Short-term effect of increasing nitrogen deposition on CO2, CH4 and N2O fluxes in an alpine meadow on the Qinghai-Tibetan Plateau, China

Chunming Jiang a,b,d, Guirui Yu a, Huajun Fang a, Guangmin Cao c, Yingnian Li c

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A B S T R A C T

An increasing nitrogen deposition experiment (2 g N m−2 year−1) was initiated in an alpine meadow on the Qinghai-Tibetan Plateau in May 2007. The greenhouse gases (GHGs), including CO2, CH4 and N2O, was observed in the growing season (from May to September) of 2008 using static chamber and gas chromatography techniques. The CO2 emission and CH4 uptake rate showed a seasonal fluctuation, reaching the maximum in the middle of July. We found soil temperature and water-filled pore space (WFPS) were the dominant factors that controlled seasonal variation of CO2 and CH4 respectively and lacks of correlation between N2O fluxes and environmental variables. The temperature sensitivity (Q10) of CO2 emission and CH4 uptake were relatively higher (3.79 for CO2, 3.29 for CH4) than that of warmer region ecosystems, indicating the increase of temperature in the future will exert great impacts on CO2 emission and CH4 uptake in the alpine meadow. In the entire growing season, nitrogen deposition tended to increase N2O emission, to reduce CH4 uptake and to decrease CO2 emission, and the differences caused by nitrogen deposition were all not significant (p > 0.05). However, we still found significant difference (p < 0.05) between the control and nitrogen deposition treatment at some observation dates for CH4 rather than for CO2 and N2O, implying CH4 is most susceptible in response to increased nitrogen availability among the three greenhouse gases. In addition, we found short-term nitrogen deposition treatment had very limited impacts on net global warming potential (GWP) of the three GHGs together in term of CO2-equivalents. Overall, the research suggests that longer study periods are needed to verify the cumulative effects of increasing nitrogen deposition on GHG fluxes in the alpine meadow.

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1. Introduction

Anthropogenic nitrogen deposition, mainly originating from fertilizer application, fossil fuel combustion and legume cultivation, drastically increased since the industrial revolution (Matson et al., 2002) and the increasing trend is considered to be enhanced in the next few decades (Galloway et al., 2004). The increase of nitrogen availability will exert great influences on the ecosystem processes, including stimulating plant growth, changing species composition and diversity and altering greenhouse gas (GHG) fluxes (Matson et al., 2002). The effect of anthropogenic nitrogen deposition on GHG fluxes has caused great concerns because GHGs (CO2, CH4 and N2O) in the atmosphere play an important role in regulating the global climate (Conrad, 1996; Dalal and Allen, 2008).

Soils are important sources or sinks of the GHGs (Conrad, 1996). CO2 emission is through root respiration, rhizomicrobial respiration and soil organic matter decomposition; CH4 is produced by methanogens and consumed by methanotrophs; Nitriﬁcation and denitriﬁcation are the main processes causing N2O production from soils (Liu and Greaver, 2009). Environmental factors governing GHG production and consumption include availability and amount of substrates, temperature, soil water content, ion deficiencies and toxicities, atmospheric nitrogen deposition and so on (Dalal and Allen, 2008). Experimental additions of nitrogen to soil typically result in decreased CO2 emission and CH4 consumption and increased N2O emission (Burton et al., 2004; Mo et al., 2008; Mosier et al., 1991; Scheer et al., 2008), although contrary effects and lacks of response also have been reported (Ambus and Robertson, 2006; Bodelier and Laanbroek, 2004; Bradford et al., 2001; Hall and Matson, 1999).
Simulated atmospheric nitrogen deposition experiments have conducted in many natural ecosystems. However, the impact of nitrogen deposition on the production or consumption of GHGs is not well understood and has not been assessed in the alpine meadow on the Qinghai-Tibetan Plateau. The plateau covers nearly one-quarter of the area of China and represents one of the largest alpine grasslands in the world (He et al., 2006). More valuably, the alpine meadow of Qinghai-Tibetan Plateau, which is very far away from the industrial area and remains relatively undisturbed by humans, receives much smaller of atmospheric nitrogen deposition (wet deposition is only 0.46 g N m^{-2} year^{-1}, the data is not published) relative to the nitrogen pollution regions in Eastern United States and Western Europe (typically greater than 2.5 g N m^{-2} year^{-1}) (Holland et al., 2005; MacDonald et al., 2002). Consequently, the alpine meadow on the Qinghai-Tibetan Plateau is an ideal region in which to verify the actual consequences of increasing nitrogen deposition for GHG fluxes. Moreover, the experiment of increasing nitrogen deposition on the alpine meadow can contribute to reducing the uncertainty of Qinghai-Tibetan plateau in global GHG budgets. The specific objects of the study are: (1) to quantify short-term effects of increasing nitrogen deposition on GHG fluxes in the alpine meadow; (2) to investigate the responses of GHG fluxes to changes of environmental factors.

