Original Research

Nitrous Oxide Emission Rates over 10 Years in an Alpine Meadow on the Tibetan Plateau

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Abstract

The alpine grassland ecosystem covering about 85% of the Tibetan plateau is fragile and sensitive to natural and anthropogenic disturbances. However, it is unclear how nitrous oxide (N_2O) has varied over the last 10 years. In order to accurately estimate the regional N_2O emissions budget, the N_2O levels, environmental factors, and biomass were investigated on a yearly, monthly, and seasonal basis from 2000 to 2014 in an alpine meadow. The results showed that there was an overall declining trend in emissions over 10 years, during which the two maximum emission rates were 64.8±11.1 and 41.8±18.2 µg m^{-2} h^{-1} (in 2001 and 2006). The average N_2O emissions rate was about 38.4±3.3 µg m^{-2} h^{-1}. Pearson correlation demonstrated that soil and air temperature exerted a crucial influence on N_2O, followed by precipitation and aboveground biomass, but the effect of soil moisture at a depth of 10 cm was negative. Multiple linear regressions showed a good relationship between N_2O and all environmental factors. Future scenarios of wetter and warmer weather would noticeably increase alpine meadow N_2O emissions on the Tibetan Plateau.

Keywords: N_2O emissions, alpine meadow, temperature, precipitation, aboveground biomass

Introduction

Nitrous oxide (N_2O) is a powerful greenhouse gas with an atmospheric lifetime of more than 120 years. Its warming potential is reported to be 310 times higher than that of carbon dioxide [1]. Globally, 6% of all global warming is caused by N_2O emissions, and those N_2O levels in the atmosphere are increasing by 0.8 ppbv a^{-1} every year. The current level of N_2O in the atmosphere is 324 ppbv. [2]. N_2O production is largely attributed to terrestrial soil processes, which account for about 60-70%

of total N_2O emissions [3]. Therefore, it is particularly necessary to focus on N_2O production in terrestrial ecosystems.

The dominant ecosystem on the Tibetan Plateau is alpine meadow [4]. This unique ecosystem is characterized by a long, cold dormancy period and short, mild growing season [4]. Consequently, N_2O emissions are estimated inaccurately under these arid and polar climatic conditions [5]. Massive efforts have been carried out to assess N_2O emissions in agricultural management systems, but few studies have focused on alpine meadows [2-5]. There are inconsistent views about the variations in N_2O emissions at diverse time scales (monthly, yearly, and between growing season and non-growing season).

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Previous studies have demonstrated that N_2O emissions in the growing season are far higher than those during the non-growing season in alpine meadows [6]; however, the opposite trend was also reported [**7**]. There is a lack of long-term data on N_2O emissions at different temporal scales.

N_2O is released mostly from microorganisms involved in nitrification and denitrification, which depend on the soil physiochemical properties and external environmental conditions [8]. Although several studies have confirmed that the main factors affecting N_2O emissions include precipitation, temperature, and biomass, many uncertainties still exist in these relationships, which are also affected by temporal changes. Hence, in order to accurately reveal temporal variations in N_2O emissions and examine their effects on environmental factors in alpine meadows on the Tibetan Plateau, our research is based on long-term studies using different time scales can provide scientific proof to estimate emissions and further develop practical mitigation strategies in the future.

The aims of this study were as follows:

1) Investigate the trends in N_2O emissions at different time scales, including yearly, monthly, and seasonal patterns.
2) Analyse the relationship between aboveground biomass and N_2O emissions.
3) Identify the key environmental factors influencing N_2O emissions and further predict variations in N_2O emissions based on future climatic scenarios.

Material and Methods

Study Site

Our research was conducted in an alpine meadow on the Tibetan Plateau situated in the Haibei Alpine Meadow Experimental Ecosystem Research Station of the Chinese Academy of Sciences (37°36'N, 101°12'E, 3,250 m above sea level). It is located in a valley encircled by the Qilian Mountains. The region has a typical plateau continental climate, with no significant distinction between the four seasons; instead, there are patterns of warmth and cold. The average annual air temperature is -1.7ºC. The alpine meadow receives approximately 560 mm precipitation annually, and the maximum precipitation usually occurs in summer during the height

of the growing season (the fifth to ninth month of the year), accounting for 80% of yearly rainfall. The clay loam soil is permanently wet and up to 65 cm thick. The total nutrient of soils defined as Mat Cry-gelic Cambisol is rich but barren of nutrients. The physical and chemical properties of the soil are shown in Table 1. The main plant communities in the region are *Kobresia*, *Gentiana*, *Saxifraga*, and *Anemone*; together, they have a coverage of nearly 75-80% [9].

