SOIL RESPIRATION IN RESPONSE TO SHORT-TERM NITROGEN ADDITION IN AN ALPINE STEPPE IN NORTHERN TIBET

ABSTRACT: Northern Tibet has a vital role in global ecological security. This study determined the effect of environmental factors on the soil respiration of an alpine steppe. Short-term nitrogen addition (2 g N m\(^{-2}\) yr\(^{-1}\)) was performed in an alpine steppe in Northern Tibet in June, 2011. Soil respiration was observed during the growing season of 2011 using LI-8100. The results were as follows. First, soil respiration had clear seasonal patterns, and significant differences existed between the control (CK) and nitrogen addition (ND) treatments \((P = 0.004)\). Second, soil respiration was more sensitive to soil temperature \((R^2 = 0.988, ND; R^2 = 0.05, CK)\) than soil moisture \((R^2 = 0.0003, ND; R^2 = 0.038, CK)\), and the relationship between soil temperature and soil respiration in ND treatment was more significant than that in CK. Third, the relationships between soil chemical properties and root biomass in CK were greater than that in ND plots, especially the relationship of root biomass with the available nitrogen and nitrate nitrogen. These results indicated that differences among geographical conditions resulted in different phenomena of gas emissions, immature soil, and extremely short plant growing time, which may all be remarkably influenced in an alpine steppe.

KEY WORDS: nitrogen addition, soil respiration, Alpine steppe, Northern Tibet

1. INTRODUCTION

Northern Tibet does not only have a vital role in improving the ecosystem and achieving sustainable development in China, but also contributes to safeguarding Asian, and even global ecological security (Lu et al. 2011). Alpine grasslands are the dominant ecosystem occupying about 94% of Northern Tibet. The natural environment of the region is extremely harsh, and the soil is generally quite thin. Once the vegetation is disturbed or degraded, recovery is difficult (Zhang et al. 1998), especially since the climate of this region is characterized by a long period of frost and a relatively short growing season (Yu et al. 2010). Furthermore, the serious situation has detrimentally affected the environment and ecology of the Tibetan Plateau. This area suffers from excessive utilization, overgrazing, deforestation, and the harvesting of many herbs commonly used in traditional medicines (Wang and Cheng 2000). A fragile ecosystem, the alpine grassland ecosystem in Northern Tibet is extremely sensitive to climate change and human activities (Gao et al. 2009).

Presently, carbon dioxide from human activities appeared to be the main cause of
global warming. The surface temperature of the Earth has increased by 0.6°C in the past century due to elevated atmospheric greenhouse gases, and the temperature will continue to increase by 1.4°C to 5.8°C over the 21st century (Intergovernmental Panel on Climate Change – IPCC – 2001). Carbon dioxide from soils is an important component of the global carbon (C) cycle because the C storage in the soil is fairly greater than the C stored in the atmosphere. The gentle variation of C in the soil could result in an intensive change of carbon dioxide concentration in the atmosphere. Carbon dioxide is emitted from soil through root respiration, rhizomicrobial respiration, and soil organic matter decomposition. Meanwhile, in the increasing experiments on nitrogen (N) addition in different ecosystems, some studies demonstrated that soil C emission can be increased (Tranvik et al. 2009, Ullah and Zinati 2006). In contrast, N fertilization has also been observed to suppress soil respiration, resulting in an increase in C contained in organic soil (Burton et al. 2004). Therefore, the effects of N deposition on soil C emission in the grassland ecosystem are still controversial.

In alpine grasslands, N is a limited element (Zhang et al. 2011), thus, increasing rates of fertilizer application are performed to explore the biochemical process of soil mass cycling. However, a thorough documentation of the links between N inputs and soil respiration is strongly needed. Despite the increasing number of studies on the relationships, several issues on the alpine steppe in Northern Tibet still remain unaddressed. More valuably, the alpine steppe of the region, which is very far from the industrial area and remains relatively undisturbed by humans (Jia et al. 2009), receives a much smaller amount of atmospheric N deposition (2.36 kg ha\(^{-1}\) a\(^{-1}\)) and are generally considered an ideal region to verify the actual consequences of N addition for soil respiration (Jiang et al. 2010).

