

## A point-by-point reply

Dear Associate Editor, Anonymous Referee #1 and #3:

Thank you for your comments and the reviewers' comments concerning our manuscript entitled " Different responses of ecosystem respiration, CH<sub>4</sub> uptake, and N<sub>2</sub>O emissions to seasonally asymmetric warming in an alpine grassland of the Tianshan Mountains " (MS No.: bg-2020-396). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied those comments carefully and have made a point to point reply and correction. Revised portions are marked in red color in this manuscript. Specific corrections and responses to the Referee's comments are listed as follows:

### General comments

The manuscript titled "Different responses of ecosystem respiration, CH<sub>4</sub> uptake, and N<sub>2</sub>O emissions to seasonally asymmetric warming in an alpine grassland of the Tianshan Mountains" by Gong et al. talks about the responses of the three GHG fluxes viz., CO<sub>2</sub> (ecosystem CO<sub>2</sub> efflux), CH<sub>4</sub> and N<sub>2</sub>O to different seasonal (growing and non-growing season) and annual experimental warming across 3 years in an alpine grassland on southern Tianshan mountain.

The manuscript investigates an important question with a strategic experimental design and intensive data collection. Most questions raised so far have been answered and revisions made are acceptable. In spite of this the manuscript in the current state has significant drawbacks due to lack of information at some places. At other places information is given without providing the context for the same.

The manuscript requires further revision and the general concerns are given below:

- Lack of information in methodology regarding OTC installation strategy, selection of sampling time of GHG fluxes, microclimatic parameters measured and few data analyses.

**Response:** Thank you for your precise comment. We supplemented the information in methodology regarding OTC installation strategy, see line no. 126 and 127 "After the warming in the NGW or GW, the tempered glass was removed and the frame was retained." Samples were taken once or twice a week, See line no. 143. Microclimate parameters were provided in the appendix, as shown in Figure S1-S3. "Soil temperature and air temperature were increased about 2.3 °C and 4 °C by the warming treatment, respectively (Figure S1 and S3). Soil moisture was reduced about 5 % by the warming treatment (Figure S2)." See line no. 132-134.

- The discussion does not flow from results and at places results are written in discussion section (example line no. 251-260). The discussion section focuses on response rates (RR) of GHG fluxes but the results section does not mention RR.

**Response:** Thank you for your precise comment. This section (line no. 251-260) has been moved to the results, see line no. 197-208. Response rates (RR) of GHG fluxes are mainly used to reveal the effect of temperature change on GHG flux. It is more valuable to clarify the relationship between temperature change and the GHG flux change in the Discussion. In response to the previous comment, we simply analyzed the microclimate variation in our experimental method, so we did not mention the RR value of seasonal asymmetric warming in our results.

- It is suggested to compute  $Q_{10}$  which is a direct and widely used parameter to assess temperature sensitivity (see Zhou et al., 2016 “Experimental warming of a mountain tundra increases soil CO<sub>2</sub> effluxes and enhances CH<sub>4</sub> and N<sub>2</sub>O uptake at Changbai Mountain, China”).

**Response:** Thank you for your precise comment. In future research work, we will pay more attention to the calculation of  $Q_{10}$  to evaluate the temperature sensitivity of greenhouse gas flux. Thanks again.

- The magnitude of temperature increase (both air and soil) inside open-top chambers should be mentioned. The study is based on the premise of significant warming within the OTC; however, figures indicate otherwise. The air temperature did not significantly increase during non-growing season in any of the plots (GW, NGW and AW) whereas the soil temperature did not significantly increase in any of the season (both growing and non-growing) and plots in the entire 3-year study. Though non-significant, there is an increasing trend in temperatures inside OTCs which should be discussed.

**Response:** Thank you for your precise comment. Through three years of experimental monitoring, it is found that open-top warming significantly increases the air temperature in growing season ( $P < 0.05$ , Figure S3 a); The air temperature increases in non-growing season, but not significantly, and the temperature increase range is about 4 °C in general. Compared with Non-warming, annual warming, warming in non-growing season only, and warming in growing season only all achieved warming effects (Figure S3 b). Similarly, soil temperature was changed by different warming treatments, but the increase rate was lower than that of air temperature. In both the growing season and non-growing season, the increase rate was between 1.5 °C - 3 °C, with no significant change. However, the warming effect required by the study was

achieved (Figure S1).

- Findings indicate strong influence of moisture on the GHG fluxes and should be discussed. Both  $R_e$  (during growing season) and  $N_2O$  uptake varied interannually, coinciding with the variations in moisture. The study area is comparatively drier in comparison to other alpine grasslands of the world hence moisture is likely to be a limiting factor. Moisture reduction inside OTCs can have significant influence on microbial enzyme activities and eventually on uptake and emission of GHGs.

