



1 **Different responses of CO₂, CH₄, and N₂O fluxes to seasonally asymmetric**
2 **warming in an alpine grassland of Tianshan Mountains**

3 Yanming Gong¹, Ping Yue², Kaihui, Li¹, Anwar Mohammad^{1*}, Yanyan Liu^{1*}

4 ¹State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and
5 Geography, CAS, Urumqi, 830011, China

6 ²Urat Desert-Grassland Research Station, Northwest Institute of Eco-Environment
7 and Resources, CAS, Lanzhou, 730000, China

8 *Correspondence: Anwar Mohammad and Yanyan Liu, Email: liuyany@ms.xjb.ac.cn

9 **Abstract:**

10 An experiment was conducted to investigate the effect of seasonally asymmetric
11 warming on CO₂, CH₄, and N₂O fluxes in alpine grassland of Tianshan Mountains of
12 Central Asia, from October 2016 to September 2019. Our results indicated that the
13 CO₂, CH₄ and N₂O fluxes varied in the range 0.56–98.03 mg C m⁻² h⁻¹, -94.30–0.23
14 μg C m⁻² h⁻¹, and -1.28–10.09 μg N m⁻² h⁻¹, respectively. The CO₂ and N₂O fluxes
15 were negatively correlated with soil temperature, but the CH₄ fluxes decreased with
16 the increase in temperature. Furthermore, the variation in greenhouse gas flux under
17 seasonally asymmetric warming was different between the growing season (June to
18 September) and the non-growing season (October to May). In addition, the response
19 rates of CO₂ and N₂O fluxes to temperature increases was significantly reduced due to
20 warming throughout the year. Warming during the growing season led to a significant
21 decrease in the response rate of CO₂ flux to temperature increases. However, warming
22 during the non-growing season caused a significant increase in the response rate of



23 CO₂ flux to temperature increases. The response rate of CH₄ flux was insensitive to
24 temperature increase under seasonally asymmetric warming. Thus, the main finding
25 of our results was that seasonally asymmetric warming resulted in different responses
26 in the fluxes of individual greenhouse gases to rising temperatures in the alpine
27 grassland.

28 **Keywords:** Greenhouse gas flux; Extreme climatic event; Temperature
29 sensitivity; Warming of open-top chamber

30 **1. Introduction**

31 Since the industrial revolution, human activities have intensified global warming.
32 The global surface temperature increased by about 0.85°C from 1880 to 2012, and it
33 is expected that the surface temperature will increase by about 1.1–6.4°C by the end
34 of this century (IPCC, 2007; IPCC, 2013). Moreover, the fastest climate warming
35 occurs in the middle latitude of the northern hemisphere, with an average warming
36 rate greater than 0.4°C per decade (Ji et al., 2014). The rise in atmospheric
37 temperature is not continuous on the temporal scale, but there is asymmetrical
38 warming during the seasons and between daytime and nighttime (Peng et al., 2013;
39 Xia et al., 2014). The 3th assessment report of the Inter-Governmental Panel on
40 Climate Change (IPCC) proposed that, against the backdrop of global warming, the
41 temperature change will show that the warming amplitude in winter is greater than
42 that in summer, and the warming amplitude at high latitude is greater than that at low
43 latitude (IPCC, 2001). In addition, the 4th assessment report of IPCC further clarified
44 that the warming rate in winter is much higher than that in the summer, and confirmed



45 that the warming shows asymmetric trends on a seasonal scale (Easterling et al., 1997;
46 IPCC, 2007).

47 Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) in the
48 atmosphere are the three major greenhouse gases (GHGs) in the atmosphere that
49 directly cause global climate warming, and their contributions to global warming are
50 20 %, 60 %, and 6 % (IPCC, 2007; IPCC, 2013), respectively. At present, most
51 studies focus on the influence of annual warming on GHG flux in terrestrial
52 ecosystems (Li et al., 2011; Keenan et al., 2014; Yang et al., 2014). Nevertheless, data
53 on the influence of asymmetric warming on the GHG flux on a temporal scale is
54 scarce. A study of the Alaskan tundra found that the increase in summer temperature
55 significantly increased the CO₂ flux in the growing season by about 20% (Natali et al.,
56 2011). Compared with the effect of increased temperature in winter on the CO₂ flux in
57 the growing season, the CO₂ flux in the nongrowing season increased by 50 % (Natali
58 et al., 2011). Studies have shown that the response of soil CH₄ absorption rates to
59 temperature increases in alpine meadows of the Qinghai-Tibet Plateau were not
60 consistent seasonally, with CH₄ absorption in the nongrowing season being more
61 sensitive to temperature (increasing by 162%) than the corresponding value in the
62 growing season (Lin et al., 2015). The study on the alpine grassland ecosystem
63 indicated that the response of N₂O flux to warming showed clear seasonal differences,
64 with the N₂O flux in the growing season showing significant differences between the
65 warming treatments, whereas the response of N₂O flux to the warming treatments in
66 November was not obvious (Cantarel et al., 2012).



