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Nitrogen Addition Decreases Soil Respiration without Changing the Temperature Sensitivity in a Semiarid Grassland

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Abstract: The mechanisms underlying the response of soil respiration (Rs) to nitrogen (N) addition remain to be explored in semiarid ecosystems. This study was conducted to determine the effect of N addition on soil microbial composition, Rs and the temperature sensitivity of Rs (Q_{10}). The N addition experiment was carried out in a semiarid grassland in China, with N fertilizer application rates of 0, 2, 4, 8, 16, or 32 g N m^{-2} yr^{-1}. Microbial phospholipid fatty acids (PLFAs), Rs and Q_{10} were measured, and their relationships with soil properties were determined for three growing seasons. The results showed that N addition significantly increased the content of soil dissolved organic carbon (DOC) and inorganic nitrogen (IN), and decreased soil pH. With respect to soil microbes, N addition reduced soil PLFAs, reduced the fungi to bacteria ratio (F:B) and increased the gram-positive bacteria to gram-negative bacteria ratio (G+:G–). Rs under the N2, N4, N8, N16 and N32 treatments decreased by 2.58%, 14.86%, 22.62%, 23.97% and 19.87%, respectively, compared to the N0 (control) treatment. The results of structural equation models showed that N addition reduced Rs by lowering soil PLFAs and altering the microbial composition. However, N addition had no significant effect on either Q_{10}, soil total organic carbon (TOC) or total nitrogen (TN), indicating that N addition alleviated soil carbon loss and was unlikely to change the potential for a bigger loss under global warming.

Key words: nitrogen deposition; soil CO_2 flux; Q_{10}; phospholipid fatty acid; soil properties; Inner Mongolia grassland

1 Introduction

Nitrogen (N) is one of the basic elements for organisms, and anthropogenic N deposition has become the largest N input at the global scale (Gruber and Galloway, 2008). Enrichment of N in terrestrial ecosystems will affect the cycle of soil carbon (C). Soil respiration (Rs) is the main route for C to be released from terrestrial ecosystems into the atmosphere, and it is a key process of the ecosystem C cycle (Ali et al., 2018). Extensive studies on the effects of N addition on Rs have been carried out in forests, farmland and other ecosystems, but the effects of N addition on Rs in grasslands remain to be elucidated (Cenini et al., 2015), as various studies have reported positive (Xu et al., 2008), negative (Ramirez et al., 2010) or non-linear changes (Zhu et al., 2016) due to N addition. These differences in the research results are mainly related to the levels and duration of N addition, soil C and N availability, and vegetation and microbial compositions. The temperature sensitivity of soil respiration (Q_{10}) largely determines the feedback relationship between global climate change and the C cycle, so studying the linkage between Q_{10} and N addition will help to clarify the responses and adaptation of underground ecological processes to climate change (Yang et al., 2011). However, such linkages have not been adequately explored and the response is inconsistent among recent studies (Zhang et al., 2014; Zhong et al., 2016; Fang et al., 2018).

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Rs includes both autotrophic and heterotrophic respiration. Autotrophic respiration mainly derives from roots, mycorrhizal fungi, and rhizosphere microorganisms. Therefore, N addition can increase Rs by enhancing root biomass (Zhou et al., 2014). Heterotrophic respiration is the process by which soil microorganisms chemically modify the composition of dead organisms and release CO_2 during the decomposition. It is closely related to the amount, composition and activity of microorganisms (Chapin and Matson, 2002). Studies in grasslands have shown that N addition reduces Rs by inhibiting microbial biomass (Riggs and Hobbie, 2016; Yue et al., 2016) and suppressing exoenzymes (Li et al., 2018). Moreover, N addition affects the components of Rs by changing soil properties. Previous studies have found that various microbial taxonomic groups display different life strategies and preferences for abiotic factors, such as soil moisture conditions (Lennon et al., 2012; Manzoni et al., 2012), pH values (Chen et al., 2015; Zhalnina et al., 2014), and nutrient contents (Kopáček et al. 2013). Moreover, severe soil acidification by the addition of NH_4^+ from fertilizers (such as ammonium nitrogen and urea) (Tian and Niu, 2015) was reported to modify the microbial composition and Rs (Chen et al., 2015; Li et al., 2018). At the same time, soil temperature and moisture are important factors affecting soil respiration and should not be ignored. Similarly, Q_{10} is also regulated by a variety of factors including soil plants and microorganisms, soil substrates, and environmental factors (Yang et al., 2011). Therefore, all of these factors should be comprehensively considered in discussing Rs and Q_{10} under the impact of N addition.

Long-term experiments with large ranges of N addition concentrations are scarce in grasslands in China. As reviewed by Fu et al. (2015), most N addition experiments used N addition amounts lower than 15 g N m^{-2} yr^{-1}, and lasted less than 3 years. To comprehensively understand the effects of N addition on grassland Rs, we conducted Rs observation experiments on a long-term N application field for three consecutive growing seasons. Rs, soil temperature and moisture, aboveground biomass, microbial phospholipid fatty acids (PLFAs), and other soil properties were measured. The objectives of this study were to determine the responses of microbial communities, soil respiration and its temperature sensitivity to the different concentrations of added N, and to explore the underlying mechanisms of the regulation of soil C by N addition in semiarid grasslands.

2 Materials and methods

2.1 Study area

The study area is in Duolun County, Inner Mongolia, China (42.02°N, 116.17°E; 1341 m). The mean annual temperature in this region is 2.1 ℃, and the mean annual precipitation is 385.5 mm, most of which falls between May and September (Niu et al., 2009). The typical soil in this region is chestnut (Soil Taxonomy Research Group, 2001), corresponding to Calcic Petrocalcids based on the U.S. Soil Taxonomy (Soil Survey Staff, 2014). The soil bulk density is 1.31 g cm^{-3}. The area is dominated by perennial bunch grass *Stipa krylovii*, perennial rhizome grass *Leymus chinensis*, perennial semi-shrub *Artemisia frigida*, and perennial forbs such as *Agropyron cristatum* and *Allium bidentatum* (Niu et al., 2009).

