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Original article

Urea addition and litter manipulation alter plant community and soil microbial community composition in a *Kobresia humilis* meadow

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ABSTRACT

Overgrazing and climate change strongly affect alpine meadows by decreasing the plant community biomass and deteriorating the soil environment. To understand how the plant community and soil microbial community structure respond to grazing and N deposition, we conducted an experiment to remove or maintain the plant litter under the chronic addition of N in the Haibei Alpine Meadow in 2005. The experiment included four treatments: added N (+N, 20 g m^{-2}) with the litter removed (LR), +N with the litter left intact (LI), LI without N addition (−N), and LR with −N. Soil samples were collected at depths of 0–10 and 10–20 cm, and the following parameters were measured: 1) aboveground biomass (AGB) and litter biomass and 2) microbial community composition and content. Overall, the AGB and litter biomass significantly increased by 45.85% and 50.42% in response to N addition, whereas litter removal increased the AGB by 52.96%. The addition of urea N significantly decreased the PLFA content of the bacterial, gram-positive (G^+), gram-negative (G^-) at a soil of 0–10 cm in the LI and LR treatments by 64.87%, 61.82%, 76.07% and 64.86%, 53.02%, 51.44%, respectively. However, the PLFA content of the bacterial increased from 37.09 to 53.54 nmol g^{-1} and from 37.09 to 62.05 nmol g^{-1} at a depth of 10–20 cm for the +N + LI and +N + LR treatments compare to the LR+(−)N treatment, respectively. In addition, the total PLFAs in the LR+(−)N treatment significantly increased by 50.61% at a depth of 0–10 cm but decreased from 121.62 to 42.31 nmol g^{-1} at a depth of 10–20 cm relative to the LI+(−)N treatment. Using PLFA as a biomarker, we detected that G^- bacteria and total PLFA generally increased with increasing soil depth in the +N plots. However, the content of G^- was the highest at a depth of 0–10 cm and the lowest at a depth of 10–20 cm in the LR plots. The modification of soil microbial biomass at a depth of 0–10 cm was induced by the bottom-up effect of changes in soil nutrient contents and using ability, which were driven by N addition and litter manipulation. Thus, different soil depths with different soil nutrient conditions resulted in a strong microbial community composition gradient.

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1. Introduction

Global estimates have indicated that inputs to the terrestrial nitrogen (N) cycle have doubled in the last century due to anthropogenic activities, particularly fertilizer use and fossil fuel combustion [1]. Global N addition [2] can reduce the effects of N limitations on plant growth and profoundly affect the plant community structure and composition [3,4].

A growing body of research is beginning to demonstrate the effects of adding urea N on the soil microbial communities, productivity of plant communities, and litter accumulation. For example, the addition of available N favors a few species, such as grasses, that can quickly exploit available resources [5]. The effects of N addition on an arbuscular mycorrhizal fungal community primarily resulted from the altered soil characteristics and the modified plant community in an alpine meadow ecosystem [6]. Tian et al. [7] found that the addition of high concentrations of N in different forms significantly increased the abundance of ammonia-oxidizing bacteria in a Tibetan Plateau alpine meadow. The N input negatively affected soil microbial diversity [8,9] and the total

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abundance of phospholipid fatty acids (PLFA) [10].

External N inputs in alpine meadows result in high litter production. Litter accumulation and decomposition play important roles in C cycling and the flow of energy [11,12]. Dynamics in the accumulation of litter in alpine meadows can promote seminal germination and influence plant community composition and succession. Soil nutrients are provided by plants as litter decomposition and root secretions, which can result in the concerted evolution of plants and microorganisms and promote soil microbial diversity. For example, bacteria are more likely to use litter that is rich in carbohydrates, whereas fungi are more likely to use litter with greater phenol concentrations [13–15]. Furthermore, different soil organisms can alter the physical, chemical, and biological characteristics of soils in a given ecosystem and are important for soil nutrient cycling and soil structure [16,17]. High litter production and accumulation contribute to C sequestration and increased soil fertility. Thus, as one of the main terrestrial ecosystems, grassland ecosystems are generally more sensitive to changes in N fertilization than to changes in animal density [18].

Worldwide, most grasslands have suffered different degrees of degradation. Degradation in alpine meadows is largely reflected by aboveground plant growth, changes in the plant community structure, and changes in multiple belowground processes. One approach for restoring meadow degradation is the application of N fertilization. However, in addition to the positive effects described above, N inputs also have negative effects. Plant litter is the primary energy source for heterotrophic microbial growth in soil. Consequently, changes in the amounts and types of organic substrates that enter the soil (induced by N addition) could indirectly affect the composition and function of microbial communities. Soil nutrients and the turnover rate of soil organic matter are influenced by litter quality and intensively influence soil biological characteristics [19–21]. Soil microorganisms have a high turnover rate and can be affected by N addition over a relatively shorter period than plant communities [22]. Thus, the competitive abilities of soil microorganisms depend on the quantity of available substrate, such as tissue (e.g., litters), and on the available nutrient concentrations [23,24].