67 In summary, the GHG flux in terrestrial ecosystems shows significant interannual,
68 seasonal and diurnal variations, and its response to warming also varies in different
69 temporal scales. After long-term uniform warming, the biotic and abiotic factors have
70 adapted to the temperature increase, and the GHG flux response to increasing
71 temperature is reduced, compared with that in the early stage of warming. Moreover,
72 climate warming is often unstable, most of it occurring as extreme events (Jentsch et
73 al., 2007). The heterogeneity of warming may change the adaptability of GHG fluxes
74 to warming, and thus affect the carbon and nitrogen cycles in terrestrial ecosystems.
75 Therefore, more uniform (continuous) heating experiments are carried out, and the
76 research results may underestimate the impact of global change on GHG emissions in
77 terrestrial ecosystems, making it impossible to accurately grasp the changed law of
78 GHG flux. Therefore, the current short communication will help to evaluate the
79 uncertainty with respect to GHG flux in response to increasing temperatures against
80 the backdrop of global climate change, by carrying out seasonally asymmetrical
81 warming studies in alpine grasslands.

82 **2. Materials and methods**

83 The experiment was conducted from October 2016 to September 2019 at the
84 Bayinbuluk Grassland Ecosystem Research Station, Chinese Academy of Sciences
85 (42°52.76' ~ 42°53.17' N, 83°41.90' ~ 83°43.12' E, 2460 m above sea level), which is
86 located in the southern Tianshan mountains of Central Asia, Xinjiang Uygur
87 Autonomous Region of China. The mean annual temperature was -4.8°C per decade,
88 with the lowest monthly temperature in January (-27.4 °C) and the highest in July



89 (11.2 °C), and the annual precipitation amounted to 265.7 mm, with 78.1% occurring
90 during the growing season, from June to September (Geng et al., 2019). Variations in
91 soil temperature are shown in Fig. S1. Ungrazed since 2005, all the plots were
92 dominated by *Stipa purpurea*, *Festuca ovina*, *Oxytropis glabra*, and *Potentilla*
93 *multifida*. The soil was subalpine steppe soil, and the parent material of the soil was
94 Loess.

95 There were four simulated treatments using open-top chambers: warming
96 throughout the year (AW), warming in the nongrowing season (October to May) only
97 (NGW), warming in the growing season (June to September) only (GW) and no
98 warming (NW). Three replicate plots were established for each treatment, each plot
99 measuring 1 m × 1 m, with a 3-m wide buffer zone between adjacent plots, making a
100 total of 12 plots.

101 CO₂, N₂O, and CH₄ fluxes were measured using static chambers that were made
102 of PVC tube with the dimensions of diameter 0.25 m and height 0.17 m in all 12 plots.
103 Gas samples were collected from the headspace of each static chamber at 0, 10, 20,
104 and 30 min after closing the chamber between 12:00 and 14:00 (GMT+8). Gas
105 samples were collected once or twice a week. Concentrations of individual gases in
106 samples were measured using a gas chromatograph (GC) (Agilent 7890A; Agilent
107 Technologies, Santa Clara, CA, USA). Fluxes were calculated according to Chen et al.
108 (2013).

