

Variations of N₂O fluxes in response to warming and cooling in an alpine meadow on the Tibetan Plateau

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Received: 6 June 2016 / Accepted: 3 May 2017 / Published online: 19 May 2017
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Abstract Little is known about the impacts of climate change especially for cooling on N₂O emissions from alpine meadows on the Tibetan Plateau. Along a slope of Qilian mountains, China, we transferred intact soil cores covering different vegetation types (graminoid, shrub, forb, and sparse vegetation) downhill (warming) and uphill (cooling) across a 600-m elevation gradient to examine the responses of soil-atmosphere N₂O exchange rates to climate warming and cooling. N₂O fluxes were measured during two growing seasons from May to October in 2008 and 2009. The Tibetan alpine meadow acted as a net N₂O source at an average rate of

Electronic supplementary material The online version of this article (doi:10.1007/s10584-017-1987-z) contains supplementary material, which is available to authorized users.

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5.2 $\mu\text{g m}^{-2} \text{h}^{-1}$ (ranging from 2.0 to 11.5 $\mu\text{g m}^{-2} \text{h}^{-1}$). In situ N_2O emission generally decreased with elevation increase except for sparse vegetation, but significant differences were only found between graminoid and other three vegetations in 2008 and between graminoid and shrub vegetation in 2009. Warming averagely increased mean N_2O fluxes by 219% (ranging from 126 to 287%) while cooling decreased it by 75% (ranging from 57 to 95%) across four vegetation types over the variation of soil temperature from 1.3 to 5.5 °C. However, opposite effects were also observed in some cases due to modification of variations in soil moisture. Soil temperature and moisture had a positive effect on N_2O fluxes and explained 48 and 26% of the variation in mean N_2O fluxes across the four vegetation types, respectively. No relationship was found between mean N_2O fluxes and aboveground biomass. Our results suggest that more N_2O -N would be released from soil in a warmer future and that less N_2O emission during cool and dry years is expected in the Tibetan alpine meadow.

1 Introduction

Nitrous oxide (N_2O) is both a powerful greenhouse gas and a contributor to the depletion of ozone (IPCC 2007; Ravishankara et al. 2009). Variations in historical atmospheric N_2O concentration are closely paralleled with patterns of climate fluctuation (Pfeiffer et al. 2013; Schilt et al. 2010) with important feedbacks to the global climate (Wuebbles 2009; Xu et al. 2012). Recent manipulative field experiments have also shown that soil-atmosphere N_2O exchange is sensitive to climate change (Cantarel et al. 2011; Dijkstra et al. 2013; Flechard et al. 2007; Hu et al. 2010; Teh et al. 2014).

N_2O is produced in soils during the processes of nitrification and denitrification and is regulated by soil C and N availability and abiotic factors such as temperature and moisture (Davidson et al. 2000; Chapuis-Lardy et al. 2007; Dijkstra et al. 2013; Teh et al. 2014). For instance, plant biomass and C availability vary with altitude on the mountainous Tibetan Plateau (Hu et al. 2016a, b; Wang et al. 2008). Although the plateau is undergoing obvious climate warming (Hansen et al. 2006; Thompson et al. 2000), higher and lower temperature spells with variable rainfall are common on the plateau (Du et al. 2004). However, there are many uncertainties in the evaluation and prediction of N_2O budgets from Tibetan alpine meadows due to scarce available data, especially with regard to N_2O fluxes under climate change (Hu et al. 2010; Shi et al. 2012).

Many previous studies have found that warming can increase C and N availability through enhancement of plant biomass and N mineralization (Hu et al. 2016b; Rui et al. 2011; Rustad et al. 2001) that may enhance N_2O emission (Davidson et al. 2000; Dijkstra et al. 2013). However, increased plant N uptake due to stimulated plant growth under warming (Rustad et al. 2001; Wang et al. 2012) may also limit mineral N supply to nitrification and denitrification that may reduce N_2O production. Experimental warming had no effects on annual N_2O emission from a Tibetan alpine meadow over 3 years (Hu et al. 2010), which was ascribed to drier soil conditions caused by warming (Hu et al. 2010; Shi et al. 2012). In contrast, reduced temperature (cooling) may have opposite effects on N_2O emission that may be modified by variations in soil moisture but that remain unclear and largely unexplored.

The use of an elevation gradient is a particularly powerful tool to understand responses of ecosystems to global changes (Malhi et al. 2011). Here, we conducted a reciprocal translocation experiment to imitate climate warming (uphill translocation) and cooling (downhill translocation) by using an elevation gradient along a slope of the Qilian mountains in the

northeastern Tibetan Plateau. N₂O fluxes were measured over two growing seasons from May to October in 2008 and 2009. Our research objectives were to investigate variations of N₂O fluxes along the elevation gradient and examine warming and cooling effects on N₂O fluxes in the Tibetan alpine meadow. We hypothesized that (1) N₂O exchange rates would decrease with elevation increase, and (2) there would be a positive feedback of warming (i.e., soil brought down to lower elevation) on N₂O emission while cooling (i.e., soil brought up to higher elevation) would have the opposite effect.

