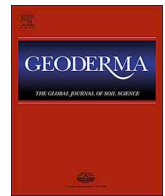




ELSEVIER

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Saturated N₂O emission rates occur above the nitrogen deposition level predicted for the semi-arid grasslands of Inner Mongolia, China

Xueying Gu^{a,f}, Yihui Wang^b, Hendrikus J. Laanbroek^{c,d}, Xiaofeng Xu^b, Bing Song^e, Yawen Huo^a, Shiping Chen^a, Linghao Li^{a,*}, Lihua Zhang^{a,b,**}

^a State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

^b Biology Department, San Diego State University, San Diego, CA 92182, USA

^c Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), the Netherlands

^d Ecology and Biodiversity Group, Utrecht University, the Netherlands

^e School of Resources and Environmental Engineering, Ludong University, Yantai 264025, China

^f University of Chinese Academy of Sciences, 100094, Beijing, China

ARTICLE INFO

Handling Editor: Junhong Bai

Keywords:

N₂O flux

Nitrogen deposition

Soil parameters

pH value

SEM

N₂O emission balance hypothesis

ABSTRACT

Nitrous oxide (N₂O) is one of the most important greenhouse gases emitted by the semi-arid grasslands of Northern China. The majority of previous studies focused on nitrogen (N) deposition and its impacts on N₂O emission rates in this region, while the mechanisms and controls of N₂O emission and the evidence for a “balance point” following increasing N deposition remained unclear. In this study, we investigated during 2013–2015 the environmental and plant controls over N₂O emission rates within a long-term N addition experiment with nitrogen levels of 1, 2, 4, 8, 16, 32, 64 g N m⁻² yr⁻¹, respectively, by using an in situ static chamber method. The results showed that N₂O emission rates increased with increasing nitrogen addition rates. The emission rates showed significantly positive linear correlations with soil temperature, NO₃⁻-N, NH₄⁺-N, inorganic N, microbial biomass carbon, microbial biomass N, total soluble N, below-ground plant biomass and above-ground plant biomass, but a significantly negative correlation with soil pH. A structural equation modeling analysis showed that N addition affected in particular the pH value and subsequently N cycling and soil N₂O emission. Meteorological factors impacted N₂O emission rates through affecting soil environment and N cycling processes. The formulated “N₂O emission balance hypothesis” was supported and the balance point at which the N₂O emission rate became saturated was somewhere between 32 and 64 g N m⁻² yr⁻¹. The plant N usage efficiency was highest when the rate of N addition was 2 g N m⁻² yr⁻¹. The increased knowledge of environmental and plant control over soil N₂O emission provides a better understanding of N cycling within the semi-arid temperate grassland ecosystem and will be fundamental for quantifying N₂O budgets at various scales.

1. Introduction

The trace gas nitrous oxide (N₂O) is one of the most important greenhouse gases (GHG) contributing to atmospheric radiative forcing (Klein et al., 2014) and hence to global warming. It is increasing at a rate of 0.2–0.3% per year (Granli and Bockman, 1994) and deteriorates the atmospheric environment more and more (Mosier et al., 1998). Due to its long residence time in the atmosphere (100–200 years), the legacy of increasing N₂O concentrations will remain dramatic, even when the present mitigation strategies become effective (Davidson, 2009).

Nitrogen (N) deposition refers to the process, in which land and

water are enriched with N elements from the atmosphere in the form of NH_x and NO_x. Based on data from 1990 to 1995, Klimont et al. (2001) presumed that by 2030 emissions of NO_x and NH₃ may increase by 95–100% in East Asia. Hence, it indicated that N deposition may be nearly doubled by 2030. Large and continuous NH_x deposition can acidify soils leading to N₂O rather than N₂ as the final product of denitrification (Chapuis-Lardy et al., 2010), and can indirectly affect N₂O emission by its impacts on the vegetation and environment especially in N limiting regions (Vitousek et al., 1997). Numerous studies indicated that N₂O emission increased along with N deposition in semi-arid grasslands of Northern China (Zhang et al., 2016; Jäger et al., 2011; Xu

* Corresponding author.

** Correspondence to: L. Zhang, State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.

E-mail addresses: llinghao@ibcas.ac.cn (L. Li), zhanglihua@ibcas.ac.cn (L. Zhang).

<https://doi.org/10.1016/j.geoderma.2019.01.002>

Received 10 October 2018; Received in revised form 20 December 2018; Accepted 2 January 2019

Available online 18 January 2019

0016-7061/ © 2019 Published by Elsevier B.V.

et al., 2008). However, it is still unclear how long-term N deposition affects N₂O emission, whether a “balance point” exists with the increasing N deposition, and which factors mainly regulate this.

The Intergovernmental Panel on Climate Change (IPCC) proposed the concept of a N₂O emission factor (EF) in order to estimate the N deposition-induced N₂O emission. EF is the percentage of fertilizer N applied that is transformed into fertilizer-induced emissions and is calculated as the difference in emission between fertilized and unfertilized soil under otherwise identical conditions (Shcherbak et al., 2014). Based on EF, IPCC used the so-called tier 1 method to estimate N₂O emission. In this approach, direct N₂O emission from N inputs is calculated as EF × the total amount of N input. The total amount of N input includes the annual amount of synthetic N fertilizer and organic N applied to the soil, and the annual amounts of N in crop residues and of N mineralized from soil organic nitrogen. A constant EF of 1%, which assumes a linear relation between N input and N₂O emission, has been widely used by the IPCC.

However, a meta-analysis of N₂O emissions from N-fertilized soils showed that an EF of 1% would likely underestimate the emission from regions predominantly fertilized at high N inputs, while the emission from modestly fertilized areas would likely be overestimated (Shcherbak et al., 2014). This is because EF is not constant, but decreases with increasing N input (Philibert et al., 2012). So the relation between N₂O emission and N fertilization was nonlinear (Mcswiney and Robertson, 2010). Philibert et al. (2012) also concluded that the relation between N input and N₂O emission is nonlinear, and an exponential function of applied N was the best fit for it. So, does the N₂O emission increase infinitely with the increasing N input?

This does not seem likely as we know from the N saturation model that a N-limited system initially retains anthropogenic N by using it for plant and microbial growth (Matson et al., 2002). When the amount of N applied is in excess of the N saturation point of plant and microbial demands, the redundant N will partly be transformed into N₂O and subsequently released into the atmosphere (Ma et al., 2010; Hoben et al., 2011). Once the amount of N applied is over the N saturation point, the N₂O emission will exponentially increase with increasing N availability due to microbial growth. It means that after N saturation point the relative amount of N that is consumed by plants and microbes decreases with increasing levels of N deposition, while plant and microbial N usage efficiencies seem depressed. Then N₂O emission will be increasing rapidly.

However, N₂O is the product of microbial metabolism, which needs not only N but also other nutrients, and its emission should also follow the N saturation model. Since EF is the usage efficiency of N applied that is transformed into fertilizer-induced N₂O emissions, we can infer that N₂O emission will be enhanced before other nutrition limitations, for example by limitations of carbon (Morley and Baggs, 2010) and phosphorus (Mehnaz and Dijkstra, 2016), and the N₂O EF is at the same time depressed. But when the N assimilation by microbial cells levels off, the level of nitrate-reducing enzymes will in theory become constant as will the amount of N₂O produced. In the following, we call this the “N₂O emission balance hypothesis”.