Current methodological advances, such as PLFA analyses, allow to obtain detailed information regarding soil microbial activities and community structure [25]. A range of PLFAs can be extracted from soils and are indicative of major microbial groups (e.g., eukaryotes, G^+ and G^- bacteria, and actinomycetes) [26]. Therefore, changes in the PLFA profile can correspond with changes in the total soil microbial community and could be used to compare different grassland management techniques, such as N addition. However, it is unclear whether the soil microbial structure and composition are affected by AGB and litter biomass under long-term N addition conditions. The aims of this study were to 1) determine how chronic urea N additions change the annual litter production and accumulation, the abundance, and the composition of microbial communities in an alpine meadow and 2) estimate the mixing effect due to the removal or maintenance of plant litter under chronic N addition on the litter production and soil microbial community structure.

2. Materials and methods

2.1. Field site

This study was conducted at the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences (37°32′ N, 101°15′ E, altitude 3240 m a.s.l.). The average annual precipitation recorded at the station between 1976 and 2001 was 560 mm, and 85% of that rainfall occurred during the growing season from May to September. The average annual air temperature from 1976 to 2001 was −1.7 °C. Species richness is typically high, at 25–40 species per 1 m^2. The dominant species at the study site included short *Kobresia* (*Kobresia humilis*), linear-leaf *Kobresia* (*Kobresia capillifolia*), and dusky-brown Oriole (*Carex atrofusca*), with many accompanying species, such as Kentucky bluegrass (*Poa pratensis*) and moderate Fescue (*Festuca modesta*). The grass community typically persists in 1–2 layers, with the tallest grasses reaching 45–60 cm and an overall ground cover of 60–95%. The soils at the study site were classified as Cryosols according to WRB [27].

2.2. Experimental design and setup

In this study, alpine meadow degradation was restored and managed following human-induced disturbances (e.g., grazing). In mid-April of 2005, we established 12 experimental blocks (4 m × 3 m) in the *K. humilis* meadow that were arranged in two parallel columns that were separated by 2 m. The blocks within each column were separated by 1-m buffer zones. Within each block, four treatment plots (1 × 1 m) were established with a 0.5-m buffer between each plot. Two levels of N fertilization (0 g m^{-2} and 20 g m^{-2}) and two levels of litter manipulation (litter removed and litter left intact) were applied to each plot in a factorial design. For the plots with N addition of a commercial $CO(NH_2)_2$ fertilizer in pellet form (46.65% N) was applied in late May each year from 2005 to 2010.

To exclude the current year's production that could have fallen in the plots, the litter was removed from some of the plots each November from 2005 to 2010 after clipping around the perimeter of the plots. Once collected, the litter was shaken to reduce the seed content. The total litter production from 2005 to 2010 included the accumulated biomass.

Four treatment combinations were used for each of the study sites: N addition (+N) with the litter removed (LR): +N + LR, +N with the litter left intact (LI): +N + LI, LI without N addition (−N): LI+(−N), and LR with −N: LR+(−N) [28].

2.3. Sample collection and processing

2.3.1. Litter sampling

Litter samples were collected from two 25 cm × 25 cm sampling quadrats within each of four 1 m × 1 m plots. After the AGB was harvested by clipping, the amount of litter collected by hand was weighed using an electronic balance (MP2000B, Shanghai Liangping Instrument Co, Ltd). The quadrats were randomly selected and located at least 50 cm from the side of the plots to avoid edge effects. All vegetative materials were dried (48 h at 65 °C) and weighed.

2.3.2. Soil sampling

Six soil samples were collected from two quadrats. For each quadrat, five soil cores (5 cm diameter) were collected and mixed to produce a single soil sample for that quadrat. Three soil samples per year were collected from areas where the vegetation had recently been removed. These samples were split into 0–10 cm and 10–20 cm sections in early/mid/late August each year from 2008 to 2010. Next, the samples were mixed by quadrat and depth, stored in iceboxes, and transported to the molecular biology laboratory at Southwest University for Nationalities, China. After removing roots and stones by passing the samples through a 2-mm mesh sieve, the samples were divided into two subsamples. One subsample was homogenized by passing through a 2-mm mesh sieve and frozen at −70 °C for PLFA analysis. The second subsample was air-dried, finely ground, and sieved through a 0.1-mm mesh sieve to

measure the soil organic C (SOC), total N (TN), total phosphorus (TP), available N (AN), available phosphorus (AP), and pH according to the standard methods described in the soil analysis manual [29,30].

