



Response of carbon dioxide emissions to sheep grazing and N application in an alpine grassland – Part 2: Effect of N application

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Received: 27 May 2013 – Published in Biogeosciences Discuss.: 22 July 2013

Revised: 19 December 2013 – Accepted: 27 February 2014 – Published: 3 April 2014

Abstract. Widespread nitrogen (N) enrichment resulting from anthropogenic activities has led to great changes in carbon exchange between the terrestrial biosphere and the atmosphere. Grassland is one of the most sensitive ecosystems to N deposition. However, the effect of N deposition on ecosystem respiration (R_e) in grasslands has been conducted mainly in temperate grasslands, which are limited largely by water availability, with few studies focused on alpine grasslands that are primarily constrained by low temperatures. Failure to assess the magnitude of the response in R_e outside the growing season (NGS) in previous studies also limits our understanding of carbon exchange under N deposition conditions. To address these knowledge gaps we used a combination of static closed chambers and gas chromatography in an alpine grassland from 2010 to 2011 to test the effects of N application on ecosystem respiration (R_e) both inside and outside the growing season. There was no significant change in CO₂ emissions under N application. R_e outside the growing season was at least equivalent to 9.4 % of the CO₂ fluxes during the growing season (GS). Annual R_e was calculated to be 279.0–403.9 g CO₂ m⁻² yr⁻¹ in Bayinbuluk alpine grasslands. In addition, our results indicate that soil temperature was the dominant abiotic factor regulating variation in R_e in the cold and arid environment. Our results suggest that short-

term N additions exert no significant effect on CO₂ emissions in alpine grassland.

1 Introduction

Widespread nitrogen (N) enrichment resulting from anthropogenic activities such as N deposition and fertilization has greatly changed ecosystem processes, structure, and functioning (Vitousek et al., 1997; Galloway et al., 2004; Liu et al., 2013). Ecosystem respiration (R_e) is an important source of carbon emissions in terrestrial ecosystems and it plays an important role in global carbon cycling (Raich and Schlesinger, 1992; Schlesinger and Andrews, 2000). Nitrogen fertilization will stimulate plant growth (Hooper and Johnson, 1999; LeBauer and Treseder, 2008; Xia and Wan, 2008) and accelerate the decomposition of light soil carbon (Neff et al., 2002) and this will also facilitate carbon exchange between the terrestrial biosphere and the atmosphere. It is assumed that N-induced variation in the R_e rate will produce a large impact on global carbon cycling and redistribution (Luo and Weng, 2011). However, the response patterns of R_e and its underlying mechanisms to N deposition vary considerably among ecosystems, and some knowledge gaps

remain in our understanding of the soil respiration response under N deposition conditions.

In particular, some observations in tropical and temperate ecosystems have revealed stimulation of R_e and its major component (i.e., soil respiration) by N deposition through increased plant biomass (Cleveland and Townsend, 2006; Fang et al., 2012; Tu et al., 2013). Other studies in tropical and temperate ecosystems have indicated that N deposition reduced soil respiration because fine root biomass and soil microbial activity were depressed by excessive N fertilization (Bowden et al., 2004; Burton et al., 2004; Olsson et al., 2005; Mo et al., 2008) or had no significant effect on soil respiration despite N fertilization altering microbial community composition (Allison et al., 2008). These contrasting response patterns and mechanisms among different ecosystems suggest that the response pattern and magnitude of R_e to N fertilization is texture-dependent and further studies in other ecosystems are required.

Grasslands are one of the largest biomes on earth and their response magnitude of R_e to N addition will exert a great influence on global carbon exchange between the terrestrial biosphere and the atmosphere. However, the effect of N deposition on carbon flux in grasslands has been examined mainly in temperate grasslands with few studies on alpine grasslands. Xia et al. (2009) and Niu et al. (2010) reported that N addition significantly increased net ecosystem CO_2 exchange during the growing season in temperate grassland and the response magnitude was regulated by annual N addition. Alpine grasslands may respond to N addition (unlike temperate grasslands) because the environmental factors and soil conditions differ markedly between the two types of ecosystem. For example, plant growth is limited mainly by water and N availability in temperate grasslands (Knapp et al., 1993; Briggs and Knapp, 1995; Paruelo et al., 1999; Bai et al., 2004) but plant growth is constrained primarily by low temperatures in alpine grasslands (Ma et al., 2012; Merbold et al., 2012). In addition, the soil organic matter content in the surface soil (0–20 cm) of alpine grasslands is higher than in temperate grasslands (Ni, 2002; Budge et al., 2011; Shi et al., 2012). Moreover, most studies of the effects of N fertilization on R_e have been conducted during the growing season when N-induced changes in biotic factors (i.e., community structure, microbial composition and plant growth strategy) and abiotic factors (i.e., soil water content and soil temperature) together determine the response pattern and magnitude of R_e (Xu and Wan, 2008; Xia et al., 2009; Chen et al., 2011). Outside the growing season the influence of biotic factors on R_e is minor and abiotic factors play a dominant role in regulating R_e (Wang et al., 2011). Failure to assess the R_e response pattern and magnitude outside the growing season will lead to underestimates of annual carbon emissions from the terrestrial biosphere and also limit our understanding of the mechanism of the R_e response under N deposition conditions.