To test the N₂O emission balance hypothesis, we explored the N₂O emission rates in a multi-level N (0, 1, 2, 4, 8, 16, 32 and 64 g N m⁻² yr⁻¹) addition experiment. The highest two of these levels (32, 64 g N m⁻² yr⁻¹) were used to verify the “N₂O emission balance hypothesis” in semi-arid grassland, where are an important source of N₂O (Denmead et al., 1979; Mosier et al., 1991), and undoubtedly play an important role in regulating the feedbacks of the global climate system. In order to better understand soil and plant controls on the mechanism of N₂O emission after long-term N addition, we will show the chain of effects of N addition on N₂O emission and the main controlling factors based on a structural equation model (SEM) analysis.

So, we hypothesized that (1) N addition can directly and/or indirectly impact soil pH, soil nutrients, soil environment, N cycling and plant performance, leading to changes in N₂O emission; (2) after a long-

time period of N addition, the N₂O EF becomes depressed; and (3) the N₂O emission balance exists in semi-arid grassland.

2. Materials and methods

2.1. Experimental site and design

The experimental site is located in Duolun County (N 42°02', E 116°17') in the typical semi-arid temperate grassland area of the Inner Mongolia Autonomous Region, China. Nowadays this region is mainly an agropastoral ecotone. The annual mean precipitation is 383 mm with peaks in July and August, and the annual mean air temperature is 2.1 °C ranging from -17.5 °C in January to 18.9 °C in July. The soil type is a chestnut soil (USDA) with 63% sand, 20% silt and 17% clay at a depth of 0–10 cm. The bulk density at the time of sampling was 1.3 g cm⁻³ and the pH value was 7.1. The sampled grassland was dominated by perennial plants, such as *Stipa sareptana* var. *krylovii*, *Agropyron cristatum* (L.) Gaertn., *Cleistogenes squarrosa* (Trin.) Keng, *Potentilla acaulis* (L.), and *Artemisia capillaris* Thunb. (Niu and Wan, 2008).

The nitrogen addition experiment had started in 2003, and include eight treatments with eight replicates each, which were in a complete Latin Square design. Each plot was 10 × 15 m², with 1 m away from the others. N was annually added as urea in the middle of July and the levels of addition amounted to 0, 1, 2, 4, 8, 16, 32 and 64 g N m⁻² yr⁻¹, respectively. We selected three out of eight replicates, which we continued to use for all our measurements throughout the whole experiment.

2.2. Gas sampling and measurements

N₂O emission rates were measured in each selected plot once a month during the growing season (from May to September) in 2013–2015, using the opaque static chamber method described in detail previously (Zhang et al., 2014). The measurements were usually carried out at 9 am local time when the emission rate is assumed to represent a one-day average flux (Mosier et al., 1997). Gas samples were taken from the chamber headspace at 0, 10, 20 and 30 min after closing of the chamber using a 60 mL plastic syringe, and stored in 100 mL air bags (Hede Inc., Dalian, China). The N₂O concentrations of the gas samples were analyzed within one week using gas chromatograph (Agilent 7890A, Agilent Technologies Limited Co., USA), and the N₂O emission rates were calculated according to Zhang et al. (2010). During gas sampling, the soil temperature (ST) and moisture (SM) at 10-cm soil depth were measured in each selected plot with a geothermometer and a portable soil moisture meter (DELTA-T ML2x, England), respectively.

2.3. Soil and plant sampling

During gas sampling, soil samples were collected from the upper 10 cm. Three soil cores were taken randomly in each plot and mixed completely. After removing plant roots and large stones by sieving (sieve mesh 2 mm), the mixed soil was divided into two parts. One part was air-dried for measuring the pH value, using a combined glass electrode (soil/water ratio 1:2.5). The other part was kept fresh for the measurement of soil ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) concentrations, using a flow injection autoanalyzer (Auto Analyzer 3, Seal Analytical Inc., Germany) after extraction of the soil samples with 1 M KCl. The inorganic nitrogen (IN) was the total of NH₄⁺-N and NO₃⁻-N. Soil microbial biomass carbon and nitrogen (MBC and MBN) were determined using the chloroform fumigation-extraction method (Vance et al., 1987). The dissolved organic carbon (DOC) and total soluble nitrogen (TSN) were measured after extraction with 0.5 M K₂SO₄ (soil/solution ratio 1:2.5). The soil solutions were shaken at 250 rpm for 30 min on a mechanical shaker. Then, the soil mixture was centrifuged at 8000 rpm for 5 min, and the supernatant was filtered through a 0.45-

μm filter membrane. The solutions with DOC and TSN were analyzed using a total organic carbon/total nitrogen analyzer (Analytikjena multi N/C® 3100, An Endress + Hauser Company, Germany). Ammonia volatilization rates were measured according to Wang et al.'s (2002) venting method. In summary, a PVC tube (13 cm-height and 7.5 cm-inner diameter) was placed in the soil with 3 cm of the tube in the soil and 10 cm above. Two sponges (8 cm-diameter and 2 cm-height) were moistened with 15 mL phosphoric acid-glycerinum liquor (50 mL + 40 mL + 910 mL distilled water). One sponge, which was used to measure the ammonia volatilization from the soil, was placed into the PVC tube at 5 cm above the soil, and the other, which was used to correct for ammonia from air, was placed at the 1 cm above the first sponge. The upper opening of the tube was sealed with a film, that allows gas to pass but not water. The sealing film and the upper sponge were replaced at a week interval, and the lower sponge was collected once a month and extracted for ammonium ($\text{NH}_4^+\text{-N}$) with 1 M KCl. The mineralization rate was determined using the in situ soil core incubation method (Hook and Burke, 1995). The samples for measuring the mineralization rate were also collected monthly during the N_2O emission measurement periods.

At all experimental plots, the above-ground plant biomass (AGB) was clipped and measured within a $1 \times 1 \text{ m}^2$ area in the middle of August when it was at the peak. The below-ground plant biomass (BGB) was determined using the root ingrowth core method. In April, one soil core of 8-cm diameter and 30-cm depth was taken randomly in each plot and removed from plant roots and large stones by sieving. The non-root soil was put into a $1 \text{ mm} \times 1 \text{ mm}$ aperture net-bag, and buried below-ground. After the AGB was removed, the net-bags were collected, and only the roots were kept by removing the soil with water. Then the roots and AGB samples were oven-dried at 65°C for 48 h and weighted. The standing death biomass and litter (SDB) were collected at end of October, using the same method as described for AGB.

2.4. Statistical analyses

The means ($n = 3$) and standard errors (s.e.) of N_2O emission rates and plant biomass were calculated for each sampled plot with Microsoft Excel 2010 (Microsoft Corporation). One-way ANOVA and Duncan tests were used to examine the effects of N addition on monthly N_2O emission rates, on N_2O emission rates summarized over the growing season, and on plant biomass at a significance level of $P < 0.05$ (SPSS software 21.0). The structural equation model (SEM) of the IBM SPSS Statistics Amos 21 software package (IBM Corporation) was used to determine the effects of different levels of N addition and meteorological factors (precipitation and air temperature) on N_2O emission rates via vegetation, soil environment (pH, SM and ST), and soil nutrients ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, IN, MBC, MBN, DOC, and TSN). The figure of Pearson correlations between all variables was created with the R 3.4.3 package (R core team), and other figures were produced with SigmaPlot 10.0 (Systat Software, Inc.). We calculated the N_2O emission factor (EF_N) for every nonzero N addition (N) as the difference between N_2O emission rates (E_N) at the application amount and the control (E_0) divided by N applied: $\text{EF}_\text{N} = (E_\text{N} - E_0) / \text{N}$ (Shcherbak et al., 2014).