Therefore, the effect of environmental factors (soil temperature, soil moisture, soil nutrients, and soil microbial biomass) on the soil respiration of an alpine steppe in Northern Tibet was examined. Specifically, the study aims (1) to identify and estimate the spatial (soil profile) and temporal (key growth period) patterns of soil respiration on an annual scale; (2) to analyze the difference of soil respiration between the control (CK) and nitrogen addition (ND) treatments; and (3) to diagnose the main environmental factors associated with soil respiration.

Fig. 1. Experimental site.
2. MATERIALS AND METHODS

2.1. Site Description

The study was performed in the permanent plots of the Alpine Steppe Ecosystem Observation and Experiment Station (N 30°57′, E 88°42′, 4675 m a.s.l.) located in Xainza County, Northern Tibet, China (Fig. 1), which has a cold and semi-arid plateau monsoon climate. The mean annual air temperature is 0°C, the mean air temperature during January is –10.1°C, and during July is 9.6°C. The mean annual precipitation is 300 mm, and precipitation mainly occurs from May to September. The annual mean time of solar radiation is 2915.5 h. The vegetation coverage of the alpine steppe is about 20%, which is dominated by Stipa purpurea and Carex moorcroftii. The companion species are Stellera chamaejasme Linn., Leontopodium alpinum, and Oxytropis microphylla, among others (Lu et al. 2011).

2.2. Field experiment

A similar environmental condition was selected in an alpine steppe. Six plots with 4 × 4 m dimensions were separated by 1 m wide buffer zones with two treatments (ND and CK) and three replications. In the ND treatment, NH₄NO₃ (2 g N m⁻²) was fertilized on June 10, 2011. The amount of fertilizer applied was twice the average value in China (12.89 kg N ha⁻² yr⁻¹) (Lü and Tian 2007). A polyvinyl chloride collar (15 cm in diameter and 35 cm in height) was inserted in the soil for each plot.

2.3. Measurements of soil respiration

Soil respiration was measured by an automated soil CO₂ efflux measurement system (LI-8100, LI-COR, NE, USA). Different parts of the living plants in the collar were removed by scissors at least a day before the measurement to avoid the inclusion of the aboveground respiration of plants. The measurements of the soil surface respiration were conducted from July to September in the forenoon (about 10 o’clock). Measurements proceeded from 7:00 to 19:00 on the 22nd of August. The soil cores were separated into six increments on the 20th of September: 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm depths, and then soil respiration of different soil depths was simultaneously observed.

2.4. Measurements of soil temperature and moisture

Soil temperatures at depths of 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm were monitored using a thermocouple probe (LI-COR 6000-09TC). Soil moisture at depths of 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm were measured using a smart neutron moisture gauge. Both instruments were simultaneously connected to the LI-COR 8100 while soil respiration was measured.

2.5. Root biomass and soil sampling

Root biomass measurements at depths of 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm were performed by the harvest method (Stinson et al. 2006) at each plot on the 20th of September. Soil samples at depths of 0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm of six cores were also collected.

The collected root samples were placed in paper envelopes and dried under the sun. These samples were oven-dried at 65°C in the laboratory. All components from each sampled plant were weighed as dry biomass. After removing the roots and stones by sieving with 2 mm mesh, soil samples from the six-soil cores were air-dried, sieved, and chemically analyzed. Part of the soil were stored on ice and subsequently transferred to the insulated box for microbial analysis. Soil nutrients (including available nitrogen, nitrate nitrogen, and ammonium nitrogen) and biotic properties (microbial biomass nitrogen) at the typical red soil area of the Yingtan Stations of Jiangxi Province, China (Institute of Soil Science, Chinese Academy of Sciences) followed all standard protocols (Bao 2000, Wu et al. 2006).

2.6. Statistical analysis

The soil sampling depths are the main factors in analyzing the soil properties and respiration. One-way ANOVA was used to test the differences of soil properties and soil res-
piration among the different sampling blocks, and the least significant difference test was used to distinguish differences at $P = 0.05$. All analyses were performed using the SPSS 16.0 statistical software package (SPSS Inc., USA).

3. RESULTS

3.1. Environmental factors

The dynamics of precipitation, air temperature, and relative humidity produced one-peak patterns (DOY 0-300), which were higher in summer and lower during the spring and fall (Fig. 2). Total precipitation in 2011 coincided with the data in the meteorological station (300 mm). The mean air temperature over the entire growing season (DOY 0-300) in 2011 was 2.02°C, the mean maximum temperature was 7.3°C, and the mean minimum temperature was close to –3°C. The mean relative humidity, the maximum humidity, and the minimum humidity were 33.1%, 52.8%, and 16.4%, respectively.

