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Response of carbon dioxide emissions to sheep grazing and nitrogen application in an alpine grassland

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Abstract

Previous work has failed to address fully the response of (autotrophic and heterotrophic) respiration to grazing and nitrogen (N) addition in different ecosystems, particularly in alpine grasslands outside the growing season. From 2010 to 2011, we combined two methods (static closed chambers and a closed dynamic soil CO_2 flux system) in a controlled field experiment in an alpine grassland in the Tianshan Mountains. We examined the effects of grazing and N application on ecosystem respiration (R_e) both outside (NGS) and during (GS) the growing season and determined the pattern of R_e in relation to climate change. There was no significant change in CO_2 emissions under grazing or N application. Heterotrophic respiration (R_h) accounted for 78.5 % of R_e. R_e, R_h and autotrophic respiration (R_a) outside the growing season were equivalent to 12.9, 14.1 and 11.4 % of the respective CO_2 fluxes during the growing season. In addition, our results indicate that precipitation (soil water content) plays a critical role in R_a in this cold and arid environment. Both R_h and R_e were sensitive to soil temperature. Moreover, our results suggest that grazing and N addition exert no significant effect on CO_2 emissions in alpine grassland but may alter soil carbon stocks in alpine grassland.

1 Introduction

Global carbon dioxide (CO_2) emissions have increased from 6.1 ± 0.3 $\mathrm{Pg\,C\,yr^{-1}}$ in 1990 to 9.5 ± 0.5 $\mathrm{Pg\,C\,yr^{-1}}$ in 2011 and will increase by 9.7 ± 0.5 $\mathrm{Pg\,C\,yr^{-1}}$ in 2012 or 2.6 % above 2011 levels (Le Quéré et al., 2012; Peters et al., 2013). Accurate assessment of anthropogenic CO_2 emissions and their redistribution among the atmosphere, oceans, and terrestrial biosphere can therefore help to better understand the global carbon (C) cycle, support the climate policy process, and project future climate change (Le Quéré et al., 2012).

Grasslands cover approximately 37 % of the Earth's land surface (O'Mara, 2012) and play an important role in moderating the global C cycle and mitigating global warming. Soil C flux was found to range from 400 to 500 $\mathrm{g\,C\,m^{-2}\,yr^{-1}}$ in grassland (Raich and Schlesinger, 1992). Yet the role of grasslands in local and regional C fluxes is still unclear. Given the large uncertainties as to whether grasslands act as a source or a sink of CO_2 (e.g. Janssens et al., 2003; Novick et al., 2004), it is important to understand the response of ecosystem CO_2 component fluxes to global change, including changes in climate and grassland management. Ecosystem respiration (R_e), including autotrophic respiration (R_a, the respiration in the above-ground parts of plants and root respiration) and heterotrophic respiration (R_h, microbial respiration), is a major determinant of the C balance (Valentini et al., 2000). In addition, soil contributes the largest part of CO_2 emissions in grasslands (Yan et al., 2010). Consequently, grassland ecosystems represent a significant component of global C cycle (Wei et al., 2012). The magnitude of CO_2 emissions from grasslands can be influenced by global climate change and the utilization of grassland resources (Xia and Wan, 2012). Numerous studies have reported the high sensitivity of soil respiration in grasslands to variation in biomass (C) supply induced by grazing or clipping (Craine and Wedin, 2002; Wan and Luo, 2003).

Grazing reduces the litter input into soil and decreases CO_2 emissions from soil root biomass (Johnson and Matchett, 2001) and labile C from microorganisms and roots (Stark and Grellmann, 2002). It has been reported that grazing reduces the CO_2 flux by 19–50 % in grassland ecosystems (Craine et al., 1999; Wan and Luo, 2003). There is therefore a direct and dynamic relationship between soil respiration and the photosynthetic substrates supplied by the above-ground parts and this has been verified by previous studies. For example, root and soil respiration changed with variation in the grazing behavior of ungulates (Ruess et al.,1998), nutrient availability (Burton et al., 2000), sunlight (Graine et al., 1999) and other factors to control plant C acquisition. Extensive studies have also revealed that grazing can directly impact plant production and thereby soil C inputs. Most work has confirmed that grazing management can reduce CO_2 emissions by slowing below-ground C cycling through allocation of less C to roots than to shoots (Johnson and Matchett, 2001). Another assumption that has been supported is that grazing has no significant effect on CO_2 emissions, indicating that C flows in grassland will not be influenced by the grazing of large migratory herds of ungulates (Wei et al., 2012). However, several studies have indicated that grazing can elevate CO_2 flux by increasing productivity, speeding up nutrient cycling, and mitigating the negative effects of warming on plant biomass production and quality (Stark et al., 2002; Klein et al., 2007).