Response: Thank you for your precise comment. General linear analysis was carried out between soil moisture and GHG fluxes, Figure S6 was drawn and added to the Appendix. And the relevant sentences are added as “General linear analyses were used to identify significant linear correlations and regressions between soil temperature and moisture variations and the responses of  $R_e$ ,  $CH_4$  uptake, or  $N_2O$  emissions, respectively.” See line no. 164-170.

“However,  $R_e$ ,  $CH_4$  uptake and  $N_2O$  emission were no significant linearly correlated with soil moisture, respectively ( $P \geq 0.05$ ; Figure S6).” See line no. 217, 218.

In future studies, we will focus on the effect of soil moisture reduction caused by warming on microbial enzyme activities and the consequent change of greenhouse gas fluxes.

## Section wise comments for major revision

### Introduction

1. The hypothesis does not directly relate to the objectives or the results of the work as the study focuses on seasonally asymmetric warming and continuous measurement of  $R_e$  and  $CH_4$ - $N_2O$  fluxes over 3-years.

Response: Thank you for your precise comment for the Introduction. We have revised this sentence as “we hypothesize the stimulatory effect of warming during the non-growing season on  $R_e$ ,  $CH_4$  uptake and  $N_2O$  emissions, the response rates of  $R_e$ ,  $CH_4$  uptake and  $N_2O$  emissions were gradually attenuated by long-term annual warming and warming over the growing season, respectively.” See line no. 97-100.

### Methodology

2. When were the OTCs installed or removed? Please clarify? For example, “for continuous annual warming OTCs remained installed since the beginning of the study while for growing season warming, these were installed at the onset of growing season

and removed at the end of growing season....”

Response: Thank you for your precise comment. For the installation and removal of OTCs, we briefly explained in the Methodology, see line no. 132-136.

3. Why was the sampling performed only between 12:00 and 14:00 (GMT + 8) every day (line no. 133-134)? Was this time standardisation based on time interval coinciding with mean of diurnal (over 24 hrs) flux rates?

Response: Thank you for your precise comment. Yes, the sampling performed between 12:00 and 14:00 (GMT + 8) every day which based on time interval coinciding with mean of diurnal (over 24 hrs) flux rates. The diurnal variation of greenhouse gases flux rates is shown in the figures below:

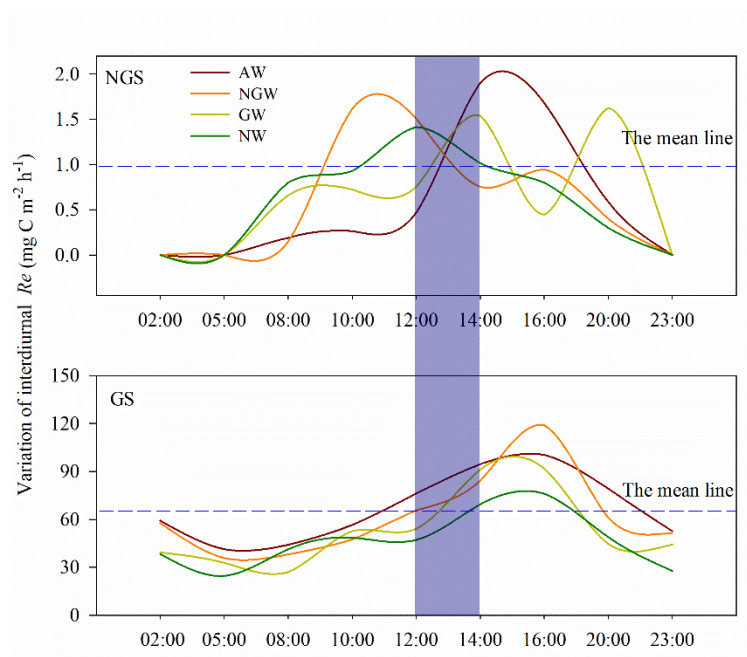


Figure R1 Diurnal variation of CO<sub>2</sub> flux in the non-growing season (NGS) and growing season (GS). AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming.

