Different responses of ecosystem CO_2 and N_2O emissions and CH4 uptake to seasonally asymmetric warming in an alpine grassland of the Tianshan Mountains

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Abstract:

An experiment was conducted to investigate the effect of seasonally asymmetric warming on ecosystem respiration (*Re*), CH_4 uptake, and N_2O emissions in alpine grassland of the Tianshan Mountains of Central Asia, from October 2016 to September 2019. The annual mean of *Re*, CH_4, and N_2O fluxes in growing season were 42.83 mg C m^{-2} h^{-1}, −41.57 μg C m^{-2} h^{-1}, and 4.98 μg N m^{-2} h^{-1}, respectively. Furthermore, warming during the non-growing season increased *Re* and CH_4 uptake by 7.9% and 10.6% in growing season, 10.5% and 9.2% in non-growing seasons, respectively. However, the increase in N_2O emission in the growing season was mainly caused by the warming during the growing season (by 29.7%). the warming throughout the year and warming during the non-growing season increased N_2O emissions by 101.9% and 192.3% in non-growing seasons, respectively. The *Re*, CH_4 uptake, and N_2O emissions were positively correlated with soil temperature. Our results suggested that *Re*, CH_4 uptake and N_2O emissions were regulated by soil temperature, rather than soil moisture, in the case of seasonally asymmetric warming. In addition, the response rate was defined by the changes in greenhouse gas fluxes driven by warming. In our field experiment, we observed the stimulatory effect of warming during the non-growing season on *Re* and CH_4 uptake. In contrast, the response rates of *Re* and N_2O emissions were gradually attenuated by long-term annual warming and the response rate of *Re* was also weakened by warming over the growing season. These findings highlight the importance of warming in the non-growing season in regulating greenhouse gas fluxes, a finding which is crucial for improving our understanding of C and N cycles under the scenarios of global warming.

Keywords: Alpine steppe; Extreme climatic event; Greenhouse gas fluxes; Warming of open-top chambers

1. Introduction

Since the industrial revolution, human activities have intensified global warming. The global surface temperature increased by about 0.85°C from 1880 to 2012 (IPCC, 2013). Furthermore, the temperature is expected that the surface temperature will increase by about 1.1–6.4°C by the end of this century (IPCC, 2007, 2013). The rise in atmospheric temperature over the year is not continuous on the temporal scale but there is asymmetrical warming across the seasons (Xia et al., 2014). The 3rd and 4rd Assessment Report of the Inter-Governmental Panel on Climate Change (IPCC) proposed that, against the backdrop of global warming, the temperature change shows that the warming amplitude in the winter is greater than that in the summer, with the warming amplitude at high latitude being greater than that at low latitude, and confirmed that the warming shows asymmetric trends on a seasonal scale (Easterling et al., 1997; IPCC, 2001, 2007).

Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are three of the major greenhouse gases (GHGs) in the atmosphere that directly cause global climate warming, with their contributions to global warming being 60%, 20%, and 6%, respectively (IPCC, 2007, 2013). Experimental warming is known to influence ecosystem respiration (*Re*), CH_4 uptake, and N_2O emission (Pärn et al., 2018; Treat et al., 2018; Wang et al., 2019). Information on *Re*, CH_4 uptake, and N_2O emission will enhance our understanding of ecosystem C and N cycling processes and improve our predictions of the response of ecosystems to global climate change (Li et al., 2020; Wang et al., 2019).

At present, most studies focus on the influence of warming on GHG flux in terrestrial ecosystems during the summer months (Keenan et al., 2014; Li et al., 2011; Yang et al., 2014). Nevertheless, data on the influence of asymmetric warming on the GHG flux on a seasonal scale are scarce. A study of the Alaskan tundra found that summer warming (using open-top chambers to increase air temperatures in the growing season) significantly increased *Re* in the growing season by about 20% (Natali et al., 2011). Compared with the slight effect of winter warming on the ecosystem respiration in the growing season, warming increased ecosystem respiration during the snow-covered non-growing season by more than 50% (Natali et al., 2011). Lin et al. (2015) reported that the response of soil CH_4 uptake rates to temperature increases in alpine meadows of the Qinghai-Tibet Plateau were not consistent seasonally, with CH_4 uptake in the non-growing season being more sensitive to temperature (increasing by 162%) than the corresponding value in the growing season. A study by Cantarel et al. (2012) in an alpine grassland ecosystem showed that the response of N_2O emission to warming showed clear seasonal differences, with the N_2O emission in the growing season showing significant differences between the warming treatments, whereas the response of N_2O emission to the warming treatments in November was not obvious. A recent study showed that seasonal variations in carbon flux were more closely related to air temperature in the meadow steppe (Zhao et al., 2019). Another study found that experimental warming enhanced CH_4 uptake in the relatively arid alpine steppe, but had no significant effects on CH_4 emission in the moist swamp meadow (Li et al., 2020). Furthermore, soil CH_4 uptake was not significantly affected by warming in the alpine meadow of the Tibetan Plateau (Wu et al., 2020). In contrast, a global meta-analysis showed that experimental warming stimulates ecosystem respiration in grassland ecosystems, and the response of ecosystem respiration to warming strongly varies across the different grassland types, with greater warming responses in cold than in temperate and semi-arid grasslands (Wang et al., 2019). Across the data set, Li et al. (2020) demonstrated that N_2O emissions were significantly enhanced by whole-year warming treatments. In contrast, no significant effects on soil N_2O emissions were observed by in short-season warming.