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). Ecosystem N cycling is originally derived from biological N fixation, mineralization, and atmospheric N deposition (Bobbink et al., 2010). It is well known that the rate of substrate decomposition can increase along with the growing input of N into the soil. However, soil organic matter tends to be more recalcitrant in soils to which N has been added because condensation reactions are driven by the abundant N compounds and phenolics and this will lead to a weakening in soil respiration and microbial activity (Gullege et al., 1997). Nitrogen fertilization can enhance plant dark respiration, promote the per unit root respiration rate, increase the root biomass (Lutze et al., 2000), and stimulate primary production to supply more substrate for soil respiration (West et al., 2006). Nevertheless, excessive N fertilizer can also reduce root carbon distribution and root-to-shoot ratio, thus inhibiting the respiration of root and rhizosphere microorganisms (Olsson et al., 2005). If there is no N deficiency in the soil, N addition may aggravate the “N saturation” situation leading to nitrogen leaching and loss and may also lead to a small change in soil respiration. However, the effects of N addition on the quantity and quality of litter and SOM are currently in dispute (Hoosbeek et al., 2002). Nitrogen addition promotes cellulose decomposition of litter and soil organic matter or other compounds that are more readily decomposed and restrains the decomposition of lignin and other more recalcitrant materials. This makes it difficult to differentiate between the effects of N addition on soil respiration and the effects of other confounding factors such as geographic location, soil type and vegetation cover.

Evaluation of C dynamics in grassland ecosystems is of great concern worldwide in the context of global climate change and growing human perturbation (Niu et al., 2010; Volk et al., 2011; Perry et al., 2012). Most studies on CO_2 emissions in grasslands have been conducted in North America, Africa and Australia, Inner Mongolia and on the Tibetan Plateau (Cao et al., 2004; Niu et al., 2010; Jenkins and Adams, 2010; Perry et al., 2012). To date, related studies in alpine grassland in central Asia have been scarce. As a consequence, evaluating the C dynamics in response to CO_2 emissions and grazing regime in such ecosystems is of critical importance (Knorr et al., 2005; West et al., 2006). One major shortcoming in previous studies is that the CO_2 efflux rate outside the growing season, which is essential for evaluating C dynamics and C budgets, has rarely been monitored in situ. Related data on annual or seasonal CO_2 flux in alpine grasslands in central Asia are also rare (Li et al., 2012). Estimating CO_2 flux under grazing and N addition within and outside the growing season is important for predicting the effects of future climate change and human activities on CO_2 fluxes or C budgets in alpine grassland (Li et al., 2012). Hence, the objectives of the present study were to clarify the effects of short- and long-term grazing exclusion and N addition on CO_2 fluxes in an alpine grassland, to evaluate whether or not CO_2 emissions outside the growing season constitute an indispensable portion of ecosystem respiration on a year-round basis, to demonstrate the effects of soil temperature and soil water content (both to 10 cm depth) on soil respiration (e.g. R_e, R_h and R_a), and to determine the response of CO_2 emissions to global climate change under grazing management and N application in alpine grassland.

2 Materials and methods

2.1 Study sites

The study was conducted at the Bayinbuluk Grassland 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 km^2. 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 % falling during the growing season from 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). General characteristics of the sites are shown in Table 1.

Five grazing management sites and five N fertilizer treatment sites were established. All sites are dominated by *Stipa purpurea* and are a cold and dry grassland type. There were two grazing regimes, namely short- and long-term grazing exclusion. The short-term grazing exclusion sites comprised site LG_B grazed by 4.3 sheep ha^{-1} in a full year (100 ha), site UG_5 ungrazed since 2005 (10 ha), and site UG_8 ungrazed since 2003 (0.25 ha). The long-term grzing exclusion sites were site UG_{27} ungrazed since 1984 (0.25 ha) and site LG_A lightly grazed by two sheep ha^{-1} in winter (October to April; 100 ha). 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 m × 8 m with an 1-m-wide buffer zone) and the N addition experiments were conducted using ammonium nitrate (NH_4NO_3) 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 two methods. The first was a static closed chamber method (opaque, static, manual stainless steel chambers, each 50 cm × 50 cm × 10 cm) at sites UG_{27}, LG_A, N_0, N_{10}, N_{30}, N_{90} and N_{150}. The external surface of each chamber was covered with white plastic foam to minimize any impact of direct radiative heating during sampling. The chamber was placed on a collar (50 cm × 50 cm × 10 cm) with a groove to prevent leakage during gas sampling. Each site had four replicate chambers. Gas samples were taken from inside the chamber 0, 15 and 30 min after chamber closure using a 60 mL plastic syringe and transferred immediately into 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 h, eastern eight zones) from May 2010 to September 2011 (no sampling in January or February 2011 because of the very low temperatures of about −40 °C) 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 8 April, about 182 days) at the seven sites. CO_2 samples, which were stored in specific air bags, were analyzed by gas chromatography (Agilent 4890D, Agilent Technologies, Wilmington, DE) within a week. Calculation of CO_2 fluxes followed the description of Zhang et al. (2005).

