Studies on the variation of CO$_2$ fluxes and its characterization with soil temperature, moisture and dissolved organic carbon under different sulfur levels from alpine grassland in the Tibetan Plateau

**Abstract**

**Aim**: The effect of sulfur deposition on the carbon dynamics of alpine grasslands has received little attention. The present study was carried out to determine the influence of sulfur addition on temporal variation of CO$_2$ fluxes and characterize the relationships between CO$_2$ fluxes and soil temperature, moisture and dissolved organic carbon from alpine grassland in the Tibetan Plateau.

**Methodology**: Based on a multi-level S (0, 2 and 6 g S m$^{-2}$ yr$^{-1}$) addition experiment, soil CO$_2$ fluxes were monitored by static chamber and gas chromatograph technique within Tibetan alpine grassland during the growing seasons in 2013 and 2014. Soil temperature, moisture, dissolved organic carbon, microbial carbon and nitrogen and enzyme activities were measured to examine the key driving factors of soil CO$_2$ fluxes.

**Results**: No significant differences in CO$_2$ fluxes between treatments were observed during almost all the sampling periods, but sulfur deposition increased mean soil dissolved organic carbon concentrations. Sulfur deposition tended to inhibit soil microbial carbon and nitrogen and enzyme activities. Regardless of sulfur treatment, soil temperature was the primary control on seasonal variation of CO$_2$ fluxes in both 2013 and 2014, but these fluxes were not limited by soil moisture in 2013.

**Interpretation**: The result indicated that CO$_2$ fluxes from Tibetan alpine grasslands resulted from mineralization of soil dissolved organic carbon and the potential increasing atmospheric sulfur deposition could have limited effects on CO$_2$ emission from the alpine grasslands.
Atmospheric sulfur deposition, originating mostly from fossil-fuel combustion and other anthropogenic activities, has been recognized as a worldwide environmental problem (Wang et al., 2004; Liang et al., 2013). Although sulfur deposition in developed countries has stabilized or declined in recent years, it is still increasing in many developing countries, especially in Asia (Lu et al., 2010; Song et al., 2013). It is well known that sulfur deposition rates have affected biogeochemical cycles of many terrestrial and aquatic ecosystems, including greenhouse gas emissions from soil (Oulehle et al., 2011). Previous studies of the impacts of sulfur deposition on ecosystem CO\(_2\) emission have focused on forests (Kuzyakov, 2006; Chen et al., 2012; Liang et al., 2013) and freshwater wetlands (Vile and Bridgham, 2003; Vile et al., 2003), and have generally shown that sulfur deposition can suppress CO\(_2\) emission. However, the relationship between CO\(_2\) emission and sulfur deposition in other ecosystems like alpine grasslands remains unknown, even though these ecosystems are experiencing increasing rate of sulfur deposition.

The Tibetan Plateau is the largest geomorphic feature of the Eurasian continent and plays an important role in global climate and environmental change. Alpine grasslands are the dominant ecosystem type in the area (Cao et al., 2004). These grasslands contain large soil carbon stocks and are currently major carbon sinks because of their high productivity and low decomposition rates (Cao et al., 2004, Yang et al., 2008). The Tibetan Plateau is currently receiving elevated atmospheric sulfur deposition (Song et al., 2013; Gao et al., 2014), but the effect of sulfur deposition on the carbon dynamics of alpine grasslands has received little attention, and less information is available about the impact of sulfur deposition on CO\(_2\) fluxes within these ecosystems.

To understand the effects of sulfur deposition on CO\(_2\) fluxes in the alpine meadows of the Tibetan Plateau, a multi-level sulfur addition experiment was carried out with the aim to determine the influence of sulfur addition on temporal variation of CO\(_2\) fluxes from alpine grasslands and to characterize the relationships between CO\(_2\) fluxes and soil temperature, moisture and dissolved organic carbon under different sulfur addition levels.

**Materials and Methods**

**Study site:** The present study was conducted in Hongyuan County, located on the eastern Tibetan Plateau, China. The study area is 3500 m above sea level, and experiences a harsh continental climate, with a mean annual temperature of 1.1 °C. Annual precipitation averages 752 mm, with about 86 % received from May to September. Soils within the study plots are classified as Mat Crygelic Cambisol (Chinese Soil Taxonomy Research Group, 1995), and average 49.6 g kg\(^{-1}\) organic C, 4.6 g kg\(^{-1}\) total N, pH of 6.1, and 0.94 g cm\(^{-2}\) bulk density at 10 cm depth (Gao et al., 2015b) Site vegetation is typical of alpine meadow in the region, and is dominated by Kobresias tchwanensis Hand.-Mazz., and Elymus nutans Griseb., accompanied by Festuca ovina Linne, Poa pachyandha Keng and Aster alpinum Linne.