In summary, the GHG fluxes in terrestrial ecosystems shows significant interannual, and seasonal variations, and its response to warming also varies over different temporal scales. After long-term uniform warming, the biotic and abiotic factors have adapted to the temperature increase, and the GHG fluxes response to increasing temperature is smaller than that in the early stages of warming. For example, over longer time periods of warming, accelerated carbon decomposition and increased plant N uptake may decrease soil organic C and N pools (Wu et al., 2012), and the microbial community with variable C use efficiency may reduce the temperature sensitivity of heterotrophic respiration (Zhou et al., 2012). Moreover, climate warming is often unstable, with most of it occurring as extreme events (Jentsch et al., 2007). The heterogeneity of warming may change the adaptability of GHG fluxes to warming, and thus affect the carbon and nitrogen cycles in terrestrial ecosystems. In this study, we hypothesize the stimulatory effect of warming during the non-growing season on *Re*, CH_4 uptake and N_2O emissions. However, the response rates of *Re*, CH_4 uptake and N_2O emissions were gradually attenuated by long-term annual warming and warming over the growing season, respectively.

2. Materials and methods

The experiment was conducted from October 2016 to September 2019 at the Bayinbuluk Grassland Ecosystem Research Station, Chinese Academy of Sciences (42°52.76′ ~ 42°53.17′ N, 83°41.90′ ~ 83°43.12′ E, 2460 m above sea level), which is located in the southern Tianshan mountains of Central Asia, Xinjiang Uyghur Autonomous Region of China. Permafrost is present in the Bayinbuluk alpine grassland, with the average maximum frozen depth (from 2000 to 2011, Zhang et al., 2018) being more than 250 cm. The mean annual temperature was −4.8 °C per decade, with the lowest monthly temperature in January (−27.4 °C) and the highest in July (11.2 °C), and the mean annual precipitation amounted to 265.7 mm, with 78.1% occurring during the growing season, from June to September (Geng et al., 2019). Variations in soil temperature, soil moisture, air temperature and precipitation are shown in Figure S1, S2, S3 and S4, respectively. The site was fenced since 2005, all the plots were dominated by *Stipa purpurea*, *Festuca ovina*, *Oxytropis glabra*, and *Potentilla multifida*. The soil was sub-alpine steppe soil, the parent material of the soil was Loess, and the average annual soil moisture was 5.9% (2017-2019).

The open-top chambers (OTCs) were made of 5 mm thick tempered glass. To reduce the impact of precipitation and snow, the OTC was constructed with a hexagonal round table which was 100 cm high, and the diagonals of the bottom and top were 100 cm and 60 cm, respectively. Four treatments were simulated using OTCs: warming throughout the year (AW), warming in the non-growing season (October 1 to May 31 of the next year) only (NGW), warming in the growing season (June 1 to September 30) only (GW) and no warming (NW). After the warming in the NGW or GW, the tempered glass was removed and the frame was retained. Three replicate plots were established for each treatment, each plot measuring 1 m × 1 m, with a 3-m wide buffer zone between adjacent plots, making a total of 12 plots. Soil temperature and soil moisture were measured at a frequency of every half an hour by an outdoor temperature and humidity data recorder (at 10 cm depth; HOBO U23-001; Onset

Computer Corporation, Bourne, USA). The air temperature inside the OTCs is also recorded at a frequency of every half an hour using HOBO Pro RH/TEMP Data LOGGERS (hanged in the center of the OTCs, 50cm above the surface; Onset Computer Corporation, Bourne, USA). Soil temperature and air temperature were increased about 2.3 ^{o}C and 4 ^{o}C by the warming treatment, respectively (Figure S1 and S3). Soil moisture was reduced about 5% by the warming treatment (Figure S2).

