Altered precipitation regimes mitigate N2O flux response to nitrogen addition in an alpine steppe

Yang Yang[1], Yuanming Xiao[1], Li Changbin[2], Bo Wang[1], Gao yongheng[3], and Zhou Guoying[1]

[1]Northwest Institute of Plateau Biology Chinese Academy of Sciences
[2]Qinghai University
[3]Institute of Mountain Hazards and Environment, Chinese Academy of Sciences

June 3, 2021

Abstract

Anthropogenic-driven global change, including changes in atmospheric nitrogen (N) deposition and precipitation patterns, is dramatically altering N cycling in soil. How long-term N deposition, precipitation changes, and their interaction influence nitrous oxide (N2O) emissions remains unknown, especially in the alpine steppes of the Qinghai-Tibetan Plateau (QTP). To fill this knowledge gap, a platform of N addition and altered precipitation experiments was established in an alpine steppe of the QTP in 2013. N addition significantly increased N2O emissions, and alterations in soil NO3?-N, pH, temperature, and belowground biomass modulated N2O emissions. In addition to abiotic parameters, ammonia-oxidizing bacteria dominated N2O emissions in nitrification compared with ammonia-oxidizing archaea. Changes in the denitrifying microbial community, namely a high ratio of (nirS+nirK) gene-containing to nosZ gene-containing organisms, were responsible for N2O emissions in denitrification. Altered precipitation did not affect N2O emissions. This unexpected finding, which is inconsistent with the conventional view that N2O emissions are controlled by soil water content, indicates that N2O emissions are particularly susceptible to N deposition in the alpine steppes. Notably, whereas N2O emissions were affected by N addition as a single factor, they were not significantly affected by the combination of precipitation changes and N addition, indicating that altered precipitation patterns may mitigate the positive feedback effect of N addition on N2O emissions. Consequently, our study suggests that the response of N2O emissions to N deposition in future global change scenarios will be affected by precipitation regimes in the alpine steppes.

Altered precipitation regimes mitigate N_2O flux response to nitrogenaddition in an alpine steppe

Yang Yang[1,2], Yuanming Xiao [1,2], Changbin Li[4], Bo Wang[1,2], Yongheng Gao[1,5], Guoying Zhou[1,3*]

[1]Northwest Institute of Plateau Biology, Chinese Academy of Science, Xining, China

[2] University of Chinese Academy of Science, Beijing, China

[3] Key Laboratory of Tibetan Medicine Research, Chinese Academy of Sciences, Xining, China

[4]College of Agriculture and Animal Husbandry, Qinghai University, Xining, China

[5] Institute of Mountain Hazards and Environment, Chinese Academy of Science, Chengdu, China

*** Corresponding author** :

Guoying Zhou, Northwest Institute of Plateau Biology, Chinese Academy of Science, No. 23 Xinning Road, Xining, Qinghai 810008, China. Email: zhougy@nwipb.cas.cn.

Abstract

Anthropogenic-driven global change, including changes in atmospheric nitrogen (N) deposition and precipitation patterns, is dramatically altering N cycling in soil. How long-term N deposition, precipitation changes, and their interaction influence nitrous oxide (N_2O) emissions remains unknown, especially in the alpine steppes of the Qinghai-Tibetan Plateau (QTP). To fill this knowledge gap, a platform of N addition and altered precipitation experiments was established in an alpine steppe of the QTP in 2013. N addition significantly increased N_2O emissions, and alterations in soil NO_3^--N, pH, temperature, and belowground biomass modulated N_2O emissions. In addition to abiotic parameters, ammonia-oxidizing bacteria dominated N_2O emissions in nitrification compared with ammonia-oxidizing archaea. Changes in the denitrifying microbial community, namely a high ratio of (*nirS+nirK*) gene-containing to *nosZ*gene-containing organisms, were responsible for N_2O emissions in denitrification. Altered precipitation did not affect N_2O emissions. This unexpected finding, which is inconsistent with the conventional view that N_2O emissions are controlled by soil water content, indicates that N_2O emissions are particularly susceptible to N deposition in the alpine steppes. Notably, whereas N_2O emissions were affected by N addition as a single factor, they were not significantly affected by the combination of precipitation changes and N addition, indicating that altered precipitation patterns may mitigate the positive feedback effect of N addition on N_2O emissions. Consequently, our study suggests that the response of N_2O emissions to N deposition in future global change scenarios will be affected by precipitation regimes in the alpine steppes.

Keywords

N_2O emissions, functional genes, nitrogen deposition, precipitation regimes, long-term experiment, alpine steppe

Introduction

Nitrous oxide (N_2O), a non-carbon dioxide (CO_2) greenhouse gas, has a global warming potential nearly 300-fold greater than that of CO_2 over a 100-year lifespan (Dijkstra et al., 2013). The accumulation of N_2O in the atmosphere will deplete stratospheric ozone and contribute to global warming (Ravishankara et al., 2009). The main sources of atmospheric N_2O are closely associated with soil nitrogen (N) cycling (i.e., nitrification and denitrification) of terrestrial ecosystems, which contribute to ˜56–70% of global N_2O emissions (Butterbach-Bahl et al., 2013). Grasslands host one of the most widely distributed vegetation types on earth, and grassland ecosystems are the main component of terrestrial ecosystems (Scurlock et al., 2002). On the Qinghai-Tibetan Plateau (QTP), alpine grassland ecosystems (e.g., alpine meadows and alpine steppes) are huge nitrogen (N) reservoirs because of sluggish microbial decomposition (Yang et al., 2018; Zhang et al., 2020). However, the substantial labile N stored in alpine soils, which is a large source of N_2O, is often neglected (Mao et al., 2020). Global change, particularly atmospheric N deposition and changing precipitation regimes, has considerable consequences for storage and patterns of N in alpine ecosystems (Fu et al., 2017; Lin et al., 2016). Given that alpine grasslands may possess the capacity for N_2O release and are sensitive to global change (Xiao et al., 2020), understanding how alpine soil N_2O emissions respond to N deposition and precipitation changes is crucial for predicting future atmospheric N_2O concentrations.