**Experimental setup and measurement:** Twelve plots were established in early May 2012, each measuring 3×3 m. Four replicate plots were randomly assigned to each of three treatments: control (without sulfur); low sulfur (2 g S m\(^{-2}\) yr\(^{-1}\)) and high sulfur (6 g S m\(^{-2}\) yr\(^{-1}\)). Sulfur was applied monthly during the growing season (May to October), from 2012 to 2014, as Na\(_2\)SO\(_4\) dissolved in 2 l of water. Control plots received the same volume of water during each application.

Fluxes of CO\(_2\) were measured using a soil gas flux chamber (50×50×50 cm) attached to permanent stainless steel soil collars inserted to a depth of 10 cm in the center of each plot. Approximately, 100 ml of gas was collected from the chamber headspace at 0, 10, 20 and 30 minutes after chamber closure, and stored in a gas bag (LB101, Delin gas packing Co, Ltd, China). Gas samples were taken between 9:00 and 11:00 hr local time, five or six times per month, from May to October in 2013 and 2014. CO\(_2\) concentration of gas samples were determined with a gas chromatograph (Agilent 7890A, Agilent Technologies, Inc). Soil CO\(_2\) fluxes were calculated as slope of linear regression between CO\(_2\) concentration and time.

On each sampling date, soil temperature and volumetric water content at 10 cm depth were measured in each plot with a digital thermometer (JM624, Jinming Instrument Co. Ltd., China) and time domain reflectometer (TDR 300, Spectrum Technologies Inc., USA). Soil samples from the upper 10 cm were also collected for analysis of dissolved organic carbon, pH and microbial activity. Fresh soil samples were extracted with 2 M KCl for 1 hr and dissolved organic carbon was determined with a TOC analyzer (Shimadzu TOC-VCSH/TN, Kyoto, Japan). Soil pH and microbial activity were analyzed at the end of August in each year. Soil pH was measured in 1:1 soil-water slurries (Multiline F/SET-3, Germany). Carbon and nitrogen concentrations in microbial biomass was determined by chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). The soil invertase and urease activity were assayed on the basis of glucose and NH\(_4\)+ release. Soil samples were incubated with 8% sucrose solution and 10% urea solution in a suitable buffer solution for 24 hrs at 37°C and prior to spectrophotometric estimation (Xu, 1986). Catalase activity was estimated by titration method (Xu, 1986).

**Statistical analyses:** Repeated measures ANOVA was used to examine the effects of sulfur addition over time on soil variation and CO\(_2\) fluxes. Differences among treatments were assessed by LSD method. Linear regression analysis was used to examine the relationship between CO\(_2\) fluxes and soil temperature, water
content and DOC concentration. Statistical analyses were performed by SPSS 16.0 (SPSS Inc., Chicago, USA).

Results and Discussion

Mean soil temperature during the growing season was 12.1±0.7°C in 2013 and 12.3±0.6°C in 2014. During both years, soil temperature increased from early May and reached maximum in late July, and then declined in October (Fig. 1a, b). Mean soil moisture during the study period was 44.6±1.2% in 2013 and 44.1±1.1% in 2014 and soil moisture varied from 28% to 57% in 2013 and from 21% to 52%, respectively (Fig 1c, d). Soil temperature and moisture did not differ significantly among treatments (Table 1).

DOC Soil concentration in control plots ranged from 19 to 95 mg kg\(^{-1}\) in 2013 and 2 to 103 mg kg\(^{-1}\) in 2014 (Fig. 1e, f). During both years, soil DOC concentrations differed among treatments (p<0.001), and was significantly higher in high sulfur treatment than in control plots. Addition of sulphate caused minor decrease in soil microbial carbon and nitrogen, enzymes and pH, but these differences were not significant (Table 2).

