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Soil microbial community composition and its driving factors in alpine grasslands along a mountain elevational gradient

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Abstract: Understanding the vertical distribution patterns of soil microbial community and its driving factors in alpine grasslands in the humid regions of the Tibet Plateau might be of great significance for predicting the soil microbial community of this type of vegetation in response to environmental change. Using phospholipid fatty acids (PLFA), we investigated soil microbial community composition along an elevational gradient (3094~4131 m above sea level) on Mount Yajiageng, and we explored the impact of plant functional groups and soil chemistry on the soil microbial community. Except for Arbuscular Mycorrhizal fungi (AM fungi) biomarker 18:2ω6,9 increasing significantly, other biomarkers did not show a consistent trend with the elevational gradient. Microbial biomass quantified by total PLFAs did not show the elevational trend and had mean values ranging from 1.64 to 4.09 μmol per g organic carbon (OC), which had the maximum value at the highest site. Bacterial PLFAs exhibited a similar trend with total PLFAs, and its mean values ranged from 0.82 to 1.81 μmol (g OC)$^{-1}$. The bacterial to fungal biomass ratios had the minimum value at the highest site, which might be related to temperature and soil total nitrogen (TN). The ratios of Gram-negative to Gram-positive bacteria had a significantly negative correlation with soil TN and had the maximum value at the highest site. Leguminous plant coverage and soil TN explained 58% of the total variation in the soil microbial community and could achieve the same interpretation as the whole model. Other factors may influence the soil microbial community through interaction with leguminous plant coverage and soil TN. Soil chemistry and plant functional group composition in substantial amounts explained different parts of the variation within the soil microbial community, and the interaction between them had no impact on the soil microbial community maybe because long-term grazing greatly reduces litter. In sum, although there were obvious differences in soil microbial communities along the elevation gradient, there were no clear elevational trends found in general. Plant functional groups and soil chemistry respectively affect the different aspects of soil microbial community. Leguminous plant coverage and soil TN had important effects in shaping soil microbial community.

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Introduction

Understanding microbial community structure and its function is essential as soil microorganisms play vital roles in regulating ecosystem function and influence a variety of important ecosystem processes related to soil organic matter turnover and biogeochemical cycling (Van Der Heijden et al. 2008). Recent studies demonstrated the importance of soil microbial communities in N and C cycles in the alpine meadow (Zhang et al. 2013) and Tibetan grasslands (Yang et al. 2013). In recent years, the spatial pattern of microbial communities in montane regions has attracted considerable interest with the advent of molecular techniques. The dramatic environmental gradients over short distances in montane regions provide a unique opportunity to assess the effect of high turnover of aboveground vegetation, local soil conditions and climate regimes on spatial patterning of microbial communities along elevational gradients (Margesin et al. 2009). However, little is known regarding the shift in microbial community structure and activities along an alpine climosequence.

Several studies have noted consistent trends in microbial community composition with altitude. For example, in the Austrian Central Alps, the fungal population and relative amount of Gram-negative bacteria increased with increasing elevation (Margesin et al. 2009). The results have demonstrated contrasting elevational patterns of plant and bacterial taxon richness and phylogenetic diversity in the Colorado Rocky Mountains (Bryant et al. 2008). Djukic et al. (2010) obtained evidence for the soil microbial community to co-vary with vegetation and soil chemical properties along an elevational gradient in the European Alps. However, Shen et al. (2013) did not find any elevational gradient in soil bacterial richness/diversity on Mount Changbai, China. Männistö et al. (2007) found that microbial community composition in tundra soils was relatively similar at different elevations, as long as the soil pH was similar. The contradictory results are attributed to many factors, including both biotic factors and abiotic factors. However, under field conditions, abiotic and biotic site parameters interact with microorganisms and can mask or modify the effect of changing temperature on the microbial community's composition. Disentangling these complex biotic and abiotic interactions is important for understanding and predicting how soil microorganisms and their activities will respond to climate change (Bardgett et al. 2008).

Plants interact with the soil microbial community in many ways. Nutrients required by soil microbes often come from plant litter or through root exudation (Grayston et al. 1998). Certain soil microorganisms form host-specific relationships with plant groups or species (Bever 2003), such as legumes and nitrogen-fixing bacteria. The characteristic plant traits that define different plant functional groups (such as differences between legumes and graminoids in their C:N ratio) are likely to influence their differing effects on a variety of soil properties that could feed back to the soil microbial community's composition. In a removal experiment on reestablishing vegetation in experimental bare patches in New Zealand pastures, there was an idiosyncratic relationship between the soil microbial community structure and function and plant functional group diversity and composition (Wardle et al. 1999). However, Wardle et al. (2003) and Niklaus et al. (2006) detected no change in microbial biomass with plant species or functional group diversity in microcosm communities.

