



Effects of warming and grazing on N₂O fluxes in an alpine meadow ecosystem on the Tibetan plateau

Yigang Hu ^{a,d}, Xiaofeng Chang ^{a,d}, Xingwu Lin ^c, Yanfen Wang ^d, Shiping Wang ^{a,b,*}, Jichuang Duan ^{a,d}, Zhenhua Zhang ^{a,d}, Xiaoxia Yang ^{a,d}, Caiyun Luo ^{a,d}, Guangping Xu ^{a,d}, Xinquan Zhao ^a

^a Key Laboratory of Adaptation and Evolution of Plateau Biota, Haibei Alpine Meadow Ecosystem Research Station, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China

^b Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100085, China

^c Institute of Soil Science in Nanjing, Nanjing 21008, China

^d Graduate University, Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

A great deal of uncertainty is associated with estimates of global nitrous oxide (N₂O) emissions because emissions from arid and polar climates were not included in the estimates due to a lack of available data. In particular, very few studies have assessed the response of N₂O flux to grazing under future warming conditions. This experiment was conducted to determine the effects of warming and grazing on N₂O flux at different time scales for three years under a controlled warming-grazing system. A free-air temperature enhancement system (FATE) using infrared heaters and grazing significantly increased soil temperatures for both of growing (average 1.8 °C in 2008) and no-growing seasons (average 3.0 °C for 3-years) within 20-cm depth, but only warming reduced soil moisture at 10-cm soil depth during the growing season during the drought year of 2008. Generally, the effects of warming and grazing on N₂O flux varied with sampling date, season, and year. No interactive effect between warming and grazing was found. Warming did not affect annual N₂O flux when grazing was moderate during the growing season because the tradeoff of the effect of warming on N₂O flux was observed between the growing season and no-growing season. No-warming with grazing (NWG) and warming with grazing (WG) significantly increased the average annual N₂O flux (57.8 and 31.0%) compared with no-warming with no-grazing (NWNG) and warming with no-grazing (WNG), respectively, indicating that warming reduced the response of N₂O flux to grazing in the region. Winter accounted for 36–57% of annual N₂O flux for NWNG and NWG, whereas only for 5–8% of annual N₂O flux for WNG and WG. Soil temperature could explain 5–35% of annual N₂O flux variation.

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1. Introduction

Nitrous oxide (N₂O) is one of the major greenhouse gases (IPCC, 2007). Intensive studies in temperate grassland/steppe ecosystems and agricultural land in Euro-Asia, North America, Australia, and New Zealand have revealed that N₂O fluxes vary with vegetation types, soil properties, climate conditions, and land uses, and that the role of N₂O emissions from grasslands in the world is an important consideration in the global N₂O budget (Mosier et al., 1991, 1996, 1998, 2002; Velthof and Oenema, 1995; Flessa et al., 1996;

Mummey et al., 1997, 2000; Ball et al., 1999; Breuer et al., 2000; Billings et al., 2002; Xu et al., 2003a,b; Saggar et al., 2004; Wang et al., 2005; Du et al., 2006; Ma et al., 2006; Holst et al., 2007; Maljanen et al., 2007; Barton et al., 2008; Brümmer et al., 2008). Stehfest and Bouwman (2006) recently calculated that 1.8 Tg N₂O–N yr⁻¹ is emitted globally from grasslands. However, a great deal of uncertainty is associated with estimates of global N₂O emissions because emissions from arid, polar, and boreal climates were not included in the estimates due to a lack of available data, especially data during the winter (Bouwman et al., 2000; Stehfest and Bouwman, 2006).

In temperate ecosystems, particular interest has focused on winter fluxes of N₂O because much of the annual flux appears to occur during winter and during the transition from winter to spring, when freeze-thaw events are common (Brumme et al., 1999; Groffman et al., 2000, 2006; Butterbach-Bahl et al., 2002).

* Corresponding author at: Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100085, China. Tel.: +86 971 6106617; fax: +86 971 6143282.

E-mail address: wangship2008@yahoo.cn (S. Wang).

Most studies in global change biology have focused on the growing season. However, there is ample evidence of marked changes in climatic conditions during winter (Schwartz and Reiter, 2000; Dye, 2002; Hodgkins et al., 2003; Wolfe et al., 2005) and evidence that this change has implications for fluxes in greenhouse gases in grassland ecosystems (Groffman et al., 2000, 2006). In the original DeNitrification–DeComposition (DNDC) model, N_2O fluxes to the atmosphere were assumed to be zero when the soil was snow covered or frozen in any of the layers of 0–30 cm soil profiles (Li, 2000). This assumption is inconsistent with many published field measurements (Sommerfield et al., 1993; Röver et al., 1998; Xu et al., 2003a,b), and winter dynamics have been shown to be particularly important for soil-atmosphere fluxes of greenhouse gases (Sommerfield et al., 1993; Brooks et al., 1997; Alm et al., 1998; Brumme et al., 1999; Groffman et al., 2006). Field measurement results showed that up to 40% of the annual N_2O losses occurred during the no-growing season and confirmed the importance of spring and autumn periods for assessment of total N_2O losses from semi-arid temperate grasslands (Xu et al. 2003a,b; Groffman et al., 2006). Röver et al. (1998) also reported a significant increase in N_2O concentrations below the snow cover, which indicates a restricted diffusion of N_2O through the snow.

The Tibetan plateau accounts for about 25% of the total country area in China. Approximately 40% of the Tibetan plateau is alpine meadow, which is widely used for grazing. Evidence shows that the Tibetan plateau is experiencing climatic warming (Thompson et al., 1993, 2000). Many studies from tundra ecosystems (Jonasson et al., 1993; Schmidt et al., 1999, 2002) suggest that altered N cycling in alpine ecosystems may be a key response to climate and grazing perturbations. However, there have been very few studies assessing the response of N_2O flux to variation in winter conditions (either natural or manipulated) (Schimel et al., 2004; Groffman et al., 2001, 2006), especially to grazing in warming conditions. To improve our knowledge of global terrestrial N_2O losses, we need to understand N_2O emissions from grazing in a warming ecosystem in this region.