2 Materials and methods

2.1 Study site and experimental design

The study was conducted at the Heibei Alpine Meadow Ecosystem Research Station (37° 37' N, 101° 12' E) of the Chinese Academy of Sciences. The mean annual air temperature and precipitation from 1981 to 2000 were -1.7 °C and 561 mm, respectively. The soil is classified as Gelic Cambisols according to FAO classification system. Our study consisted of four sites (aspect $<3^\circ$) within 9 km in distance from each other. These sites covered a 600-m elevation gradient and contained four different vegetation types. At 3200 m, the vegetation is dominated by graminoids including *Kobresia humilis*, *Festuca ovina*, *Elymus nutans*, *Poa* spp., *Carex* spp., *Scirpus distigmaticus*, *Gentiana straminea*, *Gentiana farreri*, *Leontopo diumnanum*, and *Potentilla nivea*. At 3400 m, the vegetation is dominated by alpine shrubs including *Potentilla fruticosa*, *Kobresia capillifolia*, *Kobresia humilis*, and *Saussurea superba*. At 3600 m, the vegetation is dominated by *K. humilis*, *Saussurea katochaete* Maxim, *P. nivea*, *Thalictrum alpinum*, *Carex* spp., *Poa* spp., and *P. fruticosa*. At 3800 m, the vegetation is sparse and include *K. humilis*, *L. odiumnanum* and *Poa* spp. We refer to these four vegetation types as graminoid, shrub, forb, and sparse vegetation, respectively (Zhang et al. 2011). Plant biomass and physico-chemical properties differed from each other (Table 1). Soil depth decreased with increasing elevation with a mean thickness of 0.65, 0.7, 0.5, and 0.3 m for graminoid, shrub, forb, and sparse vegetations, respectively.

A detailed description about the experimental design can be found elsewhere (Wang et al. 2014; Zhang et al. 2011). Briefly, in early May 2007, 12 1-m long * 1-m wide * 0.3–0.4-m deep (with 30 cm depth for sparse vegetation due to a shallower soil layer) intact soil blocks with attached vegetation from each site were manually cut off for translocation. Three of these soil blocks were reinstated in situ as control blocks (home monoliths) while the other nine soil blocks were transferred to the other three elevation sites (translocated monoliths). All intact soil blocks were fully randomly translocated and arranged, and surrounded by plastic film to prevent exchange and invasion of roots with/from the ambient soil environment. Thus, there was only uphill translocation (cooling) for graminoid vegetation, both uphill and downhill translocation (warming) for shrub and forb vegetation, and only downhill translocation for sparse vegetation. There were a total of 48 intact soil blocks (4 elevations * 4 vegetations * 3 replicates) in our study.

2.2 Soil temperature and soil moisture

A HOBO weather station (Onset Computer Corporation, Cape Cod, MA, USA) was installed with temperature sensors of Model S-TMB-M002 and soil moisture smart sensors of model S-

Table 1 Characteristics of selected sites along the slope of the Qilian mountains in the northeastern Tibetan Plateau

Site name	Elevation (m a.s.l.)	Landforms	Latitude (N)	Longitude (E)	Bulk Density A horizon (g cm^{-3})	AGB ^a (g m^{-2})	BGB ^a (kg m^{-2})	Soil C A horizon (%)	Soil N A horizon (g kg^{-1})	Soil C/N A horizon
Graminoid	3200	Slope, flat	37° 36' 42.3"	101° 18' 47.9"	0.6	277.7	3.4	6.2	4.6	13.5
Shrub	3400	Slope, flat	37° 39' 55.1"	101° 19' 52.7"	0.8	164.4	2.8	5.7	4.1	13.7
Forb	3600	Slope, flat	37° 41' 46.0"	101° 21' 33.4"	0.7	85.7	2.5	5.4	4.6	11.8
Sparse	3800	Flat, basin	37° 42' 17.7"	101° 22' 09.2"	0.8	52.1	1.9	5.0	4.6	10.9

^a AGB and BGB mean aboveground biomass and belowground biomass within 20 cm soil depth in 2009

SMC-M005 ECH2O (Onset Computer, Bourne, MA, USA) at the center of the fenced experimental area outside of the blocks at each site. The sensors were connected to a CR1000 datalogger, data of soil temperature and soil moisture at 20 cm depth were measured every 1 min, and then 30-min averages were stored.

2.3 N₂O fluxes

During the growing seasons from May to September in 2008 and 2009, N₂O fluxes were measured by using static chambers and gas chromatography techniques. Details about the chambers and methods of the gas sampling can be found in the study by Lin et al. (2009). Briefly, the static chamber consisted of a bottom anchor (0.4 m length * 0.4 m width * 0.1 m height) that was permanently inserted into the soil about 10 cm below the soil surface and a removable cover box (0.4 m length * 0.4 m width * 0.4 m height) with a fan (0.1 m in diameter) attached to the inside wall and a white cover on the outside wall to reduce warming inside the chamber. The cover box was placed on the bottom anchor during sampling and sealed by adding water into the groove of the bottom anchor. Samples were taken between 9:00 and 11:00 a.m. local time every 7–10 days depending on weather conditions (measurements of N₂O fluxes were delayed due to heavy rain events and continued when the rain stopped). Four gas samples (about 100 ml for each sample) were manually taken from the closed chamber every 10 min by using 100-ml plastic syringes. The N₂O concentration was analyzed by using gas chromatography (HP Series 4890D, Hewlett Packard, USA) within 24 h after sampling. The N₂O fluxes were calculated as the slope of linear regressions from the measured gas concentrations with time (Dijkstra et al. 2013).

Here, we made an assumption that variations in N₂O fluxes of translocation monoliths were caused by changes in temperature and moisture; influences from other factors like wind and solar radiation were ignored. Variations of aboveground biomass in response to warming and cooling were reported in details by Hu et al. (2016a).