The second method was a closed dynamic soil CO_2 flux system (Li-Cor 8100, model 8100-101 or 8100-104, Li-Cor, Lincoln, NE) at sites LG_B, UG_5 and UG_8. R_e was measured by inserting three polyvinyl chloride (PVC) collars (10.2 cm inside diameter, 6 cm height) 3 cm into the soil at each site in July 2009. The collars were arranged 1 m apart to form a triangle. All living plants were maintained intact. Heterotrophic respiration (R_h, microbial respiration) was also determined by inserting three PVC collars into the soil in each site. Living roots were removed from the soil and the soil was replaced, maintaining the original horizons of the profile. A diaphragm was inserted into the soil outside the root zone to prevent regrowth. Soil CO_2 fluxes (R_h) were measured at least one day after exclusion of the living roots (Hanson et al., 2000). Autotrophic respiration

(R_a, the belowground parts of the plant and root respiration) was calculated as follows, (Piao et al., 2010):

$$R_p = R_e - R_m \tag{1}$$

In order to minimize disturbance to the plots when installing the PVC columns, we measured the CO_2 efflux rate at least 1 week after the installation/application. A soil CO_2 flux chamber attached to a Li-8100 was placed on each collar for 3 min to measure R_h(or R_e) and then moved to the next collar. R_e and R_h were measured during the same time period (11:00–20:00 h) from August 2010 to July 2011 (no sampling in January or February 2011), four times per month during the growing season, and twice per month outside the growing season at sites LG_B, UG_5 and UG_8.

Air temperature and soil temperature at 10 cm depth and soil water content at 10 cm depth were monitored during the process of gas sampling (sites UG_{27}, LG_A, N_0, N_{10}, N_{30}, N_{90} and N_{150}) and CO_2 flux measurement (LG_B, UG_5 and UG_8) (Auto Weather Station, Campbell Scientific, Logan, UT). Soil temperature (Wuqiang Inc., Hengshui, Hebei, China) and water content (gravimetric moisture content) were also measured at each site from May 2010 to September 2011.

Aboveground biomass of different species was obtained in July 2010 (1 m × 1 m plots and $n = 3$ at each site). Belowground biomass was obtained based on estimation using root cores (8 cm dia; $n = 5$ at each site) to a depth of 30 cm. Plant (aboveground and belowground) biomass was determined by oven drying at 60 °C for 24 h. Plant cover was determined by visual measurement. Soil bulk density was measured using 100 cm^3 soil wreath knives to 10 cm depth ($n = 5$ at each site). Soil samples were collected from each site ($n = 5$) to determine pH (1 : 5) and soil EC to a depth of 10 cm (Table 1).

2.3 Calculations and statistical analysis

Statistical analysis was carried out with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and SigmaPlot (SigmaPlot for Windows, Version 10, SyStat Software Inc., San Jose, CA). One-way analysis of variance was performed to determine the significant differences in CO_2 fluxes among grazing sites and N treatment sites within and outside the growing season. Non-linear curve fitting was performed with SigmaPlot software to identify significant correlations between environmental variables and CO_2 fluxes.

3 Results

3.1 CO_2 fluxes with long-term grazing exclusion

Long-term grazing enclosure management (ungrazed for 27 yr) had no significant effect on the CO_2 emissions of ecosystem respiration (R_e) in GS 2010 ($P = 0.896$), 2011 ($P = 0.960$) or NGS ($P = 0.462$) (Fig. 2b). Across the entire period of observations (May 2010–September 2011) R_e fluxes showed clear seasonal fluctuation. The maximum monthly R_e fluxes were 122.8 $\mathrm{mg\,m^{-2}\,h^{-1}}$ at UG_{27} and 103.7 $\mathrm{mg\,m^{-2}\,h^{-1}}$ at LG_A in June 2011. The minimum values were 2.03 $\mathrm{mg\,m^{-2}\,h^{-1}}$ at UG_{27} and 2.62 $\mathrm{mg\,m^{-2}\,h^{-1}}$ at LG_A in December 2010 (Fig. 2a). In addition, total R_e emissions were 179.1 and 12.8 $\mathrm{g\,m^{-2}}$ at UG_{27} and 165.3 and 10.6 $\mathrm{g\,m^{-2}}$ at LG_A in GS and NGS, respectively (Fig. 2b). R_e during NGS accounted for 7.1 and 6.4 % compared with GS in 2010 and 2011, respectively. One conclusion that can be drawn is that if the R_e emission for NGS is not taken into account, then the annual CO_2 emissions can be underestimated by 6.3 %.

3.2 CO_2 fluxes with short-term grazing exclusion

There were also no significant changes in R_e($P = 0.931$, 0.915), R_h($P = 0.990$, 0.859) or R_a($P = 0.192$, 0.842) emissions under short-term grazing enclosure management in GS and NGS at UG_8, UG_5 and LG_B (Fig. 3b, d and f). R_e, R_h and R_a flux results, based on year-round observation (August 2010–June 2011), showed substantial seasonal change. The maximum monthly R_e values were 314.9, 327.7 and 316.2 $\mathrm{mg\,m^{-2}\,h^{-1}}$ at

UG_8, UG_5 and LG_B, respectively. The maximum monthly R_h fluxes were 305.3, 263.4, and 270.6 $mg\,m^{-2}\,h^{-1}$ at UG_8, UG_5 and LG_B, respectively. The maximum monthly R_a fluxes were 76.2, 88.7 and 52.2 $mg\,m^{-2}\,h^{-1}$ at UG_8, UG_5 and LG_B, respectively. The minimum monthly R_e, R_h and R_a CO_2 fluxes were 9.6, 10.7 and 0 $mg\,m^{-2}\,h^{-1}$ at UG_8; 10.3, 14.5 and 0 $mg\,m^{-2}\,h^{-1}$ at UG_5; 2.72, 5.01 and 0 $mg\,m^{-2}\,h^{-1}$ at LG_B, respectively (Fig. 3a, c and e). Furthermore, the ranges of the total R_e, R_h and R_a emissions were 584.2–644.1, 483.4–504.2 and 98.9–169.6 $g\,m^{-2}$ under short-term grazing exclusion in GS, and 67.5–85.8, 58.0–80.8 and 12.6–20.9 $g\,m^{-2}$ in NGS, respectively (Fig. 3b, d and f).

