

RESEARCH ARTICLE

Five-year study of the effects of simulated nitrogen deposition levels and forms on soil nitrous oxide emissions from a temperate forest in northern China

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Abstract

Few studies have quantified the effects of different levels and forms of nitrogen (N) deposition on soil nitrous oxide (N₂O) emissions from temperate forest soils. A 5-year field experiment was conducted to investigate the effects of multiple forms and levels of N additions on soil N₂O emissions, by using the static closed chamber method at Xi Mountain Experimental Forest Station in northern China. The experiment included a control (no N added), and additions of NH₄NO₃, NaNO₃, and (NH₄)₂SO₄ that each had two levels: 50 kg N ha⁻¹ yr⁻¹ and 150 kg N ha⁻¹ yr⁻¹. All plots were treated to simulate increased N deposition on a monthly schedule during the annual growing season (March to October) and soil N₂O emissions were measured monthly from March 2011 to February 2016. Simultaneously, the temperature, moisture, and inorganic N contents of soil were also measured to explore how the main factors may have affected soil N₂O emission. The results showed that the types and levels of N addition significantly increased soil inorganic N contents, and the accumulation of soil NO₃⁻-N was significantly higher than that of soil NH₄⁺-N due to N addition. The three N forms significantly increased the average N₂O emissions (*P* < 0.05) in the order of NH₄NO₃ > (NH₄)₂SO₄ > NaNO₃ by 355.95%, 266.35%, and 187.71%, respectively, compared with control. The promotion of N₂O emission via the NH₄⁺-N addition was significantly more than that via the NO₃⁻-N addition, while N addition at a high level exerted a stronger effect than at the low-level. N addition exerted significantly stronger effects on cumulative N₂O emissions in the initial years, especially the third year when the increased cumulative N₂O emission reached their maximum. In the later years, the increases persisted but were weakened. Increasing inorganic N concentration could change soil from being N-limited to N-rich, and then N-saturated, and so the promotion on soil available N effect increased and then decreased. Moreover, the soil NH₄⁺-N, NO₃⁻-N, temperature, and water-filled pore space were all positively correlated with soil N₂O emissions. These findings suggest that atmospheric N deposition can significantly promote soil N₂O emission, and that exogenous NH₄⁺-N and NO₃⁻-N inputs into temperate forests can have synergic effects on soil N₂O

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emission. In future research, both aspects should be better distinguished in the N cycle and balance of terrestrial ecosystems by using ¹⁵N tracer methods.

Introduction

Nitrous oxide (N₂O) is not only a potent greenhouse gas whose global warming potential is 298- and 21-fold that of CO₂ and CH₄, but it also contributes to stratospheric ozone depletion [1]. Emissions of N₂O from soil have been identified as the primary source (57%) of total global N₂O emissions [2].

Nitrification and denitrification are the two main processes that produce N₂O in soils and both can occur simultaneously (Fig 1). N₂O is produced by denitrifying bacteria during the reduction of NO₃⁻ or NO₂⁻ to N₂O and N₂, or released as an intermediate product when nitrifying bacteria oxidize NH₄⁺-N to NO₃⁻ and NO₂⁻ [3]. These two processes may be affected by soil water content, temperature, N availability and pH, as well as other particular biotic or abiotic properties [4–6]. Inorganic N is a key factor regulating soil N₂O emission [4–5, 7–8] (Fig 1). In general, increasing available mineral N in soils leads to enhanced N₂O formation and emission via increased nitrification and denitrification rates [9]. Soil N₂O emission is also driven by soil temperature and water content [10]. Some previous studies indicated soil N₂O emissions were increased under conditions of higher soil water content and soil temperature [10]. The latter may regulate soil N₂O emission by influencing N₂O-producing microorganisms, such as nitrifying and denitrifying bacteria [11]. Furthermore, low soil moisture can reduce the temperature sensitivity of soil microbes, so that the diffusion of extracellular enzymes in the substrate are lowered [12].

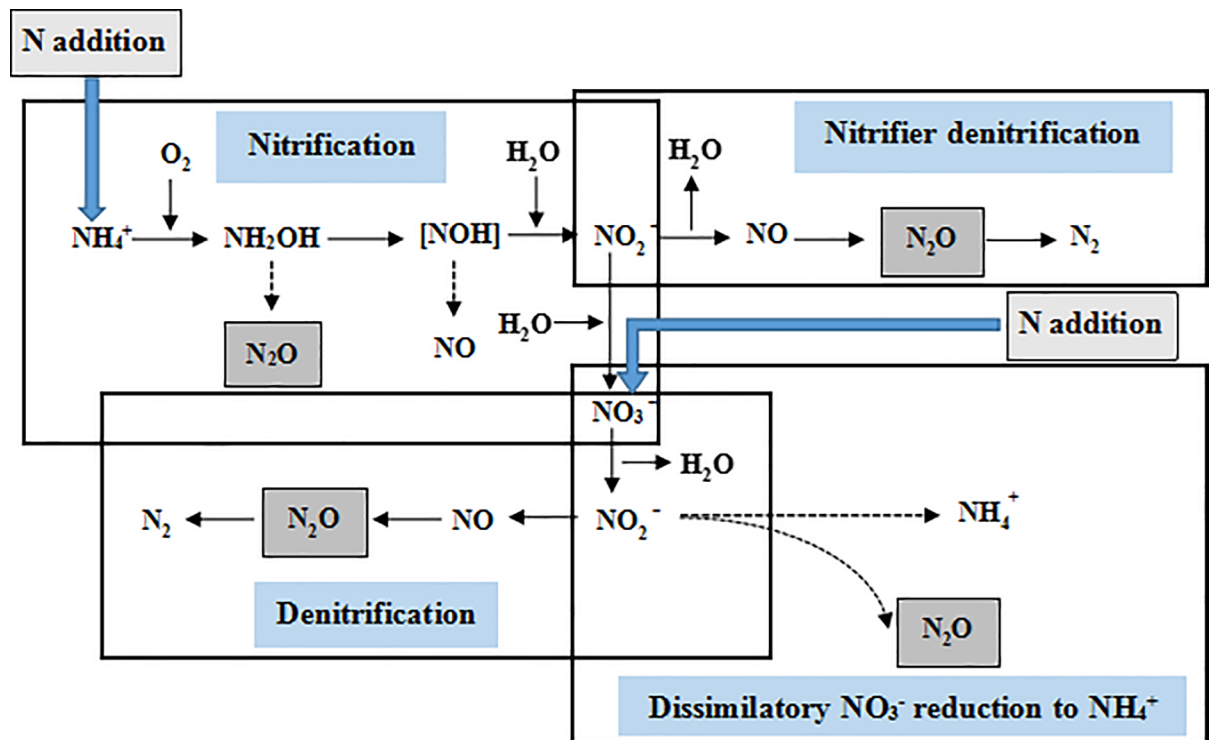


Fig 1. Main processes produce N₂O in soils.

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China is now ranked third behind Europe and North America in terms of the scale of anthropogenic reactive N emissions, and has been experiencing a dramatic increase in anthropogenic reactive N due to its rapid economic development [10]. The average N deposition in our study area was 13.2 kg N ha⁻¹ yr⁻¹ in the 1980s and 21.1 kg N ha⁻¹ yr⁻¹ in the 2000s [13]. Alongside increases in N deposition there have been decreases in the ratio of NH₄⁺-N to NO₃⁻-N deposition, from approximately 5 to 2, from the 1980s to the 2000s, although NH₄⁺-N remains the dominant form of N deposition [13]. Nationally, N deposition is a more serious issue in the north compared with the other regions of China [14].

