Nutr Cycl Agroecosyst (2020) 117:29–42
https://doi.org/10.1007/s10705-020-10062-0

ORIGINAL ARTICLE

CO_2, CH_4 and N_2O fluxes in an alpine meadow on the Tibetan Plateau as affected by N-addition and grazing exclusion

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Received: 20 July 2019 / Accepted: 16 March 2020 / Published online: 25 March 2020

Abstract Limited understanding of the effects of enhanced nitrogen (N) addition and grazing exclusion (E) on greenhouse gases fluxes (GHGs: CO_2, CH_4, and N_2O) in grasslands constrains our ability to respond to the challenges of future climate change. In this study, we conducted a field experiment using a static closed opaque chamber to investigate the response of GHG fluxes to N addition (69 kg N ha^{-1} $year^{-1}$ applied in 3 splits) and grazing exclusion in an alpine meadow on the Tibetan Plateau during the growing seasons from 2011 to 2013. Our results showed that winter grazing significantly raised soil temperature (ST), while grazing exclusion (E) had no effect on soil moisture (SM), and N fertilizer (F) had no effect on ST or SM. Aboveground biomass (AB) and root biomass (RB) were not significantly affected by E in 2011-2013 ($p > 0.05$), but F significantly affected AB and RB ($p < 0.05$). Compared with winter grazing, only E substantially reduced seasonal mean CO_2 emissions (by about 20.1%) during the experimental period. E did not significantly directly affect CH_4 uptake, whereas N addition reduced seasonal mean CH_4 uptake by about 6.5%, and N addition changed seasonal average absorption of N_2O into an emission source. CO_2 flux is the major contributor to CO_2 equivalent emissions in this area. Our results indicate that exclosure from livestock grazing might be a promising measure to reduce CO_2 emissions, while enhanced N addition might reduce CH_4 uptake and increase N_2O emission in the alpine meadow under future climate change.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10705-020-10062-0) contains supplementary material, which is available to authorized users.

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Keywords Grazing exclusion · Nitrogen addition · Greenhouse gas flux · Alpine meadow

Introduction

Increases in atmospheric nitrogen (N) deposition (Ndep) from human activities can strongly affect the exchange of greenhouse gases (GHG, i.e. CO_2, CH_4, and N_2O) between terrestrial ecosystems and the atmosphere (Reay et al. 2008; Vitousek et al. 1997). Since the Industrial Revolution, the emission of reactive N into the atmosphere is estimated to have increased by nearly 11.47 times, from 15 Tg N year^{-1} in 1860 to 187 Tg N year^{-1} in 2005, and is expected to reach 200 Tg N year^{-1} by 2050 (Galloway et al. 2008). After America and Europe, China now has the third highest rates of N deposition, with significant increases also found on the Tibetan Plateau, and rates of N deposition are expected to continue to increase in the coming decades due to economic development (Galloway and Cowling 2002; Liu et al. 2013a, b). Meanwhile, some alpine grasslands on the Tibetan Plateau have become degraded in recent decades due to overgrazing driven by population growth and food demand (Wei et al. 2012). Exclosure from livestock grazing has been widely employed by China's national and local authorities as a management practice aimed at restoring and protecting these fragile ecosystems (Luo et al. 2015; Zhang et al. 2012; Zou et al. 2014). Although some studies have explored the separate effects of Ndep and grazing exclusion on GHG fluxes (Chen et al. 2016; Hu et al. 2017; Liu et al. 2013a, b; Zhang et al. 2012), research on their combined effects on GHG fluxes in alpine meadows is scarce.

Previous studies have shown that compared with grazed plots, grazing exclusion significantly decreased soil respiration (Rs) by 23.6% over the growing season and by 21.4% annually, but grazing exclusion increased the temperature sensitivity (Q_{10}) of seasonal and annual soil respiration by 6.5% and 14.2%, respectively (Chen et al. 2016). Some studies in the region suggest that grazing exclusion and N fertilization have no effect on CO_2 emissions (Hu et al. 2017; Zhang et al. 2012), but that they can significantly reduce average CH_4 uptake (Hu et al. 2017; Jiang et al. 2010). Grazing exclusion has little effect on N_2O emissions (Hu et al. 2017), whereas N fertilizer significantly increases average N_2O emission (Jiang et al. 2010). These inconsistent and even contradictory responses of GHG fluxes to Ndep and grazing exclusion could be due to different mechanisms. For example, Ndep directly increases soil N availability, which promotes plant productivity (Bala et al. 2013; LeBauer and Treseder 2008) and thus increases inputs of nutrients into soils through decomposition from increased root and litter biomass. However, grazing exclusion decreases soil temperature (Luo et al. 2010) and increases plant productivity through shifts in plant composition towards tall grasses with deep roots (Xiong et al. 2016; Zhang et al. 2015; Zhu et al. 2016b). Therefore, the lack of data on GHG fluxes under different Ndep rates combined with grazing exclosure limits our mechanistic understanding of the relationship between GHG fluxes and Ndep, rendering predictions of the net strength of grassland GHG sinks in the future highly uncertain. We hypothesized that (1) N fertilization and grazing exclosure would increase CO_2 due to increased plant production; (2) N fertilization would increase N_2O emission, but grazing exclosure would decrease it; and (3) N fertilization would decrease CH_4 uptake due to changes in soil inorganic N, whereas grazing exclosure would increase CH_4 uptake due to decreased soil compaction. Here, measurements and analysis of GHG fluxes were carried out in grazed and fenced areas of an alpine meadow on the Tibetan Plateau during the growing seasons from 2011 to 2013. The aims of the study were to (1) investigate the effects of grazing exclusion and experimental N addition on key GHG fluxes, and (2) identify which abiotic environmental factors (i.e., soil temperature and moisture) drive changes in GHG fluxes in the alpine region.

