Spatial variability of soil microbial biomass and its relationships with edaphic, vegetational and climatic factors in the Three-River Headwaters region on Qinghai-Tibetan Plateau

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Abstract This study aims to investigate the level of soil microbial biomass (MB) and analyze the relationships between soil MB and edaphic, vegetational and climatic factors at high elevation sites (>3000 m). We collected soil samples from 0-10 cm soil depth in 259 plots at 55 sites across 6 biomes in Three-River Headwaters (TRH) region at 3280 to 5127 m elevation. Soil microbial biomass carbon and nitrogen (MBC and MBN) were measured with the chloroform fumigation-extraction method. Multivariate stepwise regression analyses were used to analyze the combined effects of edaphic, vegetational and climatic factors on soil MB. We found: (1) soil MBC and MBN had an average of 30.95 mmol C/kg dry soil and 5.84 mmol N/kg dry soil in TRH, respectively, and their values were found to be negatively correlated with elevation; (2) soil MB was found to be significantly different among different biomes, and this spatial variation could be explained by the levels of soil organic carbon and belowground plant biomass. Our results indicate that the MB at high elevation region might be very low, and the increasing trend of soil microbial biomass with elevation could reverse at higher elevations.

Key words: carbon; nitrogen; alpine ecosystem; biogeochemical cycle; stoichiometry; global warming.

Introduction

Soil microbial biomass (MB), defined as the living microbial component of the soil organic matter, mainly consists of bacteria, archaea, and fungi with a size smaller than $5 \times 10^3 \mu\text{m}$ in diameter (Jenkinson and Ladd, 1981; Wardle, 1992). Microbial biomass carbon and nitrogen (MBC and MBN) are the primary elemental components of MB. They are closely correlated with each other, and often follow a stoichiometric relationship just like the “Redfield ratio” in marine plankton and other organisms (Cleveland and Liptzin, 2007; Redfield, 1958; Sterner and Elser, 2002; Yang et al., 2014). Although MBC and MBN only contribute to small parts of soil organic matter, MB plays a key role in litter decomposition, nutrient cycling and energy flow in soils (Anderson and Domsch, 1989; Falkowski et al., 2008; Fierer et al., 2009; Xu et al., 2014). Soil MB has become an important ecological indicator due to its close associations with many biotic and abiotic parameters (Chu and Grogan, 2010; Li et al., 2005; Li et al., 2006) and its rapid response to environmental changes (Hargreaves et al., 2003; Li et al., 2004; Marinari et al., 2006; Powlson et al., 1987).

Many studies on soil MB have been conducted in the past few decades (Dempster et al., 2012; Kaschuk et al., 2010; Xu et al., 2013), and the focus has recently changed from site/ecosystem level (Shen et al., 2014, Wright and Coleman, 2000; Zak et al., 1994) to regional (Dequiedt et al., 2011; Drenovsky et al., 2010) and global scales (Serna-Chavez et al., 2013; Xu et al., 2013). These large-scale studies examined microbial biomass storage and the mechanisms determining its spatial patterns. For example, a recent study (Xu et al., 2013) found that soil MB showed substantial variation across various biomes at global scale and the spatial patterns of MBC and MBN were in line with those of soil C and N with higher densities in northern high latitudes and lower densities in low latitudes and the Southern Hemisphere. Serna-Chavez et al. (2013) suggested that the global pattern of soil MB was driven by moisture and soil nutrient contents, not temperature. Using meta-analysis, Fierer et al. (2009) showed that soil MB was highly correlated with soil organic C and belowground plant NPP across global biomes. Nevertheless, MB studies have rarely been conducted above 3000 m in elevation, therefore, previous studies estimating the global soil MB pattern and its dominant factors lack support from high elevation sites (Serna-Chavez et al., 2013; Wardle, 1992; Xu et al., 2013).

Many studies have investigated the elevation gradients of soil MB at sites below 3000 m (Arunachalam and Pandey, 2003; Djukic et al., 2010; Fierer et al., 2011). These studies conducted on Mt. Da-an in Central Taiwan (Huang et al., 2014), Mt. Kilimanjaro in Tanzania (Pabst et al., 2013) and

Cuenca Mountains in Spain (Lucas-Borja et al., 2012), suggested that soil MB at 2000-3000 m elevation is generally higher than those at lower elevations (<1000 m). Yet, it is still unclear whether such positive relationship holds true for higher elevations (>3000 m). Evaluating soil MB at different elevations could also provide indirect evidences for global warming effects based on the “space-for-time” assumption (Huang et al., 2014; Pabst et al., 2013; Smith et al., 2002; Sundqvist et al., 2013).

The Three-River Headwaters (TRH) region, the source water for Yangtze, Yellow and Mekong rivers, is located on the Qinghai-Tibet Plateau at 2578-6824 m in altitude. A field survey was conducted here to 1) investigate the level of soil MB; 2) explore the role of elevation on the spatial variation of MB and 3) analysis the relationships between soil MB and edaphic, vegetational and climatic factors. According to the patterns found in previous studies, we hypothesized that soil MB in TRH region would be the highest among all previous records.