2.4. PLFAs of the soil microorganisms

The single-phase extraction method [31,32] was used to isolate the total lipids from the soil organic matter. PLFAs have been extracted according to the method of Wilkinson [33], and non-adecanoic acid methyl ester (Sigma Aldrich, USA) has been used as internal standard (c = 33 μg ml^{-1}). Quantity and quality identifications of PLFAs have been done by a Hewlett Packard HP7890 gas chromatograph equipped with a capillary column (60 m × 0.32 mm × 25 μm [film thickness]). The injector was set at 230 °C, and the oven was held at 50 °C for 1 min after injection. Next, the oven temperature was increased to 180 °C at 12 °C min^{-1} and held for 2 min, increased to 220 °C at 6 °C min^{-1} and held for 2 min, and then increased to 240 °C at 15 °C min^{-1} and held for 1 min. Finally, the oven temperature was increased to 260 °C at 15 °C min^{-1} and held for 15 min. The transfer line was held at 280 °C throughout analysis. Electron ionization mass spectrometry (Hewlett Packard HP5975 Mass Selective Detector) with helium as a carrier gas was used to identify the FAMEs. The electron impact energy was set at 70 eV.

The abundances of the individual FAMEs were expressed as an nmol g^{-1} soil. The fatty acid nomenclature described by Frostegård et al. [34,35] was used. The 10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 26:0 fatty acids were chosen to represent the general bacterial PLFAs [36,37]; gram-positive (G^+) bacteria were identified by using the PLFAs a14:0, i15:0, a16:0, i16:0, and i17:0 [38,39]; G^- were identified by using the PLFAs 16:1ω7c, 16:1ω9c, cy16:0, 18:lω11t, 18:lω9c, and cy18:0 [37,40]; and 18:2ω9,12t was used as an indicator of fungal PLFA [41]. Monounsaturated fatty acids (MONO) were chosen to represent fungi and G^-, and normal saturated fatty acids (SAT) were used as indicators of the general bacterial and G^+ communities. The total PLFA was calculated as the sum of all PLFAs [35–38,42–44].

2.5. Calculations and statistical analyses

For the vegetation samples (12 replicates), the mean measurements obtained for each quadrat in each plot were used to calculate the treatment means. The treatment effects of litter biomass, litter accumulation, and AGB were examined using a one-way analysis of variance (ANOVA). Tests for significant differences among the treatments were performed using ANOVA with Duncan's multiple range test (DMRT) at a significance level of $P = 0.05$. In addition, PLFA profiles (3 repeats) were analyzed using principal component analysis (PCA) to identify the differences in the soil microbial community structure that were induced by N addition. Correlations between the soil PLFA content and the litter biomass or AGB were determined using a linear Pearson's coefficient (r). PCA was performed using CANOCO for Windows, version 4.02 [45]. All other analyses were conducted using SPSS 16.0 software (SPSS Inc., version 16.0).

3. Results

3.1. AGB and litter biomass

In the *K. humilis* meadow, the addition of urea N increased the plant community biomass, which was reflected by increases in the AGB and litter biomass, especially in the +N + LR treatment (Fig. 1a, b). Furthermore, significant effects were noted in the meadow

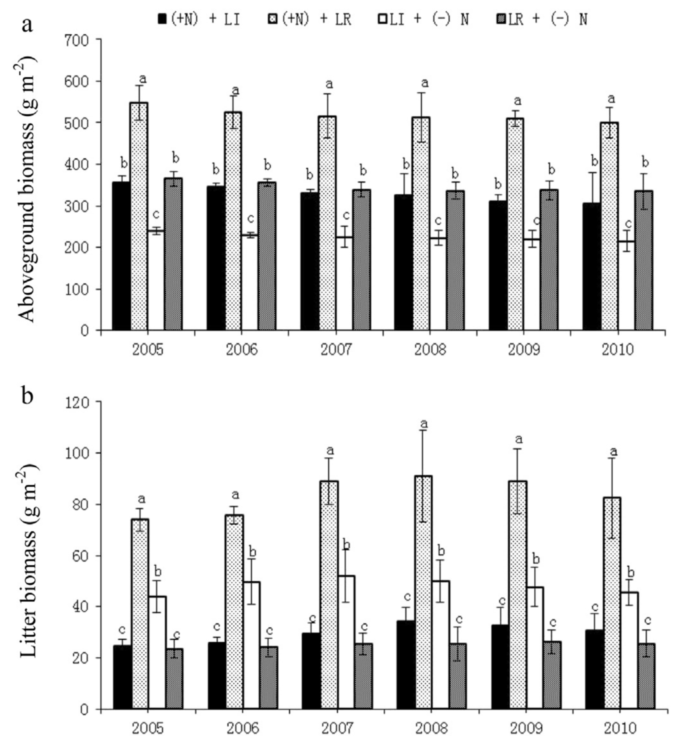

Fig. 1. Aboveground biomass (a) and litter biomass (b) over six years in experimental treatment plots with urea N addition and litter manipulations. Error bars indicate ±1 SE. n = 12 for each treatment. Different letters on the pillar indicate significant differences between treatments at 0.05 level.

treatments with removed litter ($F_3 = 976.911$, $P < 0.0001$) and intact litter ($F_{23} = 814.917$, $P < 0.0001$) (Table 1). Litter removal significantly increased the AGB ($F_5 = 8.846$, $P < 0.0001$) and decreased the litter biomass ($F_{23} = 8.933$, $P < 0.0001$) (Fig. 1a, b). However, large interannual variations occurred across the years (Table 1). The interactive effects of adding urea N were significant for litter biomass ($F_{23} = 2.046$, $P = 0.013$) but not for AGB ($F_1 = 0.413$, $P = 0.975$) (Table 1).

