



Short Communication

Aridity and NPP constrain contribution of microbial necromass to soil organic carbon in the Qinghai-Tibet alpine grasslands

Xinying Zhang^{a,b}, Juan Jia^a, Litong Chen^c, Haiyan Chu^{b,d}, Jin-Sheng He^{e,f}, Yangjian Zhang^{b,g}, Xiaojuan Feng^{a,b,*}^a State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China^b College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100049, China^c Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, 810008, China^d State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China^e State Key Laboratory of Grassland Agroecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730000, China^f Institute of Ecology, College of Urban and Environmental Sciences, Peking University, Beijing, 100871, China^g Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China

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ABSTRACT

Microbial necromass carbon (MNC) is key to soil organic carbon (SOC) storage. However, mechanisms regulating MNC accumulation on large scales are poorly understood. Here we provide the first batch of regional-scale MNC data based on amino sugars for the Qinghai-Tibet Plateau alpine grasslands. We show that Qinghai-Tibet grasslands have similar microbial biomass carbon (MBC) but lower MNC concentrations in SOC than Mongolian and other grasslands. The low contribution of MNC to SOC is mainly attributed to high aridity and low net primary productivity of the Qinghai-Tibet grasslands. Our findings highlight climatic and plant influences on MNC accumulation at regional scales.

Soil organic carbon (SOC) stabilization plays a critical role in regulating atmospheric CO₂ levels (Lal, 2018). Soil microbes, while mineralizing SOC to CO₂, convert a fraction of degradable organic matter into microbial biomass and byproducts, which build up as relatively stable microbial necromass carbon (MNC) with iterative community turnover (Liang and Balsler, 2011; Miltner et al., 2012). This process, named soil ‘microbial carbon pump’, is considered to be a key SOC stabilization mechanism (Cotrufo et al., 2013; Liang et al., 2017). However, our understanding of the drivers of MNC accumulation has remained rudimentary (Liang et al., 2019; Ni et al., 2020). Investigating the regional distribution of MNC and its regulating factors may help identify controls on MNC abundance in the soil.

Using amino sugars as microbial necromass markers in soils (Amelung et al., 2008), Ma et al. (2018) have previously demonstrated that MNC increases with increasing SOC across Mongolian temperate grasslands. By comparison, alpine grassland soils on the Qinghai-Tibet Plateau are characterized by a relatively high abundance of plant-derived compounds in an undegraded state (Dai et al., 2018; Ma et al., 2019), despite temperature-constrained plant inputs (i.e., low net

primary productivity, NPP; Gao et al., 2013; Zhang et al., 2014; Sun and Du, 2017). We hence hypothesize that the contribution of MNC to SOC is lower in alpine than in temperate grasslands due to constrained microbial activity and turnover.

To test the above hypothesis, we analyzed amino sugars in surface soils (0–10 cm) of 28 evenly distributed sites along the Qinghai-Tibet grasslands (Fig. S1; detailed information in Dai et al., 2018). At each site, three random soil cores were taken and homogenized to one composite sample. Amino sugars were analyzed according to Zhang and Amelung (1996). For comparison, we compiled published data of amino sugars for surface soils of four additional Qinghai-Tibet sites, 60 Mongolian (0–10 cm) and 72 other grassland sites (Ni et al., 2020; 0–20 cm; Table S1). Concentrations of MNC were estimated according to Liang et al. (2019). To account for the influence of microbial community size on MNC accumulation, we also measured microbial biomass carbon (MBC) content for 16 soil samples from the Qinghai-Tibet grasslands (where samples were available) and compiled 11 measured MBC data from the literature for the other grasslands. For grasslands without measured MBC values, MBC was calculated according to Xu et al. (2013)

* Corresponding author. State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China.
E-mail address: xfeng@ibcas.ac.cn (X. Feng).