Materials and methods

Study site

This study was carried out at an alpine meadow at Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37° 36′ N, 101° 12′ E, and 3250 m above sea level) in Qinghai Province during the growing seasons (from June to September) of 2011–2013. The local climate is characterized by long, cold winters and short mild summers, with multi-year mean annual precipitation of 580 mm (from 1981 to 2012), of which 80% is concentrated in the growing season from June to September. Mean annual air temperature is 1.7 °C with monthly mean air temperature ranging from − 15 °C in January to 10 °C in July. The plant community of the natural alpine meadow at the experimental site is dominated by grasses and forbs, *Lancea tibetica, Pedicularis Linn., Gentianopsis paludosa, Potentilla anserina L., Aster yunnanensis Franch., Gentiana straminea* and *Saussurea pulchra*. Prior to the experiment, the experiment site was freely grazed by sheep during winter (from the end of September to May of the following year) each year with almost no litter biomass remaining aboveground. The soil has a clay-loam texture with an average depth of 0.65 m, and is classified as Mat-Gryic Cambisol (Chinese Soil Taxonomy Research Group 1995), corresponding to Gelic Cambisol (WRB 2006). Basic soil properties were as follows: total organic carbon (C) 55.8 g kg^{-1}, potassium (K) 13.0 g kg^{-1}, phosphorus (P) 0.70 g kg^{-1}, nitrogen (N) 5.37 g kg^{-1}, pH of 8.2 determined in distilled water, and bulk density in the 0-10 cm soil layer is 1.05 g cm^{-3} (Zhang et al. 2012).

Experiment design

The experiment site was surrounded by eight fenced squares of 4 × 4.5 m. Before exclusion of livestock from the freely-grazed meadow, vegetation and other environmental conditions inside and outside the fence were almost homogeneous. Outside of the fenced meadow, grassland was still freely grazed by local herders' animals during winter, i.e. from the end of September to the end of May the following year. Eight plots (each of 4 m × 4.5 m dimension) were assigned to two treatments (i.e., N addition and control) with four replications inside the fence and four replications outside the fence. The treatments were laid out randomly. A completely randomized design was used for two exclosures and their fertilizer management practices with four replicate plots (4 m × 4.5 m) of four treatments as follows: grazing without N addition (G^+-N^0), grazing with N addition (G^+-N^+), no-grazing without N addition (G^0-N^0) and no-grazing with N addition (G^0-N^+). Local atmospheric N deposition is estimated to range from 8.7 to 13.8 kg N ha^{-1} year^{-1} (Lü and Tian 2007). The N fertilization experiment was initiated in May 2011 and the simulated future climate change scenario used about six times the amount of N deposition. N fertilization (69 kg N ha^{-1} year^{-1} as urea applied three times) was added on 20 June, 20 July and 20 August each year in 2011, 2012 and 2013, respectively.

Field sampling and measurements

GHG fluxes were measured using a static dark chamber and the gas chromatography method. A stainless steel square box [without a top or bottom, 0.4 m (length) × 0.4 m (width) × 0.08 m (height)] with a water groove to make the chamber airtight was placed 0.20 m away from the edge of each plot and inserted into the soil to a depth of 0.08 m. Four air samples were collected from 09:00 to 11:00 a.m., representing 1-day average flux as described in previous reports (Jiang et al. 2010; Lin et al. 2009). The gas samples were collected every 7–10 days from June to September during the growing seasons in 2011, 2012, and 2013. GHG fluxes were monitored on the first or second day after fertilizer application, and then monitored with the same frequency as prior to fertilization. For all observations, average gas fluxes and standard errors were calculated from four replicates. Seasonal GHG fluxes in 2011, 2012 and 2013 were estimated by calculating average fluxes over an experimental period with 16 flux observations in each period.

Air temperature (1.5 m above ground) data were recorded simultaneously using an automated weather station (AWS, Vaisala Corp., Holland), and precipitation was measured using a rain gauge. Soil temperature and soil water content data (0.05 m) were measured simultaneously to the air sampling process at each chamber using a digital temperature sensor (JM624 digital thermometer, Living–Jinming Ltd., China) and a Time Domain Reflectometer (JS-TDR300, Meridian Measurement, USA).

Peak aboveground biomass was estimated by clipping vegetation to the soil surface using a 0.5 × 0.5 m quadrat placed 0.2 m away from the plot edge in each plot in late August each year. At the center of each quadrat, two soil cores of 0–0.2 m depth were collected using a 0.08 m diameter soil auger. All samples with four replicates were taken on the same day. Samples of soil cores were washed in the laboratory with tap water to remove the soil so as to estimate root biomass. All samples were oven-dried at 65 °C to a constant weight.