The main regulatory factors for plant communities and soil ecological processes in grasslands are N and water. Field simulations of the impact of atmospheric N deposition on N_2O emissions are not scarce, especially in the alpine grasslands of the QTP. However, reports of the effects of N addition in these ecosystems are inconsistent. N addition has been shown to significantly increase soil N_2O emissions, because N input elevates the concentration of inorganic N and the abundance of functional microbes in the soil (Geng et al., 2019; Peng et al., 2018; Wu et al., 2020; Yan et al., 2018). In addition, a greater labile carbon (C) supply (e.g., litter decomposition or root exudation) under N enrichment provides substrate C for heterotrophic denitrifiers, thereby stimulating N_2O emissions (Brown et al., 2012; Dijkstra et al., 2013). However, Zhu et al. (2015) showed that N input did not affect N_2O emissions. A possible interpretation of this finding is

that low temperature and inadequate soil moisture limit the activities of microorganisms associated with N cycling in cold conditions (Banerjee et al., 2016; Curtis et al., 2006; Schaufler et al., 2010). Despite this work on grasslands, the response of N_2O emissions to long-term N deposition on the QTP remains understudied.

Soil N_2O emissions are also susceptible to hydrologic variations (Knapp et al., 2002). Generally, changes in soil water content influence N mineralization and organic matter degradation, which then affect the provision of N and C reactants for N cycling processes. On a global scale, elevated precipitation in grassland ecosystems accelerates N_2O emissions while decreased precipitation mitigates N_2O emissions. These processes are predominantly regulated by shifts in soil water availability (Li et al., 2020). By contrast, Liu et al. (2014) showed that short-term water increment did not affect N_2O emissions from semiarid steppes. Even increased precipitation decreased N_2O emission in arid grasslands (Cai et al., 2016). This finding may be attributable to soil leaching and run-off events caused by the increased rainfall, which intensified the loss of inorganic N in soil and thereby limited soil N cycling (Cregger et al., 2014). Little is known about how long-term precipitation changes impact N_2O emissions on the QTP. Both N and water affect soil biogeochemical cycles. N deposition and variation in precipitation usually occur simultaneously; thus, their effects are interdependent (Harpole et al., 2007). The combined effect of N deposition and altered precipitation on N_2O emissions is also unknown. N-cycling microbiomes play a crucial role in regulating soil N dynamics and global climate stabilization. On the QTP it is also unclear how pivotal N-cycling functional microorganisms respond to global change and which microbes better explain N_2O emissions.

Due to multifactorial climate change and intensive interventions targeting anthropogenic activities, the environmental conditions of the QTP have undergone dramatic changes in the past few decades (Gong et al., 2017). The amount, frequency, and intensity of precipitation increased from 1975 to 2014 (Ge et al., 2017). The QTP is also confronting pronounced N deposition, with an average of ˜8 kg N ha-1 year-1 (Lü et al., 2007). The alpine steppes, the largest grassland ecosystem on the QTP, are extremely sensitive to global change (Ding et al., 2016; Wang et al., 2011). Therefore, understanding the effects of N enhancement and altered precipitation on N_2O emissions in the alpine steppes is essential. This study consists of altered precipitation and N addition manipulation experiments that were conducted in an alpine steppe on the QTP in 2013. We monitored the N_2O flux during the 2020 growing season (May to October) based on in-situ experiments. To identify the key abiotic and biotic factors regulating N_2O emissions, we measured N_2O flux on six consecutive days in mid-August (during peak plant growth). Soils were also collected to measure abiotic parameters and functional microbes, including nitrifiers (ammonia-oxidizing bacteria: AOB; ammonia-oxidizing archaea: AOA) and denitrifiers (*nirS* -,*nirK* -, and *nosZ* gene-containing microorganisms). The objectives of the study were to (1) assess whether N_2O emissions were altered by long-term N addition, precipitation changes, and their interaction; and (2) identify the mechanisms that regulated N_2O emissions under N addition and altered precipitation patterns.

Materials and Methods

Site description and experimental design

The study area is a typical alpine steppe, which is situated in the northeastern Qinghai - Tibetan Plateau (QTP), China (37°18'N, 100deg15'E). The study site exhibits a plateau continental climate. The average annual temperature is ˜0.1degC. The mean annual precipitation is ˜390 mm, most of which occurs from June to August. The vegetation is mainly dominated by grasses, such as *Stipa purpurea*Grisebach, *Leymus secalinus* (Georgi) Tzvel, and *Poa crymophila* Keng (Xiao et al., 2020). The growing season is from May to October, and peaks in August. At the beginning of the experiments, the chemical properties of the soil (0–30 cm depth) were as follows: total N, 2.5 g kg^{-1}; NO_3^--N, 11.5 mg kg^{-1}; NH_4^+-N, 5.1 mg kg^{-1}. The topsoil was mainly composed of chestnut soil with a pH of 8.3.

The experimental platform was established in 2013 (Figure 1). The experiments consisted of six different treatments (NP: ambient nitrogen with ambient precipitation; NP-: ambient nitrogen with 50% reduced precipitation; NP+: ambient nitrogen with 50% increased precipitation; N+P: nitrogen addition with ambient precipitation; N+P-: nitrogen addition with 50% reduced precipitation; N+P+: nitrogen addition with 50%

increased precipitation). These 30 plots (2.7 m x 3.3 m each) were randomly established in a 5x6 block design and were each separated by a buffer zone (2 m wide). Concave sunlight-pervious polyvinyl chloride (PVC) boards without slots were placed above the 50% reduced precipitation treatments to intercept rainfall. The collected water (50% ambient precipitation) was immediately transferred to the 50% increased precipitation treatments after the rain (sprinkling evenly). To avoid light differences between the treatment plots, the same PVC boards with slots were also installed on the ambient precipitation and 50% precipitation increment treatment plots. N fertilizer (NH_4NO_3: 10 g $m^{-2}yr^{-1}$) was dissolved in 1 L water and was evenly applied two times to the N supply plots (June and July every year). Identical amounts of water without N fertilizer were sprayed evenly on the ambient treatment plots. A previous study indicated that the N saturation level was 8 g m^{-2} yr^{-1} in this study area (Peng et al., 2017). Therefore, the current N input level (10 g m^{-2} yr^{-1}) should be sufficient to simulate N saturation of the alpine grasslands.

