



JEB™

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDPDOI: <https://doi.org/10.22438/jeb/38/3/MRN-443>

Studies on the variation of CO₂ fluxes and its characterization with soil temperature, moisture and dissolved organic carbon under different sulfur levels from alpine grassland in the Tibetan Plateau

Authors Info

X. Zeng¹ and Y. Gao^{2*}

¹Department of Landscape Architecture, Sichuan College of Architectural Technology, Chengdu-610399, China

²Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu-610041, China

*Corresponding Author Email : yhgao@imde.ac.cn

Key words

CO₂ fluxes,
Dissolved organic carbon
Microbial activity
Soil temperature,
Sulfur deposition

Publication Info

Paper received : 29.08.2016
Revised received : 26.12.2016
Accepted : 23.01.2017

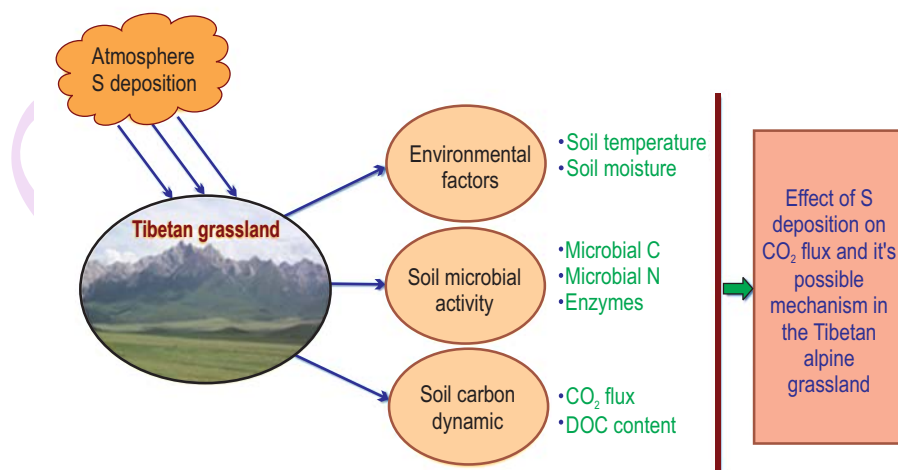
Abstract

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 CO₂ fluxes and characterize the relationships between CO₂ 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 CO₂ 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 CO₂ fluxes.

Results : No significant differences in CO₂ 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 CO₂ fluxes in both 2013 and 2014, but these fluxes were not limited by soil moisture in 2013.

Interpretation : The result indicated that CO₂ 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 CO₂ emission from the alpine grasslands.



Introduction

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 CO₂ emission have focused on forests (Kuz'yakov, 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 CO₂ emission. However, the relationship between CO₂ 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 sink 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 CO₂ fluxes within these ecosystems.

To understand the effects of sulfur deposition on CO₂ 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 CO₂ fluxes from alpine grasslands and to characterize the relationships between CO₂ 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⁻³ bulk density at 10 cm depth (Gao *et al.* 2015b) Site vegetation is typical of

alpine meadow in the region, and is dominated by *Kobresiasetchwanensis* Hand.-Mazz., and *Elymus nutans* Griseb., accompanied by *Festuca ovina* Linne, *Poapachyantha* 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 Na₂SO₄ dissolved in 2 l of water. Control plots received the same volume of water during each application.

Fluxes of CO₂ 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. CO₂ concentration of gas samples were determined with a gas chromatograph (Agilent 7890A, Agilent Technologies, Inc). Soil CO₂ fluxes were calculated as slope of linear regression between CO₂ 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 NH₄⁺-N 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 CO₂ fluxes. Differences among treatments were assessed by LSD method. Linear regression analysis was used to examine the relationship between CO₂ fluxes and soil temperature, water

