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## Short communication

## Response of soil microbial biomass to short-term experimental warming in alpine meadow on the Tibetan Plateau

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## ABSTRACT

In order to understand the response of soil microbial biomass to warming, field warming experiments using open top chambers (OTCs) were conducted in three alpine meadow sites on the Northern Tibetan Plateau since May 2010. Soil samples for microbial biomass carbon (MBC) and nitrogen (MBN) measurements were collected in July, August and September 2011. Generally, experimental warming had no obvious effect on MBC, MBN and MBC/MBN ratio across the three sampling dates. However, experimental warming tended to decrease MBC and MBN but increase MBC/MBN ratio. The negative effect of experimental warming on microbial biomass may be related to warming-induced decline in soil water content. Our findings suggested that short-term experimental warming may have no obvious effect on microbial biomass for the alpine meadow on the Tibetan Plateau.

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## 1. Introduction

Microorganisms play important roles in carbon and nitrogen cycling and nutrient transformation (Murata et al., 1999; Wardle, 1992). Soil microbial biomass responds quickly to changes in soil water content (Skopp et al., 1990) and soil temperature (Alvarez et al., 1995). Inconsistent results on the responses of microbial biomass carbon (MBC) and nitrogen (MBN) to climate warming have been observed with respect to vegetation types and initial soil characteristics (Belay-Tedla et al., 2009; Biasi et al., 2008; Liu et al., 2009). Elevated temperature generally stimulates microbial activity when water availability is not limited (Belay-Tedla et al., 2009). On the other hand, decreased soil water content under warming may restrain microbial activity (Liu et al., 2009). Therefore, the net effect is contingent on which is more limited (Belay-Tedla et al., 2009; Liu et al., 2009).

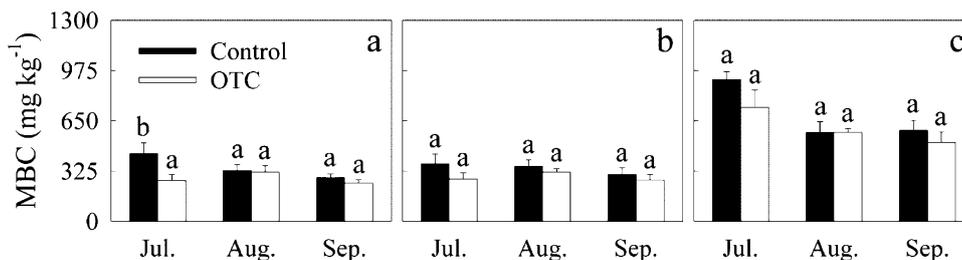
Global surface temperature is predicted to increase by 1.8–4 °C by the end of this century (IPCC, 2007). The Tibetan Plateau is one of the most sensitive regions to climate warming (Yao et al., 1991). Moreover, it is experiencing significant climatic warming and the warming trend is much greater than the average (IPCC, 2007). The alpine meadow is one of the most sensitive terrestrial ecosystems to climate warming. In alpine meadow, understanding the effect of

warming on soil microbial biomass is crucial for predicting future changes in carbon and nitrogen cycling. However, information on how microbial biomass of alpine meadow responds to warming is scarce on the Tibetan Plateau. Therefore, open top chambers (OTCs) were used to investigate the effects of experimental warming on microbial biomass in three alpine meadow sites on the Northern Tibetan Plateau.

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. Climate in this region is characterized by high irradiation, low air temperature, high diurnal fluctuation and low annual variation of air temperature and low atmospheric pressure. 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 precipitation is around 476.8 mm, with over 80% concentrated in the period from June to August. The soil freezing duration is from November to January. The soil is classified as meadow soil with sandy loam. The soil layer is 0.5–0.7 m thick. Soil organic matter content is 0.3–11.2%, total nitrogen 0.03–0.49% and pH 6.0–6.7. The vegetation surrounding the study area is *Kobresia*-dominated alpine meadow.