N_2O flux measurements

In 2013, a 40 cm x40 cm square stainless steel collar was permanently inserted into the topsoil (˜ 10 cm), which located in the dynamic monitoring area of each plot. The in-situ N_2O flux was measured using static chamber with insulation materials and gas chromatography techniques. During gas collection (between 8 am and 12 noon), a chamber (30 cm tall) with an electric fan (to mix the air) was placed on the collar. Gas samples (100 mL) were collected by medical syringes at intervals of 0, 10, 20 and 30 min and then promptly injected into multi-layer foil sampling bags (Delin Inc., Dalian, China). In 2020, we collected gas samples three times per month (May to October). Furthermore, we conducted that gas samples collection during six consecutive days in mid-August (plant growth peak). The collected gas samples were immediately transferred to the laboratory and then determined for N_2O concentration using a GC-7890B gas chromatograph (Agilent Technologies Limited Co., Chengdu, China). While collecting gas (plant growth peak), the soil volumetric water content (VWC) and temperature in the top 10 cm were measured in each plot adjacent to the collar using a hand-held moisture probe and a digital thermometer, respectively. The N_2O flux was calculated as follows:

$$\boldsymbol{F}= \ \rho \times \frac{V}{A} \mathrm{x} \frac{T0}{T} \mathrm{x} \frac{P}{P0} \mathrm{x} \frac{\mathrm{dc}}{\mathrm{dt}}$$

where F is the N_2O flux (μg N_2O m^{-2} h^{-1}); ρ is the standard status N_2O density; V is the volume of the static chamber (m^3); and A is the base area of the static chamber (m^2). T 0 and T are the standard temperature (273 K) and the static chamber temperature (K), respectively. P 0 and $P$are the standard pressure (1,013 hPa) and the air pressure (hPa), respectively. The rate of increase in the N_2O concentration in the static chamber ($10^{-6}h^{-1}$) is dc/dt .

Soil and plant sampling and chemicalanalyses

To identify the mechanisms regulating N_2O flux responses to N input and altered precipitation, plant and soil samples were collected at the peak of plant growth. First, three 25 cm × 25 cm quadrants were randomly placed in each plot, and then all living plants were clipped as aboveground biomass. After removal of the aboveground plants, three root cores (internal diameter 8 cm and depth 10 cm) were collected and then mixed. The mixed root cores were washed with water in a 0.4 mm sieve. The live roots were selected by their color and were used as belowground biomass. The collected aboveground and belowground biomasses were oven-dried at 60°C to a constant mass and were then weighed.

Three more soil cores (internal diameter 3 cm and depth 10 cm) were collected near each collar (for a total of 90 soil cores) and were then homogenized to acquire one compound sample (for a total of 30 soil samples). The collected soil samples were separated into three subsamples by a sieve (2 mm). The first subsample was immediately preserved at -80degC for DNA extraction and also analysis of the abundances of key microbial functional genes. The second subsample was stored at 4degC to determine the soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations. The third subsample was air-dried to determine the soil pH. The available N (NH_4^+-N and NO_3^--N) concentrations in soil were determined using a flow injection analyzer (Autoanalyzer 3 SEAL, Bran and Luebbe, Norderstedt, Germany) after extracting fresh soil with 1 M KCl solution. The pH of the air-dried soil was measured using a pH electrode (soil-to-deionized water ratio of

1:2.5).

Soil DNA extraction and real-time quantitative PCR (qPCR)

Soil DNA was extracted from 0.5 g frozen soil using a kit (E.Z.N.A.(r) DNA Kit, Omega Bio-tek, Norcross, GA, U.S.A.) based on the manufacturer's instructions. The DNA extract was checked on 1% agarose gel. The quality of the DNA was evaluated with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, U.S.A.). The nitrification-related *amoA* gene in ammonia-oxidizing bacteria (AOB) and archaea (AOA) was determined. The *nirS* , *nirK* , and *nos* Z genes, which are associated with denitrification, were also determined in denitrifying microorganisms. The functional gene copy numbers were amplified using an ABI 7300 Real-Time PCR System (ABI, CA, U.S.A.). PCR reactions were performed in triplicate. The PCR mixtures contained 10 μL 2X ChamQ SYBR Color qPCR Master Mix, 0.8 μL forward primer 5 (μM), 0.8 μL reverse primer (5 μM), 2 μL template DNA, 0.4 μL 50 X ROX Reference Dye 1, and 6 μL ddH_2O. The functional genes, primers, and sequences used for PCR reactions are summarized in Table 1. More detailed PCR thermal cycling conditions are listed in Table S1. The standard curve of each amplified gene was constructed using a 10-fold dilution of plasmid DNA (containing the target gene). The PCR efficiency was between 89% and 101%; the R^2 ranged from 0.98 to 0.99.

Statistical analyses

Before statistical analysis, we examined whether the data conformed to a normal distribution (Shapiro–Wilk test) and tested for homogeneity of variance (Levene's test). We conducted data analysis according to the following four steps. First, a Two-way Analysis of variance (ANOVA) was used to examine the effects of N addition, altered precipitation, and their interaction on the following: soil abiotic parameters (soil temperature, moisture, pH, NH_4^+-N, and NO_3^--N); plant properties (aboveground and belowground biomass); N_2O flux (emission peak); and the functional gene abundance related to nitrification (AOA, AOB) and denitrification (*nirS* , *nirK* , and*nos* Z). Second, a repeated-measures ANOVA was performed to assess the effects of treatments on N_2O flux during the growing season. Significant differences of the above-mentioned parameters were assessed using post-hoc tests (Duncan's test at $P < 0.05$). Third, linear regression analysis was used to evaluate the relationships among N_2O flux and soil abiotic factors and plant properties. Finally, a Pearson correlation analysis was used to investigate the correlation between functional gene abundances and N_2O flux. These statistical analyses were carried out in SPSS version 21.0 (SPSS, Chicago, IL, U.S.A.) and were visualized using Sigmaplot 12.5 software (Systat Software Corporation, U.S.A.).

