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

Y. M. Gong<sup>1</sup>, A. Mohammat<sup>1</sup>, X. J. Liu<sup>2</sup>, K. H. Li<sup>1</sup>, P. Christie<sup>3</sup>, F. Fang<sup>4</sup>, W. Song<sup>1,5</sup>, Y. H Chang<sup>1,5</sup>, W. X. Han<sup>2</sup>, X. T. Lü<sup>6</sup>, Y. Y. Liu<sup>1</sup>, and Y. K Hu<sup>1</sup>

<sup>1</sup>Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China

<sup>2</sup>College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China

<sup>3</sup>Agri-Environment Branch, Agri-Food and Biosciences Institute, Belfast BT9 5PX, UK

<sup>4</sup>State Key Laboratory of Earth Surface Processes and Resource Ecology, Institute of Resources Science, College of

Resources Science and Technology, Beijing Normal University, Beijing 100875, China

<sup>5</sup>Graduate University of the Chinese Academy of Sciences, Beijing, 100039, China

<sup>6</sup>State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110164, China

Correspondence to: A. Mohammat (anwar@ms.xjb.ac.cn) and Y. K. Hu (gongym@ms.xjb.ac.cn)

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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  $R_{\rm e}$  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 CO<sub>2</sub> emissions under N application. Re outside the growing season was at least equivalent to 9.4 % of the CO<sub>2</sub> fluxes during the growing season (GS). Annual Re was calculated to be 279.0–403.9 g  $CO_2$  m<sup>-2</sup> yr<sup>-1</sup> 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 shortterm N additions exert no significant effect on CO<sub>2</sub> 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  $R_{\rm e}$  rate will produce a large impact on global carbon cycling and redistribution (Luo and Weng, 2011). However, the response patterns of  $R_{\rm e}$  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  $R_{\rm e}$  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<sub>2</sub> 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  $R_{\rm e}$  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  $R_{\rm e}$ (Xu and Wan, 2008; Xia et al., 2009; Chen et al., 2011). Outside the growing season the influence of biotic factors on  $R_{\rm e}$ is minor and abiotic factors play a dominant role in regulating  $R_{\rm e}$  (Wang et al., 2011). Failure to assess the  $R_{\rm e}$  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  $(42^{\circ}53.1' \text{ N}, 83^{\circ}42.5' \text{ E})$ . 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 km<sup>2</sup>. 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  $-4.8 \,^{\circ}\text{C}$ , with the lowest mean monthly temperature in January ( $-27.4 \,^{\circ}\text{C}$ ) and the highest in July (11.2  $^{\circ}\text{C}$ ).

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<sub>0</sub> (0kg N ha<sup>-1</sup> yr<sup>-1</sup>), N<sub>10</sub> (10kg N ha<sup>-1</sup> yr<sup>-1</sup>), N<sub>30</sub> (30kg N ha<sup>-1</sup> yr<sup>-1</sup>), N<sub>90</sub> (90 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and N<sub>150</sub> (150 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Each treatment comprised four blocks (each  $4 \times 8$  m with an 1 m-wide buffer zone) and the N addition experiments were conducted using ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) 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<sub>2</sub> fluxes were measured using a static closed chamber method (opaque, static, manual stainless steel chambers, each  $50 \times 50 \times 10$  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  $(50 \times 50 \times 10 \text{ cm})$  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 preevacuated 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

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the five sites.  $CO_2$  samples, which were stored in separate air bags, were analyzed by gas chromatography (Agilent 4890D, Agilent Technologies, Wilmington, DE) within 1 week. Calculation of  $CO_2$  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  $(1 \times 1 \text{ m plots} \text{ and } n = 3 \text{ for each treatment})$ . 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 N<sub>0</sub>, N<sub>10</sub>, N<sub>30</sub>, N<sub>90</sub> and N<sub>150</sub>).

### 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 CO<sub>2</sub> fluxes with combined N fertilization as treatment. Monthly mean CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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<sup>-1</sup>, 84.0 g kg<sup>-1</sup> and 6.0 g kg<sup>-1</sup>, which were 2.0, 1.5 and 1.2 times higher than that in 2010, respectively.

### 3.2 CO<sub>2</sub> 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.** CO<sub>2</sub> fluxes of ecosystem respiration ( $R_e$ ) at sites N<sub>0</sub> (ungrazed since 2005), N<sub>10</sub> (10 kg Nha<sup>-1</sup> yr<sup>-1</sup>), N<sub>30</sub> (30 N kg ha<sup>-1</sup> yr<sup>-1</sup>), N<sub>90</sub> (90 N kg ha<sup>-1</sup> yr<sup>-1</sup>) and N<sub>150</sub> (150 N kg ha<sup>-1</sup> yr<sup>-1</sup>) 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).  $R_{\rm e}$  fluxes reached their maximum values of 194.1, 222.1, 281.6, 274.2 and 266.6 mg m<sup>-2</sup> h<sup>-1</sup> at N<sub>0</sub>, N<sub>10</sub>, N<sub>30</sub>, N<sub>90</sub> and N<sub>150</sub> in June 2011, respectively (Fig. 1a). Similarly, the Re 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<sup>-2</sup> h<sup>-1</sup> at N<sub>0</sub>, N<sub>10</sub>, N<sub>30</sub>, N<sub>90</sub> and N<sub>150</sub> 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  $23.1-34.8 \text{ g m}^{-2}$ , respectively (Fig. 1b).

