Impacts of long-term nitrogen addition on nitrous oxide in a temperate grassland

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Abstract: Atmospheric nitrogen (N) deposition has increased dramatically due to increased human activities since the industrial revolution. However, it is still unclear what the responses of soil nitrous oxide (N_2O) is to long-term elevated N deposition in a temperate grassland. Here, we conducted an *in situ* field experiment to investigate these responses to long-term high N addition on a temperate steppe in Inner Mongolia, China, from April 2017 to October 2018. Soil N_2O emissions significantly increased by long-term N addition, use of structural equation modeling (SEM) showed that topsoil (0-5 cm) NH_4^+-N content was the most important limiting factor for N_2O emission. Our results indicate that long-term high N addition showed a significantly increase in N_2O emission in this temperate grassland.

1 Introduction

Anthropogenic nitrogen (N) enrichment and global warming are primary components of global change. N deposition to terrestrial ecosystems has increased from 17.4 Tg N yr^{-1} in late 1860s to 187 Tg N yr-1 in the 2005, and is expected to increase to 200 Tg N·yr^{-1} in 2050[1]. This in natural ecosystems has many negative effects on ecosystem services: biodiversity loss, nutrient imbalance, and increased greenhouse gases (GHGs) emissions[2,3]

In Inner Mongolia grassland occupies a large area of northern China and 13% of the global grassland area[4]. However, models of N_2O emission with the N deposition shows N_2O emission increased as exponential and unimodal response[5,6], when the N reached biological threshold, N_2O emission transitions to the N saturation stage, at this stage, higher N could not increase N_2O emission, including in some temperate and subtropical grasslands[7].

N saturation model believes that in natural ecosystems, enhanced N deposition would produce more soil N_2O[8], but recent study on steppe showed that influence of significant climatic factors on N_2O depends on the ability to alleviate N_2O emission limited by soil "nitrogen deficiency"[9,10]. Therefore, the effects of long-term N addition on N_2O emission are still uncertain. In another way, N enrichment was always accompanied by the changes in soil properties like soil acidification, available N and C states, as well as vegetation of ecosystem. Therefore, it is also valuable to find the complex response of N_2O fluxes of soil to variations in multiple indirect factors caused by N deposition of grassland[11].

Therefore, we set up a long-term *in stiu* experiment in Inner Mongolia, China in 2005, which has caused severe soil acidification after 13 years of N fertilization, and significant changes in soil properties[12]. To assess how these changes have affected soil N_2O emission, we measured N_2O fluxes with high frequency from May 2017 to October 2018, with the objectives of: (i) quantifying the response of the N_2O emission to long-term increased N addition, and (ii) identifying the main controlling factors of N_2O caused by long-term increased N addition.

2 Experimental methods

2.1 Site description

This study was conducted in a semi-arid temperate grassland in Duolun County (116°17E, 42°02N, elevation 1324 m), northeastern of Xilingol of Inner Mongolia, China. The rainfall and air temperature average 316 mm and 3.3°C between 2006 to 2016, and 81% of total

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precipitation in growing season (May 10th to August 30th; Fig.1(a)). The local N deposition has increased to 15.4 kg N ha^{-1} yr^{-1} in 2015, consist with 58% as $NH_4^+$and 42% as NO_3^-. 0-10 cm layer soil pH and bulk density are 7.12 and 1.31 g cm^{-1}. Soil total N and total P contents are 12.3 g kg^{-1} and 1.3 g kg^{-1}, organic carbon and C:N are 0.28 g kg^{-1} and 21, respectively.

2.2 Experimental treatments

The experiment was designed with three N addition treatments of four replicates that were arranged randomly. Plot area was 5×5 m with a 1 m wide buffer zone. Ammonium nitrate was applied as foliar fertilizer at simulated N deposition rates of N_0 (0 kg N ha^{-1} yr^{-1}), N_{30} (30 kg N ha^{-1} yr^{-1}) and N_{60} (60 kg N ha^{-1} yr^{-1}). The fertilizer was divided into 2 equal portions, and sprayed evenly in mid-June and mid-July every year (15th June and 17th July in 2017). N_2O was measured from May 2017 to October 2018.