CO_2 equivalent emissions

IPCC (2007) estimated that N_2O and CH_4 have global warming potentials that are 296 and 23 times higher than CO_2, respectively, and these values were used in the conversion of N_2O and CH_4 to CO_2 equivalent warming potential after addition of CO_2 emissions. The formula used was as follows:

$$\begin{aligned}&\text{Ecological system } CO_2 \text{ equivalent emissions}\\&\quad = CO_2 \text{ annual average flux} + 296\\&\qquad \times N_2O \text{ annual average flux} + 23\\&\qquad \times CH_4 \text{ annual average flux.}\end{aligned}$$

Statistical analysis

General Linear Model (GLM) Repeated Measures Analysis of Variance (RMANOVA), with exclusion as the main factor (between-subject) and sampling date as the within-subject factor including interactions, was applied to test the effects of the main factor on monthly soil temperature, monthly soil water content, daily GHG fluxes and annual average GHG fluxes. The same analysis was performed using N fertilization as the main factor under the same treatment and with sampling date as the within-subject factor, including interactions. Multi-comparison of least standard difference (LSD) was conducted for all measured variables within each sampling date using one-way ANOVA. In order to assess how N fertilizer affected the magnitude of the effect of exclosure on GHG flues, soil temperature and moisture, aboveground and root biomass, multi-comparison of least standard difference (LSD) was conducted using one-way ANOVA to assess the relative differences in GHG fluxes, soil temperature and moisture, aboveground and root biomass under no-fertilizer and with-fertilizer management practices. All statistical analyses were performed with SPSS (SPSS 16.0, SPSS Inc., Chicago, IL, USA) using the GLM procedure and Type III sum of squares. We further fitted a piecewise structural equation model (SEM) to estimate the direct and indirect effects of grazing exclosure (E) and nitrogen fertilizer (F) on seasonal cumulative CO_2 emission, CH_4 uptake and N_2O emission using the piecewise SEM package in R software (R 3.1.3, R Development Core Team 2014).

Results

Environmental conditions

Air temperature showed a typical pattern that increased from June, peaked in August, and declined in September in each of the 3 years (Fig. S1). Mean air temperature and total rainfall during the growing seasons from 1 June to 30 September in 2011, 2012, and 2013 were 8.9, 9.0, and 9.6 °C, and 326.8, 285.6, 356.0 mm, respectively. The seasonal rainfall distributions and temperatures are shown in Fig. S1.

Soil temperature and soil moisture

The effect of exclusion on soil temperature (ST) and soil moisture (SM) varied with sampling date and year, and there were significant interactions between exclusion and year and sampling date ($p < 0.05$). In general, ST under grazing was significantly higher than under exclusion ($p < 0.001$), but exclusion had no effect on SM ($p = 0.21$), while fertilization (F) had no significant effect on ST or SM ($p > 0.05$). Average annual ST (Fig. 1a) was greater for G^+-N^0 and G^+-N^+, and the annual SM of G^0-N^0 and G^0-N^+ were significantly lower than for G^+-N^0 and G^+-N^+ in 2011, but there were no significant differences in 2012 or 2013 (Fig. 1b). In general, N fertilizer did not significantly affect ST or SM for G^+-N^0 and G^0-N^0 during the 3 year experiment (Fig. 1a, b).

Aboveground and root biomass

Although aboveground biomass (AB) and root biomass (RB) were not significantly affected by exclosure (E) in 2011–2013 ($p > 0.05$), nitrogen fertilizer (F) significantly affected AB and RB ($p < 0.05$). There were no interaction effects between E and F on AB or RB ($p > 0.05$). Inter-annual variation in AB and RB was not significant ($p > 0.05$). In general, AB in the G^+-N^0 treatment was lower than in the G^+-N^+, G^0-N^0 and G^0-N^+ treatments, and RB in the G^+-N^0 treatment was higher than in the G^+-N^+ and G^0-N^+ treatments in 2011–2013 (Fig. 2).

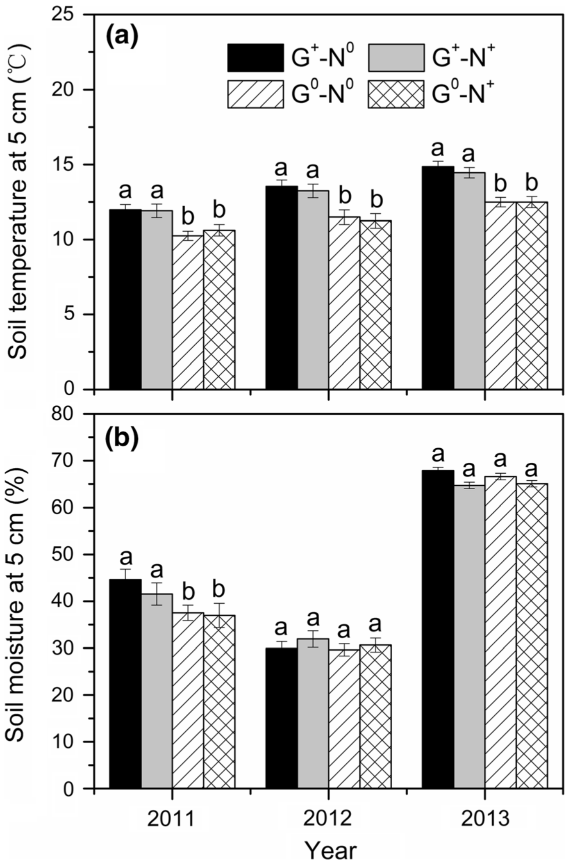

Fig. 1 Seasonal average soil temperature (**a**) and soil moisture (**b**) at 5 cm depth under four treatments from 2011 to 2013. G^{+}: grazing; N^{+}: nitrogen fertilizer; G^{0}: no-grazing; N^{0}: without N addition. Bars indicate mean ± 1SE. Different letters indicate significant differences at $p = 0.05$ level