2.4 Statistical analysis

Normality distribution of N₂O fluxes was tested in one-sample Kolmogorov-Smirnov with SPSS version 16.0. Linear mixed models with repeated measurements were used for analysis of variance (ANOVA); type III SS was adopted because of missing data at 3600 m elevation in 2009. To examine variation among the home monoliths (in situ vegetation) at the different elevations, vegetation was taken as the between-subject factor. To examine warming and cooling effects, vegetation type and elevation were between-subject factors. For all ANOVAs, date and year were within-subject factors. Pearson simple correlation, stepwise linear regressions, and a quadratic term were used to test the possible dependency of N₂O fluxes on soil temperature, soil moisture, and aboveground biomass. Differences were considered to be significant at $P < 0.05$.

3 Results

3.1 N₂O fluxes in situ

In general, soil temperature increased with a decrease in elevation (Fig. 1). Average soil temperatures during the sampling period were 9.5, 7.3, 5.9, and 4.5 °C in 2008 and 10.1, 7.8,

6.5, and 5.3 °C in 2009 at 3200, 3400, 3600, and 3800 m, respectively. Average soil moistures were 33.2, 26.9, 36.2, and 12.7% in 2008 and 35.3, 28.5, 43.0, and 16.3% in 2009 at 3200, 3400, 3600, and 3800 m, respectively.

For the home monoliths with in situ vegetation, N₂O fluxes were significantly affected by date, elevation and their interaction (Table 2). N₂O fluxes did not show a clear variation pattern across the 2-year period. The N₂O fluxes were small except for several small “bursts” in the early spring that depended on vegetation, and sometimes were negative which suggested soil uptake of N₂O. Mean N₂O fluxes decreased with elevation increase except at the highest elevation with values of 11.5, 4.1, 2.2, and 3.0 $\mu\text{g m}^{-2} \text{h}^{-1}$ at 3200, 3400, 3600, and 3800 m in 2008 and 8.5, 2.0, and 4.8 $\mu\text{g m}^{-2} \text{h}^{-1}$ at 3200, 3400, and 3800 m in 2009, respectively. However, there was no significant difference among the 3400, 3600, and 3800 m elevations over 2 years (Fig. 1).

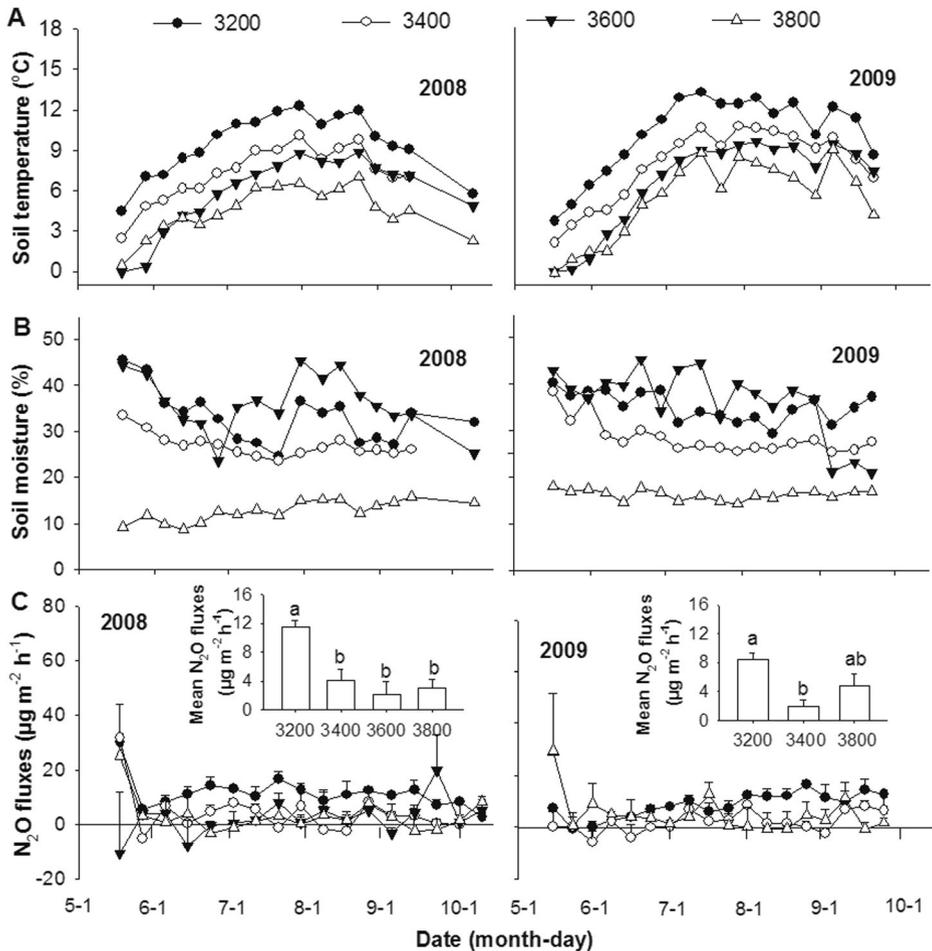


Fig. 1 Soil temperature (a), soil moisture (b), and N₂O fluxes (c) for graminoid (3200), shrub (3400), forb (3600), and sparse vegetation (3800) in situ during the growing seasons in 2008 and 2009. Panels inside the figures show mean N₂O fluxes under no-transferring. Error bars indicate standard error. Different letters indicate significant differences at $P < 0.05$ level. In 2009, N₂O fluxes at 3600 m (forb vegetation) were not recorded due to damage to monoliths from pikas (*Ochotona curzoniae*)

