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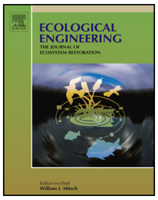

Research Article

The effect of nitrogen deposition rather than warming on carbon flux in alpine meadows depends on precipitation variations

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ABSTRACT

Uncertainties remain regarding the effects of climate warming and increasing nitrogen (N) deposition on greenhouse gas (GHG) flux in alpine grasslands due to a lack of knowledge about how hydrological characteristics control GHG fluxes. Therefore, a simulated warming and N fertilization experiment was conducted in a non-wetland (alpine meadow, AM) and a wetland (alpine swamp meadow, SM). We measured and analysed the key GHG fluxes (ecosystem respiration [Re], CH_4 and N_2O) of each treatment during two contrasting hydrological growing seasons. The results showed that: (i) warming increased the Re in both the AM and SM, warming increased the CH_4 uptake in the AM but had no effect in the SM, and warming increased the N_2O emissions from the AM and resulted in a change of the SM from a N_2O sink into a source; (ii) N fertilization decreased the Re of the AM during the dry growing season and of the SM during the wet growing season, increased the CH_4 uptake of the AM during the dry growing season, and had no effect on the CH_4 and N_2O fluxes of the SM; and (iii) the interaction between warming and N fertilization increased the CH_4 uptake of the AM over the two growing seasons while increasing the CH_4 uptake and N_2O emissions of the SM during the dry growing season. Our results suggest that (i) the GHG flux of wetland ecosystems is more sensitive to precipitation variations than that of non-wetlands and (ii) precipitation controls the carbon (Re and CH_4) flux response to increasing N deposition of these alpine meadows.

1. Introduction

Approximately 40% of the Qinghai-Tibet plateau (QTP) is alpine meadow (Hu et al., 2010). These areas provide important ecosystem services, such as protection of species diversity (Tibetan antelope, Tibetan wild donkey) and tourism (Lu et al., 2017), and they are also fundamental means of production for the survival of local herdsmen. These alpine meadows not only regarded as a very sensitive climate change trigger in the Asian monsoon region, but also have pronounced feedbacks to climate change and human activities (Li et al., 2015). Climate change and anthropogenic activities are the primary driving forces affecting alpine meadows in the QTP (Chen et al., 2014). During the past five decades, the mean annual temperature and precipitation in the QTP have increased by 0.3 °C and 9.1 mm per decade (Piao et al., 2012), respectively. Additionally, anthropogenic activities leading to inorganic nitrogen (N) deposition in the QTP have been increasing since the mid-20th century (Liu et al., 2015a). These climate changes strongly affect the carbon and N dynamics in alpine meadows. For example, the exchange of greenhouse gases (GHG) between the biosphere and atmosphere is a distinct way in which alpine meadows respond to climate change (Chen et al., 2013).

Numerous *in situ* experiments have been performed to investigate the effects of warming and N fertilization on the GHG flux in alpine grasslands. Warming increased ecosystem respiration (Re) (Zhu et al., 2015; Hu et al., 2016) or had no significant effect on the Re (Zhao et al., 2017). N fertilization increased (Peng et al., 2014b; Sun et al., 2013), decreased (Lozanovska et al., 2016; Gao et al., 2014) or had no impact on the Re (Wei et al., 2014; Zhu et al., 2015). These results often depend on variations in soil moisture (Lagomarsino et al., 2016). Increasing the soil temperature and available N will synergistically enhance the activity of microorganisms (Xiong et al., 2016), such as methanotrophs, nitrifiers and denitrifiers. Therefore, warming and N fertilization also have posi-

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tive impacts on the CH_4 uptake and N_2O emissions in non-wetland alpine grasslands (Hu et al., 2010; Liu et al., 2015b). Few studies have been conducted on wetland alpine grasslands, such as alpine swamp meadows. The QTP has approximately 100,000 km^2 of natural wetlands of which more than 50% are alpine swamp meadows (Wei et al., 2015). Thus, uncertainties remain about the effects of climate warming and increasing N deposition on GHG fluxes in alpine grasslands due to a lack of knowledge of how hydrological characteristics control GHG fluxes in both wetlands and non-wetlands. In particular, global warming will make wet/drought events more frequent (Cook et al., 2004; Manabe et al., 2004).

Some researches about GHG flux have been conducted in hydrological contrast years. Bubier et al. (2005) reported that wetlands had a strong CH_4 flux sensitivity to small changes in precipitation because the rainfall controlled the waterlogged condition, which affects air permeability of soils and then affects CH_4 oxidation or other processes. Peng et al. (2014a) also supported that hydrological variations controlled the GHG flux since drought events reduced root biomass by 50.2% thus suppressed soil respiration. Besides, the Re of an alpine meadow (the average soil moisture during the growing season was approximately 15%) was positively correlated with the soil moisture during the dry growing season(the range of soil moisture is from 7.5% to 12.5%) but negatively correlated with soil moisture during the wet growing season(the range of soil moisture is from 11.0% to 22.0%), which indicates that the soil moisture will influence the ecosystem respiration. However, Xia et al. (2009) supported the idea that hydrological variations cannot affect the Re of a temperate steppe (the soil moisture was lower than 10%), because drought would restrain ecosystem C uptake more than C release. These different conclusions are probably still due to the differences in soil moisture among wetlands, non-wetlands and semiarid grasslands. Another reason is that precipitation variations are often accompanied by changes in the air temperature. The long-term observation showed that average air temperature tends to be higher during dry years from 1990 to 2010 (Chen et al., 2014, Fig. 5a). Thus, variations in hydrological conditions will have a complex impact on the GHG flux.