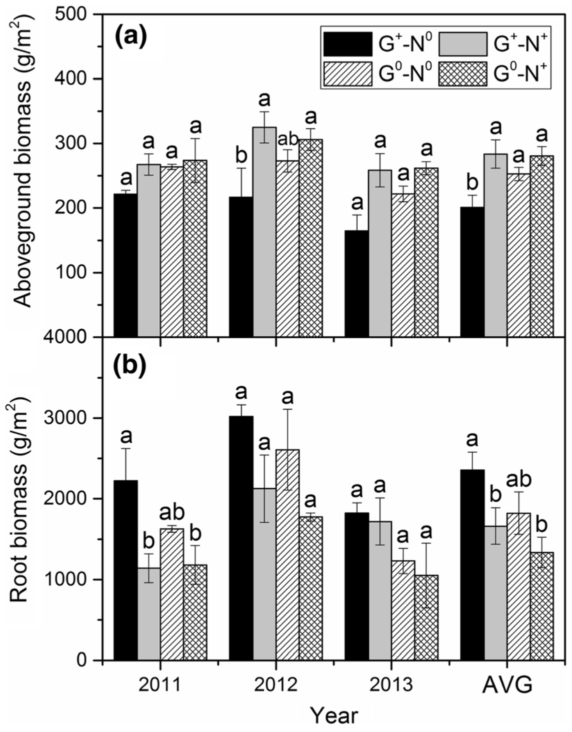

Fig. 2 Average aboveground biomass (AB) and root biomass (RB) under four treatments from 2011 to 2013. G^{+}: grazing; N^{+}: nitrogen fertilizer; G^{0}: no-grazing; N^{0}: without N addition. Bars indicate mean ± 1SE. Different letters indicate significant differences at $p = 0.05$ level

GHG fluxes

CO_2 emission followed a clear seasonal pattern with the highest rates in the vigorous growth period and the lowest rates in the early and late growing seasons in each of the 3 years (Fig. 3; Table 1). The highest value appeared in mid-July to mid-August. Regardless of fertilization, compared with the native alpine meadow, grazing exclusion reduced CO_2 emissions by an average of 20.1% over the 3-year period, whereas N addition had no significant effect on CO_2 emissions (Tables 1, 2). There were no significant interactive effects between N addition and grazing exclusion ($F = 0.022$, $p = 0.887$, Table 1). Considering the strong collinearity among affecting factors, we fitted a piecewise structural equation model (SEM) to evaluate the causal relationships among these factors in order to estimate the direct and indirect effects of exclosure and nitrogen fertilizer on cumulative seasonal CO_2 emission, CH_4 uptake and N_2O emission (Fig. 6a–c). The influence of exclosure on seasonal CO_2 emission was mainly mediated through soil temperature. There was a strong relationship between exclusion and RB ($\beta = -0.63$, standardized coefficient), and although there were significant relationships between N fertilizer and AB and RB (Fig. 6a), there were weaker relationships between AB, RB and CO_2 emissions. Grazing exclusion led to significant change in seasonal average CO_2 emission in 2011, 2012 and 2013 (Fig. 6a; Table 1).

The seasonal dynamics of CH_4 were very significant (Fig. 4; Table 1). Grazing exclusion (E) had no significant effect on CH_4 uptake (Fig. 6b; Table 1), whereas N addition reduced CH_4 uptake (Table 2). There were no significant interactive effects on CH_4 between N addition and grazing exclusion ($F = 0.191$, $p = 0.673$, Table 1). The alpine meadow soil was a CH_4 sink in each growing season (Fig. 4; Table 2).

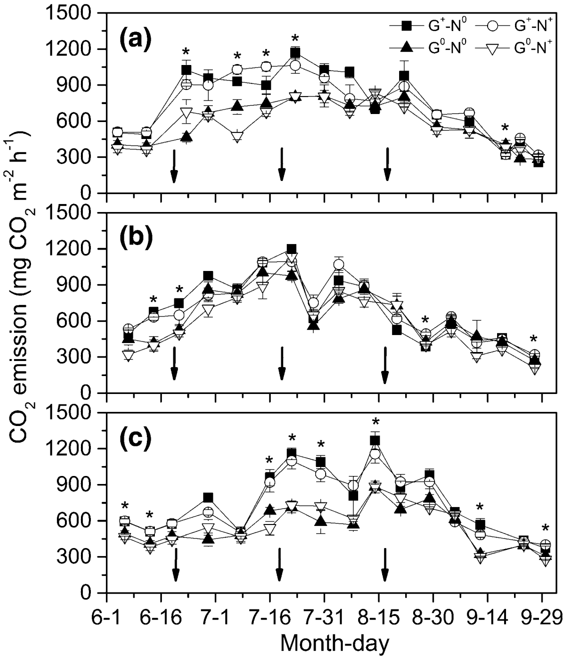

Fig. 3 Daily CO_2 flux under four treatments in 2011 (**a**), 2012 (**b**) and 2013 (**c**). G^+: grazing; N^+: nitrogen fertilizer; G^0: no-grazing; N^0: without N addition. Bars indicate mean ± 1SE. Asterisk indicates significant difference between treatments at $p < 0.05$ level. Mean ± SE (n = 4) are shown in the figure. Arrow indicates the date of N fertilization

There were strong relationships between CH_4 uptake and soil moisture, soil moisture and soil temperature, exclusion and soil temperature and weaker relationships between CH_4 uptake and soil temperature, CH_4 uptake and exclusion (Fig. 6b). However, nitrogen fertilizer had a significant effect on CH_4 uptake (Fig. 6b).

