



## Short term effect of precipitation amount change on greenhouse gas emissions from alpine grassland in the eastern Qinghai-Tibetan Plateau

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### Abstract

The effects of a change in the precipitation amount on greenhouse gases (GHGs) emission from the alpine grassland ecosystem have not yet to be elucidated. In this research, the GHGs (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) fluxes in the eastern Qinghai-Tibetan Plateau were measured with an artificially increased precipitation (increase of 200 mm) and a decreased precipitation (decrease of 200 mm) by using the static chamber meteorological chromatography method in a growing season (from May to September). Under both increased precipitation (IP) and decreased precipitation (DP) experimental treatments, the alpine grassland functioned as the source of CO<sub>2</sub> and N<sub>2</sub>O and as the CH<sub>4</sub> sink. Compared to the control check (CK), the IP slightly enhanced the average CO<sub>2</sub> and N<sub>2</sub>O emission fluxes by 4.2% (P>0.05) and 17.2% (P>0.05), respectively, but it declined the average CH<sub>4</sub> absorption flux by 21.9% (P<0.05). In contrast, the DP decreased the average CO<sub>2</sub> emission fluxes by 10.2% (P<0.05), slightly enhanced the average N<sub>2</sub>O emission fluxes by 4.6% (P>0.05), and increased the average CH<sub>4</sub> absorption flux by 15.9% (P<0.05).

**Keywords:** Alpine grassland, Climate change, Greenhouse gases, Precipitation amount, Qinghai-Tibetan Plateau,

**Abbreviations:** CK: Control check; DP: Decreased precipitation; GHGs: Greenhouse gases; IP: Increased precipitation

### Introduction

Global climate change will include changes in the size of precipitation events (Easterling *et al.*, 2000), which also could affect the greenhouse gases (GHGs) budget. GHGs emission from ecosystems can influence the global energy balance which may often cause variations in water availability across different terrestrial landscapes (Smith *et al.*, 2003). Thus, water as well as temperature

plays important roles in influencing the GHGs flux and the balance of the carbon and nitrogen budget of an ecosystem (Ghosh and Mahanta, 2014). The Intergovernmental Panel on Climate Change (IPCC, 2007; Chaturvedi *et al.*, 2016) have indicated that an increase in the amount of precipitation and extreme precipitation events will occur in high altitude areas as a result of global warming caused by an increase in greenhouse gases content. Meanwhile, many high-altitude ecosystems are on the cusp of hydrological and biogeochemical changes resulting from less rainfall (Seager *et al.*, 2007), as well as increased evapotranspiration caused by global warming (Fu *et al.*, 2012). Therefore, studying the effects of different precipitation intensities on ecosystem GHGs emissions can lead to a greater understanding of the GHGs dynamic and their effects on precipitation pattern changes.

The Qinghai-Tibetan Plateau's average altitude is higher than 4000 m; thus, it is known as "the roof of the world" (Gao *et al.*, 2015). Xu *et al.* (2008) demonstrated that precipitation in the Plateau has increased in the eastern and central parts over the past several decades. However, only a few studies have investigated the effect of precipitation change on the alpine ecosystem GHGs flux (Zhao *et al.*, 2006). The main type of vegetation on the Qinghai-Tibetan Plateau is the alpine grassland. This ecosystem is a large C pool (Ni, 2002), and it is regarded as highly sensitive to climate change, important to the C budget, and highly crucial for indicating the effect of climate change on ecosystems (Zhou *et al.*, 2006). Nevertheless, there have been very few reports on the influence of precipitation pattern change on GHGs flux. Therefore, we designed an artificial experiment at alpine grassland in the eastern Qinghai-Tibetan Plateau, China. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes were surveyed at the increased precipitation, decreased precipitation and control check plots during the vegetation period (May 1<sup>st</sup> to September 30<sup>th</sup>) in 2015. The aim of the study was to characterize

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the influence of increased and decreased natural precipitation on GHGs flux from alpine grassland in the Qinghai-Tibetan Plateau.