Therefore, we conducted a simulated warming and N fertilization experiment in a non-wetland (alpine meadow, AM) and a wetland (alpine swamp meadow, SM) in the hinterlands of the QTP. We measured and analysed the key GHG fluxes (ecosystem respiration [Re], CH_4 and N_2O) of each treatment during two contrasting hydrological growing seasons in 2014 (wet with total precipitation at 37.2% above the long-term mean) and 2015 (dry with total precipitation at 16.2% below the long-term mean)to investigate the impacts of hydrological variations on GHG flux responses to warming and N fertilization. Previous studies reported that simulated warming decreased surface soil moisture in the AM by 2% (Chen et al., 2017b) while increased in the SM by 7.0% (Chen et al., 2017a). Therefore, drought events will probably magnify and reduce the effects of warming on surface soil moisture in the AM and SM, respectively. Our hypotheses are: (i) GHGs fluxes of alpine meadows response to warming will be affected by drought events; (ii) GHGs fluxes of the AM response to drought events will be different from the SM. We intend to use this study to enhance our understanding of how hydrological conditions control the responses of GHG flux in alpine meadows to future warming and higher N deposition.

2. Materials and methods

2.1. Study site

The experiment was conducted in the Fenghuo Mountains region in the hinterlands of the Qinghai-Tibetan Plateau, China. The mean annual temperature is −5.2 °C, the relative humidity is 57% and the mean annual precipitation is 310.7 mm, 80% of which falls during the growing season (from May to September) (Wang et al., 2008). The average air temperatures were 2.79 and 3.17 °C, and the rainfall levels were 292.3 and 245.9 mm for the 2014 (total 426.3 mm) and 2015 (total 267.4 mm) growing seasons, respectively (Chen et al., 2017). The annual precipitation in 2014 was higher (37.2%) than the long-term mean annual precipitation, but it was lower (16.2%) than the long-term mean in 2015. The air pressure was approximately 570 h Pa. The area has a continental alpine cold and dry climate, and the freezing period lasts from September to April of the next year. The study site is underlain by permafrost with an active layer of 0.8–1.5 m. The alpine meadow ecosystem consists primarily of cold meso-perennial herbs that grow under conditions in which a moderate amount of water is available. This ecosystem's primary vegetation consists of *Kobresia pygmaea* (C. B. Clarke), *Kobresia humilis* (C. A. Meyer ex Trautvetter) Sergievskaja, *Kobresia capillifolia* (Decaisne) (C. B. Clarke), *Kobresia myosuroides* (Villars) Foiri, *Kobresia graminifolia* (C. B. Clarke), *Carex atrofusca* Schkuhr subsp. (minor (Boott) T. Koyama), and *Carex scabriostris* (Kukenthal). Alpine swamp meadows are populated by hardy perennial hygrophilous or hygro-mesophilic herbs under waterlogged or moist soil conditions, which primarily occur in patches or strips in the mountains, wide valley terraces and rounded hills, and they represent a small portion of the study region. These areas are dominated by *Kobresia tibetica* Maximowicz, *Stipa aliena* Keng and *Festuca* spp (Li et al., 2011). The vegetation and soil characteristics are shown in Table 1.

2.2. Experimental design

This experiment was conducted using a comparative trial design in the AM (34°43′43.9″N, 92°53′45.3″E, 4754 m a.s.l.) and the SM (34°43′43.9″N, 92°53′34.1″E, 4763 m a.s.l.) (Fig. 1), both of which have vegetation coverage of above 70%. During the warming experiment, we followed the methods of the International Tundra Experiment and used open-top chambers (OTCs) as passive warming devices to generate an artificially warmed environment (Marion et al., 1997; Yang et al., 2011). In June of 2012, we installed twelve OTCs in the AM and the SM, and this experiment is still running. Twelve unwarmed plots (1.5 m × 1.5 m, 2.25 m^2) were established in the vicinity of each OTC in both the AM and the SM. The distance between the OTCs and adjacent unwarmed plots was between 3 m and 6m, and the distance between the replicate blocks ranged from approximately 6–8 m. During the N fertilization experiment, NH_4NO_3 ($4\,g\,N\,m^{-2}\,a^{-1}$) was sprayed onto the N fertilization plots in the AM and the SM in May from 2012 to 2015, with non-N fertilization plots receiving the same amount of water (approximately 0.5 mm). Thus, we set up the following four treatments: a control (C), N fertilization (N), warming (W), warming and N fertilization (WN). Six replicates from each treatment were randomly distributed throughout six warmed plots and likewise in the adjacent unwarmed plots in both the AM and SM (for 24 plots in total). All the treatments were applied for two years (2012–2013) before the start of this experiment. All the plots are enclosed by livestock enclosures to avoid grazing disturbances of the GHG flux.

2.3. GHG measurement

Three replicates from each treatment were randomly selected for GHG flux measurements. The greenhouse gas (GHG) fluxes (Re, CH_4, and N_2O) were measured using the static chamber technique (Werner et al., 2006). The chamber cover (30 × 30 × 30 cm, for the convenience of handling the OTCs; we ultimately reduced the bottom area of the chamber) were manually mounted onto the base collars for GHG flux measurements and removed after the mea-

Table 1
Comparison of soil (5 cm depth) and vegetation properties in the alpine meadow (AM) and the alpine swamp meadow (SM) (means ± SD, n = 4).