3.2. PLFA profiles

Soils under different treatments contained a variety of PLFAs that were composed of saturated, unsaturated, branched, and cyclopropane fatty acids (SI). Overall, 42 PLFAs and 44 PLFAs with chain lengths of C11 to C26 and from C10 to C19, respectively, were identified, and the relative abundance of fatty acids varied significantly in the 0–10 cm and 10–20 cm soil layers for the addition of urea N to the litter treatments (Table 2; SI). The dominant fatty acids ($F_{8,24} = 1056.672$, $P = 0.000$) and the relative abundance of

Table 1
Results of the two-way ANOVA showing the effect of experimental treatments (+N + LR, +N + LI, −N + LR, and −N + LI), years (2005–2010), and their interactions on the AGB[a] and litter biomass in a *Kobresia humilis* meadow over six years (2005–2010).

Source	Dependent Variable	df	F	Sig.
Treatment	Aboveground biomass (g m^{-2})	3	976.911	0.000
	Litter biomass (g m^{-2})	3	814.917	0.000
Year	Aboveground biomass (g m^{-2})	5	8.846	0.000
	Litter biomass (g m^{-2})	5	8.933	0.000
Treatment × year	Aboveground biomass (g m^{-2})	15	0.413	0.975
	Litter biomass (g m^{-2})	15	2.046	0.013

[a] Aboveground biomass.

Table 2
Saturated, unsaturated, branched saturated, and cyclopropane fatty acids in the experimental treatments.

Items	LR+(−)N		LI+(−)N		+N + LI		+N + LR	
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
Saturated fatty acids	C12:0C14:0C17:0C15:0C16:0C18:0C19:0	C11:0C12:0C13:0C14:0C15:0C16:0C17:0C18:0C19:0	C15:0C14:0C16:0C17:0C19:0C26:0	C10:0C14:0C15:0C16:0C17:0C18:0C19:0	C11:0C14:0C15:0C16:0C18:0C19:0	C14:0C15:0C16:0C19:0	C14:0C15:0C16:0C17:0C18:0C19:0	C11:0C14:0C15:0C16:0C18:0C19:0
G^+ bacteria	a14:0 br14:0 i14:0 a15:0 br15:0 i15:0 i16:0 a16:0 a17:0 i17:0 br18:0	15:0 a15:0 i16:0 a16:0 br17:0 a17:0 br19:0 i18:0	i16:0 br16:0 br17:0 a17:0 i17:0 br18:0 i18:0 i14:0 br15:0 a15:0	cy12:0 i12:0 i14:0 br15:0 a15:0 i16:0 a17:0 br18:0 i18:0 br19:0	br11:0 a13:0 a17:0 br17:0 a18:0 i18:0 i16:0 br15:0	i14:0 br15:0 a15:0 i16:0 a17:0 i17:0 br17:0 br18:0 i18:0 br19:0	br11:0 i14:0 br15:0 a15:0 i16:0 br17:0 a17:0 a18:0	i14:0 a15:0 i16:0 a17:0 i17:0 br18:0 i18:0 i19:0
G^- bacteria	16:1ω11 16:1ω7t 16:1ω9c 16:1ω5 18:1ω10 19:1ω13 cy17:0 cy19:0	16:1ω7t 16:1ω11 18:1ω9c	16:1ω11 16:1ω7t cy17:0 18:1ω11	16:1ω7t 16:1ω11 18:1ω12 18:1ω6 18:1ω9c	16:1ω7t 16:1ω9t 18:1ω7	16:1ω7 16:1ω9 17:1ω1 cy17:0 18:1ω1 18:1ω11	18:1ω12 16:1ω7t 16:1ω11	16:1ω9t 16:1ω9c 16:1ω7t 16:1ω5 cy17:0 16:1ω9
Fungi	18:1ω7c 18:1ω9t	18:1ω8c 18:1ω9t	18:1ω7c 18:1ω9t	18:1ω8c 18:1ω12t	18:1ω11t	18:2ω912 18:1ω9t 18:1ω7t	18:1ω9t 18:1ω7t	18:1ω10t 18:1ω9t 18:1ω10c
Total PLFA	28	22	22	24	18	23	19	23

fatty acids ($F_{8,24} = 411356.853$, $P = 0.001$) in the soil both varied at soil depths of 0–10 cm and 10–20 cm in the different treatments. In addition, the interactions between the treatments and soil depth were all significant ($F_{8,24} = 434.930$, $P = 0.000$). The relative abundance of fatty acids significantly increased in the 0–10 cm soil layer but decreased in the 10–20 cm soil layer in the LR+(−)N treatment. However, the opposite trend was observed for the LI+(−)N treatment. The relative abundance of fatty acids decreased in the 0–10 cm soil layer but increased in the 10–20 cm soil layer in the +N + LI and +N + LR treatments.

