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No significant nitrous oxide emissions during spring thaw under grazing and nitrogen addition in an alpine grassland

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Abstract

A recent study (Wolf *et al*., 2010) suggests that short—lived pulses of N_2O emission during spring thaw dominate the annual N_2O budget and that grazing decreases N_2O emissions during the spring thaw. To verify this we conducted year—round N_2O flux measurements from June 2010 to May 2011 in Tianshan alpine grassland in central Asia. No pulse emissions of N_2O were found at grazing management sites and nitrogen addition sites during the spring thaw. The contribution of the spring thaw to the total annual N_2O budget was small and accounted for only 6.6% of the annual fluxes, with winter emissions accounting for 16.7% and growing season emissions accounting for 76.7%. The difference in N_2O emissions attributable to grazing management was not significant ($P > 0.05$). Nitrogen input tended to increase N_2O emissions at N addition sites during the grass growing season compared with those at unfertilized sites. N_2O fluxes showed a significant correlation with air temperature and also with both soil temperature and soil water content at 10 cm depth.

Keywords: grassland, grazing, nitrogen fertilization, nitrous oxide emissions, spring thaw, Tianshan mountains

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Introduction

Soil spring thaw processes occur regularly in temperate grassland ecosystems and pulse emissions of nitrous oxide (N_2O) from thawing soil are often observed (Wolf *et al*., 2010; Yao *et al*., 2010). Measurements during spring thaw in various ecosystems have revealed that N_2O fluxes can be of major importance for the calculation of annual budgets. A full evaluation of the contribution of spring thaw to annual N_2O fluxes will require measurements covering at least an entire year (Holst *et al*., 2008). In some cases more than 50% of the annual emissions are found in the thaw period (Teepe & Ludwig, 2004; Matzner & Borken, 2008; Wolf *et al*., 2010) and there are two explanations for such significant N_2O emission phenomena. The first explanation is that N_2O produced in the unfrozen subsoil is suddenly released if the gas diffusion barrier is disrupted during the thawing period and the second is that conditions favorable to denitrification during the thawing period result in a burst of N_2O emissions due to greater C and N availability for microbial activity (Nyborg *et al*., 1997). Although numerous studies have measured N_2O emissions from soils during spring thaw, the results also indicate some uncertainty arising from soil heterogeneity and the complex interactions among chemical, physical and biological factors. In some cases the effects of soil thaw have been pronounced (Sulkava & Huhta, 2003) but in others they have been relativity subtle and have even decreased during soil thaw (Prieme & Christensen, 2001). A decrease would suggest either depletion of available nutrients for microorganisms or damage to soil microbes.

Studies of N_2O emissions have mainly involved indoor controlled experiments during freezing–thawing processes and the relevance of many of these studies to naturally occurring freeze–thaw cycles has been diminished by experimental artifacts (Henry, 2007). More field observations under natural conditions are therefore required. Recent studies have shown that increased microbial activity at nongrazed sites where snow accumulation influences soil temperature and hydrology during the winter season is consistent with higher rates of N cycling (Del Grosso, 2010). Grazing decreased N_2O emissions from

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semi–arid grasslands that experience soil thaw cycles during the spring season (Wolf *et al.*, 2010). Anthropogenic activities, mainly fertilizer applications, have greatly increased N_2O emissions (Galloway *et al.*, 2008; Guo *et al.*, 2010; Liu *et al.*, 2011). Although nitrogen (N) addition experiments have been conducted in forest, swamp and agricultural ecosystems in China (Yan *et al.*, 2003; Zhang & Han, 2008; Zhang *et al.*, 2008; Lin *et al.*, 2009; Song *et al.*, 2009), the impact of N fertilization on N_2O is not well understood in alpine grassland (Jiang *et al.*, 2010). It remains unclear whether significant N_2O emissions during spring thaw can be found with different N fertilizer inputs. Although N_2O emissions from natural grasslands worldwide have been investigated (Schulze *et al.*, 2010; Vilain *et al.*, 2010), few studies have been conducted on the contribution of the spring thaw to annual N_2O fluxes. Moreover, most measurements have been short—term and have been carried out only during the growing season (Filippa *et al.*, 2009; Jiang *et al.*, 2010). N_2O fluxes during spring thaw and winter are far from negligible on an annual basis but are missing when measurements have not been made during these parts of the year. Clearly, there is a need to measure N_2O fluxes throughout the year in grassland ecosystems. To better understand the regulation of N_2O emissions during spring thaw and winter in alpine grassland in the Tianshan mountains of central Asia, four grazing management sites and four N treatments sites were established. This study focused on the following three aspects:

1 Whether or not pulse emissions of N_2O can be found during spring thaw in alpine grassland sites with different grazing management and N treatments.
2 Whether or not the contribution of spring thaw to annual N_2O fluxes is significant in alpine grassland.
3 How N_2O fluxes of short—term grazing enclosures, long—term grazing enclosures and lightly grazed alpine grassland under different N fertilization treatments compare.