Soil chemistry has also been shown to be key in explaining changes in the soil microbial community (Bååth and Anderson 2003; Hackl et al. 2005; Williamson et al. 2005; Högberg et al. 2007), and certain of the major influences of soil chemistry on the soil microbial community have been summarized by Wardle (1992). The soil microbial community generally increases with pH and soil moisture. Increased soil pH has been shown to cause the soil community to change from fungi-dominated to bacteria-dominated (Bååth and Anderson 2003; Högberg et al. 2007). Previous studies from other types of ecosystems suggest that in fertile conditions, the vegetation produces high quality, N rich organic matter, while the soil community is dominated by a bacterial-based soil food web, where in infertile conditions, the

dominant plants produce low quality litter and tend to support a fungal-based food web (Coleman et al. 1983; Wardle 2002; Nilsson et al. 2005; Van Der Heijden et al. 2008).

There are many subalpine grasslands and alpine grasslands forming under grazing conditions in the Tibet Plateau that play important roles in socioeconomic development, environmental conservation (especially biodiversity conservation) and water source conservation (Long 2007). The harsh geographic and climatic conditions contribute to one of the most challenging environments for plants and microorganisms, and the fragile ecosystem is very sensitive and vulnerable to climate change and anthropogenic perturbation (Chen et al. 2013). Understanding the vertical distribution patterns of microbial communities can provide fundamental knowledge regarding microbes at high elevational areas, which might be of great significance for predicting the microbial community's response to environmental change. We selected alpine grasslands of Mount Yajiageng to investigate the spatial patterns of the microbial community's structure and explore the impact of plant functional groups and soil chemistry on soil microbial community composition. Mount Yajiageng is situated at the eastern fringe of the Tibetan Plateau and mean annual precipitation in the study area is high. We hypothesized that plant functional group composition and soil chemistry respectively had an important impact on the soil microbial community.

1 Materials and Methods

1.1 Site description

The study was conducted during August of 2013 at Mount Yajiageng on the eastern fringe of the Tibetan Plateau. We selected four sampling sites in the natural open grasslands along the western slope of Mount Yajiageng at altitudes of 3094 to 4131 m a.s.l. The area is characterized by pronounced temperature gradients. From low to high sites, the mean daily temperatures from June to August in 2013 were, respectively, 16.75, 13.62, 12.99, and 11.65, and precipitation amounts were 738.2, 739.4, 577.4, and 506.0. The sites are named, respectively, low site (L), middle site (M), alpine site (A) and high site (H). The vegetation of the four sites is all alpine grasslands formed under grazing disturbance. Site L and M are located below the nature tree line, and site A and H are located in the nature shrub and grass ecotone.

1.2 Plant community and soil chemical analysis

The sites with approximately 400 m^2 at each chosen altitude were fenced to avoid animal disturbance. Five blocks of approximately 5 m × 5 m in each site were chosen to be as similar as possible in terms of vegetation structure and slope. Within each block, three 25 cm × 25 cm plots were randomly placed to investigate the aboveground and belowground properties. We estimated the percentage cover of all vascular species in each plot, and the mean of three plots was used as the value of the block. Thus, we obtained five replications of the plant community per altitude. Plant species were divided into three functional groups (graminoids, forbs, legumes). We calculated the species number and coverage of each functional group in each block and named them Gramsp, Forbsp, Legsp and Gramcov, Forbcov, Legcov. We also calculated the relative species number and coverage of the graminoids, which were named R Gramsp and R Gramcov.

After the investigation of the plant functional group composition, the aboveground part of the plant community was cut, and the corresponding soil was collected. Topsoil (0-10 cm) was collected at four points of each plot, and the soil from the same block was pooled as one sample. We yielded five composite soil samples per altitude. The soil was placed in polyethylene bags, stored on ice and transported to the laboratory for further treatment. The soil samples were sieved (<2 mm) to remove visible stones, animals, root fragments and plant material before freeze-drying in a lyophilizer, and stored at -20°C prior to lipid extraction. For bulk soil analysis, one subsample was used for the measurement of plant's available nitrogen (AN) (NH_4^+-N + NO_3^--N) by 2 M KCl-extraction and for the measurement of gravimetric soil water content. The second soil subsample was air-dried for the measurement of total C (TC) and N (TN) with a C/N analyzer (Multi-N/C 2100, Analytik Jena AG, Germany), pH (in water; 1:2.5 w/v), total P (TP)

(ignition and dissolution in 0.5 M sulfuric acid), and available phosphorus (AP) (bicarbonate extractable). Soil organic carbon (SOC) was measured by wet oxidation, followed by titration with ferrous ammonium sulfate, which is used to calculate soil microbial PLFA concentration.

