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Short Communication

Increased Nitrous Oxide Emissions Resulting from Nitrogen Addition and Increased Precipitation in an Alpine Meadow Ecosystem

Yangong Du[1, 2*, 3], Xiaowei Guo[2, 4], Guangmin Cao[2] ,Yikang Li[2]

[1]Key Laboratory of Mountain Surface Processes and Ecological Regulation,
Institute of Mountain Hazards and Environment, Chinese Academy of Sciences,Chengdu 610041,China
[2]Key Laboratory of Adaptation and Evolution Plateau Biota, Northwest Institute of Plateau Biology,
Chinese Academy of Science, Xining 810008, China
[3]NSW Department of Industry, Skills and Regional Development, Wagga Wagga Agricultural Institute,
NSW 2650, Australia
[4]Graham Centre for Agricultural Innovation
(an alliance between NSW Department of Industry and Charles Sturt University,
Wagga Wagga, NSW 2650, Australia)

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Abstract

The effects of nitrogen (N) addition and increased precipitation on nitrous oxide (N_2O) emissions in alpine meadow ecosystems are still unclear. In this study, we measured N_2O fluxes on the Tibetan plateau under interactions of moderate atmospheric N deposition and increased precipitation using a closed chamber method. Under all applied treatment conditions, the alpine meadow ecosystem acted as a source of N_2O. The N_2O emission rate reached a maximum of 74.83±14.40 μg m^{-2} h^{-1}, with a significant increase in emission rate of 68.76% following N addition when compared with the control plot ($p<0.05$). Increased precipitation, and its interactive combination with N deposition, enhanced the N_2O emission rate by 53.90% and 44.52%, respectively. However, there was no significant difference between these two treatments. Increased precipitation would help to mitigate N_2O fluxes under global nitrogen deposition conditions.

Keywords: nitrous oxide, N deposition, increased precipitation, alpine meadow

Introduction

An increase in the quantity of reactive nitrogen in the atmosphere, together with increased precipitation, alters soil chemistry and the physiology of soil microbes, and influences the balance of the greenhouse gas N_2O [1,2].

Annual bulk N deposition across China increased by approximately 7.9 kg ha^{-1} between 1980 and 2010, with a value of 21.1 kg ha^{-1} during the latter year [2]. Glaciers melted and shrank by 7% (about 3.79×10^3 km^2) during the period 1960-2005, and precipitation gradually increased during peak growing seasons, with a notable 14% increase in precipitation per decade in Tibet [3]. Under long-term medium soil moisture conditions, N_2O is dominant, with N_2 mostly emitted from saturated soil [1]. Aerobic

*e-mail: ygdu@nwipb.cas.cn

nitrification and anaerobic denitrification are responsible for soil N_2O production [1, 4].

Nitrogen deposition had been shown to increase N_2O emissions; the relationship between the two variables was not found to be significant on alpine meadows but had been shown to be significant in alpine grasslands during the growing season [5, 6]. Average N_2O flux had increased by 25.56% following the application of 10 g N m^2 y^{-1} in semi-arid grassland [7]. Irrigation was found to have only a small effect on yearly emissions during normal years, but had enhanced emissions by 70% during dry years [1]. Rainfall-stimulated N_2O emissions on corn farmland and increased precipitation could exert a greater influence on N_2O emissions in grassland soils [1, 6, 7].

The Tibetan Plateau is known as the birthplace of the Asian primary rivers, and it is in the part of the world with even fastest change in terms of global change, then alpine meadow, as one of the most important dominant vegetation types, expanded 35% of the Tibetan Plateau [4]. The interactive effects of N deposition and precipitation on N_2O fluxes in alpine meadow are still poorly understood [1]. It is possible that increased ammonia and nitrate, combined with precipitation, directly promote soil redox potential and influence the microbial-mediated process of N_2O production and emission [6].

To verify whether this is the case, we conducted N_2O flux measurements from May to October 2012 on an alpine *Kobresia humilis* meadow on the Tibetan plateau. This study aims to further scientific understanding of N deposition and of the impact of increased precipitation on N_2O balance, thus aiding in the evaluation of N deposition effects and climate change.