The mean soil temperatures for the three growing seasons of 2013, 2014 and 2015 were 19.4 ℃, 19.8 ℃, and 18.8 ℃, respectively. The mean soil moisture levels (soil volumetric water content, VWC) for the three growing seasons were 13.4%, 10.4%, and 11.9%, respectively. Soil volumetric water content was closely associated with precipitation events (Fig. 1).

2.2 Experimental design

Atmospheric N deposition plus fertilizer application in this area are about 5 g N m^{-2} yr^{-1} (Wang et al., 2015). Eighteen 15 m × 10 m plots were established in the site, separated from one another by 4-m-wide buffer zones. Six N addition treatments, of 0 (N0), 2 (N2), 4 (N4), 8 (N8), 16 (N16), and 32 (N32) g N m^{-2} yr^{-1} with three replicates for each level were randomly assigned to the eighteen plots. Since July 2003, a dry urea N fertilizer ($CO(NH_2)_2$, 46.67% N) has been manually spread on the surface of the plots. Based on the local weather forecast, N addition was conducted just hours before the rain in mid-July (during the rainy season) each year.

2.3 Soil respiration

A PVC collar (20 cm diameter, 14 cm height) was inserted 5–6 cm deep at random locations in each plot to measure soil respiration (Rs, μmol of CO_2 m^{-2} s^{-1}). Aboveground biomass in the collar was clipped and removed 24 h before the measurement. All collars were removed after the measurement, and new ones were randomly insert into the soil during the next year. Respiration was measured during approximately 14-day periods in the mid-growing period, from 23 July to 4 August in 2013, from 27 July to 8 August in 2014, and from 26 July to 5 August in 2015. The Rs measurements would be postponed for 1–2 days after precipitation to avoid CO_2 flux pulses. During each period, the 18 plots were each observed only once. The Rs values in three plots with six collars were measured on each date. The respiration rate was measured with a soil C-flux automatic measurement system (LI-COR, NE, USA), and data were recorded once every 1 h for each 24-h measurement period. The annual mean of Rs was used for subsequent analysis. In the meantime, soil temperature (℃) and moisture (%) in the top 0–10 cm layer of the soil near the collar were measured with an auxiliary sensor attached to the LI-COR 8150.

A soil sensor attached to a data logger (EM50; ICT International, Australia) in the experimental site recorded the soil temperature and volumetric water content at a 10-cm

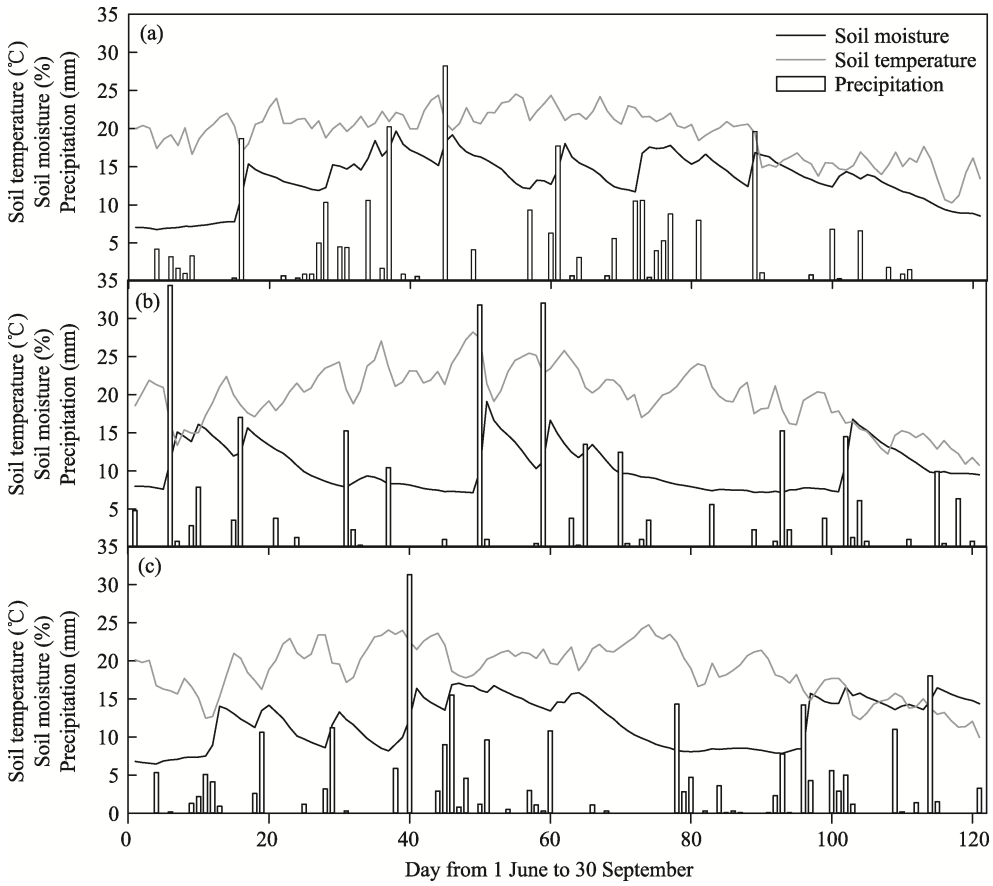

Fig. 1 Dynamics of soil temperature and soil moisture for 0–20 cm surface soil and precipitation during the growing seasons of 2013 (a), 2014 (b), and 2015 (c).

depth. Data were recorded every hour during the growing season (June to September). Precipitation events at the site were recorded by the staff working in the Duolun Restoration Ecology Research Station.