2.3 Measurements method

Static closed chamber technique was used to measure N_2O fluxes. The chambers were made of removable top and previously-installed stainless steel (into soil for 10 cm). Sampling was conducted between 10:00 am and 12:00 pm. Gases fluxes were collected once or twice a week. Gas samples were injected into 50 ml, and were analyzed using a gas chromatograph (GC; Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with an electron capture detector for determination of N_2O[13,14].

Fresh soil samples were extracted in 0.01 mol L^{-1} $CaCl_2$ solution and shaken for 1 hour. The suspension was analyzed for nitrate and ammonium concentration using a continuous-flow auto-analyzer (Seal AA3, Germany). Dissolved organic carbon (DOC) was extracted from the fresh soil sub-samples by shaking with deionized water, and filtered at 0.45 μm and the filtrate analyzed with a TOC analyzer (multi N/C 3100, Jena, Germany). Soil pH was measured using a pH Meter (Seven Easy, Mettler-Toledo, Switzerland). Soil water content and temperature in the 0-5 cm layer were measured using a TDR probe.

3 Statistical analyses

Treatment enrichment effects on soil pH, NO_3^--N, NH_4^+-N content, DOC, total emission of N_2O were assessed using a least significant difference test (LSD, $P < 0.05$). In addition, the relationships between fluxes of N_2O with soil moisture, soil NO_3^--N and NH_4^+-N contents, pH were analyzed using structural equation models (SEM). One-way repeated measures analysis of variance was used to analysis. All statistical analyses were conducted using SPSS (version 20.0) with statistically significant differences set at $p < 0.05$. SEM analyses were carried out using AMOS 22.0. All figures were drawn using Sigmaplot (version 13.0).

4 Results

Long-term high N addition increased significnatly soil NO_3^--N, DOC content (except in N_{30} Treatment), and decreased pH significantly by long-term N addition, but has no effect on NH_4^+ content.

Small N_2O emissions were observed from N_0 plots, ranging from 0.59 to 16.32 μg N m^{-2} h^{-1} (average annual 4.45 ± 0.34 μg N m^{-2} h^{-1}) (Fig. 1b). Long-term increased N addition significantly increased the N_2O emissions ($P < 0.001$) (Fig 1b; Table 3), with average annual emissions from N_{30} and N_{60} plots of 9.49 ± 0.79 and 20.35 ± 3.23 μg N m^{-2} h^{-1}, respectively (Fig 1b). The SEM showed that soil moisture had a significantly positive correlation with soil N_2O emissions both directly and indirectly, and that soil NH_4^+-N content was the most important influencing factor (Fig. 2). Notably, neither soil pH or DOC had a significant impact on N_2O emissions (Fig. 2).

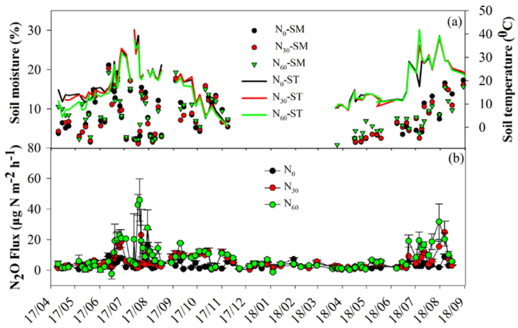
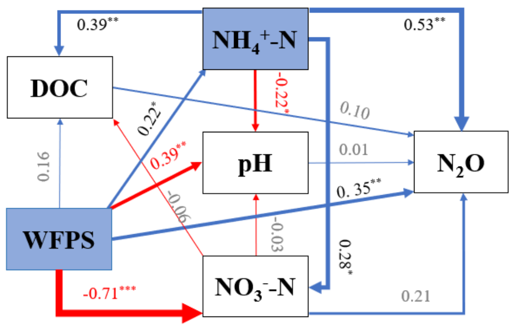


Figure 1. The Variation from April 2017 to October 2018 in soil moisture and soil temperature (°C) (a), and fluxes in N_2O (b) (mean, n=4) in an Inner Mongolian grassland.