1.3 PLFA analysis

We assessed soil microbial community composition by using the phospholipid fatty acid (PLFA) technique (Bligh and Dyer 1959); different microbial fatty acids correspond to different components of the microbial community. A subsample of 1.5 g from each sample was used, and lipids were extracted as described by Frosteg ärd et al. (1991). Extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on silicic acid columns by successive eluation with chloroform, acetone and methanol. The methanol fraction (containing phospholipids) was subjected to mild alkaline methanolysis to transform the fatty acids into free methyl esters and analyzed on a gas chromatograph (GC), equipped with a flame ionization detector using a 50-m HP5 capillary column (phenylmethyl silicone).

We used the fatty acid nomenclature described by Frostegård et al. (1993). Microbial community size was estimated by the total amount of extracted microbial phospholipid fatty acids (total PLFAs; μmol (g OC)$^{-1}$). The iso- and anteiso-branched saturated fatty acids (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0) represent Gram-positive bacteria (Kaur et al. 2005), whereas cyclopropyl (cy17:0, cy19:0), the monounsaturated 16:1ω7c and the straight chain fatty acids 14:0, 15:0, 17:0 represent Gram-negative bacteria (Kourtev et al. 2002). We calculated the sum of the PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 14:0, 15:0, 16:1ω7c, cy17:0, 17:0, cy19:0, 10Me16:0 and 10Me17:0 as an index of bacterial biomass (Frostegård and Bååth 1996). The PLFAs 18:2ω6,9, 10Me18:0 and 16:1ω5c were used as indicators of fungal biomass, actinomycetes and AM fungi, respectively (Kroppenstedt 1985; Olsson 1999). The ratio of bacterial PLFAs to 18:2ω6,9 was taken to represent the ratio of bacterial to fungal biomass (Federle 1986; Frostegård and Bååth 1996). The ratios of bacterial to actinomycetal PLFAs and Gram-negative to Gram-positive bacterial PLFAs were also calculated. The PLFAs i14:0, 15:0, and 17:0 were not observed in our research.

1.4 Statistical analysis

Data were calculated as arithmetic means with standard deviations. Duncan's test was used for comparison between pairs of means at the $p<0.05$ level. Correlations between variables were calculated using the Pearson correlation coefficient. In our ordination analysis, the relative abundance of PLFA biomarkers ascribed to bacteria, actinomycetes, fungi and AM fungi was used as species data, and plant functional group composition (biotic factors) and soil chemistry (abiotic factors) were used as environment data. We conducted a principal component analysis (PCA) of species data to observe the main difference of soil microbial PLFA composition along the elevational gradient. Then we conducted a redundancy analysis (RDA) of species data and environment data to assess the impact of environmental factors on the soil microbial community. First, we tested the significance of each environmental factor's impact on the soil microbial community. Second, we tested the significance of the Pearson correlation coefficient between biotic factors or abiotic factors to judge whether the co-line relationship exists and determine whether the forward selection is conducted before RDA. Finally, we conducted variance partitioning to assess the different and same part of the variation explained by two types of factors in the soil microbial community. ANOVAs and Pearson correlations were performed using the SPSS 18.0 software package for Windows (SPSS Inc., USA); PCA and RDA were conducted in R version 3.1.1 (R Core Team 2014).

2 Results

2.1 Soil chemistry and plant community

Only soil TC, TN and C:N ratios had a significant difference along the elevational gradient. However, they did not show elevational trends (Table 1). Soil TC and TN at site H were significantly lower than those at other sites (Table 1). Other soil chemistry factors neither had a

Table 1 Site information and soil chemistry (0-10 cm depth). Values for soil properties are arithmetic means with standard deviation given in parentheses (n=5). Different letters in the same column indicate the significant difference among sites (P<0.05).

Site	L	M	A	H
Elevation(m)	3094	3520	3873	4131
pH	4.82(0.15)a	4.87(0.10)a	4.81(0.16)a	4.90(0.32)a
TC(mg/g)	111.84(18.73)a	114.96(6.65)a	113.06(9.96)a	89.89(4.06)b
TN(mg/g)	8.85(1.21)a	8.35(0.67)a	9.43(0.78)a	7.02(0.44)b
C:N	12.63(1.21)a	13.80(0.89)b	11.99(0.48)a	12.83(0.50)ab
AN(mg/kg)	69.46(15.47)a	49.46(8.09)a	69.95(11.74)a	57.09(21.75)a
TP(g/kg)	0.87(0.25)a	0.80(0.28)a	1.05(0.37)a	0.97(0.05)a
AP(mg/kg)	11.25(5.17)a	8.43(3.61)a	14.05(6.07)a	9.36(7.59)a
C:P	139.21(49.59)a	160.66(61.72)a	121.58(50.38)a	92.83(9.45)a
N:P	10.81(2.89)a	11.49(3.76)a	10.20(1.95)a	7.25(0.72)a

Notes: TC-Total C; TN- Total N; AN-Available N; TP-Total P; AP- Available P.

significant difference nor showed elevational trends because of a small difference between sites or a large variation in each site. The soil was acid with a small pH value range (Table 1).