Results

Soil environment factors and plant properties

The soil abiotic parameters and plant attributes significantly differed by treatment (Table 2). N addition significantly reduced soil pH but had no effect on soil temperature and humidity. In contrast, N input significantly increased available N concentrations in soil and aboveground and belowground biomass. Increased precipitation significantly increased soil moisture and aboveground biomass. Precipitation reduction significantly reduced soil moisture and soil NH_4^+-N. However, soil pH, NO_3^--N, and belowground biomass were not affected by precipitation changes. Except for soil temperature, the combination of N addition and precipitation changes did not affect other environmental factors.

Responses of N_2O flux and functional genes to nitrogen addition and altered precipitation

N_2O emissions showed a significant seasonal dynamic, with the maximum flux appearing in August (Figure 2A). Although the average flux was relatively small, the alpine steppe presented as the source of N_2O ($F > 0$) during the growing season under different treatment conditions (Figure 2B). The addition of N resulted in a significant increase in N_2O emissions (317%) (Figure 2B-C). However, N_2O emissions were not significantly influenced by altered precipitation or the interaction between N addition and precipitation changes (Figure 2B). To a certain extent, the coupling of N and water alleviated the effect of N input on N_2O emissions

(178% and 100% vs 317%) (Figure 2B-C). Similarly, N_2O flux during the emission peak was only affected by N addition (Figure 2D).

The *amo* A gene abundance of the nitrifier AOA was significantly affected by both N supply and precipitation changes (Figure 3A). However, the AOB *amo* A gene abundance was significantly elevated only by N supply (Figure 3B). Although the denitrifier *nirS* and *nirK* genes regulate the same step in denitrification (nitrite reduction: NO_2^-- NO), only the *nirS* gene abundance was significantly affected by the interaction of N addition and precipitation changes (Figure 3C). The abundance of the *nirK* gene did not significantly differ among the treatments (Figure 2D). The abundance of the *nosZ* gene was reduced under N addition, and altered precipitation did not significantly affect the *nosZ* gene abundance (Figure 3E).

Linking N_2O flux to abiotic and biotic factors under nitrogen addition and altered precipitation

N_2O flux was positively correlated with soil NO_3^--N ($R^2 = 0.38$, $P < 0.05$) and belowground biomass ($R^2 = 0.13$, $P < 0.05$) but was negatively correlated with soil pH ($R^2 = 0.17P < 0.05$) and soil temperature ($R^2 = 0.16$, $P < 0.05$) (Figure 4). Soil moisture ($P = 0.77$), NH_4^+-N ($P = 0.18$), and aboveground biomass ($P= 0.15$) were not correlated with N_2O flux (Figure 4). In addition, N_2O emissions were also affected by multiple biological factors (Table 3). N_2O flux was negatively correlated with AOA (r = -0.435, $P < 0.05$) and*nosZ* (r = -0.484, $P < 0.01$) and positively correlated with AOB (r = -0.428, $P < 0.05$). Although N_2O flux did not significantly affect *nirS* and*nirK* , it was nonetheless positively correlated with the ratio of (*nirS+ nirK*)/*nosZ* (r = 0.484, $P < 0.05$).

Discussion

The results from our field experiments show that the alpine steppe was a net source of N_2O. N addition significantly increased N_2O emissions (Figure 2). Most terrestrial ecosystems, especially grassland ecosystems, are widely limited by N (Geng et al., 2019; Lu et al., 2011). N enrichment increases N available in soil, even reaching N saturation, and available N directly affects N_2O emissions (Peng et al., 2018). In our experiment, N addition significantly increased inorganic N in soil (Table 2). N_2O emissions occurred mainly due to the supply of substrate NO_3^--N, independent of NH_4^+-N (Figure 4), which indicates that denitrification may be the predominant pathway of N_2O emissions in this alpine steppe. A possible explanation for this finding is that the N supply may lead to plants and microorganisms competing for NH_4^+-N (as a substrate for nitrification) in N-limited grassland ecosystems. Liu et al. (2013) discovered that N input promotes plant N uptake, especially NH_4^+-N. In this case, nitrification might have been inhibited due to lack of substrates. We also found that changes in abiotic factors such as soil temperature and pH regulated N_2O emissions. Generally, soil N cycling largely depends on soil temperature in alpine ecosystems. In particular, warming was found to drive N_2O production and emissions (Griffis et al., 2017). However, rising temperatures negatively affected N_2O emissions in our study (Figure 4). It is possible that higher temperatures aggravate evapotranspiration and decrease soil water availability, thereby limiting various microbial N cycling processes (Shi et al., 2012). Previous studies have also shown that soil acidification caused by N saturation limits microbial growth, thus restraining N_2O emissions (Oertel et al., 2016; Treseder, 2008). In contrast, we found that lower pH contributed to N_2O emissions (Figure 4). A possible explanation for this discrepancy is that even though N addition significantly decreased soil pH, the soil was still alkaline (Table 2) and therefore microbial activity was not negatively affected. It is worth noting that plant biomass is also a key driver of N_2O emissions. Soil labile C via root secretion may accelerate N_2O emissions because denitrification is commonly driven by high available C as a source of energy (Li et al., 2020). This phenomenon is consistent with our conclusion that the increase of belowground biomass boosted N_2O emissions (Figure 4).

Changed precipitation regimes also play an important role in modulating soil N cycling (Chen et al., 2013; Cregger et al., 2014; Lin et al., 2016). Li et al. (2020) demonstrated that increased precipitation exacerbated N_2O emissions in grassland ecosystems while reductions in precipitation mitigated N_2O emissions. In this study, however, we observed that altered precipitation patterns did not affect N_2O emissions (Figure 2). On the one hand, water addition may diminish soil N pools (soil inorganic N) by promoting plant N uptake and soil leaching, neither of which are conducive to nitrification and denitrification (Austin et al., 2004; Kruger

et al., 2021; Lin et al., 2016). On the other hand, water reduction (i.e., prolonged drought treatment) had little effect on N_2O emissions, possibly because the alpine steppe itself belongs to an arid grassland ecosystem and is insensitive to drought treatment (Dijkstra et al., 2013). The interaction between altered precipitation regimes and N addition did not significantly affect N_2O emissions in our experiment (Figure 2). There are several mechanisms that could contribute to this finding. Ordinarily, N and water co-limitation is a typical feature of arid grassland ecosystems (Austin et al., 2004; Lü et al., 2009). The responses of grassland ecosystems to N deposition are strongly regulated by precipitation patterns (Harpole et al., 2007). Increased precipitation, particularly under the background of N addition, could increase plant access to soil inorganic N resources (Li et al., 2019), so the effect of N addition on N_2O emissions may be alleviated by water addition. In addition, decreased precipitation may suppress microbial activity, leading to inefficient N assimilation, despite the presence of large amounts of N substrates in the soil (Homyak et al., 2017; Li et al., 2020). Overall, precipitation changes attenuated N_2O flux responses to N addition, thus mitigating N_2O emissions on the QTP.