2. Materials and methods

2.1. Site description

The research was conducted in an alpine meadow, located in Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37°37’N, 101°120’E and at an altitude of 3250 m above sea level). The local climate is characterized by strong solar radiation with long, cold winters, and short, cool summers. The average annual air temperature was –1.7°C. The mean, maximum and minimum of averaged air temperature were 8.7, 15.6 and 2.5 °C, respectively, in summer and –13.2, –2.2 and –22.1 °C, respectively, in winter. Annual mean precipitation is 580 mm, and about 80% of precipitation is concentrated in the growing season from May to September from May to September in 2008. In the sample date, six chambers were placed over the bases filled with water in the groove to ensure air tightness and the gas samples were taken simultaneously in the six plots. Gas sample inside the chamber was taken for every 10 min over a 30 min period by using 100 ml plastic syringes (total of four samples). CO₂, N₂O and CH₄ concentrations of gas samples were analyzed with gas chromatography (HP Series 4890D, Hewlett Packard, USA) within 24 h following gas sampling. The gas chromatography was equipped with an electron capture detector for N₂O analysis and a flame ionization detector for CH₄ and CO₂ analysis. The gas chromatography configurations for analyzing concentrations of CO₂, N₂O and CH₄ were according to the method of Wang and Wang (2003). The calculation of GHG flux followed the description of Scheer et al. (2008). Negative flux values indicate GHG uptake from the atmosphere, and positive flux values indicate GHG emissions to the atmosphere.

In order to evaluate the net global warming impact of the three GHGs together caused by increasing nitrogen deposition, the cumulative fluxes of CO₂, CH₄ and N₂O during the observation period was estimated according to the method of Gü et al. (2007) and then the net global warming potential (GWP) of the three GHGs was calculated (Nykanen et al., 1995).

Air temperature (Tₐ), 5 cm soil temperature (Tₛ) and 10 cm volumetric soil moisture (%) were monitored at each chamber during gas sample collection. Tₐ and Tₛ (°C) was measured using a digital thermometer (SN2202, China). Soil volumetric soil

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the alpine meadow soil*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>Organic matter (%)</td>
</tr>
<tr>
<td>0–10</td>
<td>12.07</td>
</tr>
<tr>
<td>10–20</td>
<td>8.64</td>
</tr>
<tr>
<td>20–50</td>
<td>3.09</td>
</tr>
<tr>
<td>50–70</td>
<td>1.29</td>
</tr>
<tr>
<td>70–110</td>
<td>3.54</td>
</tr>
</tbody>
</table>

* Data are from Zhang and Cao (1999).
moisture (%, m$^3$ H$_2$O m$^{-3}$ soil) was determined using a time domain reflectometry (MPKit, China). The volumetric soil moisture was transformed to water-filled pore space (WFPS): WFPS = volumetric soil moisture/(1-bulk density/2.65).