3.2 N₂O fluxes of translocation

After being translocated, date, elevation, vegetation type, and the interactions of elevation and/or vegetation type with date and year significantly affected N₂O fluxes (Table 1). Variations in N₂O fluxes were similar to that of home monoliths with in situ vegetation, with no clear variation patterns and several small bursts in the early spring that depended on vegetation type and elevation (Fig. S1). The effects of warming and cooling varied with vegetation type and elevation based on mean N₂O fluxes (ranging from -4.2 (N₂O uptake) to $17.4 \mu\text{g m}^{-2} \text{h}^{-1}$) (Fig. 2). Compared with home monoliths, warming increased mean N₂O emission in most combinations of vegetation type and elevation across the 2-year period, with increases ranging between 126 (sparse vegetation when moved down from 3800 to 3600 m) and 287% (sparse vegetation when moved down from 3800 to 3200 m), but slightly decreased it at other two occasions by 69 (forb vegetation when moved from 3600 to 3400 m) and 58% (sparse vegetation when moved down from 3600 to 3400 m). In contrast, cooling decreased N₂O emission in most cases, with decreases ranging between 75 (graminoid vegetation when moved from 3200 to 3600 m) and 95% (shrub vegetation when moved from 3400 to 3800 m), but increased it by 73% for shrub vegetation when moved from 3400 to 3600 m (Fig. S2).

3.3 Relationships of N₂O fluxes with environmental factors

Although the correlations between N₂O fluxes and soil temperature and moisture were significant, the r values were small except for shrub, forb, and sparse vegetation with warming. In these cases, soil temperature and moisture explained 14–23% of the variation in N₂O fluxes. On the other hand, soil temperature and moisture only explained 2–12% of the variation in N₂O fluxes for the different vegetation types with cooling and pooled vegetation types (Table 1S). Based on mean N₂O fluxes over the growing season, a quadratic relationship

Table 2 Summary of linear mixed models of variance (ANOVA) on N₂O fluxes for home monoliths (in situ) and pooled home and translocated monoliths (translocation) over two growing seasons in 2008 and 2009

Gradient	Source	Df	<i>F</i>	Sig.
In situ	Year (Y)	1	0.792	0.374
	Date (D)	17	2.330	0.003
	Vegetation (V)	3	15.799	<0.001
	Y * D	16	1.643	0.059
	Y * V	2	2.181	0.115
	D * V	50	1.891	0.001
	Y * D * V	31	1.027	0.433
	Year (Y)	1	0.280	0.597
Translocation	Date (D)	17	2.959	<0.001
	Elevation (E)	3	3.530	0.015
	Vegetation (V)	3	92.449	<0.001
	Y * D	16	2.218	0.004
	Y * E	3	1.943	0.121
	Y * V	2	9.051	<0.001
	D * E	51	1.788	0.001
	D * V	50	2.325	<0.001
	E * V	9	4.081	<0.001
	Y * D * E	48	1.967	<0.001
	Y * D * V	31	2.107	<0.001
	Y * E * V	6	2.770	0.011
	D * E * V	150	1.324	0.009
	Y * D * E * V	93	1.445	0.005

Values of significance at $P < 0.05$ are shown in italic.

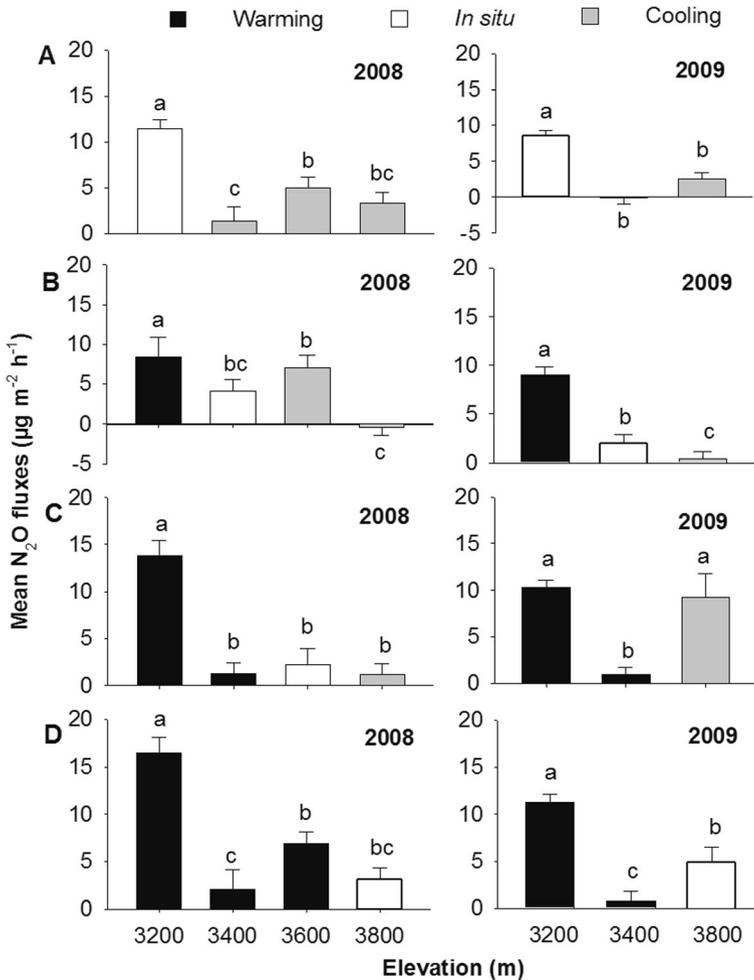


Fig. 2 Effects of warming and cooling on N₂O fluxes for graminoid (a), shrub (b), forb (c), and sparse vegetation (d) during the growing seasons in 2008 and 2009. Error bars indicate standard error. Different letters indicate significant differences at $P < 0.05$ level under different treatments. In 2009, N₂O fluxes at 3600 m for all vegetations were not recorded due to damage to monoliths from pikas (*Ochotona curzoniae*)