Materials and methods

Study site

The Three-River Headwaters region, the headwaters of three major rivers in China, the Yellow River, the Yangtze River and the Lancang River (upper reach of Mekong River), is known as "the water tower of China" (Fig. 1). The TRH region is located in southern Qinghai Province (30°39'-36°12' N, 89°45'-102°23' E) and covers a total area of about 363,000 km², slightly larger than the area of Germany. The altitude stretches from ~2578 m in the east to ~6824 m in the west with an average of ~4500 m. The study area features a typical plateau continental monsoon climate. The annual average temperature and precipitation in this area are 3.96 °C and 500 mm, respectively. Grassland is the primary biome in TRH which accounts for 65.37% of the total area, of which, 76.18% is classified as alpine meadow and 23.36% is classified as alpine steppe. Other biomes in this region include bare land (12.70%), desert (8.57%), wetland (8.40%), forest and shrubland (4.71%) and cropland (0.25%) (Xu et al., 2008).

Experimental design and Field investigation

This study was conducted at 55 sites in 6 biomes in TRH region: 7 sites in forest (F), 8 in shrubland (S), 16 in alpine meadows (M), 8 in alpine steppe (P), 7 in desert (D), and 9 in wetland (W) (Fig. 1). These sites were selected based on the area and distribution of each biome, and they varied from 3280 m to 5127 m in elevation. The snow line in this region is at 5200~5600 m in elevation (Zhang et al.,

2010). Specifically, forests and shrublands are located in the southeast of the region at lower elevations (3280 m~4390 m). Alpine meadows are located at 3360 m to 4513 m. Alpine steppes are mainly found in the west part of the region at 3454 m~5127 m. Considering the high homogeneity and poor accessibility, we selected a smaller number of sites from deserts relative to the total desert area. All the deserts in TRH region are classified as cold deserts and the altitudes of the selected desert ecosystems are all higher than 4000 m (4248 m~4586 m). The wetlands in our study contained various types, including rivers, lakes, and swamps, scattering across the TRH region (3605 m~4580 m) (Tong et al., 2014).

Field investigation was done during the plant growing season in August, 2013. In the field, we sampled vegetation and soils with a square plot of 800m² for each forest and shrubland site. In each plot, we measured understory shrubs in three sub-plots (5 m×5 m) and sampled understory herbs and soils in 9 sub-plots (1 m×1 m) located at the four corners, mid-points and the center of each 800 m² plot. For the other biomes (alpine meadow, alpine steppe, desert, and wetland), we established transects of 100-200 m in length, depending on the variation of topography and vegetation conditions at each site. Along each transect, we set up 3-5 plots (1 m×1 m) at 50m intervals, to measure vegetation biomass and soil properties. Three cores of soil samples along the diagonal were collected at each 1m×1m plot with a soil auger of 7 cm in diameter. Although we collected soil samples from multiple depths (up to 100 cm), we only measured soil MBC and MBN in the top layer (0-10 cm) for the current study because most of the soils are very shallow and MB mostly presents in the top layer. Another reason that we choose the top soil layer of 10 cm depth is to compare with other studies published in the literature. In total, we collected 777 cores of top soils in 259 1 m×1 m sub-plots. In the field, the fresh soil samples collected from each site were mixed and sieved with a 2 mm soil mesh to remove plant roots and coarse rocks. The rocks, plant roots, and meshed soil were weighted separately with a scale of 0.1 g in accuracy. Then, the meshed soil samples were divided into two sub-samples: one of which (about 110 g) was taken to the laboratory using a cooler as soon as possible for soil moisture measurement and MB analysis, the other one were stored separately under ambient temperature for analyzing physical and chemical properties.

In each forest plot, we measured the diameter at breast height (DBH) for all trees over 5 cm in DBH. The aboveground and belowground biomasses of these trees were estimated with allometric equations by species (Luo et al., 1999; Luo et al., 2002). For each shrub plot in forest and shrubland biomes, we harvested and weighted the fresh aboveground shrub biomass. We determined the root-shoot ratio of each shrub species by removing and measuring 5 individuals. For each grass plot in all biomes,

we removed and measured all aboveground biomass and collected 5 root cores (0-20 cm in depth) using a 10 cm diameter soil auger. Soil was washed away from the roots and the roots were weighted after air dried for a few hours. After determining the fresh weight of the roots and aboveground plant biomass, we extracted about 20 g for leaf, stem, and root samples for each forest, shrub and grass plot to determine the plant water and nutrient contents.

Laboratory analysis

We measured several physical and chemical variables at the laboratory using the collected soil and plant samples. For each sample, we took one measurement. And reference samples (known concentration), one in every 15 samples, were used to maintain measurement accuracy. In case of abnormal results, we repeated measurements on the same sample to confirm. For each soil and forest sample, the measuring result represented the correspondent value in each site. But for grass and shrub samples, we analyzed the samples for each plot (shrub plot: 3 for forests and shrublands; grass plot: 9 for forests and shrublands, 3~5 for alpine meadows, alpine steppes, deserts and wetlands), then we calculated the average value for each site.