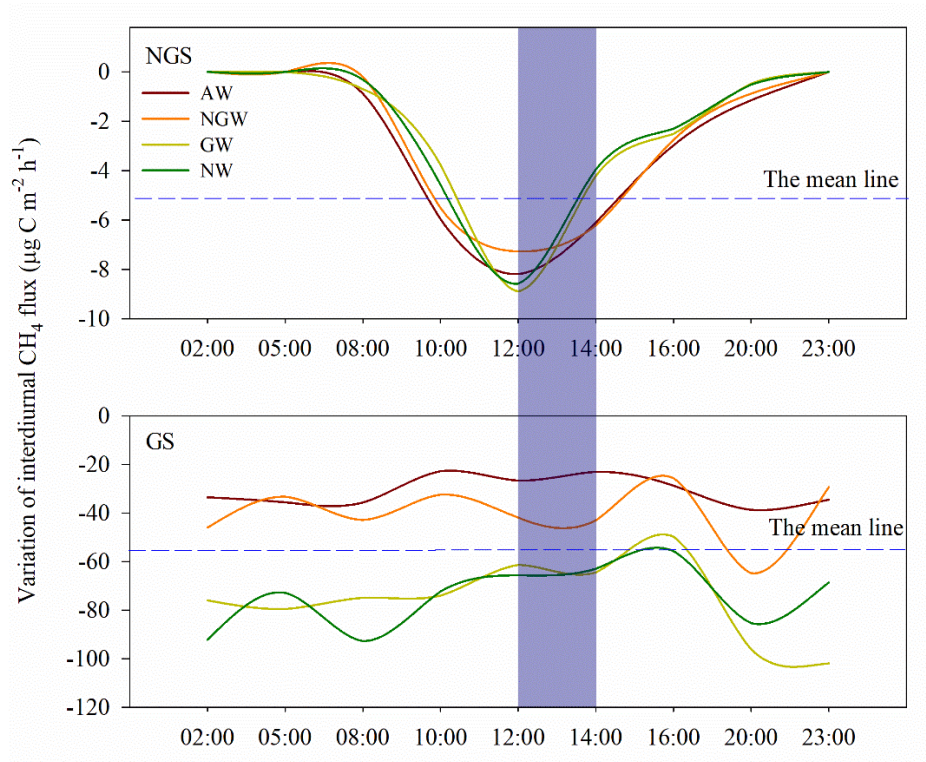


Figure R2 Diurnal variation of CH<sub>4</sub> flux in the non-growing season (NGS) and growing season (GS). AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming.

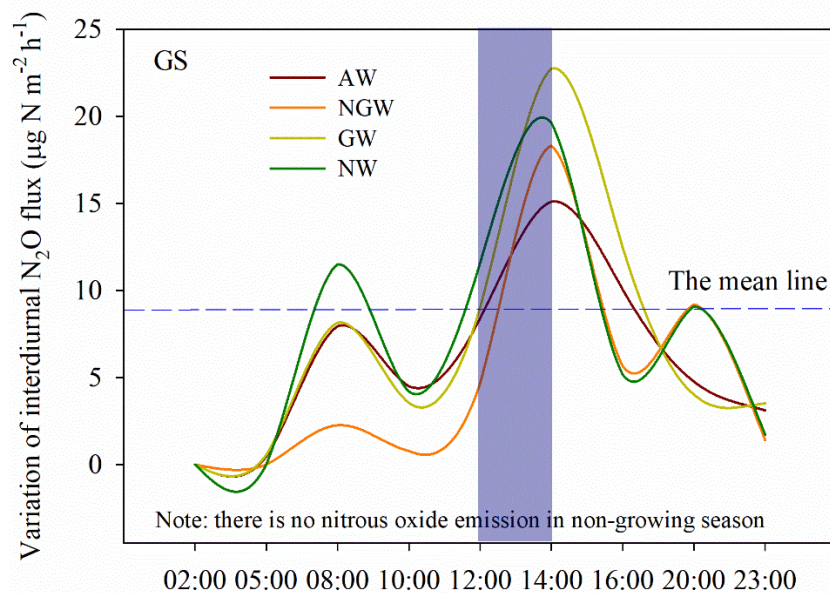


Figure R3 Diurnal variation of N<sub>2</sub>O flux in the growing season (GS). AW, warming throughout the year; NGW, warming in the non-growing season only; GW, warming in the growing season only; NW, non-warming.

4. Line no. 116 states that all the plots were ungrazed since 2005, how was this achieved?  
I assume the plots or the entire site was fenced. Please clarify?

Response: Thank you for your precise comment. We revised the sentence as “The site was fenced since 2005,…” See line no. 125.

5. Measurement of soil temperature and soil moisture at 10 cm depth by data loggers were made at what frequency? hourly or daily? How air temperature was measured or recorded inside all the 4 experimental plots and at what height?

Response: Thank you for your precise comment. Soil temperature and moisture (10cm) were measured at a frequency of every half an hour. The air temperature and humidity 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 OCTs, 50cm above the surface). We revised this sentence. See line no. 138-146.

6. Line no. 134 states that the gas samples were collected every day while line no. 139, in contrast, states that they were collected once or twice a week. Clarify.