To contribute to our understanding of N cycling and N trace gas exchange, and especially to the role of the alpine ecosystem in global N_2O budgets in future warming conditions, a study was conducted in an alpine meadow on the Tibetan plateau. This study was conducted with warming and grazing by means of closed-chamber measurements of soil–atmosphere N_2O exchange in the field from 2006 to 2009. The specific aims of this study were, for the first time, to (1) observe temporal variation in N_2O flux at different time scales (i.e., daily, monthly, seasonally, and annually), especially for the contribution of N_2O emission during the no-growing seasons; (2) evaluate the effects of warming and grazing on N_2O flux through a controlled warming with grazing experiment; and (3) investigate the relationships between N_2O flux and soil temperature and soil moisture.

2. Materials and methods

2.1. Controlled warming–grazing experiment

A detailed description of the experimental site, the design of the controlled warming with grazing experiment heated by the free-air temperature enhancement system (FATE), and measurements of soil temperature and soil moisture can be found in Zhao and Zhou (1999), Kimball et al. (2008), and Luo et al. (2009a,b). In brief, in May 2006 eight hexagonal arrays of Mor FTE (1000 W, 240 V) infrared heaters were deployed over vegetation canopy that had previously been heavily grazed by sheep during cool seasons from October to May of prior years at the Haibei Alpine Meadow Ecosystem Research Station with eight dummy arrays over reference plots. The heaters were controlled using the proportional-integral-derivative-outputs

(PID) control system so as to ensure constant warming between heated and reference plots. The setpoint differences between heated and corresponding reference plots were 1.2 °C during daytime and 1.7 °C at night during the growing season (from May to September). During no-growing season, (from October to April), because some infrared thermometers were not working, the power outputs of the heaters were manually set at 1500 W per plot. A two factorial design (warming and grazing) was used with four replicates of each of four treatments: no-warming with no-grazing (NWNG), no-warming with grazing (NWG), warming with no-grazing (WNG), and warming with grazing (WG). In total, 16 plots of 3-m diameter were used in a complete randomized block distribution in the field. One adult Tibetan sheep was fenced in the grazing plots in the morning of 17 August 2006 for approximately 2 h. The canopy height was about 8–9 cm before grazing and 4–5 cm after grazing. The stocking rate roughly corresponded to a moderate stocking rate in the region. Similarly, two adult Tibetan sheep were fenced for approximately 1 h in the grazing plots in the mornings of 12 July, 3 August, and 12 September in 2007, and 8 July and 20 August in 2008. Mean temperature and total rainfall during the growing seasons from 1 May to 20 September in 2006, 2007, and 2008 were 8.4, 8.5, and 8.1 °C, and 449.2, 397.6, and 339.4 mm, respectively. The seasonal rainfall distribution and grazing time are shown in Fig. 1.

At 50 cm inside the edge of each plot, type-K thermocouples (Campbell Scientific, Logan, Utah, U.S.A) were used to automatically measure soil temperature at depths of 5, 10, and 20 cm every 1 min, and 15 min averages were stored. Soil moisture at depths of 10, 20, 30, and 40 cm was manually measured through a tube in the ground down to a 40 cm depth using a frequency domain reflectometer (FDR; Model Diviner-2000, Sentek Pty Ltd., Australia) at 8:00, 14:00, and 20:00 every day. The soil moisture was expressed as a volume percentage (%) or mm/10 cm. All data were collected from 26 May 2006 to 30 April 2009.

2.2. N_2O sampling and analysis

N_2O fluxes were measured by opaque, static, manual stainless steel chambers (Lin et al., 2009). The dimension (40 cm × 40 cm × 40 cm) and architecture of the chambers were the same as those reported by Ma et al. (2006). Gas samples were taken every 3–5 days depending on weather conditions during the growing season in 2006 and every 7–10 days during the growing seasons from May to September in 2007 and 2008. There were 26 sampling occasions from 9 June to 17 September in 2006, and 19 sampling occasions from May to September for both 2007 and 2008. During the two no-growing seasons, there were 8 sampling occasions from October 2007 to April 2008 and 5 sampling occasions from October 2008 to April 2009, which were at almost one-month intervals depending on weather conditions. The N_2O flux between 9:00 a.m. and 11:00 a.m. local time represents one-day average flux (Tang et al., 2006). Chambers were closed for half an hour and gas samples (100 ml) were collected every 10 min using plastic syringes. Gas samples of N_2O concentrations were analyzed with gas chromatography (HP Series 4890D, Hewlett Packard, USA) within 24 h following gas sampling. The gas chromatography configurations for analyzing concentrations of N_2O and the methods of calculating each gas flux were the same as those described by Wang and Wang (2003) and Ma et al. (2006).

2.3. Statistical analysis

Repeated-measures analyses of variance (ANOVA), with warming and grazing as the main factors (between-subject factors) and with sample date and/or soil depth as within-subject factors including interactions, was applied to test the effects of the main factors on soil temperature, soil moisture, and N_2O fluxes