Site	pH	BD ($g\,m^{-3}$)	SOC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)	MBC ($mg\,kg^{-1}$)	MBN ($mg\,kg^{-1}$)	DOC ($mg\,kg^{-1}$)	NH_4^+-N ($mg\,kg^{-1}$)	NO_3^--N ($mg\,kg^{-1}$)	AGB ($g\,m^{-2}$)	BGB ($Kg\,m^{-2}$)
AM	7.79 ± 0.08	0.89 ± 0.03	27.39 ± 4.16	1.62 ± 0.32	86.40 ± 8.04	13.91 ± 4.38	13.15 ± 3.34	3.65 ± 4.84	18.84 ± 5.57	169.5 ± 12.7	1.9 ± 0.1
SM	7.42 ± 0.03	0.63 ± 0.03	66.11 ± 7.96	2.85 ± 0.20	223.85 ± 111.93	38.11 ± 17.03	35.19 ± 3.70	34.37 ± 6.56	14.09 ± 3.22	553.3 ± 54.7	6.4 ± 0.7
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.89	<0.01	<0.01

Fig. 1. The OTCs in the alpine meadow (left) and alpine swamp meadow (right).

surements. Four air samples were collected at 10-min intervals (0 min, 10 min, 20 min, and 30 min) after the chamber was closed and the air temperature was recorded with an electric thermometer (JM224, Jinming Crop., Tianjin, China) at the same time. Gas samples were collected using a 50 ml plastic syringe fitted with three-way stopcocks via a Teflon tube connected to the chamber, and they were stored in 100 ml plastic bags (Delin Tech, China). The GHG was measured every 3–10 days from 9:00–11:00 a.m. (UTC/GMT +8) from 1st June to 15th October during the growing seasons of 2014 and 2015. The results from Zhang et al. (2015) indicated that the soil CO_2 flux measured during 9:00–11:00 a.m. could represent the average daily soil CO_2 flux in this area. The total sampling frequencies were 30 and 31 times in 2014 and 2015. The gas samples were taken to the lab and measured using a gas chromatograph (Agilent GC-7890B, Agilent Co., Santa Clara, CA, USA) equipped with N_2 carrier gas (there was a pre-column prior to the analytical column (Agilent OT3-2, 500 cm^3) to remove O_2 and water vapour) and mixed make-up gas (10% CH_4 and 90% N_2), a flame ionization detector (FID) and an electron capture detector (ECD).

To calculate the GHG flux, the equation $F_i = \rho_i\,(V/A)\,(P/P_o)\,(T_o/T)\,(dC_i/d_t)$ was used, where F_i is the flux rate, ρ_i is the density under standard conditions, V is the chamber volume, A is the bottom area, P is the air pressure, P_o is the standard air pressure, T is the air temperature, T_o is the standard temperature, and dC_i/d_t is the growth rate for the accumulation of GHGs (Wei et al., 2012).

2.4. Auxiliary measurement

The daily rainfall (52202-L30, R. M. Young, USA) and air temperature (HMP155-L15, Vaisala, Finland) were recorded at 30 min intervals by data loggers (CR1000, Campbell, USA). In the OTCs and control plots of the AM and SM, continuous air temperature measurements were recorded at 15 cm above the ground by a Decagon ECT sensor fitted with a radiation shield (Decagon Devices, Pullman, Washington, USA) as were the soil temperature and soil moisture (v/v) at 5 cm using Decagon 5TM and EC-TM sensors every 10 min in the alpine meadow and swamp meadow, respectively. The results were auto-transmitted to recorders (Em50G, Decagon, USA).

2.5. Statistics

All the data are displayed as the means ± standard error, unless otherwise stated. The van't Hoff equation ($y = ae^{bt}$) was used to analyse the temperature sensitivity (Q_{10}) of the Re ($Q_{10} = e10^b$). Linear and non-linear regressions were applied to test the relationship between the GHG flux and the soil temperature/moisture. A one-way ANOVA was used to examine the treatments effects on average seasonal GHG flux (the results were labelled in Fig. 4). A three-way ANOVA was used to examine the effects of the year, warming, N fertilization and their possible interactions on the average seasonal ecosystem GHG flux, followed by the least significant difference (LSD, $p < 0.05$). If there is significant inter-annual variability (year effect $p < 0.05$) (Table 2), repeated-measure ANOVAs were used to examine all of the treatment effects on the GHG flux during the 2014 and 2015 growing seasons. All of the statistical analyses were performed in SPSS 19.0 (SPSS, Inc., Chicago, IL, USA).

Table 2
Results of (*p*-values) three-way ANOVA on the effects of warming (W), N fertilization (N), year (Y), and their interactions on average seasonal Re, CH_4 and N_2O fluxes.

Site	Treatment	Re	CH_4 flux	N_2O flux
AM	W	<0.01	<0.01	<0.01
	N	0.20	0.08	0.65
	W × N	0.61	<0.01	0.16
	Y	0.32	<0.01	<0.01
	Y × W	0.62	0.61	0.97
	Y × N	0.80	0.33	0.80
	Y × W × N	0.79	0.83	0.73
–				
SM	W	<0.01	0.76	<0.01
	N	0.30	0.97	0.95
	W × N	0.53	0.17	0.24
	Y	<0.01	<0.05	<0.05
	Y × W	0.17	0.76	0.44
	Y × N	0.69	0.92	0.79
	Y × W × N	0.84	0.67	0.90

3. Results

3.1. Environmental conditions

In the AM, the OTCs reached 4.5 °C (the mean of the two growing seasons) of warming for the air temperature (Fig. 2a). This warming increased the soil temperature at the 5 cm depth by 4.93 and 5.50 °C (Fig. 2c) during the 2014 and 2015 growing seasons, respectively, while decreasing the soil moisture levels at the 5 cm depth by 5.24% and 1.57% (Fig. 2e). In the SM, the OTCs reached 6.2 °C in warming for the air temperature (Fig. 2b). This warming increased the soil temperature at the 5 cm depth by 2.85 and 2.44 °C (Fig. 2d) during the 2014 and 2015 growing seasons, respectively, while increas-