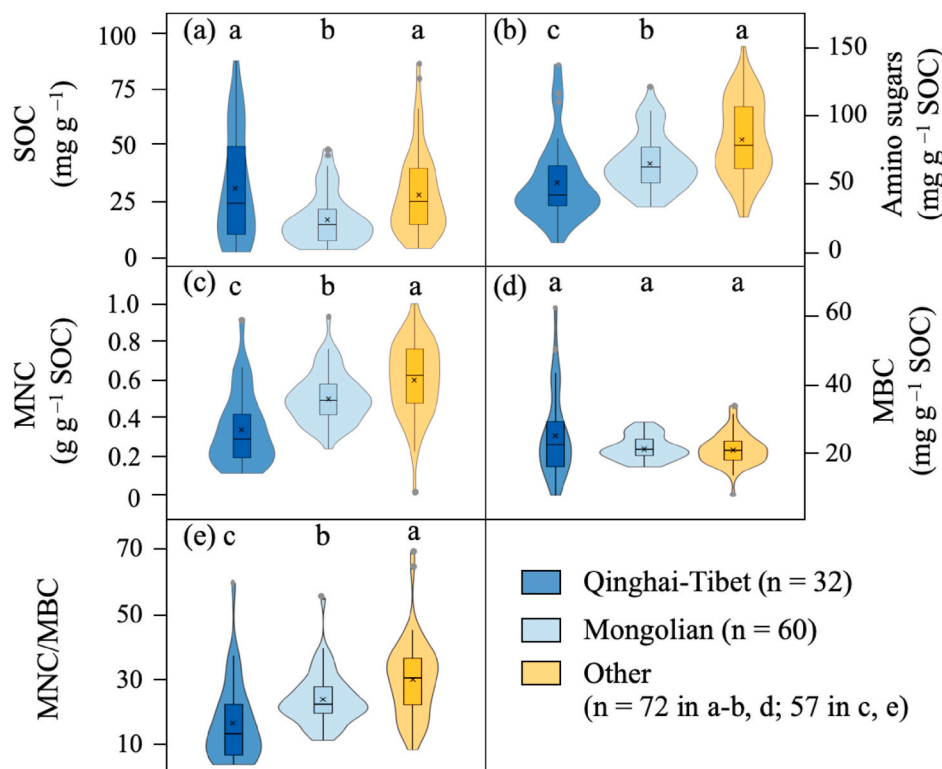


Fig. 1. Concentrations of soil organic carbon (SOC; a), amino sugars (b), microbial necromass carbon (MNC; c), microbial biomass carbon (MBC; d) and ratios of MNC to MBC (e) in surface soils of different grasslands. For amino sugar, 28 soils from the Qinghai-Tibet grasslands were analyzed in this study while the rest were compiled from the literature (details in Table S1). For MBC, 16 soils of the Qinghai-Tibet grasslands were measured in this study and 11 were compiled from the literature. For grasslands without measured MBC values, MBC was calculated according to Xu et al. (2013). Due to the lack of specific amino sugar concentrations for some sites in the other grasslands, MNC cannot be calculated, resulting in different numbers of samples for amino sugars and MNC. Solid line and cross in the box mark the median and mean of each dataset, respectively. The upper and lower ends of boxes denote the 0.25 and 0.75 percentiles, respectively. The upper and lower whisker caps denote the maximum and minimum values, respectively. Dots denote outliers. Shapes of the violin represent the distribution pattern of the corresponding parameters. Letters indicate significant differences among different grasslands ($p < 0.05$).