### Materials and Methods

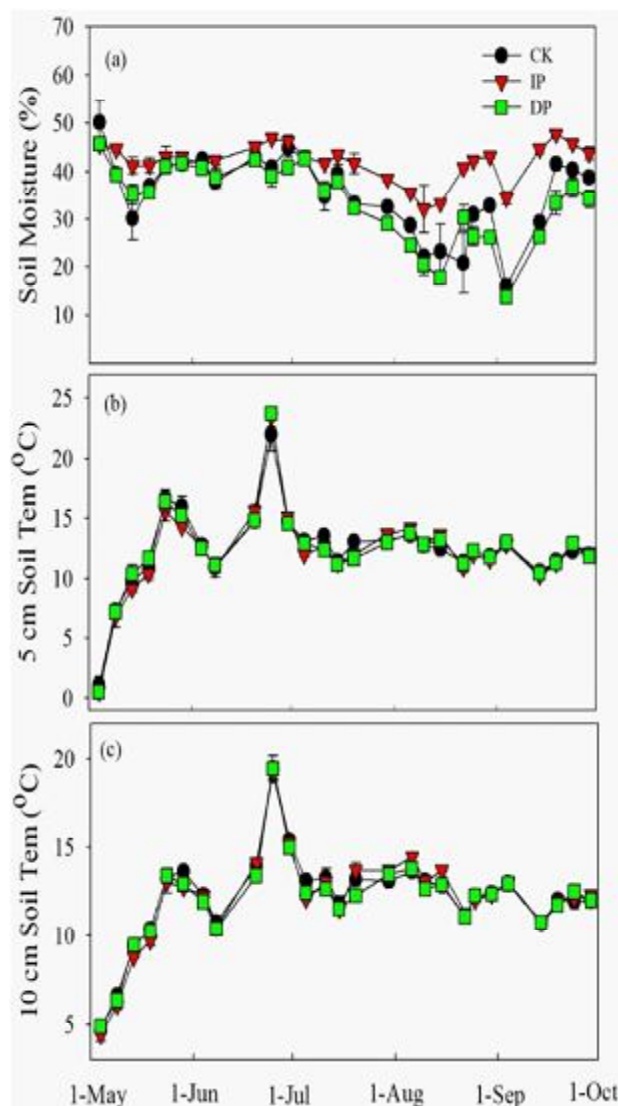
**Study site:** The study was conducted in Hongyuan County (33° 54' N, 10° 36' E, 3507m) located on the eastern Tibetan Plateau. The mean annual temperature is about 1.3 °C and annual average precipitation is 753 mm, with about 80% (nearly 600 mm) received from May to September, (Gao *et al.*, 2016). The vegetation in the alpine grassland was primarily dominated by *Kobresia setchwanensis*, *Kobresia pygmaea*, *Elymus nutans* with alpine grassland soil (Table 1) (Gao *et al.*, 2015).

**Field experiment and sampling:** Three treatments were used in this study: the increased precipitation (IP), decreased precipitation (DP) and control check (CK), which totaled 800 mm, 400 mm and 600 mm precipitation, respectively. Each treatment included four replicates. In total twelve plots (2 m × 1.5 m) were randomly selected and permanently marked in April 2015, before the growing season began. The IP was added by an addition of 6.7 mm of precipitation and then the addition of 200 mm of precipitation through artificial irrigation thirty times. The DP was reduced by 200 mm of precipitation by using a “V-type” transparent organic glass plate to cover one third of the plot area. Moreover CK group did not undergo any treatments.

The GHGs (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) emissions were measured according to Chen *et al.* (2013). The chamber was made of iron and included two parts, a stationary base (without the top and bottom, 50 cm × 50 cm and a 2 cm high water channel was laid into each plot at a depth of 10 cm ) and a removable covering box (without bottom, 50 cm × 50 cm × 40 cm). The covering box was placed on the stationary base top for sampling and was sealed airtight by water in the water channel.

In the afternoon at 18 o'clock before the sampling day, 6.7 mm of water was distributed within the IP plots by using a watering can. All GHGs samples were measured during the morning between 9 and 11 o'clock every five days from May 1 to September 30, 2015. Before sampling the boxes were closed for 10 minutes to build an equilibrium state. Four 100 ml syringes of gas were collected after 10, 20, 30 and 40 minute intervals. The soil samples were randomly collected 0-10 cm below the soil surface four times by a soil auger (2 cm diameter) on the gases sampling dates. Soil temperatures were

recorded by two soil thermometers at depths of 5 cm and 10 cm below the soil surface, and the soil moisture was determined by a TDR (Time Domain Reflectometer) (Fig 1).