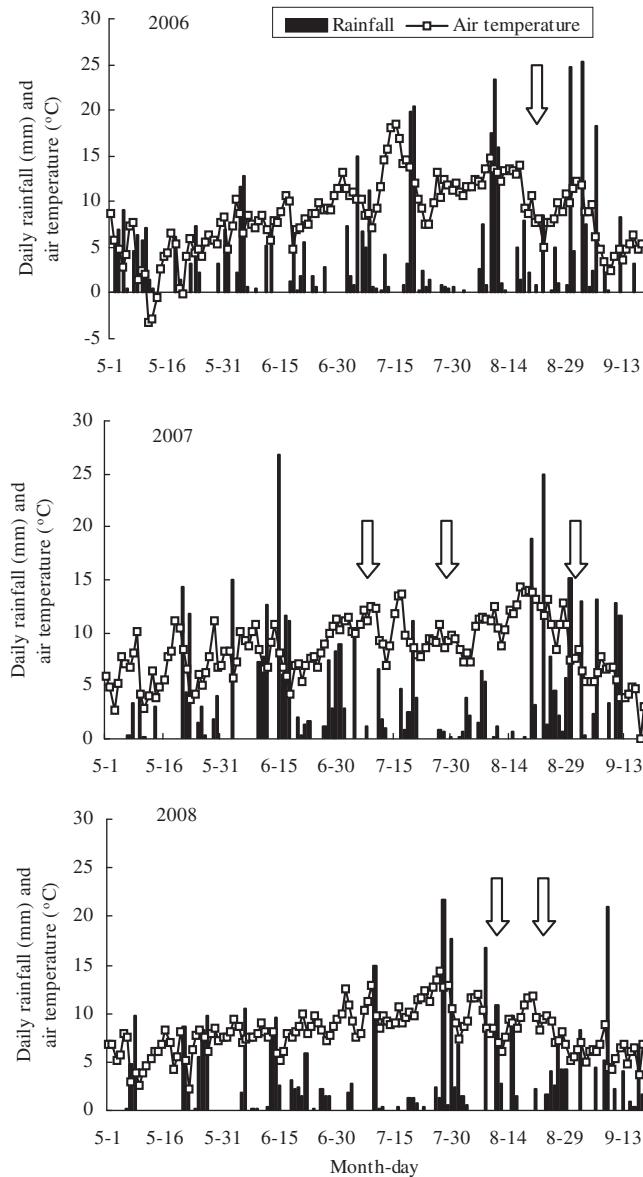


Fig. 1. Distributions of rainfall, air temperature, and grazing time during growing seasons from 2006 to 2008. □ means grazing time in the Figures.

(repeated-measures) by date and soil depth as described by Klein et al. (2007) using SPSS Version 12.0. Multi-comparison of least standard difference (LSD) was conducted for all measured variables within each sampling date and each soil depth using a two-way ANOVA. Because no-grazing treatment was applied on all plots before 16 August 2006, the data during the growing season before and after 16 August 2006 were analyzed separately. The influence of warming and grazing on mean monthly, seasonal, and annual N₂O fluxes during growing seasons and no-growing seasons were investigated using a two-way ANOVA, in which warming and grazing were crossed (Klein et al., 2007). All statistical analyses were performed with SPSS using the GLM procedure and type III sum of squares. Simple correlation analysis was used to measure the relationships between N₂O fluxes and the corresponding mean soil temperature and soil moisture at different soil depths between 9:00 a.m. and 11:00 a.m. during growing seasons and no-growing seasons from 2007 to 2009, respectively, due to a different grazing times in 2006. All significant differences were at $p = 0.05$ level.

3. Results

3.1. Soil temperature and soil moisture

Similar to the previous reports (Luo et al., 2009a,b), warming and grazing significantly increased average soil temperature by 1.8 °C at 20 cm during the growing season in 2008, in particular, the average soil temperatures at 20 cm during the no-growing seasons were increased by 3 °C (range from 1.9 to 4 °C) by heaters in 2007–2008 and 2008–2009 (Fig. 2) due to higher power outputs than during the growing seasons. However, inconsistent with previous results of 2006 and 2007, only in 2008 warming significantly decreased soil moisture at 10 cm by approximately 16% during the growing season (Fig. 3). Both warming and grazing did not affect soil moistures at any soil depths during no-growing seasons because the ground was frozen (data not shown).

3.2. Effects of warming and grazing on temporal variation in N₂O fluxes

Generally, warming, grazing, and the interactions of warming and/or grazing and sampling date significantly affected N₂O fluxes, and their effects varied with season and year (Table 1). The peak of daily N₂O flux occurred during July–August for all treatments except in 2008 (Figs. 4 and 5). In 2008, there was a "burst" of N₂O flux during early May (Fig. 5). Warming significantly decreased the average N₂O flux by 27.4% before grazing compared with no-warming (137.1 $\mu\text{g m}^{-2} \text{h}^{-1}$), but neither warming nor grazing affected N₂O flux after grazing during the growing season in 2006 (Fig. 4). However, warming and grazing significantly increased N₂O flux by 23.6 and 28.5% during the growing season in 2007, respectively. Warming did not significantly affect N₂O flux but grazing increased it by 55.0% during the growing season in 2008 (Fig. 5). Average seasonal N₂O fluxes were 4.8 (ranged 4.5–5.1 $\mu\text{g m}^{-2} \text{h}^{-1}$), 7.1 (ranged 6.9–7.2 $\mu\text{g m}^{-2} \text{h}^{-1}$), 5.7 (4.6–6.8 $\mu\text{g m}^{-2} \text{h}^{-1}$), and 7.6 (ranged 7.0–8.2 $\mu\text{g m}^{-2} \text{h}^{-1}$) $\mu\text{g m}^{-2} \text{h}^{-1}$ during the two growing seasons (5 months from May to September) in 2007 and 2008 (Fig. 5), and 3.3 (ranged 1.8–4.8 $\mu\text{g m}^{-2} \text{h}^{-1}$), 5.5 (ranged 5.2–5.7 $\mu\text{g m}^{-2} \text{h}^{-1}$), 0.2 (ranged –0.7–1.1 $\mu\text{g m}^{-2} \text{h}^{-1}$), and 0.5 (ranged –0.8–1.8 $\mu\text{g m}^{-2} \text{h}^{-1}$) $\mu\text{g m}^{-2} \text{h}^{-1}$ during the two no-growing seasons (7 months from October to April) in 2007–2008 and 2008–2009 (Fig. 6) for NWNG, NWG, WNG, and WG, respectively. The contributions of total N₂O emissions during no-growing seasons to total annual N₂O emissions were 49.0 (ranged 35.9–56.9%), 52.0 (ranged 44.9–50.6%), 4.8