The soil sample (about 30 g) was oven dried at 105 °C for 24 h to determine the gravimetric water content. Soil pH was measured with a pH meter (Delta 320, Mettler-Toledo, Switzerland) at a soil to water ratio of 1:2.5 (w/v). Soil bulk density (BD) was calculated as the ratio of the total weight of rocks, dried soil and plant roots relative to the total volume of the cores. Soil texture was measured with hydrometer method (Bao, 2008) and classified with USDA standard: sand (2-0.05 mm), silt (0.05-0.002 mm) and clay (<0.002 mm). A Vario Max CN analyzer (Elementar, Hanau, Germany) was used to measure soil total carbon (TC), total inorganic carbon (TIC) and total nitrogen (TN). The difference between soil total carbon and soil total inorganic carbon is soil total organic carbon (TOC). The soil atomic TOC/TN ratio was calculated based on the measurement of TOC and TN.

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform fumigation-extraction method (Beck et al., 1997; Jenkinson et al., 2004; Wu et al., 1990). The fumigated and unfumigated soil samples were both extracted with 0.5M K₂SO₄. The extractions were filtered through 0.45 µm filter membrane and analyzed with a total organic carbon analyzer (Liqui TOC II, Elementar, Hanau, Germany) and a continuous flow analyzer (Integral Futura, Alliance Instruments, France) to obtain the extractable organic carbon and total nitrogen. The extra C and N released from lysed

soil microbes were converted to MBC and MBN using a converting factor of 0.45 (Fu et al., 2012a; Fu et al., 2012b). Soil microbial biomass was expressed both in weight and area based scale as mmol C/ kg dry soil, mmol N/ kg dry soil, and mol C/m², mol N/ m² by considering the sampling volume and bulk density. The atomic MBC/TOC ratio, MBN/TN ratio and MBC/MBN ratio were calculated as soil MBC divided by soil TOC, soil MBN divided by soil TN and soil MBC divided by soil MBN, respectively.

Meanwhile, the plant water content was determined by oven drying at 105°C for 30min and 70°C for 48h. And a Vario Max CN analyzer (Elementar, Hanau, Germany) was used to measure the carbon ratio of the plant tissues. Using the plant water content and the carbon ratio, we converted fresh plant biomass into plant carbon content. At each site, the total aboveground and belowground plant carbon contents in every square meter represent the aboveground and belowground plant biomass, and the total plant biomass is the sum of the aboveground and belowground plant biomass.

Data analyses

In the current study, annual mean temperature (T_a) and annual precipitation (P_a) of each site were derived from World-clim with 30 seconds resolution (<http://www.worldclim.org/current>) (Hijmans et al., 2005). To compare the level of MB in TRH with published results for other parts of the world, we also selected the measurements for the same soil depth (0-10cm) from the microbial biomass database in Xu et al. (2013), the most comprehensive microbial biomass database in the world. Within the total 3422 measurements in the database, we chose 373 measurements which were also reported in the top layer of 10cm in depth. These selected microbial biomass data were categorized into five biomes, namely forest, shrubland, grassland, desert, and wetland.

Soil physical-chemical variables, soil microbial biomass and plant biomass related variables and climatic variables were all grouped by biomes, and the non-parametric Kruskal -Wallis test was used to test for significance. The spatial variances within the same variable were analysed using standard deviation (SD) and the variances among different variables were determined using the coefficient of variances (CV). Correlation analysis was carried out to determine the relationships among soil microbial biomass related variables and other variables within each biome. Regression analysis was used to determine the relationships among soil microbial biomass related variables and other variables among the biomes. We also used multiple stepwise regression analyses to quantify the combined effects of edaphic, vegetation, and climatic variables on soil microbial biomass related variables in all 55 sites in TRH. Soil

MBC, MBN, MBC/MBN ratio, MBC/TOC ratio and MBN/TN ratio were used as response variables and were analyzed individually. Edaphic variables included TOC, TN, TOC/TN ratio, pH, BD, sand content (SAC), silt content (SIC) and clay content (CLC). Vegetation variables contain aboveground plant biomass (AP), belowground plant biomass (BP), total plant biomass (TP) and biome type variables. To consider the effect of the biome type on soil microbial biomass, we also included six biome type variables: forest variable (F_{or}), shrubland variable (S_{or}), alpine steppe variable (S_{or}), alpine meadow variable (M_{or}), desert variable (D_{or}) and wetland variable (W_{or}). F_{or} was set to 1 in the forest and was set to 0 in other biomes. Other biome type variables were assigned with the same rule. Climatic variables included T_a and P_a . Elevation was not included because it was a compound variable. We log-transformed soil MBC, MBN, MBC/TOC ratio, MBN/TN ratio, TOC, TN, TOC/TN ratio, CLC, AP, BP, and TP to approach normal data distribution and improve the performances of the regression equations. The multivariate models were chosen according to the adjusted coefficient of determination (R_{adj}^2), and the impacts of each factor on the response variables were compared with the standardized coefficients. The Kruskal -Wallis test was performed with R 3.1.0 software (R development Core Team, 2014) and other statistical analyses were achieved using the SPSS 16.0 software (SPSS Inc., Chicago, USA).