Materials and methods

Study site

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 in the Tianshan mountains basin at a mean altitude of 2500 m a.s.l. From local meteorological data (1980–1999), mean annual precipitation is 265.7 mm with 78.1% falling during the growing season from May to August and 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.

Four grazing management sites and four N fertilization treatment sites were established. The four grazing management sites were SW site grazed by 1.7 sheep per hectare in winter (from October to April; 100 ha), UGI site ungrazed since 2005 (10 ha), UGII site ungrazed since 1984 (0.25 ha) and LG site lightly grazed by two sheep per hectare in winter (from October to April; 100 ha). SW site is dominated by *Carex melantha* and is a seasonally inundated wetland, and UGI, UGII and LG sites are dominated by *Stipa purpurea* and are a dry grassland type. The four N fertilizer treatment sites were: N_{10} site (10 kg ha^{-1} year^{-1}), N_{30} site (30 kg ha^{-1} year^{-1}), N_{90} site (90 kg ha^{-1} year^{-1}) and N_{150} site (150 kg ha^{-1} year^{-1}). Each treatment comprised four blocks (each 4 × 8 m with an 1 m wide buffer zone) and the N addition experiments were conducted with ammonium nitrate (NH_4NO_3) in late May and June each year from 2009 to 2011. All the N fertilization sites are dominated by *Stipa purpurea* and have been ungrazed since 2005.

Measurement method

N_2O fluxes were measured using opaque, static, manual stainless steel chambers (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. The 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 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). N_2O fluxes were sampled during the same time period (12:00–14:00 hours) from June 2010 to May 2011 (no sampling in January or February 2011 because of the very low temperatures, about −40 °C) and four times per month during the growing season (from 10 May to 8 October, about 152 days), and two times per month during winter (from 9 October to 8 April, about 182 days) at all sites. During spring thaw (from 9 April to 9 May, about 31 days), N_2O fluxes were sampled at 2–3 days intervals (total of 10 times). N_2O concentrations in gas samples were analyzed by gas chromatography (Agilent 4890D; Agilent Technologies, Wilmington, DE, USA) within 1 week. The gas chromatograph was equipped with an electron capture detector for N_2O analysis and was configured for analysis of N_2O concentration according to the method of Wang & Wang (2003). Calculation of N_2O flux followed the description of Song *et al.* (2009). Air temperature (Air T), soil temperature at 10 cm depth (Soil T) and soil water content at 10 cm depth (SWC) were monitored during gas sample collection (Fig. 1; Auto Weather Station, Campbell Scientific, Logan, UT, USA). Moreover, Soil T

Table 1 Characteristics of the eight alpine grassland sites in the Tianshan mountains, central Asia

Site	Latitude (N)	Longitude (E)	Altitude (m)	Aboveground biomass (July) ± S.E (g m^{-2})	Plant cover (July) ± S.E (%)	Soil water content at 10 cm depth (July) ± S.E (%)	NH_4-N concentration (September, 0–10 cm) ± S.E (kg ha^{-1})	NO_3-N concentration (September, 0–10 cm) ± S.E (kg ha^{-1})	Soil bulkdensity (0–10 cm) ± S.E (g cm^{-3})	pH (0–10 cm)	Soil organic carbon (0–10 cm) (g kg^{-1})	Grazing intensity (sheep unitsha^{-1})
SW	42°53.024′	83°43.285′	2462	296.5 ± 13.2	100 ± 0	98.5 ± 5.6	No data	No data	0.41 ± 0.03	7.74	252.0	1.7
UGI	42°53.128′	83°42.442′	2468	107.2 ± 15.8	74.4 ± 10.1	24.5 ± 3.1	6.6 ± 0.9	32.3 ± 7.4	1.07 ± 0.02	7.87	59.9	0
UGII	42°52.802′	83°42.173′	2472	97.6 ± 5.2	77.5 ± 6.6	36.3 ± 2.0	No data	No data	0.96 ± 0.02	8.01	60.6	0
LG	42°52.832′	83°42.125′	2473	72.4 ± 9.7	55 ± 2.9	36.1 ± 2.2	No data	No data	0.94 ± 0.01	7.99	62.7	2.0
N_{10}	42°53.128′	83°42.442′	2468	99.4 ± 10.0	82.6 ± 12.8	24.5 ± 3.1	5.8 ± 0.6	33.7 ± 11.7	1.07 ± 0.02	7.64	51.5	0
N_{30}	42°53.128′	83°42.442′	2468	112.4 ± 11.2	84.6 ± 9.3	24.5 ± 3.1	6.4 ± 0.8	36.9 ± 11.7	1.07 ± 0.02	7.85	58.2	0
N_{90}	42°53.128′	83°42.442′	2468	113.7 ± 14.0	85.8 ± 9.1	24.5 ± 3.1	7.0 ± 0.9	35.6 ± 9.7	1.07 ± 0.02	8.05	37.0	0
N_{150}	42°53.128′	83°42.442′	2468	123.3 ± 8.5	98.9 ± 4.4	24.5 ± 3.1	6.4 ± 0.6	53.8 ± 13.8	1.07 ± 0.02	7.99	40.3	0