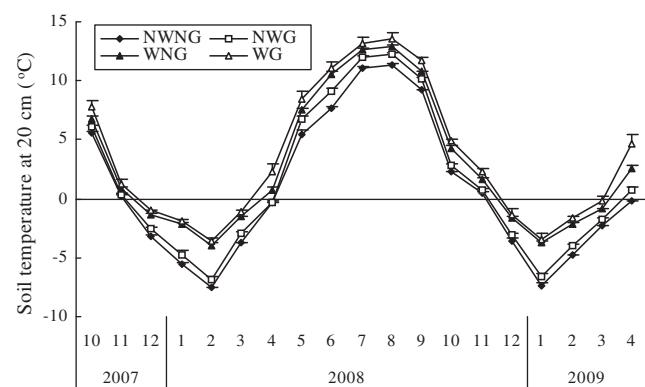


Fig. 2. Dynamics of soil temperature at 20 cm under different treatments from October 2007 to April 2009. NWNG: no-warming with no-grazing, NWG: no-warming with grazing, WNG: warming with no-grazing, and WG: warming with grazing. Bars were standard error. Note: the soil temperatures at 5 and 10 cm are not shown here because of the same trends as that at 20 cm under different treatments.

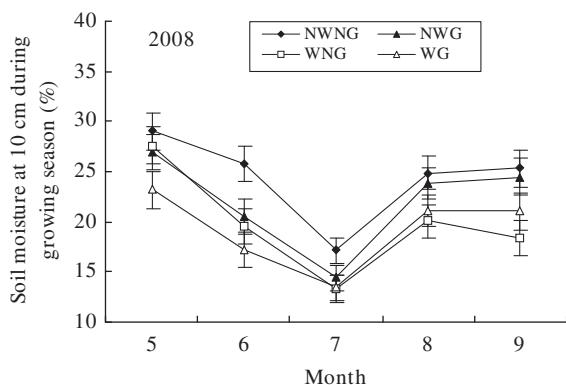


Fig. 3. Dynamics of soil moisture at 10 cm during growing season under different treatments in 2008. NWNG: no-warming with no-grazing, NWG: no-warming with grazing, WNG: warming with no-grazing, and WG: warming with grazing. Bars were standard error. Note: there were no effects of warming and grazing on soil moistures below 10 cm (data not shown).

(−27.1–18.5%), and 8.4% (−19.0–23.5%) for NWNG, NWG, WNG, and WG treatments, respectively. Therefore, warming with grazing or without grazing greatly reduced the contribution of N_2O emission during the no-growing season to annual emission, whereas grazing

Table 1

Summary of the analysis of variance on N_2O fluxes from repeated-measure ANOVAs using month and day as repeated-measures conducted separately for growing and no-growing season from 2006 to 2009.

Year	Period	Model	F	P
2006	Before grazing	Warming (W)	5.96	0.028
		Date (D)	63.24	<0.001
		W × D	2.88	<0.001
		W	0.36	0.557
		Grazing (G)	1.06	0.324
		W × G	0.91	0.359
		D	58.64	<0.001
		W × D	1.95	0.072
		G × D	1.84	0.090
		W × G × D	3.79	0.001
2007–2009	During growing season	W	5.30	0.040
		G	45.86	<0.001
		W × G	0.23	0.639
		Year (Y)	15.54	0.002
		W × Y	7.91	0.016
		G × Y	2.45	0.143
		W × G × Y	0.05	0.823
		D	24.50	<0.001
		W × D	4.50	<0.001
		G × D	7.42	<0.001
		W × G × D	3.59	<0.001
		Y × D	21.14	<0.001
		W × Y × D	3.95	<0.001
		G × Y × D	9.04	<0.001
		W × G × Y × D	1.24	0.228
	During no-growing season	W	12.10	0.005
		G	3.97	0.070
		W × G	0.30	0.591
		Year (Y)	11.24	<0.001
		W × Y	2.01	0.157
		G × Y	0.85	0.439
		W × G × Y	0.97	0.392
		D	2.52	0.039
		W × D	7.93	<0.001
		G × D	2.24	0.061
		W × G × D	1.60	0.174
		Y × D	2.53	0.008
		W × Y × D	6.60	<0.001
		G × Y × D	3.89	<0.001
		W × G × Y × D	2.87	0.003

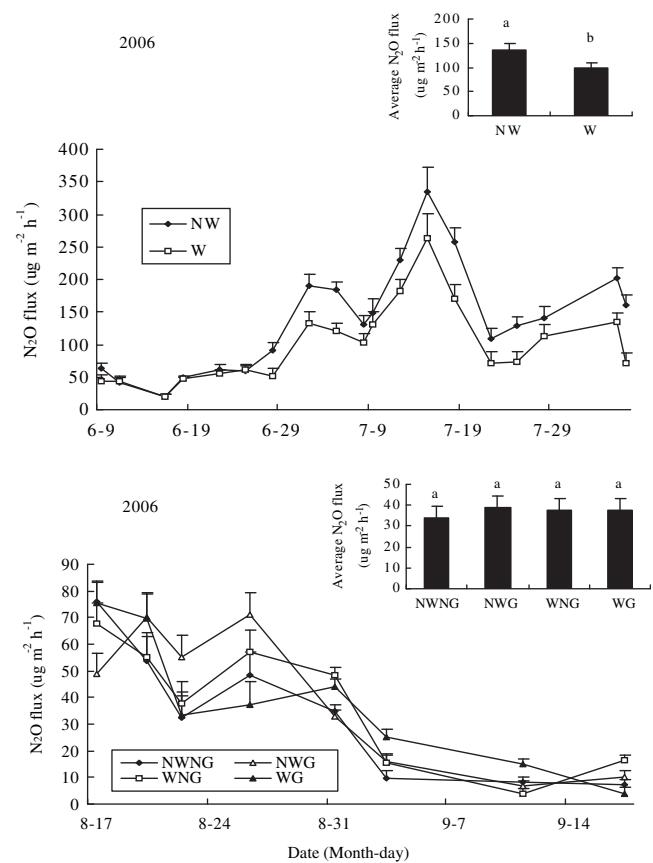


Fig. 4. Effects of warming and grazing on N_2O fluxes before and after grazing during the growing season in 2006. NWNG: no-warming with no-grazing, NWG: no-warming with grazing, WNG: warming with no-grazing, and WG: warming with grazing. Panels inside the Figures were average values of N_2O fluxes under different treatments. Bars were standard error. Different letters mean significant differences at $p = 0.05$ level under different treatments.

did not affect its contribution during the no-growing season to annual N_2O emission, regardless of warming in the region.