The experiment was set up on a south-facing slope on the Nyainqentanglha Mountains along an altitudinal gradient (i.e., at 4313 m, 4513 m and 4693 m). Four open top chambers (OTCs) were randomly established at each alpine meadow site in May 2010. The OTCs remained on the plots year round. One control plot was randomly set up in the vicinity of each OTC. There was about 3 m distance between plots. The OTCs were 1.5 m and 1.0 m in bottom

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**Fig. 1.** Effects of experimental warming on microbial biomass carbon (MBC) in the three alpine meadow sites located at altitude 4313 m (a), 4513 m (b), and 4693 m (c), respectively. Error bars represent standard errors ( $n=4$ ). Different letters on the error bars indicated  $P<0.05$  within each sampling date.

**Table 1**

Comparisons of soil temperature ( $^{\circ}\text{C}$ ) at the depth of 0.05 m and soil water content ( $\text{m}^3 \text{m}^{-3}$ ) at the depth of 0.10 m between open-top chambers (OTCs) and control plots (July–September in 2011).

Altitude (m)	Soil temperature		Soil water content	
	OTC	Control	OTC	Control
4313	15.22	13.96*	0.157	0.194*
4513	14.38	13.40*	0.185	0.220*
4693	12.52	11.15*	0.161	0.204*

\*  $P<0.05$ .

and top diameters and 0.40 m in height, which resembled those described by Klein et al. (2004).

Soil temperature at the depth of 0.05 m, soil water content at the depth of 0.10 m, air temperature and relative humidity at the height of 0.15 m were continuously monitored using a meteorological tower. All the channels were connected to a data logger (HOBO weather station, Onset Computer, Bourne, MA, USA). Daily mean soil temperature and air temperature during the study period from July to September in 2011 decreased with increasing altitude along the study altitudinal gradient (4313–4693 m) (Table 1).

Soil samples (0–20 cm depth) were collected (with a probe of 3.0 cm diameter) along the altitudinal gradient on July 7, August 9 and September 10, 2011. For each of the four replicates, five soil subsamples were randomly sampled and composited into one soil sample at each plot. The soil samples were immediately stored in an icebox and then transferred to laboratory. Each composited soil sample was passed through a sieve (1 mm diameter) and any visible roots were picked out from the sieved soil. Then the sieved soil samples were used to measure MBC and MBN.

MBC and MBN were determined using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, the fumigated and the unfumigated soil samples were both extracted using  $\text{K}_2\text{SO}_4$ . Then  $\text{K}_2\text{SO}_4$  extracts were filtered through 0.45  $\mu\text{m}$  filter membrane. The extractable organic carbon and total nitrogen in the  $\text{K}_2\text{SO}_4$  extracts were analyzed by Liqui TOC II elemental analyzer (Elementar Liqui TOC, Elementar Co., Hanau, Germany) and UV-1700 PharmaSpec visible spectrophotometer, respectively. The

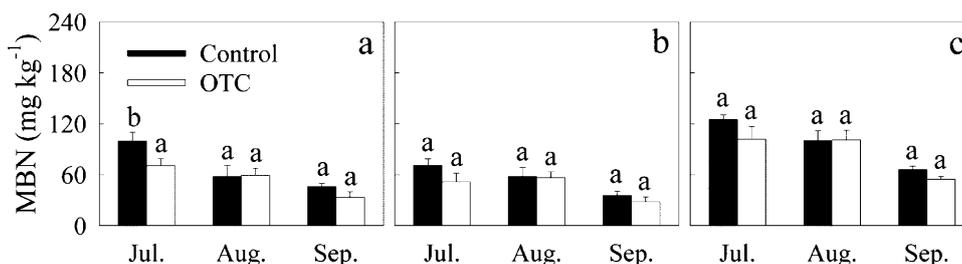
extractable carbon and nitrogen were converted to MBC and MBN using conversion factors ( $K_{\text{ec}}$  and  $K_{\text{en}}$ ) of 0.45 both (Xu et al., 2010).

For a specific site, repeated-measures analysis of variance was used to estimate the main and interactive effects of sampling date and experimental warming on MBC, MBN and the ratio of MBC and MBN (MBC/MBN ratio), respectively. For each treatment, Student–Newman–Keuls multiple comparisons were performed among the three alpine meadow sites. All the statistical tests were performed using the SPSS software (version 16.0; SPSS Inc., Chicago, IL).