measurements were also made by thermometer (Wuqiang Inc., Hengshui, Hebei, China) and soil water content (gravimetric moisture content) was also measured at each site during the growing season. Average gas fluxes and standard errors were calculated from four replicates at each site. Statistical analysis was carried out with SPSS 11.0 (SPSS Inc., Chicago, IL, USA) and Origin 7.5 (ORIGINLAB Co., Northampton, MA, USA). One–way ANOVA analysis and independent sample *t*-test were performed to determine the significant differences in N_2O fluxes among N treatment sites and grazing management sites. Linear regression analysis was used to identify significant positive or negative correlations between environmental variables and N_2O fluxes.

Results

N_2O fluxes during spring thaw

The soil surface began to thaw in the middle of April. No significant increases in N_2O flux were observed during the spring thaw at any site (Fig. 2). Daily mean N_2O fluxes were calculated to vary within the range from 1 to 11.9 $\mu g\ m^{-2}\ h^{-1}$ at SW site, 1.7 to 23.4 $\mu g\ m^{-2}\ h^{-1}$ at UGI site, 1.8 to 11.0 $\mu g\ m^{-2}\ h^{-1}$ at UGII site, 0.7 to 11.1 $\mu g\ m^{-2}\ h^{-1}$ at LG site, 1.9 to 21.5 $\mu g\ m^{-2}\ h^{-1}$ at N_{10} site, 1.2 to 25.0 $\mu g\ m^{-2}\ h^{-1}$ at N_{30} site, 1.6 to 25.0 $\mu g\ m^{-2}\ h^{-1}$ at N_{90} site and 1.4 to 28.3 $\mu g\ m^{-2}\ h^{-1}$ at N_{150} site. Mean N_2O fluxes were 4.6, 10.5, 6.7, 6.6, 12.4, 13.2, 13.4 and 16.0 $\mu g\ m^{-2}\ h^{-1}$ for SW, UGI, UGII, LG, N_{10}, N_{30}, N_{90} and N_{150} sites, respectively, during the period. Independent sample *t*-test analysis (95% confidence intervals) indicates no significant differences among all sites (all $P > 0.1$).

Contribution of spring thaw to annual N_2O fluxes

Across the entire period of observations (June 2010–May 2011) the mean annual N_2O fluxes were from 0.732 to 1.46 kg N ha^{-1} and mean fluxes were from 0.034 to 0.119 kg N ha^{-1} during spring thaw for all sites. The contribution of spring thaw fluxes to the total annual N_2O budget was not significant and on average accounted for 6.6% of the annual fluxes for all sites (Table 2). The contribution of winter fluxes to the total annual N_2O budget accounted for 22.3%, 16.7%, 20.3%, 20.5%, 15.9%, 12.1%, 13.2% and 13.1% of the annual fluxes at SW, UGI, UGII, LG, N_{10}, N_{30}, N_{90} and N_{150} sites, respectively, and on average accounted for 16.7% of the annual fluxes for all sites. Different from expectations, growing–season emissions dominated the total annual N_2O budget and accounted for 74.8%, 76.3%, 73.3%, 72.8%, 77.2%, 80.3%, 79.9% and 78.7% for SW, UGI, UGII, LG, N_{10}, N_{30}, N_{90} and N_{150} sites, respectively, and on average accounted for 76.7% of the annual fluxes across all sites.