**Fig 1.** Soil moisture (a) soil 5 cm (b) and 10 cm temperature (c) subjected to different precipitation treatment in alpine grassland in Qinghai-Tibetan Plateau

**Samples analyses:** An Agilent 7890A gas chromatograph was used to analyze the GHGs concentrations in the gas samples. The GHGs flux rates were calculated by the following formula (Chen *et al.*, 2013):

$$J = \frac{dc}{dt} \cdot \frac{M}{V_0} \cdot \frac{P}{P_0} \cdot \frac{T_0}{T} \cdot H$$

where  $J$  is the GHGs flux;  $dc/dt$  is the rate of concentration change;  $M$  is the molar mass of GHGs;  $P$  is the atmosphere pressure of the sampling site;  $T$  is the absolute temperature during the time of sampling;  $V_0$ ,  $P_0$ , and  $T_0$  are the molar volume, atmosphere pressure, and absolute temperature, respectively, under the standard condition; and  $H$  is the sampling box height over the water surface.

The soil samples were analyzed for the concentration of dissolved organic carbon (DOC), dissolved total nitrogen (DTN), dissolved organic nitrogen (DON), ammonia ( $\text{NH}_4^+\text{-N}$ ), and nitrate ( $\text{NO}_3^-\text{-N}$ ). The DOC,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$  contents in the extracted solution were determined by using an AA3 continuous flow analytical system (Seal, Germany). The DTN concentration was oxidized to  $\text{NO}_3^-\text{-N}$  by potassium persulfate in an alkaline solution and determined according to the above method, and the DON value was calculated by the follow formula:

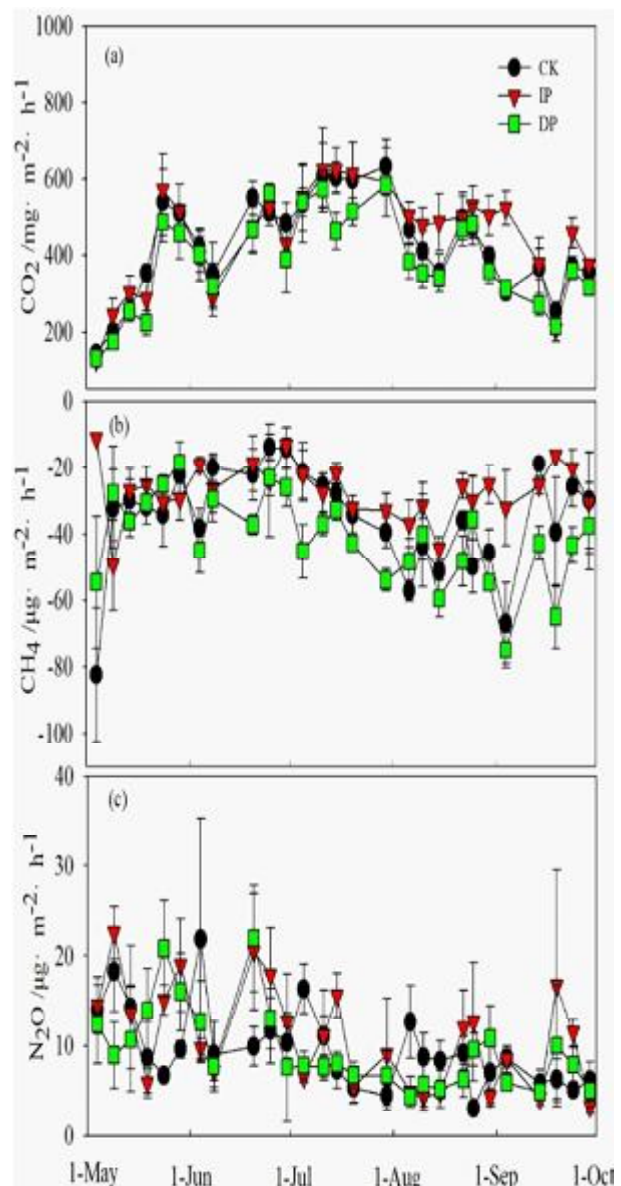
$$\text{DON} = \text{DTN} - (\text{NH}_4^+\text{-N}) - (\text{NO}_3^-\text{-N}).$$

