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Effects of Nitrogen Addition on Alpine Kobresia tibetica Swamp Meadow and Soil Microbial Community: Postprint

Authors: Wang Changting, Wang Genxu, Li Xiangzhen, Wang Yong, Zi Hongbiao, Adeluji

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Abstract

Using the Kobresia tibetica swamp meadow as the study object, phospholipid fatty acid (PLFA) analysis was employed to investigate the effects of six consecutive years of nitrogen addition on aboveground vegetation community quantitative characteristics and soil microbial community structure. The results showed that: The community biomass and litter of Kobresia tibetica swamp meadow exhibited no significant response to fertilization treatments, and Cyperaceae plants demonstrated low absorption and utilization efficiency of soil nitrogen. Fertilization increased the PLFA richness of soil microbial groups in the 0-10 cm layer, particularly for bacterial and Gram-positive bacterial PLFAs, while decreasing PLFA richness in the 10-20 cm layer. The ratios of saturated to monounsaturated phospholipid fatty acids and bacterial to fungal PLFAs increased with soil depth. In the 0-10 cm soil layer, the contents of Gram-positive bacterial and fungal PLFAs were significantly positively correlated with pH, soil available phosphorus, available nitrogen, and soil organic matter ($P< 0.05$ or $P< 0.01$); in the 10-20 cm soil layer, the contents of bacterial, Gram-positive bacterial, fungal, and total PLFAs were significantly positively correlated with soil organic matter content ($P< 0.05$ or $P < 0.01$). These findings indicate that the response of microbial PLFA content and richness to fertilization in Kobresia tibetica swamp meadow exhibited a significant soil layer gradient effect, and that soil microbial PLFA content and richness were primarily influenced by the initial nutrient content of the surface soil.

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Full Text

Effects of Nitrogen Addition on Plant and Soil Microbial Communities in Alpine *Kobresia tibetica* Swamp Meadow of the Qinghai-Tibet Plateau

Changting Wang[1], Genxu Wang[2], Xiangzhen Li[3], Yong Wang[1], Hongbiao Zi[1], Deluji A[1] [1]College of Life Science and Technology, Southwest University for Nationalities [2]Institute of Mountain Hazards and Environment, Chinese Academy of Sciences [3]Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

Abstract

This study investigated the effects of six-year continuous nitrogen addition on quantitative characteristics of aboveground vegetation and soil microbial communities in *Kobresia tibetica* swamp meadow using field surveys and phospholipid fatty acid (PLFA) analysis. The experiment included four manipulative treatments involving both nitrogen and litter: +N with litter removed (LR), +N with litter left intact (LI), -N with LR, and -N with LI. Soil samples were collected at depths of 0–10 cm and 10–20 cm. Aboveground biomass (AGB), litter biomass, microbial community composition, and nutrient content were measured. The results showed that: (1) Fertilization had no significant effects on AGB and litter biomass. (2) N addition significantly increased microbial group PLFAs abundance at 0–10 cm soil depth, particularly bacterial PLFAs (B-PLFAs) and Gram-positive bacterial PLFAs (G-PLFAs), but decreased microbial abundance at 10–20 cm depth. (3) The ratios of normal saturated fatty acids/monounsaturated fatty acids (SAT/MONO) and B-PLFAs/fungal PLFAs (F-PLFAs) increased with soil depth. (4) G-PLFA and F-PLFA contents were significantly positively correlated with pH, available phosphorus, available nitrogen, and soil organic matter at 0–10 cm depth. The contents and abundance of fatty acids were mainly regulated by initial nutrient levels in the topsoil after short-term N addition. These results indicate that microbial communities in this alpine tundra are sensitive to both litter and N inputs, and that their vertical structures are particularly vulnerable.

Keywords: *Kobresia tibetica* meadow; phospholipid fatty acid (PLFA); microbial community; nitrogen addition

Introduction

Grassland fertilization is an important management practice for maintaining nutrient balance in grassland ecosystems. It can supplement soil nutrients, increase plant community primary productivity, and reduce species richness and community diversity [1–6]. Since microorganisms participate in underground ecological processes such as litter decomposition, nitrification, and denitrification, and their abundance, activity, and community structure are affected by

increased nitrogen inputs [7], the effects on soil microbial characteristics remain highly uncertain [8]. Some studies have shown that nitrogen addition reduces soil microbial biomass [9–10], while others have found it increases actinomycetes. Nitrogen input not only affects grassland community primary productivity but more importantly influences soil microbial composition and diversity and its interactions with plants [11].