Increasing N deposition could influence the production and emission of N₂O by disturbing the balance between microbial N mineralization and immobilization, with the consequences for the relative availability of soil NH₄⁺-N and NO₃⁻-N [15] (Fig 1). Most studies report that raising N addition levels could linearly stimulate soil N₂O emissions [4–5, 16–17]. A meta-analysis of global N addition experiments showed that N additions increased soil N₂O emissions by an average of 134% in terrestrial ecosystems [18]. Some plausible mechanisms have been proposed to clarify the promotion effect of N addition for soil N₂O emissions: (1) Without additional N, the N retention in soil is mainly used by plants and microorganisms to maintain biomass and growth, so less N becomes lost as gaseous N [19]; (2) The amount of additional N greatly exceeds the atmospheric N deposition, thus leading to N accumulation in forest soil, which can benefit nitrifying and denitrifying bacteria [20] which would stimulate the nitrification rate and N₂O emissions [21]. However, some studies indicated that N addition has no significant effect on soil N₂O emission, which might be attributed to particular N addition threshold level for increased N₂O emissions [7, 22]. Thornton and Valente [23] found that the increased rate of soil N₂O emissions was low at high N-addition levels; this may have occurred because the high level N addition to soil drove other limitations, such as carbon availability, thereby decreasing the C/N ratios that regulate the status of N saturation, which likely had a strong influence on N₂O emission [24]. Furthermore, some studies have shown denitrification to be the main source of soil N₂O emissions [25–26], whereas other studies reported that nitrification were primarily responsible for soil N₂O emissions [7, 27–28]. Clearly then, how soil N₂O emissions respond to additional N appears to be inconsistent.

The NH₄⁺-N/NO₃⁻-N ratio showed a decreasing trend in our study area [29], and so clarifying the response of soil N₂O emission to different forms and levels of N addition now is necessary. However, several previous studies that stimulated N deposition only considered NH₄NO₃ [2, 4, 6], while others that did examine N deposition in varied N forms only reported their short-term effects on N₂O emission [30]. In addition, some studies have focused on soil core incubations in the laboratory [31], which are conditions that differ greatly from those in the field. Therefore, from both a scientific and management perspective, further examination of the characteristics of different levels and forms of N addition is critically important for better understanding how N deposition affects soil N₂O emissions in temperate forest soils.

In our study, we report the results of continuous measurements of soil N₂O emissions over a 5-year period from a temperate forest in northern China. Based on the above analysis, we hypothesized that (1) N addition could increase soil N₂O emission and that this promotion effect likely increased with the N addition level; (2) Applying NO₃⁻ and NH₄⁺-N in combination could promote soil N₂O emission more than would their respective single applications.

Materials and methods

Study area

The study was conducted in a temperate forest of the Xi Mountain Experimental Forest Station (31°54'32" N, 110°68'08" E, 133 m a.s.l.) in Beijing, northern China. The station belongs to

Beijing Forestry University. The study area is characterized by a temperate continental monsoon climate with a maximum air temperature of 31 °C in July and a minimum of -9 °C in January. Mean annual temperature is 11.6 °C and the average annual precipitation is 630 mm. During the 5-year experimental period, the yearly maximum and minimum temperatures were, respectively, 31, 31, 32, 33, 31 °C and -9, -8, -8, -5, -5 °C, while the total precipitation received annually was 721, 759, 508, 500, and 459 mm. At this research station, *Quercus liaotungensis* is the zonal vegetation with an average age of 62 years. The diameter at breast height, canopy closure, average height, and density were 9.7 cm, 69%, 8.4 m, and 2963 trees ha⁻¹. The soil here is classified as Chromic Luvisols (WRB Soil Classification) composed of 51% sand, 40% silt, and 9% clay. The thickness of the soil humus horizon (A horizon) is approximately 3–5 cm, and the O horizon thickness < 3 cm. Before starting the experiment, soil samples from the upper 10 cm of soil in each plot (with three replicates) were collected by using corers in March 2011. Initial soil properties were measured and showed no significant differences among the plots (Table 1).

Experimental design

The experiments were performed from March 2011 to February 2016. Seven 10 m × 10 m N addition plots, with three replicates each (n = 21 plots in total), were randomly established and distributed on a flat ground dominated by the *Quercus liaotungensis* community at the research station. To ensure plot independence, 1.5-m buffer strips were set up between adjacent plots. As deposition of NH₄⁺-N and NO₃⁻-N showed great variation from month to month in the study area [30], three N-addition forms, namely NaNO₃, (NH₄)₂SO₄, and NH₄NO₃, were used to simulate the effects of deposited NH₄⁺-N, NO₃⁻-N, and their combination. According to the current level of atmospheric N deposition (30.6 kg N ha⁻¹ yr⁻¹) at the experimental site [30], two N-addition levels referred to as low N (L: 50 kg N ha⁻¹ yr⁻¹) and high N (H: 150 kg N ha⁻¹ yr⁻¹) were used to simulate a future increase in atmospheric N deposition by 1.5-fold and 5-fold. A control (0 kg N ha⁻¹ yr⁻¹) was used to calculate the net effect of naturally occurring N addition to the soil. From 2011 to 2015, additional N was evenly sprayed on the soil surface in plots by using sprayers, with eight equal applications made from March to October (i.e., the growing season). If it rained, the scheduled N addition was postponed to 1 day after the rain day. To reduce the effect of additional water on the experiment, control plots received an equivalent deionized water treatment.

Gas sampling and measurement

Soil N₂O emission measurements were performed three times in the first week of each month, from March 2011 to February 2016. Soil N₂O emissions were measured using a static closed

Table 1. Soil properties of the sampling area.

Variable	Treatment						
	Control	NaNO ₃		(NH ₄) ₂ SO ₄		NH ₄ NO ₃	
		L	H	L	H	L	H
pH	7.18±0.28a	7.02±0.39a	7.16±0.49a	7.01±0.35a	7.20±0.28a	7.19±0.39a	7.12±0.21a
Organic C (g kg ⁻¹)	29.97±0.88a	30.14±1.45a	29.45±1.73a	28.37±1.65a	29.15±0.83a	30.33±1.38a	31.06±1.67a
Total N (g kg ⁻¹)	2.43±0.68a	2.35±0.84a	2.36±0.72a	2.40±0.68a	2.54±0.60a	2.39±0.66a	2.44±0.48a
NH ₄ ⁺ -N (mg/kg)	2.82±0.20a	2.51±0.46a	2.37±0.43a	2.45±0.22a	2.41±0.36a	2.47±0.44a	2.55±0.36a
NO ₃ ⁻ -N (mg/kg)	11.83±1.01a	12.80±1.02a	12.87±1.18a	13.05±1.09a	12.78±1.26a	13.06±1.22a	13.15±1.36a

L: 50 kg N ha⁻¹ yr⁻¹; H: 150 kg N ha⁻¹ yr⁻¹.

Treatments with same letter mean no significant difference in the whole row parameters.

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opaque chamber and gas chromatography method [32]. The chamber was made of stainless steel and consisted of a fixed base and a removable top (without bottom, length × width × height = 50 cm × 50 cm × 50 cm). Before measurement, the base, which supported the sampling chamber, was installed into the soil at a depth of 20 cm for the entire experiment to avoid soil disturbance. Soil temperatures were measured in each plot at a depth of 5 cm nearby the chamber before and after collecting gas samples. And the average temperature value was used for emission calculation. The fixed base frame was free of vegetation. When collecting the gases, we inserted the removable top into the fixed base. The chamber was covered with thermal insulation cotton to reduce the impact of direct radiative heating in the chamber and a digital thermometer in the chamber was used to record its air temperature. Two fans were used to increase mixing and uniformity of air in the chamber.

Gas samples were collected three times, from a sampling outlet at the top of the chamber, from 09:00 to 11:00 AM local time on the first, fourth, and seventh day after N addition in each month from March 2011 to February 2016. If unpredicted extreme weather occurred, such as heavy rain or snow, this gas sampling was rescheduled. Gas samples were taken using 100 mL plastic syringes at intervals of 0, 10, 20, and 30 minutes after closing the chamber and inserting polyethylene-coated aluminum bags for soil N₂O concentration analysis. Gas samples were analyzed within 6 h in a gas chromatograph (Agilent 7890A, Agilent Technologies Inc., Palo Alto, CA, USA) [33].