3.3. *Microbial PLFA contents*

Microbial communities can be classified into different groups based on their PLFA compositions. The addition of urea N and litter manipulation significantly decreased the PLFA content of the bacterial, G^+, G^-, fungal, and total microbial communities at a soil depth of 0–10 cm in the LI and LR treatments (Table 3 and Fig. 2a). Conversely, removing the litter significantly increased the PLFA contents of the bacterial, G^+, G^-, fungal, and total microbial communities at a soil depth of 0–10 cm in the LR+(−)N treatment (Table 3 and Fig. 2a). The treatments with intact litter and fertilization had significantly greater contents of PLFA than the LR+(−)N in the bacterial, G^+, G^-, fungal, and total microbial communities at a soil depth of 10–20 cm (Table 3 and Fig. 2b). The removal of the litter significantly decreased the amounts of PLFA in the bacterial, G^+, G^-, fungal, and total microbial communities at soil depths of 10–20 cm in the LR+(−)N treatment (Table 3 and Fig. 2b). The interactions between the addition of urea N and both litter treatments significantly affected the PLFA contents in the bacterial, G^+, G^-, fungal, and total microbial communities (Table 3). Thus, the addition of urea N significantly affected the PLFA contents of each microbial group and the total PLFA content of the microbial community.

3.4. *The soil microbial community structure*

To distinguish the individual PLFA patterns of the soil microorganisms, a PCA was conducted for each treatment. The PCA of the PLFA showed dissimilarities in the soil microbial community composition at soil depths of 0–10 and 10–20 cm in the +N + LI, +N + LR and LR+(−)N, LI+(−)N treatments. The first principal component (PC1) (x axis) and the second principal component (PC2) (y axis) explained 81.5%, 16.1% and 49.8%, 42.0% of the overall variance in the data (Fig. 3a, b). In this study, PC1 had more power than PC2. As shown in Fig. 3a and b, the PCA of the loadings on the separate patterns of PC2 were clearly observed at a soil depth of 0–10 cm (a) in the +N + LI, +N + LR and LR+(−)N, LI+(−)N treatments. The PCA of the loadings on the separate patterns of PC1 were clearly observed at a depth of 10–20 cm (b) in the +N + LI, +N + LR and LR+(−)N, LI+(−)N treatments, which indicated a significant difference in the microorganisms between the treatment soils. This analysis also indicated that the soil nutrient concentrations following the addition of urea N and the changes in the litter biomass affected the individual PLFAs, the PLFA contents at different soil depths. For example, the relative abundance of saturated, monounsaturated fatty acids decreased at the 0–10 cm soil layer in the +N + LI, +N + LR and LI+(−)N treatments, but increased in the 10–20 cm soil layers (Table 2; SI).

3.5. *Correlations between the litter biomass, AGB, and PLFA contents*

Correlations between the soil microbial group PLFA contents, litter biomass, and AGB showed that the litter biomass was

Table 3
ANOVA for soil microbial groups in the 0–10 and 10–20 cm soil layers under varying urea N addition and litter manipulations in a *Kobresia humilis* meadow.

Factors	Bacterial PLFA			Gram-positive bacterial PLFA			Gram-negative bacterial PLFA		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Treatment	3	30.123	<0.001	3	104.594	<0.001	3	4.073	<0.05
Depth	1	20.901	<0.001	1	59.818	<0.001	1	15.361	<0.01
Treatment × depth	3	127.031	<0.001	3	123.516	<0.001	3	133.831	<0.001

Factors	Fungal PLFA			Total PLFA		
	df	*F*	*P*	*df*	*F*	*P*
Treatment	3	63.761	<0.001	3	15.564	<0.001
Depth	1	844.132	<0.001	1	21.110	<0.001
Treatment × depth	3	199.487	<0.001	3	125.169	<0.001

significantly and negatively correlated with the PLFA in the soil microbial group at a soil depth of 0–10 cm (Table 4). The AGB was significantly and positively correlated with the fungal PLFA at a soil depth of 0–10 cm in the treatments with added urea N and intact litter ($r = 0.594$, $P = 0.042$; Table 4). Litter biomass was positively correlated with the soil microbial PLFA at a soil depth of 10–20 cm. Finally, the AGB was significantly and negatively correlated with the G^+ PLFA at a soil depth of 10–20 cm in the treatments with urea N addition and intact litter ($r = -0.741$, $P = 0.006$; Table 4). These results indicated that PLFAs content is closely related with soil C content which is induced by N fertilization and litter decomposition. Soil C might play important role in soil N cycling processes at different soil layers in alpine meadow (Table 5).