CO\(_2\) fluxes from the Tibetan alpine grassland site varied from 75 to 1020 mg m\(^{-2}\) hr\(^{-1}\) in 2013 and from 114 to 897 mg m\(^{-2}\) hr\(^{-1}\) in 2014, with maximum values occurring in late July (Fig 1e, f). Sulfur addition caused slight increase in CO\(_2\) fluxes throughout the measurement period. Cumulative CO\(_2\) emissions in high...
sulfur treatments exceeded those in control plots by 19.6% in 2013 and 20.2% in 2014, while emissions in low sulfur treatments were 6.7 to 9.1% higher than controls (Table 2). CO₂ fluxes were positively correlated with soil temperature and negatively correlated with soil dissolved organic carbon, but negatively correlated with soil moisture in 2014 (Fig. 2). These relationships were not affected by sulfur addition treatments.

The magnitudes of CO₂ flux ranged from 75 to 1012 mg m⁻² h⁻¹ in this study, which were comparable with the previous studies carried out in Tibetan alpine grasslands (Lin et al., 2009; Jiang et al., 2010). Soil temperature and moisture are often the primary physical constraints on soil CO₂ fluxes (Jiang et al., 2010; Liu et al., 2015). In the present study, CO₂ fluxes were strongly dependent on soil temperature, but exhibited inconsistent correlations with soil moisture. Soil moisture did not significantly affect CO₂ fluxes in 2013, probably because soil moisture on most sample dates (45-55%) was not low enough to limit CO₂ production. Similar relationships between CO₂ fluxes and soil moisture have been observed in other Tibetan alpine grasslands (Lin et al., 2009; Jiang et al., 2010).

Fig. 2: Relationships between CO₂ fluxes and soil temperature, soil moisture and dissolved organic carbon (DOC) in alpine meadow with different sulfur treatments during 2013 and 2014.
Effect of S deposition on CO₂ fluxes in the alpine meadows

Soil DOC is mainly released from litter decomposition, root exudation and mineralization of soil organic matter (Don and Schulze, 2008; Gao et al., 2015a). Lower soil DOC in the plots during early summer likely resulted from increased carbon mineralization driven by elevated soil temperatures, while higher soil DOC in early autumn might have been caused due to decreased microbial activity and increased litter inputs associated with plant senescence (Cleveland et al., 2004; Don and Schulze, 2008; Kalbitz et al., 2000). Previous studies of DOC dynamics in grassland soils have reported similar seasonal dynamics (Don and Schulze, 2008).

Soil DOC has been proposed as the primary labile carbon source contributing to microbial CO₂ fluxes (Bengtson and Bengtsson, 2007; Yang et al., 2013). The negative relationship between seasonal variation of CO₂ fluxes and soil DOC concentration in this study suggests that DOC provided labile carbon for microbial CO₂ production. The concentrations of SO₄²⁻ added in the present study had no significant effects on soil microbial biomass carbon and nitrogen and enzyme activity, but increased the production of soil DOC availability. SO₄²⁻ additions increased soil DOC, which is in agreement with the findings of Monteith et al. (2007). The possible reason is SO₄²⁻ addition stimulated plant growth through S nutrient supplying and increased litter and root input (Tallec et al., 2008).

Sulfate addition induced minor increase in CO₂ fluxes from the Tibetan alpine grassland plots. To our knowledge, this is the first published information on the effects of SO₄²⁻ on CO₂ emissions from alpine grasslands. Previous studies from forested ecosystems have found that SO₄²⁻ addition inhibited CO₂ emissions from forest soils, because decreased soil pH suppressed soil microorganism activity (Kuzyakov, 2006; Chen et al., 2012). In the present study, changes in soil pH were not responsible for variation in CO₂ fluxes, since soil pH did not differ significantly among treatments. Soil CO₂ fluxes are driven by soil microbial respiration and root respiration (Zhang et al., 2014). It was noted that SO₄²⁻ addition did not increase microbial activity, suggesting that small increase in CO₂ flux with SO₄²⁻ addition might be due to simulated root respiration, instead of microbial respiration. Root respiration can contribute more than 80% of CO₂ fluxes in alpine grasslands (Geng et al., 2012).

Overall, sulfur deposition is often considered to have negative consequences for soil CO₂ emission in several ecosystems. Contrary to this view, the present study revealed that S addition resulted in small increase in soil CO₂ fluxes from alpine grasslands. Further research is needed to investigate the effects of S deposition on long-term CO₂ emissions in alpine grasslands in the Tibetan Plateau.