**Statistical analyses:** Repeated measures ANOVA was used to examine the effects of precipitation treatments over time on the GHGs fluxes and soil parameters. The Duncan multiple comparison test was used to determine which precipitation treatments were different at a significance level of  $P < 0.05$ . Liner regression analysis was used to analyze the relationships between the soil properties and GHGs fluxes, and significance was accepted at the  $P < 0.05$  level of probability.

## Results and Discussion

**Soil moisture and temperature:** Soil moisture reached maximum (about 50%) in early May (Fig 1a), while soil temperature reached maximum ( $24^\circ\text{C}$ ) at the end of June (Fig 1b,c). The variation of soil moisture was more stable than that of soil temperature. Generally, IP soil moisture was higher than CK and DP ( $P < 0.05$ ), but no significant changes on soil temperature were found between treatments.

**$\text{CO}_2$  flux:** In our research, the average  $\text{CO}_2$  fluxes for the CK, IP, and DP treatments were 427.1, 460.6 and 398.3  $\text{mg}/\text{m}^2\cdot\text{h}$ , respectively, and the average  $\text{CO}_2$  flux from the DP treatment was significantly lower than those of the CK and IP treatments (Fig 2a). The IP plot's accumulated  $\text{CO}_2\text{-C}$  emission value was 4505.8  $\text{kg C}/\text{ha}$ , which was 202.1  $\text{kg C}/\text{ha}$  more than CK, an increase of 4.7%. In contrast, the DP plot's accumulated  $\text{CO}_2\text{-C}$  emission value was 3818.1  $\text{kg C}/\text{ha}$ , which was 485.6  $\text{kg C}/\text{ha}$  less than CK, a decreased of 11.3% (Table 2).



**Fig 2.** The  $\text{CO}_2$  (a),  $\text{CH}_4$  (b) and  $\text{N}_2\text{O}$  flux (c) subjected to different precipitation treatment in alpine grassland in Qinghai-Tibetan Plateau

In general, the alpine grassland is the source of  $\text{CO}_2$  and  $\text{N}_2\text{O}$ , and is a  $\text{CH}_4$  sink (Shi *et al.*, 2012; Chen *et al.*, 2013). Soil  $\text{CO}_2$  emission is mainly caused by soil microbial activities; they can absorb the organic matter to supply their lives and to transfer soil organic carbon to inorganic and emission carbon-containing gases (Kuzayakov and Blagodatskaya, 2015). While the soil microbial activities are affected by numerous environmental factors, the water content is a key factor (Dharumarajan *et al.*, 2017). Bouma and Bryla (2000) found that changes in the soil water content could

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**Table 1.** Soil properties in study site (Means  $\pm$  SD.)

Organic C (g/kg)	Total N (g/kg)	Total P (g/kg)	C/N	Bulk Density (g/cm <sup>3</sup> )	pH
9.59 $\pm$ 3.35	4.56 $\pm$ 0.29	0.96 $\pm$ 0.05	10.92 $\pm$ 1.24	1.04 $\pm$ 0.13	6.07 $\pm$ 0.11

**Table 2.** The accumulated emission flux of GHGs with different month in alpine grassland in the Qinghai-Tibetan Plateau

Months	CH <sub>4</sub> (kg C/ha)			CO <sub>2</sub> (kg C/ha)			N <sub>2</sub> O (kg N/ha)		
	CK	IP	DP	CK	IP	DP	CK	IP	DP
May	-0.217b	-0.152a	-0.182ab	568.5a	586.4a	499.9b	0.063b	0.085a	0.082a
Jun	-0.129a	-0.171b	-0.188b	974.1a	931.8ab	862.9b	0.074a	0.073a	0.071a
Jul	-0.164a	-0.158a	-0.226b	1188.0a	1156.2a	1032.2b	0.04ab	0.048a	0.033b
Aug	-0.265b	-0.186a	-0.279b	917.5b	1031.4a	839.6c	0.031a	0.032a	0.030a
Sep	-0.224a	-0.281b	-0.296b	655.6b	800.0a	583.5c	0.031a	0.029a	0.026a
Total	-1.0ab	-0.948a	-1.171b	4303.7a	4505.8a	3818.1b	0.239b	0.268a	0.241b