3.3 CO_2 fluxes under different N addition rates

There were no significant differences in R_e flux under different N addition rates in GS (2010 and 2011) or NGS ($P = 0.601$, 0.559, 0.461) (Fig. 4b). Throughout the study (May 2010–September 2011) R_e showed clear seasonal fluctuation. In June 2011 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 N_0, N_{10}, N_{30}, N_{90} and N_{150}, respectively (Fig. 4a). Similarly, under N addition the R_e fluxes reached minimums values of 2.03 (March 2011), 3.09 (November 2010), 2.04 (November 2010), 2.81 (March 2011) and 2.48 (March 2011) at N_0, N_{10}, N_{30}, N_{90} and N_{150}, respectively (Fig. 4a). R_e increased at N_0, N_{10}, N_{30}, N_{90} and N_{150} with increasing soil N content. The ranges of 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. 4b).

3.4 Sensitivity of CO_2 emissions to climate change under grazing and N addition

Air temperature (AT), soil temperature at 10 cm depth (ST) and soil water content at 10 cm depth (SWC) showed clear seasonal variation throughout the observation period

(Fig. 1). The annual AT, ST and SWC reached maximum values of 17.9 °C (early August 2010), 15.8 °C (late July 2010) and 26.4 % (June 2011) and minimum values of −38.3 °C (January 2010), −10.8 °C (late January 2010) and 5.6 % (March 2011), respectively. Average AT, ST and SWC in GS were 9.6 °C, 10.2 °C and 18.4 %, respectively, and −18.5 °C, −4.8 °C and 9.7 % in NGS, respectively.

The relationships between CO_2 fluxes and fluctuating environmental conditions were analyzed from May 2010 to September 2011 (the duration of short-term grazing exclusion sites was from August 2010 to July 2011). CO_2 fluxes of R_e, R_h and R_a were growth exponentially with air temperature, soil temperature and soil moisture ($P < 0.0001$) (Fig. 6).

4 Discussion

4.1 Effects of long-term grazing exclusion on CO_2 flux

Plant cover and above- and belowground biomass increased by 41, 187 and 14 % under long-term grazing (27 yr) exclusion (Table 1). These changes may increase the ecosystem respiration flux (Craine and Wedin, 2002). Nevertheless, the cold and dry environment at our sites inhibited microbial activity and this, coupled with the higher soil pH may have lowered heterotrophic respiration (Xu and Qi, 2001). Although substrate input increased significantly because of positive and negative feedback mechanisms, R_e flux showed no significant change under long-term grazing exclusion management. Under long-term grazing exclusion, the fluctuating R_e fluxes (+18.5 % (GS 2011) to −3.9 %) were subject to changes in precipitation and temperature. However, the R_e fluxes during NGS increased 20.1 % under long-term grazing. Consequently, ecosystem respiration was greatly affected by long-term enclosure in NGS in this alpine grassland (Figs. 5a, 6g, h and i).

4.2 Effects of short-term grazing exclusion on CO_2 flux

Plant cover increased 0.6–1.0 times under short-term grazing exclusion, aboveground biomass increased 0.9–1.2 times, and root biomass increased about 60.0 % (Table 1). Although changes in the cold and dry conditions may have elevated ecosystem respiration, these factors may have limited the soil root and microbial activities to relatively low levels. In addition, the influence of the feedback mechanisms (e.g. increasing pH), R_h and R_a showed no significant change in the short-term grazing exclusion management.

Over the whole year total R_e accounted for 78.5 % of R_h and 21.5 % of R_a(percentages calculated by the averages of UG_8, UG_5 and LG_B). Furthermore, CO_2 emissions of R_h and R_a in NGS were 69.7, 15.8 $g\,m^{-2}$ and 14.1 %, 11.4 % in GS, respectively. At UG_8, UG_5 and LG_B, R_e during NGS accounted for 13.9–13.3 and 11.6 % of that in GS, respectively. R_h in GS was reduced 1.2 % by short-term zero-grazing management. In contrast, the R_e emissions rate in NGS increased 24.3 % under short-term grazing exclusion (Fig. 5c). Moreover, short-term zero-grazing affected R_a more, with enhancement by 61.1 and 38.9 % in GS and NGS, respectively (Fig. 5d). R_e increased 26.8 % in NGS and 5.6 % in GS (Fig. 5c). The effects of R_h and R_a emissions from NGS under grazing management therefore merit further investigation.