The stoichiometric relationships between MBC and MBN across all sites and for each biome were examined using standardized major axis (SMA) regression estimation (Cleveland and Liptzin, 2007; Warton et al., 2006). Unlike the ordinary least-squares regression, the SMA regression technique fits the slope and intercept for a linear model by assessing the "best fit bivariate line" between two variables instead of predicting one variable from the other (Warton et al., 2012; Warton et al., 2006). If the slope does not significantly differ from one, two variables are described to follow an isometric relationship which indicates that they would change with the same pace. Otherwise, the two variables were thought to follow the allometric relationship. In this study, stoichiometric relationship was analysed on a log-log scale because only did the log-transformed soil nutrient and microbial biomass related variables follow the normal distribution ($\text{Log}(Y) = a + b \cdot \text{Log}(X)$). The SMA regression analysis was conducted using the SMATR 3 package in the R 3.1.0 environment (R development Core Team, 2014; Warton et al., 2012).

Results

Variability of soil MBC and MBN

Our results showed high variability of soil MB at 0-10cm soil depth across the TRH region (Fig.2). The weight-based MBC ranged from 0.97 to 122.06 mmol C/kg dry soil with a mean value of 30.95 and a CV of 88.73% (Fig.2a). The area-based MBC varied from 0.10 to 7.42 mol C/m² with an average of 2.58 and a CV of 72.53%. Meanwhile, the weight-based MBN varied from 0.32 to 18.32 mmol N/kg dry soil with a mean value of 5.84 and a CV of 77.10% (Fig.2b). And the area- based MBN varied from 0.03 to 1.39 mol N/m² with an average of 0.50 and a CV of 61.40%. The mean weight-based MBC in forests, shrublands, and alpine meadows were all significantly higher than those in deserts, and forests also had significantly higher mean weight-based MBC than alpine steppes (Fig. 2a). Specifically, forests showing the highest mean weight-based MBC (53.34mmol C/kg dry soil), followed by shrublands (42.28 mmol C/kg dry soil), alpine meadows (36.16 mmol C/kg dry soil), wetlands (33.35 mmol C/kg dry soil), alpine steppes (10.02 mmol C/kg dry soil) and deserts (4.55 mmol C/kg dry soil) (Fig. 2a). A similar pattern was found with the mean area-based MBC among the six biomes except that wetlands showed the highest mean, and alpine meadows showed insignificantly higher mean value than shrublands. On the other hand, soil MBN shared similar spatial patterns as soil MBC among the biomes except that wetlands had relatively low soil MBN contents (Fig. 2b).

We found that soil MBC and MBN were closely related with each other across the TRH. Nevertheless, according to the SMA regression analysis, the slope of the linear relationship between log-transformed MBC and MBN was 0.86 which was significantly lower than 1, suggesting that surface soil MBC and MBN followed an allomeric relationship with soil MBC increasing faster than MBN (Table S1). Meanwhile, the stoichiometry relationship between soil MBC and MBN was biome dependent (Table S1). The slope of the SMA linear regression between log-transformed MBC and MBN was significantly lower than 1 in alpine meadow, while the slopes in forest, shrubland, alpine steppe, desert and wetland were not significantly different from 1 (Table S1). We also found that the atomic MBC/MBN ratio ranged from 1.38 to 11.76, with an average of 4.95 and a medium CV of 40.42% (Fig. 2c). And the spatial variation of soil MBC/MBN ratio was also biome dependent. The averaged soil MBC/MBN ratios were 8.05, 4.93, 5.01, 4.58, 3.19, and 3.80, respectively, for wetland, forest, shrubland, alpine meadow, alpine steppe, and desert. Wetlands had significantly higher soil MBC/MBN ratio than alpine steppes and deserts (both $P < 0.001$) (Fig. 2c).

Soil MBC and MBN only accounted for small proportions of the soil organic carbon and soil total nitrogen in TRH. The MBC/TOC ratio ranged from 0.12% to 3.91% with a mean of 1.06% and a CV

of 63.09% (Fig. 3a), whereas the MBN/TN ratio varied from 0.67% to 6.79% with an average of 2.50% and a CV of 53.16% (Fig. 3b). There was no significant difference in soil MBC/TOC ratio and MBN/TN ratio among the biomes in TRH (Fig. 3).

Variability of soil microbial biomass at different elevations

The data of the current study supported the claim that soil microbial biomass varied with elevation. Specifically, both soil MBC and MBN were significantly and negatively related to elevation for all biomes (Fig. 4a & 4b). Similarly, negative relationship also existed between soil MBC/MBN ratio and elevation across biomes when wetland was excluded (Fig. 4c & 4d). In contrast, soil MBC/TOC ratio and soil MBN/TN ratio were not significantly related to elevation (Fig. 4e & 4f). Elevation could explain 92% and 89% of the spatial variances of soil MBC and MBN among the biomes, respectively (Fig. 4a & 4b). Meanwhile, elevation only explained 8% of the variances of soil MBC/MBN ratio among all biomes, but explained 89% of the variances of soil MBC/MBN ratio when wetlands were excluded (Fig. 4c & 4d). All in all, high elevation sites of the TRH region generally showed lower levels of soil MBC and MBN compared with the sites at lower elevations, and they also showed lower soil MBC/MBN ratios (except wetlands). And according to the relationships between elevation and MBC, MBN and MBC/MBN ratio, as elevation increases by 1000m, soil MBC and MBN would decrease by 52 mmol C /kg dry soil and 9 mmol N / kg dry soil in the six biomes, and soil MBC/MBN ratio would decrease by 2 in the biomes except for wetlands (Fig. 4a-d).