2.4 Soil properties and aboveground biomass

Soil sampling was conducted during the soil respiration measurements in each year. Three 0–20 cm surface soil samples were randomly collected from each plot to form a composite sample. The soil pH, dissolved organic carbon (DOC), ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N) were measured. Fresh soil was sieved through 2-mm-pore filters and stored at 4 ℃ for PLFAs, pH, DOC, NH_4^+-N, and NO_3^--N measurements. To measure the DOC, a 0.5 M K_2SO_4 solution was used to extract soil organic carbon. The extracts were measured with a TOC analyzer (Liqui TOC II, Elementar, Germany). Ion chromatograph analyzers were used to measure inorganic ions such as NH_4^+-N and NO_3^--N (DX-600 and ICS-2100, Dionex, CA, USA), and inorganic nitrogen (IN) was calculated as the sum of NH_4^+-N and NO_3^--N. The other portion of each soil sample was air dried and then ground for soil total organic carbon (TOC) and total nitrogen (TN) measurements. TOC was measured by the potassium dichromate-oxidation method, and TN was analyzed with an elemental analyzer (Perkin-Elmer, MA, USA). Aboveground biomass (ABM) in one quadrat (1 m × 1 m) of each plot was clipped and determined as described by Fang et al. (2012).

2.5 Soil phospholipid fatty acids

The microbial composition was measured in fresh soil using PLFAs as described by Bossio and Scow (1998). For comparisons to be valid among years, 18 biomarkers (i14:0, i15:0, a15:0, i16:0, a16:0, 16:1w5c, 16:0, i17:0, a17:0, 17:1w8c, cy17:0, 10Me17:0, 18:2w6c, 18:1w9c, 18:1w7c, 18:1w5c, 18:0, and 10Me18:0) that existed in all three years were selected, and the proportions of the individual PLFA biomarkers (i.e., the ratios of the selected biomarker to the total PLFAs that existed in a growing season) were used for further analysis. According to previous studies, i14:0, i15:0, a15:0, i16:0, a16:0, 16:0, 16:0, i17:0, a17:0, cy17:0, 17:1w8c, 18:1w7c, 18:1w5c, and 18:0 indicate bacteria; 18:2w6c and 18:1w9c indicate fungi; 10Me17:0 and 10Me18:0 indicate actinomycetes; i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 indicate gram-positive bacteria; a16:0, 17:1w8c, cy17:0, 18:1w7c, and 18:1w5c indicate gram-negative bacteria; and 16:1w5c indicates arbuscular mycorrhizal fungi (AMF) (Bi et al., 2012; Bossio and Scow,

1998; Wei et al., 2013). The ratios of fungi: bacteria (F : B ratio) and gram-positive bacteria: gram-negative bacteria (G^+ : G^- ratio) were considered as potential tools for assessing soil quality and environmental stress (Kaur et al., 2005; Canarini et al., 2017). Thus, these ratios were calculated according to the PLFA biomarkers mentioned above.

2.6 Statistical analysis

Temperature sensitivity of soil respiration (Q_{10}) was calculated by the following exponential functions:

$$Rs = ae^{bT} \quad (1)$$

$$Q_{10} = e^{10b} \quad (2)$$

where Rs is the 24-h dynamic soil CO_2 flux rate (μmol CO_2 m^{-2} s^{-1}) in the PVC collar, and T is the 24-h dynamic soil temperature (℃) near the collar. Parameter a indicates the basal respiration rate, and b is the exponent used to calculate Q_{10}.

Two-way ANOVA was used to evaluate the effects of N addition and interannual variability on Rs, Q_{10}, soil properties and microbial composition. Pairs of mean values were compared with the least significant difference (LSD) test. Pearson correlations were used to analyze the correlations of soil properties, microbial composition, Rs and Q_{10}.

SPSS 20.0 was used to perform a principal component analysis (PCA) on microbial composition (measured by the proportions of individual PLFA biomarkers) in soil samples in the six N addition levels and three years. Graphs were created using SigmaPlot 12.5 (Systat Software, CA, USA).

A structural equation model (SEM) was used to assess the direct and indirect effects of N addition on microbial community, soil respiration, and Q_{10}. The model included nine categories of variables, which were "N addition", "soil temperature", "soil moisture", "soil pH", "C and N availability", "aboveground biomass", "Rs", "Q_{10}", and "microbial community". Soil DOC, TOC, and TN were considered as potential indicators for "C and N availability". In light of the complexity of defining the microbial community, we quantified such variables in each model as follows: "PLFAs" and "microbial composition". Prior to the path analysis, PCA was performed for the indicators representing "microbial composition" and "C and N availability", respectively, then the loading of PC1 was used in the model (Table 1). A bootstrapping with 200 resamples was performed, and the 95% bootstrap confidence interval was used to judge whether the estimated path coefficients were significant in SEM. The criteria for evaluating model fit, such as chi-square, P values, and root mean square errors of approximation (RMSEA) were adopted according to Schermelleh-Engel et al. (2003). The SEM was carried out using Amos 21.0 (Smallwater Corporation, Chicago, IL, USA).