The community composition and diversity of N cycling microbes are directly involved in N_2O production and emissions. Microbial functional genes associated with N cycling encode some key oxidoreductases and are therefore used as genetic markers for nitrifying and denitrifying microorganisms (Mushinski et al., 2021). The functional genes of AOA and AOB usually regulate the rate-limiting step (ammonia oxidation: NH_3 - NH_2OH) in nitrification (Hu et al., 2015; Lu et al., 2015). Some studies have indicated that N_2O emissions were promoted by increased abundances of both AOA and AOB (Brin et al., 2019; Linton et al., 2020). However, we found that N addition only significantly increased the abundance of AOB (Figure 3), and the functional genes of AOB rather than those of AOA dominated the N_2O emissions from nitrification (Table 3). Di et al. (2009) also showed that N_2O emissions are driven by AOB and not AOA in N-enriched grassland ecosystems. Previous investigations demonstrated that AOA and AOB occupy different niches. AOA and AOB play a dominant role in acidic and alkaline soils, respectively, and pH is the chief factor for niche separation (Hu et al., 2015; Tzanakakis et al., 2019). The alkaline conditions in this study may be more conducive to the activity of AOB, which further supports our conclusion that AOB controlled the N2O emissions in nitrification. The key step of denitrification (NO_2^- - NO) is generally mediated by*nirS* - or *nirK* -encoding nitrite reductase (Butterbach-Bahl et al., 2013). In this study, N_2O emissions were not related to the abundance of *nirS* and *nirK* (Table 3). This finding can be explained by other environmental factors such as soil temperature, pH, labile carbon, and oxygen concentration dominating the underlying ecological process (Li et al., 2020). The nitrous oxide reductase encoded by *nosZ* promotes N_2O reduction (N_2O - N_2), thereby reducing N_2O emissions (Butterbach-Bahl et al., 2013; Hu et al., 2015). Decreased *nosZ* abundance is unfavorable to the reduction of N_2O, thus aggravating N_2O emissions (Bowen et al., 2020). We found that N addition decreased *nosZ*abundance to some extent, and N_2O flux was negatively correlated with *nosZ* . Thus, the lower *nosZ* abundance may be responsible for the increased N_2O emission in denitrification. Although changes in *nirS* and *nirK* had no effect on N_2O emission, the high ratios of (*nirS+nirK*)/*nosZ* induced N_2O emissions (Table 3). Given that the high ratios of (*nirS+nirK*)/*nosZ*represented a strong N_2O emissions capacity (Hu et al., 2015), the *nirS* - or *nirK* -containing denitrifiers cannot be ignored in future work on N cycling.

Conclusions

Our field experiments show that the alpine steppe was a net source of N_2O. Our results also demonstrate that N addition intensified N_2O emission, while altered precipitation and its interaction with N addition did not affect N_2O emission. Changes in N_2O flux were attributable to the synergy between soil abiotic parameters and functional microorganisms. Most importantly, however, altered precipitation attenuated the response of N_2O flux to N addition, thereby mitigating N_2O emissions. This study provides necessary insight to predict the future responses of N_2O emissions to long-term N deposition and precipitation alterations in alpine grasslands.

Acknowledgments

This work was jointly supported by the Natural Science Foundation of Qinghai Province (2019-ZJ-910), the

National Key Research and Development Program of China (2019YFC0507404), and International Exchange and Cooperation Project of Qinghai Province (2019-HZ-807).

Conflict of Interest

The authors have no conflict of interest.

Author Contributions

Y.Y and G.Y.Z designed experiments. Y.Y., Y.M.X., C.B.L., and B.W. participated in field data collection. Y.Y. processed and analyzed the data. Y.Y wrote the manuscript with G.Y.Z., and Y.H.G.

Data Accessibility Statement

We had uploaded our data to the data to the Dryad.

References

Austin, A. T., Yahdjian, L., Stark, J. M., Belnap, J., Porporato, A., Norton, U., . . . Schaeffer, S. M. (2004). Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* ,*141* (2), 221-235. doi:10.1007/s00442-004-1519-1

Banerjee, S., Helgason, B., Wang, L. F., Winsley, T., Ferrari, B. C., & Siciliano, S. D. (2016). Legacy effects of soil moisture on microbial community structure and N_2O emissions. *Soil Biology & Biochemistry* , *95* , 40-50. doi:10.1016/j.soilbio.2015.12.004

Bowen, H., Maul, J. E., Cavigelli, M. A., & Yarwood, S. (2020). Denitrifier abundance and community composition linked to denitrification activity in an agricultural and wetland soil.*Applied Soil Ecology* , *151* , 13. doi:10.1016/j.apsoil.2020.103521

Brin, L. D., Goyer, C., Zebarth, B. J., Burton, D. L., & Chantigny, M. H. (2019). Linking changes in snow cover with microbial nitrogen cycling functional gene abundance and expression in agricultural soil.*FEMS Microbiology Ecology* , *95* (7), 14. doi:10.1093/femsec/fiz073

Brown, J. R., Blankinship, J. C., Niboyet, A., van Groenigen, K. J., Dijkstra, P., Le Roux, X., . . . Hungate, B. A. (2012). Effects of multiple global change treatments on soil N_2O fluxes.*Biogeochemistry* , *109* (1-3), 85-100. doi:10.1007/s10533-011-9655-2

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? P*hilosophical Transactions of the Royal Society B-Biological Sciences* , *368* (1621), 13. doi:10.1098/rstb.2013.0122

Cai, Y. J., Chang, S. X., Ma, B., & Bork, E. W. (2016). Watering increased DOC concentration but decreased N_2O emission from a mixed grassland soil under different defoliation regimes.*Biology and Fertility of Soils* , *52* (7), 987-996. doi:10.1007/s00374-016-1135-3