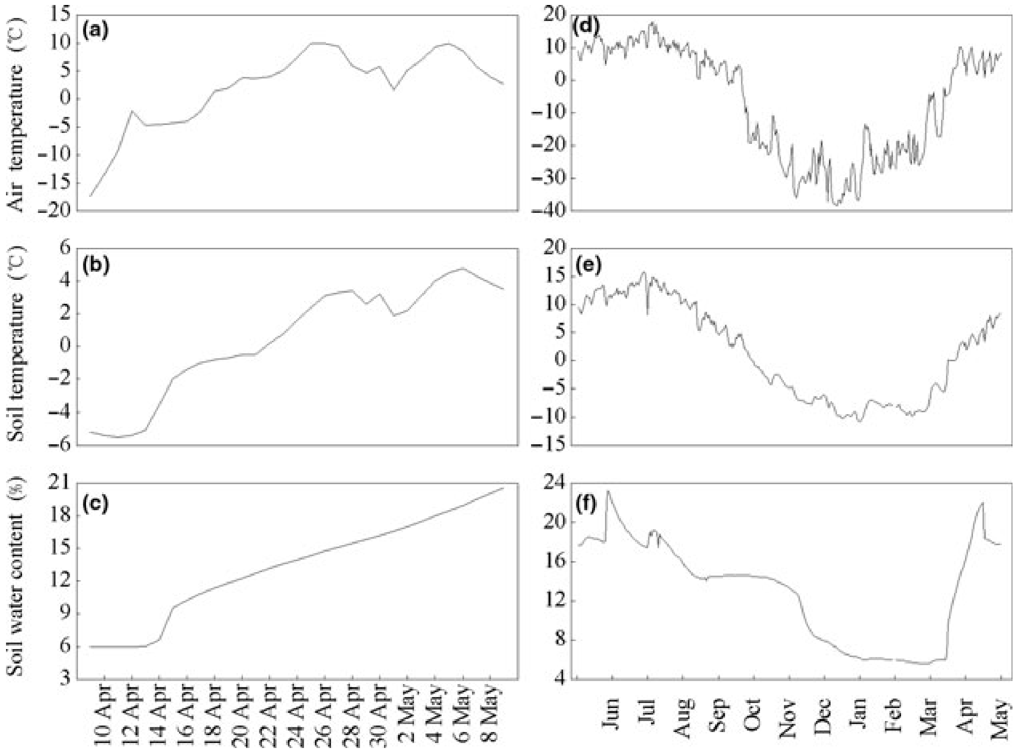

Fig. 1 Air temperature (a), soil temperature at 10 cm depth (b) and soil water content at 10 cm depth (c) during spring thaw. Air temperature (d), soil temperature at 10 cm depth (e) and soil water content at 10 cm depth (f) from June 2010 to May 2011 in alpine grassland of the Tianshan mountains.

N_2O fluxes of different grazing management practices

During the entire period of measurements the N_2O emission was higher at short—term grazing enclosure (UGI, ungrazed since 2005) as compared to long—term grazing enclosure grassland (UGII, ungrazed since 1984) or lightly grazed grassland, and the N_2O flux at SW site was higher than at UGII site and LG site. However, no significant differences were found among SW, UGI, UGII and LG. Even during the growing season there were no significant differences in N_2O emission between UGI and UGII ($P = 0.19$) or between UGI and LG ($P = 0.14$) or between UGII and LG sites ($P = 0.82$) excluding the SW site which was significantly higher than the LG site ($P = 0.04$).

N_2O fluxes of different N addition treatments

N_2O fluxes of different N addition treatments showed a clear seasonal pattern with the mean daily fluxes in the following order: growing season > spring thaw > winter. Total annual mean N_2O fluxes were 14.4, 13.8, 15.7 and 15.9 μg m^{-2} h^{-1} at N_{10}, N_{30}, N_{90} and N_{150} sites, respectively. The application of fertilizer N tended to significantly increase N_2O emissions at N_{10}, N_{30}, N_{90} and N_{150} sites during the growing season when compared with the unfertilized control site (UGI) but no significant differences were found across all sites during spring thaw or winter (Fig. 2).

Environmental variables

During the entire observation period, air temperature (Air T), soil temperature at 10 cm depth (Soil T) and soil water content at 10 cm depth (SWC) showed clear seasonal patterns in this area (Fig. 1). The annual mean temperature was −7.1 °C. Monthly mean Air T reached a maximum (11.1 °C, July) and minimum (−33.3 °C, January), Monthly mean Soil T reached maximum (13.0 °C, July) and minimum (−9.1 °C, January) and monthly mean SWC reached a maximum (19.1%, June) and minimum (5.7%, March). The alpine grassland belongs to the seasonal spring thaw area where the soil temperature at 10 cm depth reaches values below 0 °C and spring thaw lasted for 1 month (from the middle 10 days of April to the first 10 days of May). The relationships between N_2O fluxes and environment conditions were analyzed across an entire year. At SW, UGI, UGII and LG sites, N_2O fluxes were significantly correlated with air temperature, soil temperature and soil moisture (Fig. 3).