Re, CH_4 and N_2O fluxes were measured using static chambers, made of PVC tubing with diameter 0.25 m and height 0.17 m, with one chamber in each of the 12 plots. Gas samples were taken 0, 10, 20 and 30 minutes after the lid of the static chamber was sealed in between 12:00 and 14:00 (GMT + 8), collecting once or twice a week. The rates of ecosystem respiration, CH_4 and N_2O fluxes were calculated based on the change in concentration of CO_2, N_2O and CH_4 in each chamber over time by a linear or non-linear equation ($P < 0.05$, $r^2 > 0.95$) (the positive flux values represent emission, and the negative flux values represent uptake; Liu et al. 2012; Wang et al. 2013). Concentrations of individual gases in samples were measured using a gas chromatograph (GC) (Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA).

Effects of seasonally asymmetric warming on *Re*, CH_4 uptake, and N_2O emissions were analyzed by two-way repeated-measures analysis of variance (ANOVA). One-way ANOVA was used to compare soil temperature, soil moisture and air temperature differences, respectively. Nonlinear regression analyses (Exponential Growth, Single, 3 Parameter) was used to identify the relationship between ecosystem respiration (Re) and soil temperature (at 10-cm depth) from October 2016 to September 2019. General linear analyses were used to identify significant linear correlations and regressions between soil temperature, or soil moisture variation and the responses of CH_4 uptake, or N_2O emissions. Variation-partitioning analysis was used to disentangled the influence of soil temperature and soil moisture on *Re*, CH_4 uptake and N_2O emission under the four treatments in the growing season and the non-growing season, respectively. The natural logarithm of the response ratio (RR) was used to reflect the effects of seasonally asymmetric warming on alpine grassland GHG fluxes (Hedges et al., 1999). The RR is the ratio of the mean value of the chosen variable in the warming group ($\overline{W}_t$) to that in the control group (NW; $\overline{W}_c$), and is an index of the effect of seasonally asymmetric warming on the corresponding variable (Eq. 1). All statistical analyses were conducted using SPSS (version 20.0) (IBM, Armonk, NY, USA) with the statistically significant difference threshold set at $P < 0.05$.

$$RR = \ln\left(\frac{\overline{W}_t}{\overline{W}_c}\right) = \ln\left(\overline{W}_t\right) - \ln\left(\overline{W}_c\right) \qquad (1)$$

3. Results

Our study showed that the Bayinbuluk alpine grassland exhibited a low *Re*, was a net CH_4 sink, and a negligible N_2O source. The annual mean values of *Re*, CH_4 uptake, and N_2O emissions in the growing season were 42.83 mg C m^{-2} h^{-1}, 41.57 μg C m^{-2} h^{-1}, and 4.98 μg N m^{-2} h^{-1}, respectively, from October 2016 to September 2019. One-way ANOVA results of *Re*, CH_4 uptake and N_2O emissions among the four warming treatments were not significant, with the exception that the soil CH_4 uptake in the growing season 2019 under GW treatment was significantly higher than that of the AW and NGW treatments ($P < 0.05$). Compared with the control group (NW), the *Re* was decreased by 7.5% and 4.0% in the growing season and non-growing season, respectively, under AW and decreased by 2.4% and 8.5% under GW in the growing season and non-growing season, respectively. However, compared with the control group, the *Re* under NGW increased by 7.9% and 10.5% in the growing season and non-growing season, respectively, averaged over the three years (Figure 2 a).

The AW temperature change induced a 6.4% increase in CH_4 uptake in the growing season and a 3.8% decrease in the non-growing season. The GW treatment resulted in 7.1% and 10.2% increases in CH_4 uptake in the growing season and non-growing season, respectively. On the contrary, the NGW generated a 10.6% and 9.2% decrease in CH_4 uptake in the growing season and non-growing season, respectively (Figure 2 b). The AW and NGW treatments resulted in 5.8% and 2.2% decreases, respectively, in N_2O emission in the growing season, and 101.9% and 192.3% increases, respectively, in N_2O emission in the non-growing season. Compared with the control, NW group, the N_2O emission increased by 29.7% and decreased by 24.4% under GW in the growing season and non-growing season, respectively (Figure 2 c).

