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Responses of microbial respiration to nitrogen addition in two alpine soils in the Qinghai-Tibetan Plateau

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Abstract

An incubation experiment was conducted to examine the effects of nitrogen (N) application on microbial respiration in alpine meadow and alpine shrub soils from eastern of Qinghai-Tibetan Plateau. Four different levels of nitrogen fertilization were selected in this study: control (CK, 0 mg N g^{-1}), low (LN, 0.04 mg N g^{-1}), medium (MN, 0.16 mg N g^{-1}), high (HN, 0.4 mg N g^{-1}). The results showed that microbial respiration was higher in alpine shrub than in alpine meadow soil, regardless of the rate of N application. Total microbial respiration over the course of incubation period decreased in both soils with HN and MN treatments relative to control, but no significant differences were observed in soils with LN treatments. There was significantly positive correlation between total microbial respiration and dissolved organic carbon concentration in both soils. The results indicated that DOC may be a useful indicator of microbial respiration rate in alpine soils and the increasing N inputs could drive a negative feedback to global warming effects of carbon dioxide emitted to the atmosphere in alpine soils.

Key words

Alpine shrub soil, Carbon cycle, Fertilization, Microbial CO_2, Nitrogen deposition

Introduction

Soil microbial respiration, microbial CO_2 production from soil organic carbon by microorganisms, is an important component of global carbon cycle (Schlesinger and Andrews 2000; Yang *et al*., 2013). Understanding control on soil microbial respiration is of critical importance since certain measures can be taken to decrease soil CO_2 emission and improve carbon sequestration. Nitrogen has been regarded as an important factor controlling microbial respiration in soils (Peng *et al*., 2011). Many measurements examining the effects of N addition on microbial respiration have dealt with temperate and boreal soils (Bowden *et al*., 2004; Cleveland *et al*., 2006; Janssens *et al*., 2010), but little information is available for the soils distributed in alpine regions. With increasing rates of anthropogenic N loading (Galloway *et al*., 2004), there is a strong need to understand links between N inputs and soil microbial respiration at high-altitude ecosystems.

The alpine meadows in Qinghai-Tibetan Plateau, the world's largest high-altitude region, store large amount of C in soils (18.2 kg m^{-2}; Ni, 2002), which play an important role in regional, even global, C cycling (Wang *et al*., 2002). These alpine meadows are experiencing increased N loading due to atmospheric deposition and other anthropogenic activities and the increasing trend is considered to be enhanced in the next few decades (Lü and Tian, 2007). Understanding the response of microbial respiration to N addition in these soils is better to predict the changes in alpine soil C flux and storage under global change. However, few details about the effect of N inputs on microbial respiration were measured in these soils. The objective of this paper was to experimentally examine the effect of N inputs with different levels on soil microbial respiration in two representative alpine soils: alpine meadow and alpine shrub soils, in Qinghai-Tibetan Plateau, China.

Materials and Methods

Study site and soil sampling : The study area was located at Hongyuan County of the eastern Qinghai-Tibetan Plateau, approximately 400 km from Chengdu, China. It is 3500 m above sea level, with a continental harsh climate. The study area appeared as a fairly flat glacial landscape with wide valleys and small hill range of 50-150 m height. Annual precipitation averaged 752 mm, with about 86% received from May to September. Mean annual temperature was 1.1ºC and there was not an absolute frost-free period. The highest monthly mean temperature was 10.9 °C in July and the lowest was -10.3ºC in January. Alpine meadow, dominated by *Kobresia setchwanensis* and *Elymus nutans*, and alpine shrub, dominated by *Potentilla fruticosa*, were the main type of vegetables distributed in the study area. Alpine meadow and alpine shrub soils were classified as Mat Cryic Cambisols and Mollic Cryic Cambisols, respectively.

Two stand, alpine meadow and alpine shrub, located within 10 km of each other, were selected in the present study. Soil samples were collected from four random sampling plots at each stand in early August 2012. Within each sampling plot, part of vascular plants were cut and removed using scissors, and then one composite sample of the top 15 cm of soil was taken by an auger (7 cm diameter). Roots and other impurities were removed from soil samples by hand and soils were left to air dry at room temperature. After being air-dried, soil samples were ground to pass through 2-mm sieve and were stored at 4ºC, before use. Soil properties were measured by the method described by Lu (2000).

Treatment and incubation : Incubation experiment were carried out to determine the rate of N application influences soil microbial respiration. Four level of N fertilizer, 0 (CK), 0.04 (LN), 0.16 (MN) and 0.4 (HN) mg N g^{-1}, were evaluated in this study. N fertilizer was added as ammonium nitrate (NH_4NO_3). Each treatment was replicated four times. 30 g of air-dried soil was weighed and placed in each 275 ml glass flask. Distilled water was added evenly over the soil surface with a mini-pipette to maintain moisture content to 40% water-holding capacity (WHC). The flasks were pre-incubated for 7 days at 15ºC in dark. Then ammonium nitrate solution with different N concentration was applied to pre-incubated soil and the final soil moisture content was adjusted to 60% WHC. All the flasks were incubated at 15 °C for 28 days in dark. Water content was maintained constant during incubation by adding distilled water occasionally.