Soil N₂O emissions were calculated as follows [33]:

$$F_{N_2O} = D \times H \times (\Delta c / \Delta t) \quad (1)$$

where, F_{N_2O} refers to N₂O emission ($\mu\text{g m}^{-2} \text{h}^{-1}$); D refers to the gas density of the chamber (mol m^{-3}); $D = WP/RT$; W refers to the molar mass of N₂O (g mol^{-1}); P refers to air pressure (Pa); T refers to the air temperature inside the chamber (K); R refers to the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$); H refers to the height of the sampling chamber (m); and $\Delta c / \Delta t$ denotes the linear slope of the concentration change over the measurement period.

Soil cumulative N₂O emissions were calculated by interpolating the N₂O emissions measured between sampling periods [34]. Cumulative N₂O emissions were calculated spanning the time period from March to February next year as follows [35]:

$$\text{Cumulative N}_2\text{O emissions} = \frac{\sum_{i=1}^n 0.5 \times (F_i + F_{i+1}) \times (t_{i+1} - t_i) \times 24}{100000} \quad (2)$$

where, F is the N₂O emissions ($\mu\text{g m}^{-2} \text{h}^{-1}$); i is the sampling number, i.e., samples collected in March had a value of 1 and those collected next February had a value of 12; and t is the sampling time based on the Julian day.

Soil sampling and measurement

Considering that N₂O release mainly occurred in the mineral horizon, litter was first removed from the soil surface (O horizon < 3 cm) when sampling the soil. Soil samples at 0–10 cm depth were collected from near the static chambers monthly. Soil samples were passed through a 2-mm sieve to remove roots, gravel, and stones for soil analyses. Part of the fresh soil was used for soil NH₄⁺-N and NO₃⁻-N content analyses, while the remaining portion was air-dried for pH measurement. Soil NO₃⁻-N and NH₄⁺-N concentrations were determined by the KCl extraction method [5]. Soil water content (WC) was measured using the standard oven-drying method at 105°C for 8 h. Bulk density (BD) was determined by the core method.

Water-filled pore space (WFPS) (%) was calculated based on the equation:

$$\text{WFPS} = (\text{WC} \times \text{BD}) \times \frac{100}{(1 - \text{BD}/2.65)} \quad (3)$$

where 2.65 (g cm⁻³) refers to the assumed soil particle density.

Statistical analysis

All statistical analyses were conducted by SPSS v22.0 (IBM Corp., Armonk, USA) and the significance level for all statistical tests was set at $P = 0.05$. The differences in initial soil properties between different N-addition plots were examined using one-way analysis of variance (ANOVA) and least significant difference (LSD). Repeated-measures ANOVA was used to analyze the effects of N forms, N levels, experimental years, and their interactions on the temporal variation of soil N₂O emissions, annual cumulative N₂O emissions, ST, WFPS, and inorganic N concentrations. We examined the differences in annual N₂O emissions within each single year among the N additions by one-way ANOVA and LSD testing, and the differences within each N addition throughout the 5 years. Pearson's correlation analyses and linear regression analyses were used to examine the relationships between soil N₂O emissions and environmental variables. Means and standard deviations of N₂O emissions were calculated, and the plot values represented means ($n = 3$) \pm standard error (SE).

Results

Soil N₂O emissions under N addition

During the 5-year experimental period, the temperate forest soil was a net source of N₂O. Soil N₂O emissions were higher between May and September, but the values were lower and leveled off in other times of each year. Meanwhile, the peak of soil N₂O emissions was concentrated in August of each year (Fig 2). Soil N₂O emissions were significantly influenced by N forms, N levels, and the sampling time ($P < 0.01$), but the interaction effect of N forms and levels, months and N levels or months, N forms and N levels, did not significantly influence the soil N₂O emissions ($P > 0.05$, Table 2).

Promotion effects of different N forms and levels

Different levels and forms of N addition and experimental time all significantly influenced the soil N₂O emissions ($P < 0.01$, Table 2). As for the two N-level addition treatments, the N-addition treatments significantly increased soil N₂O emissions, and this promotion effect was enhanced as the N-addition levels increased (Table 2, Fig 2). Soil N₂O emissions ranged from 1.30 $\mu\text{g m}^{-2} \text{h}^{-1}$ to 34.44 $\mu\text{g m}^{-2} \text{h}^{-1}$, with an average value of 11.55 $\mu\text{g m}^{-2} \text{h}^{-1}$ in the control plots (Fig 2). Compared to the control, the average N₂O emissions in the low- and high-level N addition plots significantly increased by 186.02% and 353.98%, respectively. The maximal emissions were obtained in August 2013 for the low and high nitrogen addition serials, which were 163.23 and 276.33 $\mu\text{g m}^{-2} \text{h}^{-1}$ in the L-NH₄NO₃ and H-NH₄NO₃ addition plots respectively, for all the three added nitrogen forms (Fig 2).

As for the N-addition treatments using the different forms of N, soil N₂O emissions were significantly increased by NH₄NO₃, (NH₄)₂SO₄, and NaNO₃ additions in the order of NH₄NO₃ > (NH₄)₂SO₄ > NaNO₃ > control for the same level of N addition (Fig 2, Table 2). Compared to the control, the average N₂O emissions in the NH₄NO₃, (NH₄)₂SO₄, and NaNO₃ addition plots significantly increased by 355.95%, 266.35%, and 187.71%, respectively (Fig 2).

There was no significant interaction between N form and N level on soil N₂O emissions ($P > 0.05$, Table 2).

Interannual soil cumulative N₂O emissions under N addition

Except for the interaction between N form and N level, year, N form and N level as well as all their interactions exerted significant effects on cumulative N₂O emissions (Table 2). In the