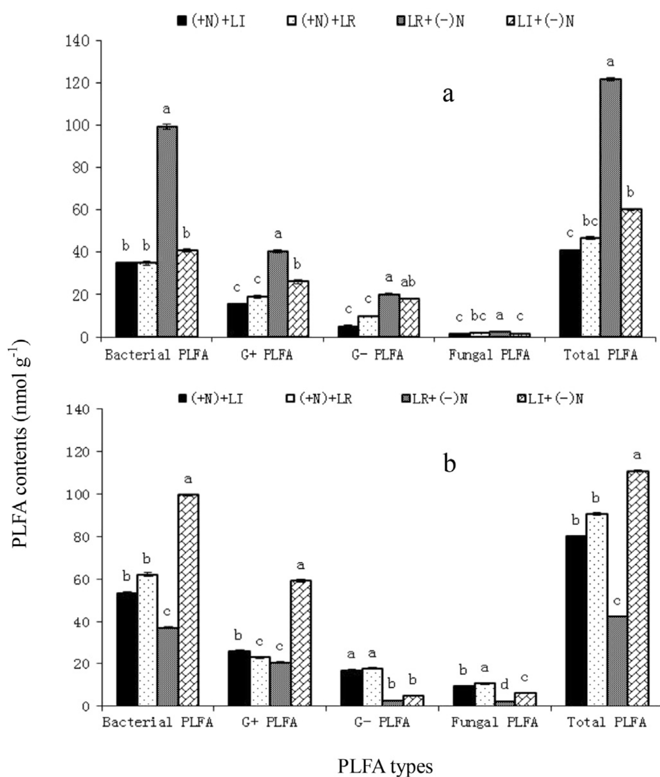

Fig. 2. Changes in PLFAs content of various microbial groups in experimental treatments with urea N addition and litter manipulations in a *Kobresia humilis* meadow in the 0–10 (a) and 10–20 cm (b) soil layers. Different letters on the pillar indicate significant differences between treatments at 0.05 level.

4. Discussion

This study showed that the litter removal increased the AGB and decreased the litter biomass accumulation in the *K. humilis* meadow. With the addition of urea N alpine meadow, the aboveground productivity and grass biomass increased [28,46]. These findings are inline with other studies where the addition of N enhanced the community productivity largely due to the increased availability of nutrients [46]. However, the interannual variations showed a different trend in AGB and litter biomass in response to N addition (Fig. 1). The AGB significantly increased in the initial years and then decreased in later years. In addition, the litter biomass slightly increased in the initial years and then decreased in later years. The addition of urea N was linked to an increase in grass height, which potentially resulted in the linear enhancement of plant density during the early stage of N addition.

The contents of fatty acids (which are related to bacteria, G^+, G^-, and fungi) significantly decreased at a soil depth of 0–10 cm and increased at a depth of 10–20 cm. In addition, the total microbial PLFA contents also decreased at a soil depth of 0–10 cm and increased at a soil depth of 10–20 cm with the addition of N and changes in the litter. Addition of N accelerated mineralization rates and hence the soil N contents (N availability), especially at a soil depth of 0–10 cm (Table 5). This increase in soil N contents alters not only the soil nutrient levels and the populations of microorganisms but also the quality and quantity of litter. Overall, compared to the surface microbial communities, higher PLFAs contents in the soil subsurface, soil nutrients are likely to be responsible for the specific changes in microbial community composition. Based on other studies, the addition of N and the accumulation of litter cause the reduction in plant diversity and plant species richness [47], change the composition of soil microbial communities [48,49], and affect coevolution and symbiotic relationships between plants and soil microorganisms.

Moreover, our results showed that the litter biomass was negatively correlated with bacterial PLFA, G^+ PLFA, and the total PLFA content at a soil depth of 0–10 cm. However, the AGB was positively correlated with fungal PLFA at the 0–10 cm soil depth in the treatments with added urea N and intact litter. Thus, the addition of N in the litter treatment plots could have resulted in lower bacterial PLFA contents and greater fungal PLFA contents. Greater N inputs could also alter the excretion of plant root exudates in soils and affect special soil fungi (e.g., AMF) that form symbiotic mutualistic relationships with plant roots [50]. Similarly, we found that litter biomass was positively correlated with various groups of microorganisms at a soil depth of 10–20 cm. In addition, the AGB was negatively correlated with G^+ PLFA at the 10–20 cm soil depth in the urea N addition and litter treatments. These findings suggest that the deeper layers of soil may contain microbial communities that are controlled litter quantity and quality

