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Differential response of microbial respiration to supplied nitrogen forms in 3 contrasting alpine meadow soils on the Tibetan Plateau

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ABSTRACT: An incubation experiment was conducted to examine the effects of nitrogen (N) applications in different forms (NH_4NO_3, NH_4Cl, and KNO_3) on microbial respiration considering 3 different alpine meadow soils (C poor soil, pH = 8.1, 1.6% C; C moderate soil, pH = 6.0, 5.0% C; C rich soil, pH = 7.1, 7.4% C) in the Tibetan Plateau. The addition of NH_4NO_3 and NH_4Cl increased the microbial respiration in C poor soil, but KNO_3 had no effect. The inorganic N forms had no effects on C rich soil, but decreased microbial respiration in C moderate soil. Soil microbial respiration levels across the different types were ordered as follows: C poor soil < C rich soil < C moderate soil, regardless of N addition. These results suggest that the effect of N on microbial respiration in alpine meadow soils is more dependent on the initial soil pH than on soil C availability.

Key words: C cycling, microbial activity, N deposition, soil properties, high-altitude region.

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INTRODUCTION

Soil microbial respiration, i.e. the production of carbon dioxide (CO_2) through soil organic carbon (C) decomposition by microbes, is an important component of the global C cycle (Schlesinger and Andrews 2000; Kaštovská et al. 2010). To reduce atmospheric CO_2 and enhance soil C sequestration, it is critical to understand the factors that drive soil microbial CO_2 dynamics (Schlesinger and Andrews 2000). The effects of nitrogen (N) on soil microbial respiration have received extensive attention as global N deposition continues to increase (Nadelhoffer et al. 1999; Bowden et al. 2004). The studies have shown disparate results, but most suggest that N application inhibits the biological activity of soil microbes and reduces soil microbial respiration rates (Bowden et al. 2004; Craine et al. 2007; Ouyang et al. 2008, Kaštovská et al. 2010; Ramirez et al. 2012). However, it is still unclear whether the response of soil microbial respiration is directly due to the increased C availability following N application, or indirectly caused by changes in chemical properties (such as pH) of soils resulting from N application (Khalil et al. 2007; Ramirez et al. 2012). Further, it is not clear if different forms of N influence soil microbial activity in the same ways (Ramirez et al. 2012). Moreover, most studies were conducted on soils in the temperate or northern climatic zones (Craine et al. 2007; Kaštovská et al. 2010; Ramirez et al. 2012), and little information is available for soils in alpine areas.

The alpine meadows in the Tibetan Plateau, the world's largest high-altitude region, store large amounts of C in soils (18.2 $kg \cdot m^{-2}$; Ni 2002) which may play an important role in regional, or even global, C cycling (Wang et al. 2002). These alpine meadows are currently experiencing increased N loads due to atmospheric deposition and other anthropogenic activities; this trend is expected to increase over the next few decades (Lü and Tian 2007). Understanding the response of microbial respiration to N addition in these soils is necessary to better predict changes in alpine soil C flux and storage during global climate changes. Therefore, the objective of this research was to experimentally examine the effects of different forms of N inputs on soil microbial respiration in 3 contrasting alpine meadow soils that vary in organic C content and pH in the Tibetan Plateau, China.

MATERIALS AND METHODS

The soil for this experiment was obtained from 3 different locations in the Tibetan Plateau, China (Table 1). The sampling locations were chosen because they provided 3 typical representations of alpine meadow vegetation, with a wide range of organic C, from 16 to 74 $g \cdot kg^{-1}$, and pH values from 6.0 to 8.0. Soil samples were collected from 4 random sampling plots at each stand in early August →

Table 1. Properties of the 3 alpine meadow sites in the Tibetan Plateau.