3 Results

3.1 Soil properties and aboveground biomass

The DOC and IN show significant differences along the N gradient (Table S1, $P < 0.001$). The DOC was promoted by N addition in 2014 and 2015 but did not change in 2013 (Fig. 2a). The DOC in 2013 was significantly higher than in 2014 and 2015 (Table S2, $P < 0.001$). The IN was significantly positively correlated with N addition for the three years (Fig. 2b), with the highest content in 2014 (Table S2). TOC and TN did not respond significantly to N addition ($P > 0.05$), but they had significant differences along the interannual gradient ($P < 0.001$) (Fig. 2d and Fig. 2e). In contrast, the pH was significantly inhibited by N addition for the three years ($P < 0.001$), but there was no significant difference between years ($P > 0.05$) (Fig. 2c). Aboveground biomass did not significantly respond to N addition for the three years ($P > 0.05$) but was significantly different between years ($P < 0.01$) (Fig. 2f).

3.2 Soil microbial phospholipid fatty acids

In the three growing seasons, the PLFA biomarkers selected in this study accounted for 64%–69% of the total nitrogen gradients, with the sum of the leading five markers (16:0, 18:1w7c, 18:1w9c, i15 :0 and i16:0, all of which are bacterial biomarkers) accounting for 36%–40% of the total. Soil

Table 1 Loadings of microbial compositions and C and N availability with PC1 in principal component analysis on soil samples with six N addition levels and three years

Factor	PC1
PLFA biomarkers	
i14:0	0.050
i15:0	–0.358
a15:0	–0.687
i16:0	–0.572
a16:0	–0.718
16:1 w7c	0.055
16:1 w5c	0.699
16:0	0.108
i17:0	–0.551
a17:0	–0.492
17:1 w8c	0.216
cy17:0	–0.474
10Me17:0	0.377
18:2 w6,9c	0.679
18:1 w9c	0.679
18:1 w7c	0.412
18:1 w5c	0.866
18:0	0.685
10Me18:0	0.701
C and N availability	
DOC	0.824
TOC	0.872
TN	0.949

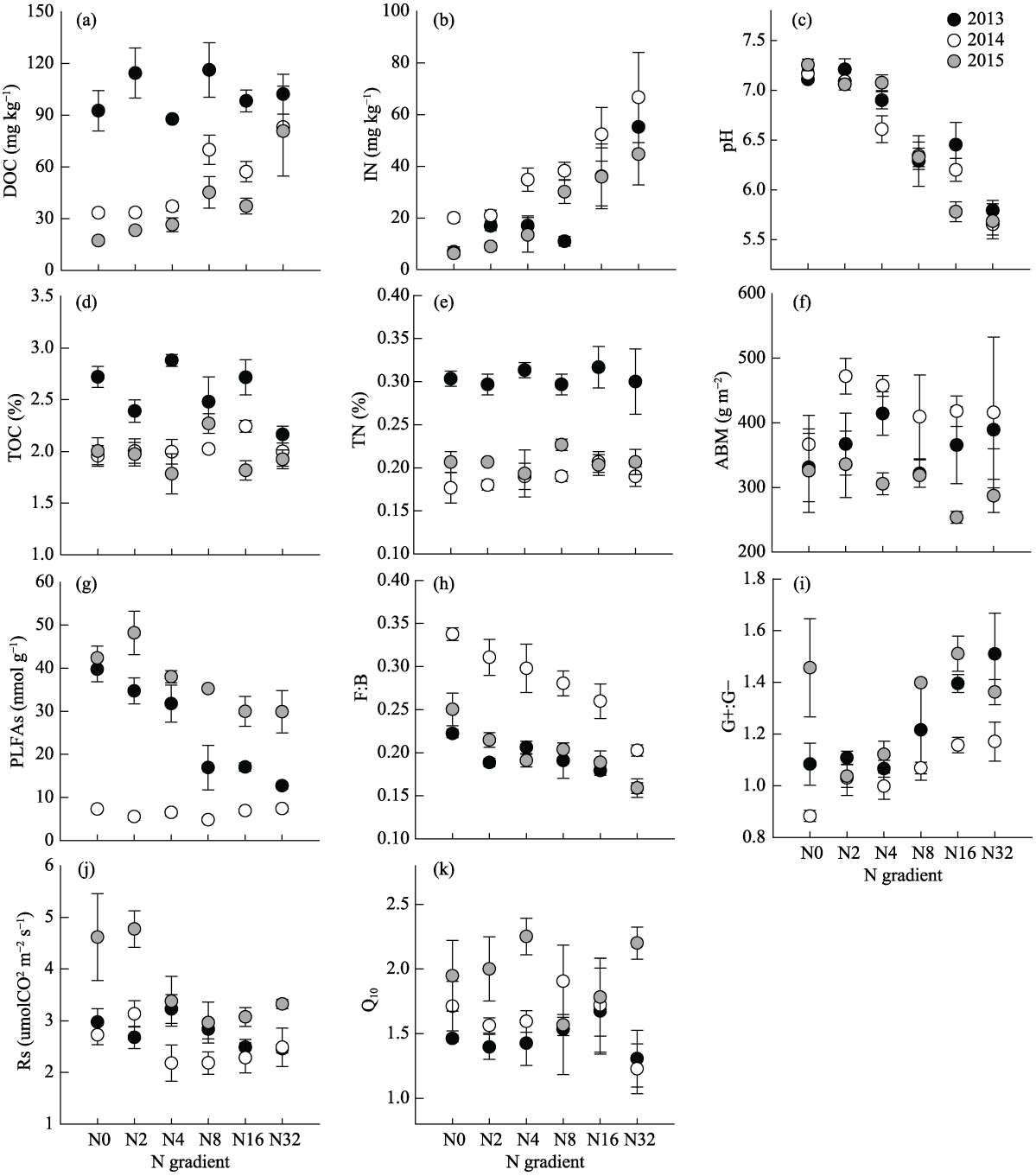

Fig. 2 Soil variables with six N gradients and three years (n=3)

Note: (a) Dissolved organic carbon; (b) Inorganic nitrogen; (c) Ph; (d) Total organic carbon; (e) Total nitrogen; (f) Aboveground biomass; (g) PLFAs; (h) Fungi to bacteria ratio; (i) Gram-positive bacteria to gram-negative bacteria ratio; (j) Soil respiration; (k) Q_{10}.