Relationships between soil MB and edaphic, vegetational and climatic variables in TRH

The spatial variations of soil MBC among the biomes were found to be correlated with many edaphic, vegetational and climatic properties in TRH (Fig. 5-8 & S1-S3). Soil TOC, TN, TOC/TN ratio, pH, and BD explained 94%, 98%, 94%, 85%, and 75% of the variances of soil MBC among the biomes, respectively (Fig. 5a-e). Soil MBC was negatively related to SAC but positively related to SIC and CLC, where SICs could explain more spatial variances of soil MBC (82%) than SACs and CLCs (Fig. 5f). Plant biomass was also significantly correlated with soil MBC, where AP, BP and TP explained 55%, 70% and 62% of the variances of MBC among the biomes (Fig. 5g-i). T_a and P_a were positively related to soil MBC among the biomes in TRH (Fig. 6c&6d) due to their negative relationships with elevation (Fig. 6a & 6b). Similarly, we found that soil MBN was significantly correlated with soil TOC, TN, TOC/TN ratio,

pH, BD, SIC, plant biomass, T_a and P_a among the biomes in TRH (Fig. 6e-f & Fig. 7). Meanwhile, soil MBC/MBN ratio among the biomes in TRH was significantly related to T_a and P_a at 95% confidence level (Fig. 6g & 6h) and was correlated with soil TOC, TN and soil TOC/TN ratio at 90% confidence level when wetlands were excluded. Soil MBC/TOC ratio was significantly related to soil TOC, TN, TOC/TN ratio, pH, BD, SIC, BP, T_a and P_a among the biomes in TRH (Fig. S1 & Fig. S2a-b), whereas none of the edaphic, vegetational and climatic variables was significantly related to soil MBN/TN ratio (Fig. S2c-d & Fig. S3).

In addition to the variations among the biomes, we also found that soil MB had considerable within-biome variations, especially for wetlands which are highly varied in water depth, water salinity and plant cover (Fig. 2). The edaphic, vegetational, and climatic variables also were significantly related to soil MB within some biomes in TRH. Specifically, soil MBC and MBN were significantly related to TOC and BD in forests; to TOC, TN, pH, BD, and SIC in shrublands; to pH in alpine meadows; to TOC and TN in deserts and to TOC, TN and pH in wetlands (Table S2&S3). Plant biomass was weakly correlated to MBC and MBN within all the biomes studied (Table S2&S3). P_a was significantly related to MBC and MBN in wetlands and alpine meadows (Table S2&S3). Meanwhile, edaphic and vegetational variables were significantly related to soil MBC/MBN ratio in forests and alpine steppes, but climatic variables significantly correlated to soil MBC/MBN ratio in wetlands and alpine meadows (Table S4). No edaphic, vegetational, and climatic variable was significantly correlated to soil MBC/TOC ratio and MBN/TN ratio within each biome in TRH except for the TOC and TN in alpine meadows (Table S5&S6).

Our multivariate analyses showed that edaphic, vegetational and climatic variables could explain the majorities of the spatial variances of MBC, MBN and soil MBC/MBN ratio among the 55 sites in TRH, but could explain little spatial variances of soil MBC/TOC ratio and soil MBN/TN ratio (Table 1). The multivariate equation of soil MBC only contained one variable, TOC, and this equation could explain 75% of the spatial variances of MBC among the 55 sites in TRH. The multivariate equation of soil MBN included TOC, T and W_{or} , but the influence of soil TOC was much higher than those of the other two variables according to the standardized coefficients. This equation could explain 81% of the spatial variances of soil MBN in TRH among the 55 sites. Meanwhile, the multivariate equation of soil MBC/MBN ratio included two variables: W_{or} and P_a . These two variables both were soil water content-related variables and they explained 66% of the spatial variances of soil MBC/MBN ratio among the 55 sites in TRH. The equation of soil MBC/TOC ratio only included SIC and that of soil MBN/TN ratio

contained T_a and W_{or} . These two equations could explain 24% and 27% of the spatial variances of soil MBC/TOC ratio and soil MBN/TN ratio among the 55 sites in TRH, respectively.

Discussion

Soil microbial biomass C and N in TRH

This study showed that soil MBC and MBN in TRH (0-10cm in depth) averaged at 30.95 mmol C/kg dry soil and 5.84 mmol N/kg dry soil, are much lower than the global means in the same soil depth (0-10 cm) (72.12 mmol C/kg dry soil and 9.24 mmol N/kg dry soil) (Table 2), as well as the global means (56.7 mmol C kg⁻¹ dry soil and 7.5 mmol N kg⁻¹ dry soil) at 0-30 cm soil depth(Xu et al., 2013) although soil microbial C and N concentrations normally decrease with soil depth (Fierer et al., 2003; Peng et al., 2005; Yu and Steinberger, 2012). We acknowledged that soil microbial properties feature high temporal variability in addition to spatial variations and the synthesized study of Xu et al. (2013) included global datasets with soil microbial biomass measured in different seasons. However, most studies on soil microbial biomass were conducted during the growing season, especially in the high latitudinal and altitudinal regions. We believed that the large difference between our result and that of the global mean was unlikely caused by seasonal variation. Our results contradict with the previous hypothesis that soil MB increases with elevation, but suggesting that this trend may reversed at high elevations (>3000 m) forming a bell-shape of soil microbial biomass along an altitudinal gradient.