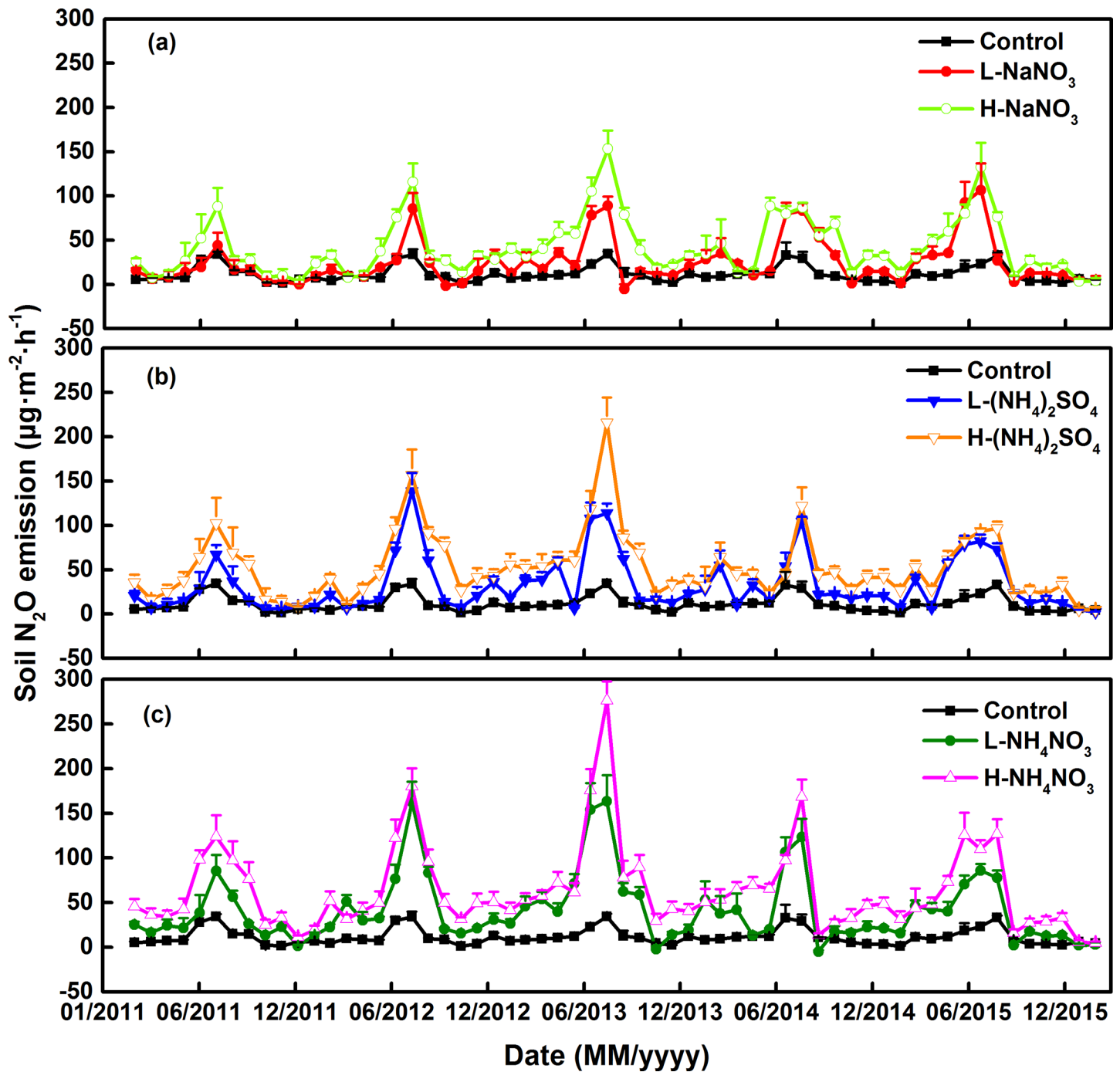


Fig 2. Variations of soil N₂O emissions applied with different forms and levels of N addition among five-year experimental period. L: 50 kg N ha⁻¹ yr⁻¹; H: 150 kg N ha⁻¹ yr⁻¹. Error bars indicate the standard error of the mean (n = 9).

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Table 2. Summary of repeated measures ANOVA results (*F* values) indicating the effects of different forms and levels of N addition and experimental time on temporal variation of soil N₂O emissions and annual cumulative N₂O emissions.

N ₂ O emissions			Cumulative N ₂ O emissions		
Subjects	d.f.	<i>F</i>	Subjects	d.f.	<i>F</i>
Between subjects					
Form	2	8.367 **	Form	2	57.816 **
Level	1	24.979 **	Level	1	172.416 **
Form × Level	2	0.165 ns	Form × Level	2	1.138 ns
Within subjects					
Month	59	28.630 **	Year	4	120.249 **
Month × Form	118	3.774 **	Year × Form	8	19.198 **
Month × Level	59	2.040 ns	Year × Level	4	10.061 **
Month × Form × Level	118	0.624 ns	Year × Form × Level	8	2.571 *

* *P* < 0.05

** *P* < 0.01 and ns *P* > 0.05.

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control plot, cumulative N₂O emissions showed no significant differences among the 5 years (Table 3). However, in the N addition plots, the promotion effect of additional N on soil N₂O emissions increased over time in the initial years, but then it decreased. As for the three N-form additions, ANOVA showed that the annual emissions were basically elevated by NH₄NO₃, (NH₄)₂SO₄, and NaNO₃ additions in the order of NH₄NO₃ > (NH₄)₂SO₄ > NaNO₃ > control (*P* < 0.05), but no significant differences were found between NaNO₃ and control plots in the first year (Table 3, *P* > 0.05).

Environmental variables and their correlation with N₂O emissions

During the 5-year period, air temperature had a clear seasonal pattern with higher temperatures in wet seasons (May to September) and lower in dry seasons (November to February). Soil temperature (ST) at the 5-cm depth fluctuated greatly, following changes in air temperature. The highest ST was 29.9°C and lowest was -7.2°C. WFPS ranged from 10.20% to 69.84% and fluctuated greatly (Fig 3). There were no significant differences among different N-addition plots on ST and WFPS (*P* > 0.05, Table 4).

Table 3. Cumulative N₂O emission (kg N ha⁻¹ yr⁻¹) from different N addition treatments plots.

Treatments	Cumulative N ₂ O emissions (kg N ha ⁻¹ yr ⁻¹)				
	2011	2012	2013	2014	2015
Control	0.98±0.02 _{a(a)}	1.01±0.09 _{a(a)}	1.08±0.08 _{a(a)}	1.04±0.15 _{a(a)}	0.97±0.14 _{a(a)}
L-NH ₄ NO ₃	2.52±0.03 _{d(a)}	4.28±0.34 _{d(d)}	5.32±0.43 _{d(e)}	3.18±0.10 _{c(c)}	2.88±0.04 _{b(b)}
H-NH ₄ NO ₃	4.73±0.04 _{f(a)}	5.82±0.75 _{d(b)}	7.52±0.77 _{f(c)}	5.22±0.46 _{e(b)}	4.59±0.74 _{d(a)}
L-(NH ₄) ₂ SO ₄	1.69±0.15 _{b(a)}	3.16±0.16 _{c(b)}	3.84±0.54 _{c(c)}	2.75±0.36 _{b(c)}	2.86±0.23 _{b(b)}
H-(NH ₄) ₂ SO ₄	3.41±0.00 _{e(a)}	5.25±0.24 _{e(c)}	6.21±0.25 _{e(d)}	4.13±0.48 _{d(b)}	3.71±0.28 _{c(b)}
L-NaNO ₃	1.15±0.18 _{a(a)}	1.89±0.20 _{b(b)}	2.57±0.23 _{b(c)}	2.65±0.25 _{b(c)}	2.63±0.32 _{b(c)}
H-NaNO ₃	2.31±0.17 _{c(a)}	3.30±0.05 _{c(b)}	4.97±0.73 _{cd(c)}	3.95±0.28 _{d(c)}	3.67±0.69 _{c(b)}

L: 50 kg N ha⁻¹ yr⁻¹; H: 150 kg N ha⁻¹ yr⁻¹.

Different superscripts of lowercase letters outside the parentheses indicate the significant differences at the level of *P* < 0.05 between the treatments within the same column, inside the parentheses indicate difference at the level of *P* < 0.05 between the experimental years within the same row.

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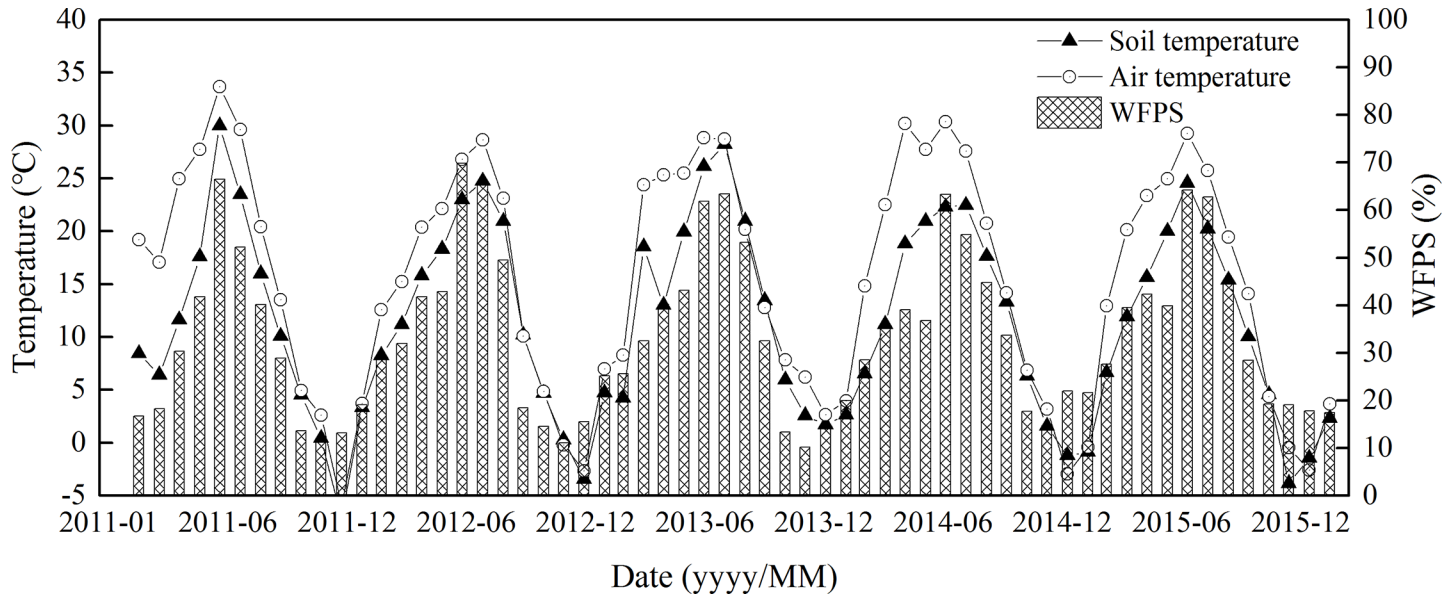


Fig 3. Water filled pore space (WFPS), soil temperature and air temperature in the observed period.