Sampling and Measurement

N_2O emission rates were measured using opaque static chambers. The stainless steel chambers consisted of two parts: a bottomless square box ($0.5 \times 0.5 \times 0.5$ m³) used to collect N_2O gases, and a stainless steel base ($0.5 \times 0.5 \times 0.1$ m³) that was plugged into the meadow soil. Two electric fans were installed on top of the chamber to mix air, and a thermo-probe was used to monitor the internal temperature of the chamber during the sampling period. In addition, the external chamber was wrapped with white plastic foam and waterproof cloth, with the aim of avoiding increasing the interior temperature of the chamber caused by solar radiant heat during the sampling period.

The N_2O samples were obtained roughly four times a month in the growing season (May to September) and twice a month in the non-growing season (October to April) from 2000 to 2014. During sampling, the opaque static chambers were completely sealed for at least 30 min between 09:00 and 10:00 local time, when N_2O flux measurement is nearly identical to the diurnal mean. N_2O samples were gathered once every 10 min using a three-way valve pumping samples into a 100 ml syringe, and were sent for laboratory analysis within 48 h. The N_2O content of the samples was analyzed by a gas chromatograph (HP4890D, Agilent) equipped with an electron capture detector. The temperature of the detector and column oven were 330°C and 55ºC, respectively, and the instrument had an analysing accuracy of ±5 ppbv.

The aboveground biomass was measured by harvesting 10 squares (0.25×0.25 m²) in the study plot once a month from April to October. The harvested biomass was dried at 65ºC for 24 h and then weighed. Soil temperature was measured at depths of 5 cm and 10 cm using a digital thermometer (JM 624), while the N_2O gas was collected. Air temperature and precipitation data were obtained from the meteorological station

Table 1. Basic soil properties of alpine meadow (means of three replicates are shown).

Layer (cm)	Organic carbon (%)	Field capacity (%)	pH	Bulk density (g cm⁻³)	NO_3^- (mg kg⁻¹)	NH_4^+ (mg kg⁻¹)
0-10	5.5	53.6	7.3	0.75	8.3	11.7
10-20	3.3	----	7.4	1.11	4.4	5.8
20-30	2.7	35.9	----	1.13	3.2	4.0
30-40	1.9	----	----	1.15	2.6	3.4

at Haibei Station. The water content of the soil was calculated using the weighting method [10].

Calculating N$_2$O Emissions

The rate of N$_2$O emissions per hour is obtained using the linear slope of the gas concentration changing with time:

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

...where F is N$_2$O emissions per hour (µg m^{-2} h^{-1}), V is chamber volume (cm^3), A is bottom area of the chamber (cm^3), C$_t$ is N$_2$O concentration of the volume mixed at moment t (10^{-9} L /(L·min)), t is time (min), ρ is N$_2$O emissions under standard conditions (g cm^{-3}), T$_0$ and P$_0$ are temperature (K) and atmospheric pressure (Pa) at sea level respectively, and T and P are temperature (K) and atmospheric pressure (Pa), respectively, during sampling in the chamber.

Statistical Analyses

Repeated measure analysis of variance (ANOVA) tests were used to explore whether the years and dates of sampling, and their interaction had an influence on N$_2$O emissions. The relationship between N$_2$O emissions and aboveground biomass was examined with simple linear regression analysis in SPSS (19.0). Multiple linear regression analyses was used to evaluate the effects of multiple variable environmental factors on N$_2$O emissions, and Pearson correlation analyses were employed to explain the connections between N$_2$O emissions and diverse factors such as precipitation, temperature (soil and air), soil moisture, and aboveground biomass.