PLFAs had significant differences between N gradients (Table S1, $P < 0.001$). Soil PLFAs were inhibited by N addition in 2013 and 2015, but did not change in 2014 (Fig. 2g). F:B was significantly inhibited by N addition for the three years (Table S1, $P < 0.001$), and the highest ratio was in 2014 (Fig. 2h). In contrast, G+:G– significantly increased by N addition ($P < 0.001$), and the highest ratio was in 2015 (Fig. 2i).

Principal component analysis was used to determine the relationships between soil microbial communities. Soil samples with decreasing N gradients were generally distributed from left to right along PC1, indicating that PC1 was related to N addition rate. In addition, samples in 2015 were allocated in the ordination plot where PC2 > 0, but the opposite was found for samples in 2013 and 2014. These patterns implied that interannual variability was associated with PC2. The two axes explained 51.56% of the variation of the microbial community composition in the soil samples (Fig. 3).

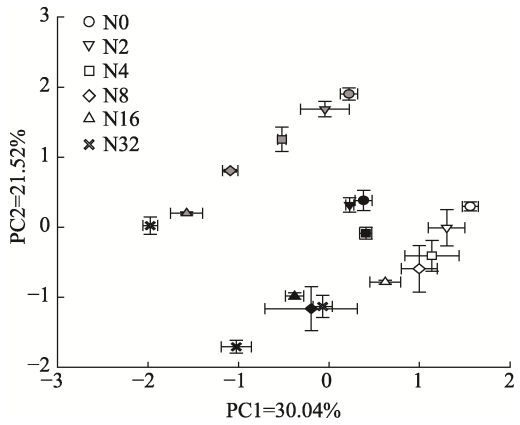

Fig. 3 Principal component analysis of microbial community composition in soil samples with six N gradients and three years (mean±SE) (*n*=3). Soil samples in the three years are presented by different colors, as black indicates 2013, white indicates 2014, and grey indicates 2015.

3.3 *R*s and Q_{10}

Rs shows significant differences between N gradients (Fig. 2j, Table S1, $P < 0.001$). Compared to the N0 treatment, Rs under the N2, N4, N8, N16 and N32 treatments decreased by –2.58%, 14.86%, 22.62%, 23.97% and 19.87% for the three growing seasons, respectively. On the other hand, Q_{10} did not significantly respond to N addition (Fig. 2k, Table S1, $P > 0.05$), with values of 1.71, 1.65, 1.76, 1.67, 1.73 and 1.58 for the six N gradients.

3.4 Relationships between soil respiration and the various impact factors

Pearson correlation analysis showed that *R*s was positively correlated with soil moisture, soil pH and PLFAs, and negatively correlated with N addition, DOC and IN. Q_{10} was significantly positively correlated with soil moisture and Rs, and negatively correlated with soil temperature, DOC and TN (Table 2).

Two SEM models were constructed using the two microbial community indicators of soil PLFAs and microbial community composition. The models explained the variation between 62% and 65% for Rs and 52% for Q_{10}, respectively. The path coefficients in the models show that N addition significantly reduces soil pH and changes soil microbial community composition. The aboveground biomass was not significantly affected by various factors of the soil, but had a significant direct effect on soil PLFAs and soil microbial community composition. Soil pH, soil moisture and C and N availability have significant positive effects on soil PLFAs, and aboveground biomass inhibits soil PLFAs. N addition, soil moisture, and C and N availability have significant direct negative effects on soil microbial community composition, and aboveground biomass has a significant positive effect on it (Fig. 4).

From the standard total effect coefficient, N addition inhibited Rs, but had less effect on Q_{10}. The main factors affecting soil PLFAs and soil microbial community composition were soil pH and N addition, respectively. Rs was more affected by soil moisture, soil pH, soil PLFAs and microbial community composition. Soil moisture was the most important factor in Q_{10} change, and other factors had less influence on Q_{10} (Table 3).

4 Discussion

4.1 The responses of soil properties and soil microbial community to N addition

After urea fertilizer is hydrolyzed to NH_4^+-N, the soil pH decreases because NH_4^+-N is transformed into NO_3^--N and H^+ is released into the soil (Kopáček et al., 2013). This

Table 2 Pearson correlation analysis of soil respiration and the impact factors

	N	ST	SM	pH	DOC	IN	TOC	TN	ABM	PLFAs	F:B	G+:G-	*Rs*	Q_{10}
N	1													
ST	0.165	1												
SM	–0.083	–0.308*	1											
pH	–0.861**	–0.038	–0.013	1										
DOC	0.352**	0.277*	–0.365**	–0.277*	1									
IN	0.729**	0.126	–0.100	–0.690**	0.209	1								
TOC	–0.137	0.309*	–0.260	0.174	0.501**	–0.143	1							
TN	0.031	0.260	–0.267	0.043	0.702**	–0.099	0.795**	1						
ABM	–0.041	0.136	–0.115	0.064	0.071	0.138	0.081	–0.101	1					
PLFAs	–0.298*	–0.091	0.395**	0.366**	–0.124	–0.493**	0.096	0.220	–0.452**	1				
F:B	–0.504**	–0.124	–0.039	0.417**	–0.469**	–0.138	–0.202	–0.500**	0.378**	–0.432**	1			
G+:G–	0.455**	–0.147	0.152	–0.472**	0.116	0.280*	–0.080	0.188	–0.259	0.153	–0.543**	1		
Rs	–0.271*	0.137	0.510**	0.323*	–0.366**	–0.334*	–0.068	–0.059	–0.179	0.608**	–0.119	0.039	1	
Q_{10}	–0.089	–0.309*	0.718**	0.058	–0.358**	–0.043	–0.243	–0.273*	–0.011	0.255	0.030	0.016	0.436**	1

Note: N—N addition; ST—soil temperature; SM—soil moisture; DOC—dissolved organic carbon; IN—inorganic nitrogen; TOC—total organic carbon; TN—total nitrogen; ABM—aboveground biomass; PLFAs—microbial phospholipid fatty acids; *Rs*—soil respiration; ** means $P<0.01$ and * means $P<0.05$. Same as below.