109 Effects of seasonally asymmetric warming on CO₂, N₂O, and CH₄ fluxes were
110 analyzed by two-way repeated-measures analysis of variance (ANOVA). One-way



111 ANOVA was used to compare soil temperature differences. General linear analyses
112 were used to identify significant linear correlations and regressions between soil
113 temperature variation and responses of CO₂, CH₄, or N₂O fluxes. The natural
114 logarithm of the response ratio (RR) was used to reflect the effects of seasonally
115 asymmetric warming on alpine grassland GHG fluxes (Hedges et al., 1999). The RR
116 is the ratio of the mean value of the chosen variable in the warming group (\bar{W}_t) to
117 that in the control group (NW; \bar{W}_c), and is an index of the effect of seasonally
118 asymmetric warming on the corresponding variable (Eq. 1). All statistical analyses
119 were conducted using SPSS (version 20.0) (IBM, Armonk, NY, USA) with
120 statistically significant difference threshold set at $P < 0.05$.

$$121 \quad RR = \ln\left(\frac{\bar{W}_t}{\bar{W}_c}\right) = \ln(\bar{W}_t) - \ln(\bar{W}_c) \quad (1)$$

122 **3. Results**

123 Our study showed that the CO₂, CH₄, and N₂O fluxes in the alpine grassland
124 varied in the range of 0.56–98.03 mg C m⁻² h⁻¹, -94.30–0.23 μg C m⁻² h⁻¹, and -1.28–
125 10.09 μg N m⁻² h⁻¹, respectively, from October 2016 to September 2019 (Fig. 1).
126 Compared with the control group (NW), the CO₂ flux decreased by 7.5% and 4.0% in
127 the growing and nongrowing seasons, respectively, under AW and decreased by 2.4%
128 and 8.5% under GW in the growing and nongrowing seasons, respectively. However,
129 compared with the control group, the CO₂ flux under NGW increased by 7.9% and
130 10.5% in the growing season and nongrowing season, respectively, averaged over the
131 3 years (Fig. S2 a). The AW temperature change induced a 6.4% increase in CH₄ flux
132 in the growing season and a 3.8% decrease in the nongrowing season. The GW



133 treatment resulted in 7.1% and 10.2% increases in CH₄ flux in the growing and
134 nongrowing seasons, respectively. On the contrary, the NGW generated a 10.6% and
135 9.2 % decrease in CH₄ flux in the growing and nongrowing seasons, respectively (Fig.
136 S2 b). The AW and NGW treatments resulted in 5.8% and 2.2 % decreases in N₂O
137 flux in the growing season, and 101.9% and 192.3% increases in N₂O flux in the
138 nongrowing season, respectively. Compared with the control, NW group, the N₂O flux
139 increased by 29.7% and decreased by 24.4% under GW in the growing season and
140 nongrowing season, respectively (Fig. S2 c).

141 The results of two-way repeated measures ANOVA showed significant
142 interannual CO₂ flux in the growing season ($P < 0.05$, Fig. 1 a), while the CH₄ flux
143 under warming treatment exhibited extremely significant differences in the growing
144 season ($P < 0.01$, Fig. 1 b), whereas the interannual N₂O flux showed significant
145 difference in both the growing and the nongrowing seasons ($P < 0.05$, Fig. 1 c). Under
146 the four warming treatments, CO₂ flux and soil temperature were significantly
147 positively linearly correlated ($P < 0.05$, Fig. S3 a), whereas CH₄ flux showed
148 significantly decreasing trends with increasing soil temperature ($P < 0.05$, Fig. S3 b).
149 On the other hand, the N₂O flux showed a significantly positive linear correlation with
150 soil temperature, but only under NGW ($P < 0.05$, Fig. S3 c).

151 4. Discussion

152 Our study found that the response rate of CO₂ flux to temperature significantly
153 decreased with the increase in soil temperature (ΔST_{AW} and ΔST_{GW}) under AW and
154 GW treatments, respectively (Figure 2 a, c; $P < 0.05$). This finding indicated that the



155 response of CO₂ flux to soil temperature became less and less sensitive with warming
156 throughout the year (or growing season) in the alpine grasslands. On the contrary,
157 NGW significantly increased the response rate of CO₂ flux to temperature change (Δ
158 ST_{NGW}), indicating that warming in the non-growing season amplified the sensitivity
159 of CO₂ flux to temperature change (Figure 2 b, $P < 0.05$). In addition, Zou et al. (2018)
160 showed that the soil fluxes of CO₂ increased exponentially with temperature, but
161 warming decreased the temperature sensitivity by 23% at the grassland. Furthermore,
162 Natali et al. (2011) also confirmed that compared with the CO₂ flux in the growing
163 season, the CO₂ flux in the non-growing season was more sensitive to the temperature
164 increase.