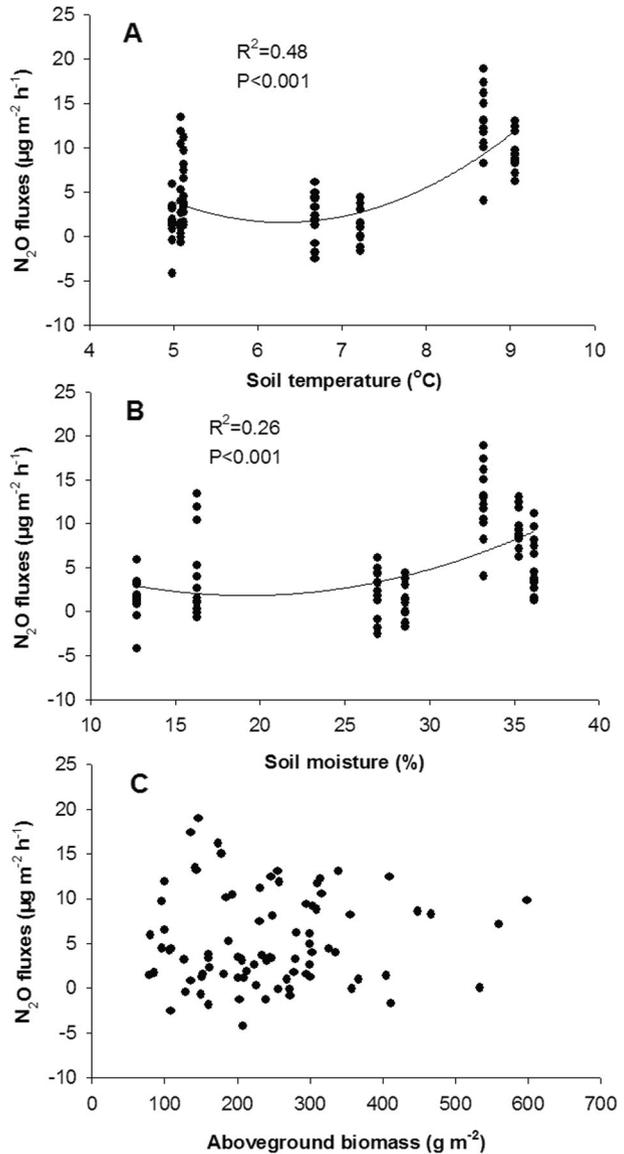
was found between N₂O fluxes and soil temperature and moisture, which explained 48 and 26% of the variation in N₂O fluxes for the pooled vegetation types, respectively. There was no relationship between N₂O fluxes and aboveground biomass (Fig. 3).

4 Discussion

4.1 Temporal and spatial variations of N₂O fluxes

Similar to the results of previous studies (Hu et al. 2010; Jiang et al. 2010), variations in N₂O fluxes showed no clear pattern across all vegetation types over a 2-year period. Both emission (positive value) and uptake of N₂O (negative value) were observed across all vegetations and

Fig. 3 N₂O fluxes as a function of soil temperature, soil moisture, and aboveground biomass. Each data point is the mean N₂O flux, average soil temperature, and soil moisture or aboveground biomass. Regression lines are only shown when significant ($P < 0.05$)



elevations, which was also reported by other studies (Cantarel et al. 2011; Dijkstra et al. 2013; Jiang et al. 2010; Pei et al. 2003; Teh et al. 2014). The uptake of N₂O might result from denitrification (Chapuis-Lardy et al. 2007), where denitrifying bacteria might use atmospheric N₂O as an alternative electron acceptor when nitrate is in short supply (Rosenkranz et al. 2006). N₂O fluxes also showed large temporal and spatial variability (Flecharth et al. 2007; Jiang et al. 2010), with an average variation coefficient (CV) of 202% across all monoliths. Several small bursts of N₂O emission in the early growing season were likely caused by freeze-thaw processes (Burton and Beauchamp 1994; Elberling et al. 2010; van Bochove et al. 2000) and originated from (1) release of N₂O accumulated beneath frozen soil layers (Burton

and Beauchamp 1994; van Bochove et al. 2001) and (2) freezing-induced release of available C and N that stimulates N_2O emission (DeLuca et al. 1992; van Bochove et al. 2000). However, these small bursts of N_2O may have depended on vegetation type and elevation, and it is likely that we missed some of these events with our sampling frequency.

In support of our first hypothesis, mean N_2O fluxes decreased with elevation increase except for the in situ sparse vegetation (home monoliths) at the highest elevation, which was also found in other studies (Neto et al. 2011; Teh et al. 2014). Many studies found that C and N availability, particularly nitrate, might play a vital role in limiting N_2O emission (Dijkstra et al. 2012; Jiang et al. 2010; Teh et al. 2014). The decline in N_2O emission in our study also coincided with reduced C and ammonium availability with increasing elevation (Hu et al. 2016b, c). Decreased labile C might have caused C constraints on denitrification thereby causing a decrease in N_2O emission (Dijkstra et al. 2012), while reduced ammonium likely limited nitrification. Higher N_2O emission for sparse vegetation than forb or shrub vegetation may be linked to both bursts of N_2O emission resulted from freeze-thaw processes due to its cold weather condition and higher soil nitrate concentrations (Hu et al. 2016c), providing denitrification with more N substrates (Davidson et al. 2000). It is further possible that high moisture contents and low ammonium concentrations favored N_2O uptake at the elevation with forb vegetation (Chapuis-Lardy et al. 2007).