4.3 Effects of N fertilizer application on CO_2 fluxes

Plant cover increased from 74.4 to 98.9 % with increasing soil N content in the present study. Moreover, above- and below-ground biomass increased from 107 $g\,m^{-2}$ and 9.0 g 50 cm^{-3} to 123 $g\,m^{-2}$ and 11.5 g 50 cm^{-3}, respectively (Table 1). However, because of the restricted continuous substrate supply the effects of the low temperatures and drought stress on microbial activity and the positive and negative effects of the soil internal offset function on CO_2 flux, R_e was not significantly increased by N fertilization ($P = 0.601$ (GS 2010), 0.559 (GS 2011), 0.461 (NGS)) (Fig. 4b).

Via N addition, 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 (Fig. 4b). In NGS, R_e increased 20.1–51.0 % under the four different levels of N addition and the increment in NGS was two times than the growth of R_e from GS. 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 51 % by the high level of N addition (150 kg N ha^{-1} yr^{-1}) (Fig. 5g). These results indicate that more studies are required on CO_2 emissions from NGS under elevated N deposition or N addition.

In summary, N addition affects soil CO_2 efflux through altering plant growth and soil C turnover. The response of soil respiration to N fertilization and N deposition changed greatly and was largely dependent on fertilizer type, quantity, application frequency and local conditions. Several studies have reported stimulation of soil CO_2 efflux by fertilization or deposition (Craine et al., 2001; Fang et al., 2012). Soil N availability influences plant growth, root activity, net primary productivity, and litter decomposition (Carreiro et al., 2000; Xu and Wan 2008), all of which can affect the supply of C substrate for plant roots and soil microorganisms. Thus N fertilization would be expected to lead to enhanced soil respiration by increasing plant growth, net primary productivity, the quantity of litter, and decomposition in terrestrial ecosystems. However, numerous other studies have reported little or no significant change in CO_2 emissions (Zak et al., 2003) on account of biomass production with N fertilization without a negative environmental impact. In contrast to the two standpoints above, a growing number of studies have indicated that N fertilization suppresses soil respiration, fine root biomass and soil microbial biomass, and enhances root tissue N concentrations and increases root respiration rate (Jia et al., 2010). Furthermore, N addition increased the soil pH and this might result in reduced CO_2 emissions. In addition, atmospheric N deposition tends to inhibit soil CO_2 efflux and promote ecosystem C sequestration (Magnani et al., 2007). The inhibitory mechanisms include a decrease in litter decomposition (Janssens et al., 2010) and a decline in fine roots and soil microbial biomass and their activities (Mo et al., 2008). Jassal et al. (2010) showed that N fertilization resulted in a significant short-term (over 3–4 months) increase in forest-floor CO_2 efflux due to an increase in autotrophic (or rhizospheric) soil respiration followed by a small decrease in heterotrophic soil respiration.

4.4 Effects of grazing and N addition on carbon budget

According to the CO_2 flux calculation, R_e emission decreased by 4 % owing to long-term grazing exclusion which increased the above-ground biomass by a factor of three. Thus, there was more litter decomposition to enhance the accumulation of soil organic C with long-term grazing exclusion in the alpine grassland. However, short-term grazing exclusion produced increases inR_e emissions of about 10 and 5 % and in above-ground biomass of 96 and 118 % in UG_5 and UG_8, respectively. Therefore, soil C stocks may be augmented by short-term grazing exclusion although R_e emissions may or may not increase. Consequently, there was a net C fixation or sequestration with grazing exclusion in alpine grassland.

In the present study, through the calculation of CO_2 flux at different sites (N_0, N_{10}, N_{30}, N_{90} and N_{150}) the responses of ecosystem respiration to N addition were examined. Compared to zero N addition (N_0), N addition (N_0, N_{10}, N_{30}, N_{90} and N_{150}) increased R_e emission by 10, 13, 19, and 20 %, respectively. N addition (N_0, N_{10}, N_{30}, N_{90} and N_{150}) also increased above-ground biomass by only −7, 5, 7, and 15 %, respectively. It was therefore not possible to determine whether or not N deposition increased soil C content in alpine grassland. Further studies will focus on the linkage between soil C stocks and grazing intensity and or^{-1} N addition in this alpine grassland.

5 Conclusions

In summary, our results confirm no significant changes in R_e, R_h and R_a under short- or long-term grazing exclusion and N addition in GS and NGS in our alpine grassland. R_h accounted for 78.5 % of R_e. Nevertheless, CO_2 emissions from NGS cannot be ignored because during NGS, R_e, R_h and R_a account for 12.9, 14.1 and 11.4 % of the values from GS, respectively (the percentages are the mean values of the corresponding sites). Under grazing management or N addition, CO_2 emissions are more sensitive in NGS than in GS. Our observations strongly indicate that grazing exclusion

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and N addition played a critical role in accumulation of soil organic C in this frigid and arid environment in the Tianshan Mountains.

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Table 1. Characteristics of the ten alpine grassland sites in the Tianshan mountains of central Asia.