Neither graminoid coverage nor forb coverage had a significant difference along the elevational gradient (Table 2). Relative graminoid species number had a significantly higher value at site L; however, relative graminoid coverage showed no significant difference (Table 2). Leguminous species number and coverage both had a significantly higher value at site L and great variability at sites A and H (Table 2). Leguminous species did not appear at site M.

2.2 Microbial community size and composition

Other biomarkers did not show a consistent trend with elevational gradient, except for the AM fungi biomarker 18:2ω6,9 increasing significantly (Figure 1). Biomarker cy19:0 had the maximun value at L site, whick had high relative abundance in Gram-positive bacteria. Biomarker i15:0 had the maximun value at H site, which accounted for a large portion in Gram-negative. The relative abundance of Actinomycetes biomarker 10Me18:0 were highest at site A. Fungi biomarker 18:2ω6,9 had the maximun value at H site. Microbial biomass quantified by total PLFAs did not show an elevational trend and had mean values ranging from 1.64 to 4.09μmol (g OC)$^{-1}$ (Figure 2a). Total PLFAs had the highest value at site H and the lowest value at site M. Total PLFAs had no significant correlation with soil C:N ratios or pH (R^2=0.1009, P=0.172, N=20; R^2=0.1481, P=0.104, N=20). Bacterial PLFAs had a similar trend with total PLFAs along the elevational gradient because bacterial PLFAs make up a large portion of total PLFAs (Figure 2a). The mean values of bacterial PLFAs ranged from 0.82 to 1.81μmol (g OC)$^{-1}$. The bacterial to fungal biomass ratios had mean values ranging from 15.24 to 24.56, with the least value at site H (Figure 2b). The ratios of bacterial to actinomycetal PLFAs had mean values ranging from 8.94 to 15.49 (Figure 2b). The ratios of Gram-negative to Gram-positive bacteria had mean values ranging from 0.65 to 1.15 (Figure 2c). Gram-negative bacteria were more abundant than Gram-positive bacteria only at site H. The relative abundance of PLFA biomarkers was subjected to PCA, where the first principal component (PC1) explained 49% and the second (PC2) 26% of the variance (Figure 3). We plotted the PC1 and PC2 score values for all analyzed samples along the studied elevational gradient, which resulted in a clear separation of the four studied sites, except for very few points (Figure 3). The main characteristics of PLFA biomarkers at each site were shown in the

Table 2 Plant community functional group characteristics. Values are arithmetic means with standard deviation given in parentheses (n=5). Different letters in the same column indicate the significant difference among sites (P<0.05).

Site	Gramsp	Forbsp	Legsp	Legcov(%)	Forbcov(%)	Gramcov(%)	R Gramcov	R Gramsp
L	4.40(1.14)a	6.20(2.59)a	1.00(0.00)a	10.60(3.29)a	71.10(14.89)a	24.02(4.88)a	0.26(0.07)a	0.43(0.15)a
M	5.80(0.45)b	13.60(0.89)c	0.00(0.00)b	0.00(0.00)b	65.46(19.77)a	30.44(13.08)a	0.31(0.09)a	0.30(0.02)b
A	4.40(0.55)a	10.80(0.84)b	0.20(0.45)b	0.20(0.45)b	65.30(12.82)a	28.40(10.21)a	0.30(0.10)a	0.29(0.02)b
H	4.80(0.84)ab	12.40(2.51)bc	0.40(0.55)b	1.80(2.49)b	58.92(17.48)a	26.52(11.25)a	0.31(0.10)a	0.28(0.04)b

Notes: Gramsp-Graminoid species number; Forbsp-Forb species number; Legsp-Leguminous species number; Legcov-Leguminous species coverage; Forbcov-Forb species coverage; Gramcov- Graminoid species coverage; R Gramsp-Relative graminoid species number; R Gramcov -Relative graminoid species coverage.