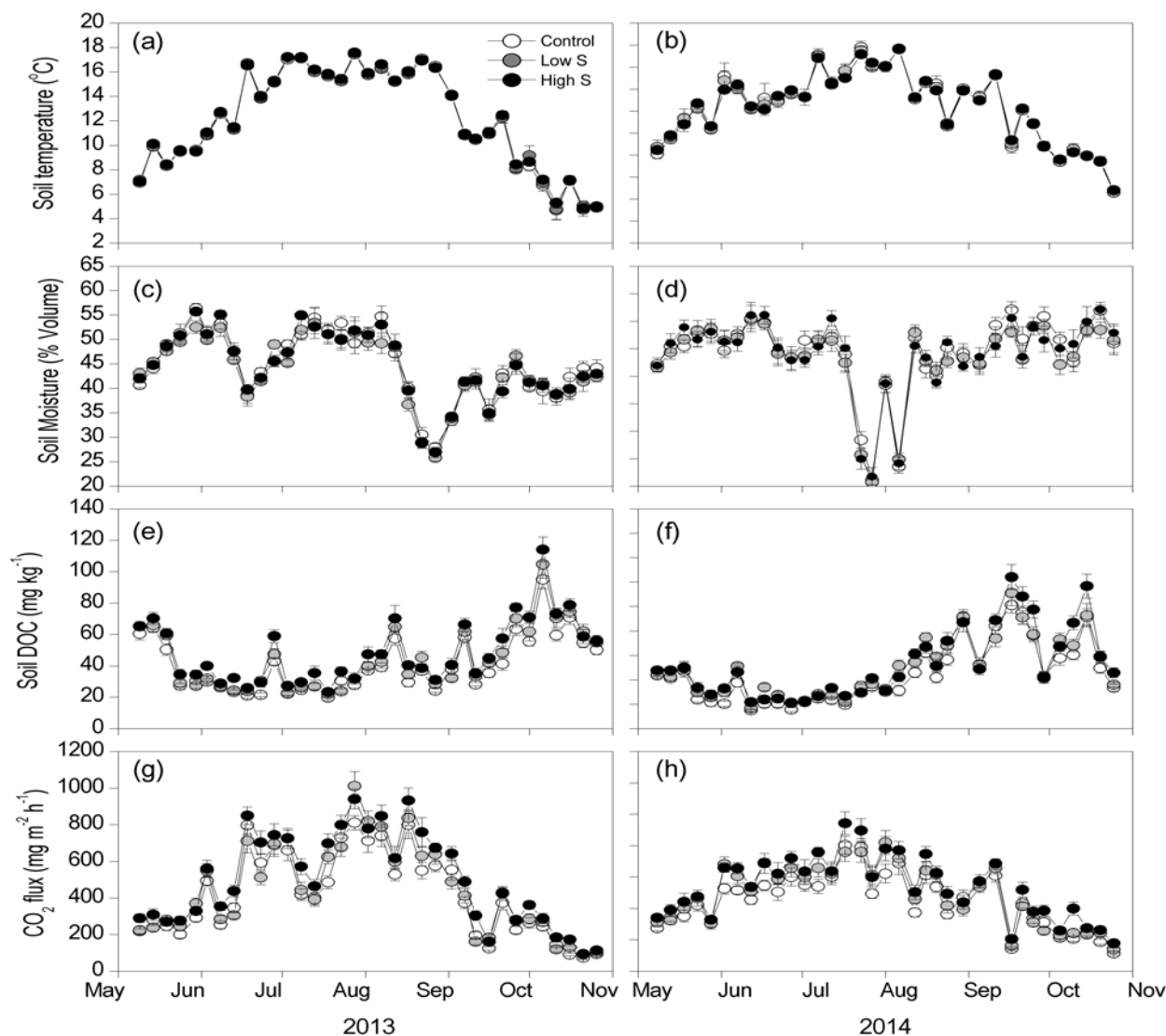


Fig. 1: Seasonal dynamics in soil temperature, moisture, dissolved organic carbon and CO₂ fluxes in alpine meadow with different sulfur (S) treatments during 2013 and 2014. Vertical bars indicate standard errors of four replications

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 $44.6 \pm 1.2\%$ in 2013 and $44.1 \pm 1.1\%$ 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).