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Soil NH₄⁺-N and NO₃⁻-N concentrations exhibited significant seasonal variation, with a single peak value in the N addition plots. The maximum value appeared between June and August, while the minimum was observed from November to March (Fig 4, Fig 5).

Soil NO₃⁻-N accumulated significantly in N addition plots and its concentration ranged from 15.24 to 53.33 mg kg⁻¹. N level had a significant promotion effect on soil NO₃⁻-N concentrations, with those under the and high level N addition had a significantly greater promotion on it compared with that of low level (*P* < 0.05, Table 4). The concentrations from low- and high-level N addition plots were, respectively, 142.14% and 172.90% greater than those from the control (12.03 mg kg⁻¹).

Soil NH₄⁺-N significantly accumulated in the N addition plots and its concentration ranged from 2.32 to 6.74 mg kg⁻¹. The accumulation of NH₄⁺-N caused by N addition was less than that of NO₃⁻-N in soil. Soil NH₄⁺-N concentration was significantly influenced by the

Table 4. Summary of repeated measures ANOVA results (F values) indicating the effects of different forms and levels of N addition and experimental time on soil temperature at 5 cm soil depth (ST), water-filled pore space (WFPS), and the concentrations of soil inorganic N (NO₃⁻ and NH₄⁺).

	d.f.	ST		WFPS		NO ₃ ⁻		NH ₄ ⁺	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Between subjects									
Form	2	0.054	0.947	0.082	0.921	3.096	0.051	6.928	0.002
Level	1	0.000	0.996	0.004	0.947	7.633	0.007	1.680	0.199
Form×Level	2	0.020	0.981	0.011	0.989	0.008	0.992	0.273	0.762
Within subjects									
Date	59	6.498	< 0.001	39.766	< 0001	10.772	< 0.001	4.253	0.002
Date×Form	118	0.542	0.824	0.251	0.980	1.740	0.089	1.158	0.325
Date×Level	59	0.422	0.793	0.249	0.910	1.289	0.274	0.630	0.641
Date×Form×Level	118	0.358	0.942	0.609	0.770	0.916	0.504	0.259	0.978

Significant effects (*P* < 0.05) are highlighted in bold.

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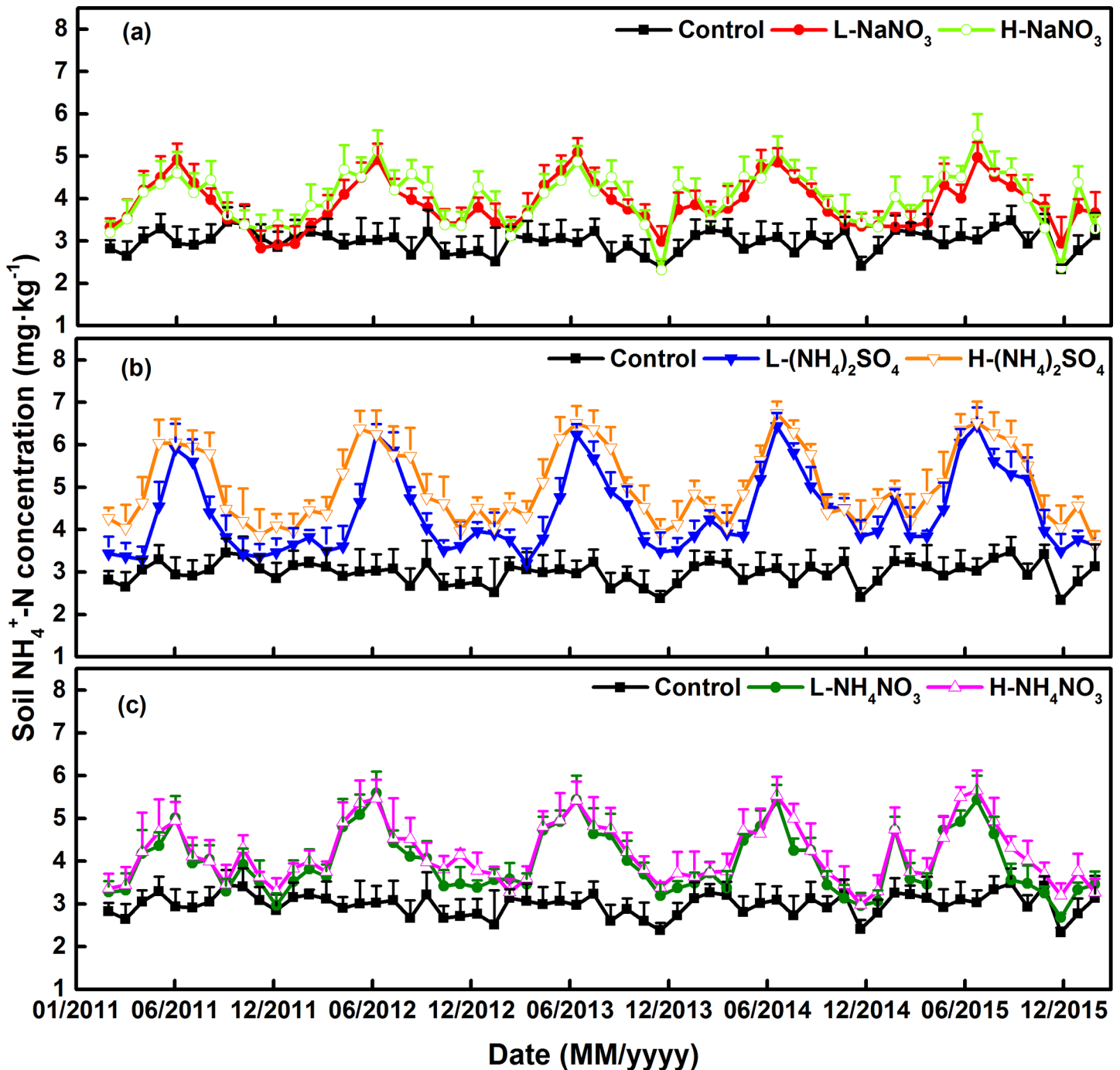


Fig 4. Variations of soil NH₄⁺-N concentrations applied with different forms and levels of N addition among five-year experimental period (a) NaNO₃ addition plots; (b) (NH₄)₂SO₄ addition plots; (c) NH₄NO₃ addition plots. L: 50 kg N ha⁻¹ yr⁻¹; H: 150 kg N ha⁻¹ yr⁻¹. Error bars indicate the standard error of the mean (n = 9).

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three N forms, which increased NH₄⁺-N in the order of (NH₄)₂SO₄ > NH₄NO₃ > NaNO₃ and by 57.40%, 36.27%, and 31.84% when compared with the control (2.98 mg kg⁻¹), respectively (Fig 4, Fig 5).