Response: Thank you for your precise comment. We revised this sentence as “..., collecting once or twice a week.” See line no. 153,154.

7. Line no. 138-139 states that “A total of 232 samples were taken, collecting once or twice a week” however figure 2 shows that  $n = 232$  only for the growing season of 2017 whereas  $n = 192$  for GS 2018 and  $n = 128$  for others. Kindly correct.

Response: Thank you for your precise comment. We deleted the sentence because it was inaccurate and superfluous.

8. One-way ANOVA was performed to compare only soil temperature (line no. 144). As Figures S2 and S3 indicate that you performed ANOVA for soil moisture and air temperature also, correct your statement.

Response: Thank you for your precise comment. We revised this sentence as “One-way ANOVA was used to compare soil temperature, soil moisture and air temperature differences, respectively.” See line no. 163, 164.

9. General linear analysis was carried out between soil temperature and GHG fluxes only. The same analysis could be repeated for soil moisture also.

Response: Thank you for your precise comment. General linear analysis was carried out between soil moisture and GHG fluxes, Figure S6 was drawn and added to the Appendix. And the relevant sentences are added as “General linear analyses were

used to identify significant linear correlations and regressions between soil temperature and moisture variations and the responses of  $Re$ ,  $CH_4$  uptake, or  $N_2O$  emissions, respectively.” See line no. 164-170.

“However,  $Re$ ,  $CH_4$  uptake and  $N_2O$  emission were no significant linearly correlated with soil moisture, respectively ( $P \geq 0.05$ ; Figure S6).” See line no. 217, 218.

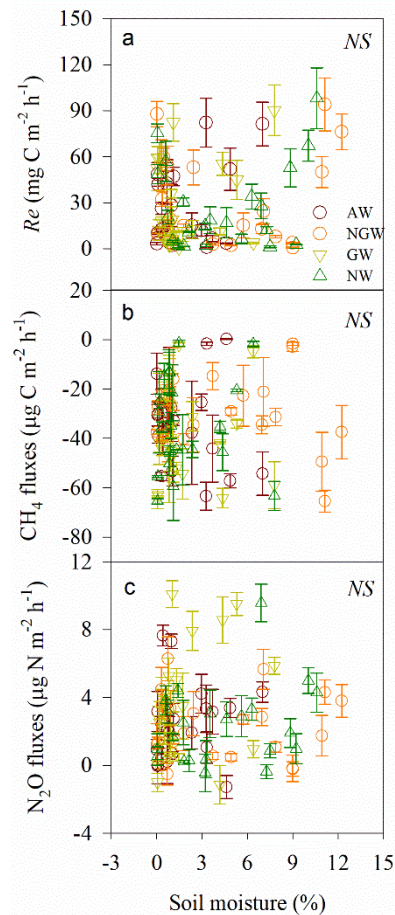


Figure S6 The relationship between ecosystem respiration ( $Re$ ),  $CH_4$  uptake and  $N_2O$  emissions and soil moisture (at 10-cm depth) from October 2016 to September 2019. AW, warming throughout the year; NGW, warming in the nongrowing season only; GW, warming in the growing season only; NW, non-warming.

10. Use of variation partitioning analysis in figure 4 should be mentioned under methodology.

Response: Thank you for your precise comment. We added the sentence to our methodology, “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.” See line no. 170-172.

## Results

11. Results of all the GHG fluxes under warming have been given in terms of increase or decrease however, the ANOVA results do not show significant difference, which 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” add “however non-significant” Alternatively, write line no. 175-179 (stating ANOVA results) before line no. 160, so as to report in the beginning only, that the differences were not significant.

Response: Thank you for your precise comment for the Results. We put the sentence of line no. 175-179 before line no. 160. See line no. 186-189.

12. Line no. 172: increase in N<sub>2</sub>O emission by 101.9% and 192.3% under AW and NGW in the growing season seems very high. Please check.

Response: Thank you for your precise comment. We checked and recalculated the data to make sure it was correct. Refer to the data in Table R1. To view the detailed data set, please visit the website: <http://doi.org/10.5281/zenodo.4244207>

Table R1 N<sub>2</sub>O emissions in NGS 2016-2017, NGS 2017-2018 and NGS 2018-2019. AW, warming throughout the year; NGW, warming in the nongrowing season only; GW, warming in the growing season only; NW, non-warming (Control group).