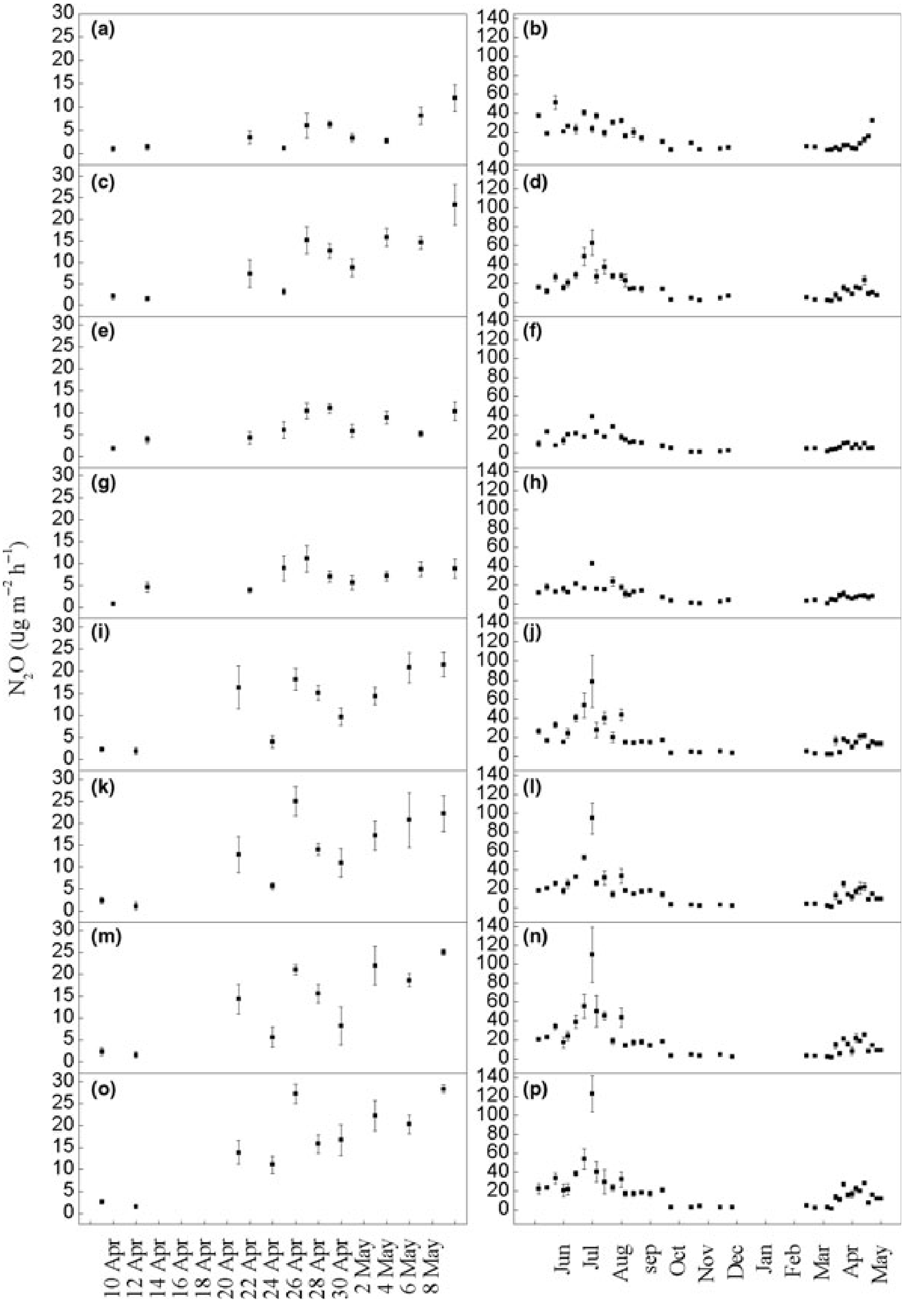

Fig. 2 N_2O emissions at SW site (grazing in winter, a, b), UGI site (ungrazed since 2005, c, d), UGII site (ungrazed since 1984, e, f), LG site (grazed in winter, g, h), N_{10} site (i, j, 10 kg ha^{-1} y^{-1}), N_{30} site (k, l, 30 kg ha^{-1} y^{-1}), N_{90} site (m, n, 90 kg ha^{-1} y^{-1}) and N_{150} site (o, p, 150 kg ha^{-1} y^{-1}) in alpine grassland of the Tianshan mountains.