Soil microbial respiration rates (taken as CO_2 emission rates) were measured 10 times throughout the incubation period of 28 days. 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) for CO_2 analysis by gas chromatography after the flasks had been closed with a butyl rubber stopper for 0 and 2 hr. Soil microbial respiration was calculated from the linear changes in CO_2 concentration with the headspace volume for each soil flasks. Total microbial CO_2 production from each flask was calculated by linear interpolation between sampling dates (Ni *et al.*, 2012). At the end of incubation, dissolved organic carbon (DOC) was extracted with 0.5 M K_2SO_4 from the incubation soil and was measured using a TOC/TN analyzer (multi N/C 3000, Analytik, Jena, Germany).

Statistical analysis : Statistical analyses were done using SPSS 16.0 (SPSS Inc., Chicago). Data were tested for normality and log-transformed before analysis of variance where appropriate. A repeated measures two-way ANOVA with nitrogen levels as factors, and with time as the repeated factor were performed to test the effects of the addition of nitrogen on soil microbial respiration rates. Least significant difference tests (LSD) were used for comparison of means between treatments. A linear regression was used to examine the relationship between total microbial respiration and soil DOC concentration. Significance was tested at the $P < 0.05$ level of probability.

Results and Discussion

Soil nutrient properties at 0-15 cm depth were significantly different between alpine meadow and alpine shrub (Table 1). Soil organic carbon and total nitrogen contents in alpine shrub were 1.55 and 1.46 times higher than alpine meadow, but there were no significant differences observed in C/N ratio between these two alpine soils. The clay and silt content of top 15 cm soil layer in alpine meadow was lower than that of alpine shrub. In contrast, soil sand content in alpine meadow was 20.5 % higher than in alpine shrub (Table 1).

Soil microbial respiration in both alpine meadow and alpine shrub was highest on first day and then generally decreased toward the end of incubation period, regardless of the rate of N application (Fig. 1). In alpine meadow, soil microbial respiration ranged from 0.79 mg CO_2-C kg^{-1} h^{-1} to 2.69 mg CO_2-C kg^{-1} h^{-1}, whereas in alpine shrub, it ranged from 1.35 mg CO_2-C kg^{-1} h^{-1} to 4.75 mg CO_2-C kg^{-1} h^{-1} (Fig. 1). Treatment with N, except LN

Table 1: Chemical and physical properties of soil studied

	SOC (g kg^{-1})	TN (g kg^{-1})	C/N	pH (2.5:1)	Clay (%)	Silt (%)	Sand (%)
Alpine meadow	46.42±3.60	4.22±0.50	11.07±0.83	6.12±0.13	17.16±0.75	28.74±1.88	54.09±1.68
Alpine shrub	72.06±6.83	6.17±0.22	11.68±1.05	5.73±0.17	23.32±1.76	33.67±1.61	43.00±1.95

SOC is soil organic carbon; TN is total N; Values are mean of four replicate ± SD

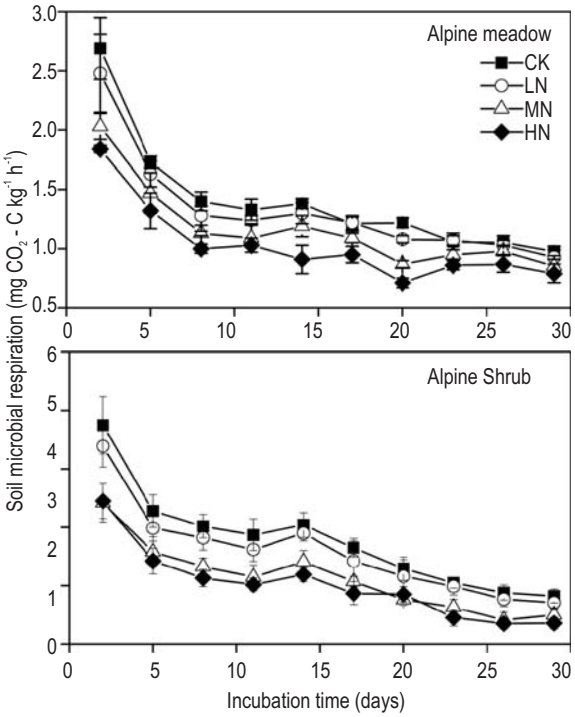

Fig. 1 : Changes of microbial respiration in alpine meadow and alpine shrub soils with different N treatments. Vertical bars represent the standard deviations of the means (n=4)