Based on a monthly scale (Fig. 7), the effects of warming and grazing on N_2O flux were opposite (i.e., warming and grazing decreased and increased monthly average N_2O flux by 37.2 and 65.4%, respectively), and there was no interaction between them when excluding the data during the growing season in 2006. In particular, there was no significant difference between NWNG (monthly average $3.1 \mu\text{g m}^{-2} \text{h}^{-1}$) and WG (monthly average $3.2 \mu\text{g m}^{-2} \text{h}^{-1}$), whereas NWG and WNG increased by 70.5 and 34.4% of monthly N_2O flux compared with NWNG, respectively.

Average annual N_2O fluxes were $4.2, 6.5, 4.2$, and $5.5 \mu\text{g m}^{-2} \text{h}^{-1}$ for NWNG, NWG, WNG, and WG treatments. Warming did not affect average annual N_2O flux, whereas grazing significantly increased average annual N_2O fluxes, and no interactions between warming and grazing on N_2O flux were found for all years. Moreover, NWG and WG significantly increased average annual N_2O flux 57.8 and 31.0% compared with NWNG and WNG, respectively, indicating that warming reduced the response of annual N_2O flux to grazing on the alpine meadow ecosystem in the region.

3.3. Relationships between N_2O flux and soil temperature and soil moisture

Generally, although the correlations between N_2O flux and soil temperature at different soil depths were significant, their values

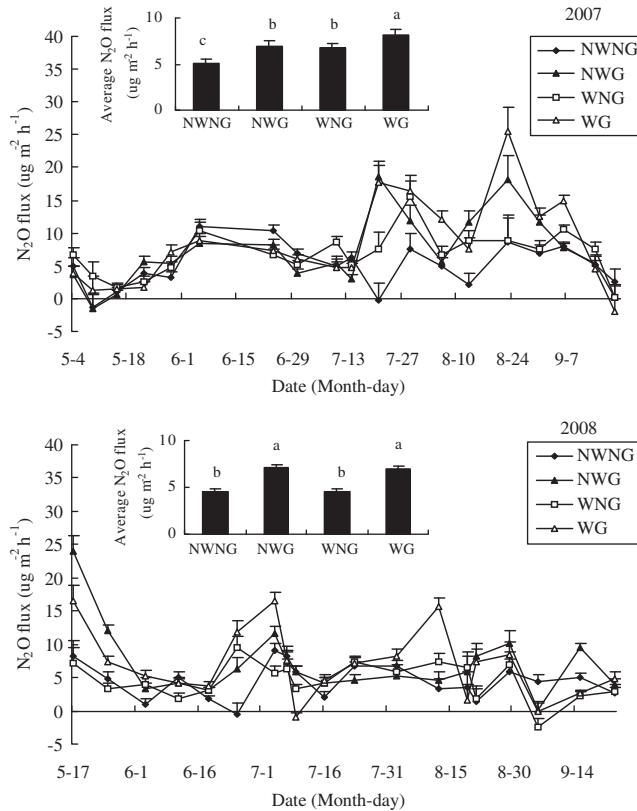


Fig. 5. Effects of warming and grazing on N_2O fluxes during the growing season in 2007 and 2008. NWNG: no-warming with no-grazing, NWG: no-warming with grazing, WNG: warming with no-grazing, and WG: warming with grazing. Panels inside the Figures were average values of N_2O fluxes under different treatments. Bars were standard error. Different letters mean significant differences at $p = 0.05$ level under different treatments.

were small except in 2007. In this case, soil temperature explained about 35% of the variation in N_2O flux during the growing season (Table 2). The effects of soil moisture at different soil depths on N_2O flux were small during the growing season ($r^2 < 0.05$), and there were no significant correlations between N_2O flux and soil temperature and soil moisture during the no-growing season (data not shown).

4. Discussion

The processes of N_2O formation are very complicate. N_2O is formed during the nitrification process when O_2 is limiting and during denitrification (Saggar et al., 2004; Pérez et al., 2006). Nitrification explained about 64–88% of the variation of N_2O in Inner Mongolia steppe (Xu et al., 2003a). The intensity of nitrate reduction in soils depends mainly on soil parameters that control the oxygen state of soils. Among these parameters, available C, temperature, and the soil water content seem to be the most important (Maag and Vinther, 1999). Generally, soil water content causes a increase in denitrification (Maag and Vinther, 1999; Xu et al., 2003a). However, in our study, we did not measure these processes. Therefore, it is a little difficult to discuss them deeply because some processes are mixed each other. For example, some studies show that both of increases of soil water and temperature increased denitrification (Maag and Vinther, 1999), whereas usually soil warming cause decrease of soil water content which will increase aerobic state in soils, in this case, we do not know which factor is main controlling factor for N_2O formation in our study.