In grassland ecosystems, soil available nitrogen is the form that plants can utilize. Previous studies have explored and summarized the availability of soil organic nitrogen, plant absorption capacity, and the mechanisms of organic nitrogen uptake by roots [12–15]. In alpine meadows, although plant uptake of organic nitrogen is low, soils contain large amounts of free amino acids [16–18], and plants annually uptake nitrogen far exceeding the mineralization rate. The mineralization rate of soil organic nitrogen is low, and nitrogen supply is insufficient, especially in alpine swamp wetland ecosystems. Different plant species show differential and diversified characteristics in nitrogen absorption [19–21]. The diversified nitrogen uptake by plants in terrestrial ecosystems challenges current understanding of nitrogen cycling [22–24]. Nitrogen input affects soil microorganisms more in terms of community structure and function, while changes in microbial biomass, activity, and community structure affect important soil ecosystem functions and processes such as litter decomposition, nutrient cycling, and nitrogen utilization [25].

Based on biochemical method development, phospholipid fatty acid (PLFA) analysis provides a rapid, reliable, and reproducible method for analyzing soil microbial community structure, overcoming limitations of traditional cultivation methods. It can comprehensively understand microbial community structure under various conditions and characterize dominant soil microbial communities quantitatively [26–30]. This study used alpine *Kobresia tibetica* meadow as the research object, analyzing soil PLFA content and composition to understand the effects of exogenous nitrogen addition on aboveground vegetation, the competitive relationship between plants and soil microorganisms for nitrogen nutrients, and soil microbial community spatial distribution patterns. The goal was to clarify the relationships between plant community biomass, community structure, and soil nutrients in alpine grassland ecosystems and provide theoretical basis for nutrient utilization.

1. Study Area Overview

The study was conducted at the Luanhaizi experimental site of the Haibei Alpine Meadow Ecosystem Research Station, Chinese Academy of Sciences, in mid-July. The site is located at 37°37 N, 101°18 E, at an altitude of 3,200 m. The mean annual temperature is -1.7°C, with maximum temperature of 23.7°C and minimum of -37.1°C. Mean annual precipitation is 560 mm, concentrated in May–September, accounting for about 80–95% of annual rainfall. The *Kobresia tibetica* swamp meadow community has simple structure with only herbaceous layer, total coverage of 80–95%, and average height of 25 cm. The dominant species

is *Kobresia tibetica*, with companion species including *Blysmus sinocompressus*, *Carex pamirensis*, *Carex atrofusca*, *Trollius pumilus*, *Primula tangutica*, *Saussurea stella*, and *Koeleria cristata*. Soil type is organic cryic gleysol [22–33]. Soil total nitrogen content is 14.45 g/kg, available nitrogen 31–39 mg/kg, available phosphorus 69–114 mg/kg, and organic matter 0.7–1.1%.

2. Methods

2.1 Experimental Design and Sample Collection In mid-July 2005, a flat area of 2–3 hm^2 with uniform, non-degraded *Kobresia tibetica* meadow was selected within a fenced plot and protected. Four parallel experimental plots (50 m × 50 m) were established with 4 m spacing between plots. Each plot contained 12 subplots (4 m × 3 m) marked with wooden stakes at corners, with 0.5 m spacing between subplots. A two-factor randomized block design was used for fertilization treatments. Each subplot had two litter treatments: litter removal (LR) and litter intact (LI). Fertilization treatments began in late July 2005, with urea applied at 46.65% N content. The four treatment combinations were: (1) +N+LR, (2) +N+LI, (3) -N+LR, and (4) -N+LI. Litter was manually removed from LR subplots in October each year, while LI subplots accumulated litter from 2005–2010.

2.2 Community Survey In each 1 m × 1 m subplot, plant community characteristics were surveyed including species coverage, height, frequency, and total community coverage. Plants were clipped at ground level by species, dried at 65°C for 48 hours, and weighed for aboveground biomass (AGB). Litter was collected by hand from a 50 cm × 50 cm area. Root biomass was sampled from a 25 cm × 25 cm area along soil profiles at 0–10 cm and 10–20 cm depths.

2.3 Soil Sample Collection and Analysis Soil samples were collected from 0–10 cm and 10–20 cm depths in each subplot. Each sample was divided: one portion air-dried for pH, organic matter, total nitrogen, and total phosphorus analysis; another refrigerated at -70°C for microbial PLFA analysis. pH was measured with a pHS-3 pH meter (1:2.5 soil:water). For PLFA analysis, 6.0 g freeze-dried soil was extracted using a modified Bligh-Dyer method [35]. Phospholipids were separated by silica gel column chromatography, methylated to phospholipid fatty acid methyl esters, and analyzed by GC-MS (HP7890-HP5975). The temperature program: initial 180°C for 2 min, increased at 12°C/min to 220°C for 1 min, then at 6°C/min to 240°C for 15 min. GC-MS interface temperature was 260°C, ion source 280°C, with helium carrier gas (1 mL/min). Electron ionization (EI) at 70 eV was used. Qualitative identification used mass spectral libraries and published reports [37]. Quantification used internal standard n-nonadecanoic acid methyl ester.