The alpine meadow soil acted as a weak N_2O source in the growing seasons under the G^+-N^+ and G^0-N^+ treatments, but was a weak N_2O sink in the growing seasons for G^+-N^0 and G^0-N^0 (Table 2). Exclusion had no effect on N_2O exchange capacity (Fig. 6c; Table 1). However, N_2O exchange was converted from absorption to emission by N fertilizer application (Table 2). The relationship between N_2O and nitrogen fertilizer was very weak (Fig. 6b), but the combination of all direct and indirect effects was significant (Table 1). We detected significant seasonal dynamics in N_2O emissions (Fig. 5; Table 1), which was highly variable. N addition significantly increased N_2O release ($p < 0.001$), resulting in net N_2O emissions (Fig. 6c). The strongest relationships were between N_2O emission and soil moisture, soil moisture and soil temperature, exclosure and soil temperature, and aboveground biomass and nitrogen fertilizer (Fig. 6c),

Table 1 Results of repeated measures ANOVAs on the effects of nitrogen addition (F), year (Y), sampling date (D), exclusion (E) and their interactions on seasonal integrals of CO_2, CH_4 and N_2O flux

Model	CO_2		CH_4		N_2O	
	F	*p*	*F*	*p*	*F*	*p*
Exclusion (E)	67.012	< **0.001**	0.390	0.550	0.609	0.458
Fertilizer (F)	0.497	0.501	5.829	**0.042**	259.723	< **0.001**
E × F	0.022	0.887	0.191	0.673	1.448	0.263
Year (Y)	0.513	0.494	17.672	**0.003**	925.980	< **0.001**
Y × E	1.012	0.344	12.514	**0.008**	0.270	0.617
Y × F	0.028	0.872	11.639	**0.009**	175.545	< **0.001**
Y × E × F	0.517	0.493	0.399	0.545	8.551	**0.019**
Day (D)	89.200	**< 0.001**	49.894	< **0.001**	379.155	< **0.001**
D × E	14.803	**0.005**	2.057	0.189	10.998	**0.011**
D × F	0.554	0.478	3.833	0.086	189.871	< **0.001**
D × E × F	0.206	0.662	2.703	0.139	7.764	**0.024**
Y × D	339.971	< **0.001**	99.500	< **0.001**	0.804	0.396
Y × D × E	74.871	< **0.001**	0.900	0.371	1.483	0.258
Y × D × F	1.890	0.206	2.496	0.153	213.299	< **0.001**
Y × D × E × F	0.516	0.493	2.998	0.122	4.835	0.059

Bold indicates a significant difference

Table 2 Seasonal average CO_2, CH_4, N_2O and CO_2 equivalent emissions under four treatments from 2011 to 2013

Treatments	CO_2 flux	CH_4 flux	N_2O flux	CO_2 equivalent
G^+-N^0	733.76 ± 23.66 a	− 33.67 ± 1.22 ab	− 1.40 ± 0.52 b	732.57 ± 23.65 a
G^+-N^+	723.76 ± 21.68 a	− 32.13 ± 1.33 b	2.98 ± 0.80 a	723.91 ± 21.71 a
G^0-N^0	589.89 ± 17.82 b	− 35.43 ± 1.25 a	− 1.48 ± 0.50 b	588.63 ± 17.82 b
G^0-N^+	574.64 ± 17.96 b	− 32.51 ± 1.26 b	3.37 ± 0.68 a	574.89 ± 18.02 b

G^+: grazing; N^+: nitrogen fertilizer; G^0: no-grazing; N^0: without N addition. Mean ± SE (n = 48) are shown in the Table. Different letters in rows indicate significant difference at 0.05 level

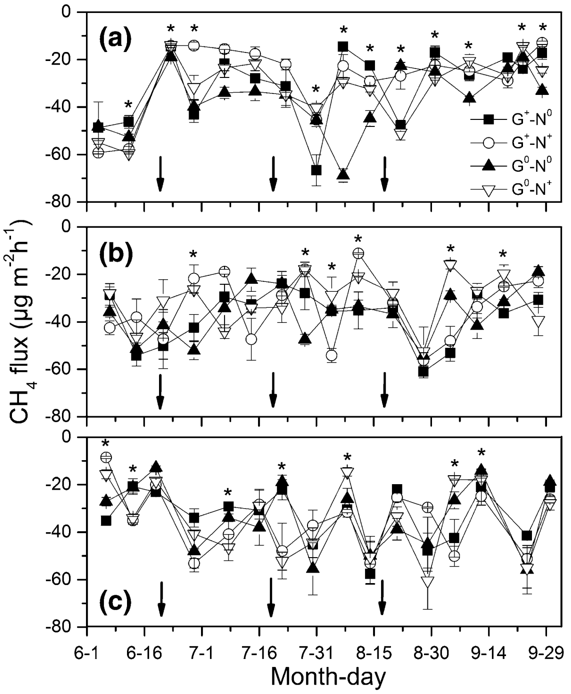

Fig. 4 Daily CH_4 flux under G^+-N^0, G^0-N^0 and nitrogen fertilizer in 2011 (**a**), 2012 (**b**) and 2013 (**c**). G^+: grazing; N^+: nitrogen fertilizer; G^0: no-grazing; N^0: without N addition. Bars indicate mean ± 1SE. Asterisk indicates significant difference between treatments at $p < 0.05$ level. Mean ± SE (n = 4) are shown in the figure. Arrow indicates the date of N fertilization