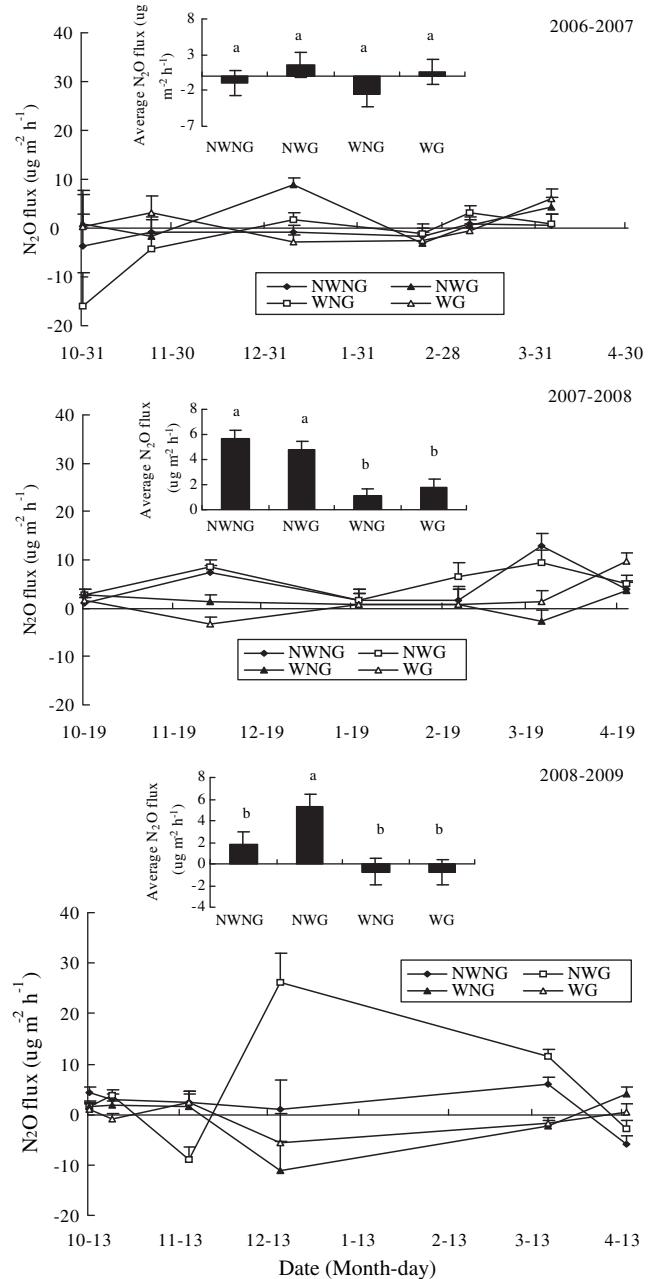


Fig. 6. Effects of warming and grazing on N_2O fluxes during the no-growing season in 2006–2007, 2007–2008, and 2008–2009. NWNG: no-warming with no-grazing, NWG: no-warming with grazing, WNG: warming with no-grazing, and WG: warming with grazing. Panels inside the Figures were average values of N_2O fluxes under different treatments. Bars were standard error. Different letters mean significant differences at $p = 0.05$ level under different treatments.

Similarly, grazing increases excrete patches which increase NO_3-N in soils (Ma et al., 2006; Lin et al., 2009). Thus, denitrification of NO_3-N increases N_2O emission. Meanwhile, grazing also increases soil temperature in our study. All of these processes are most important for us to clearly explain why and how temperature and grazing affect N_2O emission.

4.1. Temporal variation of N_2O fluxes

Similar to the results of Lin et al. (2009), a great yearly variation in N_2O fluxes between 2006 (maximum $137.1 \mu\text{g m}^{-2} \text{h}^{-1}$) and

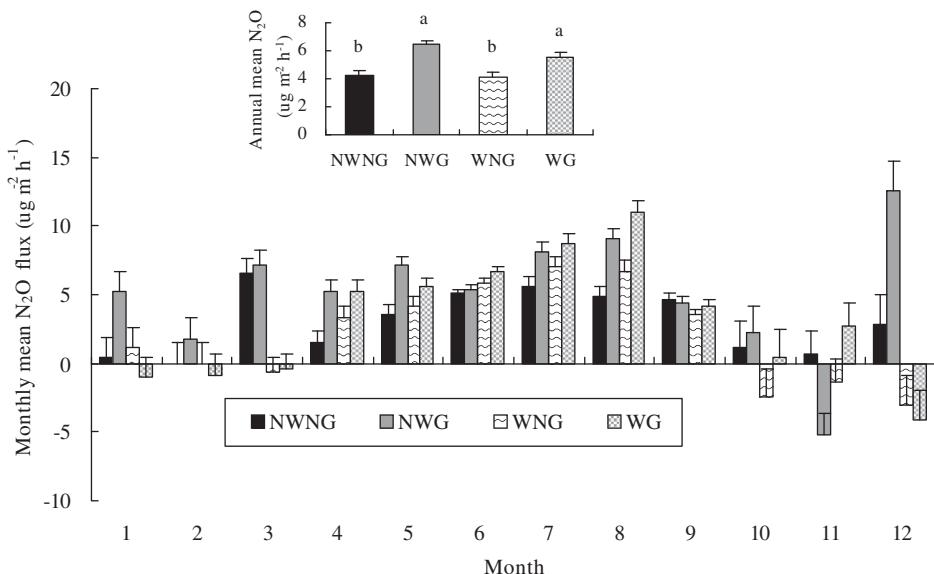


Fig. 7. Effects of warming and grazing on monthly and annual N_2O fluxes. Bars were standard error. Different letters mean significant differences at $p = 0.05$ level under different treatments.

2007–2008 (maximum $25.5 \mu\text{g m}^{-2} \text{h}^{-1}$) was found in our study (Figs. 4 and 5), probably because the study site was a winter grazing area until June 2006. Some patches of fresh sheep dung covered the meadow evenly before the experiment in 2006, which may result in greater soil NO_3^- content in 2006 compared with 2007 and 2008 (Lin et al., 2009). Therefore, the substantive N_2O emissions may come from the sheep excreta patches and the process of denitrification of NO_3^- in the soil in 2006 (Ma et al., 2006; Lin et al., 2009). The results suggest that heavy grazing intensity during the no-growing season (Zhou et al., 2005), which removes almost all litters and deposits many excreta patches on the meadow, could cause great N_2O emissions during the growing season in the region (Lin et al., 2009). However, the experimental site was fenced after 2006, and grazing intensity for the grazing treatment was moderate for two or three days during the growing season, which greatly reduced excreta patches in the grazing plots.