3. Results

3.1. Seasonal changes in meteorological factors during the study period

Similar seasonality was observed in the three years of our study period with maximum monthly mean air temperature and precipitation in July and with minimum values during December to February (Fig. 1). The maximum monthly mean air temperatures were around 18.9°C , and the total annual precipitations were 300 mm, 254 mm and 297 mm in 2013, 2014 and 2015, respectively (Fig. 1). Air temperature declined to its minimum values during December to February, while rainfall rarely happened during the same period (Fig. 1).

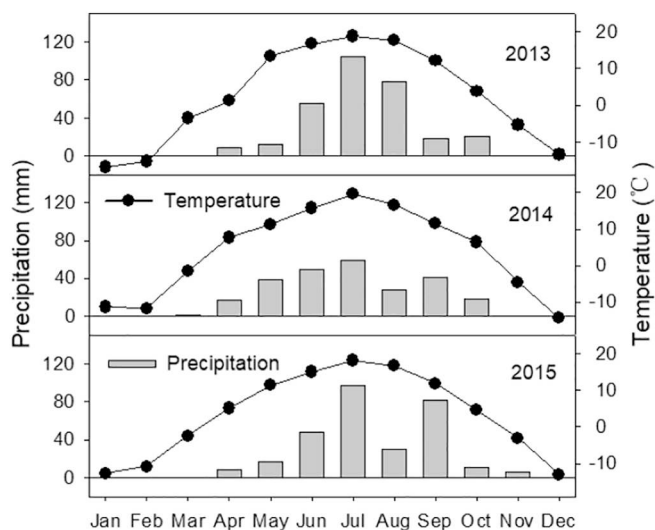


Fig. 1. Monthly total precipitation and mean air temperature in 2013–2015 for the experimental N additions site.

3.2. Responses of plant and N_2O emission rates to different levels of N addition

The above-ground plant biomass (AGB) in all three years and the below-ground plant biomass (BGB) in 2014 significantly increased with the levels of N addition, while the standing dead biomass (SDB) did not show a clear tendency in 2013 and 2014 (Table 1). The annual mean AGB tended to increase during the whole study period. In 2014, the AGB was less than the BGB across the N deposition levels, especially when the level of N addition was larger than $4 \text{ g N m}^{-2} \text{ yr}^{-1}$, BGB increased then to be 4–5 times the value of AGB. The BGB was greater in 2014 than in 2015, and it increased along the levels of N addition but declined at the highest level of $64 \text{ g N m}^{-2} \text{ yr}^{-1}$. The BGB at the highest level of N application was significantly less than that at 8, 16 and $32 \text{ g N m}^{-2} \text{ yr}^{-1}$ levels ($P < 0.05$). Only in 2015, the SDB significantly ($P < 0.05$) increased with increasing levels of N addition. In addition, there were no differences in vegetation coverage among all the treatments ($P > 0.05$, Table 1).

The highest monthly N_2O emission rate measured in the different years was $640.27 \mu\text{g N m}^{-2} \text{ h}^{-1}$ at $64 \text{ g N m}^{-2} \text{ yr}^{-1}$ in August 2013, $432.96 \mu\text{g N m}^{-2} \text{ h}^{-1}$ at $32 \text{ g N m}^{-2} \text{ yr}^{-1}$ in August 2014 and $115.82 \mu\text{g N m}^{-2} \text{ h}^{-1}$ at $64 \text{ g N m}^{-2} \text{ yr}^{-1}$ in June 2015. During our study period, the semi-arid grassland was a net source of N_2O among all the treatments and the N_2O emission rates increased with increasing levels of N addition (Fig. 2A). The observed correlation between N_2O emission rates and above-ground plant biomass for the different levels of N addition showed that lower emission rates were associated with lower biomass, and that emission rates increased rapidly while the change in plant biomass was small (Fig. 2B).

3.3. Effects of environmental and plant characteristics on N_2O emission as a result of N deposition

The N_2O emission rate was affected by various factors, including soil nutrients, soil environment and plant characteristics. The correlations between any two factors are shown in Fig. 3. Soil N_2O emission rates showed significantly positive linear correlations with soil temperature ($R^2 = 0.1524$, $P = 0.0128$), $\text{NO}_3^-\text{-N}$ ($R^2 = 0.1012$, $P = 0.0455$), $\text{NH}_4^+\text{-N}$ ($R^2 = 0.8354$, $P < 0.0001$), IN ($R^2 = 0.2401$, $P = 0.0013$), MBC ($R^2 = 0.1160$, $P = 0.0178$), MBN ($R^2 = 0.1045$, $P = 0.0250$), TSN ($R^2 = 0.0988$, $P = 0.0295$), BGB ($R^2 = 0.4570$, $P = 0.0003$), and AGB ($R^2 = 0.2500$, $P = 0.0129$), whereas a significantly negative correlation was observed with soil pH ($R^2 = 0.1524$,

Table 1

AGB (above-ground plant biomass), BGB (below-ground plant biomass), SDB (standing dead plant biomass + litter) and vegetation coverage in grasslands along the levels of N addition in 2013–2015.

	N addition	AGB (g m ⁻²)	BGB (g m ⁻²)	SDB (g m ⁻²)	Coverage (%)
2013	Control	96.30 ± 2.90 ^f		321.38 ± 34.91	75.63 ± 1.99
	1 g N m ⁻²	120.64 ± 3.37 ^{de}		246.26 ± 34.61	77.50 ± 1.88
	2 g N m ⁻²	129.53 ± 2.04 ^{cd}		249.43 ± 32.49	79.38 ± 1.99
	4 g N m ⁻²	112.26 ± 5.85 ^{ef}		261.08 ± 42.62	74.38 ± 2.40
	8 g N m ⁻²	141.95 ± 5.31 ^{bc}		317.92 ± 38.04	81.25 ± 1.83
	16 g N m ⁻²	155.20 ± 3.18 ^{ab}		281.36 ± 39.27	79.38 ± 1.75
	32 g N m ⁻²	154.38 ± 9.73 ^{ab}		256.59 ± 38.44	75.63 ± 1.47
	64 g N m ⁻²	168.21 ± 4.19 ^a		311.08 ± 48.86	77.50 ± 2.50
2014	Control	202.84 ± 5.41 ^e	487.60 ± 5.46 ^g	454.86 ± 40.81	65.00 ± 1.33
	1 g N m ⁻²	220.33 ± 1.67 ^{de}	604.43 ± 7.36 ^f	403.20 ± 31.44	64.38 ± 1.48
	2 g N m ⁻²	216.39 ± 4.32 ^{de}	676.10 ± 10.50 ^e	388.96 ± 32.08	64.38 ± 2.40
	4 g N m ⁻²	216.06 ± 13.61 ^{de}	744.52 ± 14.58 ^d	390.31 ± 21.65	65.00 ± 1.64
	8 g N m ⁻²	245.27 ± 5.25 ^c	1172.73 ± 12.45 ^c	498.52 ± 60.33	63.13 ± 1.32
	16 g N m ⁻²	235.55 ± 7.30 ^{cd}	1187.07 ± 18.22 ^c	482.18 ± 29.25	63.13 ± 1.88
	32 g N m ⁻²	337.03 ± 4.09 ^b	1616.52 ± 4.49 ^b	414.65 ± 36.68	63.13 ± 2.10
	64 g N m ⁻²	384.85 ± 5.80 ^a	1883.51 ± 35.31 ^a	456.01 ± 33.00	64.38 ± 0.63
2015	Control	209.16 ± 9.44 ^c	62.13 ± 1.45 ^f	187.27 ± 6.37 ^f	75.63 ± 1.75
	1 g N m ⁻²	220.63 ± 11.23 ^c	69.10 ± 3.75 ^f	212.57 ± 2.81 ^e	75.00 ± 1.64
	2 g N m ⁻²	279.53 ± 2.36 ^b	103.75 ± 2.41 ^e	213.33 ± 11.14 ^e	77.50 ± 1.89
	4 g N m ⁻²	275.51 ± 11.90 ^b	116.32 ± 4.08 ^d	290.47 ± 2.48 ^b	77.50 ± 0.94
	8 g N m ⁻²	290.90 ± 3.14 ^b	147.65 ± 3.73 ^b	269.20 ± 7.34 ^{cd}	76.25 ± 1.83
	16 g N m ⁻²	292.97 ± 3.11 ^b	163.12 ± 3.72 ^b	248.60 ± 3.01 ^d	76.25 ± 2.06
	32 g N m ⁻²	325.09 ± 2.91 ^a	166.52 ± 3.42 ^a	312.20 ± 6.59 ^{ab}	76.88 ± 1.62
	64 g N m ⁻²	337.40 ± 10.60 ^a	129.87 ± 0.45 ^e	325.17 ± 13.49 ^a	77.50 ± 1.34