4.2 Warming and cooling effects

Previous studies found that warming increased (Cantarel et al. 2011; Larsen et al. 2011; Shi et al. 2012) or had no effect (Hart 2006; Hu et al. 2010) on N_2O emission. In our study, mean N_2O emission was positively related to soil temperature (Fig. 3), and as expected, warming increased mean N_2O emission while cooling reduced it in most cases (Fig. 2S), supporting our second hypothesis. N_2O fluxes seemed to be minimized at about 6 °C and the implied mechanism was unknown, but it is the best fit for the relationship between mean N_2O fluxes and soil temperature over the growing season. This was also in accordance with the decreased trend in the mean N_2O fluxes with elevation of the in situ vegetation types (home monoliths), suggesting that N_2O fluxes of this alpine meadow had a rapid response to climate warming and cooling. This finding was paralleled with similar responses of aboveground biomass and labile C (Hu et al. 2016a, c). As discussed above, the warming-induced increase in labile C (Hu et al. 2016b; Rui et al. 2011) reduced C constraints on denitrification leading to an increased N_2O emission (Dijkstra et al. 2012). It is also possible that higher levels of mineral N in the soil due to warming-induced increases in N mineralization, nitrification, and denitrification may have increased N_2O emission (Hart, 2006; Larsen et al. 2011; Rustad et al. 2001).

There are very few field studies focused on the response of N_2O fluxes to climate cooling. According to a soil transfer study from Hart (2006), cooling (mean annual 2.5 °C) had no significant effects on N_2O fluxes. Our results showed that cooling commonly, but not consistently, decreased N_2O fluxes. Probably, the reduced soil labile C in response to cooling (Hu et al. 2016c) increased C constraints on denitrification (Dijkstra et al. 2012). Alternatively, cooling may have reduced nitrification by limiting aerobic ammonia oxidation due to the decreased abundance of ammonia-oxidizing archaea and bacteria (Zheng et al. 2014). Hart (2006) has found that warming significantly increased but cooling had no significant effects on mean N_2O emission when soil moisture condition was almost the same between in situ and translocation, suggesting that N_2O fluxes seems to be more sensitive to warming than cooling. In our study, soil moisture highly varied among different elevations and we were unable to

distinguish soil moisture and temperature effects. As a result, our work failed to compare the temperature sensitivity of N₂O fluxes to warming and cooling. Given the importance of N₂O fluxes to climate feedback (Wuebbles 2009; Xu et al. 2012) and its higher variations in response to soil temperature and moisture conditions, direct manipulations of soil water content would be need in the future soil transfer studies.

Mean N₂O fluxes were positively related to soil moisture, indicating that increased soil moisture had a positive effect (Dijkstra et al. 2013; Hart 2006), while drought can lead to a reduction in N₂O emission (Goldberg and Gebauer 2009; Larsen et al. 2011; Shi et al. 2012). Soil moisture affected N₂O fluxes by changing oxygen conditions in the soil (Goldberg and Gebauer 2009; Hartmann and Niklaus, 2012). Higher soil moisture could create anaerobic conditions that are beneficial to denitrification (Maag and Vinther 1999) and lower soil moisture lead to a reduction of N₂O emission by denitrifiers (Goldberg and Gebauer, 2009; Hartmann and Niklaus, 2012). Therefore, the decrease in N₂O emission of forb vegetation when moved to 3400 m and the increase N₂O emission of shrub vegetation when moved to 3600 m could be explained by drier and wetter soil conditions, respectively, indicating that soil moisture could modify warming and cooling effects on N₂O fluxes. These results affirmed that the direct stimulatory effect of warming on the N₂O flux could be offset by the indirect inhibitory effect of reduced soil moisture (Bijoor et al. 2008; Shi et al. 2012).

4.3 Loss of N as N₂O emission

Most reported loss of N as N₂O emission was below 6.5 μg N m⁻² h⁻¹ or smaller than 0.6 kg N ha⁻¹ year⁻¹ (Chen et al. 2000; Epstein et al. 1998; Hu et al. 2010; Mosier et al. 1996, 2002; Mummey et al. 2000) except for (sub)tropical forests and pastures where N₂O emissions can be higher (ranging from 1.5 to 5.7 kg N ha⁻¹ year⁻¹) (Hadi et al., 2000; Keller and Reiners, 1994; Melillo et al. 2001; Mosier and Delgado 1997). In our study, the average loss of N as N₂O emission from alpine meadow was 0.34 ± 0.09 kg N ha⁻¹ year⁻¹ across the four vegetation types. Taking the area of alpine meadow (35% of the plateau area) into consideration, the Tibetan alpine meadow acted as a net source of N₂O emission with a mean total annual flux of 0.30 ± 0.07 Tg N, which is lower than estimated for temperate and tropical grasslands (0.59–1.52 Tg N year⁻¹) based on an empirical model study (Xu et al. 2008), and accounted for 1.6–3.6% of the annual global N₂O emission (8.2–18.4 Tg N year⁻¹) according to estimates from model studies (Xu et al. 2008; Xu et al. 2012). By considering positive effects of temperature on N₂O emission, more N₂O-N is expected to be released from the Tibetan alpine meadow soil under warmer and wetter climate conditions. However, less N₂O emission in cool and dry years should also be considered to evaluate N₂O budget from the Tibetan Plateau.