2.4. Statistical analyses

The distributions of GHG fluxes and environmental parameters in the growing season were all tested for normality with the Shapiro–Wilk test. Mann–Whitney U-tests were used to analyze differences of GHG fluxes between control and increasing nitrogen deposition plots (values in every sample date and cumulative values in the growing season). To explore the effect of sample date and the changes of treatment effect with sample date, repeated-measures ANOVA was also employed with sample date as the repeated factor. Correlation analyses and stepwise linear regression analyses were used to examine the relationships between GHG fluxes and the measured environmental variables. The van’t Hoff equation ($y = a \exp(bT_s)$) was established to calculate the temperature sensitivity ($Q_{10} = \exp(10b)$) of GHG fluxes to the changes of $T_s$. SPSS13.0 was used for all statistical analyses.

3. Results and discussion

3.1. Environmental variables

$T_a$, $T_s$ and WFPS showed clear seasonal patterns in the alpine meadow. $T_a$ and $T_s$ reached maximum (about 15 °C) (Fig. 1a) while WFPS reached minimum (below 30%) (Fig. 1b) in the middle of July. The variation of $T_s$ was more stable than that of $T_a$. The mean values of the growing season were 10.3 °C for $T_a$, 9.7 °C for $T_s$ and 55.6% for WFPS. The total precipitation of the growing season was 269.1 mm.

3.2. CO$_2$ flux

The alpine meadow soil was a source of CO$_2$ and displayed clear seasonal pattern. The magnitude of CO$_2$ increased very sharply in the middle of June and the maximum flux occurred in the middle of July (Fig. 2a). The CO$_2$ fluxes were positively correlated with $T_a$ ($p < 0.001$) and $T_s$ ($p < 0.001$), and negatively correlated with WFPS ($p < 0.001$). The WFPS was excluded in the stepwise linear regression, indicating temperature was the main controlling factor for temporal variation of CO$_2$ emissions in the alpine meadow. The $Q_{10}$ of CO$_2$ emissions was 3.79 (Table 2). The nitrogen deposition did not affect the seasonal varying pattern of CO$_2$. Before August, the CO$_2$ fluxes were nearly identical between control and nitrogen deposition plots and nitrogen deposition tended to suppress the CO$_2$ emissions in August and September (Fig. 2a). In the entire growing season, there was no significant difference ($p = 0.82$) of CO$_2$ emissions between the control and nitrogen deposition plots.
plots and the mean CO2 fluxes of nitrogen deposition and control plots were 535.7 ± 45.74 mg m⁻² h⁻¹ and 548.5 ± 47.35 mg m⁻² h⁻¹, respectively. The interactive effect between sample date and nitrogen deposition on CO2 flux was also not observed (p = 0.73) (Table 3).