The results of two-way repeated measures ANOVA showed significant interannual differences of *Re* in the growing season ($P < 0.05$, Figure 1 a), whereas the CH_4 uptake under the warming treatment exhibited significant differences in the growing season ($P < 0.01$; Figure 1 b), and the interannual N_2O emission showed significant differences in both the growing season and non-growing season ($P < 0.05$, Figure 1 c). Therefore, interannual variation was larger than the impact of the warming treatment (for *Re* and N_2O emissions, Figure 1), whereas the warming treatment had a significant impact on CH_4 uptake. Under the four warming treatments, *Re* exhibited exponential growth, respectively ($P < 0.05$; Figure S5 a). we observed increasing CH_4 uptake with increasing soil temperature ($P < 0.05$; Figure S5 b). On the other hand, the N_2O emission showed a significantly positive linear correlation with soil temperature, but only under NGW ($P < 0.05$; Figure S5 c).

The soil moisture was reduced by warming in the alpine grassland (Figure S2). However, *Re*, CH_4 uptake and N_2O emission were not significant linearly correlated with soil moisture, respectively ($P \geq 0.05$; Figure S6). We disentangled the influence of soil temperature and soil moisture on *Re*, CH_4 uptake, and N_2O emission by variation-partitioning analysis under the four treatments in the growing season and the non-growing season (Figure 4). Under the NGW treatment, *Re*, CH_4 uptake, and N_2O emission in the non-growing season were more influenced by soil temperature than by soil moisture. Under the GW treatment, there was the single effect of soil temperature on CH_4 uptake and N_2O emission in the non-growing season. In contrast, there were the joint effects of soil temperature and moisture on *Re* in the non-growing season under the GW treatment. *Re* in the growing season was influenced more by soil moisture than soil temperature under the GW treatment. Annual *Re* under the AW treatment was influenced by the joint effects of soil temperature and moisture.

4. Discussion

Our study found that the response rate of *Re* to temperature significantly decreased with the increase in soil temperature (ΔST_{AW} and ΔST_{GW}) under AW and GW treatments, respectively (Figure 3 a, c; $P < 0.05$). This finding indicated that the response of *Re* to soil temperature became less and less sensitive to soil temperature with warming throughout the year (or the growing season) in the alpine grasslands. On the contrary, NGW significantly increased the response rate of *Re* to temperature change (ΔST_{NGW}), indicating that warming in the non-growing season amplified the sensitivity of *Re* to temperature change (Figure 3 b, $P < 0.05$). In addition, Zou et al. (2018) showed that the soil fluxes of CO_2 increased exponentially with increasing temperature, but warming decreased the temperature sensitivity by 23% in the grassland. Furthermore, Natali et al. (2011) also confirmed that, compared with the CO_2 flux in the growing season, the CO_2 flux in the nongrowing season was more sensitive to the temperature increase.

Ecosystem CH_4 flux is the net result of CH_4 production and consumption, occurring simultaneously under the action of methanogenic archaea and methane-oxidizing bacteria (e.g., Mer and Roger, 2001). In this study, warming increased CH_4 uptake in the growing season, but decreased CH_4 uptake in the non-growing season in the alpine grassland, findings similar to those from other grassland ecosystems (Lin et al., 2015; Wu et al., 2020; Zhu et al., 2015). Our results also demonstrated that seasonally asymmetric warming did not significantly affect the response rate of CH_4 uptake (Figure 3 d-f, $P > 0.05$). CH_4 flux depended on temperature, pH, and the availability of substrate (e.g., Treat et al., 2015). The CH_4 uptake observed during the three growing season and non-growing season implied that the alpine grassland soil could act as an atmospheric CH_4 sink, a finding which agrees with the results of many previous studies in similar regions (Wei et al., 2015; Zhao et al., 2017). Hu et al. (2016) suggested that asymmetrical responses of CH_4 fluxes to warming and cooling should be taken into account when evaluating the effects of climate change on CH_4 uptake in the alpine meadow on the Tibetan plateau. Unlike CH_4 flux in alpine grasslands, Treat et al. (2018) confirmed that wetland was a small CH_4 source in the non-growing season, whereas uplands varied from CH_4 sinks to CH_4 sources. The latest research confirmed that warming in the Arctic had become more apparent in the non-growing season than in the typical growing season (Bao et al., 2020). Hereby, Bao et al. (2020) found that the CH_4 emissions during the spring thaw and the autumn freeze contributed approximately one-quarter of the annual total CH_4 emissions. That experimental warming is stimulating soil CH_4 uptake in the growing season implies that the grasslands of the Bayinbuluk may have the potential to remove more CH_4 from the atmosphere under future global warming conditions.