Site	Latitude (N)	Longitude (E)	Altitude (m)	Aboveground biomass (Jul 2010) ±S.E. ($g\,m^{-2}$)	Belowground biomass (Jul 2010) ±S.E. ($g\,50\,cm^{-2}$)	Plant cover (Jul 2010) ±S.E. (%)	Soil BD (0–10 cm) (Jul 2010) ±S.E. ($g\,m^{-3}$)	pH (top 10 cm)	Soil EC (0–10 cm) ($mS\,cm^{-1}$)	Grazing intensity (sheep units ha^{-1})	Method
UG_8	42°52.802′	83°42.437′	2468	122 ± 10.5	11.0 ± 3.22	97 ± 4.2	1.0 ± 0.01	7.9	0.34	0	Li-8100
UG_5	42°52.802′	83°42.442′	2468	110 ± 7.4	12.4 ± 2.60	77 ± 8.7	1.1 ± 0.03	7.7	0.31	0	
LG_B	42°52.798′	83°42.437′	2468	56 ± 4.4	7.4 ± 1.81	48 ± 5.7	1.1 ± 0.01	7.8	0.21	4.3	Static closed
UG_{27}	42°52.802′	83°42.173′	2472	207 ± 15.2	11.2 ± 2.13	78 ± 6.6	0.9 ± 0.02	8.00.33	0	chamber	
LG_A	42°52.832′	83°42.125′	2473	72 ± 9.7	9.8 ± 1.58	55 ± 2.9	1.1 ± 0.01	8.0	0.27	2.0	
N_0	42°52.802′	83°42.442′	2468	107 ± 15.8	9.0 ± 1.45	74 ± 10.1	1.1 ± 0.02	7.9	0.31	0	
N_{10}	42°52.802′	83°42.442′	2468	99 ± 10.0	9.2 ± 1.37	83 ± 12.8	1.1 ± 0.02	7.6	0.26	0	
N_{30}	42°52.802′	83°42.442′	2468	112 ± 11.2	9.9 ± 2.67	85 ± 9.3	1.1 ± 0.02	7.85	0.30	0	
N_{90}	42°52.802′	83°42.442′	2468	114 ± 14.0	11.2 ± 1.91	86 ± 9.1	1.1 ± 0.02	8.05	0.19	0	
N_{150}	42°52.802′	83°42.442′	2468	123 ± 8.5	11.5 ± 0.51	99 ± 4.4	1.1 ± 0.02	7.99	0.24	0	

NB: BD, bulk density; EC electrical conductivity

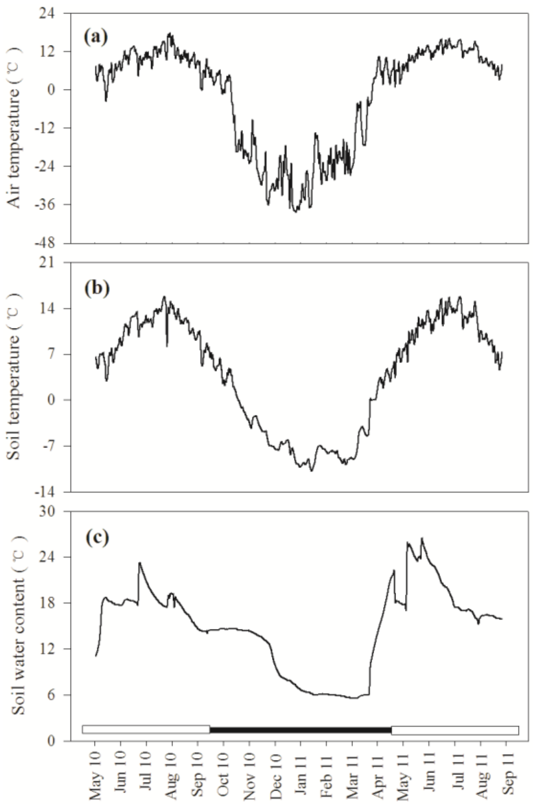

Fig. 1. Air temperature **(a)**, soil temperature at 10 cm depth **(b)** and soil water content at 10 cm depth **(c)** from May 2010 to September 2011 in an alpine grassland of the Tianshan mountains.White and black horizontal bars represent within (2010 and 2011) and outside the growing season, respectively.

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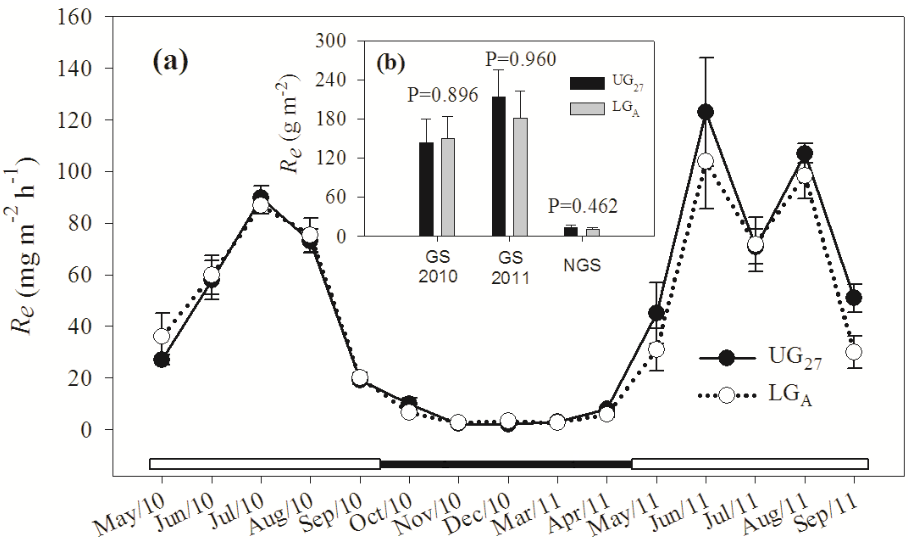