	C poor soil	C moderate soil	C rich soil
Location	lat 34°43′N, long 92°53′E	lat 32°50′E, long 102°36′E	lat 37°37′N, long 101°19′E
Altitude (m)	4,754	3,462	3,240
Mean annual air temperature (°C)	–5.3	1.1	–1.7
Mean annual precipitation (mm)	269.7	752.4	560.5
Dominant species	*Kobresia pygmaea*	*Kobresia setchwanensis*	*Kobresia humilis*
Organic carbon ($g \cdot kg^{-1}$)	15.71 ± 0.74	50.24 ± 2.63	74.33 ± 3.71
Total N ($g \cdot kg^{-1}$)	1.53 ± 0.05	4.28 ± 0.18	6.89 ± 0.40
C/N	10.27 ± 0.72	11.75 ± 0.17	10.82 ± 0.89
Dissolved organic carbon ($mg \cdot kg^{-1}$)	16.46 ± 1.21	33.28 ± 2.07	56.29 ± 3.69
Available N ($mg \cdot kg^{-1}$)	141.28 ± 3.36	378.40 ± 26.40	508.14 ± 30.55
Total P ($g \cdot kg^{-1}$)	0.65 ± 0.03	1.31 ± 0.03	1.15 ± 0.11
Available P ($mg \cdot kg^{-1}$)	5.48 ± 0.69	15.87 ± 0.71	14.75 ± 0.98
pH (H_2O)	8.06 ± 0.06	6.02 ± 0.05	7.14 ± 0.08

Values are means (± SD) for 4 replications.

2013. Within each sampling plot, the parts of vascular plants were cut and removed using scissors, and then 1 composite sample of the top 10 cm of soil was taken using a 7 cm diameter auger. Debris was removed from the soil surface prior to sample collection. Roots and other impurities were removed from the soil samples, which were left to air dry at room temperature. The sampling process was conducted according to standard methods for observation and analysis in the Chinese ecosystem research network (Liu 1996). After being air-dried, the soil samples were sieved using a 2-mm sieve and stored at 4 °C before use. Some soil properties are summarized in Table 1. The dissolved organic carbon was determined using a fresh sample, and other soil properties were measured using air-dried samples. The soil analysis method was described by Lu (2000).

Incubation experiments were conducted to determine how the supplied N forms influenced soil microbial respiration. Three different N forms, NH_4NO_3, NH_4Cl, and KNO_3, were evaluated in this study. The N application rate for each treatment was 0.04 g $N \cdot kg^{-1}$ soil. Each treatment was replicated 4 times, and 30 g of air-dried soil was weighed and placed in a 275-mL glass flask. Distilled water was added evenly over the soil surface with a mini-pipette to bring the moisture content to 40% of water-holding capacity (WHC). The flasks were pre-incubated for 7 days at 15 °C in the dark. Then, the N solution of a specific form was applied to the pre-incubated soils, and the final soil moisture content was adjusted to 60% of WHC. Control soils received only water. All the flasks were incubated at 15 °C for 28 days in the dark. The water content was maintained as constant during the incubation by adding distilled water occasionally.

Soil microbial respiration rates were taken as CO_2 emission rates and measured 14 times throughout a 28-day incubation period. At each sampling date, a 10-mL gas sample was collected from each flask using a gas-tight syringe and transferred to an evacuated gas-tight vial (12.5 mL). These flasks were closed with a butyl rubber stopper for 2 h and then underwent CO_2 analysis by gas chromatography. Soil microbial respirations were calculated from the linear changes in CO_2 concentration in the headspace volume for each soil flask. The cumulative microbial CO_2 production during the investigation period from each flask was calculated using linear interpolation between the sampling dates (Ni et al. 2012). At the end of incubation, soil pH was measured using glass electrodes in a 1:2.5 soil-to-water suspension.

The data were analyzed by ANOVA using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The effects of soil type and N forms on microbial respiration, as well as their interactions, were tested using a 2-way ANOVA. The least significant differences (LSD) were calculated from the means between the treatments.