	N <sub>2</sub> O flux ( $\mu\text{g N m}^{-2} \text{ h}^{-1}$ )			
	AW	NGW	GW	NW
NGS 2016-2017	1.12	1.70	0.51	0.50
NGS 2017-2018	1.57	2.26	0.64	0.68
NGS 2018-2019	1.68	2.37	0.49	0.99
Mean	1.46	2.11	0.55	0.72
SD	0.30	0.36	0.09	0.25
Percentage	101.9%	192.3%		
	(AW-NW)/NW	(NGW-NW)/NW		

13. The authors may fit an exponential curve to determine the relationship between *Re* and soil temperature at 10 cm depth. Figure S5 a indicate towards an exponential pattern.

Response: Thank you for your precise comment. As your suggested, an exponential curve is more appropriate than the linear fitting to determine the relationship between *Re* and soil temperature at 10 cm depth. Through the value of the exponential function, we can well judge the response difference of *Re* with temperature increase under different warming treatments. We revised the Figure S5 a as an exponential pattern. And the related sentences in the manuscript were revised simultaneously. See line no. 164-170 “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.” See line no.223-225 “Under the four warming treatments, *Re* was significantly exponential growth



correlated with soil temperature ( $P < 0.05$ ; Figure S5 a).”

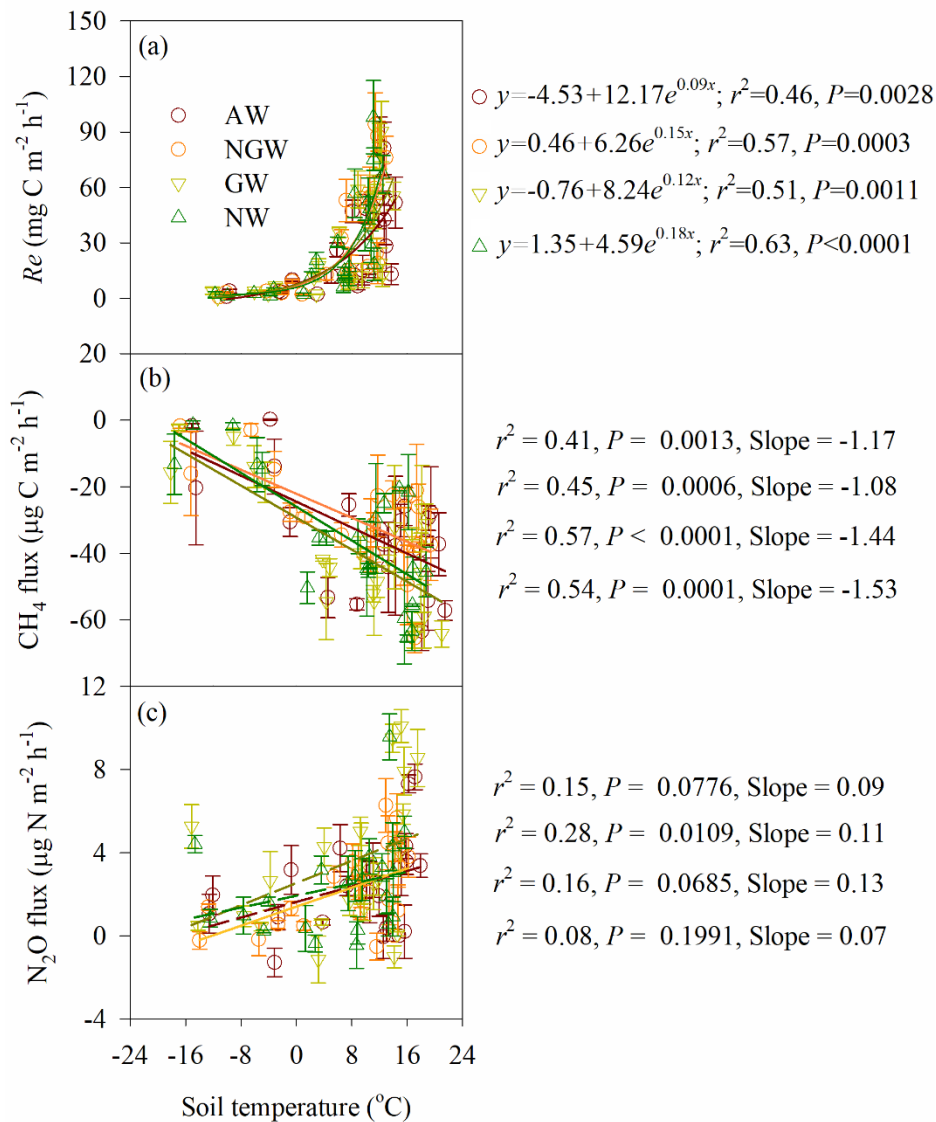


Figure S5 The relationship between ecosystem respiration ( $Re$ ),  $CH_4$  uptake and  $N_2O$  emissions and soil temperature (at 10-cm depth) from October 2016 to September 2019. AW, warming throughout the year; NGW, warming in the nongrowing season only; GW, warming in the growing season only; NW, non-warming.