Chen, W. W., Zheng, X. H., Chen, Q., Wolf, B., Butterbach-Bahl, K., Bruggemann, N., & Lin, S. (2013). Effects of increasing precipitation and nitrogen deposition on CH_4 and N_2O fluxes and ecosystem respiration in a degraded steppe in Inner Mongolia, China. *Geoderma* , *192* , 335-340. doi:10.1016/j.geoderma.2012.08.018

Cregger, M. A., McDowell, N. G., Pangle, R. E., Pockman, W. T., & Classen, A. T. (2014). The impact of precipitation change on nitrogen cycling in a semi-arid ecosystem. *Functional Ecology* ,*28* (6), 1534-1544. doi:10.1111/1365-2435.12282

Curtis, C. J., Emmett, B. A., Reynolds, B., & Shilland, J. (2006). How important is N_2O production in removing atmospherically deposited nitrogen from UK moorland catchments? *Soil Biology and Biochemistry* , *38* (8), 2081-2091. doi:10.1016/j.soilbio.2006.01.013

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'Callaghan, M., Bowatte, S., & He, J. Z. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience* , *2* (9),

621-624. doi:10.1038/ngeo613

Dijkstra, F. A., Morgan, J. A., Follett, R. F., & Lecain, D. R. (2013). Climate change reduces the net sink of CH_4 and N_2O in a semiarid grassland. *Global Change Biology* , *19* (6), 1816-1826. doi:10.1111/gcb.12182

Ding, J. Z., Li, F., Yang, G. B., Chen, L. Y., Zhang, B. B., Liu, L., . . . Yang, Y. H. (2016). The permafrost carbon inventory on the Tibetan Plateau: a new evaluation using deep sediment cores. *Global Change Biology* , *22* (8), 2688-2701. doi:10.1111/gcb.13257

Fu, G., & Shen, Z. X. (2017). Response of alpine soils to nitrogen addition on the Tibetan Plateau: A meta-analysis. *Applied Soil Ecology* , *114* , 99-104. doi:10.1016/j.apsoil.2017.03.008

Ge, G., Shi, Z. J., Yang, X. H., Hao, Y. G., Guo, H., Kossi, F., . . . Liu, J. H. (2017). Analysis of Precipitation Extremes in the Qinghai-Tibetan Plateau, China: Spatio-Temporal Characteristics and Topography Effects. *Atmosphere* , *8* (7), 16. doi:10.3390/atmos8070127

Geng, F. Z., Li, K. H., Liu, X. J., Gong, Y. M., Yue, P., Li, Y. G., & Han, W. X. (2019). Long-term effects of N deposition on N_2O emission in an alpine grassland of Central Asia.*Catena* , *182* , 6. doi:10.1016/j.catena.2019.104100

Gong, J., Li, J. Y., Yang, J. X., Li, S. C., & Tang, W. W. (2017). Land Use and Land Cover Change in the Qinghai Lake Region of the Tibetan Plateau and Its Impact on Ecosystem Services. *International Journal of Environmental Research and Public Health* , *14* (7), 21. doi:10.3390/ijerph14070818

Griffis, T. J., Chen, Z. C., Baker, J. M., Wood, J. D., Millet, D. B., Lee, X. H., . . . Turner, P. A. (2017). Nitrous oxide emissions are enhanced in a warmer and wetter world. *Proceedings of the National Academy of Sciences of the United States of America* , *114* (45), 12081-12085. doi:10.1073/pnas.1704552114

Harpole, W. S., Potts, D. L., & Suding, K. N. (2007). Ecosystem responses to water and nitrogen amendment in a California grassland.*Global Change Biology* , *13* (11), 2341-2348. doi:10.1111/j.1365-2486.2007.01447.x

Homyak, P. M., Allison, S. D., Huxman, T. E., Goulden, M. L., & Treseder, K. K. (2017). Effects of Drought Manipulation on Soil Nitrogen Cycling: A Meta-Analysis. *Journal of Geophysical Research :Biogeosciences* , *122* (12), 3260-3272. doi:10.1002/2017jg004146

Hu, H. W., Chen, D., & He, J. Z. (2015). Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. *FEMS Microbiol Reviews* , *39* (5), 729-749. doi:10.1093/femsre/fuv021

Knapp, A. K., Fay, P. A., Blair, J. M., Collins, S. L., Smith, M. D., Carlisle, J. D., . . . McCarron, J. K. (2002). Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland.*Science* , *298* (5601), 2202-2205. doi:10.1126/science.1076347

Kruger, M., Potthast, K., Michalzik, B., Tischer, A., Kusel, K., Deckner, F. F. K., & Herrmann, M. (2021). Drought and rewetting events enhance nitrate leaching and seepage-mediated translocation of microbes from beech forest soils. *Soil Biology & Biochemistry* ,*154* , 11. doi:10.1016/j.soilbio.2021.108153

Li, C., Zheng, Z., Peng, Y., Nie, X., Yang, L., Xiao, Y., & Zhou, G. (2019). Precipitation and nitrogen addition enhance biomass allocation to aboveground in an alpine steppe. *Ecology and Evolution* ,*9* (21), 12193-12201. doi:10.1002/ece3.5706

Li, L. F., Zheng, Z. Z., Wang, W. J., Biederman, J. A., Xu, X. L., Ran, Q. W., . . . Wang, Y. F. (2020). Terrestrial N_2O emissions and related functional genes under climate change: A global meta-analysis. *Global Change Biology* , *26* (2), 931-943. doi:10.1111/gcb.14847

Lin, L., Zhu, B., Chen, C. R., Zhang, Z. H., Wang, Q. B., & He, J. S. (2016). Precipitation overrides warming in mediating soil nitrogen pools in an alpine grassland ecosystem on the Tibetan Plateau.*Scientific Reports* , *6* , 9. doi:10.1038/srep31438

Linton, N. F., Machado, P. V. F., Deen, B., Wagner-Riddle, C., & Dunfield, K. E. (2020). Long-term diverse rotation alters nitrogen cycling bacterial groups and nitrous oxide emissions after nitrogen fertilization. *Soil Biology & Biochemistry* , *149* , 12. doi:10.1016/j.soilbio.2020.107917