treatment, significantly decreased microbial CO_2 production over the course of incubation period in both the two soil. Total microbial CO_2 production during the investigation period in alpine meadow soil for CK, LN, MN and HN treatments were 0.65, 0.74, 0.83 and 0.88 g CO_2-C kg^{-1} and correspondingly in alpine shrub soil were 1.27, 1.35, 1.64 and 1.75 g CO_2-C kg^{-1}, respectively (Fig. 2). Total microbial CO_2 production decreased approximately by 26% in HN and 17% in MN in alpine meadow soil, and 27% in HN and 23% in MN in alpine shrub soil relative to control (CK) (Fig. 2).

Some previous studies have shown that addition of N to temperate and boreal soils have variable effects on soil microbial respiration, including increase, decrease, or unchanged rates after additional N inputs (Bowden *et al*., 2004; Traoré *et al*., 2007; Janssens *et al*., 2010). In the present study, application of N decreased soil microbial respiration in both alpine meadow and shrub soils. The negative effect of N on microbial respiration in these two soils could be explained by the following presence of additional N may inhibit production of specific enzymes due to decrease in the pH of soil after addition of NH_4NO_3, and thus decrease the rate at which soil microbes can decompose organic matter and produce CO_2 (Fog, 1988; Lee and Jose, 2003); soil microbes may react to increase in available N by switching their focus from respiration to reproduction which would decrease measured respiration (Moscatelli *et al*., 2005). Our results also showed that microbial respiration in alpine shrub soil was higher than in alpine meadow soil. This result may be due to difference in the initial status of organic matter in soil (Lee and Jose, 2003).

The extractable DOC of alpine meadow soil ranged from 58.0 mg kg^{-1} under HN treatment to 82.3 mg kg^{-1} under control, while it ranged between 79.6 mg kg^{-1} (for HN) and 107.7 mg kg^{-1} (for the control) in alpine shrub soils (Fig. 3). As compared to control, HN and MH treatments decreased DOC by 29.5 % and 16.4 % in the alpine meadow soil and by 26.1 % and 16.2 % in alpine shrub soil, respectively (Fig. 3). However, there were no significant difference in DOC between LN treatment and control in both alpine soils. The amount of DOC under same treatment was higher in alpine shrub than in alpine meadow soil, for example, it was 1.3 times higher in alpine shrub soil than in alpine meadow soil without N addition (Fig. 3). Significant positive correlation was observed between extractable DOC and total microbial respirations in both alpine soils (alpine meadow soil: R^2=0.648,

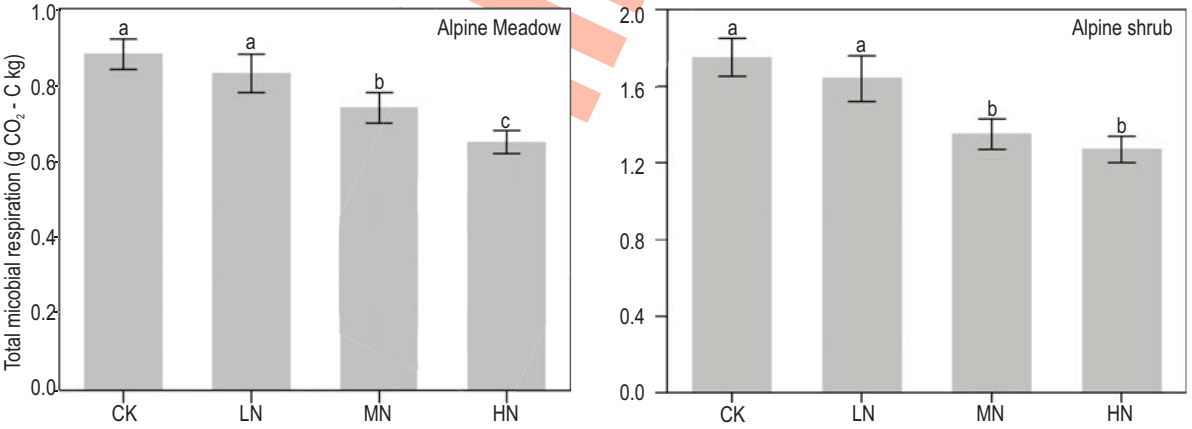

Fig. 2 : Total microbial respiration during 28 days of incubation in alpine meadow and shrub soils with different N treatments. Different superscript letters indicate a significant difference across all treatments. Vertical bars are standard deviation of the means (n=4)