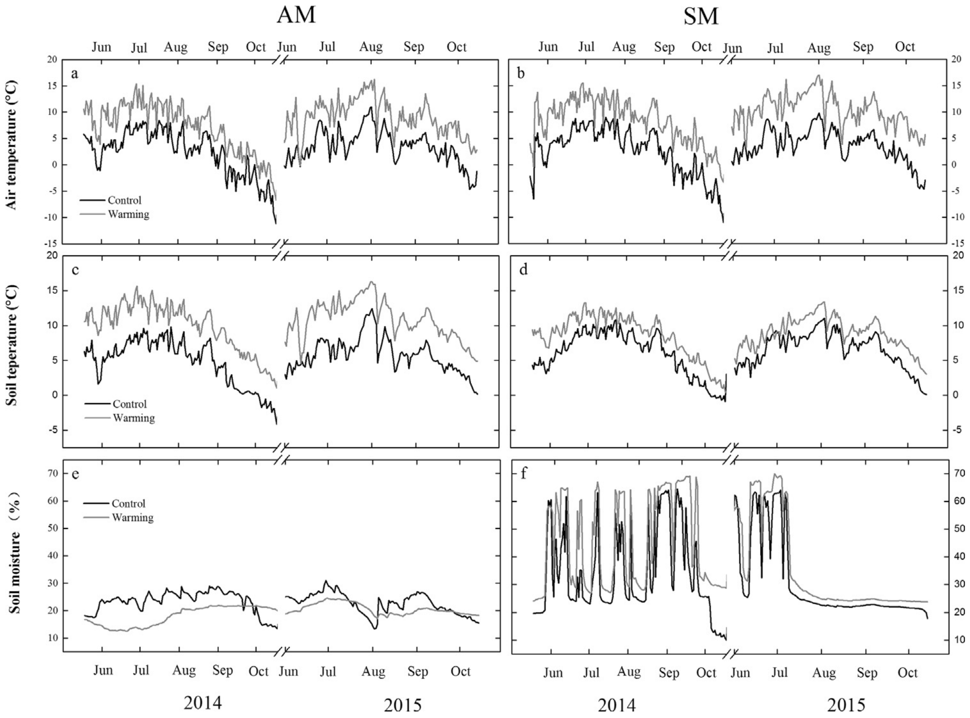

Fig. 2. Air temperature, soil temperature and soil moisture at the 5 cm depth in the warming plots (black line) and control plots (grey line) during the 2014 and 2015 growing seasons.

ing the soil moisture levels at the 5 cm depth by 9.73% and 5.11% (Fig. 2f).

3.2. Temporal variation in the GHG flux

The Re values of the AM and SM showed similar seasonal dynamics as the air temperature, which increased from mid-May, peaked in August, and decreased starting in September (Fig. 3a and b). The Re of the SM showed dramatic fluctuations during the dry growing season (2015), while no obvious difference was found between the two growing seasons in the AM. The AM is a CH_4 sink (Fig. 3c), while the SM switched between a CH_4 sink and source (Fig. 3d). The CH_4 uptake of the AM showed similar seasonal dynamics as the air temperature and with more CH_4 absorption during the dry growing season (2015). The CH_4 flux of the SM showed similar seasonal dynamics as the soil moisture (Fig. 2f). The SM was a CH_4 sink when the soil moisture decreased sharply after mid-July. The N_2O flux in the AM and SM switched between uptake and emission and did not show a clear seasonal pattern during the two growing seasons (Fig. 3e and f). This flux showed more drastic fluctuations during the dry growing season in both the AM and the SM.

3.3. Treatment effects on the GHG flux

The average seasonal Re of the AM ranged from (including all treatments and years) 214.33 ± 25.10 to 482.84 ± 61.07 mg m^{-2} h^{-1}, while the Re of the SM ranged from 287.14 ± 28.43 to 616.25 ± 63.98 mg m^{-2} h^{-1} (Fig. 4a and b). Warming increased the Re in both the AM and SM during the two growing seasons ($p < 0.01$, Table 3). N fertilization decreased the Re of the AM in 2015 ($p < 0.05$), and it decreased the Re of the SM in 2014($p < 0.05$, Table 3). The interaction between warming and N fertilization for the Re was only found in 2014 for the SM ($p < 0.05$).

Table 3
Results of (p-values) repeated-measures ANOVA on the effects of warming (W), N fertilization (N), date (D) and their interactions on Re, CH_4 and N_2O fluxes.

Year	Site	Treatment	Re	CH_4 flux	N_2O flux
2014	AM	W	<0.01	<0.01	<0.01
		N	0.08	0.38	0.84
		W × N	0.64	<0.01	0.09
		D	<0.01	<0.01	<0.01
		D × W	<0.01	0.97	0.07
		D × N	<0.01	0.90	0.99
		D × W × N	0.88	0.89	0.96
	SM	W	<0.01	0.06	<0.05
		N	<0.05	0.64	0.31
		W × N	<0.05	0.83	0.14
		D	<0.01	<0.01	<0.01
		D × W	0.48	<0.01	<0.01
		D × N	0.37	0.99	0.59
		D × W × N	0.01	0.97	<0.01
2015	AM	W	<0.01	<0.01	<0.01
		N	<0.05	<0.05	0.28
		W × N	0.33	<0.01	0.12
		D	<0.01	<0.01	<0.01
		D × W	<0.01	0.12	0.07
		D × N	<0.01	0.14	0.94
		D × W × N	0.68	0.98	0.99
	SM	W	<0.05	0.99	<0.01
		N	0.14	0.91	0.61
		W × N	0.63	<0.01	<0.01
		D	<0.01	<0.01	<0.01
		D × W	<0.01	<0.01	<0.01
		D × N	0.33	0.93	0.67
		D × W × N	0.84	0.81	0.74

