some studies showed that grazing had no obvious effects on microbial biomass [12–14].

In China, alpine meadow ecosystem covers about 6.4×10^5 km^2 and is concentrated in the western and south-western regions, most in the Qinghai-Tibet Plateau, containing 35.4 Pg of C (i.e., 26.4% of total C in grassland of China) [15]. Moreover, the alpine meadow plays a critical role in the regional carbon budget of China [16]. To our knowledge, although pasture for domestic sheep and yak is a common land use type on the Tibetan Plateau, few studies are available about the response of soil microbial biomass to grazing especially above 4300 m. Therefore, here we investigated the grazing effects on MBC, microbial biomass N (MBN) and the ratio of MBC to MBN, respectively, in three alpine meadow sites located at elevation 4313 m, 4513 m and 4693 m on the Northern Tibetan Plateau.

The main objective of this study was to examine the grazing effects on microbial biomass in an alpine meadow along an elevation gradient on the Northern Tibetan Plateau. We hypothesized that grazing could decrease microbial biomass and that microbial biomass of the upper sites were higher in comparison with the lower sites.

The study area (30°30′–30°32′ N, 91°03′–91°04′ E) was located at Damxung Grassland Observation Station, Tibet Autonomous Region in China. The annual mean sunlight is 2880.9 h and the annual mean solar radiation is 7527.6 MJ m^{-2}. Annual mean air temperature is 1.3 °C, ranging from the lowest value (−10.4 °C) in January to the maximum (10.7 °C) in July. Annual mean

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precipitation is around 476.8 mm, with over 80% occurring in the period from June to August. The annual potential evapotranspiration is about 1725.7 mm. The soil freezing duration is from November to January. The soil is classified as meadow soil with sandy loam (sand 67.02%; silt 18.24%; clay 14.74%). The soil layer is 0.5–0.7 m thick, with organic matter of 0.3–11.2% and total N of 0.03–0.49%. The vegetation surrounding the study area is *Kobresia*-dominated alpine meadow. Three sampling sites were established on a south-facing slope on the Nyainqentanglha Mountains along an elevation gradient (i.e., at 4313 m, 4513 m and 4693 m). One fenced enclosure (about 20 m × 20 m) was made at each sampling site in July 2008. Daily mean soil temperature at the depth of 0.05 m from July to September in 2011 was 13.96 °C, 13.40 °C and 11.15 °C at elevation 4313 m, 4513 m and 4693 m, respectively. Management practices were different before enclosure (i.e., winter pasture for elevation 4313 m, summer pasture for elevation 4513 m and 4693 m) [17,18]. After 3-year enclosure, soil compaction (0–20 cm depth) outside the fenced enclosure was 28.1% higher than that inside the fenced enclosure at elevation 4313 m ($P < 0.001$), whereas there were no statistically significant differences at elevation 4513 m and 4693 m. However, total vegetation covers outside the fenced enclosures were 50.6%, 33.5% and 10.5% lower than those inside the fenced enclosures at elevation 4313 m, 4513 m and 4693 m, respectively ($P < 0.001$). The dominant species were different (*Stipa capillacea*, *Carex montis-everestii* and *Kobresia pygmaea* for elevation 4313 m and 4513 m, *K. pygmaea* for elevation 4693 m).

Soil samples (0–20 cm depth) outside and inside the fenced enclosures were collected (with a soil auger of 3.0 cm diameter) at each site on July 7, August 9 and September 10, 2011 (the three dates were chosen as repeated measures). For each of the four replicates, five soil subsamples were randomly sampled and composited into one soil sample. The soil samples were stored in an icebox and then transferred to laboratory.

MBC and MBN were determined using the chloroform fumigation–extraction method [19]. Briefly, the fumigated and unfumigated soil samples (20 g) were both extracted using 100 ml 0.5 M K_2SO_4 (soil/extractant ratio 1:5). Then K_2SO_4 extracts were filtered through 0.45 μm filter membrane. The extractable organic C and total N in the K_2SO_4 extracts were analyzed on a Liqui TOC II elementar analyzer (Elementar Liqui TOC, Elementar Co., Hanau, Germany) and UV-1700 PharmaSpec visible spectrophotometer, respectively. The extractable C and N were converted to MBC and MBN using conversion factors (K_{ec} and K_{en}) of 0.45 both [20].