Material and Methods

Site Description

The study site was located at the *Haibei* national alpine meadow research station (37°32′ N, 101°15′ E; 3250 m altitude) within an alpine *K. humilis* meadow located at the northeast edge of the Qinghai-Tibetan plateau. The climate of the site was dominated by the southeast monsoon and by the high-pressure system of Siberia. Annual average air temperature was approximately -1.7ºC, with extreme maximum and minimum temperatures of 27.6ºC and -37.1ºC, respectively. Annual precipitation at the *Haibei* station for the normal year reached about 560 mm, 80% of which fell during the short summer growing season from May to September. Average annual sunlight was 2462.7 h, representing 60.1% of total possible sunshine [8]. Average annual precipitation in 2012 was approximately 380 mm.

The soil within the study area was a clay loam Mat-Gryic Cambisol, with a mean thickness of 0.65 m and high organic content of approximately 5.5% and 3.3% at 0-10 cm and 10 -20 cm soil depths, respectively. Soil bulk density was 0.75 g cm^{-3} and 1.11 g cm^{-3} at 0-10 cm and 10-20 cm soil depths, respectively (Table 1).

The study site was used as winter pasture for local sheep and yaks, with an average grazing intensity of 2.55 sheep ha^{-1} since 1982. Average aboveground plant living biomass was 340 g m^{-2} in August [8, 9]. The biotic community included more than 40 species m^{-2} and was dominated mainly by the perennial sedges *Kobresia humilis* Serg.*,* *Stipa aliena* Keng.*,* *Festuca ovina* Linn.*,* *Poa* spp. Linn.*,* *Gentiana straminea* Maxim.*,* *Elumus nutants* Griseb.*,* and *Polygonum viviparum* Linn.

We state clearly that no specific permissions were required for our locations or activities, and provide details on why this is the case, and confirm that the field studies did not involve endangered or protected species. This research did not refer to vertebrate studies.

Experimental Treatments

Four treatments, with three replications in each case, were set up on the *Kobresia humilis* meadow in May 2012. The first treatment (N) involved simulated nitrogen deposition at 2 g N m^{-2} of NH_4NO_3 and most closely approximated the perennial averaged observation of nitrogen deposition at this station. The second treatment (Pre) involved the addition of 20% of the amount of perennial averaged precipitation. The third treatment (N×Pre) comprised the interaction of the first two treatments, while the fourth treatment (CK) was a control, i.e., no actual treatment was applied. Each plot covered an area of 1 m^2 and was divided into two equal portions, one for the growing season (May to September) and the other for the period of dormancy. Over the growing season, nitrogen and water were added on the last day of every month in six equal doses, while during the dormancy period all applications were provided on a single occasion in October. During each application, NH_4NO_3 was weighed, dissolved in water, and applied to each plot using a sprayer. Two passes were conducted to ensure even distribution. The control plot received the same amount of

Table 1. Basic soil properties of alpine meadow.

Soil depth (cm)	pH	Organic C (%)	Field WHC (%)	Bulk density (g cm^{-3})
0 – 10	7.3±0.4	5.5	53.6	0.75±0.05
10 – 20	7.4±0.5	3.3		1.11±0.09
20 – 30		2.7	35.9	1.13±0.04
30 – 40		1.9		1.15±0.03

water as the experimental plots but with no addition of nitrogen. To avoid disturbing other elements, dissolved phosphorus and potassium were simultaneously used for fertilization in adequate amounts.

Sample Collection and Measurement

Nitrous oxide emissions were measured using a static chamber method and gas chromatograph (HP4890D, Agilent) with electron capture detector [4]. Pre-experimental sampling at 2 h intervals showed that the flux rate between 0900 and 1000 h local time was close to that of the diurnal average [9]. Gas samples were collected from the chambers every 10 min over a 30 min period using 100 ml plastic syringes; measurements took place immediately after. The accuracy of the sample analysis was approximately $\pm 5\times 10^{-9}$ L L^{-1} in the case of N_2O measurements.

Calculation and Statistical Analysis of Data

Hourly N_2O emissions (μg m^{-2} h^{-1}) were calculated based on the slope of linear increase in concentrations over the sampling period, as follows:

$$F_{N_2O} = \rho \times \frac{V}{A} \times \frac{P}{P_0} \times \frac{T_0}{T} \times \frac{dC_t}{dt}$$

…where F is the hourly emission rate (μg m^{-2} h^{-1}) and V and A are the volume and ground area covered by the chamber, respectively. C_t denotes N_2O concentration in the chamber at time t, and ρ is air density inside the chamber. T_0 and P_0 represent air pressure and temperature under standard conditions (with values of 1.103×10^4 Pa and 273 K, respectively). P and T are local air pressure and temperature in the chamber, respectively.