For NWNG and NWG treatments in our study, fluxes of N_2O showed no clear seasonal pattern, with production during the no-growing season similar to fluxes during the growing season (i.e., winter accounted for approximately 36–57% of annual N_2O flux), which was consistent with other reports (Brumme et al., 1999; Xu et al., 2003a, b; Groffman et al., 2006). Mosier et al. (1996) also measured high N_2O fluxes ($>5 \mu\text{g-N m}^{-2} \text{h}^{-1}$) during winter in Colorado short grass steppe. Even Kaiser et al. (1998) and Röver et al. (1998) found higher N_2O fluxes (ca. $50 \mu\text{g-N m}^{-2} \text{h}^{-1}$) in frozen soils (soil temperature -4°C) under arable management in Germany. Therefore, the fact that approximately 40% of the average annual N_2O losses occurred during the no-growing season confirmed the importance of spring and autumn periods for

assessment of total N_2O losses in the region. High fluxes during these periods have been attributed to: (1) accumulation and release of N_2O from beneath frozen soil layers (Goodroad and Keeney, 1984; Burton and Beauchamp, 1994; van Bochove et al., 2001); (2) freezing-induced microbial mortality followed by rapid regrowth and high rates of microbial transformations of N (Edwards and Killham, 1986; Christensen and Tiedje, 1990; Deluca et al., 1992; Schimel and Clein, 1996; Brooks et al., 2004; Dörsch et al., 2004); and (3) freezing-induced disruption of soil aggregates and release of available carbon that stimulates N_2O emission (Groffman and Tiedje, 1989; van Bochove et al., 2000). However, our study indicated that warming altered the seasonal pattern of N_2O flux because warming significantly reduced N_2O flux during the no-growing season; winter accounted for only about 5–8% of annual N_2O flux for WNG and WG.

4.2. Effects of warming on N_2O fluxes

We found that the effect of warming on N_2O flux varied with year and season (Table 1). For example, warming reduced, increased, and did not affect N_2O fluxes during the growing season in 2006, 2007, and 2008, respectively. These differences may have been attributed to 1) increased plant uptake to NO_3^- (Xu et al., 2004) leading to decreased denitrification rate (Zak et al., 1990; Groffman et al., 1993, 2006) due to increased plant production by warming (Luo et al., 2009b); 2) accumulation of litter and increased decomposition rate by warming (Luo et al., 2009a), which may increase releases of available C and N into soils; and 3) decreased soil moisture by warming during the growing season in 2008 (Fig. 2). In 2006, increased plant uptake for the warming treatments may be a dominant effect which reduced N_2O fluxes because of normal rainfall (449 mm) during the growing season (Fig. 1), no effect of warming on soil moisture in 2006 (Luo et al., 2009a), and little litter accumulation. In particular, warming made dung patches dry more quickly after rain, which also greatly decreased N_2O emission (Ma et al., 2006; Lin et al., 2009). However, in 2007 a little drought (398 mm rainfall during the growing season) (Fig. 1) may have limited plant uptake for N; moreover, much litter accumulation and increased decomposition by warming may have increased mineral N return to the soil (Xu et al., 2003a,b), which could be the

Table 2

Simple correlation between N_2O flux and soil temperature and soil moisture at different soil depths during the growing season.

Time	Soil temperature				Soil moisture	
	5 cm	10 cm	20 cm	40 cm	10 cm	40 cm
2006		0.233**	0.214**	0.173**	-0.148**	
2007	0.556**	0.587**	0.592**	0.460**		-0.141**
2008				0.118**		
2006–2008	0.281**	0.283**	0.274**	0.240**		

**Mean significant at $p = 0.01$. Only significant correlations were shown.

dominant contribution to seasonal increased N_2O flux for the warmed plots. On the other hand, in 2008, a heavy drought year (only 339 mm rainfall during the growing season), warming significantly decreased soil moisture, which may have limited the response of N_2O flux to warming. It is also possible that increased levels of inorganic N or lower soil moisture by warming altered the product ratios ($\text{NO:N}_2\text{O:N}_2$) during denitrification not to favor N_2O (Davidson and Verchot, 2000). Therefore, the effect of warming on N_2O flux will depend on the offsets between the positive and negative effects of warming on N_2O production processes. Xu et al. (2003b) reported that denitrification was main process for N_2O formation which explained about 64–88% of the variation of total N_2O emission in the Inner Mongolia steppe. The intensity of nitrate reduction in soils depends mainly on soil parameters that control the oxygen state of soils. Among these parameters, available C, temperature, and soil water content seem to be the most important (Beauchamp et al., 1989; Aulakh et al., 1992; Maag and Vinther, 1999). High temperature enhanced both aerobic respiration and denitrification, and aerobic respiration further enhanced denitrification by consuming oxygen, resulting in strong sensitivity of denitrification to temperature (Maag and Vinther, 1999).

Warming did not affect N_2O flux during the no-growing season in 2006–2007, but warming significantly reduced N_2O flux during the two no-growing seasons in 2007–2008 and 2008–2009. We observed that warming decreased the snow cover and caused the snow to melt earlier compared with the no-warming plots due to increased soil temperature (Fig. 3; Luo et al., 2009a,b). Release of N_2O trapped beneath ice layers in the soil, one mechanism by which soil freezing has been reported to increase N_2O flux (Goodroad and Keeney, 1984; Burton and Beauchamp, 1994; van Bochove et al., 2000, 2001, 2006), did not appear to be a major factor, as we did not observe any marked “bursts” of N_2O production during the no-growing season in 2006–2007 (Fig. 6), whereas some “bursts” were found during the no-growing seasons in 2007–2008 and 2008–2009 for NWNG and NWG treatments (Fig. 6). Groffman et al. (2006) also suggest that winter climate change that decreases snow cover and induces soil freezing will increase soil-atmosphere N_2O fluxes from northern hardwood forests. Our results suggest that in a warmer world with less soil freezing, N_2O emission from the alpine meadow on the Qinghai-Tibetan plateau may decrease, perhaps by as much as 10–20 times. Therefore, generally based on annual scale, warming did not significantly affect annual N_2O flux because of the offset between positive and negative effects on N_2O flux between growing and no-growing seasons in the region.