Furthermore, with the increased variation in soil temperature, the response rate of N_2O emission gradually decreased under AW treatment (Figure 3 g, $P < 0.05$). The response of N_2O emission to temperature increase was limited by the warming that occurred throughout the year. However, N_2O emission peaks were displayed during the freeze–thaw periods (e.g., May 2017, June 2018 and April 2019). Warming increased N_2O emissions in the thawing period due to disruption of the gas diffusion barrier and greater C and N availability for microbial activity (Nyborg et al., 1997). Wagner-Riddle et al. (2017) also demonstrated that the magnitude of the freeze/thaw-induced N_2O emissions was associated with the number of days with soil temperatures below 0°C. Pärn et al. (2018) found that N_2O emission from organic soils increases with rising soil NO_3^-, follows a bell-shaped distribution with soil moisture. Another study has shown that a whole-year warming treatment significantly increased N_2O emissions, but daytime, night-time or short-season warming did not have significant effects (Li et al., 2020). In addition, Cantarel et al. (2010) suggested that the N_2O flux from cool and upland grasslands may be driven primarily by response to elevated temperature under projected future climate conditions.

5. Conclusions

In summary, the effect of seasonally asymmetrical warming on *Re* and N_2O emission was obvious, unlike the situation with CH_4 uptake. The *Re* and N_2O emission were able to adapt to continuous warming, resulting in a reduced response rates of the *Re* and N_2O emission to temperature increase. Warming in the non-growing season increased the temperature dependence of the *Re*. Thus, we believe that the study of climate change should pay greater attention to warming in the non-growing season, to avoid underestimating the greenhouse effect on *Re* in alpine grasslands.

Data availability

The measured CO_2, CH_4 and N_2O fluxes and soil temperature and soil water content data are available in Zenodo (http://doi.org/10.5281/zenodo.4244207).

Author contributions

GYM, LYY and MA conceive the research question, designed the study approach, led the field survey, ensured data curation and conducted formal analysis. YP and LKH assisted with data collection and analysis. GYM wrote the first draft of the paper, and all co-authors contributed to writing review and editing.

Competing interests

The authors declare that they have no conflicts of interest.

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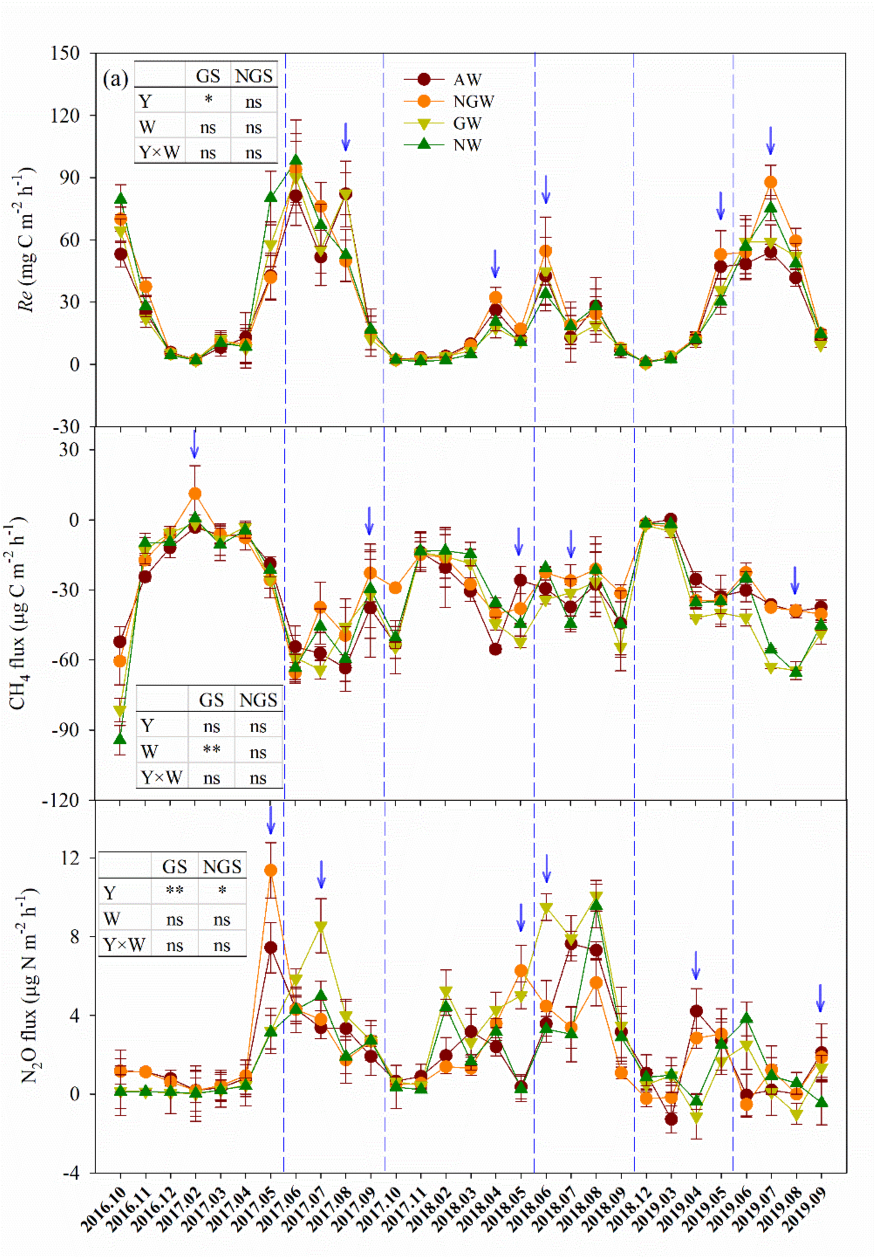