Discussion

Effects of spring thaw and winter on N_2O fluxes

Although pulse emissions of N_2O during spring thaw or winter have been characterized in continental steppe (Wolf *et al.*, 2010), marsh (Song *et al.*, 2008) and agricultural systems (Barton *et al.*, 2011), the underlying processes that occur during freezing and thawing are poorly understood. The contribution of N_2O emissions during spring thaw can amount to more than 70% of the total annual N_2O budget in temperate grassland

Table 2 Seasonal N_2O emissions from different grazing management sites and nitrogen fertilization sites in alpine grassland of the Tianshan mountains

Site	Number of chambers	SE ± S.E (kg N h^{-1})	WE ± S.E (kg N ha^{-1})	GE ± S.E (kg N ha^{-1})	AE ± S.E (kg N ha^{-1})	Ratio of SE to AE (%)
SW	4	0.034 ± 0.009	0.251 ± 0.005	0.842 ± 0.030	1.126 ± 0.190	3.0
UGI	4	0.078 ± 0.005	0.185 ± 0.004	0.845 ± 0.040	1.108 ± 0.022	7.0
UGII	4	0.050 ± 0.010	0.158 ± 0.004	0.571 ± 0.026	0.779 ± 0.014	6.4
LG	4	0.049 ± 0.012	0.150 ± 0.003	0.533 ± 0.232	0.732 ± 0.013	6.7
N_{10}	4	0.092 ± 0.015	0.210 ± 0.005	1.021 ± 0.052	1.323 ± 0.028	7.0
N_{30}	4	0.098 ± 0.016	0.156 ± 0.005	1.036 ± 0.056	1.290 ± 0.031	7.6
N_{90}	4	0.100 ± 0.021	0.193 ± 0.006	1.167 ± 0.064	1.459 ± 0.034	6.9
N_{150}	4	0.119 ± 0.015	0.191 ± 0.007	1.145 ± 0.060	1.455 ± 0.033	8.2

SE, spring thaw emissions; WE, winter emissions; GE, growing season emissions; AE, annual emissions; S.E, standard error.

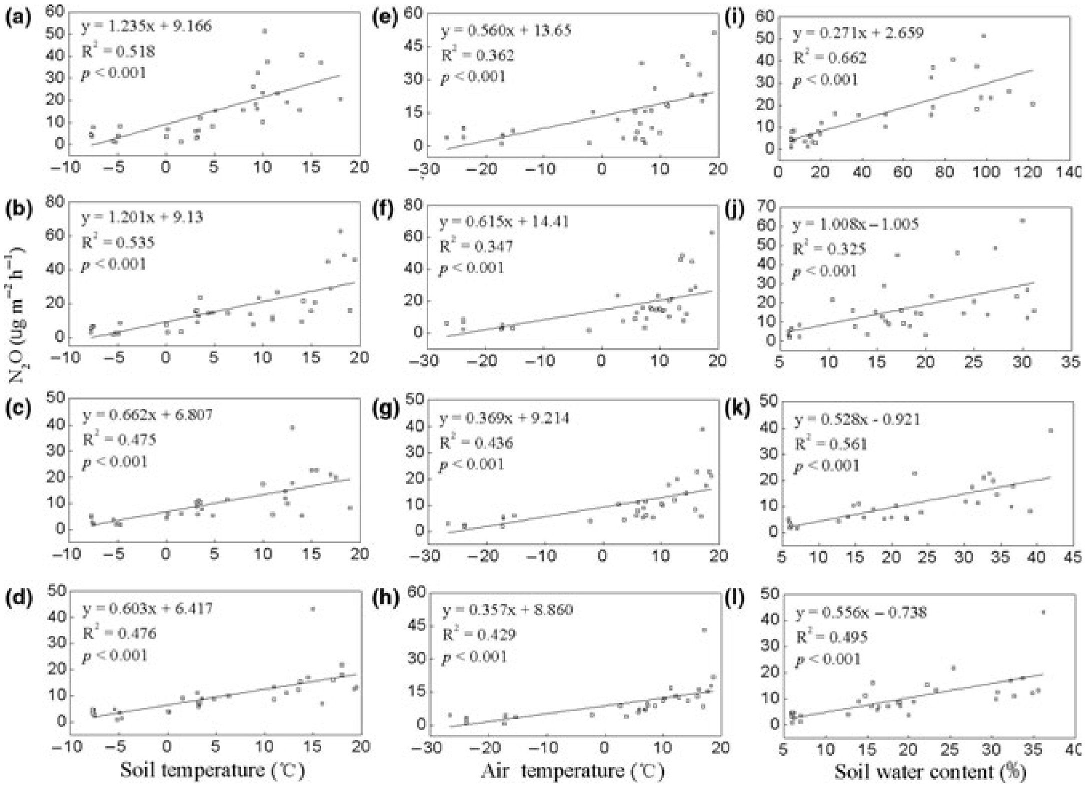

Fig. 3 Relationships between N_2O flux and soil temperature at 10 cm depth, air temperature and soil water content at 10 cm depth at SW site (a, e, i), UGI site (b, f, j), UGII site (c, g, k) and LG site (d, h, l), respectively.