**Note:** IP and DP mean increased 200 mm and decreased 200 mm precipitation in this research, respectively, the same below. Different lowercase letters mean the difference of one kind of GHG in the same month with different precipitation treatment ( $P < 0.05$ ).

influence the interpretations of root and soil measurements based on CO<sub>2</sub> emission, especially in fine particle soils. In the experiment period, the average soil moisture of the IP and DP plots were 42.1% and 33.8%, respectively, and there was a significant difference between them. The DOC contents consistently declined from May to September and had a significant negative relationship with the CO<sub>2</sub> fluxes. Thus, the changes in the CO<sub>2</sub> fluxes during the growth season may have been caused by the precipitation change's effect on microbial activities. Blankinship and Hart (2014) reported that in a subalpine grassland, the CO<sub>2</sub> flux was 159.8 and 52.7 mg C/m<sup>2</sup>·h, respectively, when the soil volumetric water content was 35.2% in July and 27.2% in September. The seasonal differences in the CO<sub>2</sub> flux may have been caused by plant growth and wither. Piao *et al.* (2012) reported that a precipitation regime change and an increase in atmospheric CO<sub>2</sub> concentration caused the net ecosystem productivity (NEP) to increase from a net C source (-0.5 Tg C/yr) in the 1960s to a net C sink in the 2000s (21.8 Tg C/yr) in the Qinghai-Tibetan plateau grassland. Therefore, with precipitation change, the alpine grassland's soil-atmospheric CO<sub>2</sub> flux will also change, and the NEP will be further affected.

**CH<sub>4</sub> flux:** The average CH<sub>4</sub> fluxes for CK, IP, and DP were -37, -28.9 and -42.9  $\mu$ g/m<sup>2</sup>·h, respectively, and the average CH<sub>4</sub> flux of the IP treatment was significantly higher than those of CK and DP ( $P < 0.05$ ) (Fig 2b). During the experiment period, the accumulated CH<sub>4</sub>-C absorption values of the CK, IP, and DP treatments were -1.0, -0.948, and -1.171 kg C/ha, respectively (Table 2). Therefore, when simulating decreased precipitation in the growth season in the future, there will be greater CH<sub>4</sub> absorption

in the alpine grassland.

In this study, it was also found that increased water could decrease the CH<sub>4</sub> uptake rates and, in contrast, decreased water could increase the CH<sub>4</sub> uptake rates, which were -29 and -43  $\mu$ g/m<sup>2</sup>·h, respectively. The former situation may be caused by declined substrate availability for CH<sub>4</sub> oxidation in the wetter grassland soil because the soil water solution functions as a barrier to gas diffusion (Wu *et al.*, 2013); therefore, in a moist soil environment, CH<sub>4</sub> oxidizing bacteria do not have sufficient CH<sub>4</sub> and O<sub>2</sub> (Liu *et al.*, 2008). The latter situation may be caused by the drought force increasing the gas diffusion in the drier soil to stimulate the CH<sub>4</sub> oxidation bacteria activities and transport more atmospheric O<sub>2</sub> and CH<sub>4</sub> in the drier soil, thus inhibiting methanogenesis (Conrad, 1996). This result is in accordance with results published in other studies (Blankinship and Hart, 2014). They found that drier soil (-0.032~-0.047 mg C/m<sup>2</sup>·h) expended roughly five times more CH<sub>4</sub> than did moist soil (-0.004~-0.01 mg C/m<sup>2</sup>·h) in a subalpine grassland. The decreased water plots uptook more CH<sub>4</sub>, which supports the former results (Lin *et al.*, 2009) of a negative relationship between CH<sub>4</sub> consumption and soil moisture in a grassland ecosystem. The soil moisture's large effect on the CH<sub>4</sub> uptake is consistent with another study (Bowden *et al.*, 1998) because CH<sub>4</sub> was consumed in an aerobic environment by methanotrophs (Conrad, 1996).