PLFA content was calculated as: PLFA (nmol/g) = 1000 × (P_PLFA × V) / (P_STD × S × M × W)

where P_PLFA and P_STD are peak areas of sample and standard, S is standard concentration (g/mL), V is sample volume (mL), M is PLFA molar mass (g/mol), and W is dry soil mass (g). PLFA biomarkers for microbial groups are shown in .

** PLFA biomarkers indicating microbial types in *Kobresia tibetica* meadow**

Microbial Group	PLFA Signatures
General Bacteria (B-PLFAs)	11:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0
Gram-positive Bacteria (G-PLFAs)	i14:0, i16:0, i17:0, i18:0, a13:0, a15:0, a17:0, a18:0, br15:0, br16:0, br17:0, br18:0, br19:0
Gram-negative Bacteria (G -PLFAs)	16:1 7t, 18:1 4, 18:1 7, 18:1 11, 18:1 12
Fungi (F-PLFAs)	18:1 9, 18:1 9t, 16:1 11, 18:1 6t
Monounsaturated Fatty Acids (MONO)	Sum of fungal and Gram-negative markers
Normal Saturated Fatty Acids (SAT)	Sum of general bacterial and Gram-positive markers

2.4 Data Analysis PLFA quantitative analysis used peak area and internal standard curve methods. SPSS 19.0 was used for ANOVA of soil microbial PLFAs, with Duncan' s test ($P = 0.05$). Pearson correlation analysis ($P = 0.05$) examined relationships between microbial PLFAs and soil nutrients. Canoco for Windows (4.5) performed principal component analysis (PCA) of soil microbial PLFA signatures.

3. Results

3.1 Aboveground Biomass and Litter Aboveground biomass and litter showed only weak variation among years ($0.05 < P < 0.10$) but no significant differences among treatments or interactions ($P > 0.05$) [Attached Fig., Attached Table].

3.2 Soil Microbial Community Changes Microbial group PLFA contents varied significantly among treatments ($P < 0.05$). At 0–10 cm depth, all microbial group PLFA contents were higher than at 10–20 cm. The ratios of SAT/MONO and B-PLFAs/F-PLFAs increased with soil depth. Fertilization and litter accumulation increased PLFA contents, with +N+LR and +N+LI treatments showing significantly higher contents than -N treatments at 0–10 cm. At 10–20 cm, +N treatments decreased microbial abundance.

Principal component analysis (PCA) of PLFA signatures revealed distinct differences among treatments along the first principal component (PC1), which explained 54.9% of variance, and second principal component (PC2), explaining

29.5% (cumulative 82.5% for 0–10 cm). At 10–20 cm, PC1 explained 24.0% and PC2 54.9% (cumulative 78.9%). The +N+LR treatment showed significantly smaller horizontal distance on PC1 axis than +N+LI, indicating litter removal had greater effect on microbial community structure than fertilization alone. Key PLFAs contributing to PC1 included C14:0, C19:0, a15:0, C11:0, a18:0, C17:0, i18:0, and 16:1 7t.

[Figure 1: see original paper] Changes of microbial group PLFAs contents at 0–10 cm and 10–20 cm soil depths in *Kobresia tibetica* meadow

[Figure 2: see original paper] Principal component analysis (PCA) of soil microbial PLFA signatures at 0–10 cm and 10–20 cm soil layers under varying urea N addition and litter manipulations

3.3 Soil Physicochemical Properties Treatment and soil depth interactions significantly affected available nitrogen (AN), available phosphorus (AP), and soil organic matter (SOM) ($P < 0.001$). Depth effects were highly significant for AN and SOM ($P < 0.001$), while treatment effects were significant for total nitrogen (TN) and AP ($P < 0.05$) .