Soil dissolved organic carbon 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 dissolved organic carbon 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).

CO₂ 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 increases in CO₂ fluxes throughout the measurement period. Cumulative CO₂ 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). CO_2 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 CO_2 flux ranged from 75 to 1012 $\text{mg m}^{-2} \text{h}^{-1}$ 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 CO_2 fluxes (Jiang *et al.*, 2010; Liu *et al.*, 2015). In the present study, CO_2 fluxes were strongly dependent on soil temperature, but exhibited inconsistent correlations with soil moisture. Soil moisture did not significantly affect CO_2 fluxes in 2013, probably because soil moisture on most sample dates (45-55%) was not low enough to limit CO_2 production. Similar relationships between CO_2 fluxes and soil moisture have been observed in other Tibetan alpine grasslands (Lin *et al.*, 2009; Jiang *et al.*, 2010).

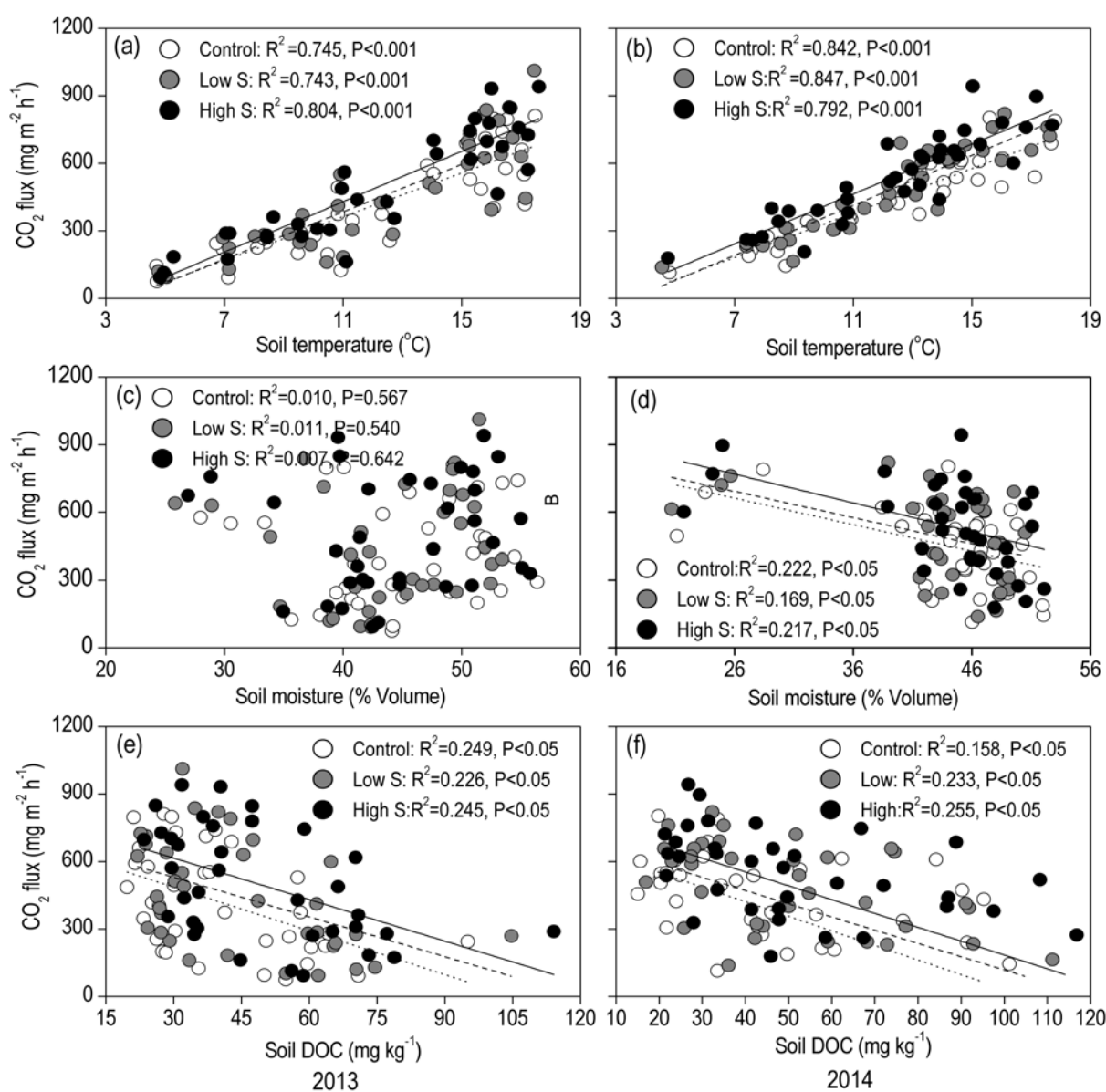


Fig. 2 : Relationships between CO_2 fluxes and soil temperature, soil moisture and dissolved organic carbon (DOC) in alpine meadow with different sulfur treatments during 2013 and 2014

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

Year	Effect	df	Soil temperature		Soil moisture		Soil DOC		CO ₂ fluxes	
			F-value	p	F-value	p	F-value	p	F-value	p
2013	Treatment	2	0.232	0.798	0.657	0.542	39.398	<0.001	2.994	0.101
	Date	34	1106.228	<0.001	77.252	<0.001	94.687	<0.001	166.257	<0.001
	Treatment × sampling date	68	0.338	0.860	0.827	0.624	0.730	0.719	1.530	0.159
