Seasonal timing of extreme drought regulates N₂O fluxes in a semiarid grassland

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ABSTRACT

Terrestrial ecosystems are important sources of nitrous oxide (N₂O), a powerful greenhouse gas which can be strongly impacted by increasing droughts in association with climate change. However, detailed information on whether and how drought timing regulates N₂O fluxes is still lacking. Here, we conducted a 3-year field experiment on a semiarid grassland in which extreme drought was imposed in either early-, mid-, or late-growing seasons repeatedly from 2014 to 2016. We found that early drought affected N₂O emission with high interannual variability (increased, decreased and unchanged N₂O emission in 2014, 2015, and 2016, respectively), coincident with changes in inorganic nitrogen (SIN), dissolve organic carbon (DOC), microbial biomass carbon (MBC), and soil functional genes (bacterial amoA, nirK, nirS, and nosZ). However, middle drought consistently suppressed N₂O emissions due to simultaneous decreases in MBC, DOC and the abundances of archaeal amoA, nirK, and narG genes, causing the largest reduction in N₂O emissions across the three years. In contrast, late drought had little effect on N₂O fluxes, even though DOC and SIN decreased and the abundance of nirK, nirS, and nosZ increased. As a result, soil organic C and mineral N availability and functional gene abundances were not always robust factors for predicting N₂O emissions under droughts across all treatments, except for abundance of AOA and nosZ. Our results highlight the vital role of seasonal timing in regulating the response of N₂O emissions to extreme droughts.

Atmospheric nitrous oxide (N₂O), a long-lived greenhouse gas, has increased by 20 percent in the atmosphere since 1750, representing a major contribution to global warming and associated climate extremes, including droughts (Berg et al., 2016; Sherwood and Fu, 2014). Terrestrial ecosystems are the single largest source of N₂O (Tian et al., 2020) via the mechanisms of nitrification and denitrification. Although the nitrogen (N) cycle including N₂O production is affected by elevated atmospheric CO₂ and increasing temperature, drought is a dominant global change consequence impacting soil microorganisms and the associated N transformation processes (Sénéca et al., 2020). Hence, understanding the effects of droughts on N₂O flux in terrestrial ecosystems could largely improve the assessment of N₂O budget in the atmosphere and its feedback to climate change.

Numerous studies have examined drought effects on N₂O flux, but the outcomes have varied from positive (e.g. Wieder et al., 2011) to negative (e.g. Bu et al., 2018). Key mechanisms identified include drought impacts on soil water and nutrient availability as well as community sizes of nitrifiers and denitrifiers (Li et al., 2022a). Seasonal timing of drought events within a growing season may be a potential explanation for the wide range of results reported, given that seasonal timing strongly regulates drought effects on other ecosystem attributes such as plant biomass and CO₂ fluxes (Li et al., 2022b). Rowlings et al. (2015) found that N₂O flux was usually lower and less sensitive to changes in precipitation/soil moisture in low temperature conditions.
than in high temperature conditions. Thus, it could be expected that N$_2$O flux may be less affected by drought occurring in early or late stages of the growing season than in the middle of the growing season. Besides, droughts in the middle of the growing season, when the temperature and evapotranspiration were higher, may cause more severe soil water stress (Huang et al., 2018), which may lead to larger suppression effects on microbial activities and resultant N$_2$O emissions. Nevertheless, early-season drought may exert larger ecological effects if the effects persist throughout the remainder of the growing season (Zhou et al., 2013). To date, empirical evidence of whether and how seasonal timing of drought influences N$_2$O flux response is scarce.

To identify how seasonal timing regulates the effects of drought on N$_2$O flux, we conducted a 3-year manipulative study in which an extreme drought (a rain-free period of 30 days) was imposed in the early-, mid-, or late-growing season in a semiarid unfertilized grassland (43°20'N, 116°400'E, 1200 m a.s.l) in Inner Mongolia from 2014 to 2016, using a randomized block layout with 3 replicates as described in Li et al. (2022b). Given ecological processes in this grassland were largely limited by precipitation, we hypothesized that: (i) carbon (C) and N substrates as well as the abundance of nitrifier and denitrifier groups would be reduced by all three drought events as a consequence of lower water availability, thereby decreasing N$_2$O emissions; and (ii) drought in the middle growing season would cause the largest reduction in N$_2$O emissions because N$_2$O emissions are usually higher during this period due to warmer and wetter conditions; and warm temperatures increase evapotranspiration, escalating drought effects on soil moisture stress.

N$_2$O fluxes were measured with the chamber and gas chromatograph method during each growing season. Cumulative N$_2$O fluxes over the measurement periods were calculated by multiplying the average flux measured on two consecutive dates by the time interval, and then

![Fig. 1.](image-url)
summed. Soil water content (SWC) of the top 20 cm was simultaneously measured. Above-ground biomass (AGB) and belowground biomass (BGB) were estimated at the end of treatments. Soils (0–20 cm) were sampled in pairs from drought and ambient control plots at the end of each drought period. Soil inorganic N content (SIN; NH₄(BGB) and microbial biomass C (MBC) and dissolved organic C (DOC) were also measured. Soil DNA was extracted and copy numbers of archaeal amoA (AOA), bacterial amoA (AOB), narG, nirK, nirS, and nosZ genes were quantified.