** Soil physicochemical properties (0–10 cm, 10–20 cm) under varying urea N addition and litter manipulations in *Kobresia tibetica* meadow (mean ± SE)**

Factor	TN (g/kg)	AN (mg/kg)	TP (g/kg)	AP (mg/kg)	SOM (g/kg)
Treatment	F = 15.354, P < 0.001	F = 5.633, P = 0.008	F = 6.353, P = 0.005	F = 10.861, P < 0.001	F = 33.139, P < 0.001
Depth	F = 4.139, P = 0.059	F = 38.577, P < 0.001	F = 59.036, P < 0.001	F = 127.538, P < 0.001	F = 65.131, P < 0.001
Treatment × Depth	F = 4.992, P = 0.013	F = 0.974, P = 0.429	F = 2.052, P = 0.147	F = 4.383, P = 0.020	F = 0.740, P = 0.544

3.4 Correlations Between Community Biomass and Microbial Groups At 0–10 cm depth, B-PLFAs were significantly positively correlated with AN ($r = 0.028$, $P < 0.05$), AP ($r = 0.011$, $P < 0.05$), and SOM ($r = 0.011$, $P < 0.01$). F-PLFAs showed significant positive correlations with pH ($r = 0.045$, $P < 0.05$), AN ($r = 0.012$, $P < 0.05$), AP ($r = 0.002$, $P < 0.01$), and SOM ($r = 0.004$, $P < 0.01$). G-PLFAs were significantly positively correlated with TN ($r = 0.000$, $P < 0.01$), AN ($r = 0.033$, $P < 0.05$), AP ($r = 0.000$, $P < 0.01$), and SOM ($r = 0.015$, $P < 0.01$).

At 10–20 cm depth, only G-PLFAs showed significant positive correlation with SOM ($r = 0.008$, $P < 0.05$). Other correlations were not significant .

** Correlation between soil nutrients and microbial PLFA contents under different treatments in *Kobresia tibetica* meadow (2008–2010)**

4. Discussion and Conclusion

Fertilization is a conventional grassland management practice to maintain ecosystem nutrient balance. It can supplement soil nutrients, stimulate plant growth, and promote degraded grassland recovery. However, excessive nitrogen application causes nutrient imbalance, soil acidification, and negatively impacts vegetation productivity [3, 48–49]. In alpine meadows, nitrogen addition shows inconsistent effects on aboveground biomass and plant total biomass [5], possibly related to community species composition, initial microbial groups, and soil nutrient content.

Kobresia tibetica meadows are dominated by perennial sedges with low nitrogen utilization efficiency. Our results showed fertilization did not significantly affect aboveground biomass or litter, consistent with findings that sedges have low nitrogen absorption rates [22]. However, nitrogen addition significantly increased microbial PLFA contents at 0–10 cm depth, particularly B-PLFAs and G-PLFAs, while decreasing microbial abundance at 10–20 cm. This vertical gradient response indicates that microbial communities in swamp meadows are sensitive to both litter and nitrogen inputs.

The ratios of SAT/MONO and B-PLFAs/F-PLFAs increased with soil depth, reflecting nutrient stress gradients. Higher ratios indicate greater microbial stress [59]. The increase in these ratios with depth suggests that deeper soil microbial metabolism is more limited by resources, while surface soils with better moisture, temperature, and aeration conditions favor microbial accumulation.

The significant positive correlations between G-PLFAs, F-PLFAs and soil nutrients (pH, AN, AP, SOM) at 0–10 cm demonstrate that microbial communities are closely linked to nutrient availability. The lack of significant correlations at 10–20 cm suggests that microbial communities in deeper layers are less responsive to short-term nitrogen addition.

Litter removal showed stronger effects on microbial community structure than fertilization alone, as evidenced by PCA results where +N+LR plots clustered separately from +N+LI plots. This suggests that litter manipulation alters carbon input, which is crucial for microbial growth in these carbon-limited systems [57].

Several mechanisms may explain these patterns: (1) Most roots and nutrients concentrate in 0–10 cm depth, so fertilization stimulates microbial growth primarily in surface layers; (2) High soil moisture in swamp meadows creates anaerobic conditions that favor bacterial and actinomycete proliferation; (3) Root exudates provide fresh carbon sources supporting microbial biomass; (4) Short-term experiments may mask long-term ecosystem responses.

In conclusion, *Kobresia tibetica* swamp meadow microbial communities are sen-

sitive to nitrogen and litter inputs, with pronounced vertical stratification in their responses. Surface soil initial nutrient levels regulate microbial content and abundance after short-term nitrogen addition. Long-term, multi-factor experiments are needed to fully understand aboveground-belowground interactions in alpine meadow ecosystems.

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Attached Figure. Aboveground biomass (a) and litter biomass (b) over six years (2005–2010) in experimental treatment plots with urea N addition and litter manipulations (Mean ± SE, n = 12 for each treatment). Error bars indicate ±1 SE. Same letters indicate no significant difference at P = 0.05 level.

Attached Table. Results of two-way ANOVA showing effects of experimental treatments (fertilization, litter), years (2005–2010), and their interactions on AGB and litter biomass in *Kobresia tibetica* meadow over six years.

Note: Figure translations are in progress. See original paper for figures.

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