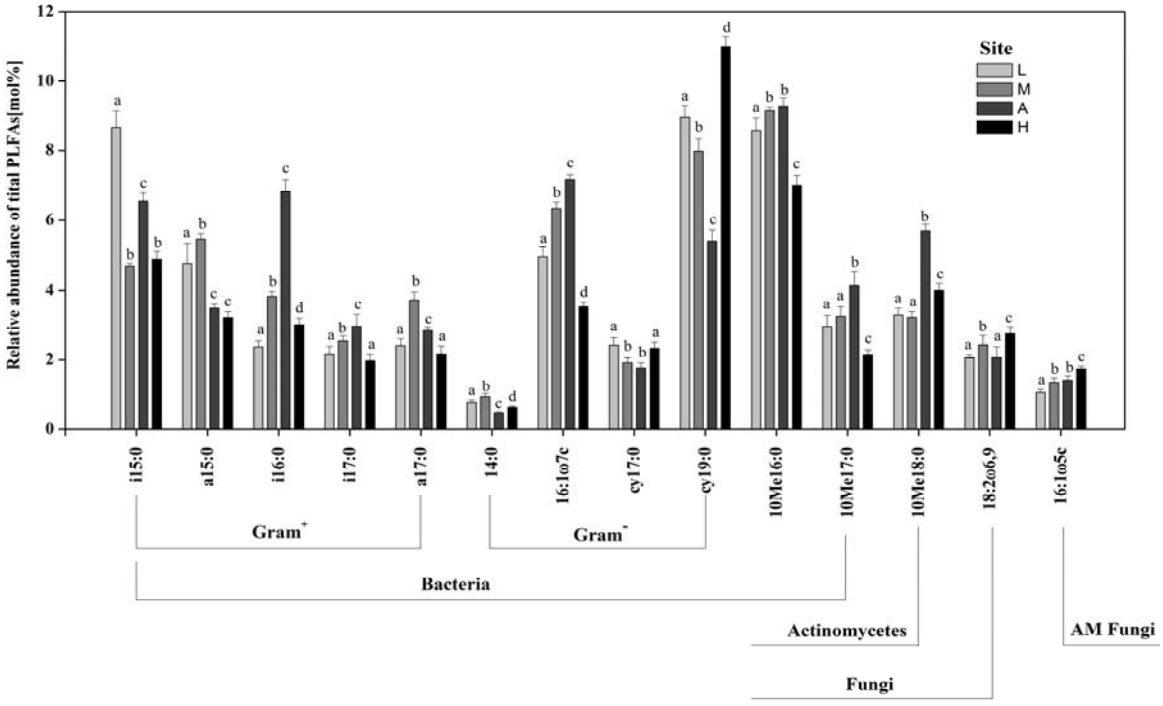

Figure 1 Relative abundance of bacterial, actinomycetal and fungal phospholipid fatty acids (PLFAs) in soils (0-10 cm depth) along the elevational gradient. Values are arithmetic means±standard deviation (n=5). Different letters indicate significant differences ($p < 0.05$; ANOVA, Duncan's test). AM=*Arbuscular mycorrhiza*.

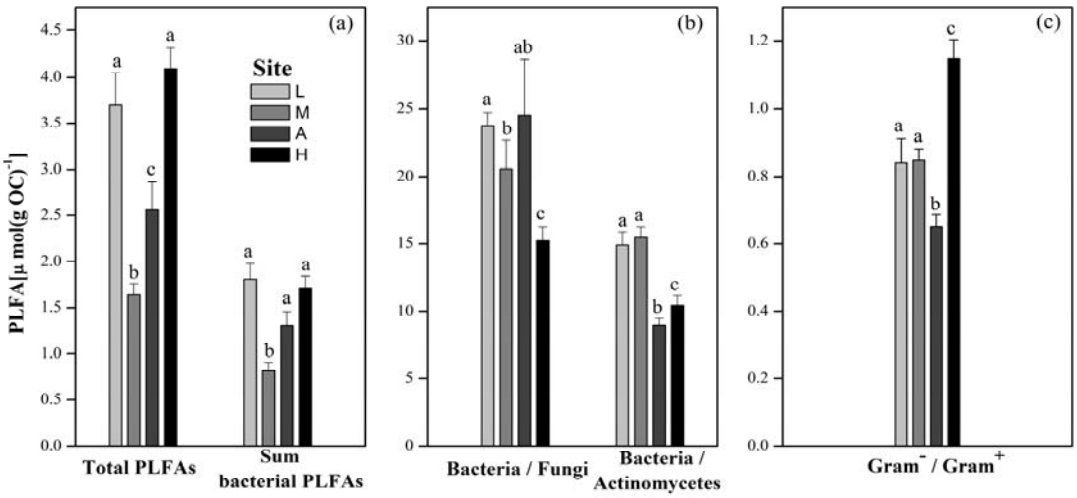

Figure 2 Sums and ratios of phospholipid fatty acids (PLFAs) of various microbial groups in soils (0-10 cm depth) along the elevational gradient. Values are arithmetic means±standard deviation (n=5). Different letters indicate significant differences ($p < 0.05$; ANOVA, Duncan's test).

biplot (Figure 3).