Nevertheless, the low microbial biomass observed in TRH is in accordance with the results of previous macro-scale studies which concluded that the spatial pattern of soil microbial biomass at broad scales was driven by macro-climate and plant traits (Fierer et al., 2009; Insam, 1990; Serna-Chavez et al., 2013; Wardle, 1992; Wright and Coleman, 2000; Xu et al., 2013; Zak et al., 1994). The TRH region has extremely harsh climate with high temperature variability (Sang et al., 2013; van Gestel et al., 2011), low precipitation (Li and Chen, 2004; Yi et al., 2013), and high UV radiation (Dahlback et al., 2007; Johnson et al., 2002), hindering the growth of the belowground microbes. Other studies have also shown that the net primary productivity (NPP) in TRH is lower than that in other places of the world (Cramer et al., 1999; Sun et al., 2013b; Yuan et al., 2014). Considering the essential roles of the substrates from the plants to microorganisms, the low NPP would fundamentally limit the abundance of microbes in TRH (Brant et al., 2006; Fierer et al., 2009; Zak et al., 1994). Thus, soil microbial biomass should positively correlate with total soil organic carbon content and belowground plant biomass. Indeed, we have found

that soil microbial biomass is highly correlated with soil organic C and plant belowground biomass across the examined biomes (Fig. 5&7), meaning that the low soil microbial biomass in TRH may be due to low plant productivity that caused low soil organic C and low substrate availability.

Our result indicated that soil MBC/MBN ratio in TRH has an average of 4.95, which is also much lower than the average ratio at global scale (8.54 ± 3.51) (Table 2). Our analyses showed that soil MBC/MBN ratio was positively correlated with the ratio of soil organic carbon to total nitrogen in forests, shrublands, alpine meadows, alpine steppes and deserts, suggesting that the soil microbial communities were not homeostasis in TRH (Fig. 5c). Similarly, many stoichiometry studies concluded that C:N stoichiometry of organisms are positively related to that of the substrate (Cleveland and Liptzin, 2007; Cleveland et al., 2003; Elser, 2000; Elser et al., 2000; Li et al., 2012), and so we believe that the low MBC/MBN ratio could also be due to the substrate limitation in TRH (Mooshammer et al., 2014a; Mooshammer et al., 2014b).

Relationships between soil microbial biomass and elevation in TRH

We found negative correlation between soil microbial biomass and elevation in the study region, which was not in line with some previous studies that reported a positive relationship between soil microbial biomass and elevation below 3000 m in elevation (Huang et al., 2014; Pabst et al., 2013; Wardle, 1992; Whitaker et al., 2014). Our result was supported by the recent finding that soil microbial biomass phosphorus decreases with elevation above 3500m on the eastern slope of Gongga Mountain, China (Sun et al., 2013a) and was helpful in refining the current understanding of soil microbial biomass distributions.

The lower turnover rate of soil microbes at higher elevations has been commonly used to explain the increase of soil microbial biomass with elevation. This is because a low turnover rate can lead to the accumulation of microbial biomass in soils (Insam, 1990; Serna-Chavez et al., 2013; Wardle, 1992; Xu et al., 2013). This explanation is only valid if temperature change along the altitudinal gradient would not decrease the substrate supply for microbes. Yet, the growth of soil microbes may be restricted by low temperatures in extremely high altitudes where the long frozen period became the limiting factor of soil microbes (Tan et al., 2012). Furthermore, the extremely low water availability in the study region may further limit the growth of soil microbes in high elevation regions (Li and Chen, 2004; Serna-Chavez et al., 2013). In addition, the extremely low temperature and precipitation also constrain the growth of plants

which is critical to maintaining soil substrate availability to soil microbes (Yang et al., 2009; Zhang et al., 2013). In TRH, NPP is known to be remarkably low above 4000 m elevation (Song et al., 2011; Wei and Wang, 2014; Zhang et al., 2009) where higher plants are mostly absent (Baniya, 2010; Baniya et al., 2012).

Our results suggested that global warming may favour carbon accumulation in the form of microbial biomass in alpine ecosystems in high elevation regions, such as the Qinghai-Tibet Plateau, contradicting with the previous claim that global warming would decrease soil microbial biomass that based on studies along altitudinal and latitudinal gradients (Liu et al., 2008; Sundqvist et al., 2013). We speculate that the response of soil microbial biomass to elevation has a bell-shaped relationship, with a positive response below a threshold and a negative response above. This complex relationship between microbial biomass and elevation could have combined the effects of many abiotic and biotic factors, such as temperature, precipitation and vegetation conditions. As previous conclusions may be not applicable to high elevation regions above 3000 m, such as the Qinghai-Tibet Plateau, care should be taken when using the elevational pattern of microbial biomass to deduce possible impacts of global warming. More studies are needed to elucidate the altitudinal pattern of microbial biomass and its controlling mechanisms for estimating global soil carbon and nitrogen pools and their feedback to global climate change, especially for high elevation ecosystems (Wardle, 1992; Xu et al., 2013).