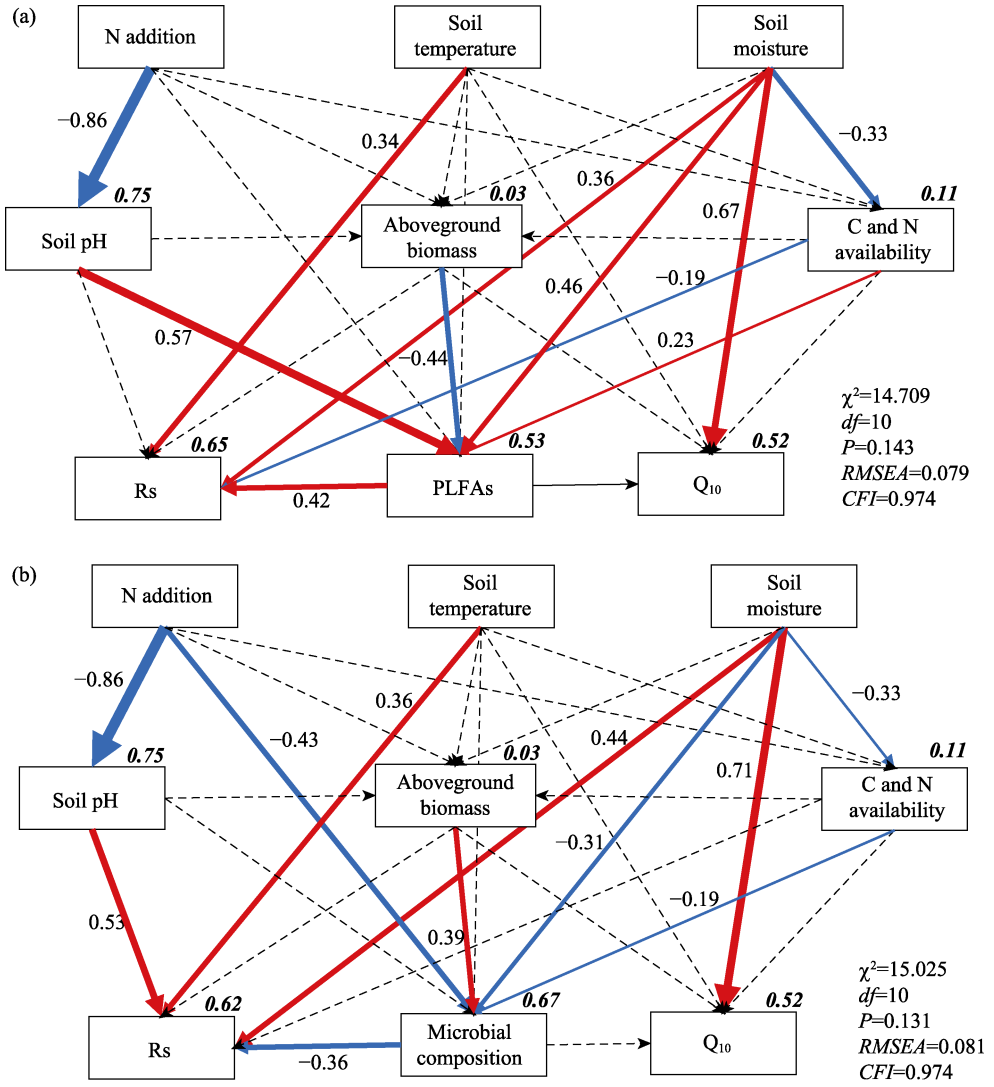

Fig. 4 Structural equation model for the effects of nitrogen addition, soil temperature and soil moisture on soil pH, C and N availability, aboveground biomass, total PLFAs, microbial composition, Rs and Q10.

Note: Dashed, red, and blue paths indicate the effect is insignificant, positive, and negative, respectively. The width of the arrow indicates the magnitude of the standardized path coefficient. Bold and italic-typed values indicate the proportions of the variance explained for each variable. χ^2—chi-square, *df*—degrees of freedom, *P*— *P* value, *RMSEA*—root mean square error of approximation, *CFI*—comparative fit index.