(Wolf *et al.*, 2010). In contrast, we found that the average contribution of N_2O emissions during the spring thaw accounted for only about 6.6% of the annual N_2O budget across different grazing management sites and N treatment sites in this study. Mean emissions from all eight sites in winter accounted for 16.7% of the annual N_2O budget. The difference between our study and that by Wolf *et al.* (2010) may be due to lower temperatures at our monitoring sites. Air temperature was not so low in winter in the study by Wolf *et al.* (2010), in which mean monthly temperatures ranged from −21.6 °C in January to 19.0 °C in July (Bai *et al.*, 2010), which may have allowed increased microbial activity in the soil. In comparison, temperatures ranged from −33.3 °C in January to 11.1 °C in July in our study. Thus, we can predict that there may have been less unfrozen water in the winter and spring thaw in the alpine grassland compared to the continental steppe studied by Wolf *et al.* (2010). Because the most biologically important feature of unfrozen water is that it makes mass transfer possible in permafrost and mass exchange is greatest in microzones with low ice contents and smallest at sites where the ice content is high or in solid ice, the physical structure of permafrost makes metabolic activity possible (Rivkina *et al.*, 2000). In addition, different winter conditions may affect N_2O emissions. Regina *et al.* (2004) reported that winter or thawing fluxes could be different even at one site and, depending on the conditions in winter, N_2O concentrations in the soil did not increase in winter, likely because the soil was frozen throughout the winter and there was no unfrozen water and thus little denitrification in the soil. Accordingly, N_2O emissions were not especially large in our study during thawing.

Bergstermann *et al.* (2011) reported significant interaction between N_2O emissions and soil moisture. We found soil moisture to be higher in the study by Wolf *et al.* (2010) compared to our study during spring thaw. Thus, high soil moisture is also an important factor which may have contributed to the differences between the two studies. Moreover, concentrations of mineral N in soil in different seasons may affect emissions of N_2O. Nitrogen mineralization rates are higher before snow melt than during the growing season (Brooks *et al.*, 1997) and there was much inorganic N during the period of snow cover because inactive plant roots cannot utilize mineralized N in winter (Brooks *et al.*, 1998). For example, soil mineral N content ranged from 15.6 to 38.9 kg N ha^{-1}, respectively, from May to September in UGI site; plants in alpine grassland will take up more mineral N due to mineralization and nitrification during the growing season while mineral N tends to accumulate after the growing season. The very low temperature and dry soil may prevent high N_2O fluxes in winter or subsequent thawing periods because of the low temperature and soil moisture limitation for denitrification, although mineral N was not a restricting factor.

Effect of grazing management on N_2O fluxes

Grassland ecosystems are often regarded as a significant source of N_2O (Williams *et al.*, 1999; Du *et al.*, 2008) and N_2O emissions can increase when grassland soils are damaged by animal treading (Thomas *et al.*, 2008). Wolf *et al.* (2010) reported that annual emissions from ungrazed sites (ungrazed for about 10 years) were higher than those from heavily grazed sites, with most of the annual emissions from the ungrazed grassland sites occurring outside the growing season, suggesting that grazing decreases rather than increases N_2O emissions. This study on alpine grassland shows that annual emissions of N_2O from the UGII site (ungrazed for about 26 years) were low and similar to those from the LG site (lightly grazed site) and the annual emissions were lower than in the UGI site (ungrazed for about 5 years) or swamp site (lightly grazed), but the differences in N_2O emissions were not significant ($P > 0.05$) among the four sites. N_2O emissions were dominated by the spring thaw period in grazing management sites in the study by Wolf *et al.* (2010) and differences in the N_2O emissions were not significant among grazing enclosure, heavy grazing, moderate grazing and lightly grazed sites. We found no significant difference in the N_2O emissions in spring thaw among light grazing, short—term grazing enclosures and long—term enclosures in our study, thus grazing management had little impact on N_2O emissions. In addition, similar soil variables resulted in little difference in N_2O emissions among grazing management sites. For example, pH, soil bulk density, organic carbon, available N and total P in the topsoil (0–10 cm depth) showed relatively small variations (from 7.87 to 8.01, 0.94 to 1.07 g cm^{-3}, 59.9 to 62.7 g kg^{-1}, 0.14 to 0.19 g kg^{-1}, 0.91 to 0.98 g kg^{-1} at UGI, UGII and LG sites, respectively). The effects of grazing on N_2O emissions seem to vary in different grassland ecosystems and deserve further field studies under natural conditions.