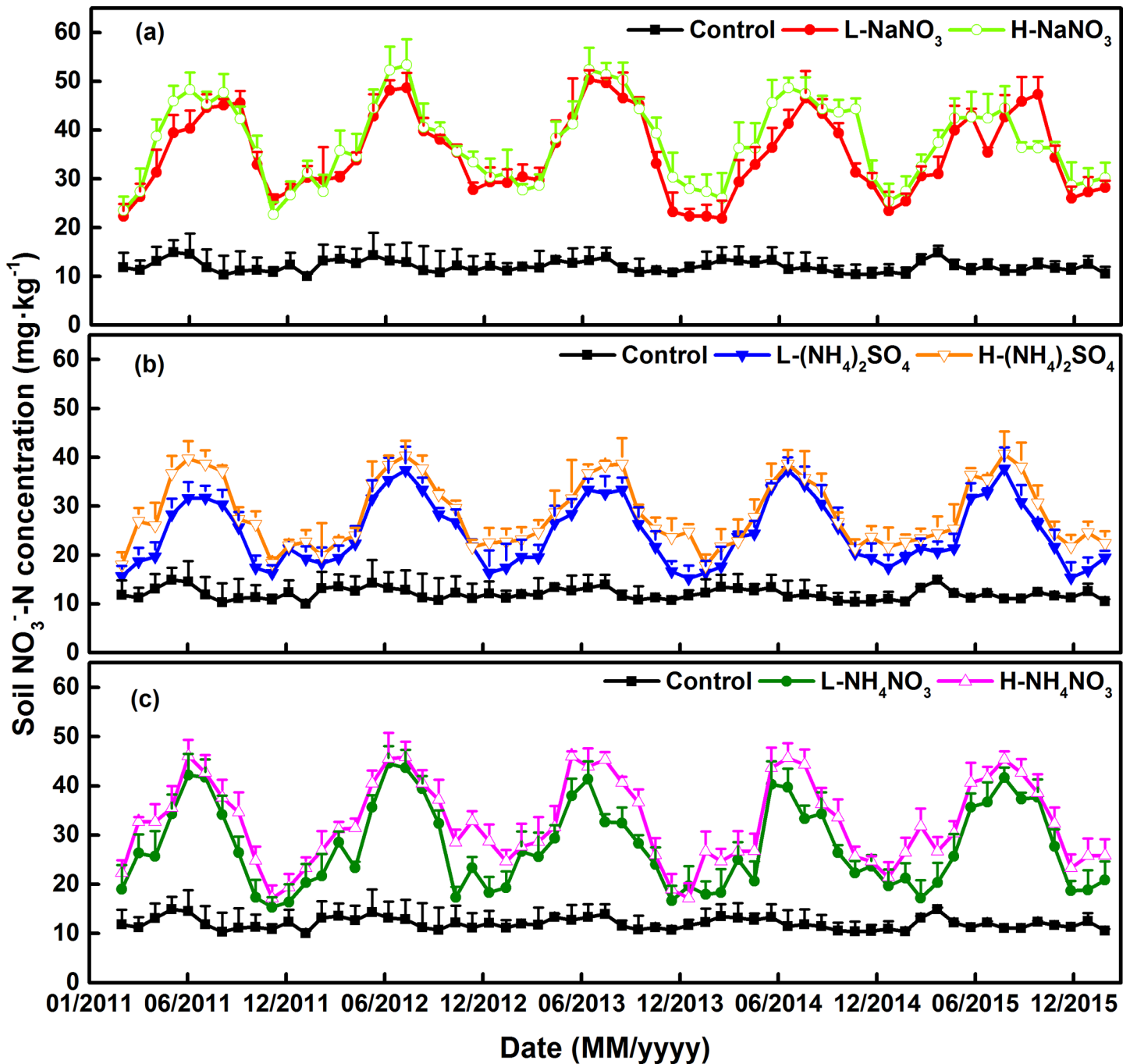


Fig 5. Variations of soil NO₃⁻-N concentrations applied with different forms and levels of N addition among five-year experimental period (a) NaNO₃ addition plots; (b) (NH₄)₂SO₄ addition plots; (c) NH₄NO₃ addition plots. L: 50 kg N ha⁻¹ yr⁻¹; H: 150 kg N ha⁻¹ yr⁻¹. Error bars indicate the standard error of the mean (n = 9).

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The correlation analysis showed that soil N₂O emissions were positively correlated with ST at 5 cm depth, WFPS at a 10-cm depth, and soil inorganic nitrogen concentration (Fig 6). In addition, a linear equation showed that soil N₂O emissions were extremely significantly ($P < 0.01$) correlated with ST, WFPS, and soil NH₄⁺-N and NO₃⁻-N (Fig 6).

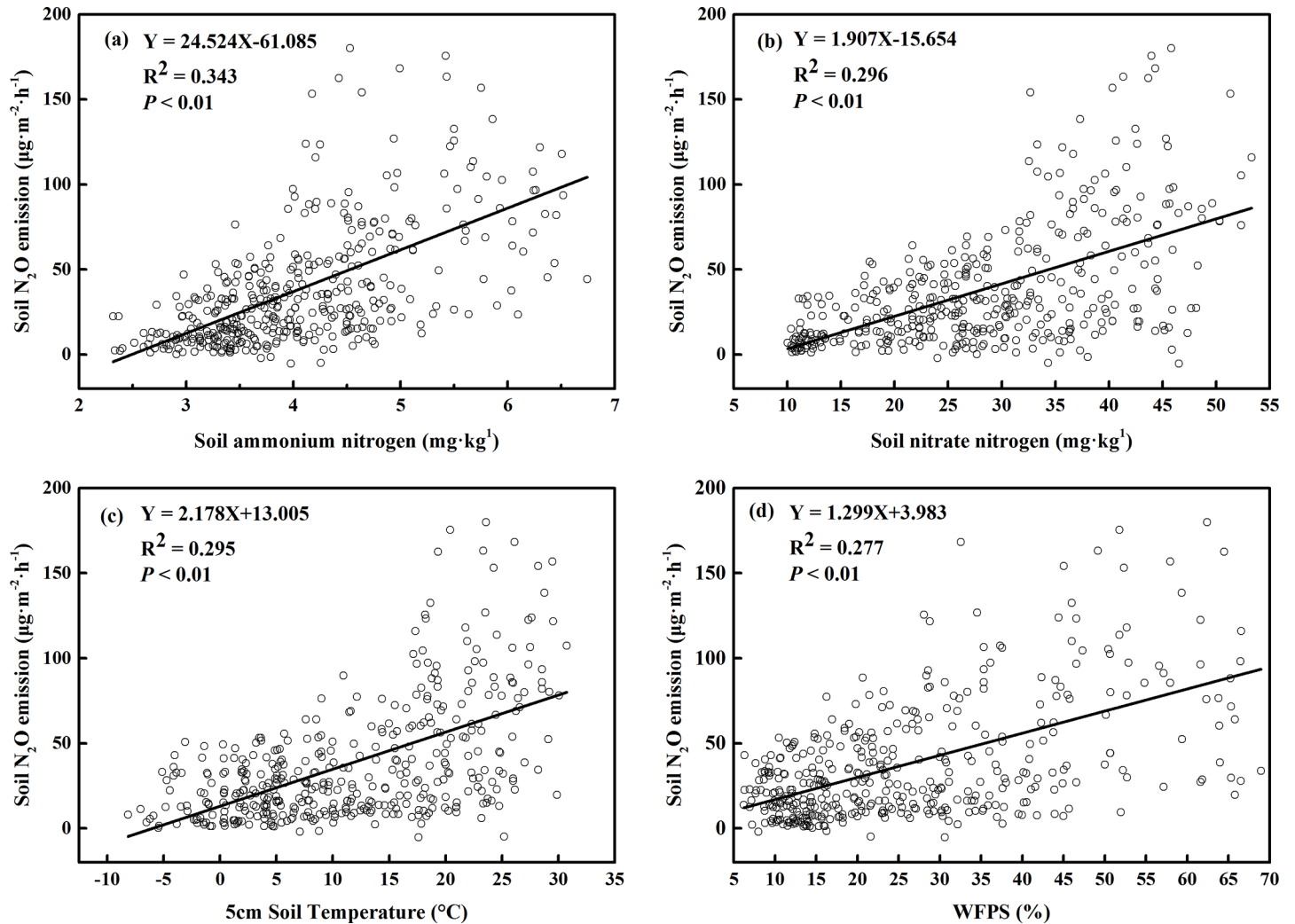


Fig 6. Relationships between soil N₂O emissions and soil NH₄⁺-N concentration (a), soil NO₃⁻-N concentration (b), soil temperature (5 cm depth) (c), WFPS (10 cm depth) (d).