Table 3 Standardized total effects in structural equation models

Factor	N	ST	SM	pH	CN	ABM	PLFAs	Microbial composition
ABM	–0.07	0.13	–0.08	0.04	–0.06	N/A	N/A	N/A
PLFAs	–0.27	–0.03	0.37	0.55	0.25	–0.44	N/A	N/A
Microbial composition	–0.61	0.10	–0.30	0.19	–0.21	0.39	N/A	N/A
Rs(a)	–0.27	0.33	0.56	0.40	–0.09	–0.18	0.42	N/A
Q_{10}(a)	–0.02	–0.08	0.71	0.02	–0.08	0.08	0.04	N/A
Rs(b)	–0.25	0.33	0.56	0.47	–0.09	–0.18	N/A	–0.36
Q_{10}(b)	–0.04	–0.08	0.71	0.01	–0.08	0.08	N/A	0.05

Note: CN—C and N availability. (a) and (b) represent the a and b structural equation models of Fig. 4, respectively. N/A means that there is no such path and corresponding value in the structural equation model.

process explains the significant correlations between N addition and soil pH. In this study, soil pH had less relative impact on microbial composition than C and N availability. This finding concurs with the review of Zhou et al. (2017), in which N addition was found to regulate the F: B ratio and G^+: G^- ratio by increased N availability rather than by

decreased soil pH. However, there are studies which considered soil acidification as the primary factor shaping the microbial composition (Chen et al., 2015) and soil respiration components (Li et al., 2018). Since the fertilizer used in these two studies was NH_4NO_3, one explanation for this contradiction is the difference in the form of N fertilizer. Although both forms induced soil acidification (Tian and Niu, 2015), the addition of urea and NH_4NO_3 were found to have inconsistent effects on other key factors, including soil respiration (Zhou et al., 2014), DOC (Yue et al., 2016), and SOC (Cheng et al., 2017). Therefore, the mechanisms of altering soil C fractions, including the soil microorganisms, might differ between two forms of N addition. The significant inhibition of total PLFAs by N addition echoed other studies (Riggs and Hobbie, 2016; Yue et al., 2016), which might be caused by soil acidification in this study, the release of toxic minerals such as Al^{3+}, Fe^{3+} and Mn^{3+} (Tian et al., 2016), and depressed enzyme activities.

As commonly occurrs in N saturation conditions, the DOC is expected to decrease when C becomes the limiting factor (Kopáček et al., 2013; Tahovská et al., 2013), but the DOC increased with N addition in this study. The sophisticated in- and outflows of labile C in the soil processes can make it difficult to untangle the lines linking N fertilization and DOC, let alone considering the effects of the different forms of N added and the changes in soil acidity (Evans et al., 2008). In their meta-analysis, Yue et al. (2016) found that DOC in the O-horizon of terrestrial ecosystems was enhanced by N addition; and the decreased microbial biomass and increased litter decomposition (Liu et al., 2010) in this study site might explain such a phenomenon (Rodriguez et al., 2014).

The groupings of the samples as explained by the first ordination axis in the PCA revealed the impact of N fertilization on the soil microbial composition in this semiarid grassland soil. In agreement with Zhou et al. (2017), when N was added to the soil, bacterial communities outcompeted saprophytic fungal communities, and gram-positive bacteria outcompeted gram-negative bacteria. Consistent with Wei et al. (2013), the decrease of the F : B ratio with N addition was mainly caused by a decrease in the fungi biomarker. The soil pH preferences among microbial communities have been inconsistent among studies (Zhalnina et al., 2014; Chen et al., 2015), and soil acidity had a more negative impact on fungi than on bacteria in this study. The inhibition of expression of genes coding for lignin decomposition enzymes, the lower C investment in mycorrhizal fungi, and decomposition prevention and toxicities due to the formation of complex-structured humus with N addition were all found to severely suppress the growth of fungi (Treseder, 2008; Zak et al., 2011). For the bacterial communities, there was a trade-off between the ability to decompose recalcitrant carbon compounds and the high N demand (Treseder et al., 2011). Gram-positive bacteria relied more on nutrients for building complex cell walls and enzymes (Schimel et al., 2007; Treseder et al., 2011), thus they thrived under the conditions of high N availability.

4.2 The responses of *Rs* and Q_{10} to N addition

Rs includes the respiration of soil microorganisms, plant roots, soil animals, etc., and was affected by many factors such as soil temperature and moisture, soil physical and chemical properties, aboveground and underground biomass of vegetation, and soil microbial community. N addition had a negative effect on Rs in this grassland soil. However, this is not necessarily inconsistent with the reviews in which Rs increased by N addition (Zhang et al., 2014; Zhong et al., 2016). Studies have shown that organic matter formed by aboveground biomass through photosynthesis will increase the substrate concentrations for root respiration and microbial respiration, and significantly increase *Rs* (Wen et al., 2014). However, the aboveground biomass in this study did not change significantly under N addition. The reviews concluded that the increased Rs was closely correlated with enhanced root biomass. However, root biomass showed no significant response to N fertilization in this study site (Song et al., 2014). Moreover, decreased soil heterotrophic respiration by N addition in grasslands was caused by reduced microbial biomass (Riggs and Hobbie, 2016). Therefore, the lack of a response of aboveground biomass and root biomass compounded by the negative response of total PLFAs to N addition might explain the decrease in Rs. N addition changed the microbial community composition, and because different microorganisms have different preferences and efficiencies for C decomposition and utilization, it also had a significant impact on Rs.

Q_{10} is the proportional rate of increase in soil respiration when soil temperature increases by 10 ℃, which largely determines the feedback of the terrestrial soil carbon cycle to global climate change (Yang et al., 2011). In this study, N addition had no significant effect on Q_{10}. The three-year result of Q_{10} was consistent with what we primarily found in 2013 (Li et al., 2015), thus N addition in this grassland might not be able to regulate Rs in a warmer temperature. However, the inhibition and stimulation of Q_{10} were both found by N addition in other grasslands (Zhong et al., 2016; Fang et al., 2018). Q_{10} is affected by many factors such as soil temperature and moisture, plant community composition, the number and composition of soil microbial communities, and the quality and quantity of soil substrates (Moyano et al., 2013), which also explains the varying results in different studies. Studies have shown that N addition promotes the formation of difficult-to-decompose organic carbon in the soil, so a higher activation energy is required during the decomposition process, leading to an increase of Q_{10} (Wang et al., 2018). Studies have also shown that a decrease in soil microbial biomass will reduce Q_{10}, and at the same time, the amount of litter and root biomass will also

have a greater effect on Q_{10} (Liu et al., 2016.). Therefore, the non-response of Q_{10} to N addition in this study may be caused by a combination of interacting factors. Multi-factor experiments found soil moisture was the primary factor in the semi-arid ecosystems (Wang et al., 2017; Zhang et al. 2015). The response of Q_{10} to N addition exhibits a parabolic relationship with soil temperature and soil moisture (Zhong et al., 2016), suggesting that N addition could increase Q_{10} when soil temperature and moisture were unsuitable, such as under an extreme climate. In this study, increased soil moisture had positive effects on $Q_{10.}$ Due to the importance and complexity of Q_{10}, the mechanisms involved in its changes need to be further explored.