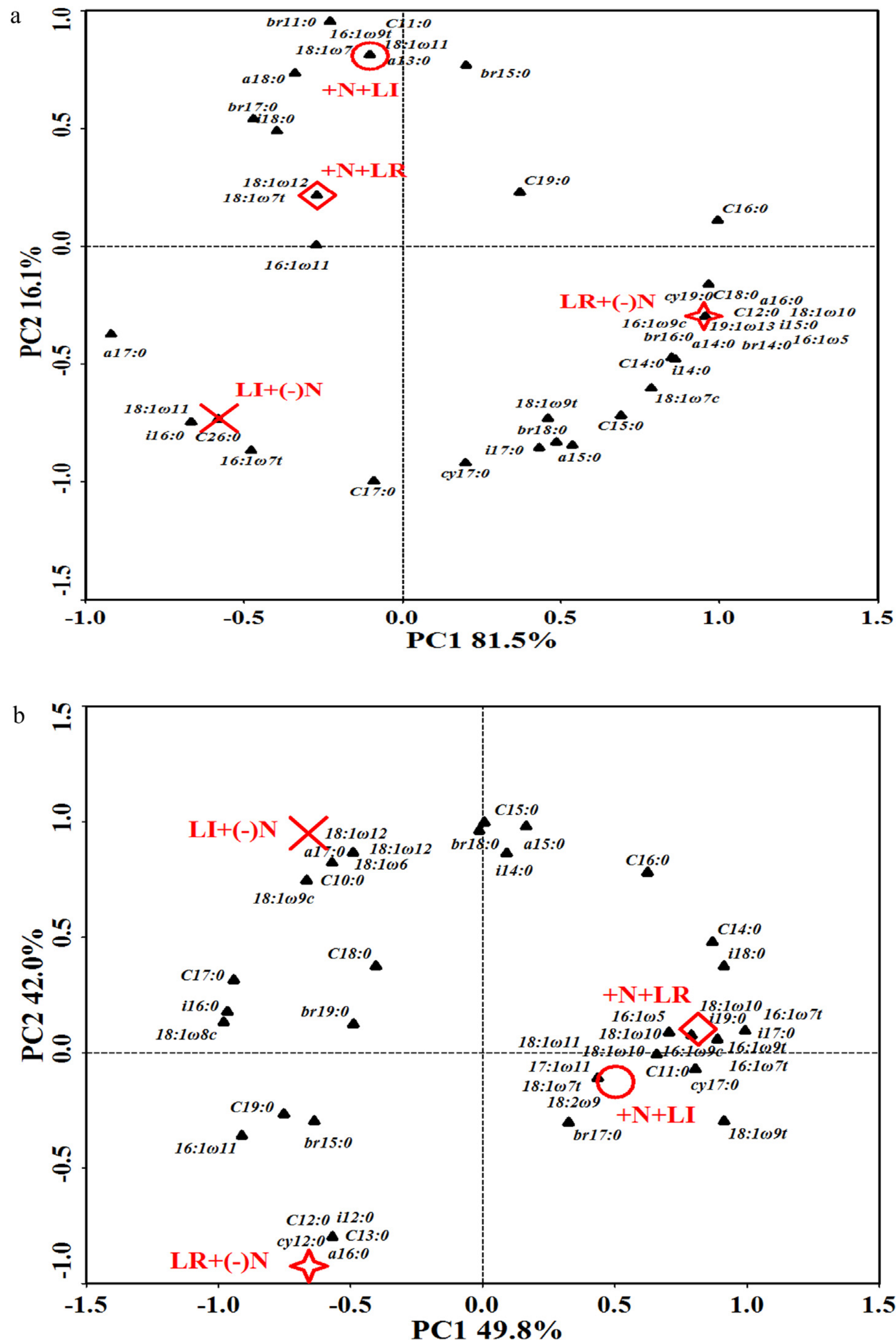

Fig. 3. Principal component analysis (PCA) plots for all phospholipid fatty acid signatures detected in the 0–10 top (a) and 10–20 cm bottom (b) soil layers in treatment plots with urea N addition and litter manipulations.

under N addition. Litter fall is an important source of soil C for bacteria, and increased plant litter can enhance the abundance of bacteria [51]. Therefore, excessive soil nutrients resulting from N enrichment could change the composition and structure of the soil microbial community [10].

Although, the importance of edaphic factors in shaping microbial communities has been demonstrated in a number of studies [6,48,52–54], the results showed that the total number of PLFAs increased with the increase of soil depth in the three treatments of +N + LI, +N + LR and LI+(−)N, but decreased in LR+(−)N treatment. The mechanisms underlying this pattern include the gradual adaptation of soil microorgnisms to N addition, which changes the soil environment and the soil microbial community composition. The decreasing content of PLFAs with increasing soil depth were related to the soil nutrient levels and physicochemical properties (Table 5), indicating the importance of depth in controlling the microbial community composition [55,56].

5. Conclusions

The results showed significant increased by 45.85% and 50.42% in AGB and litter biomass under increased N addition and litter

Table 4
Correlation between litter biomass and microbial phospholipid fatty acid (PLFA) contents in the 0–10 and 10–20 cm soil layers for sample plots (n = 12) with urea N addition and litter manipulations from 2008 to 2010.