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Discussion

Promotion effects of N addition on soil N₂O emissions

Our results showed that the temperature plantation in northern China was a source of atmospheric N₂O under natural conditions. The mean N₂O emissions value in the control was 11.55 μg N₂O-N m⁻² h⁻¹; this rate is comparable to that reported by Butterbach-Bahl et al. [16] who found that the N₂O emissions in soils of spruce forests in Germany and Ireland ranged from 3.5 to 16.4 μg N m⁻² h⁻¹. In our study, NaNO₃, (NH₄)₂SO₄, and NH₄NO₃ addition at levels of 50 and 150 kg N ha⁻¹ yr⁻¹ significantly increased soil N₂O emissions by an average of 115.26% to 260.15%, 182.92% to 349.77%, 259.89% to 452.02%, respectively. The rate of increase was lower than that for a subtropical forest of the Qianyanzhou Ecological Station, where it was increased by 403% to 762% [5]. Except for L-NaNO₃ addition, the increase in soil N₂O emission was higher than the global average (134%) [18]. On one hand, these results indicate that the temperature plantation had high turnover rates of soil N and responded to the increased N deposition. On the other hand, to measure the peak N₂O emissions in our study,

the gas samples were collected in the first, fourth and seventh days after N was added; hence, the cumulative N₂O emissions might have been overestimated since the N₂O emissions should have been measured weekly during growing season (to properly reflect an average impact over time). In our previous work, N addition significantly increased the amount of soil microbes and changed the soil microbial community structure in our study area [36]. Soil urease activities were significantly increased by N additions, which promoted soil N₂O emission [37]. Therefore, the weakened N limitation brought about by a higher litter decomposition rate and greater microbial activity could explain the increased N₂O emissions we found here [38].

N₂O emissions under different N addition forms and levels

Based on our observations over 5 years, the results supported our hypothesis that soil annual cumulative N₂O emissions increased under elevated N-addition levels. Positive correlations between N-addition levels and soil N₂O emissions have been found in many previous studies [4, 6, 39–40]. However, at our site, NaNO₃ addition at a rate of 50 kg N ha⁻¹ yr⁻¹ did not stimulate a significant increase in the cumulative N₂O emissions in the first year. Perhaps this is because of a threshold response of soil N₂O emissions to the N additions [4, 7, 41]. Specifically, such a response is determined by the competition between plants and soil microbes for available N, and thus emissions will not significantly increase until the plant N demands have been satisfied [4, 7, 42].

Considering the addition of different N forms, both NH₄⁺-N and NO₃⁻-N significantly promoted soil N₂O emission and exogenous NH₄⁺-N and NO₃⁻-N inputs into our temperate forest had synergic effects on soil N₂O emission; this result supports our hypothesis and is also consistent with the finding elsewhere that exogenous NH₄⁺-N and NO₃⁻-N additions into boreal forest soil can have a synergic effect on its N₂O emissions [43]. The promotion of NH₄⁺-N (NH₄NO₃ and (NH₄)₂SO₄) additions for N₂O emission exceeded that provided by the NO₃⁻-N addition. This result is consistent with other studies finding higher N₂O emissions from ammonium sources than from nitrate sources [7, 26]. Two potential mechanisms may be responsible for this phenomenon: (1) high immobilization of NO₃⁻-N and nitrification rates, coupled to a low denitrification potential, led more NO₃⁻-N to accumulate in soil [44]; (2) poor mobility of NH₄⁺ created depletion zones around the plant roots, leaving more N input exposed to microorganisms in soils. However, most research to date suggests that denitrification is the main process driving N₂O production [25, 45]. Yet when WFPS is in the range of 30 to 70%, nitrification can become the main process driving N₂O production, as denitrification rates increase rapidly when WFPS exceeds 60% [7]. WFPS in our research plots was at a low level for most of the 5-yr monitoring period, only exceeding 60% for a few months, which likely provided less than optimal conditions for the denitrification process [46]. Given this trend in WFPS, we indirectly conclude that NH₄⁺-N had higher conversion efficiency to N₂O than NO₃⁻-N at our forest site.

Although NH₄⁺-N was always the major N form in local actual N deposition [47], since 1980 its NH₄⁺-N / NO₃⁻-N ratio has decreased [48]. Considering the stronger promotion of N₂O emission by NH₄⁺, and the decreasing proportion of NH₄⁺ in N deposition, we expect that the increased soil N₂O emission stimulated by N deposition at our site will not persist into the future.

Interannual soil N₂O emissions under N addition

Considering the time scale, we found a sharp increase in the annual N₂O emissions in the first three years, but after this point the rate of increase diminished. Soil reaches N saturation when the N input exceeds the N demanded by plants and microorganisms [49]. Early successional

forests are always defined as N-limited, because of the limited N availability for vigorous plant growth and the lack of N-fixing plants or bacteria, whereas mature tropical forests and old-growth subtropical forests are typically grouped as being N-saturated [50]. Being N-limited is relative to being N-rich, and this necessarily depends on the soil N availability and the response of vegetation to any N addition [50]. In our study area, N was clearly a limiting factor in the initial years based on amount and stimulating effect of N addition upon tree biomass. Continuing the N addition could shift the soil from being N-limited to N-rich, and then becoming N-saturated, such that soil N₂O emissions may appear to reach a steady state at high N levels [23]. In addition, Liu and Song [51] found that soil microbial activities may be limited by carbon availability when N is abundant. The suppression of soil N₂O emissions by long-term N additions was possibly due to a lack of readily available organic carbon [52] and/or adverse effects on mineralization of organic carbon under conditions of high N addition [53]. Therefore, our field experiment highlights the importance carrying out long-term studies to avoid possibly overestimating the N addition effects on N₂O emissions from short-term observations.

Relationships between soil N₂O emissions and soil properties

In our study area, the soil concentration of NO₃⁻-N was higher than that of NH₄⁺-N, and the accumulation of NO₃⁻-N caused by N addition was more than soil NH₄⁺-N concentration. On the one hand, although the soil NO₃⁻-N concentration was directly increased by NO₃⁻-N addition, the NH₄⁺-N addition could have enhanced the activity of soil nitrifiers and led to the NO₃⁻-N accumulation in soil we found. This finding and interpretation is consistent with some previous studies carried out in tropical and subtropical forests [54–55]. On the other hand, several studies using the ¹⁵N tracing method suggest that plants in temperate forest at our site preferred NH₄⁺-N, which led to more NH₄⁺-N becoming assimilated, such that the accumulation of NH₄⁺-N in the soil was relatively little and brief [56].

We found that the soil N₂O emissions were significantly correlated with concentrations of soil NH₄⁺-N and NO₃⁻-N, suggesting soil N₂O emission was dominated by both nitrification and denitrification processes. Since atmospheric N deposition can significantly promote soil N₂O emission, and exogenous NH₄⁺-N and NO₃⁻-N inputs into temperate forests may have synergic effects on soil N₂O emission, in the future both of these aspects ought to be distinguished in the dynamics of the N cycle and balance in terrestrial ecosystems by using ¹⁵N tracer methods. High ST, together with a relatively high WFPS, tend to promote both nitrification and denitrification processes [57] and consequently, high N₂O emissions, an interpretation that is consistent with many previous findings [58–59]. In particular, high WFPS may promote microbial movement and the expansion of the soil anaerobic microbial community [43]. Warm temperatures benefit soil nitrifying and denitrifying bacteria activities [11], which may explain the seasonal variation in the relatively high N₂O emissions that occurred from May to September that we observed in this study. Many other complex factors may have played a role in determining our results, such as soil pH, soil C availability, and the microbial community structure, since they jointly influence the two key processes of nitrification and denitrification that are involved in soil N₂O production [36, 60].