To address these uncertainties and knowledge gaps we conducted a 2-year N addition experiment in alpine grass-

lands in central Asia to answer the following two questions. Firstly, how does R_e in alpine grasslands in central Asia respond to elevated N deposition both within and outside the growing season? Secondly, what is the major mechanism regulating the response pattern and magnitude of R_e in alpine grasslands in central Asia?

2 Materials and methods

2.1 Study sites

The study was conducted at the Bayinbuluk Grasslands Ecosystem Research Station, Chinese Academy of Sciences (42°53.1' N, 83°42.5' E). Bayinbuluk alpine grassland is located in the southern Tianshan mountains, Xinjiang Uygur Autonomous Region, central Asia and covers a total area of approximately 23 000 km². The grassland is at a mean altitude of 2500 m a.s.l. Local meteorological data (1980–1999) show a mean annual precipitation of 265.7 mm with 78.1 % occurring during the growing season (May to September) and the mean annual temperature is -4.8°C , with the lowest mean monthly temperature in January (-27.4°C) and the highest in July (11.2°C).

Five N fertilizer treatment sites were established. The area belongs to a cold and dry grassland type and the alpine grassland is dominated by *Stipa purpurea*. The five N fertilizer treatment sites were N_0 (0 kg N ha⁻¹ yr⁻¹), N_{10} (10 kg N ha⁻¹ yr⁻¹), N_{30} (30 kg N ha⁻¹ yr⁻¹), N_{90} (90 kg N ha⁻¹ yr⁻¹), and N_{150} (150 kg N ha⁻¹ yr⁻¹). Each treatment comprised four blocks (each 4 × 8 m with an 1 m-wide buffer zone) and the N addition experiments were conducted using ammonium nitrate (NH_4NO_3) applied in late May and June each year from 2009 to 2011. All the N fertilization sites have been ungrazed since 2005.

2.2 Measurement methods

CO_2 fluxes were measured using a static closed chamber method (opaque, static, manual stainless steel chambers, each 50 × 50 × 10 cm). The external surface of each chamber was covered with white plastic foam to minimize any impact of direct radiative heating during sampling. Each chamber was placed on a collar (50 × 50 × 10 cm) with a groove to prevent leakage during gas sampling. Each site had four replicate chambers. Gas samples were taken from inside the chambers 0, 15 and 30 min after chamber closure using a 60 ml plastic syringe and transferred immediately to a pre-evacuated 50 ml air bag (Hede Inc., Dalian, Liaoning, China). CO_2 fluxes of R_e were sampled during the same time period (12:00–14:00, GMT + 8) from May 2010 to September 2011 (no sampling in January and February 2011 because of the low mean temperatures of -33.3 and -23.0°C , respectively) and four times per month during the growing season (10 May to 8 October, about 152 days), and twice per month outside the growing season (9 October to 9 May, about 213 days) at

the five sites. CO₂ samples, which were stored in separate air bags, were analyzed by gas chromatography (Agilent 4890D, Agilent Technologies, Wilmington, DE) within 1 week. Calculation of CO₂ fluxes followed the description of Zhang et al. (2005).

Aboveground net primary biomass (ANPP) values of the different treatments were obtained in July 2010 and 2011 (1 × 1 m plots and *n* = 3 for each treatment). Soil samples were collected from each site (*n* = 5) to determine soil organic matter (SOM) and total nitrogen content (TN) to a depth of 30 cm. Air temperature and soil temperature at 10 cm depth and soil water content at 10 cm depth were monitored by an Auto Weather Station (Campbell Scientific, Logan, UT) adjacent to plots around the whole experiment (sites N₀, N₁₀, N₃₀, N₉₀ and N₁₅₀).