Results

N$_2$O Emissions Dynamics at Different Time Scales

The N$_2$O emissions showed considerable year-to-year variation, and the average rate was 38.4±3.3 µg m^{-2} h^{-1} during 10 years. The lowest rate was 29.0±2.4 µg m^{-2} h^{-1} in 2009. There was an overall downward trend between 2000 and 2014. The first peak reached 64.8±11.1 µg m^{-2} h^{-1} in 2001. Then the N$_2$O emissions dropped sharply to 37.4±6.1 µg m^{-2} h^{-1} in 2003, and fluctuated slightly around 35.2±3.2 µg m^{-2} h^{-1} from 2003 to 2005. The second peak was 41.8±18.2 µg m^{-2} h^{-1}, after which emissions remained steady at about 31.6 µg m^{-2} h^{-1} in 2013 and 2014 (Fig. 1). The N$_2$O emissions indicated a clear seasonal pattern, with an average emission rate of 49.9±3.5 µg m^{-2} h^{-1} in the growing season, which was significantly higher than (27.0±4.9 µg m^{-2} h^{-1}) in the dormant season (p<0.05). N$_2$O
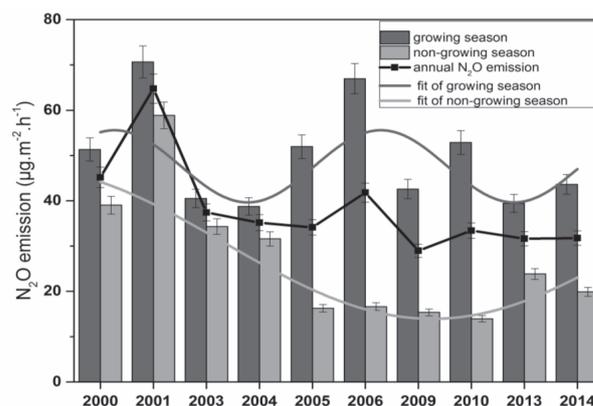
Fig. 1. N$_2$O emission dynamics in growing and dormant seasons, yearly scales (data were not measured in 2002, 2007, 2008, 2011, and 2012).

emissions showed enormous fluctuation in the growing season and presented a general declining trend in the non-growing season during 10 years (Fig. 1).

Monthly N$_2$O emissions fluxes showed obvious pulse variations (Fig. 2). The first peak appeared in March, reaching 32.8±10.7 µg m^{-2} h^{-1}. The magnitudes of the N$_2$O fluxes varied dramatically, and the second peak reached 73.7±16.3 µg m^{-2} h^{-1} in August. The lowest value was observed in February (Fig. 2).

Repeated measures of ANOVA showed a significant difference among inter-year N$_2$O emissions, different sampling dates, and their interactions (Table 2). In addition, the effect of sampling dates on emission rates was higher than on the yearly scale.

Effects of Aboveground Biomass and Environmental Factors on N$_2$O Emissions

It was revealed that aboveground biomass significantly affects N$_2$O emissions in the growing season (R^2 = 0.45, p<0.05; Fig. 3). This study demonstrated that precipitation and N$_2$O emissions showed a weak positive
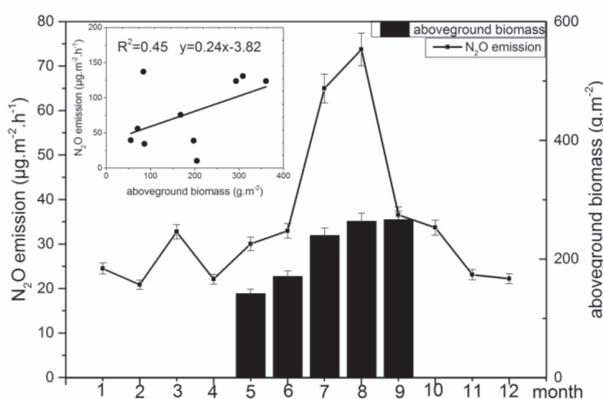
Fig. 2. N$_2$O monthly variation and aboveground variation during growing season and their correlation.

Table 2. Effect of sampling time on soil N_2O emissions of an alpine meadow.

Items	d_f	F	P
Year	9	23.12	0.01**
Date	29	139.49	0.000**
Year× date	261	65.42	0.002**

Table 3. Multiple liner regression analysis equation between N_2O emissions and environmental variables measured in the alpine meadow.

Equation	R^2	F	P
Y= - 0.07P -1.47Sm +1.75Ta +5.99Ts + 0.23B+35.46	0.43	1.71	0.19

P means precipitation, Sm means soil moisture at 10 cm depth, Ta and Ts represent air temperature and 5 cm soil temperature, and B means aboveground plant biomass.

correlation (R = 0.1; Fig. 3). However, soil moisture at 10 cm soil made a negligible contribution to N_2O emissions (R = 0.3; Fig. 3). Soil temperature at 5 cm and air temperature were positively correlated with N_2O emissions (R = 0.51, R = 0.48). Compared to precipitation and soil moisture, N_2O emissions rates were controlled to a greater extent by the soil temperature at 5 cm and air temperature. Environmental factors and biomass (R^2 = 0.43, Table 3) were also well described by multiple linear regression equations.