The magnitudes of CO2 flux ranged from 222 mg m⁻² h⁻¹ to 897 mg m⁻² h⁻¹ in our study, which were comparable to previous studies in the alpine meadow (Cao et al., 2004; Hu et al., 2008) and in a temperate steppe on the Inner Mongolia (Niu et al., 2008) and was markedly larger than magnitudes of CO2 flux in the alpine grassland of Wudaolang on the Qinghai-Tibetan Plateau (Pei et al., 2003). Temperature (especially soil temperature) was the dominant environmental variable that controlled the seasonal change of CO2 flux in our study, which has been documented in many other studies (Fang and Moncrieff, 2001; Lloyd and Taylor, 1994). Q10, the temperature sensitivity of respiration, was 3.79 in the alpine meadow, which agreed very well with the result of Kato et al. (2004), who quantified the temperature sensitivity of nighttime CO2 flux using the eddy covariance method in the same alpine meadow. The temperature sensitivity of respiration has made intensive study in global scale and across China, including various kinds of ecosystems (Chen and Tian, 2005; Peng et al., 2009; Raich and Schlesinger, 1992; Tjoelker et al., 2001; Zheng et al., 2009). The temperature sensitivity of nighttime CO2 flux ranged from 222 mg m⁻² h⁻¹ to 897 mg m⁻² h⁻¹ in our study, which was comparable to previous studies in the alpine meadow (Cao et al., 2004; Hu et al., 2008) and in a temperate steppe on the Inner Mongolia (Niu et al., 2008) and was markedly larger than magnitudes of CO2 flux in the alpine grassland of Wudaolang on the Qinghai-Tibetan Plateau (Pei et al., 2003). Temperature (especially soil temperature) was the dominant environmental variable that controlled the seasonal change of CO2 flux in our study, which has been documented in many other studies (Fang and Moncrieff, 2001; Lloyd and Taylor, 1994). Q10, the temperature sensitivity of respiration, was 3.79 in the alpine meadow, which agreed very well with the result of Kato et al. (2004), who quantified the temperature sensitivity of nighttime CO2 flux using the eddy covariance method in the same alpine meadow. The temperature sensitivity of respiration has made intensive study in global scale and across China, including various kinds of ecosystems (Chen and Tian, 2005; Peng et al., 2009; Raich and Schlesinger, 1992; Tjoelker et al., 2001; Zheng et al., 2009). Q10 values in global scale varied from 1.3 to 3.3 and had a median value of 2.4 (Raich and Schlesinger, 1992). Our result is at the high end value of global scale, indicating the increasing of temperature in the future will exert great impacts on the ecosystem respiration of the alpine meadow. WFPS is another important factor affecting CO2 emission. Positive or negative effects of WFPS on soil CO2 flux have been reported (Davidson et al., 1998, 2000; Franzluebbers et al., 2002; Sotta et al., 2006). Xu and Qi (2001) found a splitting point of soil moisture of 19% (volumetric soil moisture), above which the effect of soil water content on CO2 emission turned from positive to negative. In our study, CO2 flux was negatively correlated with WFPS, probably because overabundant soil water content inhibited plant root andmicrobeactivity or blocked CO2 diffusing from soil to atmosphere. Alternatively, decrease of CO2 emissions with increase of WFPS could be an artifact of changing soil temperature whose effect was not separated in this analysis (Kato et al., 2004). A multi-factor controlled experiment is needed to identify WFPS, soil temperature and their interactive effect on CO2 flux in the alpine meadow (Niu et al., 2008).

Before August, the CO2 fluxes were nearly identical between control and nitrogen deposition plots and nitrogen deposition suppressed CO2 emissions by only 7% in August and September. The lack of response of CO2 fluxes to short-term nitrogen deposition agreed with the finding of Mo et al. (2008) and Burton et al. (2004). With the lasting of the nitrogen deposition experiment, decreasing magnitude of CO2 emissions may be even more pronounced (Bowden et al., 2004). There are two mechanisms explaining reduction of CO2 emission after nitrogen addition: (1) Because a large fraction of soil respiration is related to nitrogen assimilation of plants, with the increasing of nitrogen availability, energetic costs of nitrogen assimilation may be reduced (Bowden et al., 2004); (2) Microbial respiration could be decreased in response to nitrogen additions indicated by that the decomposition rates of litter and soil organic matter were suppressed (Berg and Matzner, 1997; Zak et al., 2008).

### 3.3. N₂O flux

Variation of N₂O fluxes did not show clear seasonal pattern. There were two peaks of N₂O fluxes in the growing season: one at the end of May, the other at the beginning of August (Fig. 2b). We did not find correlation between the N₂O fluxes and environmental variables (Table 2). Nitrogen deposition tended to increase N₂O emission from middle of July to middle of August (Fig. 2b).