5 Conclusion

Although N₂O fluxes showed large spatial and temporal variation, the Tibetan alpine meadow showed net N₂O emission with an average annual emission rate of 0.30 ± 0.07 Tg N. Our results showed that mean N₂O emission generally decreased with elevation increase except for sparse vegetation, but significant differences were only found between graminoid and other three vegetations in 2008 and between graminoid and shrub vegetation in 2009. Warming commonly, but not consistently, increased mean N₂O emission while cooling had a similar

opposite effect depending on vegetation type and elevation. N_2O fluxes were positively related to soil temperature and moisture but not correlated to aboveground biomass. More N_2O -N is predicted to be released in the warmer future, while less N_2O emission is expected to occur during cool and dry years in the Tibetan alpine meadow. Our study has important implications for the contribution of N_2O emissions from the Tibetan Plateau and to the global N_2O budget with warmer future conditions.

Acknowledgements This work was financially supported by the National Basic Research Program (2013CB956000), the Natural Science Foundation Committee of China (41230750 and 41101081), and the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB03030403).

References

- Bijoor NS, Czimeczik CI, Pataki DE et al (2008) Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn. *Glob Chang Biol* 14:2119–2131
- Burton DL, Beauchamp EG (1994) Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. *Soil Sci Soc Am J* 58:115–122
- Cantarel AA, Bloor JM, Deltroy N et al (2011) Effects of climate change drivers on nitrous oxide fluxes in an upland temperate grassland. *Ecosystems* 14:223–233
- Chapuis-Lardy L, Wrage N, Metay A et al (2007) Soils, a sink for N_2O ? A review. *Glob Chang Biol* 13:1–17
- Chen GX, Huang B, Xu H, Zhang Y et al (2000) Nitrous oxide emissions from terrestrial ecosystems in China. *Chemosphere Global Change Sci* 2:373–378
- Davidson EA, Keller M, Erickson HE et al (2000) Testing a conceptual model of soil emissions of nitrous and nitric oxides. *Bioscience* 50:667–680
- DeLuca T, Keeney D, McCarty G (1992) Effect of freeze-thaw events on mineralization of soil nitrogen. *Biol Fertil Soils* 14:116–120
- Dijkstra FA, Prior SA, Runion GB, Torbert HA, Tian H, Lu C, Venterea RT (2012) Effects of elevated carbon dioxide and increased temperature on methane and nitrous oxide fluxes: evidence from field experiments. *Front Ecol Environ* 10:520–527
- Dijkstra FA, Morgan JA, Follett RF et al (2013) Climate change reduces the net sink of CH_4 and N_2O in a semiarid grassland. *Glob Chang Biol* 19:1816–1826
- Du M, Kawashima S, Yonemura S et al (2004) Mutual influence between human activities and climate change in the Tibetan Plateau during recent years. *Glob Planet Chang* 41:241–249
- Elberling B, Christiansen HH, Hansen BU (2010) High nitrous oxide production from thawing permafrost. *Nat Geosci* 3:332–335
- Epstein HE, Burke IC, Mosier AR et al (1998) Plant functional type effects on trace gas fluxes in the shortgrass steppe. *Biogeochemistry* 42:145–168
- Flechar C, Ambus P, Skiba U et al (2007) Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. *Agric Ecosyst Environ* 121:135–152
- Goldberg SD, Gebauer G (2009) Drought turns a Central European Norway spruce forest soil from an N_2O source to a transient N_2O sink. *Glob Chang Biol* 15:850–860
- Hadi A, Inubushi K, Pumomo E et al (2000) Effect of land-use changes on nitrous oxide (N_2O) emission from tropical peatlands. *Chemosphere Global Change Sci* 2:347–358
- Hansen J, Sato M, Ruedy R et al (2006) Global temperature change. *Proc Natl Acad Sci* 103:14288–14293
- Hart SC (2006) Potential impacts of climate change on nitrogen transformations and greenhouse gas fluxes in forests: a soil transfer study. *Glob Chang Biol* 12:1032–1046
- Hartmann AA, Niklaus PA (2012) Effects of simulated drought and nitrogen fertilizer on plant productivity and nitrous oxide (N_2O) emissions of two pastures. *Plant Soil* 361:411–426
- Hu YG, Chang XF, Lin XW et al (2010) Effects of warming and grazing on N_2O fluxes in an alpine meadow ecosystem on the Tibetan plateau. *Soil Biol Biochem* 42:944–952
- Hu YG, Jiang LL, Wang SP et al (2016a) The temperature sensitivity of ecosystem respiration to climate change in an alpine meadow on the Tibet plateau: a reciprocal translocation experiment. *Agric For Meteorol* 216:93–104
- Hu YG, Wang ZR, Wang Q et al (2016b) Climate change affects soil labile organic carbon fractions in a Tibetan alpine meadow. *J Soil Sediment*. doi:10.1007/s11368-016-1565-4