2.3 Calculations and statistical analysis

Statistical analysis was carried out using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and SigmaPlot (SigmaPlot for Windows, Version 10, SyStat Software Inc., San Jose, CA). Two-way ANOVA was used to examine the effects of N, year and their interaction on ANPP, SOM and TN using N and year as fixed factors. We also used repeated measures analysis of variance (RMANOVA) to examine interannual variation in CO₂ fluxes with combined N fertilization as treatment. Monthly mean CO₂ fluxes in each plot were calculated by averaging all measurements during the same month. If significant interactive effects between N and year were detected (i.e., *P* < 0.05 for year effects), RMANOVA was used again to examine treatment effects on CO₂ fluxes within each year. Between-subject effects were examined as N and within-subject effects were time of month and its interaction, with N linear and non-linear curve fitting performed using SigmaPlot software to examine the relationships between CO₂ fluxes and the environmental factors.

3 Results

3.1 Responses of community productivity and soil properties to N addition

Results from two-way ANOVA show that N and N × year interaction had no significant influence on ANPP, SOM or TN in the topsoil (0–30 cm) but these variables differed significantly between years (all *P* < 0.01) (Table 1). Average ANPP, SOM and TN in 2011 were 217.7 g m⁻¹, 84.0 g kg⁻¹ and 6.0 g kg⁻¹, which were 2.0, 1.5 and 1.2 times higher than that in 2010, respectively.

3.2 CO₂ fluxes under different N addition rates

Results from RMANOVA show that significant interannual variation in *R_e* fluxes was detected (*P* = 0.001) but N and N × year had no significant effect on *R_e* fluxes (*P* = 0.792

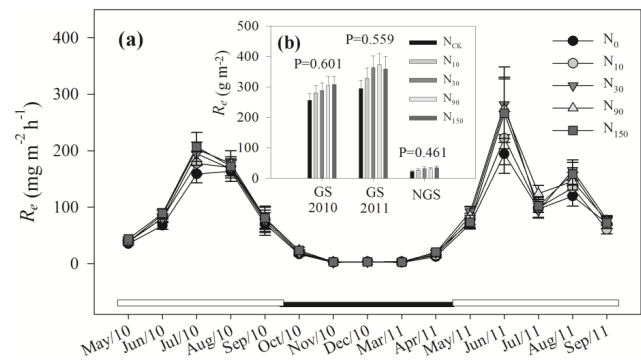


Fig. 1. CO₂ fluxes of ecosystem respiration (*R_e*) at sites N₀ (ungrazed since 2005), N₁₀ (10 kg N ha⁻¹ yr⁻¹), N₃₀ (30 kg N ha⁻¹ yr⁻¹), N₉₀ (90 kg N ha⁻¹ yr⁻¹) and N₁₅₀ (150 kg N ha⁻¹ yr⁻¹) within and outside the growing season using the static closed chamber method. White and black horizontal bars represent within (GS, 2010 and 2011) and outside (NGS) the growing season, respectively.

and 0.965, respectively). When RMANOVA was used to examine each year separately, both N and N × month had no significant effect on *R_e* fluxes (all *P* > 0.05) but significant seasonal patterns were observed in both years (*P* < 0.001). *R_e* fluxes reached their maximum values of 194.1, 222.1, 281.6, 274.2 and 266.6 mg m⁻² h⁻¹ at N₀, N₁₀, N₃₀, N₉₀ and N₁₅₀ in June 2011, respectively (Fig. 1a). Similarly, the *R_e* fluxes reached minimum values of 2.0 (March 2011), 3.1 (November 2010), 2.0 (November 2010), 2.8 (March 2011) and 2.5 (March 2011) mg m⁻² h⁻¹ at N₀, N₁₀, N₃₀, N₉₀ and N₁₅₀ under N addition, respectively (Fig. 1a). When monthly mean *R_e* fluxes were reclassified into growing season (GS) and outside growing season (NGS), N fertilization still had no significant effect on *R_e* fluxes in GS (2010 and 2011) and NGS (*P* = 0.601, 0.559, 0.461, respectively) (Fig. 1b). The ranges in *R_e* emissions in GS (2010 and 2011) and NGS were 255.9–307.7, 294.7–372.7 and 23.1–34.8 g m⁻², respectively (Fig. 1b).