In this study we observed a very high inherent spatial variability of N₂O flux (Röver et al., 1999; Velthof et al., 1996) in the experiment plots, with an average coefficient of variation (CV) of 518%, which was obviously larger than that of CO2 (15.7%) and CH4 (33.8%). The results suggest that increasing the number of spatial replicates is very necessary in the N₂O observation so that we could estimate the N₂O flux and identify the effect of nitrogen deposition more exactly. The magnitudes of N₂O fluxes in the alpine meadow was in the range of −2.05 ug m⁻² h⁻¹ to 5.65 ug m⁻² h⁻¹, which were in the very low part of the range of temperate grassland (Du et al., 2006; Glatzel and Stahr, 2001; Mosier et al., 1991; Wang et al., 2005) and tropical pasture (Garcia-Montiel et al., 2001; Passioni et al., 2003; Steudler et al., 2002). N₂O production is through nitrification and denitrification, in which microorganism use the mineral nitrogen (NH₄⁺ and NO₃⁻) as substrate (Conrad, 1996). The low N₂O emission is due to the limited mineral nitrogen in the alpine meadow soil caused by low mineralization rates of organic nitrogen (Cao and Zhang, 2001). The negative N₂O fluxes were observed in the growing season, especially from the middle of August to September. This phenomenon has been reported by many studies (Clayton et al., 1997; Kellman and Kavanagh, 2008; Rosenkranz et al., 2006; Vieten et al., 2009). Rosenkranz et al. (2006) assumed that in the case of shortage in nitrates supply denitrifying bacteria might use atmospheric N₂O as an alternative electron acceptor to nitrate. Chapuis-Lardy et al. (2007) also pointed out that N₂O uptake seemed to be favored by low mineral nitrogen and large moisture contents in soil from analysis a large number of literatures. The two peaks of N₂O in the growing season were showed by other researches in German temperature grassland (Glatzel and Stahr, 2001) and subalpine meadow of Colorado (Filippa et al., 2009). The first peak in the end of May could be attributed to the thawing process of alpine soil, during which the increasing of N₂O emissions resulted from a sudden increase of bioavailable carbon and nitrogen (Ludwig et al., 2006; Melvold et al., 2006) or a quick release of N₂O trapped by ice layer (Goodroad and Keeney, 1984; Teepe et al., 2001). The absence of N₂O emission from middle of June to middle of July was probably because that the mineral nitrogen was almost used up by plants. This speculation could be supported by the evidence that the
The global warming potential (GWP) of CO₂ is 1, while for CH₄ is 23 times that of CO₂ and for N₂O is 296. GWP indicates greater percent (%) of GHG emission in increasing the main source for N₂O production in the Inner Mongolian semi-arid steppe.

Comparison of the GWP of CO₂, N₂O and CH₄ fluxes as affected by increasing nitrogen deposition (ND) in the growing season.

<table>
<thead>
<tr>
<th></th>
<th>CO₂ flux</th>
<th>N₂O flux</th>
<th>CH₄ flux</th>
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<tbody>
<tr>
<td></td>
<td>MS</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Between subjects</td>
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<td></td>
</tr>
<tr>
<td>ND</td>
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<td>4911</td>
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<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>19</td>
<td>336 076</td>
<td>103.7</td>
</tr>
<tr>
<td>Date × ND</td>
<td>19</td>
<td>2514.2</td>
<td>0.776</td>
</tr>
</tbody>
</table>

The alpine meadow soil acted as a sink of CH₄. The CH₄ uptake gradually increased from the beginning of the growing season, reached maximum uptake value in July and then gradually declined (Fig. 2c). There was positive correlation between CH₄ uptake and Tₚ (p < 0.01) or Tₚ (p < 0.001), and negative correlation between CH₄ uptake and WFPS (p < 0.001). Among the three environment variables, WFPS was the dominant factor that controlled the CH₄ uptake in the alpine meadow from the stepwise linear regression analysis. The Q₁₀ of CH₄ oxidation was 3.29 (Table 2). From the middle of June, the CH₄ uptake capacities in the nitrogen deposition plots were always smaller than that in control. Some of these differences were significant (p < 0.05) from the statistical analysis (Fig. 2c). The value of CH₄ uptake for nitrogen deposition plots was 15.3% smaller than that for control plots in the entire growing season, however, this discrepancy was not significant (p = 0.12) (Table 4). There was no interactive effect between sample date and nitrogen deposition for CH₄ uptake during the investigation period (p = 0.47) (Table 3).

CH₄ uptakes in the alpine meadow varied from 1.4 ug m⁻² h⁻¹ to 71.9 ug m⁻² h⁻¹, which were comparable to the range of CH₄ uptake in the temperature grassland in Colorado (Mosier et al., 1991) and in Inner Mongolia (Wang et al., 2005). The Q₁₀ of CH₄ oxidation was 3.29, which was substantially larger than that in the temperature forest soil (ranged from 1 to 2) (Bowden et al., 1998; King and Adamsen, 1992; Macdonald et al., 1997). This comparison indicated soil methane consumption in the alpine meadow might be more sensitive to changes in global temperature than in the temperature regions. Higher effect of WFPS than temperature on the uptake of CH₄ was consistent with other studies (Bowden et al., 1998; Bradford et al., 2001) because CH₄ was consumed by methanotrophs in aerobic condition (Conrad, 1996; Mancinelli, 1995) and the uptake activity was mainly controlled by the diffusion rate of CH₄ into the active of methanotrophic zone in mineral soil (Steinkamp et al., 2001).