## Discussion

14. Results should report response rates (only given in discussion section). The low  $r^2$  value of linear regression in Figure 3 (where significant) should be discussed.

Response: Thank you for your precise comment. Response rates (RR) of GHG fluxes are mainly used to reveal the effect of temperature change on GHG flux. It is more valuable to clarify the relationship between temperature change and the GHG flux change in the Discussion. In response to the previous comment, we simply analyzed the microclimate variation in our experimental method, so we did not mention the RR value of seasonal asymmetric warming in our results.

The  $r^2$  value of linear regression in Figure 3 was low, but the correlation was significant (Figure 3 a, b, c, and g). It is not appropriate to use the determination coefficient ( $r^2$ ) in the Figure 3, so we revised the Figure 3 and used the value of correlation coefficient ( $r$ ) to represent the correlational relationship between soil temperature change and GHG flux response rate (RR).

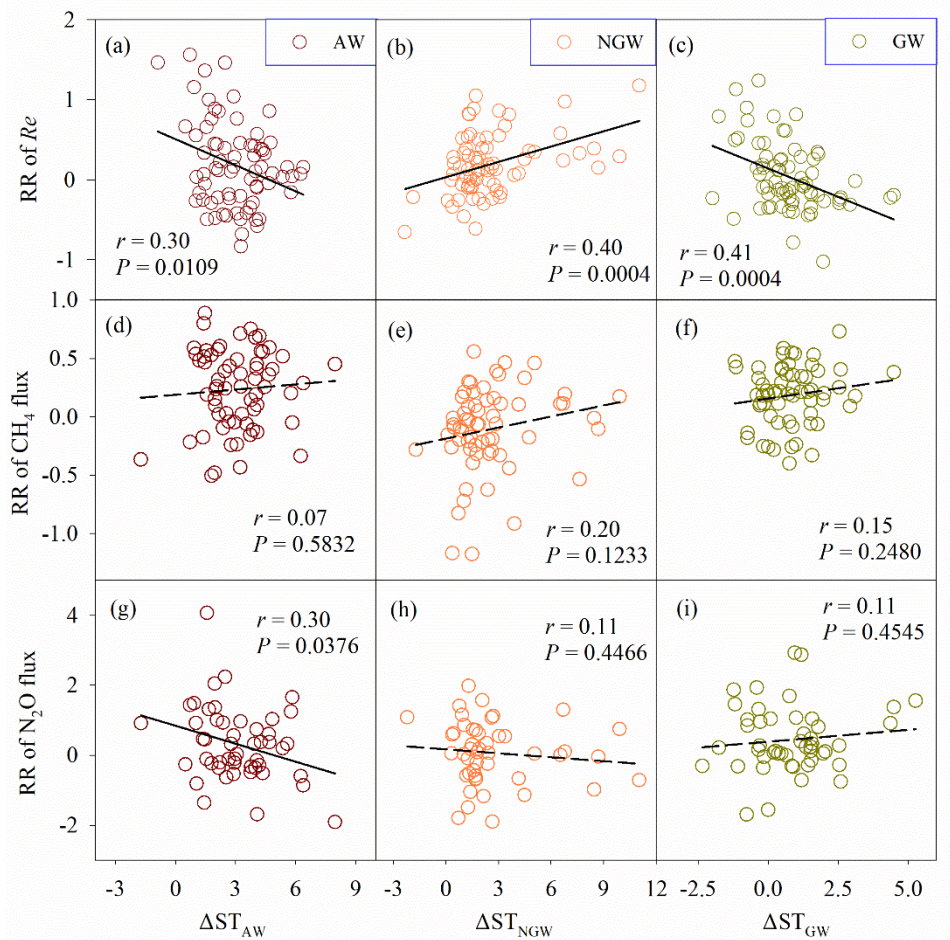


Figure 3 Response (presented by linear regression) of variation in ecosystem respiration ( $Re$ ),  $CH_4$  uptake, and  $N_2O$  emission to changes in soil temperature under AW, NGW and GW conditions in the alpine grassland, from 2016 to 2019. RR, the natural logarithm of the response ratio of the mean value of the chosen variable in the warming group to that in the control (NW) group.  $\Delta ST_{AW}$ , soil temperature of AW minus that of NW;  $\Delta ST_{CW}$ , soil temperature of NGW minus that of NW;  $\Delta ST_{WW}$ , 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.

interpretation. This analysis should be mentioned in methodology and in result section.

Response: Thank you for your precise comment. This section (line no. 251-260) has been moved to the results, see line no. 197-208.