3.3 Varying CO₂ emissions in NGS and GS under N addition

Under N addition conditions *R_e* increased from 255.9 to 358.4 g m⁻² in GS and increased from 23.1 to 34.8 g m⁻² in NGS. In NGS, *R_e* increased by 20.1–51.0% under the four different levels of N addition and the increment in NGS was twice the increase in *R_e* in GS. In addition, the *R_e* values of the five sites (N₀, N₁₀, N₃₀, N₉₀ and N₁₅₀) in NGS were equivalent to 8.4, 9.1, 9.8, 9.2 and 10.5% of the CO₂ fluxes in GS, respectively. Moreover, a low level of N addition (10 kg N ha⁻¹ yr⁻¹) did not promote *R_e* in GS or NGS. *R_e* from NGS was increased by 51.0% by the high level of N addition (150 kg N ha⁻¹ yr⁻¹). These results indicate that more studies are required on CO₂ emissions from NGS under elevated N deposition or N application.

Table 1. Effects of nitrogen (N), year (Y) and their interaction on aboveground net primary productivity (ANPP), soil organic matter (SOM) and total nitrogen (TN) in topsoil (0–30 cm) (Mean \pm SD).

Year (Y)	Nitrogen (N)	ANPP (g m^{-2})	SOM (g kg^{-1})	TN (g kg^{-1})
2010	N ₀	94.7 \pm 13.7	56.7 \pm 2.4	5.1 \pm 0.2
	N ₁₀	99.4 \pm 10.0	60.5 \pm 3.5	5.1 \pm 0.2
	N ₃₀	112.4 \pm 11.2	64.2 \pm 1.2	5.6 \pm 0.1
	N ₉₀	123.7 \pm 13.8	54.9 \pm 3.6	4.7 \pm 0.4
	N ₁₅₀	123.3 \pm 8.5	53.7 \pm 3.4	4.7 \pm 0.4
2011	N ₀	175.5 \pm 33.7	85.0 \pm 7.9	6.5 \pm 1.0
	N ₁₀	226.2 \pm 56.5	86.9 \pm 5.7	5.9 \pm 0.7
	N ₃₀	253.4 \pm 57.1	81.9 \pm 5.0	5.7 \pm 0.2
	N ₉₀	205.6 \pm 31.2	82.1 \pm 11.3	6.1 \pm 1.0
	N ₁₅₀	228.0 \pm 50.1	84.3 \pm 10.2	6.0 \pm 0.9
ANOVA		(P value)		
	N	0.605	0.891	0.944
	Y	<0.0001	<0.0001	0.012
	N \times Y	0.834	0.871	0.775

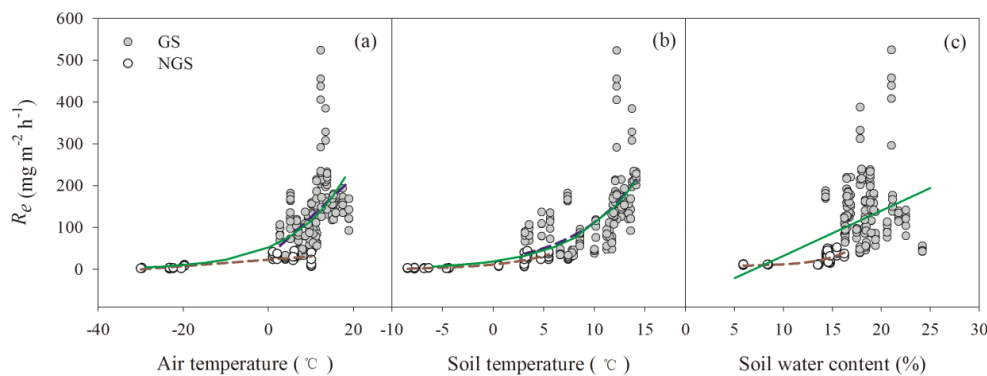


Fig. 2. Relationships between CO₂ fluxes (R_e) and air temperature (a), soil temperature at 10 cm depth (b) and soil water content at 10 cm depth (c). Green, dark red and dark blue lines represent the best fitted relationship between R_e and biotic factors over the whole year, outside the growing season (NGS) and within the growing season (GS), respectively.

3.4 Correlations between ecosystem respiration and abiotic factors

Generally, R_e increased exponentially with increasing air temperature and soil temperature and linearly with soil moisture (all $P < 0.001$) (Fig. 2). Air temperature, soil temperature and soil water content explained 40 %, 56 % and 23 % of the total R_e variation, respectively. When data were separated into NGS and GS groups, R_e in NGS and GS increased linearly with increasing air temperature and exponentially with soil temperature, but only R_e in NGS was related exponentially with soil water content (Fig. 2). Indeed, soil temperature was the dominant abiotic factor regulating R_e in NGS and GS as it independently explained 80 % and 32 % of the total variation in R_e , while air temperature was the next important factor (explaining 70 % and 19 % of total variation in R_e). Soil water content was important only in NGS and contributed 50 % of the total variation in R_e .