RESULTS

For the duration of the incubation period, there were 2 peaks in microbial CO_2 emissions that occurred in C poor and rich soils on day 1 and days 14 – 15, respectively (Figure 1). In C moderate soil, microbial respiration was →

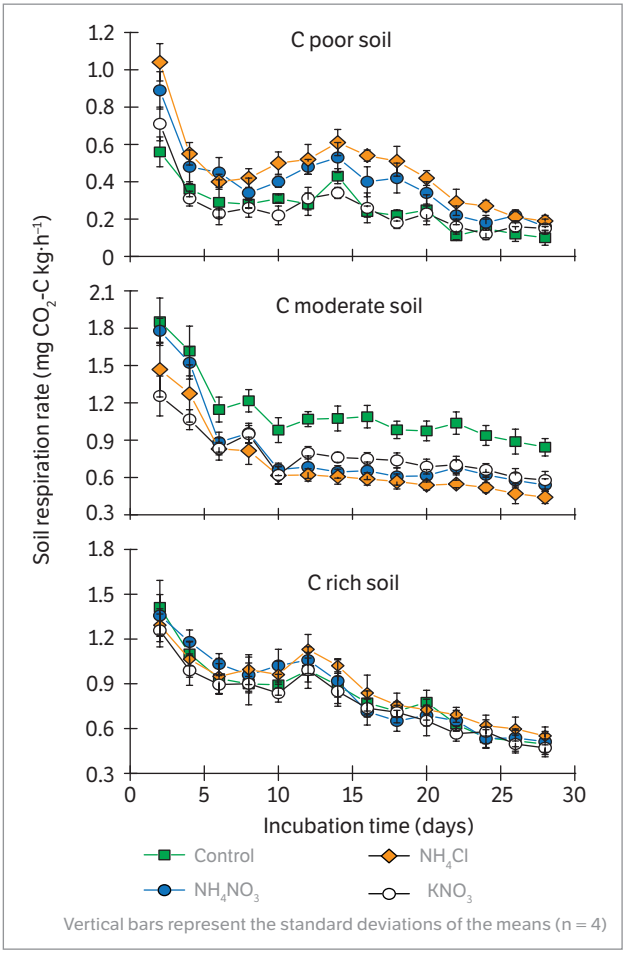

Figure 1. Changes of microbial respiration in 3 alpine meadow soils with different N treatments.

the highest on day 2 and then gradually decreased until the end of the 28-day incubation period. Daily microbial CO_2 emissions from C poor soil with NH_4Cl addition were significantly higher than those of the control. However, N addition had no significant effect on daily microbial CO_2 flux in the C rich soil. In contrast, the addition of NH_4^+-N or NO_3^--N reduced daily microbial CO_2 emissions in the C moderate soil.

The cumulative microbial CO_2 emissions during the investigation period from the C rich soil with the NH_4Cl and NH_4NO_3 additions increased 75 and 50%, respectively, when compared with the control soil (Figure 2). However, no significant difference was found between the treatments with added KNO_3 and the controls. The addition of N with different forms had no impact on cumulative microbial CO_2 in the C rich soil. Compared to the control, the addition of NH_4NO_3, NH_4Cl, and KNO_3 caused a 28, 30, and 38% decrease in the cumulative microbial CO_2 emissions in the C moderate soil, respectively. The microbial CO_2 emissions from the C moderate soil were significantly higher than those from the 2 other soils, regardless of N addition.

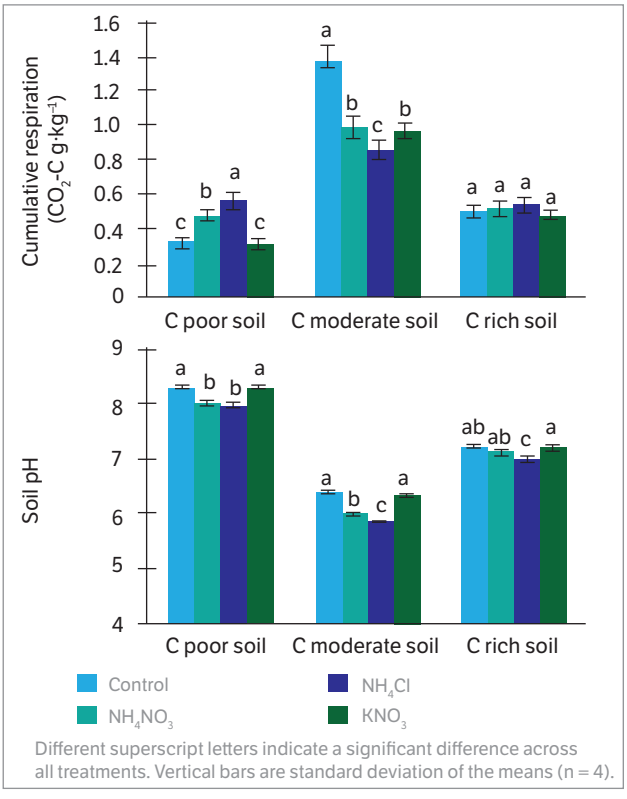

Figure 2. Cumulative microbial respiration during 28 days of incubation and soil pH in 3 alpine meadow soils with different N treatments.