2.3 Impact of biotic and abiotic factors

Soil TC and TN both had a significant impact on the soil microbial community (Table 3). Legsp, Forbsp, R Gramsp and Legcov ascribed to biotic factors, respectively, had significant impact on the soil microbial commnity (Table 3). However, a significantly positive correlation existed between TC and TN (Appendix 1), and a significant correlation existed between the four biotic factors (Appendix 2). Thus, abiotic factors and biotic factors had a co-linear relationship. We conducted

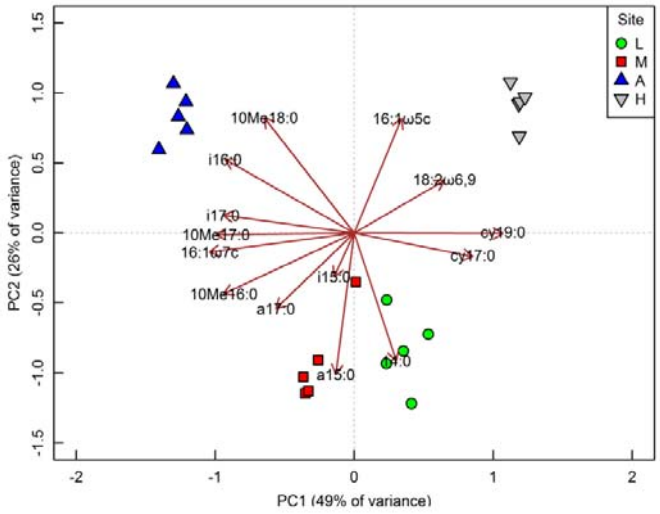

Figure 3 Principal component analysis (PCA) of microbial phospholipid fatty acids (PLFAs) in soils (0-10 cm depth) along the elevational gradient.

Table 3 Significance testing to the effect of environmental factors on microbial phospholipid fatty acids (PLFAs) composition in soils (0-10 cm depth) along the elevational gradient. The variable abbreviations are the same as in Tables 1 and 2.

	r^2	Pr(>r)
pH	0.0213	0.865
TC	0.4349	0.011*
TN	0.6422	0.002**
C:N	0.1283	0.28
AN	0.14	0.271
TP	0.0669	0.577
AP	0.0678	0.566
C:P	0.1306	0.307
N:P	0.1962	0.17
Legsp	0.4699	0.003**
Gramsp	0.0345	0.739
Forbsp	0.5324	0.004**
R Gramcov	0.0429	0.677
R Gramsp	0.4371	0.002**
Legcov	0.7961	0.001***
Forbcov	0.051	0.64
Gramcov	0.031	0.8

Notes: ***. Significant at $P<0.001$; **. Significant at $P<0.01$; *. Significant at $P<0.05$.

a forward selection for the two types of variables to resolve the co-linear problem before redundancy analysis (RDA). The simple model with only TN and Legcov can achieve the same interpretation as the entire model (Figure 4a and 4b). TN and Legcov explained 58% of the total variation in the soil microbial community (Figure 4a and 4b). TN was nearly orthogonal with Legcov, which indicated that correlation was small between two factors (R^2=0.0163, P=0.591) (Figure 4a). TN had significantly negative correlation with PLFA biomarker 18:2ω6,9 and 16:1ω5c (Figure 4a, 5a and 5b). TN also had significantly negative correlation with cy19:0 (R^2=0.4816, $P<0.01$) (Figure 5a). Legcov had significantly positive correlation with PLFA biomarker i15:0 (R^2=0.6071, $P<0.01$) (Figure 4a). TN had a significantly negative correlation with the ratios of Gram-negative to Gram-positive bacteria (Figure 5c).

To assess accurately the different parts and the same part of the variation in the soil microbial community explained by abiotic and biotic factors, we conducted a forward selection for the two types of factors, and then used variance partitioning. Soil chemistry and plant functional group composition uniquely explained 34% and 24% of the variation. The same part jointly explained by these factors was 0%, which is consistent with the nearly orthogonal relationship between TN and Legcov. The unexplained part was 42%.

3 Discussion

3.1 Soil microbial community size and composition

The total PLFAs in our study were similar to the values 1.52~3.53 μmol (g OC)$^{-1}$ (mean, 0~15 cm soil) reported by Xu et al. (2014) along the Segrila Mountain elevational gradient (3100~4600 m a.l.s.) on the Tibetan Plateau, and the values 1.79~3.83 μmol (g OC)$^{-1}$ (mean，0~5 cm soil) reported by Djukic et al. (2010) in the Austrian Limestone Alps (900~1900 m a.l.s.), though their results were from different alpine vegetation zones. Xu et al. (2014) reported that total PLFAs had the highest value at the lowest site, and the total PLFAs at the highest site with an alpine frigid meadow was 1.72 μmol (g OC)$^{-1}$. However, Djukic et al. (2010) reported that the highest total PLFAs appeared at the highest site with alpine grassland and mountain pine bushes, which is similar to our results. Our results were consistent with those of Xu et al. (2014) and Djukic et al. (2010), who also found that total PLFAs had no consistent trend