Plant biomass (g m^{-2})	Soil depth (cm)		Bacterial PLFA	Gram-positive bacteria PLFA	Gram-negative bacteria PLFA	Fungal PLFA	Total PLFA
Litter biomass	0–10	Spearson correlation	−0.735**	−0.643*	−0.361	−0.028	−0.634*
		Sig.	0.005	0.022	0.238	0.931	0.026
Aboveground biomass		Spearson correlation	0.308	0.049	−0.112	0.594*	0.154
		Sig.	0.331	0.880	0.729	0.042	0.633
Litter biomass	10–20	Spearson correlation	0.741**	0.399	0.608*	0.720**	0.776**
		Sig.	0.006	0.199	0.036	0.008	0.003
Aboveground biomass		Spearson correlation	−0.399	−0.741**	0.238	0.350	−0.364
		Sig.	0.199	0.006	0.457	0.265	0.245

**Significant at $P < 0.01$; *Significant at $P < 0.05$.

Table 5
Soil chemical properties in the 0–10 and 10–20 cm soil layers under varying urea N addition and litter manipulations in a *Kobresia humilis* meadow from 2008 to 2010. Data present mean ± SE. n = 3.

Item	Soil depth (cm)	(+N) + LI	(+N) + LR	LI + (−) N	LR + (−) N
PH	0–10	6.38 ± 0.16c	6.57 ± 0.25b	7.25 ± 0.17a	7.37 ± 0.19a
SOM[a] (g kg^{-1})		169.23 ± 12.01a	147.23 ± 4.68a	144.06 ± 10.87a	135.03 ± 13.43a
TN[b] (g kg^{-1})		8.42 ± 0.46a	8.36 ± 3.07a	6.47 ± 0.80b	5.67 ± 1.13c
AN[c] (mg kg^{-1})		50.10 ± 10.77a	38.36 ± 3.07a	15.35 ± 1.04b	12.78 ± 1.68bc
TP[d] (g kg^{-1})		0.84 ± 0.04a	0.86 ± 0.04a	0.80 ± 0.07a	0.84 ± 0.02a
AP[e] (mg kg^{-1})		10.63 ± 0.76a	10.62 ± 0.92a	10.23 ± 1.24a	9.38 ± 1.12a
TK[f] (g kg^{-1})		22.17 ± 1.37a	19.63 ± 2.06b	23.27 ± 3.55a	22.80 ± 1.49a
AK[g] (mg kg^{-1})		403.53 ± 24.42a	369.90 ± 18.54a	383.98 ± 12.88a	373.79 ± 19.75a
PH	10–20	6.42 ± 0.28c	6.86 ± 0.18b	7.29 ± 0.07a	7.37 ± 0.24a
SOM[a] (g kg^{-1})		74.53 ± 14.91a	57.87 ± 5.66a	82.67 ± 3.13a	77.47 ± 4.65a
TN[b] (g kg^{-1})		6.34 ± 2.20a	6.66 ± 0.35a	4.82 ± 0.72b	3.76 ± 0.52c
AN[c] (mg kg^{-1})		18.42 ± 2.21a	13.86 ± 2.42a	8.53 ± 0.46b	7.83 ± 1.30bc
TP[d] (g kg^{-1})		0.65 ± 0.06a	0.59 ± 0.02a	0.75 ± 0.07a	0.81 ± 0.03a
AP[e] (mg kg^{-1})		6.61 ± 0.99a	6.20 ± 0.50a	7.16 ± 1.23a	6.47 ± 1.18a
TK[f] (g kg^{-1})		21.10 ± 0.26a	16.00 ± 1.49b	18.50 ± 2.79a	21.57 ± 0.97a
AK[g] (mg kg^{-1})		264.58 ± 53.05a	256.38 ± 53.98a	252.29 ± 19.74a	271.52 ± 38.94a

Different letters in the same index row indicated significant difference at 0.05 level (n = 3, statistical treatment by Duncan's multiple range test (DMRT).
[a] SOM: soil organic matter.
[b] TN: total nitrogen.
[c] AN: available nitrogen.
[d] TP: total phosphorous.
[e] AP: available phosphorous.
[f] TK: total potassium.
[g] AK: available potassium.

manipulation. The addition of N decreased the total PLFAs content by 31.8%, 61.5% at a depth of 0–10 cm, whereas increased by 47.19%, 18.37% at 10–20 cm soil layer. PCA of the PLFA indicated that the composition of the soil microbial communities changes significantly with soil depth. Different microbial groups responded distinctly to fertilization and litter manipulation, indicating that specific microbial groups are distinctly differently affected by soil layer resource limitations. The G^{-} and total PLFAs generally increased in proportion to the increasing soil depth when N was added. Soil depth appeared to be an important factor to alter the habitat conditions with N addition and litter manipulation. Long-term fertilization may improve nutrient transfer to plants and enhance the availability of N at deep soil layers. Therefore, the interaction between plants and soil microorganisms plays an important role in improving our understanding of ecosystem functioning under conditions of global N enrichment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2015.06.003.

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