All soils with NH_4NO_3 and NH_4Cl additions showed a trend towards lower pH, but it was not significant.

DISCUSSION

Most studies examining the effect of N fertilizer on soil microbial respiration have found that N applications can reduce respiration rates (Bowden et al. 2004; Craine et al. 2007; Ouyang et al. 2008; Kaštovská et al. 2010; Ramirez et al. 2012). In this study, the application of 3 different N forms reduced the respiration rate of microbes in C moderate soil, which is consistent with the results of previous studies. The negative effects of N on soil microbial respiration are generally believed to be due to the reduction of soil pH following N addition, thus inhibiting soil microbial activity (Ouyang et al. 2008; Ramirez et al. 2012). This study showed that the application of NH_4NO_3 and NH_4Cl significantly reduced soil pH, but KNO_3 had no significant effects on it, suggesting that pH cannot fully account for the negative effects of N on soil microbe respiration.

Contrary to what was found in the C moderate soil, N application had no significant effect on the respiration rate of microbes in C- ich soil. Similar results have been reported on soils of temperate forests (Flanagan and van Cleve 1983). The result of this study indicated that the respiration rate of microbes in C rich soil may not have been limited by exogenous soil N. For C poor soil, the application of NH_4NO_3 and NH_4Cl increased the microbial respiration rate, which is consistent with other studies (Yoshitake et al. 2007; Aspray et al. 2008). It is possible that the input of exogenous N enhanced the activity of soil microbes limited by N nutrition, accelerating the decomposition of soil organic matter (Neff et al. 2002). However, the addition of KNO_3 produced no effects on the respiration rate of microbes, indicating that NH_4^+-N is the restrictive factor of microbes in C poor soil and that NO_3^--N is not a limiting factor. It is important to point out that these 2 soils have similar C/N ratios, but their responses to N input were different. This might be due to different labile C/N ratios (Alburquerque et al. 2012), i.e. DOC/TN (where: DOC means dissolved organic carbon and TN represents total N) was 10.8 for C poor soil and 8.2 for C rich soil. In addition, the respiration response pattern of the soil microbes to N application was →

consistent with the pH response pattern to the N forms, indicating that the pH decreases in C poor soil facilitate the growth activity enhancement of soil microbes. A decreased pH increased the soil microbial activity because the initial pH of C poor soil was high (at 8.0) compared to the 2 other soils.

The differential respiration response patterns of soil microbes to N input are thought to be caused by the differences in the initial pH, as well as the C and N availability of soils (Lee and Jose 2003; Khalil et al. 2007). In this study, the 3 types of alpine meadow soils produced completely different responses to N input. These types differed significantly in pH and available C and N content. Although the available C and N contents of C rich soil were higher than those of the C moderate soil, the respiration rate of microbes in C moderate soil was significantly higher than that of microbes in C rich soil. N application inhibited microbial respiration in C moderate soil, but produced no effects on C rich soil, which had high available C and N content levels. Therefore, the effects of N input on the alpine meadow soils in the Tibetan Plateau do not depend alone on the availability of soil C and N, but the initial pH of the soils may also be a major factor that influences the effects of N. In Swedish forest soils, the effects of N inputs were found to be dependent upon the initial pH soil values (Binkley and Högberg 1997).

Alpine meadows are the primary vegetation type of the Tibetan Plateau, which account for 35% of the Tibetan Plateau in the area and are rich in organic C (Wang et al. 2002). In future climate scenarios, these soils will continue to experience heightened levels of N deposition (Lü and Tian 2007). However, alpine meadows with different soil properties may have differential response patterns to N deposition, suggesting that more consideration should be given to soil properties, especially pH, in evaluating the response of C outputs (such as soil respiration) from alpine meadow soils to N input.

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