**N<sub>2</sub>O flux:** The average N<sub>2</sub>O fluxes of CK, IP and DP were 9.24, 10.83, and 9.67  $\mu$ g/m<sup>2</sup>·h, respectively, but there was no significant difference (Fig 2 c). Similar to the CH<sub>4</sub> and CO<sub>2</sub> trends, the IP plot's accumulated N<sub>2</sub>O-N emission



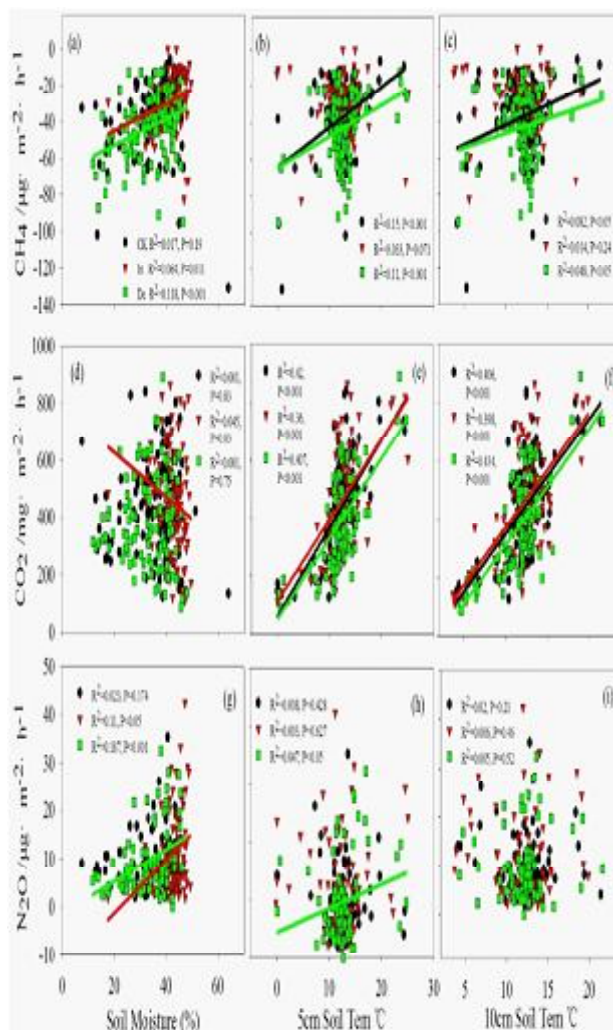
value was 0.268 kg N/ha, which was 0.029 kg N/ha more than CK and 0.027 kg N/ha more than DP, an increase of 12.1% and 11.2%, respectively (Table 2).

The soil  $N_2O$  emission primarily occurs through two pathways, one is nitrification and the other is denitrification (Horvath *et al.*, 2010). These two functions are affected by numerous factors, such as the soil pH, temperature, moisture, and so on (Bergstermann *et al.*, 2011). This research showed that the observed  $N_2O$  fluxes were not significantly different with the different precipitation conditions. This result did not agree with the previous reports. Blankinship and Hart (2014) found that the wetter soil was a net  $N_2O$  sink, and the drier soil was a net  $N_2O$  source in July in a subalpine grassland; however, the  $N_2O$  flux did not exhibit this pattern in September at the same study site. Smith *et al.* (2003) believed that moist soil might emit more  $N_2O$  to the air due to less  $O_2$  and strong denitrification. However, the drier soil condition increased  $O_2$  diffusion from the air to soil, and it may inhibit denitrifying microbes from decreasing  $N_2O$  to  $N_2$ , thus increasing the  $N_2O$  emission (Burgin and Groffman, 2012). On the other hand, the wetter soil condition may be preferable for reducing  $N_2O$  to  $N_2$ , thereby leading to less  $N_2O$  production (Avrahami and Bohannan, 2009) or net  $N_2O$  uptake, particularly when the soil nitrate nitrogen content was low (Wu *et al.*, 2013). Bollmann and Conrad (1998) suggested that rainfall was a key factor influencing the  $N_2O$  flux, and denitrification may be the first source of  $N_2O$  in an alpine grassland, because denitrification is an anoxic process during which  $O_2$  availability is the dominant regulating factor. However, Xu *et al.* (2003) found that nitrification was the main source for  $N_2O$  emission in the inner Mongolian semi-arid steppe.