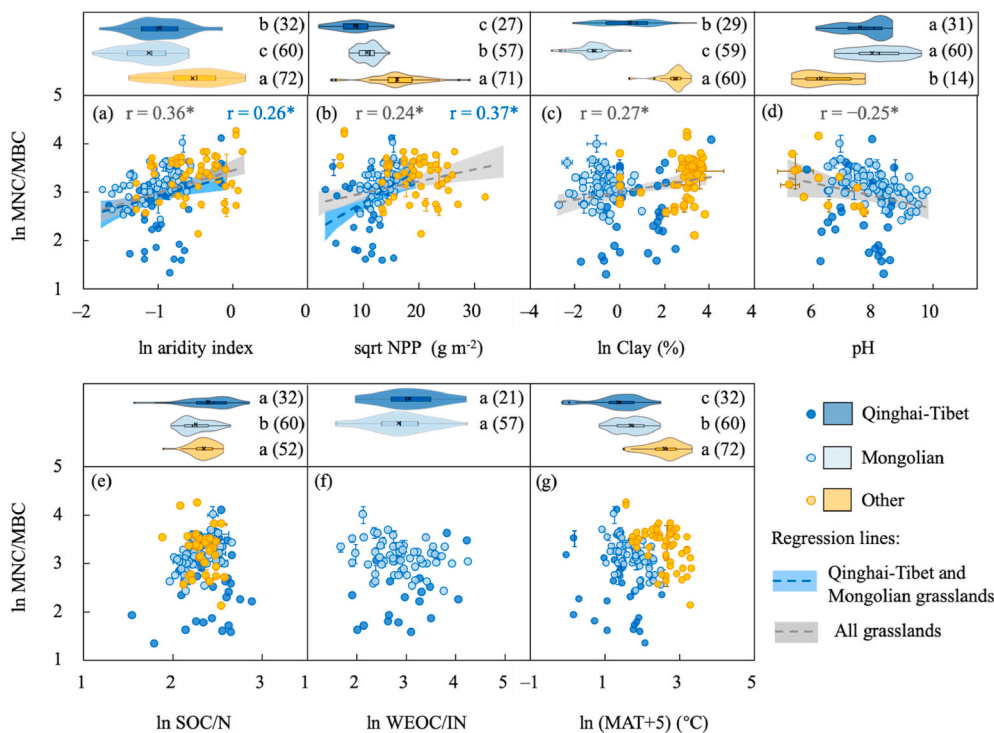


Fig. 2. Relationships between the ratio of microbial necromass carbon to microbial biomass carbon (MNC/MBC) and environmental variables among different grassland soils: (a) aridity index; (b) net primary productivity (NPP); (c) clay; (d) pH; (e) ratio of soil organic carbon to total nitrogen (SOC/N); (f) ratio of water-extractable organic carbon (WEOC) to inorganic nitrogen (IN); (g) mean annual temperature (MAT). Non-normally distributed data were Napierian logarithm (ln) or square root transformed (sqrt) before Pearson correlation. Error bars represent standard error of mean for site replicates. Symbols of the box and violin charts are defined in Fig. 1. Letters indicate significant differences among different grasslands ($p < 0.05$). * indicates a significant correlation ($p < 0.05$). Numbers in parentheses represent the number of sites.

(Fig. S2). The ratio of MNC/MBC was then calculated to estimate MNC accumulation per unit of MBC (MNC accumulation coefficient). Potential factors influencing MNC/MBC were compiled. We also measured water-extractable organic carbon (WEOC) and inorganic nitrogen (IN) in 21 Qinghai-Tibet and 57 Mongolian soils to assess the availability of carbon and N to microbes. The analytical and data details can be found in the Supplementary Information.

Differences in the (non-normally distributed) variables among different grasslands were analyzed by Kruskal-Wallis test. Consistent with our hypothesis, Qinghai-Tibet grasslands had higher or similar SOC contents but lower SOC-normalized concentrations of amino sugars and hence MNC ($343.4 \pm 34.1 \text{ mg g}^{-1} \text{ SOC}$) compared with Mongolian ($503.9 \pm 17.0 \text{ mg g}^{-1} \text{ SOC}$) and other grasslands ($607.9 \pm 15.5 \text{ mg g}^{-1} \text{ SOC}$; $p < 0.05$; Fig. 1a-c), suggesting a lower contribution of MNC to

Table 1

Values of *r* for the partial correlation analysis between environmental variables and the ratio of microbial necromass carbon to microbial biomass carbon (MNC/MBC) in grassland soils ($p < 0.05$). Numbers in parentheses represent the number of sites. NPP, net primary productivity; ln, Napierian logarithm transformation; sqrt, square root transformation; na, not applicable; ns, not significant.