2014	Treatment	2	1.014	0.075	0.047	0.955	20.089	<0.001	3.168	0.091
	Date	34	383.476	<0.001	53.238	<0.001	99.053	<0.001	86.372	<0.001
	Treatment × sampling date	68	0.686	0.643	0.775	0.653	1.442	0.162	1.228	0.283

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

Year	Treatment	CO ₂	Microbial C	Microbial N	Invertase	Urease	Catalase	pH
		gm ⁻²	mg kg ⁻¹	mg kg ⁻¹	mg glucose g ⁻¹	mg NH ₄ +N g ⁻¹	ml 0.1 N KMnO ₄ g ⁻¹	
2013	Control	1452.0±82.9a	529.4±17.9a	65.72±3.20a	441.0±15.1a	31.36±1.34a	1.72±0.08a	5.96±0.02a
	Low S	1549.4±76.7a	525.3±12.1a	61.03±2.53a	422.5±13.0a	29.49±1.21a	1.65±0.13a	5.99±0.04a
	High S	1737.3±92.0a	517.1±16.5a	59.93±1.84a	416.1±19.3a	28.09±1.69a	1.68±0.11a	5.91±0.03a
2014	Control	1556.2±70.9a	566.6±18.8a	71.89±3.05a	496.7±17.2a	37.6±1.35a	1.88±0.14a	6.08±0.03a
	Low S	1697.7±86.8a	553.3±16.1a	65.40±3.74a	460.7±13.4a	35.93±1.27a	1.79±0.08a	6.05±0.04a
	High S	1870.3±104.5a	549.7±19.5a	62.71±3.40a	441.8±20.7a	35.18±1.82a	1.70±0.11a	6.00±0.03a

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)

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 simulated 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 (Kuzuyakov, 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.

Acknowledgments

This research was supported by the National Science Foundation of China (40801089 and 41271276), the Hundred Young Talents Program of the Institute of Mountain Hazards and Environment (SDSQB-2016-02), and the Youth Innovation Promotion Association of the Chinese Academy of Sciences. We greatly appreciate Dr. Jeremy Shaw, Colorado State University, USA for editing the manuscript.