Both the AM and SM are CH_4 sinks. The average seasonal CH_4 flux of the AM ranged from −23.57 ± 1.38 to −11.27 ± 0.56 μg m^{-2} h^{-1}, while for the SM, it ranged from −7.56 ± 2.87 to −1.23 ± 2.51 μg m^{-2} h^{-1} (Fig. 4c and d). Warming increased the CH_4 uptake of the AM ($p < 0.01$), while it had no significant impact on the CH_4 flux of the SM. N fertilization increased the CH_4 uptake of the AM in 2015 ($p < 0.05$), while it also had no significant impact on the CH_4 flux of the SM. Warming combined with N fertilization interacted with theCH_4 uptake of the AM in

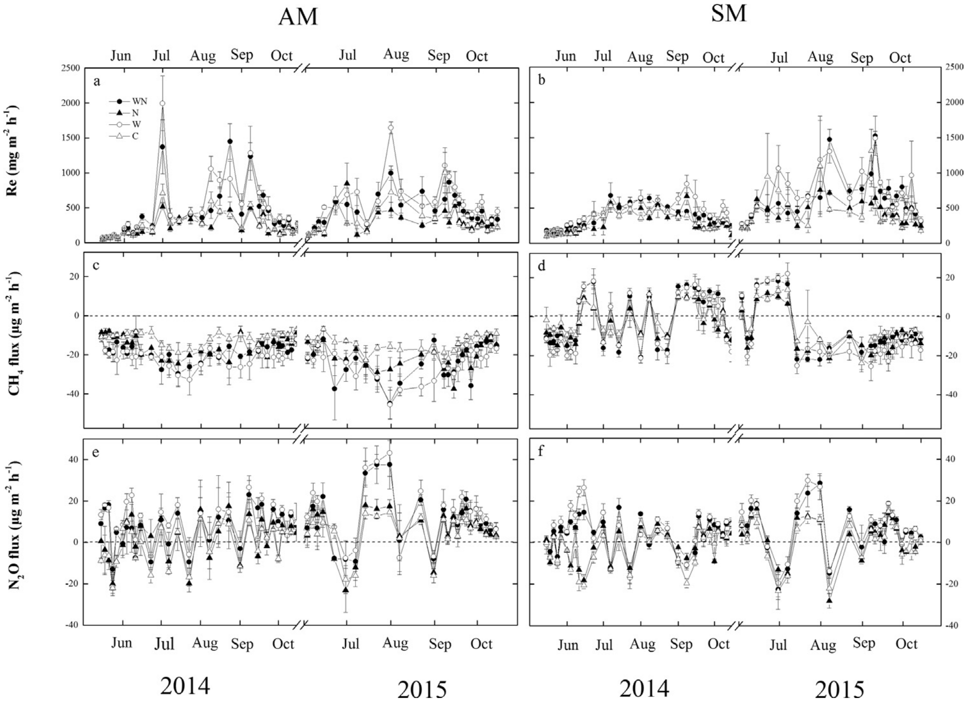

Fig. 3. Temporal variations in the Re, CH_4 and N_2O fluxes in the alpine meadow (AM) (a, c, e) and the alpine swamp meadow (SM) (b, d, f) for the 2014 and 2015 growing seasons. C: control; W: warming; N: nitrogen fertilization; and WN: warming and nitrogen fertilization. The bars indicate the standard errors (SE).

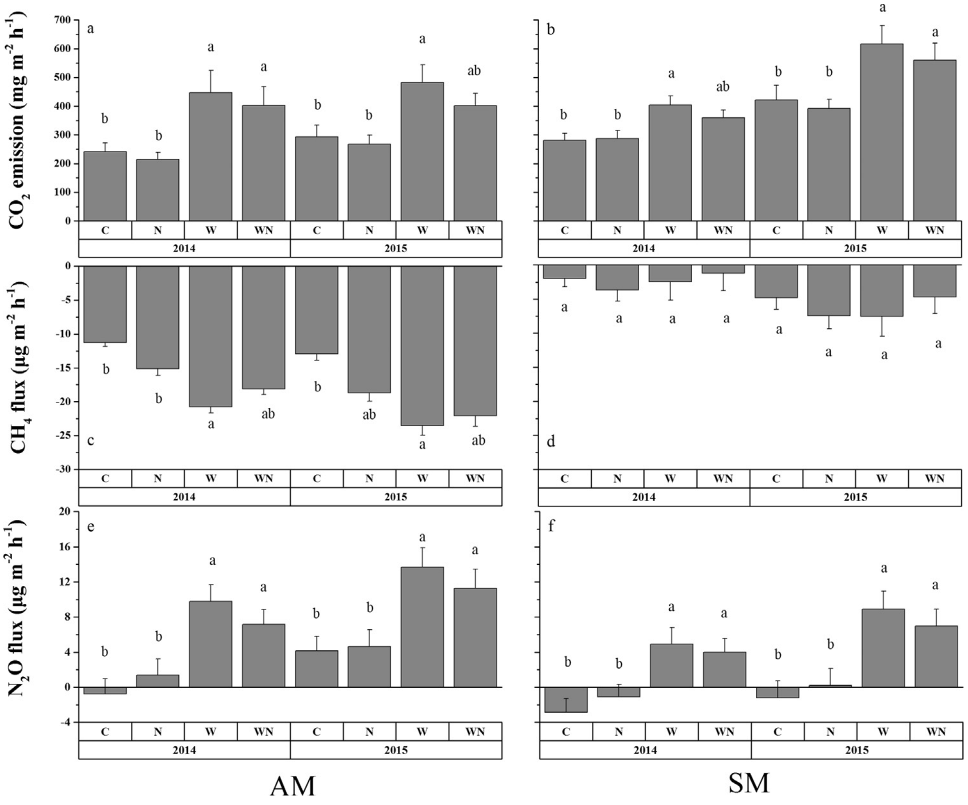

Fig. 4. Average seasonal Re, CH_4 and N_2O fluxes of the alpine meadow (AM) (a, c, e) and the alpine swamp meadow (SM) (b, d, f) for the 2014 and 2015 growing seasons. C: control; W: warming; N: nitrogen fertilization; and WN: warming and nitrogen fertilization. The bars indicate the standard errors (SE).

both 2014 and 2015 ($p < 0.01$), while their interaction with theCH_4 flux of the SM was only found in 2015 ($p < 0.01$).