Fig. 2. Rates of CO_2 emission with ecosystem respiration (R_e) **(a)** and the magnitude of CO_2 fluxes within and outside the growing season **(b)** at sites UG_{27} (ungrazed since 1984) and LG_A (grazed in winter) using the static closed chamber method. White and black horizontal bars represent within the growing season (GS, 2010 and 2011) and outside the growing season (NGS), respectively.

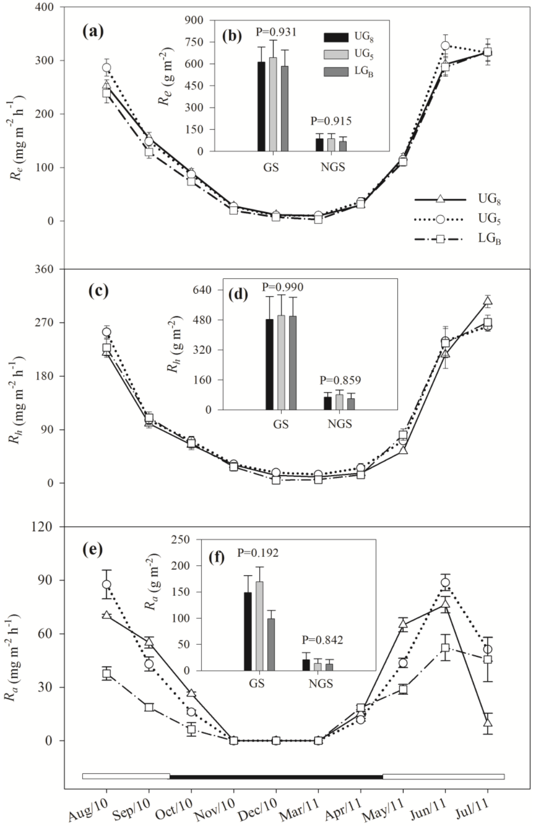

Fig. 3. CO_2 fluxes of ecosystem respiration (R_e), heterotrophic respiration (R_h) and autotrophic respiration (R_a) at sites UG$_8$ (ungrazed since 2003), UG$_5$ (ungrazed since 2005) and LG$_B$ (grazed all year) within (GS) and outside (NGS) the growing season respectively using a Li-8100. White and black horizontal bars represent within (GS, 2010 and 2011) and outside (NGS) the growing season, respectively.

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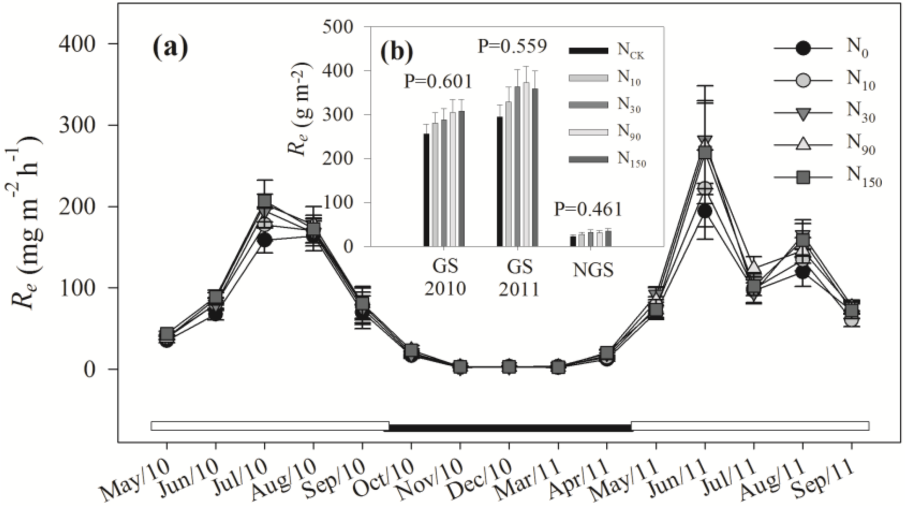

Fig. 4. CO_2 fluxes of ecosystem respiration (R_e) at sites N_0 (ungrazed since 2005), N_{10} (10 kg N ha^{-1} yr^{-1}), N_{30} (30 kg N ha^{-1} yr^{-1}), N_{90} (90 kg N ha^{-1} yr^{-1}) and N_{150} (150 kg N ha^{-1} yr^{-1}) within and outside the growing season using the static closed chamber method. White and black horizontal bars represent within (GS, 2010 and 2011) and outside (NGS) the growing season, respectively.