4 Discussion

4.1 Response of ANPP and soil properties to N addition

Our experiment at Bayinbuluk alpine grasslands shows that N fertilization did not significantly increase ANPP in two consecutive treatment years (2010–2011), which was inconsistent with the increased response patterns found in sub-alpine grasslands in the European central Alps (Bassin et al., 2012), an alpine meadow in Colorado (Bowman et al., 2006) and the Tibetan Plateau (Song et al., 2012). Given that the form of fertilizer N was similar among these studies (NH_4NO_3) and the highest N application rate in our study ($150 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) was three times or twice as high as that in other ecosystems (50 , 60 and $75 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the central Alps, Colorado and Tibetan Plateau, respectively), the absence of a significant effect of N fertilization on ANPP in our study suggests that plant growth in Bayinbuluk may be constrained by other potential factors (i.e., climatic conditions

and plant growth strategy). Firstly, previous meta-analysis has shown that the percentage change in plant biomass under N addition conditions was positively correlated with mean annual precipitation (MAP) (Xia and Wan, 2008). MAP in our study (256.7 mm) was lower than on the Tibetan Plateau (560 mm) (Song et al., 2012), in Colorado (900 mm) (Bowman et al., 2006) or in the central Alps (1000 mm) (Bassin et al., 2012), suggesting that the absence of a significant effect of N fertilization on ANPP in our study was partly due to low MAP. Secondly, the plant community in our study was dominated by a perennial grass (i.e., *Stipa purpurea*) and the growth and leaf gas exchange of perennial grass has been reported to be conservative because the soil water deficit in the dry meadow was aggravated by N addition (Bowman et al., 1995). If this response pattern occurs widely in all alpine grasslands, we can attribute the absence of a significant response of ANPP partly to the conservative growth strategy of the dominant grass species to N fertilization.

Our results also show that N fertilization had no significant effect on top SOM or TN in both treatment years and this is consistent with results from temperate grasslands in northern China (Li et al., 2013) but contrary to results from alpine meadows in Colorado (Freppaz et al., 2012). The different response patterns of SOM and TN may be explained in terms of the period over which N applications have been made. The short periods of N fertilizer addition in our study (3 years) and in temperate grassland in north China (4 years) (Li et al., 2013) were short relative to the studies on alpine meadow in Colorado (16 years) (Freppaz et al., 2012). The increased SOM and TN in the Colorado dry meadow may result from the long-term accumulation of litter inputs and N mineralization.

4.2 Effects of N fertilizer application on CO₂ fluxes

Previous studies showed that N fertilization significantly changed R_e in GS as the plant community and soil properties were altered under N fertilization conditions. However, our results show that the N had no significant effects on R_e but a clear seasonal response pattern was detected in each year, which was consistent with recent studies conducted in an Alaskan boreal forest (Allison et al., 2008) and in a Tibetan Plateau alpine meadow (Jiang et al., 2013). One possible explanation for this pattern was that R_e was closely related with ANPP and SOM, which also did not respond to N fertilization in our study. The range of seasonal maximum R_e in our study (194.1–266.6 mg m⁻² h⁻¹ in June) was similar to that in the Tibetan Plateau alpine meadow (about 129.6–259.2 mg m⁻¹ h⁻¹) (Kato et al., 2006; Jiang et al., 2013) but the seasonal minimum R_e range in our study (2.0–2.8 mg m⁻² h⁻¹ in March) was higher than that in Tibetan Plateau alpine meadow (about 1.8 mg m⁻² h⁻¹) (Kato et al., 2006; Jiang et al., 2013). Based on monthly mean R_e flux, we calculated the annual R_e in Bayinbuluk alpine grasslands to be about 279.0–403.9 g CO₂ m⁻² yr⁻¹, a lower value than

found in European grasslands (1800–6000 g CO₂ m⁻² yr⁻¹) (Gilmanov et al., 2007).

We could not determine the monthly R_e values in January and February because of the very low temperatures but a previous study demonstrated that winter R_e in grasslands was controlled mainly by air and soil temperatures (Wang et al., 2011). Given that air temperatures in January and February were extremely low (mean monthly air temperatures were –33.3 °C and –23.0 °C, respectively), we assume that the missing R_e values in our study were correspondingly low relative to the whole annual R_e . Even so, our results show that carbon fluxes outside the growing season were equivalent to at least 9.4 % of that during the growing season, suggesting that annual carbon exchange between soil and atmosphere was underestimated if only the growing season was taken into consideration.