Relationships between soil MB and edaphic, vegetational and climatic variables

In the current study, we found that soil MB was correlated with many edaphic, vegetational and climatic variables in TRH. We believed that these relationships were similar to those found in other regions of the world except for the relationship between soil MB and T. Take the soil MBC for example, we found that soil MBC was positively related to soil TOC, TN, TOC/TN ratio, SIC, AP, BP, TP, T_a and P_a , and negatively related to pH and BD among and within the major biomes in TRH although not all relationships were significant. Wardle et al. (1992) presented similar relationships between soil MBC and soil physical-chemical variables through compiling the microbial and environmental variables from published papers. Wright and Coleman (2000) and Fierer et al. (2009) both showed that soil MBC was positively related to plant biomass, particularly for the belowground plant biomass. Serna-Chavez et al. (2013) also illustrated that soil MBC was positively related to annual precipitation. However, the positive relationship between MBC and T contrast with many previous papers as forementioned.

It should be noted that the multivariate regression equations of different microbial biomass related variables in TRH were divergent, which suggested that different microbial biomass related variables had different determining factors. Specifically, we found that soil TOC was the most important variables in the equations of MBC and MBN. This result is consistent with the vast majority of previous studies, such as, Fierer et al. (2009) and Xu et al. (2013). It is because soil organic carbon is the main food resources of soil microorganisms. Nevertheless, the main determining factors of soil MBC/TMN ratio in TRH are found to be wetland factors and precipitation. This result may suggest that water availability was very important in determining soil MBC/MBN ratio in TRH. It is also found that soil MBC/MBN ratios in wetlands were significantly higher than those in other biomes in TRH. Previous studies also found similar results. For example, Xu et al. (2013) showed that soil MBC/MBN ratios in natural wetlands were much higher than those of other terrestrial biomes. Huang et al. (2013) found that soil MBC/MBN ratio increased along a chronosequence order after croplands were recovered to wetlands in Sanjiang Plain, China. In addition, the low determination coefficients of the multivariate equations of soil MBC/TOC ratio and soil MBN/TN ratio in this study may suggest that the main determining factors of soil MBC/TOC ratio and MBN/TN ratio were not included in the current study. Yet, Xu et al. (2014) found that soil MBC/TOC ratio would be profoundly influenced by soil TOC/TN ratio, and a model in Serna-Chavez et al. (2013) that included many edaphic and climatic variables could explain 50.7% of the total variances of soil MBC/TOC ratio around the world. We suggested there are many differences in the mechanisms in determining MBC/TOC ratio and MBN/TN ratio between alpine region and other regions of the world. More efforts should be made on the determining factors of MBC/TOC ratio and MBN/TN ratio, especially in the high elevation regions, to manifest the connections of soil microbial biomass with soil substrate.

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Table 1 The results of the multivariate regression analyses on soil microbial biomass related variables with edaphic, vegetation, and climatic variables among all the 55 sites in TRH

Variables	Unstandardized Coefficients	Standardized Coefficients	Sig.	VIF
(1) Log(MBC)	$R^2=0.748$	$R_{adj}^2=0.743$	$P<0.001$	
Intercept	-3.186		<0.001	
Log(TOC)	0.799	0.865	<0.001	1.000
(2) Log(MBN)	$R^2=0.807$	$R_{adj}^2=0.795$	$P<0.001$	
Intercept	-4.693		<0.001	
Log(TOC)	0.797	1.010	<0.001	1.527
T	-0.105	-0.277	0.001	1.530
W _{or}	-0.557	-0.210	0.001	1.002
(3) MBC/MBN	$R^2=0.659$	$R_{adj}^2=0.646$	$P<0.001$	
Intercept	1.327		0.033	
W _{or}	3.780	0.704	<0.001	1.001
P	0.006	0.425	<0.001	1.001
(4) Log(MBC/TOC)	$R^2=0.235$	$R_{adj}^2=0.220$	$P<0.001$	
Intercept	0.458		0.009	
SIC	-0.017	-0.485	<0.001	1.000
(5) Log(MBN/TN)	$R^2=0.270$	$R_{adj}^2=0.241$	$P<0.001$	
Intercept	0.800		<0.001	
T	-0.089	-0.428	0.001	1.002
W _{or}	-0.456	-0.312	0.012	1.002

MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBC/MBN: the ratio of soil MBC to MBN; TOC: soil organic carbon; TN: soil total nitrogen; MBC/TOC: the ratio of soil MBC to TOC; MBN/TN: the ratio of soil MBN to TN; W_{or}: the wetland variables, 0 in wetlands and 1 in other biomes; T: annual mean temperature; P: annual precipitation; VIF, variance inflation factors.