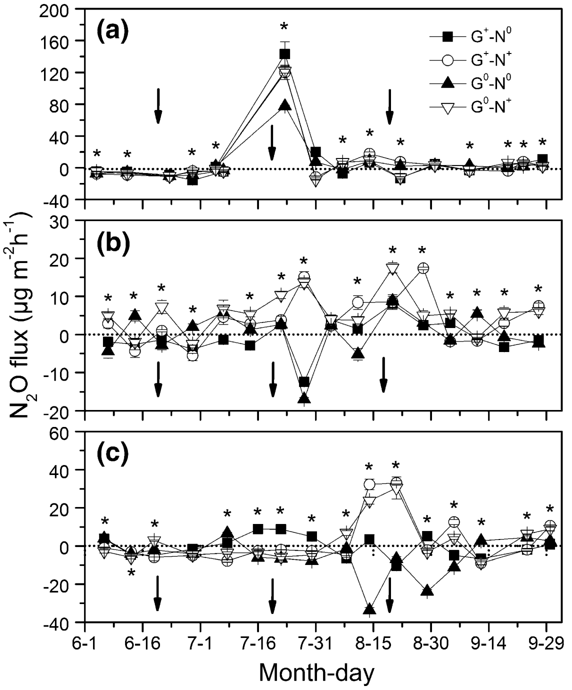

Fig. 5 Daily N_2O flux under the G^+-N^0, G^0-N^0 and nitrogen fertilizer treatments in 2011 (**a**), 2012 (**b**) and 2013 (**c**). G^+: grazing; N^+: nitrogen fertilizer; G^0: no-grazing; N^0: without N addition. Bars indicate mean ± 1SE. Asterisk indicates significant difference between treatments at $p < 0.05$ level. Mean ± SE (n = 4) are shown in the figure. Arrow indicates the date of N fertilization

and there were weaker relationships between N_2O emission and soil temperature, and exclusion and aboveground biomass. Exclusion affects SM by affecting ST, and indirectly affects emissions of N_2O (Fig. 6c).

In general, grazing exclusion had a significant impact on CO_2 equivalent emissions, while N fertilizer had no effect on CO_2 equivalent emissions (Table 2). The CO_2 equivalent emissions of grazing without N addition and grazing with N addition were significantly higher than in the no-grazing without N addition and no-grazing with N addition treatments, with a 3-year average increase of about 20%. CO_2 flux is the major contributor to CO_2 equivalent emissions. Compared with CO_2 flux, CH_4 and N_2O emissions were very small, so CO_2 flux is the decisive factor in determining CO_2 equivalent emissions.

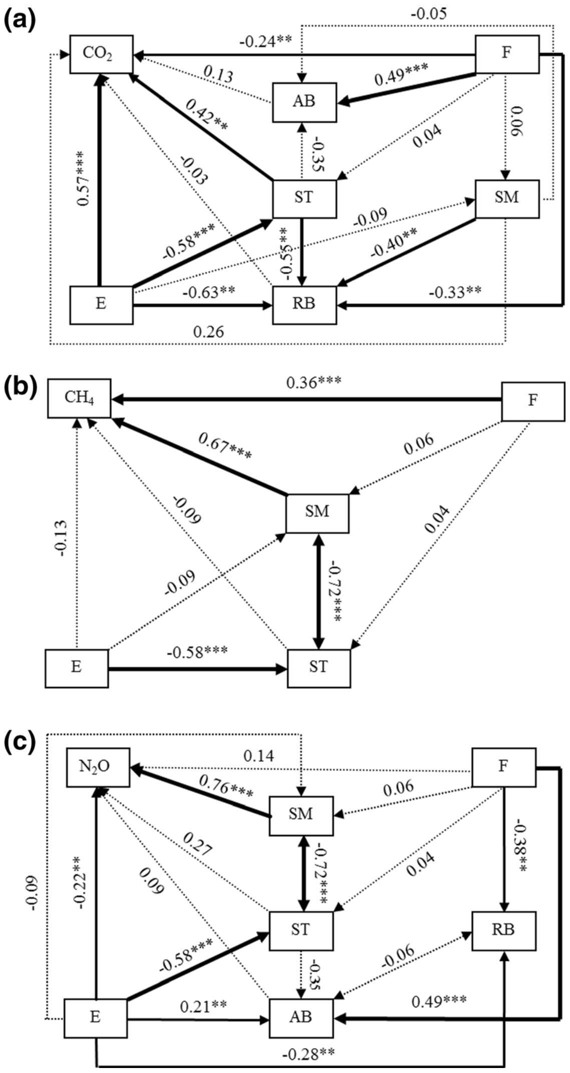

Fig. 6 A piecewise structural equation model fitted to infer the direct and indirect effects of grazing exclusion (E) and nitrogen fertilizer (F), soil temperature (ST) and moisture (SM), aboveground biomass (AB) and root biomass (RB) on seasonal average CO_2, CH_4 and N_2O emission from 2011 to 2013. Arrows represent the direction of causality. The bold black lines represent highly significant relationships [$p < 0.001$, three asterisks (***)]; the non-bold black lines represent significant relationships [$p < 0.05$, two asterisks (**)]; and the dotted lines represent non-significant relationships ($p > 0.05$). AB and RB are not shown in figure **b** because the result cannot be run after adding these variables