	GS	NGS
Y	*	ns
W	ns	ns
Y×W	ns	ns

	GS	NGS
Y	ns	ns
W	**	ns
Y×W	ns	ns

	GS	NGS
Y	**	*
W	ns	ns
Y×W	ns	ns

Figure 1 Monthly variation of a). ecosystem respiration (*Re*), b). CH$_4$ uptake and c). N$_2$O emissions under the four treatments from October 2016 to September 2019. AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming. The blue arrows indicate warming effects. The data points represent mean ± standard error, SE. The tables illustrate the tests of significance for year (Y) and warming (W) on *Re*, CH$_4$ uptake and N$_2$O emission by two-way repeated-measures analysis of variance (ANOVA) in

the growing season (GS) and the non-growing season (NGS), respectively; $^{*}P < 0.05$; $^{**}P < 0.01$; ns, non-significant.

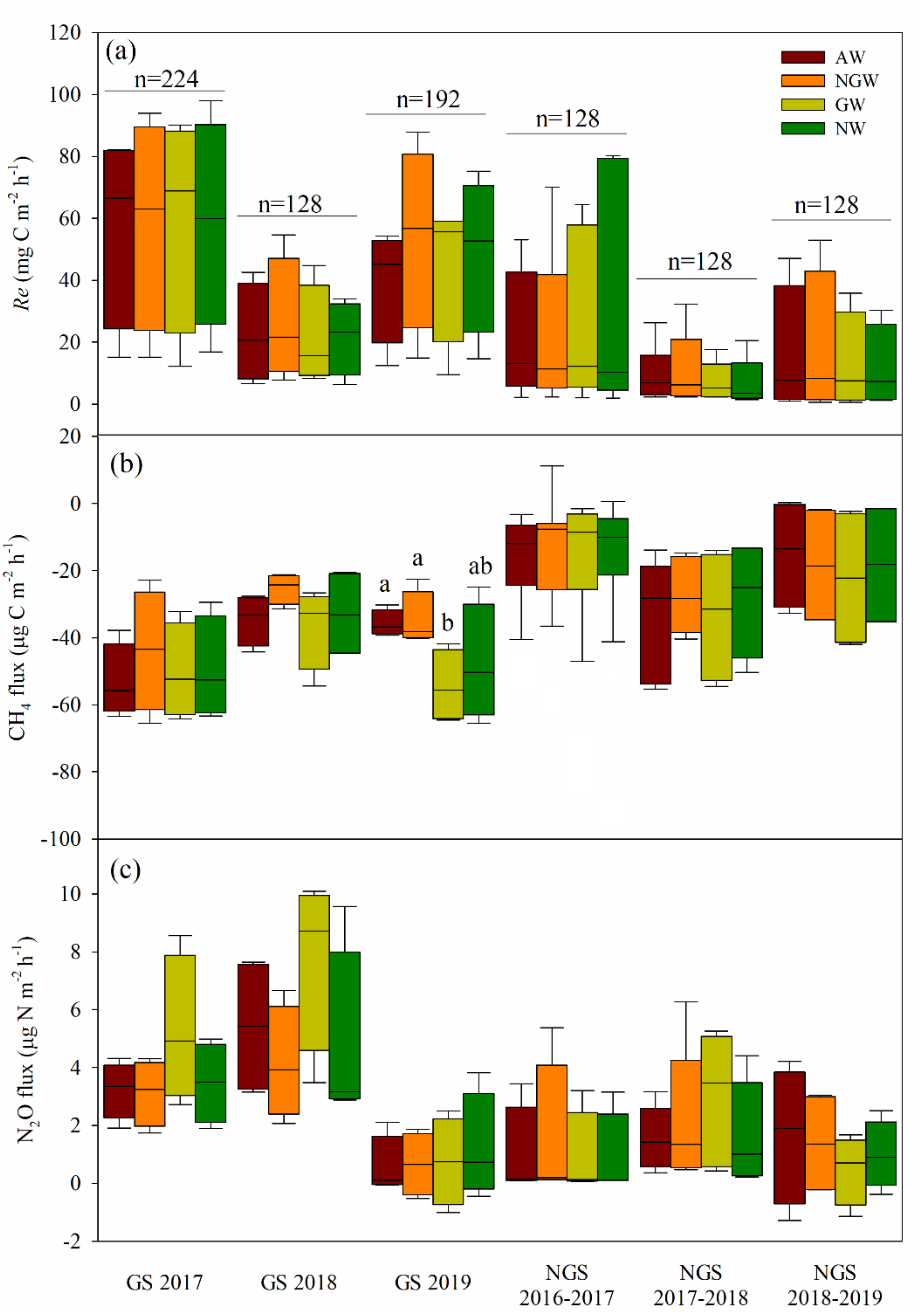