Table 1: Results of repeated measures ANOVAs on the effect of sulfur addition and sampling date on soil temperature, moisture, DOC concentration and CO₂ fluxes in alpine meadow

<table>
<thead>
<tr>
<th>Year</th>
<th>Effect</th>
<th>df</th>
<th>F-value</th>
<th>p</th>
<th>F-value</th>
<th>p</th>
<th>F-value</th>
<th>p</th>
<th>F-value</th>
<th>p</th>
<th>F-value</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>2013</td>
<td>Treatment</td>
<td>2</td>
<td>0.232</td>
<td>0.798</td>
<td>0.657</td>
<td>0.542</td>
<td>39.398</td>
<td>&lt;0.001</td>
<td>2.994</td>
<td>0.101</td>
<td>1.530</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>Date</td>
<td>34</td>
<td>1106.228</td>
<td>&lt;0.001</td>
<td>77.252</td>
<td>&lt;0.001</td>
<td>94.687</td>
<td>&lt;0.001</td>
<td>166.257</td>
<td>&lt;0.001</td>
<td>86.372</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment × sampling date</td>
<td>68</td>
<td>0.338</td>
<td>0.860</td>
<td>0.827</td>
<td>0.624</td>
<td>0.730</td>
<td>0.719</td>
<td>1.530</td>
<td>0.159</td>
<td>1.228</td>
<td>0.283</td>
</tr>
<tr>
<td>2014</td>
<td>Treatment</td>
<td>2</td>
<td>1.014</td>
<td>0.075</td>
<td>0.047</td>
<td>0.955</td>
<td>20.089</td>
<td>&lt;0.001</td>
<td>3.168</td>
<td>0.091</td>
<td>2.550</td>
<td>0.112</td>
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<tr>
<td></td>
<td>Date</td>
<td>34</td>
<td>383.476</td>
<td>&lt;0.001</td>
<td>53.238</td>
<td>&lt;0.001</td>
<td>99.053</td>
<td>&lt;0.001</td>
<td>86.372</td>
<td>&lt;0.001</td>
<td>8.777</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Treatment × sampling date</td>
<td>68</td>
<td>0.686</td>
<td>0.643</td>
<td>0.775</td>
<td>0.653</td>
<td>1.442</td>
<td>0.162</td>
<td>2.632</td>
<td>0.109</td>
<td>2.632</td>
<td>0.109</td>
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</tbody>
</table>

Table 2: Cumulative CO₂ fluxes, soil microbial carbon and nitrogen, enzymes and pH for the alpine meadow with different sulfur treatments

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>CO₂</th>
<th>Microbial C</th>
<th>Microbial N</th>
<th>Invertase</th>
<th>Urease</th>
<th>Catalase</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Control</td>
<td>1452.0±82.9a</td>
<td>529.4±17.9a</td>
<td>65.72±3.20a</td>
<td>441.0±15.1a</td>
<td>31.36±1.34a</td>
<td>5.96±0.02a</td>
<td>5.96±0.02a</td>
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<tr>
<td></td>
<td>Low S</td>
<td>1549.4±76.7a</td>
<td>525.3±12.1a</td>
<td>61.03±2.53a</td>
<td>422.5±13.0a</td>
<td>29.49±1.21a</td>
<td>5.99±0.04a</td>
<td>5.99±0.04a</td>
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<tr>
<td></td>
<td>High S</td>
<td>1737.3±92.0a</td>
<td>517.1±16.5a</td>
<td>59.93±1.84a</td>
<td>416.1±19.3a</td>
<td>28.09±1.69a</td>
<td>5.91±0.03a</td>
<td>5.91±0.03a</td>
</tr>
<tr>
<td>2014</td>
<td>Control</td>
<td>1556.2±70.9a</td>
<td>566.6±18.8a</td>
<td>71.89±3.05a</td>
<td>496.7±17.2a</td>
<td>37.6±1.35a</td>
<td>6.08±0.03a</td>
<td>6.08±0.03a</td>
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<tr>
<td></td>
<td>Low S</td>
<td>1697.7±86.8a</td>
<td>553.3±16.1a</td>
<td>65.40±3.74a</td>
<td>480.7±13.4a</td>
<td>35.93±1.27a</td>
<td>6.05±0.04a</td>
<td>6.05±0.04a</td>
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<tr>
<td></td>
<td>High S</td>
<td>1870.3±104.5a</td>
<td>549.7±19.5a</td>
<td>62.71±3.40a</td>
<td>441.8±20.7a</td>
<td>35.18±1.82a</td>
<td>6.00±0.03a</td>
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</table>

Values in parentheses are SE (n=4). Same letters in a column within each year are not significantly different at p<0.05 (ANOVA, LSD).
Acknowledgments
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References