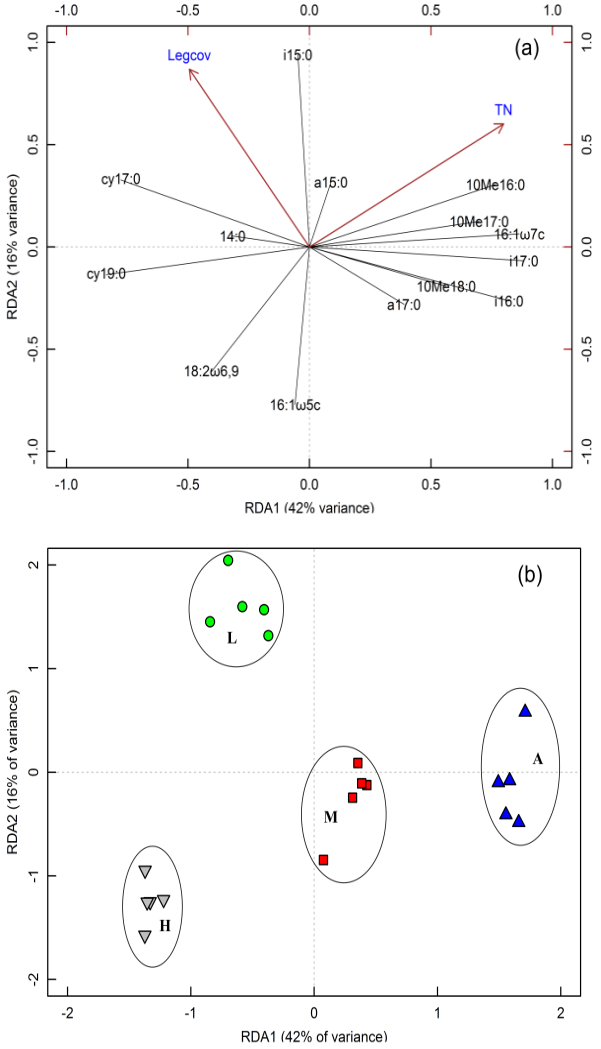

Figure 4 Redundancy analysis (RDA) of microbial phospholipid fatty acids (PLFAs) in soils (0-10 cm depth) along the elevational gradient after respective forward selection of biotic factors and soil factors. (a) Biplot of environment variables and PLFAs; (b) Score plot of samples from sites with different elevations. The variable abbreviations are the same as in Table 2.

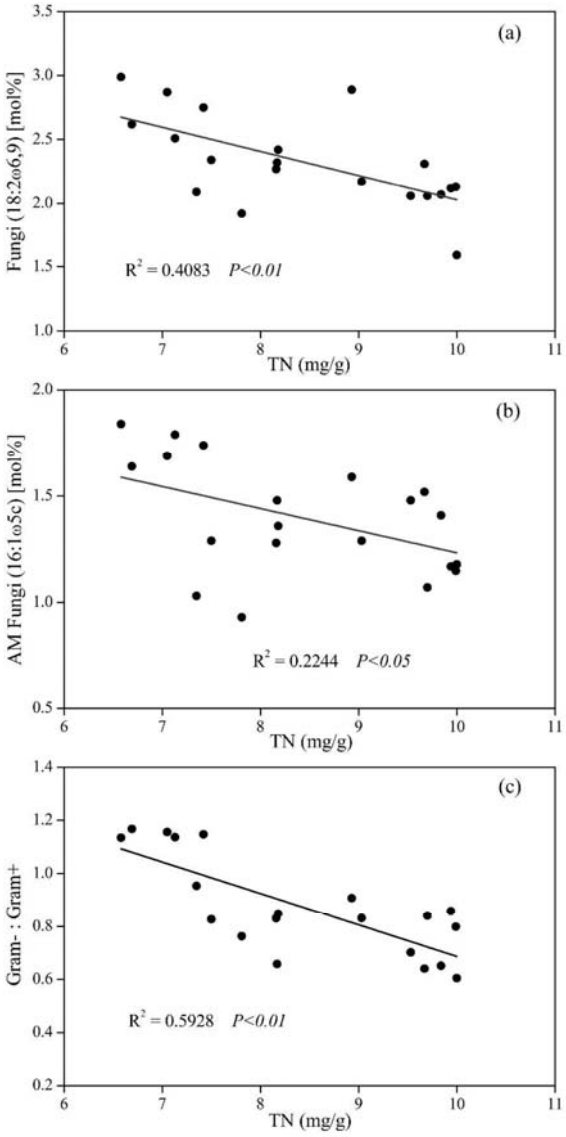

Figure 5 Correlation between soil TN and soil fungi (a), AM fungi (b) and Gram⁻: Gram⁺ ratios (c).

along the studied elevational gradient. Djukic et al. (2010) reported that the microbial community size was coupled with soil pH and the C:N ratio, along the studied vegetation gradient. Bååth et al. (2003) reported that the concentration of total PLFAs increased from a lower to higher pH in beech/beech-oak forest soils of Northern Germany (Bååth and Anderson 2003). Wardle (1992) also reported that the quality of soil organic matter can influence microbial biomass levels with a low microbial biomass at a high C:N ratio soil and vice versa. Soil C:N and pH had no significant impact on total PLFAs in our study. Microbial community size may be influenced by other factors, such as plant root biomass, soil temperature, etc.