Discussion

Previous studies have revealed that grasslands were sources of N_2O emissions [11]. However, the differences were significant among different studies performed on the Haibei Alpine Meadow; emission rates varied from 4.1 to 54 μg m^{-2} h^{-1} [5, 9, 12]. Here, we calculated values for alpine meadows more accurately using long-term field measurements.

This study also describes in detail the declining trend in N_2O emission rates, with two peaks during 10 years. This phenomenon was closely related to environmental factors [13]. Temperatures impacted microbe activity as well as N_2O emission diffusion; warming could

trigger soil nitrification and lead to the release of a large amount of N_2O [14]. N_2O emissions were mainly caused by nitrification in alpine meadows [3] and temperate meadows [15]. Nitrification occurs between 15ºC and 30ºC, but it can be inhibited at temperatures below 15ºC or over 40ºC [16-17]. The N_2O emission peaks occurred in 2001 and 2006, which were also warm years. For the last 10 years, global warming tendencies were delayed on the Tibetan Plateau [18]. Thus, the majority of N_2O emissions were trapped in soil, and little could be released to the atmosphere during cold years [19].

On the other hand, precipitation gradually increased during the last 10 years [18]. Rainfall affects the biological process of N_2O production by changing the oxygen content in the soil pores [20]. N_2O is reduced to N_2 by denitrification when the soil water content is high and soil oxygen is low. It also has been reported that humid soil is conducive to the accumulation of soil organic matter, thus increasing the available supply of substrates for microbes, further stimulating N_2O emissions [21].

This study revealed that aboveground biomass played a crucial role in N_2O fluxes in alpine meadows. A similar result was also reported for salt marshes and wheat fields [22] because the number of denitrifying bacteria and nitrification intensity were affected by plant communities, plant litter, and root exudates as a consequence of changing the soil N_2O emission fluxes [23-24]. Plant aerenchymae would also play an important role in gas transportation, leading to increased N_2O emissions from the roots to the air [25]. In addition, plant root respiration accelerates oxygen consumption and facilitates the creation of anaerobic conditions during the growing season, causing further N_2O emissions by enhancing denitrification [12, 26-27].

According to Coupled Model Intercomparison Project Phase5 (CMIP5), the Tibetan plateau experiences a wetter climate and temperatures will continue to rise at the rate of 0.26°C every decade in the next 50 years [28]. N_2O will increase by about 8% against the global background of a warmer and wetter scenario, because the rate of N cycle would increase under future climatic conditions [29]. Our research showed that N_2O emissions were controlled by temperature; the Q_{10} value was 2.21, and precipitation levels exerted a negligible influence. Therefore, we predict a steady increase in N_2O emissions in the alpine meadows of the Tibetan plateau, based on future climatic warming and wetting conditions.
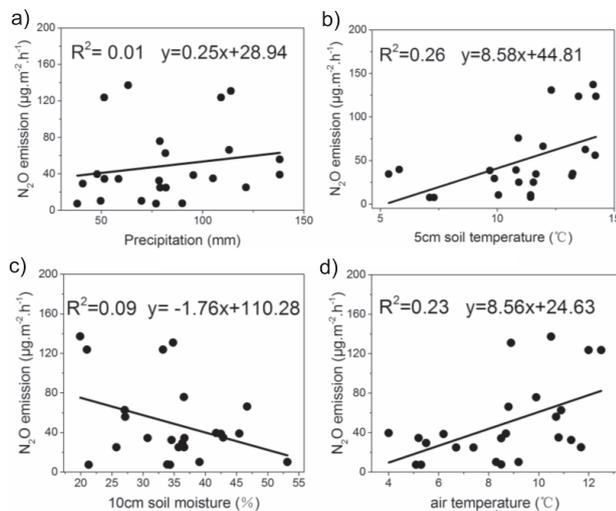
Fig. 3. Linear fitting between environmental factors (precipitation, 5 cm and 10 cm soil moisture, and air temperature) and N_2O emissions.

Conclusions

There was an overall downward trend in N_2O emission**s** over 10 years, during which the average emission rate was about 38.4±3.3 µg m^{-2} h^{-1}. And N_2O emissions in the growing season were significantly higher than in the dormant season**.** Besides, temperature (soil and air) produced a critical impact on N_2O, followed by precipitation and aboveground biomass, but the effect of soil moisture at a depth of 10 cm was negative. Therefore, it is predicted that future scenarios of wetter and warmer weather would significantly increase alpine meadow N_2O emissions on the Tibetan Plateau.

Acknowledgements

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