Liu, X. C., Qi, Y. C., Dong, Y. S., Peng, Q., He, Y. T., Sun, L. J., . . . Cao, C. C. (2014). Response of soil N2O emissions to precipitation pulses under different nitrogen availabilities in a semiarid temperate steppe of Inner Mongolia, China. *Journal of Arid Land* ,*6* (4), 410-422. doi:10.1007/s40333-013-0211-x

Liu, Y. W., Xu, R., Xu, X. L., Wei, D., Wang, Y. H., & Wang, Y. S. (2013). Plant and soil responses of an alpine steppe on the Tibetan Plateau to multi-level nitrogen addition. Plant and Soil, 373(1-2), 515-529. doi:10.1007/s11104-013-1814-x

Lu, C., & Tian, H. (2007). Spatial and temporal patterns of nitrogen deposition in China: Synthesis of observational data. *Journal of Geophysical Research* , *112* (D22). doi:10.1029/2006jd007990

Lu, M., Yang, Y. H., Luo, Y. Q., Fang, C. M., Zhou, X. H., Chen, J. K., . . . Li, B. (2011). Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytologist* , *189* (4), 1040-1050. doi:10.1111/j.1469-8137.2010.03563.x

Lu, X.-T., & Han, X.-G. (2009). Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China.*Plant and Soil* , *327* (1-2), 481-491. doi:10.1007/s11104-009-0078-y

Lu, X., Bottomley, P. J., & Myrold, D. D. (2015). Contributions of ammonia-oxidizing archaea and bacteria to nitrification in Oregon forest soils. *Soil Biology and Biochemistry* , *85* , 54-62. doi:10.1016/j.soilbio.2015.02.034

Mao, C., Kou, D., Chen, L. Y., Qin, S. Q., Zhang, D. Y., Peng, Y. F., & Yang, Y. H. (2020). Permafrost nitrogen status and its determinants on the Tibetan Plateau. *Global Change Biology* , *26* (9), 5290-5302. doi:10.1111/gcb.15205

Mushinski, R. M., Payne, Z. C., Raff, J. D., Craig, M. E., Pusede, S. E., Rusch, D. B., . . . Phillips, R. P. (2021). Nitrogen cycling microbiomes are structured by plant mycorrhizal associations with consequences for nitrogen oxide fluxes in forests. *Global Change Biology* , *27* (5), 1068-1082. doi:10.1111/gcb.15439

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils A review. *Chemie Der Erde-Geochemistry* , *76* (3), 327-352. doi:10.1016/j.chemer.2016.04.002

Peng, Y., Li, F., Zhou, G., Fang, K., Zhang, D., Li, C., . . . Yang, Y. (2017). Linkages of plant stoichiometry to ecosystem production and carbon fluxes with increasing nitrogen inputs in an alpine steppe. *Global Change Biology* , *23* (12), 5249-5259. doi:10.1111/gcb.13789

Peng, Y. F., Wang, G. Q., Li, F., Zhou, G. Y., Yang, G. B., Fang, K., . . . Yang, Y. H. (2018). Soil Temperature Dynamics Modulate N_2O Flux Response to Multiple Nitrogen Additions in an Alpine Steppe. *Journal of Geophysical Research-Biogeosciences* ,*123* (10), 3308-3319. doi:10.1029/2018jg004488

Ravishankara, A. R., Daniel, J. S., & Portmann, R. W. (2009). Nitrous Oxide (N_2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* , *326* (5949), 123-125. doi:10.1126/science.1176985

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., & Zechmeister-Boltenstern, S. (2010). Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. *European Journal of Soil Science* , *61* (5), 683-696. doi:10.1111/j.1365-2389.2010.01277.x

Scurlock, J. M. O., Johnson, K., & Olson, R. J. (2002). Estimating net primary productivity from grassland biomass dynamics measurements.*Global Change Biology* , *8* (8), 736-753. doi:10.1046/j.1365-2486.2002.00512.x

Shi, F. S., Chen, H., Chen, H. F., Wu, Y., & Wu, N. (2012). The combined effects of warming and drying suppress CO_2 and N_2O emission rates in an alpine meadow of the eastern Tibetan Plateau. *Ecological*

Research , *27* (4), 725-733. doi:10.1007/s11284-012-0950-8

Treseder, K. K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters* ,*11* (10), 1111-1120. doi:10.1111/j.1461-0248.2008.01230.x

Tzanakakis, V. A., Taylor, A. E., Bakken, L. R., Bottomley, P. J., Myrold, D. D., & Dorsch, P. (2019). Relative activity of ammonia oxidizing archaea and bacteria determine nitrification-dependent N_2O emissions in Oregon forest soils. *Soil Biology & Biochemistry* , *139* , 6. doi:10.1016/j.soilbio.2019.107612

Wang, G. X., Bai, W., Li, N., & Hu, H. C. (2011). Climate changes and its impact on tundra ecosystem in Qinghai-Tibet Plateau, China.*Climatic Change* , *106* (3), 463-482. doi:10.1007/s10584-010-9952-0

Wu, X., Wang, F. F., Li, T., Fu, B. J., Lv, Y. H., & Liu, G. H. (2020). Nitrogen additions increase N_2O emissions but reduce soil respiration and CH_4 uptake during freeze-thaw cycles in an alpine meadow. *Geoderma* , *363* , 9. doi:10.1016/j.geoderma.2019.114157

Xiao, Y., Li, C., Yang, Y., Peng, Y., Yang, Y., & Zhou, G. (2020). Soil Fungal Community Composition, Not Assembly Process, Was Altered by Nitrogen Addition and Precipitation Changes at an Alpine Steppe.*Frontiers in Microbiology* , *11* , 579072. doi:10.3389/fmicb.2020.579072

Yan, Y. L., Ganjurjav, H., Hu, G. Z., Liang, Y., Li, Y., He, S. C., . . . Gao, Q. Z. (2018). Nitrogen deposition induced significant increase of N_2O emissions in an dry alpine meadow on the central Qinghai-Tibetan Plateau. *Agriculture Ecosystems & Environment* ,*265,* 45-53. doi:10.1016/j.agee.2018.05.031