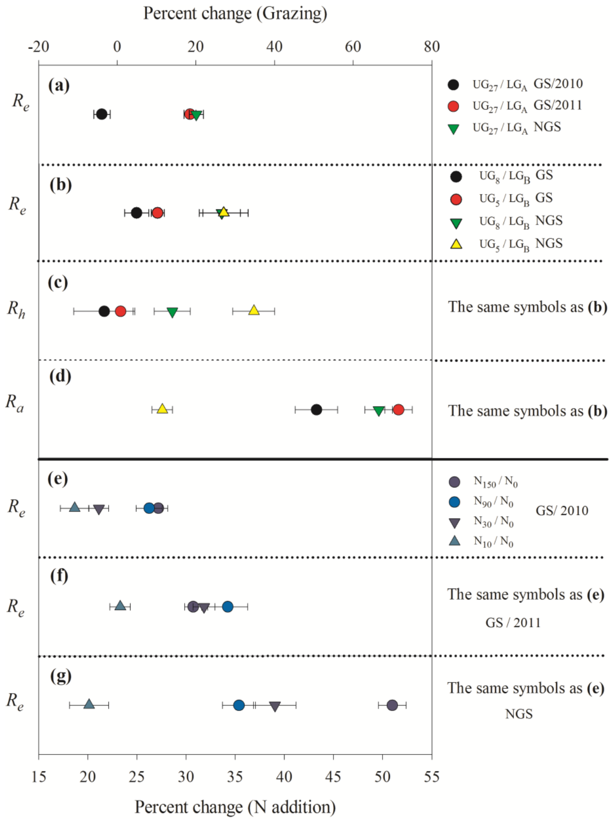

Fig. 5. Percentage change in R_e (CO_2 fluxes of ecosystem respiration) at site UG_{27} compared with LG_A and at sites N_{10}, N_{30}, N_{90}, and N_{150} compared with N_0, respectively. In addition, percentage change in R_e (CO_2 fluxes of ecosystem respiration), R_h (CO_2 fluxes of heterotrophic respiration) and R_a (CO_2 fluxes of autotrophic respiration) at sites UG_8 and UG_5 are compared with site LG_B within and outside the growing season. GS, within the growing season; NGS, outside the growing season.

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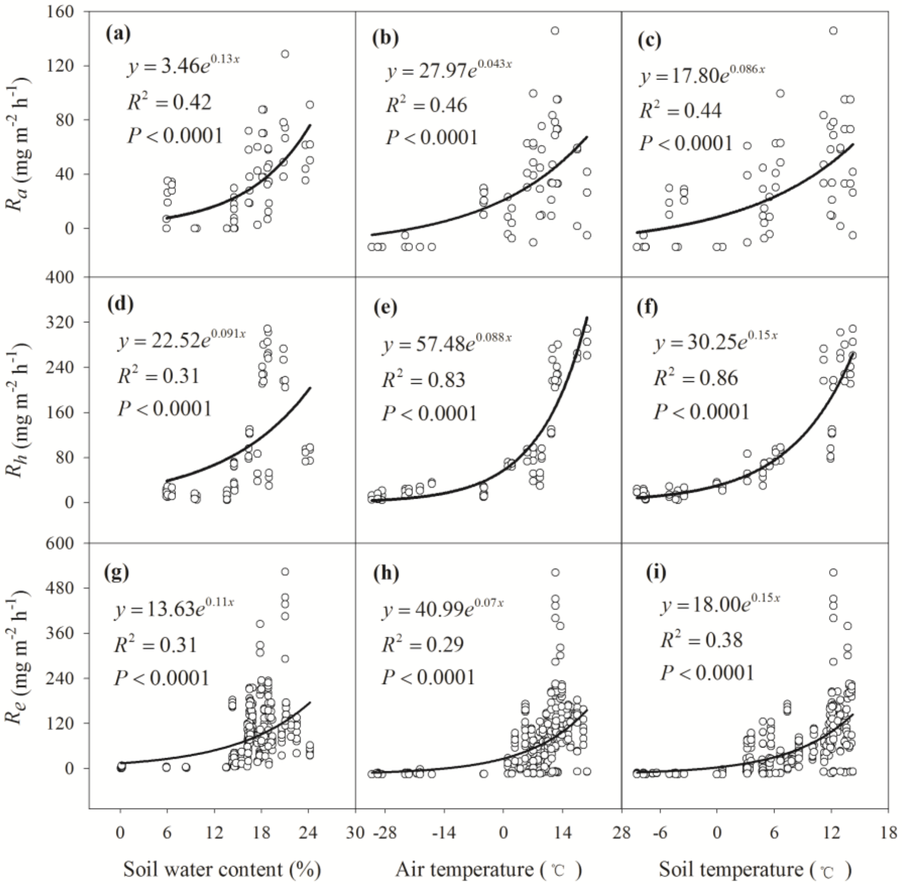

Fig. 6. Relationships between CO_2 fluxes (R_a, R_h and R_e) and soil water content at 10 cm depth (**a**, **d** and **g**), air temperature (**b**, **e** and **h**) and soil temperature at 10 cm depth (**c**, **f** and **i**). R_a, R_h: $n = 72$; R_e: $n = 366$.