4.3 Mechanism regulation carbon fluxes in alpine grasslands

Our study indicates that soil and air temperatures were the dominant abiotic factors regulating R_e variation in NGS, GS and over the whole year and this is in agreement with studies from global and regional databases (Bond-Lamberty and Thomson, 2010; Wang et al., 2011). It is believed that microbial activity in soil cohorts and mitochondrial electron transport in plants increase with increasing temperature over a given temperature range (Atkin et al., 2000; Mikan et al., 2002), which in turn results in a positive feedback on soil and ecosystem respiration. Indeed, we found that the contribution of soil temperature to ecosystem respiration was more important than air temperature (i.e., R^2 explained by soil temperature was 12–16 % higher than by air temperature). Moreover, air temperatures in GS and NGS were linearly correlated with R_e but soil temperature was exponentially correlated with R_e .

We also found no significant correlation between soil water content and R_e in GS despite the apparent linear and exponential correlations between the whole year and NGS. We assumed that in the cold grasslands of central Asia soil water content was not the primary abiotic factor limiting plant growth and soil microbial activity. A tight correlation between temperature and R_e suggests that carbon flux in GS in this cold grassland was mainly determined by temperature.

5 Conclusions

Our experiment in central Asia indicates that short-term N fertilization did not increase aboveground net primary productivity or alter soil organic matter or total N content. Ecosystem respiration also showed no significant response to N fertilization and ecosystem respiration outside the growing season was equivalent to at least 9.4 % of the CO₂ fluxes during the growing season. Moreover, annual ecosystem

respiration emissions were 279.0–403.9 g CO₂ m⁻² yr⁻¹ in the Bayinbuluk alpine grasslands. Generally, soil temperature was the primary limiting abiotic factor regulating ecosystem respiration in this cold alpine grassland.

Acknowledgements. This work was financially supported by National Basic Research Program of China (2014CB954202), the National Natural Science Foundation of China (41340041), and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA05050301-2).