Soil moisture at 5 cm depth ranged from 10.0% to 19.9% during the period of DOY 154–261, and the mean soil moisture was 15.9%. The fluctuations in precipitation caused higher soil moisture in summer and lower in fall; thus, the variable trend displayed a downward pattern. In contrast, an upward trend was observed in soil temperature, and the mean was 13.6°C with the greatest and lowest values of 18.5 and 7.2°C.

3.2. Variations of soil respirations

The maximum value occurred at 17:00 (Fig. 3A). The soil respiration of an alpine steppe displays clear seasonal patterns, and significant differences existed between CK and ND treatments ($P = 0.004$). The magnitude of CO$_2$ decreased very sharply from July to September, and the maximum rates occur in the middle of July (Fig. 3B). The season significantly affects the soil respiration, ranging from 0.4 μmol m$^{-2}$ s$^{-1}$ to 4.3 μmol m$^{-2}$ s$^{-1}$ in the ND plot, and ranking from 0.6 μmol m$^{-2}$ s$^{-1}$ to 4.0 μmol m$^{-2}$ s$^{-1}$

Table 1. Relationships of root biomass and soil respiration with soil nutrients (available nitrogen, nitrate nitrogen, and ammonium nitrogen) in the control (CK) and nitrogen addition (ND) plots at the different soil depths.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Items</th>
<th>Available nitrogen</th>
<th>Nitrate nitrogen</th>
<th>Ammonium nitrogen</th>
<th>SMBN</th>
<th>Root biomass</th>
<th>Soil respiration</th>
</tr>
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<tbody>
<tr>
<td>CK</td>
<td>Available nitrogen</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Nitrate nitrogen</td>
<td>0.936**</td>
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<tr>
<td></td>
<td>Ammonium nitrogen</td>
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<td>0.524</td>
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<tr>
<td></td>
<td>SMBN</td>
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<td>0.065</td>
<td>-0.367</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>0.899*</td>
<td>0.950**</td>
<td>0.258</td>
<td>0.306</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Soil respiration</td>
<td>0.255</td>
<td>0.109</td>
<td>0.476</td>
<td>0.338</td>
<td>0.031</td>
<td>1</td>
</tr>
<tr>
<td>ND</td>
<td>Available nitrogen</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitrate nitrogen</td>
<td>0.991**</td>
<td>1</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Ammonium nitrogen</td>
<td>0.994**</td>
<td>0.996**</td>
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<tr>
<td></td>
<td>SMBN</td>
<td>0.946**</td>
<td>0.912*</td>
<td>0.924**</td>
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<td></td>
<td>Root biomass</td>
<td>0.675</td>
<td>0.658</td>
<td>0.716</td>
<td>0.613</td>
<td>1</td>
<td></td>
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<tr>
<td></td>
<td>Soil respiration</td>
<td>-0.104</td>
<td>-0.093</td>
<td>-0.17</td>
<td>-0.13</td>
<td>-0.753</td>
<td>1</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (two-tailed). ** Correlation is significant at the 0.01 level (two-tailed).

Note: SMBN represents microbial biomass nitrogen.
Fig. 2. Variations in precipitation, air temperature, relative humidity, soil temperature, and soil moisture in the meteorological station in Xainza County, Northern Tibet, China.
in the CK plot. Fig. 3C illustrates the variations of soil respiration rates at different soil depths. The soil respiration of ND is greater than CK at each soil depth, and the differences between CK and ND plots are clear ($P = 0.013$). However, the differences of the diurnal variation between CK and ND are unclear ($P = 0.577$).

3.3. Relationships of soil respiration with soil temperature and soil moisture

The relationships between the soil moisture of two different treatments (CK and ND) and soil respiration have been analyzed. The seasonal soil respiration is positively correlated with soil temperature and soil moisture (Figs 4A and B). The diurnal variations of soil respiration with soil moisture show a negative linear correlation, whereas, a positive relationship was observed with soil temperature ($R^2 = 0.0003$, ND; $R^2 = 0.038$, CK) (Figs 4C and D). In Figs 4E and F, the results show that soil respiration is more sensitive to soil temperature ($R^2 = 0.988$, ND; $R^2 = 0.05$, CK) than soil moisture ($R^2 = 0.0003$, ND; $R^2 = 0.038$, CK) at different soil depths. Additionally, the relationship between soil temperature and soil respiration in ND treatments was more significant than that in CK.