165 In addition, seasonally asymmetric warming did not significantly affect the
166 response rate of CH₄ flux to temperature increase (Figure 2 d-f, $P > 0.05$). However,
167 Wu et al. (2020) deemed that experimental warming was stimulating soil CH₄ uptake
168 in the grasslands of the Tibetan Plateau. The results of research on alpine meadows of
169 the Qinghai-Tibet Plateau showed that the CH₄ absorption in the non-growing season
170 being more sensitive to temperature than the corresponding value in the growing
171 season (Lin et al., 2015). Furthermore, Hu et al. (2016) suggested that asymmetrical
172 responses of CH₄ fluxes to warming and cooling should be taken into account when
173 evaluating the effects of climate change on CH₄ uptake in the alpine meadow on the
174 Tibetan plateau. The latest research confirmed that warming in the Arctic had been
175 more apparent in the non-growing season than in the typical growing season (Bao et
176 al., 2020). Hereby, Bao et al. (2020) found that the CH₄ emissions during spring thaw



177 and autumn freeze contribute to about a quarter of annual total CH₄ emissions.

178 Furthermore, with the increase in soil temperature variation, the response rate of
179 N₂O flux significantly decreased gradually under AW treatment (Figure 2 g, $P < 0.05$).
180 Our results suggested that the response to temperature increase of N₂O flux was
181 insensitive due to warming occurring throughout the year. Another study has shown
182 that whole-day or whole-year warming treatment significantly enhanced N₂O
183 emissions, but daytime, nighttime or short-season warming did not have significant
184 effects (Li et al., 2019). Additionally, Cantarel et al. (2010) suggest that the N₂O flux
185 of cool and upland grasslands may be driven primarily by responses to elevated
186 temperature under projected future climate conditions.

187 **5. Conclusions**

188 In summary, the effect of seasonally asymmetrical warming on CO₂ flux was
189 obvious, with the GHG flux being able to adapt to continuous warming, resulting in a
190 reduced response rate of GHG flux to temperature increase. Warming in the
191 nongrowing season increased the temperature dependence of GHG flux. Thus, we
192 believe that the study of climate change should pay greater attention to warming in the
193 nongrowing season, so as not to underestimate the greenhouse effect of the GHG flux
194 in alpine grasslands.

195 **Data availability**

196 The measured CO₂, CH₄ and N₂O fluxes and soil temperature and soil water
197 content data are available in Zenodo (<http://doi.org/10.5281/zenodo.4244207>).