Statistical Analysis

The coefficient of variation was analyzed by means of the following equation: (standard deviation/mean value) ×100%. The effects of treatments and their interaction were analyzed using two-way analysis of variance (ANOVA). Critical least significant difference (LSD) values were calculated post-hoc using SPSS 16.0 (System Software Inc.) at a 5% level of error probability.

Result

Effect of Nitrogen Addition and Increased Precipitation on N_2O Fluxes

The plots used for alpine meadow ecosystem treatments acted as sources of N_2O. During the growing season, the emission rate from the plots to the atmosphere averaged approximately 44.34±9.67 μg m^{-2} h^{-1} (in the case of CK), 74.83±14.40 μg m^{-2} h^{-1} (N), 68.24±22.32 μg m^{-2} h^{-1} (Pre), and 64.08±22.22 μg m^{-2} h^{-1} (N×Pre) (Fig. 1). The N_2O emission rate following nitrogen deposition (N) increased significantly by 68.76% when compared with the control plot ($p<0.05$). Precipitation increases (Pre) and combined precipitation increase and nitrogen addition (N×Pre) enhanced the N_2O emission rate by 53.90% and 44.52%, respectively. However, there was no significant difference between the two treatments (Fig. 1). Precipitation increases would help to mitigate future N_2O fluxes under conditions of global nitrogen deposition.

Seasonal Variations in N_2O Emission Rates on the Alpine Meadow

Variations in N_2O emissions did not exhibit a clear seasonal pattern. There were two emission peaks, one at the end of June and the other at the beginning of August. Nitrogen deposition tended to increase N_2O emissions from late June to July and in September. On the August 6, N_2O emission rates resulting from Pre and (N×Pre) treatments were significantly higher than those produced

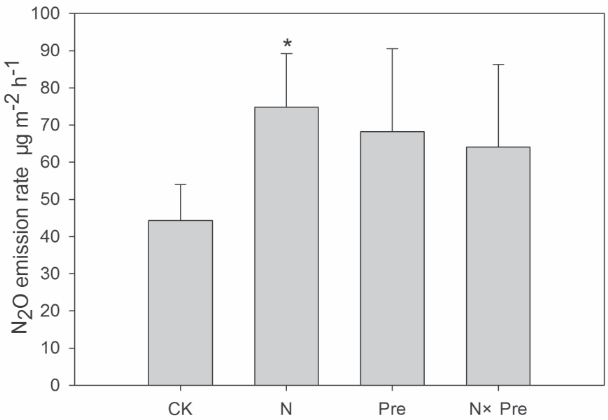

Fig. 1. Average nitrous oxide emission fluxes during the growing season under different treatment conditions. Means±standard errors are presented.
* indicates a significant difference among the four plots.

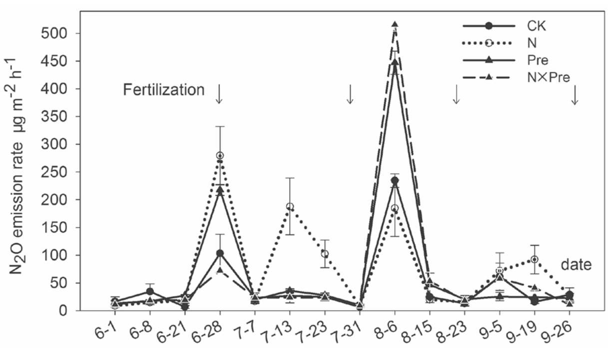

Fig. 2. Nitrous oxide emission rates under different treatment conditions on different dates. Means±standard errors are presented.

from N and CK plots ($p<0.05$). The highest emission flux recorded was 515.50±11.48 μg m^{-2} h^{-1}, and it occurred at the beginning of August and resulted from the N×Pre treatment. A significant priming effect after fertilization and precipitation increases was noted during one week in the period spanning July to September, at the time of application of the nitrogen fertilization treatment in June (Fig. 2). This study indicated very high inherent spatial variability of N_2O fluxes in all experimental plots, with an average coefficient of variation.