Yang, G. B., Peng, Y. F., Marushchak, M. E., Chen, Y. L., Wang, G. Q., Li, F., . . . Yang, Y. H. (2018). Magnitude and Pathways of Increased Nitrous Oxide Emissions from Uplands Following Permafrost Thaw.*Environmental Science & Technology* , *52* (16), 9162-9169. doi:10.1021/acs.est.8b02271

Zhang, Y., Zhang, N., Yin, J. J., Yang, F., Zhao, Y. X., Jiang, Z. Q., . . . Hu, S. J. (2020). Combination of warming and N inputs increases the temperature sensitivity of soil N_2O emission in a Tibetan alpine meadow. *Science of the Total Environment* ,*704* , 11. doi:10.1016/j.scitotenv.2019.135450

Zhu, X. X., Luo, C. Y., Wang, S. P., Zhang, Z. H., Cui, S. J., Bao, X. Y., . . . Zhou, Y. (2015). Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau. *Agricultural and Forest Meteorology* , *214* , 506-514. doi:10.1016/j.agrformet.2015.09.00

Table 1 The targeted genes, primers pairs, and sequences for PCR reactions

Target gene	**Primer name**	**(5'-3')**	**Product size (bp)**
Archaeal *amoA*	Arch-amoAF	STAATGGTCTGGCTTAGACG	635
	Arch-amoAR	GCGGCCATCCATCTGTATGT	
Bacterial *amoA*	bamoA1F	GGGGTTTCTACTGGTGGT	491
	bamoA2R	CCCCTCKGSAAAGCCTTCTTC	
nirS	cd3aF	GTSAACGTSAAGGARACSGG	425
	R3cdR	GASTTCGGRTGSGTCTTGA	
nirK	FlaCuF	ATCATGGTSCTGCCGCG	471
	R3CuR	GCCTCGATCAGRTTGTGGTT	
nosZ	CHEND-nosZ-1126F	GGGCTBGGGCCRTTGCA	255
	CHEND-nosZ-1381R	GAAGCGRTCCTTSGARAACTTG	

Table 2 Effects of nitrogen addition and altered precipitation on soil environment factors and plant properties

Treatment	**Soil temperature**	**Soil moisture**	**Soil pH**	**Soil NH_4^+-N**	**Soil NO_3^--N**	**AGB**
	(°C)	**(%)**		**mg kg^{-1}**	**mg kg^{-1}**	**g m^{-2}**

Treatment	**Soil temperature**	**Soil moisture**	**Soil pH**	**Soil NH_4^+-N**	**Soil NO_3^--N**	**AGB**
NP(control)	12.84±0.24ab	21.58±0.81b	8.20±0.03a	20.53±1.82b	41.64±2.96d	117.31±15.97
NP-??	13.08±0.30ab	18.86±0.35c	8.22±0.06a	14.33±0.53c	48.24±2.92cd	107.25±4.31c
NP+??	13.08±0.31ab	23.55±0.67a	8.15±0.04ab	20.38±0.69b	42.25±3.16d	136.00±4.75b
N+P	12.34±0.13b	21.65±0.45b	8.08±0.02bc	21.65±1.11ab	60.26±4.53ab	161.67±17.10
N+P-??	13.12±0.24a	18.24±0.17c	7.99±0.04c	19.17±0.91b	68.40±4.89a	182.67±12.68
N+P+??	12.34±0.11b	24.81±0.21a	8.13±0.02ab	23.92±0.67a	53.91±3.27bc	253.54±42.30
Two-way ANOWA	*Two-way ANOWA*					
N	0.169	0.714	**0.013**	**0.036**	**0.000**	**0.018**
P	**0.021**	**0.000**	0.631	**0.003**	0.100	**0.008**
N×P	**0.021**	0.293	0.048	0.072	0.562	0.145
Block	0.268	0.773	0.577	0.601	0.690	0.361

N: nitrogen treatment; P: altered precipitation treatment; N×P: combination of nitrogen addition and altered precipitation. AGB: aboveground biomass; BGB: belowground biomass. Data are represented by mean ± SE (n = 5). Bold values indicate that treatment effects are significant ($P < 0.05$). Different lowercase letters indicate significant difference ($P < 0.05$). The block was used as a random factor in the two-way ANOVA.

Table 3 Pearson correlation analysis of the relationships between N_2O flux and functional genes abundance (n = 30 for N_2O flux and functional gene, significance: $^*P < 0.\ 05$; $^{**}\ P < 0.01$)

		N_2O flux	**N_2O flux**
		correlation coefficients (r)	
Nitrifiers	**AOA**	–0.435*	
	AOB	0.428*	
Denitrifiers	nirS	–0.188	
	nirK	–0.018	
	nosZ	–0.484**	
	(nirS+nirK)/nosZ	0.408*	

Note: the ratio of$(nirS + nirK)/nosZ$ indicates changes in the denitrifying microbial community.

Figure legends

Figure 1 Platform for nitrogen addition and altered precipitation experiments. (A) Experimental treatments: 1, NP-; 2, NP (CK); 3, NP+ ; 4, N+P-; 5, N+P; 6, N+P+. N: ambient N deposition; P: ambient precipitation; N+: N addition; P-: 50% reduced precipitation; P+: 50% increased precipitation. (B) Experimental site.

Figure 2 Seasonal dynamics of N_2O flux and (B) seasonal average N_2O flux under different conditions. (C) Changes in seasonal average N_2O flux (compared with control) under different treatments. (D) Average N_2O flux during the peak period of plant growth. N: nitrogen treatment; P: altered precipitation treatment; N×P: combination of nitrogen addition and altered precipitation. Different letters indicate a significant difference ($P < 0.05$). Error bars represent the standard error.

Figure 3 Effects of nitrogen addition and altered precipitation on abundances of functional genes. (A) ammonia-oxidizing archaea (AOA); (B) ammonia-oxidizing bacteria (AOB); (C) *nirS* ; (D) *nirK* ; (E) *nosZ* . N: nitrogen treatment; P: altered precipitation treatment; N×P: combination of nitrogen addition and altered precipitation. Different letters indicate a significant difference ($P < 0.05$). Error bars represent the standard errors of the means (n = 5).

Figure 4 Relationships between the N_2O flux and abiotic properties. Regression lines are only shown when significant ($P< 0.05$).