References

- Bengtson, P. and G. Bengtsson: Rapid turnover of DOC in temperate forests accounts for increased CO₂ production at elevated temperatures. *Ecol. Lett.*, **10**, 783–790 (2007).
- Brookes, P.C., A. Landman, G. Pruden and D.S. Jenkinson: Chloroform fumigation chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.*, **17**, 837–842 (1985).
- Cao, G.M., Y.H. Tang, W.H. Mo, Y.S. Wang, Y.N. Li and X.Q. Zhao: Grazing intensity alters soil respiration in an alpine meadow on the Tibetan Plateau. *Soil Biol. Biochem.*, **36**, 237–243 (2004).
- Chen, S.T., X.S. Shen, Z.H. Hu, H.S. Chen, Y.S. Shi and Y. Liu: Effects of simulated acid rain on soil CO₂ emission in a secondary forest in subtropical China. *Geoderma*, **189–190**, 65–71 (2012).
- Cleveland, C.C., J.C. Neff, A.R. Townsend and E. Hood: Composition, dynamics and fate of leached dissolved organic matter in terrestrial ecosystems: Results from a decomposition experiment. *Ecosystems*, **7**, 275–285 (2004).
- Don, A. and E. Schulze: Controls on fluxes and export of dissolved organic carbon in grasslands with contrasting soil types. *Biogeochemistry*, **91**, 117–131 (2008).
- Ganjurjav, H., Q.Z. Gao, W.N. Zhang, Y. Liang, Y.W. Li, X.J. Cao, Y.F. Wan, Y. Li and L.B. Danjui: Effects of warming on CO₂ fluxes in an alpine meadow ecosystem on the central Qinghai-Tibetan Plateau. *PLoS ONE*, **10**, e0132044 (2015).
- Gao, Y.H., H. Chen and X.H. Zeng: Effects of nitrogen and sulfur deposition on CH₄ and N₂O fluxes in high-altitude peatland soil under different water tables in the Tibetan Plateau. *Soil Sci. Plant Nutr.*, **60**, 404–410 (2014).
- Gao, Y.H., G. Ma, X.Y. Zeng, S.Q. Xu and D.X. Wang: Responses of microbial respiration to nitrogen addition in two alpine soils in the Qinghai-Tibetan Plateau. *J. Environ. Biol.*, **35**, 261–265 (2015a).
- Gao, Y.H., X.Y. Zeng, Q.Y. Xie and X.X. Ma: Release of carbon and nitrogen from alpine soils during thawing periods in the eastern Qinghai-Tibet Plateau. *Water Air Soil Pollut.*, **226**, 209 (2015b).
- Geng, Y., Y. Wang, K. Yang, S. Wang, H. Zeng, F. Baumann, P. Kuehn, T. Scholten and J.S. He: Soil respiration in Tibetan alpine grasslands: Belowground biomass and soil moisture, but not soil temperature, best explain the large-scale patterns. *PLoS ONE*, **7**, e34968 (2012).
- Jiang, C.M., G.R. Yu, H.J. Fang, G.M. Cao and Y.N. Li: Short-term effect of increasing nitrogen deposition on CO₂, CH₄ and N₂O fluxes in an alpine meadow on the Qinghai-Tibetan Plateau, China. *Atmos. Environ.*, **44**, 2920–2926 (2010).
- Kalbitz, K., S. Solinger, J.H. Park, B. Michalzik and E. Matzner: Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Sci*, **164**, 277–304 (2000).
- Kuzaykov, Y. : Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biol. Biochem.*, **38**, 425–448. (2006).
- Liang, G.H., X.Z. Liu, X.M. Chen, Q.Y. Qiu, D.Q. Zhang, G.W. Chu, J.X. Liu, S.Z. Liu and G.Y. Zhou: Response of soil respiration to acid rain in forests of different maturity in southern China. *PLoS ONE*, **8**, e62207 (2013).