Analyzed dataset	Controlled variable	Tested variable			
		ln Aridity index	sqrt NPP (g m^{-2})	ln Clay (%)	pH
All grasslands	ln Aridity index	na	ns (140)	ns (138)	ns (104)
	sqrt NPP	0.29 (140)	na	0.19 (138)	ns (99)
	ln Clay	0.26 (138)	ns (138)	na	ns (101)
	pH	0.28 (104)	ns (99)	0.21 (101)	na
Qinghai-Tibet and Mongolian grasslands	sqrt NPP	ns (84)	na	na	na
	ln Aridity index	na	0.29 (84)	na	na

SOC in alpine grasslands. Qinghai-Tibet grassland soils had similar SOC-normalized concentrations of MBC compared to other grasslands (Fig. 1d), resulting in increasing ratios of MNC/MBC in the order of Qinghai-Tibet < Mongolian < other grasslands ($p < 0.05$; Fig. 1e). This finding suggests that the MNC accumulation coefficient is lowest in Qinghai-Tibet alpine grasslands, which may be related to slow microbial turnover and/or poor necromass preservation.

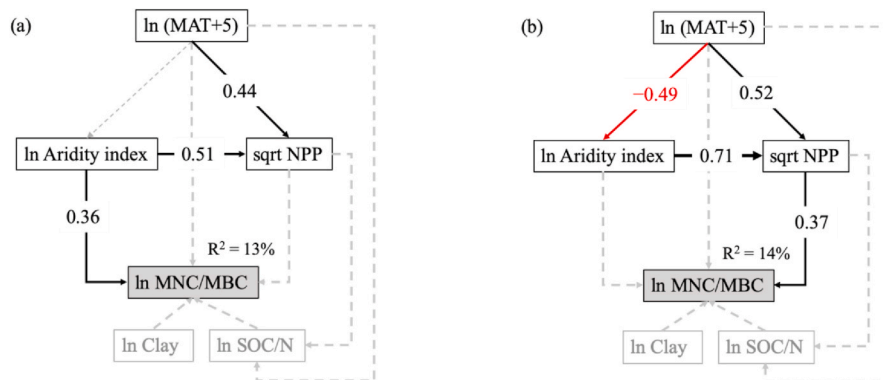
Pearson correlations between MNC/MBC and environmental variables were conducted to identify potential causes for the lowered MNC/MBC ratios on the Qinghai-Tibet and Mongolian grasslands (Fig. 2). MNC/MBC ratios were positively correlated with the aridity index (ratio of mean annual precipitation to potential evapotranspiration), NPP and soil clay content in all examined grasslands ($p < 0.05$; Fig. 2a–c). These correlations reflect enhanced MNC accumulation due to elevated microbial turnover in soils with high water and energy (i.e., NPP) supply (He et al., 2011; Jia et al., 2017; Prommer et al., 2019) and/or elevated preservation of microbial necromass in clay-rich soils (Six et al., 2006; Ma et al., 2018). In addition, lower soil pH may favor fungal relative to bacterial growth (Kamble et al., 2014; Barcenás-Moreno et al., 2016), leading to an accumulation of the more stable fungal necromass (Indorf et al., 2015) and a higher MNC/MBC ratio in the relatively acidic soils ($p < 0.05$; Fig. 2d). It should be noted that data are Napierian logarithm or square root-transformed for the regression analysis, so the correlation of MNC/MBC with aridity index, NPP and clay content is not linear.

Furthermore, partial correlation analysis showed that for all

grasslands MNC/MBC remained positively correlated with the aridity index when other variables were controlled for (Table 1). However, none of the variables had any effect on MNC/MBC when the aridity index was controlled for, suggesting a dominant impact of aridity on the MNC accumulation coefficient across all grasslands analyzed. This finding explains the lower MNC/MBC ratio in the relatively arid grasslands of Qinghai-Tibet and Mongolia (aridity index of 0.39 ± 0.01) compared with grasslands elsewhere (aridity index of 0.64 ± 0.03 ; Fig. 2a).