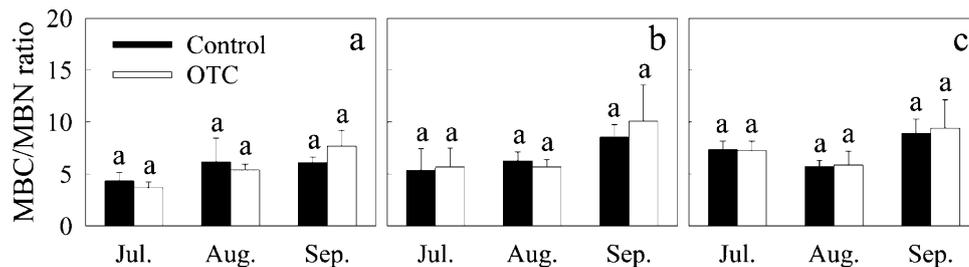
Experimental warming had no significant effects on MBC, MBN and MBC/MBN ratio across sampling dates for each site. However, significant effects of experimental warming on MBC and MBN were found in 1 of 3 sampling dates at altitude 4313 m (Fig. 1). Experimental warming caused decline in MBC by 21.1%, 16.6% and 12.4%, and MBN by 19.9%, 17.2% and 11.7% across sampling dates at altitude 4313 m, 4513 m and 4693 m, respectively. In contrast, MBC/MBN ratios were 0.8%, 6.2% and 2.7% larger in OTCs compared to control plots. Both MBC and MBN showed similar seasonal dynamics regardless of warming treatments among sites (Figs. 1–3).

Significant differences of MBC ( $F=43.684$ ,  $P<0.001$  for OTCs;  $F=22.045$ ,  $P<0.001$  for control plots) and MBN ( $F=16.810$ ,  $P=0.001$  for OTCs;  $F=28.108$ ,  $P<0.001$  for control plots) were found among sites across sampling dates. In contrast, the differences of MBC/MBN ratio among sites were relative lower ( $F=3.747$ ,  $P=0.065$  for OTCs;  $F=5.511$ ,  $P=0.027$  for control plots).

Recently, some studies with short-term (<3 years) experimental warming showed that warming had no obvious effect on microbial biomass (Biasi et al., 2008; Xu et al., 2010; Zhang et al., 2005), whereas other studies with long-term (>3 years) experimental warming indicated that warming significantly increased or decreased microbial biomass (Belay-Tedla et al., 2009; Frey et al., 2008; Liu et al., 2009; Rui et al., 2011). Liu et al. (2009) demonstrated that the negative effect of warming on microbial biomass increased with time. Besides, some studies showed that warming had a lagging effect on microbial biomass (Belay-Tedla et al., 2009; Ruess et al., 1999). Therefore, the insignificant response of microbial biomass to warming in our study may be probably due to the short period of warming treatment (<1.5 years). However, experimental



**Fig. 2.** Effects of experimental warming on microbial biomass nitrogen (MBN) in the three alpine meadow sites located at altitude 4313 m (a), 4513 m (b), and 4693 m (c), respectively. Error bars represent standard errors ( $n=4$ ). Different letters on the error bars indicated  $P<0.05$  within each sampling date.



**Fig. 3.** Effects of experimental warming on the ratio of microbial biomass carbon and nitrogen (MBC/MBN ratio) in the three alpine meadow sites located at altitude 4313 m (a), 4513 m (b), and 4693 m (c), respectively. Error bars represent standard errors ( $n=4$ ). Different letters on the error bars indicated  $P < 0.05$  within each sampling date.

warming tended to decrease microbial biomass (Figs. 1 and 2), which may be attributed to warming-induced decline in soil water content (Table 1). This finding was in line with the study conducted in a temperate steppe in Inner Mongolia (Liu et al., 2009), but inconsistent with the study conducted in an alpine meadow on the Tibetan Plateau (Rui et al., 2011). Similar with our observations, Liu et al. (2009) and Rui et al. (2011) both found warming-induced soil drying. As a consequence, the different responses of microbial biomass to warming among the three studies may be attributed to the different soil water content for control plots, which was lower than 30% in our study and the temperate steppe compared to greater than 30% in the alpine meadow located at the Haibei Alpine Meadow Ecosystem Research Station (Liu et al., 2009; Rui et al., 2011).

In conclusion, experimental warming had no significant effect on microbial biomass, although it tended to decline microbial biomass in our study.

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