- Lin, X.W., S.P. Wang, X.Z. Ma, G.P. Xu, C.Y. Luo, Y.N. Li, G.M. Liang and Z.B. Xie: Fluxes of CO₂, CH₄ and N₂O in an alpine meadow affected by yak excreta on the Qinghai-Tibetan plateau during summer grazing periods. *Soil Biol. Biochem.*, **47**, 718–725 (2009).
- Liu, Y.Y., S.K. Dong, S.L. Liu, H.K. Zhou, Q.Z. Gao, G.M. Cao, X.X. Wang, X.K. Su, Y. Zhang, L. Tang, H.D. Zhao and X.Y. Wu: Seasonal changes of CO₂, CH₄ and N₂O fluxes in different types of alpine grassland in the Qinghai-Tibetan Plateau of China. *Soil Biol. Biochem.*, **80**, 306–314 (2015).
- Lu, Z., D. G. Streets, Q. Zhang, S. Wang, G. R. Carmichael, Y. F. Cheng, C. Wei, M. Chin, T. Diehl and Q. Tan: Sulfur dioxide emissions in China and sulfur trends in East Asia since 2000. *Atmos. Chem. Phys.*, **10**, 6311e6331 (2010).
- Monteith, D. T., J. L. Stoddard, C. D. Evans, H. A. de Wit, M. Forsius, T. Högåsen, A. Wilander, B. L. Skjelkvåle, D. S. Jeffries, J. Vuorenmaa, B. Keller, J. Kopáček and J. Vesely: Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540 (2007).
- Oulehle, F., C. D. Evans, J. Hofmerster, R. Krejci, K. Tahovska, T. Persson, P. Cudlin and J. Hruska: Major changes in forest carbon and nitrogen cycling caused by declining sulphur deposition. *Glob. Change Biol.*, **17**, 3115–3129 (2011).
- Song, G.J., W.T. Qian, B. Ma and L. Zhou: Preliminary evaluation on the policies of acid rain control in China. *China Population. Res. Environ.*, **23**, 6–12 (2013).
- Tallec, T., D.C. Fauveau, M.P. Bataillé and A. Ourry: Effects of nitrogen and sulphur gradients on plant competition, N and S use efficiencies and species abundance in a grassland plant mixture. *Plant Soil*, **313**, 267–282 (2008).
- Vance, E.D., P.C. Brookes and D.S. Jenkinson: An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.*, **19**, 703–707 (1987).
- Vile, M.A. and S.D. Bridgman: Atmospheric sulfur deposition alters pathways of gaseous carbon production in peatlands. *Glob. Biogeochem. Cycle.*, **17**, 1058–1064 (2003).
- Vile, M.A., S.D. Bridgman and R.K. Wieder: Response of anaerobic carbon mineralization rates to sulfate amendments in a boreal peatland. *Ecol. Appl.*, **13**, 720–734 (2003).
- Wang, T.J., Z.Y. Hu, M. Xie, Y. Zhang, C.K. Xu and Z.H. Chao : Atmospheric sulfur deposition onto different ecosystems over China. *Environ. Geochem. Hlth.*, **26**, 169–177 (2004).
- Xu, G.H. and H.Y. Zheng: Handbook of Analysis Methods of Soil Microbiology. Agricultural Press, Beijing, China (1986).
- Yang, C.M., S. Shen, M.M. Wang and J.H. Li: Mild Stalination stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island, China. *J. Environ. Biol.*, **34**, 367–373 (2013).
- Yang, Y.H., J.Y. Fang, Y.H. Tang, C.J. Ji, C.Y. Zheng, J.S. He and B.A. Zhu: Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Glob. Change Biol.*, **14**, 1592–1599 (2008).
- Zhang, C.P., D.C. Niu, S.J. Hall, H.Y. Wen, H.D. Li, H. Fu, C.G. Wan and J.J. Elser: Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in a semi-arid grassland. *Soil Biol. Biochem.*, **75**, 113–123 (2014).