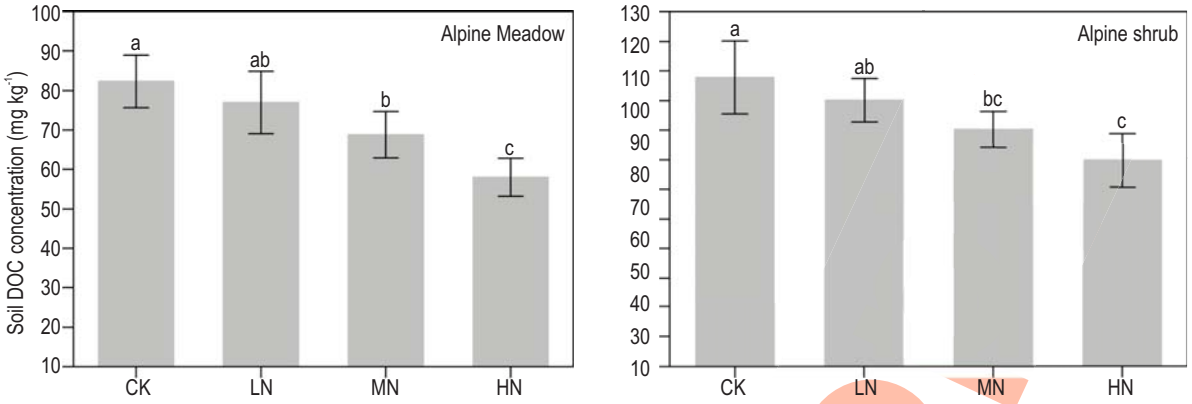

Fig. 3 : DOC concentration in alpine meadow and shrub soils with different N treatments after 28 days of incubation. Different superscript letters indicate a significant difference across all treatments. Vertical bars are standard deviation of the means (n=4)

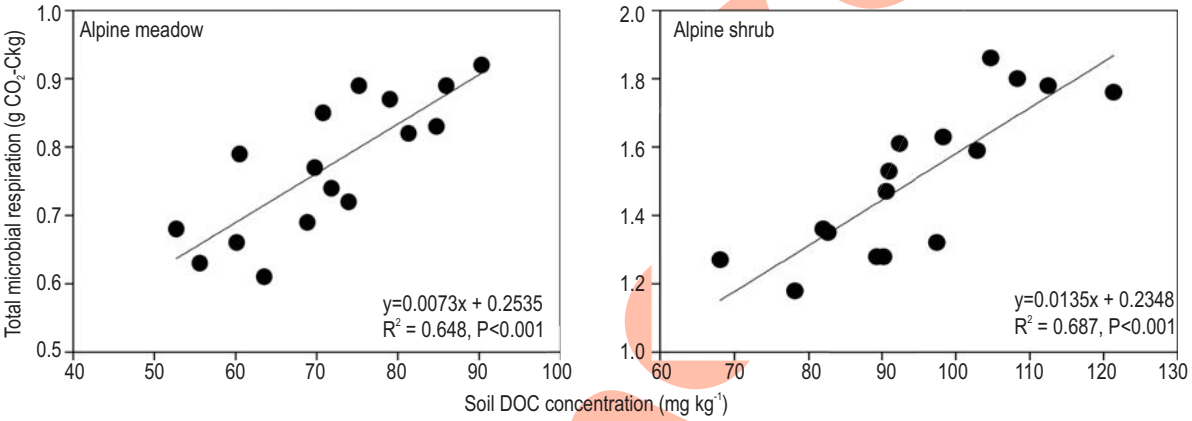

Fig. 4 : Relationship between total microbial respiration and DOC concentration in alpine meadow and alpine shrub soils

P<0.001; alpine shrub soil: R^2=0.687, P<0.001) (Fig. 4).

DOC was hypothesized to be major source of respired CO_2 in soil (Bengtson and Bengtsson, 2007), which is a product of physical, chemical and biological breakdown of soil organic matter (Fang and Moncrieff, 2005). The present study showed that there was significant relationship between microbial respiration and DOC in both alpine soils. Positive correlation between microbial respiration and DOC concentrations in soils have previously been reported (Fang and Moncrieff, 2005; Neff and Hooper, 2002). The results suggested that DOC might be a useful indicator of soil microbial respiration rate in alpine soils. Similar to the response of microbial respiration to N addition, N addition significant by reduced DOC concentration in both the two soils. Addition of N affects DOC through suppressing microbial activity and decrease organic matter mineralization, which in turn can decrease DOC production (Zech *et al.*, 1994). Changes in soil DOC after N addition also can explain, to some extent, the response pattern of microbial respiration to N addition in this study.

In conclusion, the results showed that addition of N to alpine soils had a negative effect on soil microbial respiration, at least in short term. Such a suppression of microbial CO_2 production with increasing N inputs could drive a negative feedback to global warming effects of CO_2, emitted the atmosphere in alpine soils in the Qinghai-Tibetan Plateau.

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