**Soil carbon and nitrogen:** The soil DOC, DON,  $NH_4^+$ -N and  $NO_3^-$ -N concentrations all indicated a declining trend. In particular, the DOC concentration rapidly reduced from May to June. The different precipitation treatments did not significantly influence the soil DOC, DON,  $NH_4^+$ -N and  $NO_3^-$ -N concentrations.

**Regression analysis of GHGs with soil variables:** The  $CH_4$  absorption fluxes of the IP and DP treatments had a significant negative regression relationship with soil moisture (Fig 3a). The  $CH_4$  absorption fluxes also had a significant negative regression relationship with the soil temperatures at 5 cm and 10 cm-deep in the CK and DP treatments (Fig 3b, c). The  $CO_2$  fluxes only had a significant negative regression relationship with soil

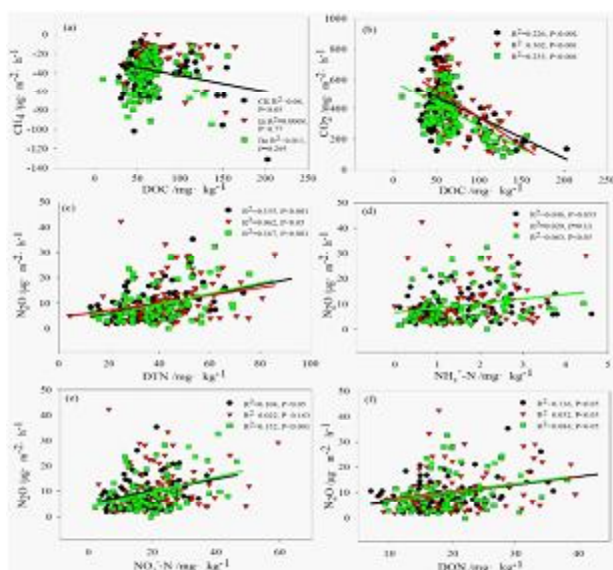
moisture in the IP treatment (Fig 3d). The  $CO_2$  fluxes in all three treatments had a significant positive regression relationship with the soil temperatures at 5 cm and 10 cm-deep (Fig 3e, f). The  $N_2O$  fluxes had a significant positive regression relationship with soil moisture in the IP and DP plots (Fig 3g).



**Fig 3.** The regression analysis of GHGs with soil moisture and temperature in alpine grassland in Qinghai-Tibetan Plateau

The  $CH_4$  absorption fluxes had a significant positive regression relationship with the soil DOC content in the IP plot (Fig 4a), but the  $CO_2$  fluxes had significant negative regression relationships with the soil DOC content in all three experiment plots (Fig 4b). The  $N_2O$  fluxes had significant positive regression relationships with the soil DTN and DON contents in all three experiment plots (Fig 4c, f), but only had a significant positive regression relationship with the soil  $NH_4^+$ -N in the DP plots and with the soil  $NO_3^-$ -N in the CK and DP plots (Fig 4d, e).

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**Fig 4.** The regression analysis of GHGs with soil DOC,  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and DON in alpine grassland in Qinghai-Tibetan Plateau

### Conclusion

This study showed that the increased precipitation causes a significant decline in the  $\text{CH}_4$  uptake flux, whereas decreased precipitation causes a significant decline in the  $\text{CO}_2$  emission flux but enhances the  $\text{CH}_4$  uptake flux. There was no significant change in the  $\text{N}_2\text{O}$  flux. Taking into consideration that the Qinghai-Tibetan Plateau is very sensitive to climate change and that the alpine grassland is a huge C pool, future research should be aimed at the effect of long-term climate change on the C and N cycle from these high altitude ecosystems, as well as the global effects from these changes.

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