3.4. Relationships of soil respiration with soil properties and root biomass

The effects of soil properties on soil respiration were analyzed in both treatments (Table 1), and the effects of soil properties on root biomass at different soil depths were explored to further clarify the differences between CK and ND treatments. The results show that the relationships between soil properties and root biomass in CK were greater than that in ND plots, especially in the available nitrogen and nitrate nitrogen. The coefficients for CK are 0.899 ($P < 0.05$) and 0.950 ($P < 0.01$), respectively, whereas the coefficients for ND are 0.675 and 0.658, respectively. The relationships of available nitrogen, nitrate nitrogen, and ammonium nitrogen with soil respiration are positive in CK plots, whereas, the relationships are negative in ND plots.

4. DISCUSSION AND CONCLUSIONS

The study aimed to provide an insight on the effect of nitrogen addition on soil respiration in an alpine steppe ecosystem.

Significant difference existed in soil respiration between CK and ND plots in the growing season. Nitrogen was believed to be the primary element for limiting plant growth, mass circle, and ecological process in ecosystems (Reich et al. 2001), especially in Northern Tibet. The addition of N does not only improve soil fertilization, but also improves grassland production and the quality of forage grass. It could also promote the production of lignin- or cellulose-degrading enzymes and decomposition of organic C, which consequently stimulates the activities...
Effect of nitrogen addition on soil respiration of soil microorganisms (Keeler et al. 2008). Jiang with co-authors (2010) found that N addition could decrease carbon-oxygen emission and prevent the decrease in microbial respiration (Ramirez et al. 2010), indicating the suppression of the decomposition rates of litter and soil organic matter. In the present study, the microbial biomass N in ND-treated plots was greater than that in the CK plot. Thus, the emission of carbon-oxygen fluxes in ND-treated plots was greater than that in CK plots.

Soil temperature and moisture are the key environmental factors responsible for variation in soil respiration (Zhang et al. 2010). Seasonal trends in soil respiration, soil temperature, and soil moisture have already been discussed (Saiz et al. 2006, Kaur et

![Fig. 4. Relationship of soil temperature and soil moisture with soil respiration (seasonal variation, A and B; diurnal variation, C and D; profile variation, E and F) in the control (CK) and nitrogen addition (ND) plots.](image-url)
During the growing season, a significant positive relationship existed between soil temperature and CO$_2$ flux. An exponential model explained the seasonal variation of CO$_2$ flux (Song and Zhang 2009). The temperature was the dominant factor that controlled seasonal variation of carbon-oxygen emission in an alpine steppe, and a similar result was observed in the alpine meadow (Jiang et al. 2010). Interestingly, the diurnal variations of soil respiration and the soil moisture show negative linear correlations in the two treatments. Several studies reported on the significant correlation between soil respiration and moisture if water was limited in arid and semi-arid regions (Jia et al. 2006, Gaumont-Guay et al. 2006). In contrast, Zhang et al. (2009) indicated that soil respiration was significantly and negatively related to soil moisture in several sites, which was consistent with the result of the present study. Precipitation was obtained from DOY 225 to DOY 234 continuously, such that soil moisture increased from DOY 225 to DOY 234 (Fig. 2). Maximum precipitation appeared on the 22nd of August (DOY 234). Thus, the changes in the diffusion of gases in soil and deficiencies of oxygen inhibit aerobic respiration at high moisture levels (Raich and Potter 1995).

Experimental studies of N addition at single geographic locations have also reported increase in aboveground biomass production (Elvir et al. 2003) and in shoot biomass (Michelsen et al. 1996). Root biomass was also increased by N addition. Pregitzer et al. (2008) demonstrated that chronic N deposition has the potential to increase C storage in both woody biomass and soil. Some studies have determined the possible effect of soil nutrient contents on root respiration through changing root-N content (Wang et al. 2010). The root respiration is a fraction of soil respiration, and soil N has a close correlation with soil respiration. Although the result of the present study contradicted these results, the relationships of available nitrogen, nitrate nitrogen, and ammonium nitrogen with soil respiration were also positive in CK plots, whereas the relationships in ND plots were negative. This result is attributed to a mechanism wherein the soil nutrients were almost utilized by the soil microbial biomass (soil microbial biomass was significantly correlated with soil nutrient, $P < 0.05$).

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5. REFERENCES


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