The SM is a N_2O sink and the AM is a N_2O sink during the wet growing season (2014) but a N_2O source during the dry growing season (2015). The average seasonal N_2O flux of the AM ranged from -0.75 ± 1.74 to 11.27 ± 2.19 $\mu g\,m^{-2}\,h^{-1}$, while the SM ranged from -2.87 ± 1.5 to 8.89 ± 2.07 $\mu g\,m^{-2}\,h^{-1}$ (Fig. 4e and f). Warming increased the N_2O emissions in both the AM and SM during the two growing seasons ($p < 0.01$). N fertilization alone had no significant impact on the N_2O flux. The interaction of warming and N fertilization increased the N_2O emissions of the SM in 2015 ($p < 0.01$), while it had no significant impact on the N_2O flux of the AM.

3.4. Relationship between the soil temperature/moisture and GHG flux

The correlations between the Re and the soil temperature are best depicted by an exponential function in both the AM and SM ($p < 0.01$). The soil temperature sensitivity of the SM (Fig. 5b, Q_{10} was 3.81 and 3.82 in 2014 and 2015, respectively) was higher than that of the AM (Fig. 5a, Q_{10} was 2.75 and 3.02 in 2014 and 2015, respectively). In fertilization, the soil temperature sensitivity of the Re in the AM for 2014 was lower than it was in 2015. The correlations between the CH_4 uptake and the soil temperature of the AM are best depicted by an exponential function (Fig. 5c), and the soil temperature sensitivity of the AM in 2014 ($Q_{10} = 2.01$) was lower than it was in 2015 ($Q_{10} = 2.26$). The correlations between the CH_4 flux and soil temperature of the SM are best depicted by a quadratic function (Fig. 5d). The correlations between the N_2O flux and the soil temperature of the AM are best depicted by a quadratic function (Fig. 5e), while there is no significant relationship between the N_2O flux and the soil temperature in the SM (Fig. 5f).

The correlations between the Re and the soil moisture were weak (the r^2 ranged from 0.03 to 0.11) in both the AM and SM (Fig. 6a and b). The Re of the SM was positively correlated with the soil moisture in 2014 ($p < 0.01$). The CH_4 flux of the AM also has a positive correlation with the soil moisture in 2014($p < 0.01$). The soil moisture controlled the CH_4 flux of the SM (the r^2values were 0.55 and 0.69 in 2014 and 2015, respectively). There was no significant relationship between the N_2O flux and the soil moisture in the AM, while the correlations between the N_2O flux and the soil moisture in the SM are best depicted by a quadratic function (Fig. 6e and f).

4. Discussion

4.1. Single effect of warming/N fertilization on GHG flux

Numerous experiments have been performed to investigate the single effect of warming/N fertilization on the GHG flux in alpine ecosystems. The results have supported the idea that warming increased the Re due to the fact that warming is beneficial for aboveground biomass (AGB) and soil organic carbon (SOC) decomposition (Chen et al., 2017a,b); therefore, warming increased both plant and soil respiration. N fertilization increased the AGB but suppressed the SOC decomposition; there are contrary effects on the Re. Therefore, N fertilization tended to decrease or had no significant effect on the Re (Sun et al., 2013; Wei et al., 2014; Zhu et al., 2015; Jiang et al., 2013). In fact, the N fertilization decreased the Re of the AM in 2015 and of the SM in 2014 (Table 3). Higher soil temperature and N availability would stimulate activity in microorganisms, such as methanotrophs, nitrifiers and denitrifiers (Liu and Tara, 2009; Karita et al., 2014; Chapuis-Lardy et al., 2007). Therefore, warming/N fertilization would increase theCH_4 uptake and N_2O emissions.

Warming decreased the soil moisture at the 5 cm depth of the AM, while it increased in the SM. Warming promoted water evaporation, and therefore, simulated warming often decreased the soil moisture (Frank et al., 2010; Zhou et al., 2007). However, warming-increased soil moisture is understandable for a permafrost region, particularly in a wetland ecosystem. The frozen soil of the SM contains a great deal of water, and warming promotes the release of deep, frozen soil moisture and its upward diffusion. Additionally, local warming causes the heat to diffuse to the surrounding environment, whereas the water of the SM in the surrounding permafrost is almost saturated. Thus, the formation of a water island in the warming plots was possible. Warming significantly increased the soil moisture at the 5 cm depth by 7% (the mean of two growing seasons); therefore, warming should decrease the soil available oxygen and suppress methane oxidation in the SM.厌氧氧化Warming increased the soil N availability (Chen et al., 2017a,b), which is beneficial to methane oxidation bacteria. Therefore, although warming increased the CH_4 uptake of the AM, the contrary effect in relation to warming had no significant effect on the SM (Table 3). Our observations highlight the importance of soil moisture in modulating the response of the CH_4 flux to climate warming.