### **Minor edits**

Line 1: Although it is known that ecosystem respiration means CO<sub>2</sub> emissions, why not specify CO<sub>2</sub> instead for coining “respiration” as done for other gases (as done in the previous draft). This will avoid ambiguity in title. for example, “Different responses of ecosystem CO<sub>2</sub> and N<sub>2</sub>O emissions and CH<sub>4</sub> uptake to seasonally asymmetric warming in an alpine grassland of the Tianshan Mountains” Also delete comma after CH<sub>4</sub> uptake.

Response: Thank you for your precise comment. We revised the Title as “Different responses of ecosystem CO<sub>2</sub> and N<sub>2</sub>O emissions and CH<sub>4</sub> uptake to seasonally asymmetric warming in an alpine grassland of the Tianshan Mountains” See line no. 1.

Line 16-19: specify percentage increase for each GHG flux.

Response: Thank you for your precise comment. We revised the sentence as “Furthermore, warming during the non-growing season increased *R<sub>e</sub>* and CH<sub>4</sub> uptake by 7.9% and 10.6% in growing season, 10.5% and 9.2% in non-growing season, respectively. However, the increase in N<sub>2</sub>O 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<sub>2</sub>O emissions by 101.9% and 192.3% in non-growing seasons, respectively.” See line no. 17-22.

Line no. 26: remove comma after annual warming.

Response: Thank you for your precise comment. We removed comma after annual warming. See line no. 32.

Line no. 31: (i) Write greenhouse gas fluxes instead of flux, (ii) as the manuscript doesn't include temperature sensitivity as objective and it has not been calculated, it is not logical to use it as a keyword.

Response: Thank you for your precise comment. we revised the Keywords as “Alpine steppe; Extreme climatic event; Greenhouse gas fluxes; Warming of open-top chambers” See line no. 37.

Line no. 35-37: Shorten the sentence as “The global surface temperature increased by about 0.85°C from 1880 to 2012 and is expected to increase by about 1.1–6.4°C by the end of this century (IPCC, 2007, 2013).

Response: Thank you for your precise comment. We shortened the sentence as “The global surface temperature increased by about 0.85°C from 1880 to 2012 (IPCC, 2013). Furthermore, the temperature is expected to increase by about 1.1–6.4°C by the end of this century (IPCC, 2007, 2013).” See line no. 41, 42.

Line no. 38: remove comma after scale.

Response: Thank you for your precise comment. We removed the comma after scale. See line no. 44.

Line no. 52: The warming or the temperature sensitivity of the GFG fluxes have not been evaluated in the study and hence “and their sensitivity to warming” may be removed.

Response: Thank you for your precise comment. We removed “and their sensitivity to warming”. See line no. 62.

Line no. 60. Remove space between numeric and percentage sign. Follow this in the entire manuscript.

Response: Thank you for your precise comment. We removed space between numeric and percentage sign in the entire manuscript.

Line no. 62: what do you mean by CO<sub>2</sub> fluxes? Is it respiration (if yes is it soil or ecosystem) or photosynthesis or both? Consider this in Line no. 78 also.

Response: Thank you for your precise comment. We revised the “CO<sub>2</sub> fluxes”, “C fluxes” as “ecosystem respiration” See line no. 73, 74, 97, 98.

Line no. 63-67: as you are stating the result of specific study (Lin et al., 2015), it is better to start the sentence as “Lin et al. (2015) reported...”. Also give the percentage increase in CH<sub>4</sub> uptake under growing season also. Alternatively, you may add more references of the studies showing similar results, in this the percentage increase may be removed.

Response: Thank you for your precise comment. We revised the sentence as “Lin et al. (2015) reported...” See line no. 86.

Line no. 85: replace GHG flux with GHG fluxes. Follow this in Line no. 88, 98, 100.

Response: Thank you for your precise comment. We replaced “GHG flux” with “GHG

fluxes” in Line no. 88, 98, 100.

Line no. 111: add space between  $-4.8$  and  $^{\circ}\text{C}$ .

Response: Thank you for your precise comment. We added space between  $-4.8$  and  $^{\circ}\text{C}$ .

Line no. 157-159: Figure 1 does not show the annual mean values of each flux but the variations during each year and hence the reference to figure 1 in this sentence is redundant.

Response: Thank you for your precise comment. We deleted the reference to figure 1 in this sentence.

### Suggestions

1. Microclimatic parameters such as air and soil temperature and soil moisture are important to understand variations in seasonal, inter-annual and the asymmetric warming effect on GHG fluxes. Hence these should be included in the main text and their methodology and results should be stated and used while interpreting warming or inter-annual effects on GHG fluxes.