Note: Different superscript letters indicate significant differences between treatments at $P < 0.05$.

$P = 0.0128$). No significant correlations were detected between soil N₂O emission rate and soil moisture ($P > 0.05$), SDB ($P > 0.05$) and DOC ($P > 0.05$). Furthermore, our results showed that the N₂O emission rates can be explained by soil temperature, NH₄⁺-N, BGB, and AGB, while their contributions to the variation in N₂O emission rates variation were > 25% (Fig. 4).

Fitting the results by Structural Equation Modeling (SEM) yielded the following parameters: Chi-square = 16.101, $P = 0.446$, DF = 16, CMIN/DF (Chi-square/degree of freedom) = 1.006, GFI (Goodness Fit Index) = 1.000, IFI (Incremental Fit Index) = 1.000, CFI (Comparative Fit Index) = 0.999, NFI (Normed Fit Index) = 0.926, RFI (Relative Fit Index) = 0.871, RMSEA (Root Mean Square Error of Approximation) = 0.013. It suggested that three significant ($P < 0.05$) variables affected soil N₂O emission most. The variable N cycling was the most important and direct factor determining soil N₂O emission (Fig. 5). The three key pathways that affected N₂O emission were: 1. levels of N addition altering soil pH, which affected N cycling, and subsequently soil N₂O emission; 2. meteorological factors affecting the soil environment, which altered N cycling, and next soil N₂O emission;

3. meteorological factors directly affecting the soil environment, and subsequently soil N₂O emission. All three pathways can explain nearly 44% of the variation in N₂O emission rates.

3.4. N₂O emission balance hypothesis and plant N usage efficiency

The best fit for the observed N₂O emission factor along the levels of N addition was a single peak model, in which the peak appeared in the plot receiving 2 g N m⁻² yr⁻¹ (Fig. 6A). Based on the equations of the peak model, the value of the N₂O emission factor can be infinitely close to zero, but never zero. This suggests that when the field received > 2 g N m⁻² yr⁻¹, the N₂O emission factor decreased with increasing levels of N addition. So, the percentage of N applied that is transformed into N addition-induced emission decreased, when the applied amount was > 2 g N m⁻² yr⁻¹. But the mean cumulative N₂O emission during the growing season in each year increased with the level of N addition when the fields received < 32 g N m⁻² yr⁻¹; above 32 g N m⁻² yr⁻¹, it seems to remain more or less constant (Fig. 6B). The maximum values of plant N usage efficiency rate to additive N was 89.22% at

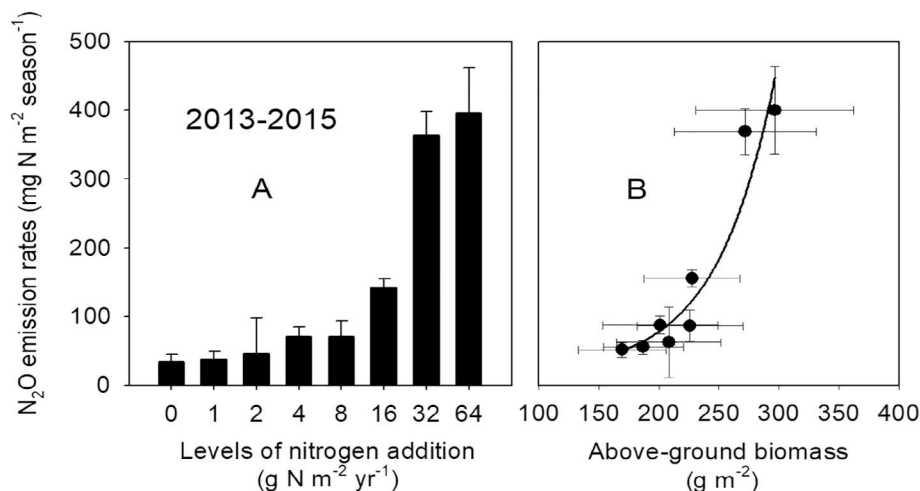


Fig. 2. (A) Average cumulative N₂O emission rates across levels of N addition in semi-arid grassland measured over the growing seasons of 2013, 2014 and 2015. (B) Relation between average cumulative N₂O emission rates and above-ground plant biomass for different levels of N addition averaged for three years. Error bars represent standard errors.