Generally, the nitrogen addition could inhibit the uptake of CH₄ (Bodelier and Laanbroek, 2004; Mancinelli, 1995) in soil. The
underlying mechanisms are: (1) NH$_4^+$ is a competitive inhibitor of CH$_4$ oxidation due to lack of specificity of methane monooxygenase (MMO) in methanotroph; (2) hydroxylamine and nitrite produced by methanotrophic ammonia oxidation are toxic to methanotrophic bacteria; (3) osmotic stress caused by added nitrogen salt can suppress the activity of methanotroph (Saari et al., 2004). In our experiment, the decreasing magnitude of CH$_4$ oxidation was much smaller than that of the nitrogen fertilizer treatments (typically ranging from 60% to 80%) (Adamsen and King, 1993; Castro et al., 1995; Macdonald et al., 1996; Mosier et al., 1991). This is because: (1) the amount of added nitrogen in our experiment was much smaller than that of the nitrogen fertilizer treatments; (2) nitrogen immobilization capacity in the alpine meadow soil was very high, which could protect methanotroph from exposure to NH$_4^+$ (Steinkamp et al., 2001). In our experiment, the decreasing magnitude of CH$_4$ oxidation was much larger than that of CO$_2$ emission (15.3% for CH$_4$, only 2.33% for CO$_2$), which was consistent with the results of Saari et al. (2004). Moreover, we detected significant difference ($p < 0.05$) between the control and nitrogen deposition treatment at some observation dates for CH$_4$ rather than for CO$_2$ and N$_2$O, implying that methanotroph might be more susceptible to the nitrogen deposition than other soil microbes relating CO$_2$ and N$_2$O emissions (Saari et al., 2004).

3.5. Greenhouse effect of GHGs as affected by nitrogen deposition

The emission of 1 kg of N$_2$O to the atmosphere is 296 times more effective than 1 kg of CO$_2$, while 1 kg of CH$_4$ is 23 times more effective than 1 kg of CO$_2$ (Liu and Greaver, 2009). By this, the GWP of the three GHGs was calculated to identify the effect of short-term nitrogen deposition treatment on global warming. Our results indicated that CO$_2$ was the overwhelmingly dominant GHG in terms of its GWP, and nitrogen deposition treatment had limited impacts on each individual GHG and net GWP of the three GHGs together in the alpine meadow (Table 4).

4. Conclusions

The GHG (CO$_2$, CH$_4$ and N$_2$O) were observed in the growing season in the alpine meadow of Qinghai-Tibetan Plateau. The results showed the temperature sensitivity ($Q_{10}$) of CO$_2$ emission and CH$_4$ uptake were relatively higher (3.79 for CO$_2$, 3.29 for CH$_4$) than that of warmer region ecosystems and soil temperature and WFPS were the dominant factors that controlled seasonal variation of CO$_2$ and CH$_4$ respectively. Increasing nitrogen deposition decreased the CH$_4$ absorption and increased the N$_2$O emission but did not strongly affect CO$_2$ flux. Among the three greenhouse gases, CH$_4$ was most susceptible in response to increased nitrogen availability. There were no interactive effects between sample date and nitrogen deposition on GHG fluxes in the observation periods. In addition, the short-term increasing nitrogen deposition (2 g N m$^{-2}$ year$^{-1}$) had very small impacts on global warming potential (GWP) in term of CO$_2$-equivalents. Overall, our study demonstrated that short-term effect of moderate nitrogen deposition on GHG fluxes was less obvious than nitrogen fertilizer experiments and longer study periods were needed to verify the cumulative effects of increasing nitrogen deposition on GHG fluxes in the alpine meadow.

Acknowledgments

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