- Hu YG, Wang Q, Wang SP et al (2016c) Asymmetric responses of methane uptake to climate warming and cooling of a Tibetan alpine meadow assessed through a reciprocal translocation along an elevation gradient. *Plant Soil* 402:263–275
- IPCC (2007) Climate change 2007—the physical science basis: working group I contribution to the fourth assessment report of the IPCC. Cambridge University Press, Cambridge and New York
- Jiang CM, Yu GR, Fang HJ et al (2010) Short-term effect of increasing nitrogen deposition on CO₂, CH₄ and N₂O fluxes in an alpine meadow on the Qinghai-Tibetan Plateau, China. *Atmos Environ* 44:2920–2926
- Keller M, Reiners WA (1994) Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. *Glob Biogeochem Cycles* 8:399–409
- Larsen KS, Andresen LC, Beier C et al (2011) Reduced N cycling in response to elevated CO₂, warming, and drought in a Danish heathland: synthesizing results of the CLIMAITE project after two years of treatments. *Glob Chang Biol* 17:1884–1899
- Lin XW, Wang SP, Ma XZ et al (2009) Fluxes of CO₂, CH₄, and N₂O in an alpine meadow affected by yak excreta on the Qinghai-Tibetan plateau during summer grazing periods. *Soil Biol Biochem* 41:718–725
- Maag M, Vinther FP (1999) Effects of temperature and water on gaseous emissions from soils treated with animal slurry. *Soil Sci Soc Am J* 63:858–865
- Malhi Y, Silman M, Salinas N et al (2011) Introduction: elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. *Glob Chang Biol* 16:3171–3715
- Melillo JM, Steudler PA, Feigl BJ et al (2001) Nitrous oxide emissions from forests and pastures of various ages in the Brazilian Amazon. *J Geophys Res* 106:34179–34188
- Mosier AR, Delgado JA (1997) Methane and nitrous oxide fluxes in grasslands in western Puerto Rico. *Chemosphere* 35:2059–2082
- Mosier AR, Parton WJ, Valentine DW et al (1996) CH₄ and N₂O fluxes in the Colorado shortgrass steppe: 1. Impact of landscape and nitrogen addition. *Glob Biogeochem Cycles* 10:387–399
- Mosier AR, Morgan JA, King JY et al (2002) Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado shortgrass steppe under elevated CO₂. *Plant Soil* 240:201–211
- Mummy DL, Smith JL, Bluhm G (2000) Estimation of nitrous oxide emissions from US grasslands. *Environ Manag* 25:169–175
- Neto ES, Carmo JB, Keller M et al (2011) Soil-atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences* 8:733–742
- Pei ZY, Hua O, Zhou CP et al (2003) Fluxes of CO₂, CH₄ and N₂O from alpine grassland in the Tibetan Plateau. *J Geogr Sci* 13:27–34
- Pfeiffer M, Van Leeuwen J, Van Der Knaap WO et al (2013) The effect of abrupt climatic warming on biogeochemical cycling and N₂O emissions in a terrestrial ecosystem. *Palaeogeogr Palaeoclimatol* 391:74–83
- Ravishankara A, Daniel JS, Portmann RW (2009) Nitrous oxide (N₂O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326:123–125
- Rosenkranz P, Bruggemann N, Papen H et al (2006) N₂O, NO and CH₄ exchange, and microbial N turnover over a Mediterranean pine forest soil. *Biogeosciences* 3:121–133
- Rui Y, Wang S, Xu Z et al (2011) Warming and grazing affect soil labile carbon and nitrogen pools differently in an alpine meadow of the Qinghai-Tibet Plateau in China. *J Soils Sediments* 11:903–914
- Rustad LE, Campbell JL, Marion GM et al (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126:543–562
- Schilt A, Baumgartner M, Blunier T et al (2010) Glacial-interglacial and millennial-scale variations in the atmospheric nitrous oxide concentration during the last 800,000 years. *Quat Sci Rev* 29:182–192
- Shi FS, Huai C, Chen HF et al (2012) The combined effects of warming and drying suppress CO₂ and N₂O emission rates in an alpine meadow of the eastern Tibetan Plateau. *Ecol Res* 27:725–733
- Teh YA, Diem T, Jones S et al (2014) Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences* 11:2325–2339
- Thompson LG, Yao T, Mosley-Thompson E et al (2000) A high-resolution millennial record of the South Asian Monsoon from Himalayan ice cores. *Science* 289:1916–1919
- van Bochove E, Prevost D, Pelletier F (2000) Effects of freeze-thaw and soil structure on nitrous oxide produced in a clay soil. *Soil Sci Soc Am J* 64:1638–1643
- van Bochove E, Theriault G, Rochette P et al (2001) Thick ice layers in snow and frozen soil affecting gas emissions from agricultural soils during winter. *J Geophys Res-Atmos* 106:23061–23071
- Wang CT, Cao GM, Wang QL et al (2008) Changes in plant biomass and species composition of alpine Kobresia meadows along altitudinal gradient on the Qinghai-Tibetan Plateau. *Sci China Ser C Life Sci* 51:86–94
- Wang SP, Duan JC, Xu GP et al (2012) Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. *Ecology* 93:2365–2376

- Wang SP, Meng FD, Duan JC et al (2014) Asymmetric sensitivity of first flowering date to warming and cooling in alpine plants. *Ecology* 95:3387–3398
- Wuebbles DJ (2009) Nitrous oxide: no laughing matter. *Science* 326:56–57
- Xu XF, Tian HQ, Hui DF et al (2008) Convergence in the relationship of CO₂ and N₂O exchanges between soil and atmosphere within terrestrial ecosystems. *Glob Chang Biol* 14:1651–1660
- Xu R, Prentice IC, Spahni R et al (2012) Modelling terrestrial nitrous oxide emissions and implications for climate feedback. *New Phytol* 196:472–488
- Zhang FW, Li YN, Cao GM et al (2011) Response of alpine plant community to simulated climate change: two year results of reciprocal translocation experiment (Tibetan Plateau). *Pol J Ecol* 59:741–751
- Zheng Y, Yang W, Hu HW et al (2014) Ammonia oxidizers and denitrifiers in response to reciprocal elevation translocation in an alpine meadow on the Tibetan Plateau. *J Soils Sediments* 14:1189–1199