Figure 2 Boxplot presentation of variations in ecosystem respiration (*Re*), CH_4 uptake, and N_2O emission under four treatments in the growing season and non-growing season from October 2016 to September 2019. The median is represented by the black line in the box. The box (the interquartile range) represents

the middle 50% of the data, whereas the whiskers represent the ranges for the bottom 25% and the top 25% of the data values, excluding outliers. GS, growing season; NGS, non-growing season; AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming. No significant differences among AW, NGW, GW, and NW were reported from ANOVA; data points are the mean ± standard error. One-way ANOVA results of *Re*, CH_4 uptake and N_2O emissions among the four warming treatments were not significant, except that the CH_4 uptake in the GS 2019 under the GW treatment was significantly higher than that of AW and NGW treatment ($P < 0.05$).

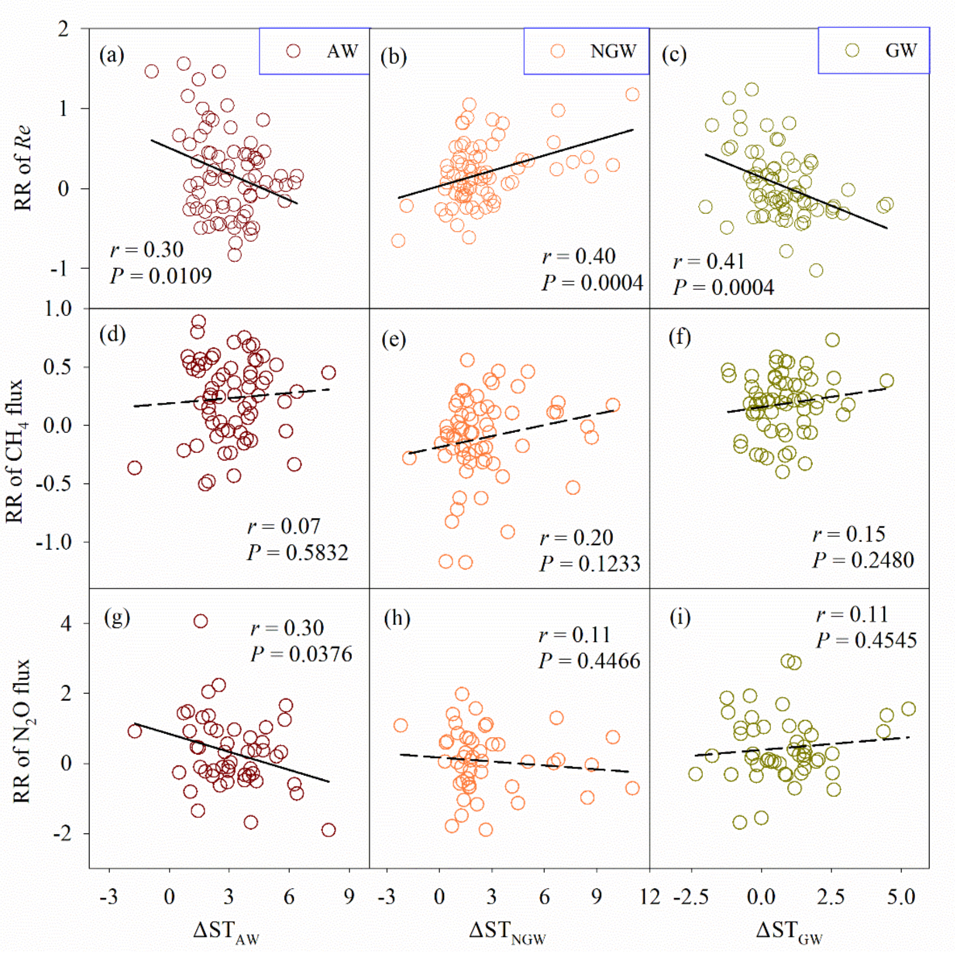
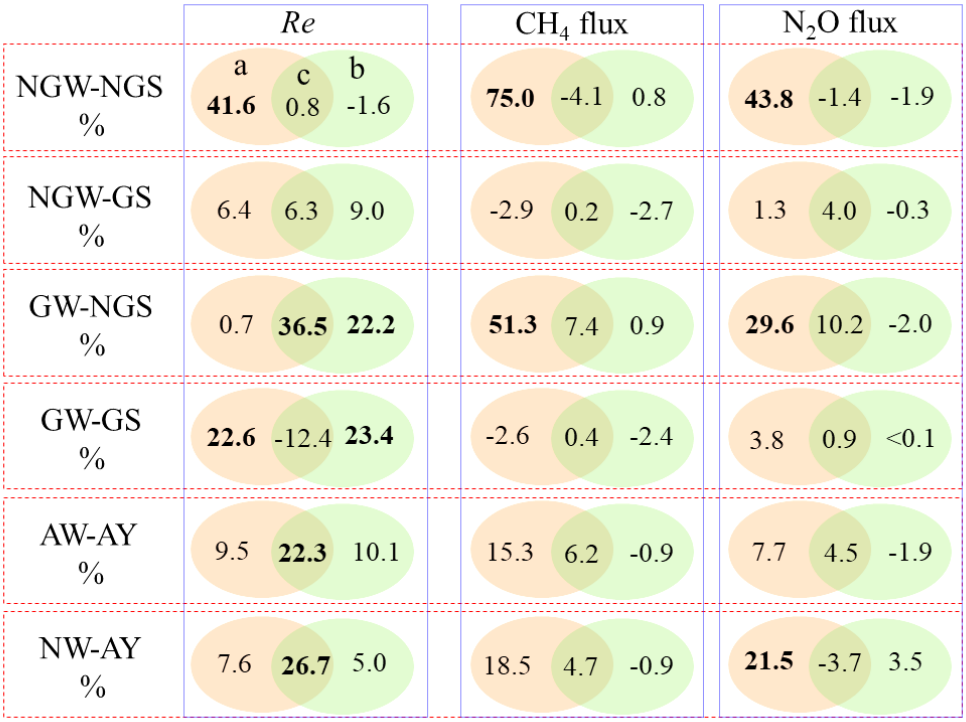