5 Conclusion

Soil microbial composition in the grassland ecosystem was changed by added N, as gram-positive bacteria grew to dominate the microbial communities by outcompeting gram-negative bacteria and saprophytic fungi. N addition reduced Rs by reducing soil pH, reducing soil PLFAs and changing microbial community composition. However, Q_{10} was not significantly affected by N addition, indicating that there is no contribution of N addition to further SOC loss under global warming in this grassland soil.

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氮添加降低了半干旱草原土壤呼吸但未改变其温度敏感性

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摘 要：半干旱生态系统土壤呼吸（Rs）对氮添加的响应机制仍有待探索。本研究在中国半干旱草原设置不同氮添加水平（0、2、4、8、16、32 g N m^{-2} yr^{-1}），测定土壤呼吸速率、土壤温湿度、微生物磷脂脂肪酸、土壤理化性质与地上生物量等指标，探讨氮添加对土壤呼吸及其温度敏感性（Q_{10}）的影响。结果表明：氮添加显著增加了土壤可溶性有机碳（DOC）和无机氮（IN）的含量，降低了土壤 pH 值，对地上生物量（ABM）无显著影响。氮添加降低了磷脂脂肪酸（PLFAs）的总量，降低了真菌细菌比（F：B），提高了革兰氏阳性阴性菌比（G+：G–）。氮添加显著降低了土壤呼吸，N2、N4、N8、N16 和 N32 处理下的土壤呼吸分别比对照 N0 变化了–2.58%、14.86%、22.62%、23.97%和 19.87%，结构方程模型表明，氮添加通过降低 PLFAs 总量和改变微生物组成降低土壤呼吸。氮添加对温度敏感性（Q_{10}）、土壤总有机碳（TOC）和总氮（TN）的影响均不显著，表明氮添加减轻了土壤碳的损失，且不会改变全球变暖背景下土壤有机碳矿化的潜力。

关键词：氮沉降；土壤 CO_2 通量；Q_{10}；磷脂脂肪酸；土壤性质；内蒙古草原

Table S1 Soil variables averaged for the three years (mean±SE) (*n*=9)

Variables	N0	N2	N4	N8	N16	N32
DOC	47.73±11.97d	57.06±15.03cd	50.45±9.55cd	77.14±11.91ab	64.25±9.43bc	88.69±8.92a
IN	11.07±2.36d	15.61±1.99cd	21.77±4.19cd	26.44±4.38c	41.46±6.32b	55.5±7.59a
pH	7.18±0.03a	7.12±0.05a	6.86±0.09b	6.32±0.09c	6.14±0.13c	5.71±0.07d
TOC	2.23±0.14a	2.12±0.19a	2.22±0.18a	2.26±0.10a	2.26±0.14a	2.03±0.06a
TN	0.23±0.02a	0.23±0.02a	0.23±0.02a	0.24±0.02a	0.24±0.02a	0.23±0.02a
ABM	341.17±28.09a	391.71±29.96a	392.4±25.46a	349.85±24.69a	345.79±30.57a	364.17±40.59a
PLFAs	29.8±5.75a	29.49±6.52a	25.44±4.99a	18.99±4.67b	17.98±3.49b	16.67±3.69b
F:B	0.27±0.02a	0.24±0.02b	0.23±0.02bc	0.23±0.02bc	0.21±0.01c	0.17±0.01d
G+:G–	1.14±0.1bc	1.06±0.03c	1.06±0.03c	1.23±0.07ab	1.35±0.06a	1.35±0.07a
Rs	3.44±0.39ab	3.53±0.35a	2.93±0.27bc	2.66±0.19c	2.61±0.16c	2.76±0.18c
Q_{10}	1.71±0.12a	1.65±0.12a	1.76±0.14a	1.67±0.14a	1.73±0.17a	1.58±0.18a

Note: Different lowercase letters in the same row mean significant differences ($P < 0.05$ by LSD test).

Table S2 Soil variables averaged for the six N gradients (mean±SE) (*n*=18)

Year	2013	2014	2015
DOC	101.92±4.64a	52.39±4.93b	38.36±6.47c
IN	23.86±4.74b	38.84±5.00a	23.23±4.34b
pH	6.63±0.13a	6.51±0.13a	6.53±0.16a
TOC	2.56±0.08a	2.04±0.04b	1.96±0.06b
TN	0.30±0.01a	0.19±0.01b	0.21±0.01b
ABM	364.79±16.61b	423.24±22.12a	304.52±14.06c
PLFAs	25.49±2.74a	6.42±0.31b	37.28±1.98a
F : B	0.19±0.01b	0.28±0.01a	0.20±0.01b
G+ : G–	1.23±0.06a	1.05±0.03b	1.31±0.05a
Rs	2.78±0.1b	2.50±0.13b	3.69±0.24a
Q_{10}	1.47±0.08b	1.62±0.09b	1.96±0.09a

Note: Different lowercase letters in the same row mean significant differences ($P < 0.05$ by LSD test).