Edited by: R. Conant

References

- Allison, S. D., Czimczik, C. I., and Treseder, K. K.: Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest, *Glob. Change Biol.*, 14, 1156–1168, 2008.
- Atkin, O. K., Edwards, E. J., and Loveys, B. R.: Response of root respiration to changes in temperature and its relevance to global warming, *New Phytol.*, 147, 141–154, 2000.
- Bai, Y. F., Han, X. G., Wu, J. G., Chen, Z. Z., and Li, L. H.: Ecosystem stability and compensatory effects in the Inner Mongolia grassland, *Nature*, 431, 181–184, 2004.
- Bassin, S., Schälajda, J., Vogel, A., and Suter, M.: Different types of sub-alpine grassland respond similarly to elevated nitrogen deposition in terms of productivity and sedge abundance, *J. Veg. Sci.*, 23, 1024–1034, 2012.
- Bond-Lamberty, B. and Thomson, A.: Temperature-associated increases in the global soil respiration record, *Nature*, 464, 579–582, 2010.
- Bowden, R. D., Davidson, E., Savage, K., Arabia, C., and Steudler, P.: Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest, *Forest Ecol. Manag.*, 196, 43–56, 2004.
- Bowman, W. D., Gartner, J. R., Holland, K., and Wiedermann, M.: Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: Are we there yet?, *Ecological Applications*, 16, 1183–1193, 2006.
- Bowman, W. D., Theodose, T. A., and Fisk, M. C.: Physiological and production responses of plant growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change, *Oecologia*, 101, 217–227, 1995.
- Briggs, J. M. and Knapp, A. K.: Interannual variability in primary production in tallgrass prairie climate, soil moisture, topographic position, and fire as determinants of aboveground biomass, *Am J Bot.*, 82, 1024–1030, 1995.
- Budge, K., Leifeld, J., Hiltbrunner, E., and Fuhrer, J.: Alpine grassland soils contain large proportion of labile carbon but indicate long turnover times, *Biogeosciences*, 8, 1911–1923, doi:10.5194/bg-8-1911-2011, 2011.
- Burton, A. J., Pregitzer, K. S., Crawford, J. N., Zogg, G. P., and Zak, D. R.: Simulated chronic NO₃⁻ deposition reduces soil respiration in northern hardwood forests, *Glob. Change Biol.*, 10, 1080–1091, 2004.
- Chen, B., Coops, N. C., Black, T. A., Jassal, R. S., Chen, J. M., and Johnson, M.: Modeling to discern nitrogen fertilization impacts on carbon sequestration in a Pacific Northwest Douglas fir forest in the first postfertilization year, *Glob. Change Biol.*, 17, 1442–1460, 2011.
- Cleveland, C. C. and Townsend, A. R.: Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere, *P. Natl. Acad. Sci. USA*, 103, 10316–10321, 2006.
- Fang, H., Cheng, S., Yu, G., Zheng, J., Zhang, P., Xu, M., Li, Y., and Yang, X.: Responses of CO₂ efflux from an alpine meadow soil on the Qinghai Tibetan Plateau to multi-form and low-level N addition, *Plant Soil*, 351, 177–190, 2012.
- Freppaz, M., Williams, M. W., Seastedt, T., and Filippa, G.: Response of soil organic and inorganic nutrients in alpine soils to a 16-year factorial snow and N-fertilization experiment, Colorado Front Range, USA, *Appl. Soil Ecol.*, 62, 131–141, 2012.
- Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R., and Vorosmarty, C. J.: Nitrogen cycles: past, present, and future, *Biogeochemistry*, 70, 153–226, 2004.
- Gilmanov, T. G., Soussana, J. E., Aires, L., Allard, V., Ammann, C., Balzarolo, M., Barcza, Z., Bernhofer, C., Campbell, C. L., Cernusca, A., Cescatti, A., Clifton-Brown, J., Dirks, B. O. M., Dore, S., Eugster, W., Fuhrer, J., Gimeno, C., Gruenwald, T., Haszpra, L., Hensen, A., Ibrom, A., Jacobs, A. F. G., Jones, M. B., Lanigan, G., Laurila, T., Lohila, A., Manca, G., Marcolla, B., Nagy, Z., Pilegaard, K., Pinter, K., Pio, C., Raschi, A., Rogiers, N., Sanz, M. J., Stefani, P., Sutton, M., Tuba, Z., Valentini, R., Williams, M. L., and Wohlfahrt, G.: Partitioning European grassland net ecosystem CO₂ exchange into gross primary productivity and ecosystem respiration using light response function analysis, *Agr. Ecosyst. Environ.*, 121, 93–120, 2007.
- Hooper, D. U. and Johnson, L.: Nitrogen limitation in dryland ecosystems: Responses to geographical and temporal variation in precipitation, *Biogeochemistry*, 46, 247–293, 1999.
- Jiang, J., Zong, N., Song, M., Shi, P., Ma, W., Fu, G., Shen, Z., Zhang, X., and Ouyang, H.: Responses of ecosystem respiration and its components to fertilization in an alpine meadow on the Tibetan Plateau, *Eur. J. Soil. Biol.*, 56, 101–106, 2013.
- Kato, T., Tang, Y. H., Gu, S., Hirota, M., Du, M. Y., Li, Y. N., and Zhao, X. Q.: Temperature and biomass influences on interannual changes in CO₂ exchange in an alpine meadow on the Qinghai-Tibetan Plateau, *Glob. Change Biol.*, 12, 1285–1298, 2006.
- Knapp, A. K., Fahnestock, J. T., Hamburg, S. P., Statland, L. B., Seastedt, T. R., and Schimel, D. S.: Landscape patterns in soil plant water relations and primary production in tallgrass prairie, *Ecology*, 74, 549–560, 1993.
- LeBauer, D. S. and Treseder, K. K.: Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed, *Ecology*, 89, 371–379, 2008.
- Li, Q., Bai, H., Liang, W., Xia, J., Wan, S., and van der Putten, W. H.: Nitrogen addition and warming independently influence the belowground micro-food web in a temperate steppe, *PLoS One*, 8, 2013.
- Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., and Zhang, F.: Enhanced nitrogen deposition over China, *Nature*, doi:10.1038/nature11917, 2013.