Other than aridity index, MNC/MBC was also positively correlated with NPP in the Qinghai-Tibet and Mongolian grassland ($p < 0.05$; Fig. 2a and b). As NPP and aridity index were interrelated ($p < 0.05$; Fig. S3), partial correlation showed that aridity index was no longer correlated with MNC/MBC when NPP was controlled for (Table 1). This suggests that NPP had a more pronounced influence on MNC/MBC than the aridity index in Qinghai-Tibet and Mongolian grasslands (Table 1). This finding is confirmed by comparative correlation analysis of MNC/MBC with NPP in grasslands under different aridity regimes (details in SI discussion and Fig. S5). Moreover, low temperature is known to constrain microbial activity and N mineralization (Carbutt et al., 2013; Wingler and Hennessy, 2016), potentially stimulating microbial re-use of its N-enriched necromass under N limitation (Jia et al., 2017). Thus, the low level of MNC on the Qinghai-Tibet and Mongolian grasslands may also result from temperature-constrained microbial turnover and/or N limitation-inhibited preservation of MNC. However, the MNC/MBC ratios in the Qinghai-Tibet and Mongolian grasslands were not correlated with either indicators of N limitation ($p > 0.05$; Fig. 2e and f), including ratios of SOC/N and WEOC/IN (Taylor and Townsend, 2010; Wickland et al., 2012; Khan and Joergensen, 2019), or mean annual temperature (MAT) ($p > 0.05$; Fig. 2g). This result suggests that low MNC/MBC in Qinghai-Tibet and Mongolian grasslands was not controlled by N limitation or temperature-constrained microbial turnover.

To confirm the above results about environmental controls on MNC/MBC ratios in grassland soils, structural equation modeling (SEM) was employed to quantify the interaction between MNC/MBC and environmental variables. The SEM was developed from a priori models (Fig. S4) based on known effects and relationships among MNC/MBC and other key drivers (Fig. S3). Non-significant χ^2 test ($p > 0.05$), high comparative fit index (CFI > 0.95) and low root mean square error of approximation (RMSEA < 0.1) indicate that our SEM have a good model fit. Based on the SEM, aridity index and NPP were direct and dominant factors affecting MNC/MBC for all grasslands (Fig. 3a) and Qinghai-Tibet and Mongolian grasslands (Fig. 3b), respectively. MAT indirectly impacted MNC/MBC by strongly affecting NPP for Qinghai-Tibet and Mongolian grasslands. These results suggest that NPP instead of MAT predominantly constrains the MNC accumulation coefficient in Qinghai-



$\chi^2 = 0.17$, $P = 0.68$, Degrees of freedom = 1, $n = 140$
 RMSEA = 0.00, CFI = 1.00, Bootstrap $P = 0.99$

$\chi^2 = 2.66$, $P = 0.26$, Degrees of freedom = 2, $n = 84$
 RMSEA = 0.063, CFI = 0.991, Bootstrap $P = 0.62$

Fig. 3. Cascading relationships for the ratio of microbial necromass carbon to microbial biomass carbon (MNC/MBC) and environmental variables in: (a) all grasslands (b) Qinghai-Tibet and Mongolian grasslands. Black and red arrows indicate positive and negative flows of causality ($p < 0.05$), respectively. Gray dotted lines indicate insignificant pathways from a priori models. Numbers on the arrow indicate significant standardized path coefficients. R^2 indicates the variance of ratios explained by the model. NPP: net primary productivity; MAT: mean annual temperature; SOC/N: ratio of soil organic carbon to total nitrogen; ln: Napierian logarithm transformation; sqrt: square root transformation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Tibet grasslands, leading to the lower contribution of MNC to SOC relative to the temperate counterparts. In other words, the soil ‘microbial carbon pump’, ultimately fueled by primary productivity, is less effective in alpine grasslands due to lack of organic matter and energy supply from plants (Cotrufo et al., 2013).

In all, we show that compared to grasslands elsewhere, Qinghai-Tibet alpine grasslands have a lower contribution of MNC to SOC, mainly constrained by low NPP and indirectly affected by low MAT and high climatic aridity. Given the wide occurrence of soil drying under global warming (Cheng and Huang, 2016), the soil ‘microbial carbon pump’ process may be curbed. Meanwhile, Qinghai-Tibet is experiencing greening (increased NPP) under climate change (Chen et al., 2013; Zhang et al., 2013; Qin et al., 2016). Further research is needed to examine how MNC accumulation responds to drying and increased plant growth in this climate-sensitive region.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108213>.

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