Figure 3 Response (presented by linear correlation) of variation in ecosystem respiration (*Re*), CH_4 uptake, and N_2O emission to changes in soil temperature under

AW, NGW and GW conditions in the alpine grassland, from 2016 to 2019. RR, the natural logarithm of the response ratio of the mean value of the chosen variable in the warming group to that in the control (NW) group. ΔST_{AW}, soil temperature of AW minus that of NW; ΔST_{CW}, soil temperature of NGW minus that of NW; ΔST_{WW}, soil temperature of GW minus that of NW; AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming.



	Re			CH_4 flux			N_2O flux		
	a	c	b						
NGW-NGS %	**41.6**	0.8	-1.6	**75.0**	-4.1	0.8	**43.8**	-1.4	-1.9
NGW-GS %	6.4	6.3	9.0	-2.9	0.2	-2.7	1.3	4.0	-0.3
GW-NGS %	0.7	**36.5**	**22.2**	**51.3**	7.4	0.9	**29.6**	10.2	-2.0
GW-GS %	**22.6**	-12.4	**23.4**	-2.6	0.4	-2.4	3.8	0.9	<0.1
AW-AY %	9.5	**22.3**	10.1	15.3	6.2	-0.9	7.7	4.5	-1.9
NW-AY %	7.6	**26.7**	5.0	18.5	4.7	-0.9	**21.5**	-3.7	3.5

Figure 4 Influence of soil temperature and soil moisture on ecosystem respiration (*Re*), CH_4 uptake, and N_2O emission by variation-partitioning analysis under four treatments in the growing season and non-growing season. a, Single effect of soil temperature (%); b, single effect of soil moisture (%); c, joint effects of soil temperature and moisture (%); NGW-NGS, greenhouse gas fluxes in non-growing season under non-growing season warming treatment; NGW-GS, greenhouse gas

fluxes in growing season under non-growing season warming treatment; GW-NGS, greenhouse gas fluxes in non-growing season under growing season warming treatment; GW-GS, greenhouse gas fluxes in growing season under growing season warming treatment; AW-AY, annual greenhouse gas fluxes under annual warming treatment; NW-AY, annual greenhouse gas fluxes without warming.