The natural log of the response ratios (the ratios of response variables under drought to those under ambient rainfall) and confidence intervals were calculated to assess the responses of soil properties and soil functional genes to drought treatments. Mixed-effects models were employed to test the individual and interactive effects of seasonal drought timing and year on cumulative N₂O fluxes while plots were included as a random effect using package lme4 in R v.3.4.4. Correlations among response ratio of above-mentioned factors were explored using package corrplot in R (Detailed information of the study site, experimental design, variable measurement, and statistical analysis were described in Text S1).

In contrast to our first hypothesis, droughts did not always result in reductions in N₂O emissions. We found that only the middle drought consistently reduced N₂O emissions across the three years (P = 0.08, Fig. 1a), mainly during the treatment periods (F. S2). However, early drought resulted in increased, decreased and unchanged N₂O emissions in 2014, 2015 and 2016, respectively (P = 0.03 for Early × year interaction, Fig. 1a) while late drought had little effect on N₂O emissions in any year (P = 0.60, Fig. 1a). Accordingly, middle drought caused the largest reduction in cumulative N₂O emissions over all three growing seasons, supporting the second hypothesis. The results suggested that responses of N₂O emissions to droughts largely depended on seasonal timing.

Across the three years, middle drought significantly reduced SIN, DOC, MBC, and the abundance of AOA, AOB, narG, and nirK (Fig. 1b and F. S3). The potential mechanisms would be that middle drought-induced decreases in soil water availability decreased the size of microbe community, the organic matter decomposition and N mineralization, and C and N availability. Additionally, middle drought largely increased root shoot ratio, reflected by decreases in AGB but increases in BGB (Fig. 1b), which might potentially increase SIN uptake by plants, resulting in the largest decrease of SIN in response to middle drought. Furthermore, low soil water availability suppressed both nitrifier (AOA and AOB) and denitrifier (narG and nirK) growth or even led to death of these microorganisms (Fig. 1b and F. S3b). Consequently, decreases in C and N substrates as well as the abundance of nitrifier and denitrifier groups jointly led to reductions in N₂O emissions under middle drought (Chen et al., 2019; Li et al., 2022), supporting the first hypothesis.

However, the late drought resulted in lower SWC, MBC, SIN, and DOC but not lower N₂O emissions compared with the ambient control (Fig. 1a and b). The poor correlation of soil water and C & N substrate availability to N₂O emission in late drought might be attributed to the possible restriction of N₂O emission by low temperature in the late growing season (14.3 °C and 19.9 °C across the three years during late drought and middle drought, respectively. F. S4), which led to the insensitivity of N₂O fluxes to the changes in soil water or/and C & N substrates. Our results were consistent with the finding that responses of N₂O fluxes to soil moisture changes were restrained by low soil temperatures in temperate grassland (Chen et al., 2003). Likewise, Wang et al. (2011) found that variation in soil water-filled pore space did not cause corresponding variation in N₂O emissions when air temperature was lower than 10 °C in an Australian subtropical cropland. Collectively, these studies indicate that N₂O flux response to water or/nutrient variation is temperature-dependent.

Interestingly, early drought effects on N₂O emission varied from negative, neutral, to positive in different years (Fig. 1a). The possible reason for the opposite responses is that early drought caused positive effects on MBC, DOC and SIN in 2014 but negative effects on these three variables in 2015 (F. S3a) even though the main effects overall were not significant (Fig. 1b). In addition, interannual variability in N₂O emissions in response to early drought might be related to changes in functional gene abundances, with increased AOB, nirK, and nirS and decreased nosZ in 2014 but on the contrary in 2015 (F. S3). This suggests that processes of ammonia oxidation to hydroxylamine, nitrite reduction to nitric oxide, N₂O reduction to N₂ could have changed correspondingly (Kuyper et al., 2018). However, current data could not explain the large interannual variation in responses of MBC, DOC and SIN to early droughts. More frequent measurements during the treatments in future may provide an insight into the underlying mechanisms.

All three droughts consistently decreased SWC over the three years as expected (Fig. 1b), but the drought effects on N₂O emissions varied across different seasonal timing and years, resulting in an unexcepted negative correlation between N₂O emission and SWC across all the treatments (Fig. 1c). Overall, both soil organic C and mineral N availability and nitrifier/denitrifier groups played vital roles in regulating N₂O emission in response to early and middle drought although the relative importance of factors was different. Conversely, N₂O emissions were not closely correlated to the above-mentioned factors under late drought. Thus, soil C and N substrate contents and functional gene abundances were not always robust factors for predicting N₂O emission dynamics in the face of droughts across all treatments in this study, except for abundance of AOA and nosZ (Fig. 1c). Instead, seasonal timing could strongly affect the relationships of N₂O emissions to soil substrates and functional microbes in the face of droughts. Besides, osmolyte accumulation and catabolism, rather than genomic and transcriptomic indicators or soil substrates, were considered to be key drivers for N₂O hot moments under droughts (Barrat et al., 2022). Across all treatments, AGB and BGB had no direct correlations with N₂O emission but had strong relationships with soil C and N substrate contents and functional gene abundances (Fig. 1c). Thus, plant biomass may potentially affect N₂O emissions through impacting soil N uptake and microbial activities.

In conclusion, the middle drought consistently reduced N₂O emissions, while the late drought had negligible effects. Meanwhile, the early drought effects on N₂O flux had large interannual variability. Soil C and N substrates availability as well as the abundance of nitrifiers and denitrifiers co-regulated N₂O dynamics during the early and the middle droughts but not the late drought due to limitation by low temperature. Our results suggest that responses of N₂O flux to drought depend on seasonal timing of drought, and an extreme drought event at the peak plant growth stage had the largest negative effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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