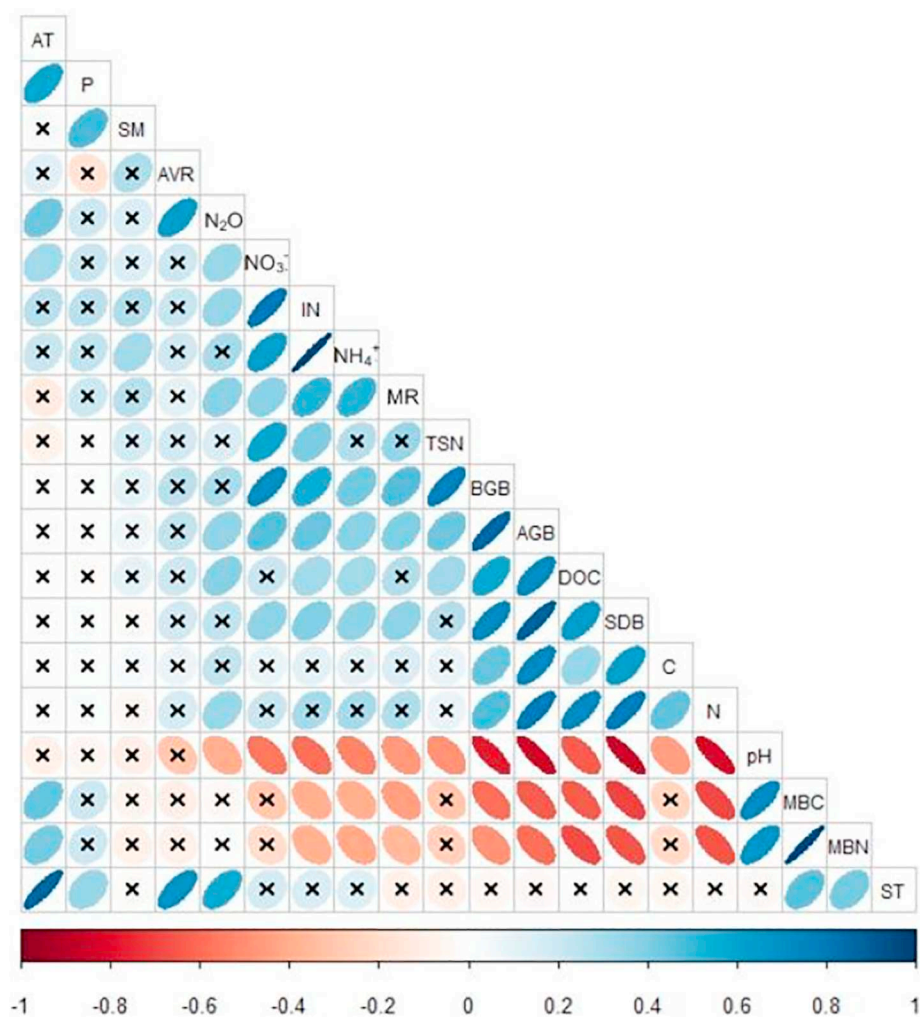


Fig. 3. Pearson correlations between pH, MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), ST (soil temperature), AT (air temperature), SM (soil moisture), AVR (ammonia volatilization rate), TSN (total soluble nitrogen), NO_3^- -N, IN (inorganic nitrogen), NH_4^+ -N, N_2O emission rates, MR (mineralization rate), BGB (below-ground plant biomass), AGB (above-ground plant biomass), SDB (standing dead plant biomass + litter), DOC (dissolved organic carbon), P (precipitation), C (vegetation coverage) and N (levels of N addition). Pearson correlation coefficients of the blue ellipses are positive, and those of the red ellipses are negative. The darker the color and the smaller the area of the ellipse, the greater the degree of correlation and the larger the absolute value of the Pearson correlation coefficient. “x” indicates no significant differences of relationship between two variables ($P > 0.05$). Variable names are on the diagonal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

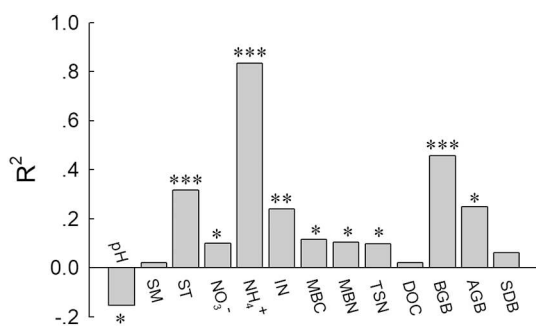


Fig. 4. R^2 of linear regression analysis between N_2O emission rates and pH value, SM (soil moisture), ST (soil temperature), NO_3^- (NO_3^- -N), NH_4^+ (NH_4^+ -N), IN (inorganic nitrogen), MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), TSN (total soluble nitrogen), BGB (below-ground plant biomass), AGB (above-ground plant biomass) and SDB (standing dead plant biomass + litter) for semi-arid grassland at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. The bars in the $R^2 > 0$ area represent positive correlation between N_2O emission rates and variables, while the bars in $R^2 < 0$ area represent negative correlation between them.

$2 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Fig. 7), which is consistent with the N application rate observed for the mean cumulative N_2O emission. Plant N usage efficiency decreased with increasing levels of N addition when the level of N was $> 2 \text{ g N m}^{-2} \text{ yr}^{-1}$. The response of TSN and MBN to applied N were the highest (3.81% and 15.40%, respectively) at $1 \text{ g N m}^{-2} \text{ yr}^{-1}$. When the level of N addition was larger than $8 \text{ g N m}^{-2} \text{ yr}^{-1}$, the

response of soil MBN added N was negative, which suggested that the soil MBN content was less than found in the control. At the highest levels of N addition, most N was not used for plant growth, and neither for microbial biomass, but the redundant N was also not transformed into soil available N.

4. Discussion

4.1. Mechanisms leading to enhanced N_2O emission by increasing N addition

In this study, N addition stimulated soil N_2O emission in semi-arid grasslands, which was consistent with the results of previous field and laboratory studies in agriculture and grassland soils (Mosier et al., 1991; Skiba and Smith, 2000; Scheer et al., 2008). The SEM analysis revealed that there were three pathways that influenced N_2O emission the most by altering the soil environment or N cycling (Fig. 5). Levels of N addition affected N_2O emission only indirectly by changing soil pH and subsequently N cycling. Thus, soil pH and N cycling were two important factors by which N addition regulated N_2O emission. Based on the two pathways affecting on N_2O emission by meteorological factors, it can be concluded soil environment was also an important factor in semi-arid grassland. Although N addition can significantly alter soil nutrients and plant community, there were no direct or indirect pathways for this factor acting on N_2O emission rates.

According to the results of SEM, there are three steps that explain the mechanisms of enhanced N_2O emission rates after increasing N addition. Firstly, N addition reduced the pH value in semi-arid