For each site, repeated-measures analysis of variance was used to estimate the main and interactive effects of sampling date and grazing on MBC, MBN and MBC/MBN ratio, respectively (Table 1). Repeated-measured analysis of variance with elevation and summer grazing as the between-subject factors and with sampling date as the within-subject factor for MBC, MBN and MBC/MBN ratio was conducted between 4513 m and 4693 m in order to examine the main and interaction of elevation and summer grazing on microbial biomass. All the statistical tests were performed using the SPSS software (version 16.0; SPSS Inc., Chicago, IL).

Repeated-measured analysis of variance between elevation 4513 m and 4693 m showed that soil MBC, MBN and MBC/MBN ratio of elevation 4513 m were 50.5%, 37.5% and 16.8% lower in comparison with those of elevation 4693 m, respectively ($P < 0.001$ for MBC and MBN, $P = 0.012$ for MBC/MBN ratio). Summer grazing significantly declined soil MBN by 11.8% ($P = 0.049$), but had no effects on soil MBC

Table 1
Repeated-measures analysis of variance for the main and their interactive effects of grazing and sampling date on microbial biomass C (MBC), N (MBN) and the ratio of MBC and MBN (MBC/MBN ratio) in the three sampling sites of alpine meadow on the Tibetan Plateau.

Elevation (m)	Model	MBC		MBN		MBC/MBN ratio	
		F	*P*	*F*	*P*	*F*	*P*
4313	Grazing(G)	5.41	0.059	25.74	**0.002****	5.75	0.053
	Date(D)	5.96	**0.016***	15.67	**0.000*****	5.97	**0.016***
	G × D	1.03	0.386	2.72	0.106	2.72	0.106
4513	Grazing(G)	0.12	0.746	0.03	0.875	0.05	0.825
	Date(D)	0.11	0.895	18.88	**0.003****	10.45	**0.002****
	G × D	1.24	0.323	0.29	0.639	0.66	0.533
4693	Grazing(G)	0.39	0.554	6.55	**0.043***	5.47	0.058
	Date(D)	16.94	**0.005****	11.11	**0.002****	18.14	**0.000*****
	G × D	10.86	**0.013***	3.85	0.051	0.40	0.677

*, ** and *** mean significant at 0.05, 0.01 and 0.001 level, respectively.