- Luo, Y. Q. and Weng, E. S.: Dynamic disequilibrium of the terrestrial carbon cycle under global change, *Trends Ecol. Evol.*, 26, 96–104, 2011.
- Ma, J., Ji, C., Han, M., Zhang, T., Yan, X., Hu, D., Zeng, H., and He, J.: Comparative analyses of leaf anatomy of dicotyledonous species in Tibetan and Inner Mongolian grasslands, *Sci. China Life Sci.*, 55, 68–79, 2012.
- Merbold, L., Rogiers, N., and Eugster, W.: Winter CO₂ fluxes in a sub-alpine grassland in relation to snow cover, radiation and temperature, *Biogeochemistry*, 111, 287–302, 2012.
- Mikan, C. J., Schimel, J. P., and Doyle, A. P.: Temperature controls of microbial respiration in arctic tundra soils above and below freezing, *Soil Biol. Biochem.*, 34, 1785–1795, 2002.
- Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., and Wang, H.: Nitrogen addition reduces soil respiration in a mature tropical forest in southern China, *Glob. Change Biol.*, 14, 403–412, 2008.
- Neff, J. C., Townsend, A. R., Gleixner, G., Lehman, S. J., Turnbull, J., and Bowman, W. D.: Variable effects of nitrogen additions on the stability and turnover of soil carbon, *Nature*, 419, 915–917, 2002.
- Ni, J.: Carbon storage in grasslands of China, *J. Arid Environ.*, 50, 205–218, 2002.
- Niu, S., Wu, M., Han, Y., Xia, J., Zhang, Z., Yang, H., and Wan, S.: Nitrogen effects on net ecosystem carbon exchange in a temperate steppe, *Glob. Change Biol.*, 16, 144–155, 2010.
- Olsson, P., Linder, S., Giesler, R., and Hogberg, P.: Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration, *Glob. Change Biol.*, 11, 1745–1753, 2005.
- Paruelo, J. M., Lauenroth, W. K., Burke, I. C., and Sala, O. E.: Grassland precipitation-use efficiency varies across a resource gradient, *Ecosystems*, 2, 64–68, 1999.
- Raich, J. W. and Schlesinger, W. H.: The Global Carbon Dioxide Flux in Soil Respiration and Its Relationship to Vegetation and Climate, *Tellus B*, 44, 81–99, 1992.
- Schlesinger, W. H. and Andrews, J. A.: Soil respiration and the global carbon cycle, *Biogeochemistry*, 48, 7–20, 2000.
- Shi, Y., Baumann, F., Ma, Y., Song, C., Kühn, P., Scholten, T., and He, J.-S.: Organic and inorganic carbon in the topsoil of the Mongolian and Tibetan grasslands: pattern, control and implications, *Biogeosciences*, 9, 2287–2299, doi:10.5194/bg-9-2287-2012, 2012.
- Song, M. H., Yu, F. H., Ouyang, H., Cao, G. M., Xu, X. L., and Cornelissen, J. H. C.: Different inter-annual responses to availability and form of nitrogen explain species coexistence in an alpine meadow community after release from grazing, *Glob. Change Biol.*, 18, 3100–3111, 2012.
- Tu, L. H., Hu, T. X., Zhang, J., Li, X. W., Hu, H. L., Liu, L., and Xiao, Y. L.: Nitrogen addition stimulates different components of soil respiration in a subtropical bamboo ecosystem, *Soil Biol. Biochem.*, 58, 255–264, 2013.
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., and Tilman, D.: Human alteration of the global nitrogen cycle: Sources and consequences, *Ecol. Appl.*, 7, 737–750, 1997.
- Wang, T., Ciais, P., Piao, S. L., Otlé, C., Brender, P., Maignan, F., Arain, A., Cescatti, A., Gianelle, D., Gough, C., Gu, L., Lafleur, P., Laurila, T., Marcolla, B., Margolis, H., Montagnani, L., Moors, E., Saigusa, N., Vesala, T., Wohlfahrt, G., Koven, C., Black, A., Dellwik, E., Don, A., Hollinger, D., Knohl, A., Monson, R., Munger, J., Suyker, A., Varlagin, A., and Verma, S.: Controls on winter ecosystem respiration in temperate and boreal ecosystems, *Biogeosciences*, 8, 2009–2025, doi:10.5194/bg-8-2009-2011, 2011.
- Xia, J., Niu, S., and Wan, S.: Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe, *Glob. Change Biol.*, 15, 1544–1556, 2009.
- Xia, J. and Wan, S.: Global response patterns of terrestrial plant species to nitrogen addition, *New Phytol.*, 179, 428–439, 2008.
- Xu, W. and Wan, S.: Water- and plant-mediated responses of soil respiration to topography, fire, and nitrogen fertilization in a semiarid grassland in northern China, *Soil Biol. Biochem.*, 40, 679–687, 2008.
- Zhang, J. B., Song, C. C., and Yang, W. Y.: Cold season CH₄, CO₂ and N₂O fluxes from freshwater marshes in northeast China, *Chemosphere*, 59, 1703–1705, 2005.