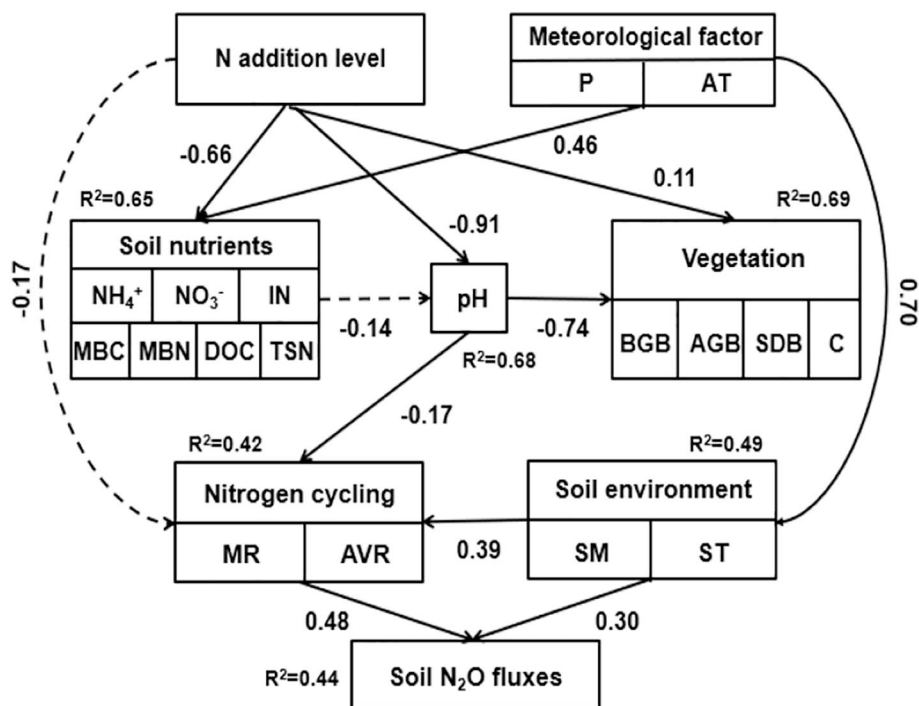


Fig. 5. Final result of structural equation modeling (SEM) of the effects of the levels of N addition and meteorological factors on soil N₂O emission via affecting the variables soil pH, vegetation, soil environment, N cycling and soil nutrients. Square boxes denote the variables included in the models. Meteorological variables include P (precipitation) and AT (air temperature). Soil nutrients variables include NH₄⁺-N, NO₃⁻-N, IN (inorganic nitrogen), MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), DOC (dissolved organic carbon) and TSN (total soluble nitrogen). Soil environment variables include SM (soil moisture) and ST (soil temperature). Nitrogen cycling variables include MR (mineralization rate) and AVR (ammonia volatilization rate). Vegetation variables include BGB (below-ground plant biomass), AGB (above-ground plant biomass), SDB (standing dead plant biomass + litter) and vegetation coverage (C). The soil meteorological factors, soil nutrients, soil environment and plant community are principal components, and total scores are calculated by using principal component analysis (PCA). Solid arrows denote the directions and effects that were significant (*P* < 0.05). Dashed arrows represent the directions and effects that were not significant (*P* > 0.05). R² values associated with response variables indicate the proportion of variation explained by the relationships with other variables. Values associated with arrows represent standardized path coefficients.

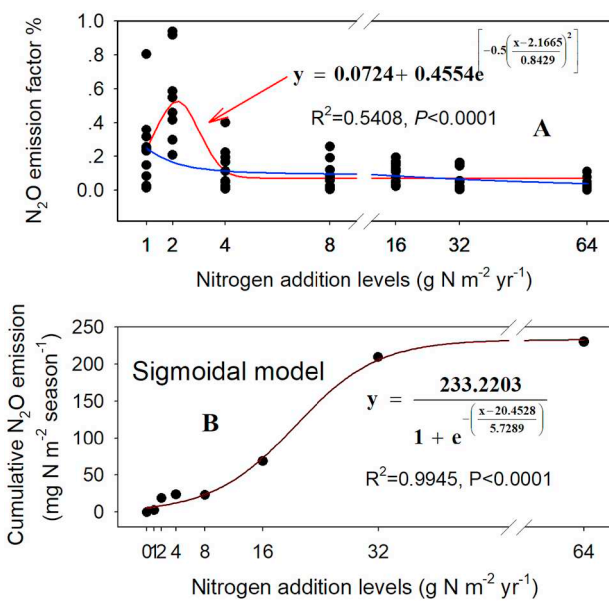


Fig. 6. (A) The red curve represents the peak model of the N₂O emission factor along the levels of N addition for three years. The blue curve is the model of N₂O emission factor along the levels of N addition with removing the 2 g N m⁻² yr⁻¹ treatment. (B) The result from regression analysis for the best fit model using the mean cumulative N₂O emission of each year during the growing seasons along the levels of N addition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

grassland soils. A previous study had also shown that long-term addition of N in the form of urea will lead to soil acidification (Goulding et al., 1998).

Secondly, lower pH can affect N cycling processes, among which the mineralization rate and the ammonia volatilization rate. The pH had no significant correlation with the ammonia volatilization rate (Fig. 3), so the main effect of pH on N cycling was likely related to the

mineralization rate. Mineralization is the process of organic N conversion into inorganic N, including the processes of ammonification and nitrification. The mineralization rate is the balance between production and consumption of inorganic N in the soil. The lower pH affected nitrification process primarily. The urea that was used in our experiment can provide more substrate for nitrification, and therefore stimulates the first step of nitrification process, which transforms ammonium into NO₂⁻. So this process results in a large number of NO₂⁻ accumulation in soil. In low pH environment NO₂⁻ toxicity is a major problem for ammonia-oxidizing bacteria (AOB), who carry on the first and N₂O-producing step in the process of nitrification (De et al., 1995).

On one hand, in an acid environment NO₂⁻ is in balance with HNO₂, which can decompose to N₂O with organic matter (Van and Samater, 1996; Mørkved et al., 2007). On the other hand, AOB who are likely the dominant N₂O-producing microorganism in N addition plot (Carey et al., 2016; Long et al., 2017), will consume the NO₂⁻ to N₂O to protect themselves from the toxicity of NO₂⁻ (Poole, 2005), which process is the nitrite-induced nitrifies denitrification. Due to above two reasons, N₂O emission rates raised substantially by impacting nitrification.

We think there is another pathway of pH affecting the N₂O emission and this is through denitrification, which reduces the consumption of N₂O. In contrast to N₂O production, which is promoted by several N-transforming, microbial processes of which the processes of nitrification and denitrification are quantitatively most important (Wrage et al., 2001), N₂O consumption is only supported by the process of denitrification (Schreiber et al., 2012). A decrease in soil pH will repress the reduction of N₂O by the process of denitrification and hence reduce the consumption of this greenhouse gas (Chapuis-Lardy et al., 2010).

In addition, based on linear regression analysis, BGB and AGB were two of the most important factors explaining N₂O emission (Fig. 4). But plant community had no remarkably direct or indirect effect on N₂O emission according to the final results of SEM in our study. Such a correlation has been explained by soil moisture constraining both parameters (Schimel et al., 1991; Merbold et al., 2014). However, our study showed that no changes in soil moisture occurred along the levels of N addition. No significant correlations were observed between soil

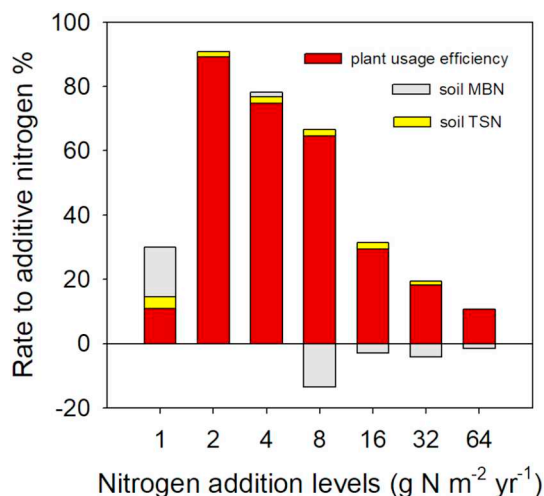


Fig. 7. Plant N usage efficiency, soil TSN (total soluble nitrogen) and MBN (microbial biomass nitrogen) partitioning as response to N addition.

moisture and plant biomass (Fig. 3). Therefore, the plant biomass did not affect N₂O emission by altering soil moisture. Possible reasons for the difference between our study and previous studies might be: 1. we avoided precipitation events when measuring the N₂O emission rates in order to focus specially on the effects of N addition; 2. the effect of the ammonium concentrations on N₂O emission rates exceeded the effects of soil moisture on these rates.

So, based on the result of SEM we come to the conclusion that by affecting the process of nitrification, soil pH plays a critical role in N₂O emission after N addition in semi-arid grasslands.