Bacteria PLFAs accounted for a large proportion in total PLFAs. In grassland ecosystems, fast-growing plant species, especially those with highly branched fine root systems, supply large quantities of exudates (Personeni and Loiseau 2004), which are favored by bacteria. Our results differ from the findings of Xu et al. (2014) and Djukic et al. (2010), who both reported that fungal biomass declined with increasing elevation. In our

study, the relative abundance of fungi had the maximum value and the ratio of bacteria to fungi was the minimum at the highest site. The difference may occur because they reported the results of different alpine vegetation zones. In their study, the lower sites were mainly composed of coniferous forest and the higher sites were mainly composed of alpine grassland. Ingham et al. (1989) reported that grassland was more strongly dominated by bacteria compared to coniferous forest. Fungal growth has been found to be less inhibited by low temperatures compared to bacterial growth (Pietikäinen et al. 2005), which may be a reason for our results. Margesin et al. (2009) argued that Gram-negative bacteria seemed to be more competitive under the prevailing conditions of low temperature and low nutrient content at high altitudes, which supported our results that the ratios of Gram-negative to Gram-positive bacteria had a significantly negative correlation with soil TN and Gram-negative bacteria were more abundant than Gram-positive bacteria only at highest site (Figure 2c and 5c).

3.2 Impact of biotic and abiotic factors

Graminoid and forb species may influence the soil microbial community by interaction with leguminous species in our study. Legumes may have a disproportionately large impact on ecosystem properties, such as vegetation cover, plant composition and nitrogen retention (Stephan et al. 2000; Spehn et al. 2005), likely due to the benefit gained by other plants from the nitrogen fixation by legumes. Our results differed from those of Marshall et al. (2011), who reported that the soil microbial community is relatively insensitive to changes in plant functional group composition in northern Canadian grassland. However, Marshall et al. (2011) thought that five growing seasons might be insufficient to detect the impact of a changing plant community on the soil microbes. The alpine grasslands in our study are formed and kept for many years under the effect of grazing disturbance, which is suitable for studying the long-term effect of the plant functional group composition on the soil microbial community.

Soil TC and TN are known to influence the soil microbial community, but the mechanisms vary (Wardle 1992). The soil microbial community tends to be directly proportional to the total C because this reflects the primary energy source maintaining the soil microbial community. Similarly, the soil microbial community generally correlates with total N because N is a necessary cell nutrient. Previous studies have shown that a close correlation existed between soil carbon and nitrogen. Although the soil community is typically thought of as carbon limited, studies have also reported their potential nitrogen limitation (Wagener and Schimel 1998; Chen and Stark 2000). Nitrogen requirements appear to vary among soil microorganisms (Schimel et al. 2005). For example, soil communities under conditions of high nutrients tend to be bacterial dominated whereas lower-nutrient soils tend to be fungal dominated communities (Wardle 2005), which was supported by our results (Figure 5a and 5b). The soil microbial community may be more N-limited than C-limited along the studied elevational gradient.

This study illustrates that soil chemistry and plant functional group composition explain, in substantial amounts, different parts of the variation within the soil microbial community, and the interaction between them had no impact on the soil microbial community. The unexplained variation suggested that there may be other drivers that are important in explaining the variation in the soil microbial community that were not measured in this study, although unexplained variation in constrained ordination is also partly due to lack-of-fit of data to the response model (Økland 1999). Soil TN generally increases with increasing legume coverage, which is different from our result that TN was nearly orthogonal with Legcov. Plant composition is known to influence soil chemistry through the chemical composition of its litter and rhizo-deposition (Miles 1985). However, grazing dramatically reduces the litter in our sites and weakens the connection between plant functional groups and soil chemistry. Previous studies also have reported that plant community characteristics including functional groups composition were mainly affected by tempature along the elevational gradients, and the impact of soil chemistry was slight, probably due to long-term grazing disturbance (Cui et al. 2015). Soil TN may be primarily influenced by other factors, such as climatic ones.

4 Conclusions

There were obvious differences in soil microbial communities along the elevation gradient. However, microbial biomass and various microbial groups did not show the elevational trend except for AM fungi. Leguminous plant coverage and soil TN had important effects on soil microbial community composition. Other factors may influence the soil microbial community through interaction with leguminous plant coverage and soil TN. Soil chemistry and plant functional group composition in substantial amounts explained different parts of the variation within the soil microbial community and respectively affect the different aspects of soil microbial community. The interaction between them had no impact on the soil microbial community maybe because long-term grazing greatly reduces litter.

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