# **3.3** Varying CO<sub>2</sub> emissions in NGS and GS under N addition

Under N addition conditions  $R_e$  increased from 255.9 to 358.4 g m<sup>-2</sup> in GS and increased from 23.1 to 34.8 g m<sup>-2</sup> 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 (N<sub>0</sub>, N<sub>10</sub>, N<sub>30</sub>, N<sub>90</sub> and N<sub>150</sub>) in NGS were equivalent to 8.4, 9.1, 9.8, 9.2 and 10.5% of the CO<sub>2</sub> fluxes in GS, respectively. Moreover, a low level of N addition (10 kg N ha<sup>-1</sup> yr<sup>-1</sup>) 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<sup>-1</sup> yr<sup>-1</sup>). These results indicate that more studies are required on CO<sub>2</sub> emissions from NGS under elevated N deposition or N application.

	Year (Y	Y) Nitrogen (N)	ANPP $(g m^{-2})$	SOM $(g kg^{-1})$	TN (g kg <sup>-1</sup> )	
	2010	N <sub>0</sub>	$94.7 \pm 13.7$	$56.7\pm2.4$	$5.1 \pm 0.2$	
		N <sub>10</sub>	$99.4 \pm 10.0$	$60.5\pm3.5$	$5.1 \pm 0.2$	
		N <sub>30</sub>	$112.4\pm11.2$	$64.2\pm1.2$	$5.6\pm0.1$	
		N90	$123.7\pm13.8$	$54.9\pm3.6$	$4.7\pm0.4$	
		N <sub>150</sub>	$123.3\pm8.5$	$53.7\pm3.4$	$4.7\pm0.4$	
	2011	N <sub>0</sub>	$175.5\pm33.7$	$85.0\pm7.9$	$6.5\pm1.0$	
		N <sub>10</sub>	$226.2\pm56.5$	$86.9\pm5.7$	$5.9\pm0.7$	
		N <sub>30</sub>	$253.4 \pm 57.1$	81.9±5.0	5.7±0.2	
		N90	$205.6\pm31.2$	$82.1 \pm 11.3$	$6.1 \pm 1.0$	
		N <sub>150</sub>	$228.0\pm50.1$	$84.3\pm10.2$	$6.0\pm0.9$	
	ANOVA	4	(P value)			
		Ν	0.605	0.891	0.944	
		Y	<0.0001	<0.0001	0.012	
		$N \times Y$	0.834	0.871	0.775	
c (mgm )	600 • GS 500 - O NGS	。 (a)		<sub>o</sub> (b)		• (c)
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	Air t	emperature (°C)	Soil temperat	hure (°C)	Soil water content	t (%)

**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).

Fig. 2. Relationships between CO<sub>2</sub> 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 GNS 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 subalpine 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 kg N ha<sup>-1</sup> yr<sup>-1</sup> 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 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 CO<sub>2</sub> 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  $R_{\rm e}$  in our study (194.1–266.6 mg m<sup>-2</sup> h<sup>-1</sup> in June) was similar to that in the Tibetan Plateau alpine meadow (about  $129.6-259.2 \text{ mg m}^{-1} \text{ h}^{-1}$ ) (Kato et al., 2006; Jiang et al., 2013) but the seasonal minimum  $R_e$  range in our study (2.0–  $2.8 \text{ mg m}^{-2} \text{ h}^{-1}$  in March) was higher than that in Tibetan Plateau alpine meadow (about  $1.8 \text{ mg m}^{-2} \text{ h}^{-1}$ ) (Kato et al., 2006; Jiang et al., 2013). Based on monthly mean Re flux, we calculated the annual  $R_e$  in Bayinbuluk alpine grasslands to be about 279.0–403.9 g  $\text{CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ , a lower value than found in European grasslands (1800–6000 g  $\text{CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$ ) (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  $-33.3 \,^{\circ}$ C and  $-23.0 \,^{\circ}$ 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 reason 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  $R_{\rm e}$  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  $R_{\rm e}$ .

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 CO<sub>2</sub> fluxes during the growing season. Moreover, annual ecosystem respiration emissions were  $279.0-403.9 \text{ g CO}_2 \text{ m}^{-2} \text{ yr}^{-1}$  in the Bayinbuluk alpine grasslands. Generally, soil temperature was the primary limiting abiotic factor regulating ecosystem respiration in this cold alpine grassland.

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