4.2. N₂O emission balance hypothesis and plant N usage efficiency

In our research, the N₂O EFs were different under multiple levels of N addition (Fig. 6A). Our EFs were lower than 1% and therefore fall within the 0–7% range mentioned in Bouwman's (1996) study. The best fit model between the N₂O EFs and N addition was not linear, but showed a maximum value at 2 g N m⁻² yr⁻¹. N₂O EFs represented the transformation efficiency of added N to N₂O emission. In general, the more N the field received, the lower the transformation efficiency was. The N addition level of 2 g N m⁻² yr⁻¹ showed the highest transformation efficiency across all the treatments. Our N₂O EF curve rose first, then dropped, so the value of ΔEF (the slope of EF) was first positive and then negative. Shcherbak et al. (2014) summarized changes in N₂O emission under N addition for 78 studies, and the ΔEFs were zero, only positive or only negative. Hence, our ΔEF was not in accordance with the patterns showed in Shcherbak et al. (2014). The difference in observations could be explained by the fact that the intervals chosen between different levels of N addition applied in the 78 studies were much larger than in our study. When we removed the points at 2 g N m⁻² yr⁻¹, the EF tend to decline constantly with increasing N addition and the value of ΔEF is negative (Fig. 6A, the blue curve). Based on the peak model, the values of N₂O EF can be infinitely close to zero, which will result in similar cumulative N₂O emission rates at higher levels of N addition. In many studies, the N₂O emission increased constantly with the level of N addition (Shcherbak et al., 2014; Philibert et al., 2012; Hoben et al., 2011; Cardenas et al., 2010). However, these studies might have reached the same conclusion that the relationship between N₂O emission and the level of applied N was nonlinear.

In our study, the cumulative N₂O emission over the growing season remained the same when the soil received > 32 g N m⁻² yr⁻¹. It suggested that somewhere between 32 g N m⁻² yr⁻¹ and 64 g N m⁻² yr⁻¹ could be the N₂O emission balance point in the semi-arid grassland. However, most studies did not find such a N₂O emission balance point

and only indicated that the maximum of N addition did not come up to the N₂O emission balance point. Before N₂O emission balance point, the N addition acts on N₂O emission through pH and then N cycling. What are the mechanisms after N₂O emission balance point or what happens at the balance point? We think after the balance point, the mechanism which exists before balance point is suppressed, and another mechanism is working. Here we suggest two successive mechanisms that may explain why a N₂O emission balance point occurred. The first one is that the nutrient-holding capacity of the soil was limited. Even with more N in soil, the soil cannot conserve it when the amount is over its saturation threshold. The redundant N will then be lost in different forms of N via abiotic pathway. The second mechanism may be the limitation of soil nutrients other than nitrogen, such as carbon (Morley and Baggs, 2010) and phosphorus (Mehnaz and Dijkstra, 2016). In the experiment of addition of different carbon compounds, Morley and Baggs (2010) found that carbon had an indirect function on N₂O production through lowering O₂ concentrations with the stimulation of heterotrophic respiration. Mehnaz and Dijkstra's (2016) studies showed that phosphorus addition played a major role in improving N₂O emission rates under high N input conditions through denitrification. So soil available N becomes less useful for plant and microorganisms when other nutrients are limited. So, the EF of 1% used by IPCC to estimated N₂O emission is inaccurate, especially when the level of N addition exceeds the N₂O emission balance point. It will then overestimate the global N₂O emissions.

The plant N usage efficiency rate had increased to maximum values at 2 g N m⁻² yr⁻¹ (Fig. 7). When soil received 1 g N m⁻² yr⁻¹, the MBN partitioning as response to added N was the highest (Fig. 7). So available N was first used for microorganism growth, and then for plant growth when the soil received < 2 g N m⁻² yr⁻¹. With levels larger than 2 g N m⁻² yr⁻¹, plant N usage efficiency tended to decline. The excess N can then be lost through nitrate leaching under wet conditions, by abiotic ammonia volatilization, and biotic N₂O emission. Nitrate leaching with rainfall has also been identified as a main sign of N saturation in N-limited temperate grasslands (Ryden et al., 1984) and forests (Peterjohn et al., 1996). Thus, plants could have the highest N usage efficiency when N deposition was 2 g N m⁻² yr⁻¹ in semi-arid grassland ecosystems. The plant biomass did not level off above 2 g N m⁻² yr⁻¹, as it still increased when the soil received over 2 g N m⁻² yr⁻¹ (Fig. 2B). The N₂O emission rates rose sharply at this range, while the change in plant biomass was small. In semi-arid grassland, N was the limiting factor for plant growth, so moderate N addition was beneficial to it. Once the large and continuous N addition is beyond the moderate range but still below the N₂O emission balance point, it does not stimulate anymore plant growth and N₂O will be released into the atmosphere.

5. Conclusion

In this study, we investigated during a period of three years (i.e. 2013–2015) the effects of environment and plant on N₂O emission in a semi-arid grassland ecosystem under long-term N additions. N₂O emission was significantly and positively correlated with soil temperature, NO₃⁻-N, NH₄⁺-N, inorganic N, microbial biomass carbon, microbial biomass N, total soluble N, above-ground and below-ground biomass, but negatively with soil pH. The mechanism of N₂O emission increasing with N addition took place through pH, and then the process of nitrification in the grasslands.

N₂O emission increased with increasing levels of N addition. N₂O EF decreased above the level of N addition larger than 2 g N m⁻² yr⁻¹. The cumulative N₂O emission over the growing season reached its maximum at levels of N addition above 32 g N m⁻² yr⁻¹. In this study, the “N₂O emission balance hypothesis” was supported and somewhere between 32 g N m⁻² yr⁻¹ and 64 g N m⁻² yr⁻¹ turned out to be the N₂O emission balance point in semi-arid grassland. The plant N usage efficiency reached the highest value when the level of N addition

amounted to $2 \text{ g N m}^{-2} \text{ yr}^{-1}$.

These findings have important implications for mitigation strategies of N_2O emission and provide the knowledge for understanding the mechanisms of the effects of N additions on N_2O emission in the semiarid grassland ecosystems of Northern China. It will help us in our investigation of the functions and processes in grassland ecosystem as affected by global climate change.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2016YFA0600803, 2016YFC0500601) and the National Natural Science Foundation of China (41371111). Xiaofeng Xu is grateful for financial supports from San Diego State University and the National Science Foundation (1702797). Financial assistance was partially provided by the SPRUCE and NGEA Arctic projects, which are supported by the Office of Biological and Environmental Research in the Department of Energy Office of Science.