Effect of N fertilization on N_2O fluxes

Nitrogen addition generally increases N_2O emissions (Saggar *et al.*, 2008; Hynst & Simek, 2009; Ma *et al.*, 2010). In this study we found increased N_2O emissions at the four N addition sites compared with the unfertilized site but there were no significant differences in N_2O emissions among the four N treatment sites. As microorganisms use mineral N to produce N_2O and temperature limits mineralization in cool climates, the difference between fertilized and unfertilized sites may be due to lower mineral N availability for microbes at unfertilized sites. N_2O emissions were also influenced by precipitation (pulses after large rainfall events following fertilization; Flechard *et al.*, 2007; Vilain *et al.*, 2010). This may explain why the N_2O fluxes occasionally did not increase with N addition in some coniferous and grassland ecosystems (Ambus & Robertson, 2006). In this study we found no significant N_2O emissions after rainfall events (precipitation in June and July accounted for 45.3% of annual precipitation) following fertilization. The plants likely suffer from N limitation and take up more N per unit of carbon. This would remove surplus N from the soil and lead to less available N for microorganisms. Water shortage, dry soil and low temperatures may also limit the growth of microorganisms in the alpine grassland. Recent field experiments suggest that significant decreases in N_2O emissions may be possible by decreasing N fertilizer inputs without affecting the economic return from grain yields (Hoben *et al.*, 2011). Significant differences in aboveground biomass were not found among N_{30}, N_{90} and N_{150} sites in our study (Table 1). Therefore, our results are consistent with the hypothesis that there is some potential to lower N_2O fluxes within a range of N fertilization that does not affect the economic return from herbage yields.

Effects of environmental variables on N_2O fluxes

Soil temperature, moisture, pH, N content, WFPS and C/N ratio are considered to be the major factors influencing N_2O fluxes. Kato *et al.* (2011) reported a negative relationship between N_2O fluxes and both soil pH and C/N ratio of surface soil in alpine meadow and grassland on the Qinghai—Tibetan plateau. Song *et al.* (2009) found that the ambient temperature in the chambers is significantly correlated with N_2O fluxes in wetland ecosystems. In this study significant relationships were found between air temperature and environmental conditions (soil temperature at 10 cm depth, soil water content at 10 cm depth and N_2O fluxes) at all eight sites. However, we did not examine the influence on N_2O fluxes of pH, N content, or soil C/N, and these may need further investigation. Moreover, Van Bochove *et al.* (2000) reported that soil water content determined the burst of N_2O emissions after frost and smaller aggregates with higher water content can enhance aggregate destruction more than macro—aggregates. We found lower soil water contents during spring thaw in the current study and this may be one of the explanations for the small contribution of N_2O emissions during the spring thaw to the annual N_2O budget. Overall, these results show that generalizations about how changes in climate and land management affect N cycling are often confounded. More accurate projections will require an improved understanding and modeling of the processes that control biogeochemical cycles (Del Grosso, 2010).

In summary, no apparent pulse N_2O emissions were observed in any of the eight sites during the spring thaw in Tianshan alpine grassland, central Asia. The cold winter conditions and dry soil resulted in low N_2O fluxes during the spring thaw. Grazing management did not significantly affect N_2O fluxes. Nitrogen fertilization tended to increase N_2O emissions at sites receiving N additions during the grass growing season. Contrary to expectations, the contribution of the spring thaw to the total annual N_2O budget was relatively small and emissions during the growing season dominated the total annual N_2O budget. N_2O fluxes showed significant correlations with air temperature, soil temperature and soil water content. Few studies on N_2O emissions have been conducted under natural conditions during the spring thaw and in winter and so the mechanisms of N_2O flux remain unclear and deserve further study, especially in terms of microbial populations and activities. This study produces approximate ratios of spring thaw and winter fluxes to annual fluxes and the magnitude of winter N_2O fluxes is not large but also not negligible in the total annual N_2O emission budgets in alpine grassland. Therefore, N_2O emissions of natural vegetation from field experiments must be investigated to provide direct evidence on the characteristics of N_2O emissions in different ecosystems during spring thaw and winter.

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