Discussion

CO_2 emission

Although positive effects of N fertilizer application on CO_2 emissions were reported by Juutinen et al. (2010), we found that N addition did not alter CO_2 fluxes under either grazing or no-grazing treatments in our 3-year study (Tables 1, 2). The first reason may be because the response of CO_2 emissions to N addition is related to the amount of N addition (Carter et al. 2011). The annual N-addition rates in our study were lower than in some studies (Kostyanovsky et al. 2018), but higher than in other studies (Jiang et al. 2010; Wang et al. 2017; Zhu et al. 2015). N-addition rates may affect N-status associated processes because high N addition could remove N constraints on microbial metabolism and improve litter quality (Carter et al. 2012). Other studies have shown that the effects of N fertilizer application on CO_2, CH_4, and N_2O emission are dependent on the form of N (Cai et al. 2007; Peng et al. 2011). Differences in soil structure and climate conditions between study sites may also have an effect, with the range of responses varying from no effect at all to positive or negative effects depending on ecosystem type, age, dominant plant species and soil chemical characteristics (Pregitzer et al. 2008; Rodriguez et al. 2014; Xiao 2017; Zhu et al. 2016a), N loads in the soil in combination with climatic conditions (Alster et al. 2013), and experiment duration (Zhou et al. 2014).

Grazing exclusion significantly decreased annual average CO_2 emission (Table 2) in our study. This was largely because exclusion lowered soil temperature (Fig. 6a), which reduced soil respiration (Chen et al. 2016), and our study found that soil temperature was positively correlated with CO_2 emissions (Fig. 6a). Studies conducted near our study site showed that grazing exclusion significantly decreased Rs, mainly because exclusion reduces soil temperature and microbial biomass carbon (Chen et al. 2016), but, as in our study, these effects are not correlated with exclusion-induced changes in aboveground and belowground biomass. Our results suggest that the effects of exclusion-induced changes in soil temperature on CO_2 emission may have overridden the effects of exclusion-induced changes in plant productivity.

Grazing exclusion can have significant effects on an ecosystem's biotic and abiotic characteristics, and these in turn can affect soil carbon fluxes (McSherry and Ritchie 2013). In the same site as the present study, Zou et al. (2016) found that accumulated litter was significantly higher under grazing exclusion (386.41 g m^{-2}) than under grazing (58.77 g m^{-2}), and because the low temperature caused by exclusion can slow down the litter decomposition rate (Luo et al. 2010), this further reduces CO_2 emissions. Temperature (especially soil temperature) was the dominant environmental variable that controlled seasonal change in CO_2 flux in our study, which has been documented in many other studies (Fang and Moncrieff 2001; Jiang et al. 2010; Lloyd and Taylor 1994; Wei et al. 2012; Zhu et al. 2015). Soil moisture is another important factor affecting CO_2 emissions. In our study, CO_2 fluxes were not positively correlated with soil moisture (Fig. 6a), probably because soil water content influenced microbial activity and litter decomposition rates and then had a combined influence on CO_2 diffusion from the soil to the atmosphere.

CH_4 uptake

Our results indicated that the natural grassland (exclosed, not exclosed, with or without N fertilizer) functioned as a sink for CH_4 during the growing seasons from 2011 to 2013. This finding is consistent with other studies (Jiang et al. 2010; Wei et al. 2012). Low precipitation and soil moisture may have contributed to the stronger CH_4 uptake in the alpine meadow (Curry 2007). In our study, CH_4 emissions were not affected by N addition under winter grazing, but no grazing with N addition significantly reduced the absorption of CH_4 (Table 2). This is likely direct influenced by the reduced activity of methanogens, as indicated by decreased CH_4 production potential (Wang et al. 2017) for the no grazing with N addition treatment.

As nitro-bacteria compete for active sites of methane monooxygenase (MMO) when oxidizing NH_4^+ and CH_4 via methane-oxidizing bacteria, the oxidation of CH_4 is inhibited (Bowman et al. 1995). A higher concentration of NH_4^+ can inhibit the growth and activity of methane-oxidizing bacteria by increasing the number of nitrifying bacteria, thereby inhibiting the oxidation of CH_4 (Jang et al. 2006). The soil NH_4^+ and NO_3^- content in different soil layers was similar between the fenced and grazed plots without N addition in our study site (Zou et al. 2016). This may be the reason for the lack of difference in CH_4 emissions between the fenced and grazed plots without N addition (Table 2). Scheutz and Kjeldsen (2004) showed that high concentration of NH_4^+ (14 mg NH_4^+/kg) significantly inhibited the oxidation of CH_4 (Scheutz and Kjeldsen 2004). Adamsen and King (1993) found in soil culture experiments that nitrate inhibited soil CH_4 oxidation (Adamsen and King 1993).