References

- Bouwman, A.F., 1996. Direct emission of nitrous oxide from agricultural soils. *Nutr. Cycl. Agroecosyst.* 46 (1), 53–70.
- Cardenas, L.M., Thorman, R., Ashlee, N., Butler, M., Chadwick, D., Chambers, B., et al., 2010. Quantifying annual N_2O emission fluxes from grazed grassland under a range of inorganic fertilizer nitrogen inputs. *Agric. Ecosyst. Environ.* 136 (3–4), 218–226.
- Carey, C.J., Dove, N.C., Beman, J.M., Hart, S.C., Aronson, E.L., 2016. Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biol. Biochem.* 99, 158–166.
- Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.L., Bernoux, M., 2010. Soils, a sink for N_2O ? A review. *Glob. Chang. Biol.* 13 (1), 1–17.
- Davidson, E.A., 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nat. Geosci.* 2 (4), 659–662.
- De, B.W., Gunnewiek, P.A.K., Laanbroek, H.J., 1995. Ammonium-oxidation at low pH by a chemolithotrophic bacterium belonging to the genus *Nitrosospora*. *Soil Biol. Biochem.* 27, 127–132.
- Denmead, O.T., Freney, J.R., Simpson, J.R., 1979. Studies of nitrous oxide emission from a grass sward. *Soil Sci. Soc. Am. J.* 43 (4), 726–728.
- Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., et al., 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. *New Phytol.* 139 (1), 49–58.
- Granli, T., Bockman, O.C., 1994. Nitrous oxide from agriculture. *Nor. J. Agric. Sci. (Suppl. 12)*, 1–128.
- Hoben, J.P., Gehl, R.J., Millar, N., Grace, P.R., Robertson, G.P., 2011. Nonlinear nitrous oxide (N_2O) response to nitrogen fertilizer in on-farm corn crops of the US Midwest. *Glob. Chang. Biol.* 17 (2), 1140–1152.
- Hook, P.B., Burke, I.C., 1995. Evaluation of methods for estimating net nitrogen mineralization in a semiarid grassland. *Soil Sci. Soc. Am. J.* 59 (3), 831–837.
- Jäger, N., Stange, C.F., Ludwig, B., Flessa, H., 2011. Emission rates of N_2O and CO_2 from soils with different organic matter content from three long-term fertilization experiments—a laboratory study. *Biol. Fertil. Soils* 47 (5), 483–494.
- Klein, C.A.D., Shepherd, M.A., Weerden, T.J.V.D., 2014. Nitrous oxide emissions from grazed grasslands: interactions between the n cycle and climate change — a New Zealand case study. *Curr. Opin. Environ. Sustain.* 9–10, 131–139.
- Klimont, Z., Cofala, J., Schöpp, W., Amann, M., Streets, D.G., Ichikawa, Y., et al., 2001. Projections of SO_2 , NO_x , NH_3 and VOC emissions in East Asia up to 2030. *Water Air Soil Pollut.* 130 (1–4), 193–198.
- Long, X.E., Shen, J.P., Wang, J.T., Zhang, L.M., Di, H., He, J.Z., 2017. Contrasting response of two grassland soils to N addition and moisture levels: N_2O emission and functional gene abundance. *J. Soils Sediments* 17 (2), 384–392.
- Ma, B.L., Wu, T.Y., Tremblay, N., Deen, W., Morrison, M.J., McLaughlin, N.B., et al., 2010. Nitrous oxide fluxes from corn fields: on-farm assessment of the amount and timing of nitrogen fertilizer. *Glob. Chang. Biol.* 16 (1), 156–170.
- Matson, P., Lohse, K.A., Hall, S.J., 2002. The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio* 31 (2), 113–119.
- Mcsweeney, C.P., Robertson, G.P., 2010. Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea mays* L.) cropping system. *Glob. Chang. Biol.* 11 (10), 1712–1719.
- Mehnaz, K.R., Dijkstra, F.A., 2016. Denitrification and associated N_2O emissions are limited by phosphorus availability in a grassland soil. *Geoderma* 284, 34–41.
- Merbold, L., Eugster, W., Stieger, J., Zahniser, M., Nelson, D., Buchmann, N., 2014. Greenhouse gas budget (CO_2 , CH_4 and N_2O) of intensively managed grassland following restoration. *Glob. Chang. Biol.* 20 (6), 1913–1928.
- Mørkved, Pål Tore, Dörsch, Peter, Bakken, L.R., 2007. The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biol. Biochem.* 39 (8), 2048–2057.
- Morley, M., Baggs, E.M., 2010. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. *Soil Biol. Biochem.* 42, 1864–1871.
- Mosier, A., Schimel, D., Valentine, D., Bronson, K., Parton, W., 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350 (6316), 330–332.
- Mosier, A.R., Parton, W.J., Valentine, D.W., Ojima, D.S., Schimel, D.S., Heinemeyer, O., 1997. CH_4 and N_2O fluxes in the Colorado shortgrass steppe: 2. Long-term impact of land use change. *Glob. Biogeochem. Cycles* 11 (1), 29–42.
- Mosier, A., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S., Ovan, C., 1998. Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle: OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr. Cycl. Agroecosyst.* 52 (2–3), 225–248.
- Niu, S., Wan, S., 2008. Warming changes plant competitive hierarchy in a temperate steppe in northern China. *J. Plant Ecol.* 1 (2), 103–110.
- Peterjohn, W.T., Adams, M.B., Gilliam, F.S., 1996. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. *Biogeochemistry* 35 (3), 507–522.
- Philibert, A., Loyce, C., Makowski, D., 2012. Quantifying uncertainties in N_2O emission due to N fertilizer application in cultivated areas. *PLoS One* 7 (11), e50950.
- Poole, R.K., 2005. Nitric oxide and nitrosative stress tolerance in bacteria. *Biochem. Soc. Trans.* 33, 176–180.
- Ryden, J.C., Ball, P.R., Garwood, E.A., 1984. Nitrate leaching from grassland. *Nature* 311 (5981), 50–53.
- Scheer, C., Wassmann, R., Kienzler, K., Ibragimov, N., Eschanov, R., 2008. Nitrous oxide emissions from fertilized, irrigated cotton (*Gossypium hirsutum* L.) in the Aral Sea Basin, Uzbekistan: influence of nitrogen applications and irrigation practices. *Soil Biol. Biochem.* 40 (2), 290–301.
- Schimel, D.S., Knapp, A.K., Seastedt, T.R., Parton, W.J., Brown, V.B., 1991. Physiological interactions along resource gradients in a tallgrass prairie. *Ecology* 72 (2), 672–684.
- Schreiber, F., Wunderlin, P., Kai, M.U., Wells, G.F., 2012. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Front. Microbiol.* 3 (372), 372.
- Scherebak, I., Millar, N., Robertson, G.P., 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci. U. S. A.* 111 (25), 9199–9204.
- Skiba, U., Smith, K.A., 2000. The control of nitrous oxide emissions from agricultural and natural soils. *Chemosphere Global Change Sci.* 2 (3–4), 379–386.
- Van, C.O., Samater, A.H., 1996. Nitrite in soils: accumulation and role in the formation of gaseous N compounds. *Fertil. Res.* 45, 81–89.
- Vance, E.D., Brooks, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass. *Soil Biol. Biochem.* 19 (19), 703–707.
- Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., et al., 1997. Technical report: human alteration of the global nitrogen cycle: sources and consequences. *Ecol. Appl.* 7 (3), 737–750.
- Wang, Z., Liu, X., Ju, X., Zhang, F., 2002. Field in situ determination of ammonia volatilization from soil: venting method. *Plant Nutr. Fertil. Sci.* 8 (2), 205–209.
- Wrage, N., Velthof, G.L., Beusichem, M.L.V., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* 33 (12), 1723–1732.
- Xu, X., Tian, H., Hui, D., 2008. Convergence in the relationship of CO_2 and N_2O exchanges between soil and atmosphere within terrestrial ecosystems. *Glob. Chang. Biol.* 14, 1651–1660.
- Zhang, Li, Wylie, B.K., Ji, L., Gilmanov, T.G., Tieszen, L.L., Laca, E.A., et al., 2010. Climate-driven interannual variability in net ecosystem exchange in the northern great plains grasslands. *Rangel. Ecol. Manag.* 63 (1), 40–50.
- Zhang, L., Huo, Y., Guo, D., Wang, Q., Yin, B., Li, L., 2014. Effects of multi-nutrient additions on GHG fluxes in a temperate grassland of northern China. *Ecosystems* 17 (4), 657–672.
- Zhang, L., Hou, L., Guo, D., Li, L., Xu, X., 2016. Interactive impacts of nitrogen input and water amendment on growing season fluxes of CO_2 , CH_4 , and N_2O in a semiarid grassland, Northern China. *Sci. Total Environ.* 578, 523–534.