4.3. Effects of grazing on N_2O fluxes

In our study, grazing consistently increased N_2O flux both during growing and no-growing seasons, except during the growing season in 2006 because of only one grazing period during the first year of the experiment. Although grazing can lead very quickly to changes in nutrient pools and fluxes (Ross et al., 1999; Augustine and Frank, 2001), in vegetation cover (Paruelo et al., 2001), and in plant community composition in grasslands (Oba et al., 2001; Wang et al., 2003), the contribution of grazing to N_2O flux may mainly come from the excreta patches which increase NO_3-N in soils (Ma et al., 2006; Lin et al., 2009). However, no significant differences in N trace gas fluxes were found between grazing histories and grazing systems in the Inner Mongolia steppe (Holst et al., 2007). Although Wang et al. (2005) also found differences in N_2O emission rates between grazed and ungrazed plots in the Inner Mongolia steppe at certain times during the growing season, especially during the flowering of the grasses, they found that grazing decreased the N_2O emission rate. As the result of

microbial related nitrification and denitrification processes, production of N_2O was usually restricted by soil temperature and moisture (Zak et al., 1990; Groffman et al., 1993). In the alpine meadow on the Tibetan plateau, lower soil temperature and higher soil moisture were observed compared with that in the Inner Mongolia steppe (Ma et al., 2006; Lin et al., 2009); therefore, different climate conditions may result in a different grazing effect on N_2O flux. In our study, grazing increased soil temperature (Fig. 2). Both increased temperature and high NO_3-N induced by excrete patches in soils may enhance the denitrification (Maag and Vinther, 1999) which enhanced N_2O emission.

4.4. Relationships between N_2O flux and soil temperature and soil moisture

Although N_2O fluxes were significantly correlated with soil moisture and soil temperature, the correlations explained less than 20% of the variance of the measured fluxes (Holst et al., 2007). Groffman et al. (2006) suggested that correlations between gas flux and soil temperature were higher for CO_2 and CH_4 than for N_2O , whereas there were no significant correlations between any gas flux and soil moisture. However, Lin et al. (2009) found that both soil temperature and soil moisture explained 34–56% of N_2O flux variation. Our results show that soil temperature only explained less than 10% of N_2O flux variation except in 2007, which explained about 35% of N_2O flux variation. Therefore, N_2O flux in the alpine ecosystem could depend more on climate conditions than in temperate grasslands/steppes because other factors contributing to N_2O flux variance could not be identified in these studies.

4.5. Loss of N as N_2O emission

Reported mean emission rates are mostly below $6.5 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ or smaller than $0.6 \text{ kg N ha}^{-1} \text{y}^{-1}$ (Mosier et al., 1991, 1996, 2002; Epstein et al., 1998; Mumme et al., 1997, 2000). The average annual N_2O emission was in the range of $0.03\text{--}0.28 \text{ kg N}_2\text{O-N ha}^{-1} \text{y}^{-1}$ in the Inner Mongolia steppe (Xu et al., 2003a,b; Wang et al., 2005; Holst et al., 2007). Du et al. (2006) reported a mean total annual N_2O flux of $0.73 \pm 0.52 \text{ kg N}_2\text{O-N ha}^{-1} \text{y}^{-1}$ in the same region. In our study, average annual values were 2.7, 4.1, 2.7, and $3\text{--}5 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ or $0.24, 0.36, 0.24$ and $0.31 \text{ kg N ha}^{-1} \text{y}^{-1}$ for NWNG, NWG, WNG, and WG when grazing was moderate during the growing season, respectively. Only 5–8% of annual 7.2 kg N deposition input (Zhang and Cao, 1999) may be offset by N_2O flux from soil to the atmosphere. Thus, the N_2O formation does not appear to be a significant pathway in the N cycle of such ecosystems which was consistent with previous report (Billings et al., 2002). However, Driscoll et al. (2003) reported that approximately 25% of this N deposition input was caused by emission by N_2O flux from the soil to the atmosphere. In our study, the data in 2006 indicated that heavy grazing in winter caused much higher annual N_2O emission (averaged $8.2 \text{ kg N ha}^{-1} \text{y}^{-1}$) from the alpine meadow due to the high density of excreta patches.

4.6. Conclusions

Generally, the effects of warming and grazing on N_2O flux varied with year, season, and sampling date. There was no interactive effect between warming and grazing. Warming did not affect annual N_2O flux due to the offset between positive effect (i.e., increased N_2O flux) during the growing season and negative effect (i.e., decreased N_2O flux) during the no-growing season when grazing was moderate during the growing season. Grazing significantly increased N_2O flux for both growing and no-growing seasons, but warming reduced the response of N_2O flux to

grazing. Fluxes in N_2O only for the no-warming treatments showed no clear seasonal pattern. There were significant correlations between N_2O flux and soil temperature at different depths, and soil temperature could explain the highest (35%) N_2O flux variation in 2007. Annual emission of an average $0.3 \text{ kg N ha}^{-1} \text{ y}^{-1}$ was found when grazing was moderate during the growing seasons regardless of warming. However, annual emission could be averaged at $8.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$ when heavy grazing occurred in the winter of 2006. These results imply that grazing intensity may be a main control factor to N_2O flux in the region. Therefore, our study has important implications for predictions about future contributions of alpine, and possibly other cold regions, to the global N_2O budget under grazing with future warming conditions.

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