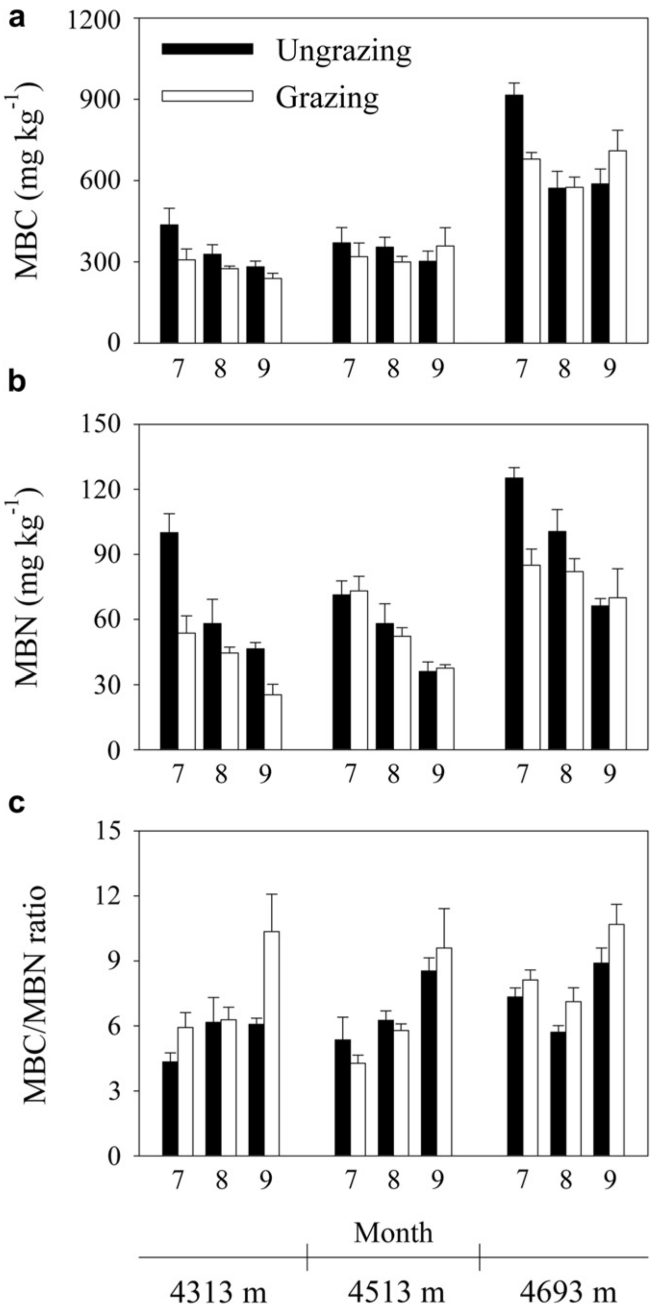

Fig. 1. Effects of grazing on soil microbial biomass C (MBC), N (MBN) and the ratio of MBC and MBN (MBC/MBN ratio) in the three alpine meadow sites located at elevation 4313 m, 4513 m and 4693 m on the Tibetan Plateau, respectively. Error bars represent standard error ($n = 4$).

and MBC/MBN ratio. There were no interactions between summer grazing and elevation on soil MBC, MBN and MBC/MBN ratio. Winter grazing marginally declined soil MBC by 21.6% and significantly decreased MBN by 39.58% across sampling dates at elevation 4313 m (Table 1). Irrespective of summer or winter grazing effect, no significant differences on MBC and MBN were found between elevation 4313 m and 4513 m, whereas they both were significantly lower than those of elevation 4693 m, respectively. However, daily mean soil temperature from July and September in 2011 at elevation 4313 m and 4513 m were 2.8 °C and 2.3 °C higher in comparison with elevation 4693 m, respectively. These results implied that climate warming and grazing probably decrease MBC and MBN, which was in line with previous studies [9,11,21].

MBC was positively correlated with SOC ($R^2 = 0.86$, $P < 0.001$ for non-grazing; $R^2 = 0.89$, $P < 0.001$ for grazing) and total N (TN) ($R^2 = 0.87$, $P < 0.001$ for non-grazing; $R^2 = 0.88$, $P < 0.001$ for grazing) along the elevation gradient. Similarly, MBN was also correlated with SOC ($R^2 = 0.51$, $P < 0.05$ for non-grazing; $R^2 = 0.78$, $P < 0.01$ for grazing) and TN ($R^2 = 0.62$, $P < 0.01$ for non-grazing; $R^2 = 0.77$, $P < 0.01$ for grazing). These results showed that the variation of soil microbial biomass along the elevation gradient may be associated with that of SOC and TN.

Grazing significantly declined soil MBN in 2 out of 3 sampling dates (July 7 and September 10) and soil MBC and MBN in 1 out of 3 sampling dates (July 7) at elevation 4313 m and 4693 m, respectively, but not at elevation 4513 m in all the three sampling dates (Fig. 1). This finding may be related to different effects of grazing on SOC and TN among the three sites for a specific sampling date. Grazing significantly decreased SOC on July 7, 2011 and marginally decreased SOC on September 10, 2011 ($P = 0.14$) at elevation 4313 m. Grazing also significantly decreased TN on July 7, 2011 at elevation 4313 m. Grazing significantly declined TN in 1 out of 3 sampling dates (July 7), although it had no statistical significant effects on SOC in all the three sampling dates at elevation 4693 m. In contrast, grazing had no effects on SOC and TN in all the three sampling dates at elevation 4513 m.

Effects of grazing on MBN were stronger than those on MBC at 2 out of 3 sites (Table 1), which was in line with previous studies [6,22]. This finding indicated that MBN may respond more rapidly to grazing than MBC.

In conclusion, climate warming and grazing probably declined microbial biomass for the alpine meadow in our study.

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