Response: Thank you for your suggestion. In our manuscript, we have paid attention to the influence of soil temperature and humidity changes on greenhouse gas fluxes, and we will analyze these good suggestions you mentioned in a more delicate way in the later research.

2. Calculate  $Q_{10}$  values (at least for ecosystem respiration). This will give you a more direct indication of temperature sensitivity changes with warming. As most studies use this approach, it will be useful for comparison.

Response: Thank you for your precise comment. Taking into account methane absorption and nitrous oxide emissions, we used RR instead of  $Q_{10}$ . Your suggestion is very good.  $Q_{10}$  is calculated in our future study on the sensitivity of ecosystem respiration to temperature caused by warming.

3. In Figure 2, it is suggested to add boxes for mean (entire study period) of each flux rates during growing and non-growing season under four treatments along with ANOVA results (as letters).

Response: Thank you for your precise comment. We revised the Figure 2 and add boxes for mean (red line) under four treatments along with ANOVA results (as letters).

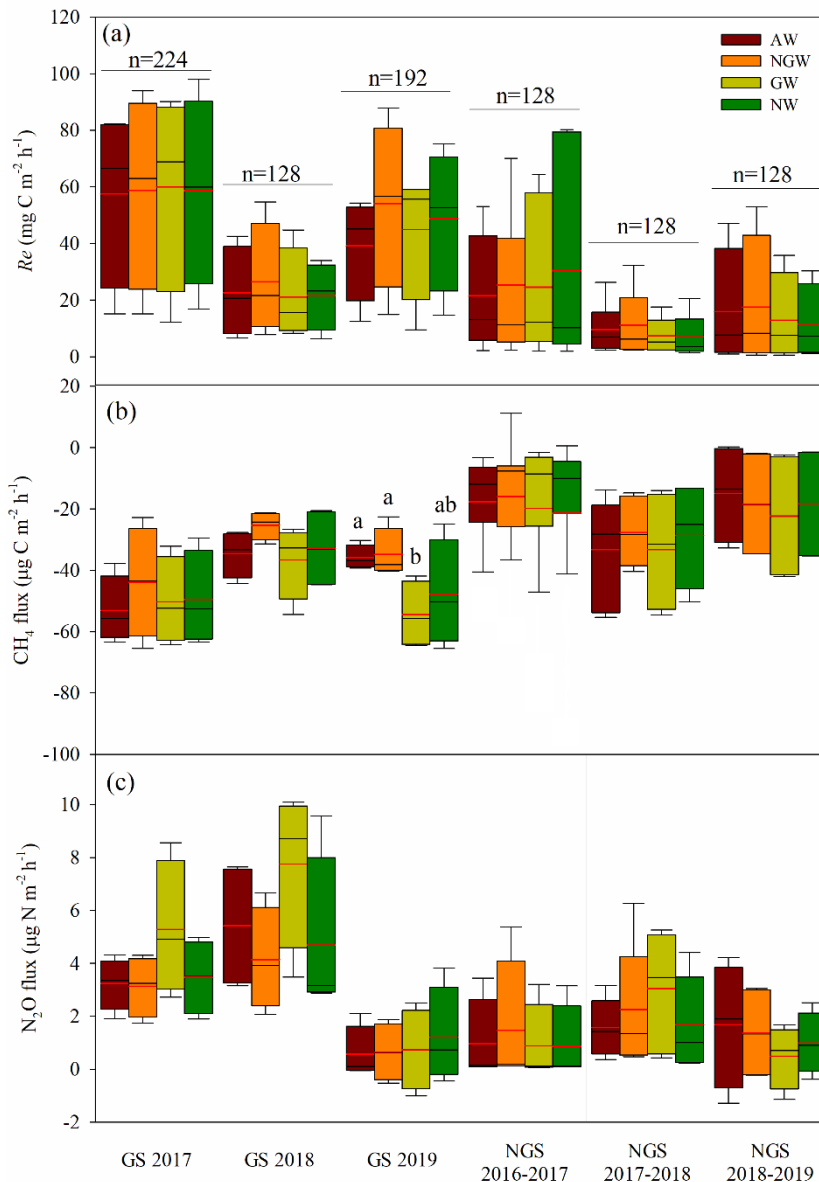


Figure 2 Boxplot presentation of variations in ecosystem respiration ( $R_e$ ),  $CH_4$  uptake, and  $N_2O$  emission under four treatments in the growing season and non-growing season from October 2016 to September 2019. The median and mean are represented by the black and red lines in the box, respectively. 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  $\pm$  standard error. One-way ANOVA results of  $R_e$ ,  $CH_4$  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 ( $P < 0.05$ ).