Discussion

Simulation of Nitrogen Deposition and Precipitation Increase on N_2O Emissions

The addition of nitrogen generally increased N_2O emission rates in grassland and meadow ecosystems [10, 11].The soil nitrogen pool is expected to play an important role in regulating N_2O emissions [12, 13]. Such a trend was also noted in this study, possibly driven by the following two factors. First, nitrogen fertilization significantly promotes grassland primary production [5], while grassland root exudation, secretion, and cell slough could provide carbon and energy sources for microorganisms [4]. In addition, alpine meadow ecosystems are limited by nitrogen availability and by the abundance of mineral nitrogen (NH_4^+ and NO_3^-) in the microorganism substrate. Both of these factors would likely stimulate alpine meadow soil N_2O fluxes [4, 14].

Summer N_2O fluxes followed an upward trend, with higher values related to precipitation in sub-alpine ecosystems [12]. This study revealed that, during the relatively dry year of 2012 (during which annual precipitation was approximately 380 mm), an increase in precipitation increased N_2O emissions from alpine meadow ecosystems by 53.90%. The soil N_2O flux increased by 70% in irrigation-enhanced Hungarian sandy and loess grasslands of a dry year [1]. When soil is well-aerated, the oxidative nitrification process dominates, resulting in nitrous oxide emissions [10]. An increase in growing season precipitation enhances grassland soil N_2O emissions through the denitrification process, due to the presence of an applied fertilizer N substrate [15]. A significant interaction between N_2O emissions and soil moisture was also reported for grassland soils [16].

Soil moisture can advance N mineralization and nitrification through its positive effects on microbial activity, provided that the soil is not saturated and remains aerobic [17]. In this study, additions of nitrogen and increases in precipitation boosted N_2O emissions by 44.52%. Drying/rewetting events induce an increase in matter cycling in soils and contribute considerably to increased N_2O emission flux [18]. Furthermore, soil moisture has a dominant role in controlling soil nitrogen cycling and is strongly positively correlated with N_2O losses [5].

The N_2O flux enhancement effect on the alpine meadow plot subject to N×Pre treatment was lower than that on plots administered either of the two treatments (i.e., N or Pre) in isolation. The soil water solution acts as a barrier to both O_2 and N_2O diffusion [19]. Agricultural soil has even been found to be an N_2O sink under anaerobic soil conditions, rich in organic matter and nitrate [18, 19]. Denitrifying bacteria may use atmospheric N_2O as an alternative electron acceptor to nitrate, with denitrification to N_2 performed as a dominant process [16].

Production Pulses of N_2O in an Alpine Meadow

The occurrence of N_2O erupted pulses after large rainfall events following fertilization of grassland ecosystems was described [13]. The emission pulses (larger than 500 μg m^{-2} h^{-1}) recorded in previous nitrogen fertilization experiments were also found to occur on the alpine meadow (Fig. 2). It was reported that both meadow marsh and fertilized grassland soil had similar peak fluxes, which were approximately 583 and 358 μg m^{-2} h^{-1}, respectively [20, 21]. This study revealed that such extreme N_2O emission release peaks were often evident immediately after each nitrogen fertilization event. On the other hand, in alpine meadows and loess grasslands, the highest pulse peak was always recorded in August [4, 5].

This discrepancy may possibly be ascribed to variations in the nutrient and oxygen concentration profiles of soils [9]. Atmospheric N_2O moves into the soil via passive diffusion and convection and is then reduced to N_2 via denitrification or to NH_3 via assimilatory reduction of N_2O [19]. Within alpine meadows, N_2O emissions are complex and possibly influenced by multiple factors [4, 9]. Nitrification has generally been reported to be the dominant process in alpine meadow soils; in theory, however, high soil moisture and optimal soil pH conditions may also provide good conditions for denitrification to N_2 [9].

Conclusion

The Tibetan Plateau plays an important function in regulating the local climate, and is vulnerable to the nitrogen deposition and precipitation variation. However, these were little data which focused on the N_2O fluxes subjected to nitrogen addition and increased precipitation in the alpine meadow ecosystem. This study indicated that alpine meadow ecosystem acted as the source of atmosphere N_2O. The nitrogen deposition increased significantly the grassland ecosystem N_2O emission flux. The significant priming effects were testified after nitrogen fertilization and precipitation application. Furthermore, the precipitation addition could be benefit in mitigating future N_2O fluxes under the scenarios of global nitrogen deposition.

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