Table 2 Summary of global soil microbial biomass data in the 0-10cm soil depth in Xu et al. (2013) and the three-river headwaters (TRH) region

Ecosystems	MBC(mmol C /kg dry soil)		MBN(mmol N /kg dry soil)		MBC/MBN ratio	
	Global	TRH	Global	TRH	Global	TRH
Total	72.12±95.08	30.95±27.47	9.24±11.97	5.84±4.51	8.54±3.51	4.95±2.00
Forest	67.82±53.42	53.34±29.76	8.81±7.46	9.71±5.78	8.89±4.06	4.93±0.74
Shrubland	42.88±47.07	42.28±22.39	2.82±1.47	8.28±4.13	8.56±0.78	5.01±1.13
Grassland	70.79±52.37	27.45±21.74	8.67±6.14	6.07±3.70	8.00±2.67	4.12±1.51
Desert	9.98±10.64	4.55±4.68	1.23±0.92	1.05±1.06	----	3.80±0.89
Wetland	327.25±287.37	33.35±37.32	38.63±33.31	3.78±3.58	8.87±2.88	8.05±2.07

MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBC/MBN ratio: the ratio of soil MBC to MBN; “global” means the microbial biomass data in the 0-10cm soil depth exacted from Xu *et al.* (2013); “TRH” meant that soil microbial biomass in the three-river headwater region; all the microbial biomass related data was expressed as “mean ± S.D.”; “Total” meant the analyzing data including forests, shrublands, alpine meadows, alpine meadows, deserts, and wetlands; “Grassland” included the alpine meadows and alpine steppes; “----” mean that no research report soil MBC and MBN in desert ecosystem simultaneously.

Figure captions

Fig.1 Locations of the sampling sites in the Three-River Headwaters region

Fig.2 Soil microbial biomass carbon (MBC) (a), microbial biomass nitrogen (MBN) (b) and the ratio between MBC and MBN (soil MBC/MBN ratio) (c) in the Three-river Headwaters Region and its major biomes (0-10cm soil depth). T: the whole region; F: forest; S: shrubland; P: alpine steppe; M: alpine meadow; D: desert; W: wetland. The bold lines represent the means (total, n=55; forest, n=7; shrubland, n=8; alpine steppe, n=8; alpine meadow, n=16; desert, n=7; wetland, n=9). The different letters above the boxes of the major biomes indicate significant difference ($P < 0.05$)

Fig. 3 The ratio of soil microbial biomass carbon to soil total organic carbon (soil MBC/TOC ratio) and the ratio of soil microbial biomass nitrogen to soil total nitrogen (soil MBN/TN ratio) in the Three-river Headwaters Region and its major biomes (0-10cm soil depth). T: the whole data; F: forest; S: shrubland; P: alpine steppe; M: alpine meadow; D: desert; W: wetland. The bold lines represent the means (total, n=55; forest, n=7; shrubland, n=8; alpine steppe, n=8; alpine meadow, n=16; desert, n=7; wetland, n=9). The different letters above the boxes of the major biomes indicate significant difference ($P < 0.05$)

Fig.4 The linear relationship between elevation and soil microbial biomass carbon (MBC) (a), soil microbial biomass nitrogen (MBN) (b), the ratio of soil MBC to MBN (soil MBC/MBN ratio) (c), the ratio of soil MBC to soil organic carbon (soil MBC/TOC ratio) (e) and the ratio of soil MBN to soil total nitrogen (soil MBN/TN ratio) (f) among six major biomes in the Three-river Headwaters Region. The linear relationship between elevation and soil MBC/MBN ratio in forest, shrubland, alpine meadow, alpine steppe and desert was showed in (d). **and * represent $P < 0.01$ and 0.05 respectively

Fig. 5 Relationships between soil microbial biomass carbon and soil total organic carbon (a), soil total nitrogen (b), the atomic ratio of soil total organic carbon to soil total nitrogen (c), soil pH (d), soil bulk density (e), soil silt content (f), aboveground plant biomass (g), belowground plant biomass (h) and total plant biomass (i) across the six major biomes in the Three-river Headwaters Region. **and * represent $P < 0.01$ and 0.05 respectively

Fig. 6 The linear relationships between elevation and annual mean temperature (a) and annual precipitation (b) among the major ecosystems in TRH and the linear relationship of climatic variables (annual mean temperature and annual precipitation) with soil microbial biomass carbon and soil microbial biomass nitrogen in six major biomes in TRH region (c-f), and with the ratio of soil microbial carbon to

microbial nitrogen in the major biomes except for wetlands (g, h). **and * represent $P < 0.01$ and 0.05 respectively

Fig.7 Relationships between soil microbial biomass nitrogen and soil total organic carbon (a), soil total nitrogen (b), the atomic ratio of soil total organic carbon to soil total nitrogen (c), soil pH (d), soil bulk density (e), soil silt content (f), aboveground plant biomass (g), belowground plant biomass (h) and total plant biomass (i) across the six major biomes in the Three-river Headwaters Region. **and * represent $P < 0.01$ and 0.05 respectively

Fig. 8 Relationships between the ratio of soil microbial biomass carbon to soil microbial biomass nitrogen (soil MBC/MBN ratio) and soil total organic carbon (a), soil total nitrogen (b), the atomic ratio of soil total organic carbon to soil total nitrogen (c), soil pH (d), soil bulk density (e), soil silt content (f), aboveground plant biomass (g), belowground plant biomass (h) and total plant biomass (i) across the five biomes (forest, shrubland, alpine meadow, alpine steppe, and desert) in the Three-river Headwaters Region. **and * represent $P < 0.01$ and 0.05 respectively

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