Re is the sum of Rs (soil respiration) and Rp (aboveground plant tissues respiration). The effects of N fertilization on the Re depend on the balance of Rs and Rp. Guo et al. (2017) reported that N fertilization will reduce soil microbial respiration of alpine grassland and then decrease the Rs. However, N fertilization also had a possibility increasing the Rs in N-limited ecosystems (Janssens et al., 2010; Xu et al., 2004). In this experiment, N fertilization did not increased AGB, because the photosynthesis was mainly limited by low temperature (average annual temperature was −5 °C) rather than soil available N in this permafrost area. Therefore, Rp did not change in this experiment. Although the alpine grasslands are N-limited ecosystems, the soil biochemical process may also primary limited by low temperature rather than soil available N. Thus N fertilization may decrease the Rs and has no impact on Rp. Therefore, N fertilization tends to decrease the Re. N fertilization is beneficial to formethanogens, methanotrophs, nitrifiers and denitrifiers, so N fertilization will increase CH_4 and N_2O fluxes levels. N fertilization decreased the Re but increased the CH_4 uptake of the AM in 2015, but it had no significant impact on the GHG flux in 2014. By contrast, N fertilization decreased the Re of the SM in 2014 but had no significant impact on the GHG flux in 2015. Therefore, drought events strengthen the N fertilization effect on GHG flux in non-wetland but weaken it in wetland. The results suggest that precipitation controls the response of the Re and CH_4 flux to increase N deposition. As study site is located in a semi-arid region (Wang et al., 2017), our results are consistent with an assumption that carbon (C) flux of alpine meadows response to N fertilization strongly depend on precipitation pattern (Harpole et al., 2007). The possible reasons as follows: (i) effects of N fertilization amendment on soil moisture (Zavaleta et al., 2003) while changes in soil moisture amplify or reduce the effect of precipitation on GHGs fluxes; (ii) precipitation effects on soil moisture were related to increases in leaf-level C exchange rates of plants associated with increased N availability (Harpole et al., 2007), therefore, although nitrogen fertilization did not alter AGB, it changed the carbon exchange of the plants together with precipitation variations. Unfortunately, we did not set up a moisture probe in the nitrogen-added plots to examine the effects of N fertilization on soil moisture. This requires us to supplement these data in the future.

4.2. Interaction of warming and N fertilization on the GHG flux

Few *in situ* experiments have determined the interaction of warming and N fertilization on the GHG flux in alpine ecosystems, particularly in alpine wetland ecosystems. In this study, their interaction on the Re was not found due to their contrary effects (warming increased but N fertilization tended to decrease the Re). In combining the results of the two growing seasons, their interaction increased the CH_4 uptake and N_2O emission in both the AM (Chen et al., 2017) and SM (unpublished result). Increasing the soil temperature and N deposition synergistically will enhance the activity of microorganisms (Xiong et al., 2016), such as methanotrophs, nitrifiers and denitrifiers. However, the annual statistical

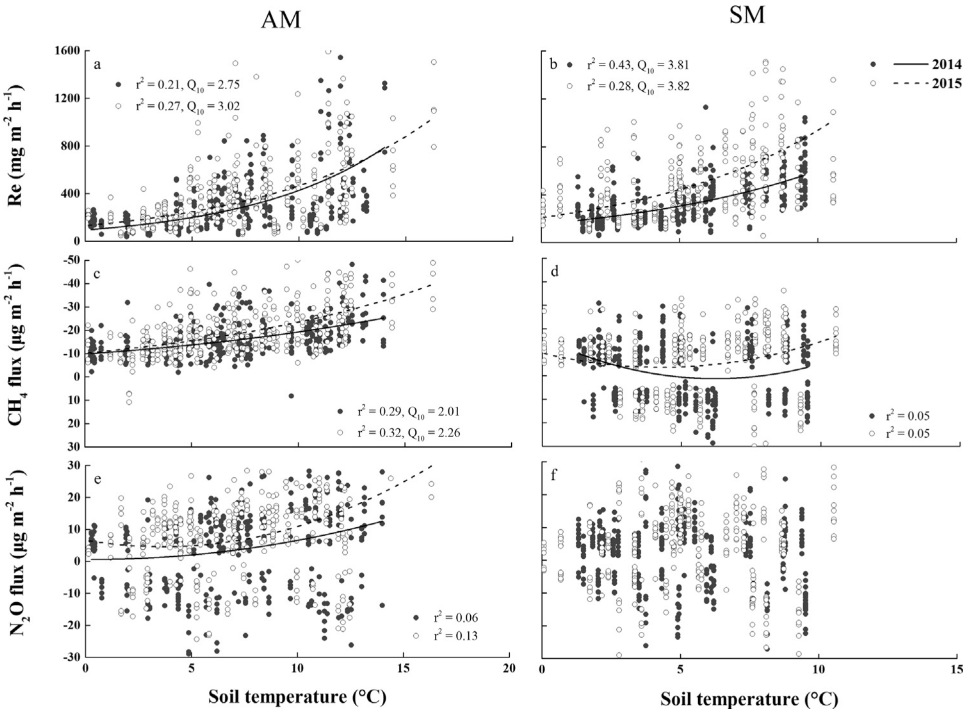

Fig. 5. Correlations between the soil temperature at the 5 cm depth and the Re (a, b), CH_4 (c, d) and N_2O (e, f) fluxes for the 2014 (black dots) and 2015 (grey dots) growing seasons.