Our results indicated that grazing reduces the absorption of CH_4 (Table 2), which is similar to the results of previous studies (Liu et al. 2007; Saggar et al. 2007). This may be because trampling resulting in soil compaction, which can decrease O_2 diffusion into the soil, thus limiting CH_4 and O_2 availability for the oxidation process (Liu et al. 2007; Saggar et al. 2007). However, Lin et al. (2015) found that summer grazing by sheep increased CH_4 absorption in the alpine region, which may be due to the difference in grazing season between their experiment and ours. With winter grazing by sheep in our experiment, grass was almost completely consumed, and was also accompanied by a large amount of fecal/urine production. Yak dung application, however, significantly increased CH_4 emissions, which was probably attributable to dissolved CH_4, large microbial populations, highly degradable organic compounds and anaerobic conditions in the fresh dung patches (Jarvis et al. 1995; Lin et al. 2009; Sherlock et al. 2002), which lowered the absorption of methane. The study of Lin et al. (2015) simulated summer grazing (Wang et al. 2012), with half of canopy height consumed by herbivores and limited trampling or fecal urine, and soil temperature was raised by grazing (Luo et al. 2009). The increase in ST can increase evaporation of SM, and SM directly affects absorption of CH_4 (Fig. 6b) and improves the permeability of soils and the activity of CH_4 oxidizing bacteria, thus promoting the absorption of CH_4 by soil (Hu et al. 2010; Zhuang et al. 2007). Hence, increased ST could offset the effects of grazing.

N_2O flux

Our results indicated that exclusion of natural grassland from livestock grazing had no effect on N_2O emission (Table 2) during the growing seasons from 2011 to 2013. This finding is different from studies that have shown that grazing may reduce N_2O release (Liu et al. 2007; Wolf et al. 2010) or may increase it (Hu et al. 2010; Jiang et al. 2010). Both emission (positive values) and uptake of N_2O (negative values) were observed across grazing exclusion and N fertilizer treatments, as has also been reported by other studies (Cantarel et al. 2011; Dijkstra et al. 2013; Jiang et al. 2010; Peng et al. 2017; Teh et al. 2014).

Previous studies showed that increased SM has a positive effect on N_2O emission (Dijkstra et al. 2013; Hart 2006), while drought can lead to a reduction in N_2O emission (Goldberg and Gebauer 2009; Larsen et al. 2011; Shi et al. 2012). Jiang et al. (2010) also reported for alpine meadow grassland that N_2O emissions peaked under higher SM conditions. However, there was a significant correlation between soil moisture and N_2O emission (Fig. 6c), suggesting that soil moisture is the main factor limiting N_2O emission. None of the treatments significantly affected soil moisture in our study, except in 2011 (Fig. 1b), indicating that soil moisture is not the main factor causing differences in N_2O emission between treatments. It is known that low temperature mainly influences microbial metabolic rates (Hulsen et al. 2016), which leads to the deterioration of nitrification (Chen et al. 2018; Delatolla et al. 2012; Hoang et al. 2014). In addition, low temperatures exert an influence on the amount of nitrifying bacteria (Siripong and Rittmann 2007). Low temperature is one of the key factors that shapes the microbial community in biological N removal systems (Urakawa et al. 2008; Zhou et al. 2016). However, there were no significant correlations between soil temperature and N_2O emission in our study (Fig. 6c). Thus, there was an interactive effect on N_2O emission between soil temperature and N addition because grazing increased soil temperature (Fig. 1a). The absence of increases in N_2O emissions could be because denitrification was limited by low temperature (Ambus and Robertson 2006; Curtis et al. 2006; Liu and Greaver 2009) for the grazing exclosure with N addition treatment. In addition, the low temperature may have reduced nitrification by limiting aerobic ammonia oxidation due to the decreased abundance of ammonia-oxidizing archaea and bacteria (Zheng et al. 2014). Our results imply that N_2O emission would increase if N addition increases with warming under future climate change. Moreover, we found that N addition significantly increased N_2O emission under grazing and exclosure conditions during the experiment period (Tables 1, 2), a similar result to that of Wang et al. (2018) in the region (Wang et al. 2018). Some studies have reported that N fertilizer application can lead to substantial N_2O emission through nitrification–denitrification processes (Loecke and Robertson 2009).

N addition reduced CH_4 uptake and increased N_2O emission. The alpine meadows of the Qinghai-Tibet Plateau are a weak sink of CH_4 and a weak N_2O source (Cao et al. 2008; Lin et al. 2009; Wang et al. 2009). CH_4 and N_2O emissions contributed little to CO_2 equivalent emissions, because CH_4 uptake and N_2O emissions are very small (Table 2), and are insufficient to fundamentally affect the ecological system CO_2 equivalent emissions (Jiang et al. 2010). CO_2 emissions are therefore the main source of global warming potential in the treatments applied in this study.

Conclusions

Our results indicated that N addition had no effect on CO_2 emission, but decreased CH_4 uptake and increased N_2O flux. Grazing exclusion decreased CO_2 emission, but had no effect on CH_4 uptake or N_2O emission. There were no interactive effects of grazing exclusion and N fertilization on CO_2, N_2O or CH_4. These results suggest that with an increase in N deposition and expansion of areas under grazing exclusion in the future, CO_2 emission and CH_4 uptake would decrease, N_2O emission would increase, and total CO_2 equivalent emissions would decrease. Therefore, increased N deposition and grazing exclusion could cause negative feedback to global warming in the alpine meadow. Our results suggest that exclusure from livestock grazing might be a promising measure to reduce ecosystem CO_2 emissions in the alpine meadow region.

Acknowledgements This work was supported by projects from the National Key Research and Development Program of China (2016YFC0501805 and 2016YFC0501905), the Science and Technology Bureau of Qinghai (2017-ZJ-716 and 2018-SF-110-1), the National Science Foundation of China (41731175), and the Qilian Mountain National Park Research Center (Qinghai) (Grant Number: GKQ 2019-01).

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