198 **Author contributions**



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

203 **Competing interests**

204 The authors declare that they have no conflicts of interest.

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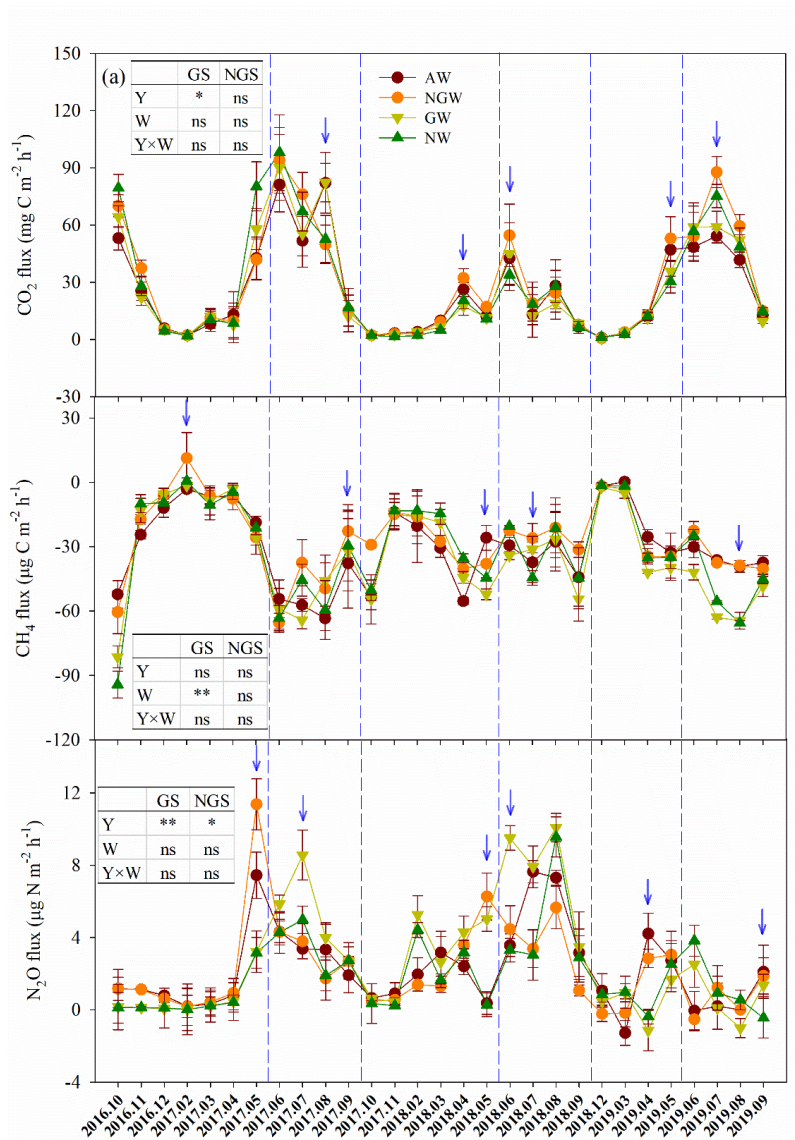
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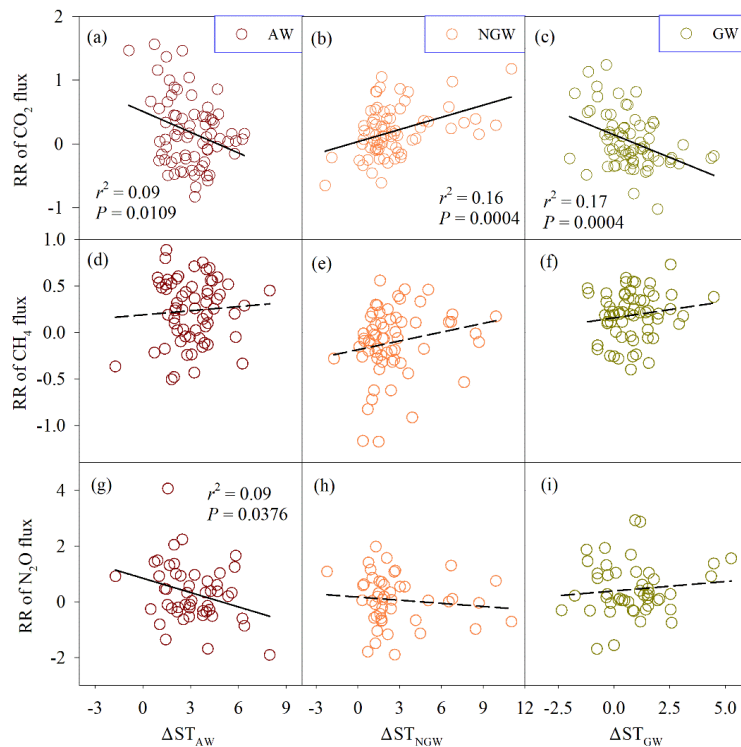


284

285 Figure 1 Monthly variation of a). CO₂, b). CH₄ and c). N₂O fluxes under the four
 286 treatments from October 2016 to September 2019. AW, warming throughout the year;
 287 NGW, warming in the nongrowing season only; GW, warming in the growing season
 288 only; NW, non-warming., The blue arrows indicate obvious warming effects. The data
 289 points represent mean ± standard error, SE. The tables illustrate the tests of



290 significance for year (Y) and warming (W) on CO₂, CH₄ and N₂O fluxes by two-way
291 repeated-measures analysis of variance (ANOVA) in the growing season (GS) and the
292 non-growing season (NGS), respectively; **P* < 0.05; ***P* < 0.01; ns, no significant. AW,
293 warming throughout the year; NGW, warming in the non-growing season only; GW,
294 warming in the growing season only; NW, non-warming.



295
296 Figure 2 Response of variations in CO₂, CH₄, and N₂O fluxes to changes in soil
297 temperature under AW, NGW and GW conditions in the alpine grassland, from 2016
298 to 2019. RR, the natural logarithm of the response ratio of the mean value of the
299 chosen variable in the warming group to that in the control (NW) group. ΔST_{AW}, soil
300 temperature of AW minus that of NW. ΔST_{NGW}, soil temperature of NGW minus that
301 of NW. ΔST_{GW}, soil temperature of GW minus that of NW. AW, warming throughout



302 the year; NGW, warming in the nongrowing season only; GW, warming in the
303 growing season only; NW, non-warming.