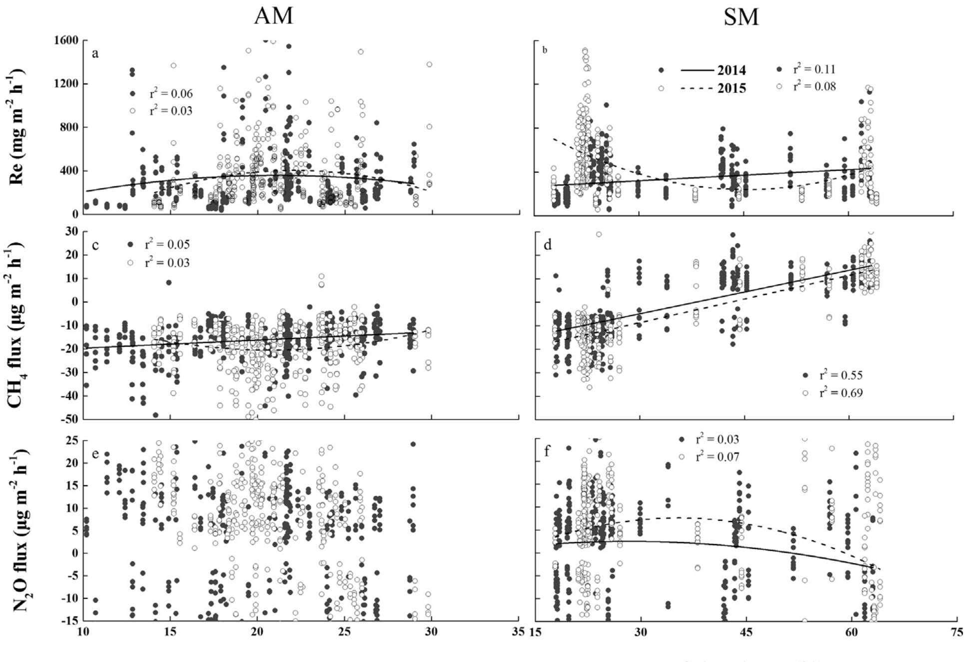

Fig. 6. Correlations between the soil moisture at the 5 cm depth and the Re (a, b), CH_4 (c, d) and N_2O (e, f) fluxes for the 2014 (black dots) and 2015 (grey dots) growing seasons.

results showed that the interaction for theCH_4 uptake N_2O flux of the SM is only found in 2015. These results suggest that the role of N fertilization in the CH_4 and N_2O fluxes of the SM in the warmed plots is affected by inter-annual variations in precipitation. The rainfall was 292.3 and 245.9 mm during the wet and dry growing season, respectively. The 50 mm differences in precipitation lead to the changes of 5.1% and 3.6% in surface soil moisture of control plots in the AM and SM, respectively. This change is considerable in this semi-arid area. The response of GHGs in the SM is more sensitive than in the AM because activities of hygrophyte and anaerobic microbe in wetland ecosystem are more sensitive to soil moisture. Another reason is that OTCs in the SM resulted in 6.2 °C warming which was much higher than that in the AM (4.5 °C). The effect of drought events on GHGs in warming plots of the AM and SM should be different. Additionally, warming effects on Rs were regulated by water availability in a semi-arid alpine meadow (Shen et al., 2015), and soil moisture affected by long-term N fertilization (Zavaleta et al., 2003). Thus, there is a possibility that four years N fertilization will affect soil moisture of the SM (high moisture content) but not enough to change the soil moisture of the AM (moderate moisture content). Although it requires us to test the effect of long-term nitrogen on soil moisture of these alpine meadows, the interacted effects of warming and N fertilization on GHGs fluxes in the AM and SM were different according to our existed results.

4.3. Effect of precipitation variation on GHG flux

This study was conducted during two hydrologically contrasting years (wet in 2014 and dry in 2015), which provided a unique opportunity to understand how wet/drought events affect GHG fluxes and their response to warming and N fertilization in alpine meadow ecosystems. Drought events increased the average seasonal Re of the control plots in the AM by 21.5% and by 49.8% in the SM (Fig. 4a and b). This finding is due to the fact that Re has a stronger relationship to the air/soil temperature than the soil moisture (Figs. 5 and 6; Zhang et al., 2015), and the Re of the SM has stronger soil temperature sensitivity than the AM. Indeed, drought events increased the air temperature by 13.6% and increased the soil temperatures in the AM and SM by 4.2% and 1.0%, ($p < 0.01$) (Fig. 2c and d), respectively. The CH_4 uptake was negatively correlated with the soil moisture (Fig. 6c and d), and drought events decreased the precipitation by 18.9%, which lead to the decreasing of soil moisture in the AM and SM by 5.1% and 3.6% ($p < 0.01$) (Fig. 2e and f), respectively. Therefore, drought events increased the average seasonal CH_4 uptake of the control plots in the AM and SM by 14.8% and 141.9%, respectively. The N_2O flux was primarily controlled by rain events rather than soil temperature/moisture (Chen et al., 2017). Because N_2O consumption often occurs on rainy days, the saturation of the water-filled pore space is beneficial for N_2O absorption by soil water, and N_2O will diffuse to deep soil layer with downward diffusion of water (Jiang et al., 2010; Zhu et al., 2014; Chapuis-Lardy et al., 2007). Thus, the control plots of the AM were N_2O sinks in 2014, but they changed into N_2O sources in 2015 (Fig. 4e), and drought events decreased the N_2O uptake of the SM by 140.4% (Fig. 4f). These results indicate that increasing precipitation would increase the Re and N_2O consumption but that it would decrease the CH_4 uptake of alpine meadows. However, we need to emphasize that this experiment only covers an alternating dry-wet process, and more research is needed to confirm these findings.

5. Conclusions

The objective of this study was to investigate the effects of hydrological conditions (including precipitation variations and different grassland type determined by soil moisture) on the GHG flux of alpine meadows under the background of climate warming and increasing N deposition. Results confirmed our second hypothesis that although the wetland ecosystem has high soil moisture, its GHG fluxes are more sensitive to inter-annual variations of precipitation than those of the non-wetland ecosystem. However, results were contrary to our first hypothesis that GHGs (Re and CH_4) fluxes of alpine meadows response to N fertilization rather than warming will be affected by precipitation variations, because drought events strengthened the N fertilization effect on the C flux (Re [CO_2] and CH_4) in non-wetlands, but weakened it in wetlands.

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