χ2=3.068, p=0.794, df=1, RMSEA = 0.00, AIC = 52.07

Figure 2. The effects on N_2O emission (n=28) of soil moisture, temperature, pH, soil NO_3^--N content and NH_4^+-N content as tested by structural equation modeling (SEM). Single-headed arrows showed that the impact of different significant controls on N_2O emission was analyzed. The blue arrows represent positive effects, and red arrows negative effects. The width of the arrows indicated the strength of the relationship. The numbers are standardized path coefficients, which show the significance of the variables in the model. Goodness-of-fit statistics for the model are shown below the model. *, ** and *** indicate significant effect at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table1. The variation in NO_3^--N, NH_4^+-N, soil pH, dissolved organic carbon (DOC) of topsoil (0-5 cm) with the treatments in August 2017. Values in the same column with different letters indicate significant differences (P < 0.05)

Treatments	NH_4^+-N (mg kg^{-1})	NO_3^-N (mg kg^{-1})	pH	DOC (mg kg^{-1})
N_0	5.30±2.82a	11.65±5.28a	6.32±0.09b	68.46±4.01a
N_{30}	5.97±0.95a	17.29±2.05a	5.91±0.07a	75.83±8.89a
N_{60}	6.79±1.30a	19.82±3.90b	5.94±0.35a	82.59±8.06b

Table2. Repeated measures ANOVA analysis of variance with F and P values of the total effects of long-term nitrogen (N) addition on N_2O emissions in long-term N addition; n indicates sample size.

Source of variation	N_2O		
	df	F	P
Date	1.834	9.861	0.009**
N	1	101.127	<0.001**
Date*N	1.834	2.603	0.142

5 Discussion

N_2O emissions of the temperate grassland (Fig. 1b; Table 3), acted as a small source of N_2O and the cumulative emission of N_2O from N_0 plots is 0.37 kg N ha^{-1} (Fig. 1b), higher than that from other ungrazed steppes in Inner Mongolia (below 0.28 kg N ha^{-1})[16]. N_2O emissions was triggered by the day following fertilization, and the peaks lasted about 20 days and the emissions fell to the baseline (Fig. 1b), which was related to the dynamics of soil mineral N (Table 1). The SEM showed that NH_4^+-N was the most important controlling factor for N_2O emission rather than NO_3^--N or pH (Fig. 2), indicated that N_2O emission was mainly as byproduct of soil ammonia oxidation. Dry weather and the sandy texture (sand 62.8%) had been keeping soil WFPS below 30% (Fig.1a) over the observed period, in that conditions N_2O by denitrification produced much less (WFPS≤70%)[17]. Recent studies suggested that in a typical steppe, long-term nitrogen fertilization and soil pH could have contrasting effects on ammonia oxidizing microorganisms[18], our result showed that pH had less influence to N_2O, that was primarily due to N-reduced pH did not dramatically shifted N inducing function and diversity of ammonia oxidizers of grassland[19].

6 Conclusion

Atmospheric N deposition has dynamically increased in recent years, which will significantly affect the N_2O balance in grassland ecosystems. Long-term (13 year) increased N addition significantly increased N_2O emission. Soil NH_4^+-N content and soil moisture were the most important limiting factors for N_2O emissions, pH had less influence to N_2O. We conclude that N_2O emission would be enhanced by long-term increased N deposition, which will have an important effect for climate change with elevating atmospheric N deposition in future.

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