Conclusions

This study emphasizes the effects of different N forms and levels on N₂O emissions from a temperate forest over 5-year experimental period. We found that the accumulation of soil NO₃⁻-N was significantly higher than that of soil NH₄⁺-N due to N addition. N addition initially promoted soil N₂O emission yet this promoting effect, although it existed, weakened in

the following years. High level N addition had a stronger promotion effect upon soil N₂O emission than did the low level N addition. Meanwhile, the combined application of NH₄⁺-N and NO₃⁻-N promotes N₂O emissions more than their single applications, and NH₄⁺-N addition had a stronger promotion effect for soil N₂O emission than did the NO₃⁻-N addition. In addition, WFPS, ST, soil NH₄⁺-N, and NO₃⁻-N were all positively related to the N₂O emissions. In the future, the long-term observation of soil N₂O emissions, and the measurement of microbial functional groups using ¹⁵N tracer methods, will be necessary to clarify the mechanisms responsible for the soil N₂O emissions.

Supporting information

S1 File. Data set underlying the findings.
(XLSX)

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Methodology: Ke Xu, Xintong Yang.

Project administration: Ke Xu, Chunmei Wang.

Resources: Ke Xu, Chunmei Wang.

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Supervision: Chunmei Wang.

Validation: Ke Xu, Xintong Yang.

Visualization: Ke Xu, Xintong Yang.

Writing – original draft: Ke Xu.

Writing – review & editing: Ke Xu, Chunmei Wang, Xintong Yang.

References

1. Wei Z, Mo J, Yu G, Fang Y, Li D, Lu X, et al. Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition. *Plant Soil*. 2008; 306(1–2):221–236.
2. Wang F, Li J, Wang X, Zhang W, Zou B, Neher DA, et al. Nitrogen and phosphorus addition impact soil N₂O emission in a secondary tropical forest of South China. *Sci Rep-UK*. 2014; 4:5615.
3. Dalal RC, Wang W, Robertson GP, Parton WJ. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Aust J Soil Res*. 2003; 41(2):165–195.
4. Liu X, Dong Y, Qi Y, Peng Q, He Y, Sun L, et al. Response of N₂O emission to water and nitrogen addition in temperate typical steppe soil in Inner Mongolia, China. *Soil Tillage Res*. 2015; 151(151):9–17.

5. Wang Y, Cheng S, Fang H, Yu G, Xu M, Dang X, et al. Simulated nitrogen deposition reduces CH₄ uptake and increases N₂O emission from a subtropical plantation forest soil in southern China. *PLoS ONE*. 2014; 9(4):e93571. <https://doi.org/10.1371/journal.pone.0093571> PMID: 24714387
6. Edith B, Wei L, Shanlong L, Jianfei S, Bo P, Weiwei D, et al. Pulse increase of soil N₂O emission in response to N addition in a temperate forest on Mt Changbai, Northeast China. *PLoS ONE*. 2014; 9(7): e102765. <https://doi.org/10.1371/journal.pone.0102765> PMID: 25079363
7. Peng Q, Qi Y, Dong Y, Xiao S, He Y. Soil nitrous oxide emissions from a typical semiarid temperate steppe in Inner Mongolia: effects of mineral nitrogen fertilizer levels and forms. *Plant Soil*. 2011; 342(1–2):345–357.
8. Rafique R, Hennessy D, Kiely G. Nitrous oxide emission from grazed grassland under different management systems. *Ecosystems*. 2011; 14(4):563–582.
9. Mosier AR, Zhu ZL. Changes in patterns of fertilizer nitrogen use in Asia and its consequences for N₂O emissions from agricultural systems. *Nutrient Cycling in Agroecosystems*. 2000; 57(1):107–117.
10. Liu X, Lei D, Mo J, Du E, Shen J, Lu X, et al. Nitrogen deposition and its ecological impact in China: An overview. *Environ Pollut*. 2011; 159(10):2251–2264. <https://doi.org/10.1016/j.envpol.2010.08.002> PMID: 20828899
11. Bjoor NS, Czimczik CI, Pataki DE, Billings SA. Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn. *Glob Change Biol*. 2008; 14(9):2119–2131.
12. Jassal RS, Black TA, Novak MD, Gaumont-Guay D, Nesic Z. Effect of soil water stress on soil respiration and its temperature sensitivity in an 18-year-old temperate douglas-fir stand. *Glob Change Biol*. 2008; 14(6):1305–1318.
13. Liu X, Zhang Y, Han W, Tang A, Shen J, Cui Z, Vitousek PJW. Enhanced nitrogen deposition over China. *Nature*. 2013; 494(7438):459. <https://doi.org/10.1038/nature11917> PMID: 23426264
14. Xu W, Luo X, Pan Y, Zhang L, Tang A, Shen J, et al. Quantifying atmospheric nitrogen deposition through a nationwide monitoring network across China. *Atmos Chem Phys*. 2015; 15(21):12345–12360.
15. Hodge A, Robinson D, Fitter A. Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci*. 2000; 5(7):304–308. PMID: 10871903
16. Butterbach-Bahl K, Gasche R, Huber C, Kreutzer K, Papen H. Impact of N-input by wet deposition on N-trace gas fluxes and CH₄-oxidation in spruce forest ecosystems of the temperate zone in Europe. *Atmos Environ*. 1998; 32(3):559–564.
17. Song C, Wang L, Tian H, Liu D, Lu C, Xu X, Zhang L, Yang G, Wan Z. Effect of continued nitrogen enrichment on greenhouse gas emissions from a wetland ecosystem in the Sanjiang Plain, Northeast China: A 5 year nitrogen addition experiment. *J Geophys Res: Biogeosciences* 2013; 118(2):741–751.
18. Meng L, Yang Y, Luo Y, et al. Responses of ecosystem N cycle to N addition: a meta-analysis. *New Phytologist*. 2011; 189(4):1040–1050. <https://doi.org/10.1111/j.1469-8137.2010.03563.x> PMID: 21138438
19. Fang YT, Gundersen P, Mo JM, Zhu WX. Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution. *Biogeosciences*. 2008; 5(2):339–352.
20. Zhang W, Mo JM, Zhou GY, Gundersen P, Fang YT, Lu XK, Zhang T, Dong SF. Methane uptake responses to nitrogen deposition in three tropical forests in southern China. *J Geophys Res*. 2008; 113(D11):3078.
21. Fang YT, Zhu WX, Gundersen P, Mo JM, Zhou GY, Yoh M. Large loss of dissolved organic nitrogen from nitrogen saturated forests in subtropical China. *Ecosystems*. 2009; 12(1):33–45.
22. Malhi SS, Lemke R, Wang ZH, Chhabra BS. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. *Soil Tillage Res*. 2006; 90(1):171–183.
23. Thornton FC, Valente RJ. Soil emissions of nitric oxide and nitrous oxide from no-till corn. *Soil Sci Soc Am J*. 1996; 60(4):1127–1133.
24. Gundersen P, Emmett BA, Kjønås OJ, et al. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecol Manag*. 1998; 101(1–3):37–55.
25. Chen GC, Tam NFY, Ye Y. Spatial and seasonal variations of atmospheric N₂O and CO₂ fluxes from a subtropical mangrove swamp and their relationships with soil characteristics. *Soil Biol Biochem*. 2012; 48(4):175–181.
26. Zhu J, Mulder J, Solheimslid SO, Dörsch P. Functional traits of denitrification in a subtropical forest catchment in China with high atmospheric N deposition. *Soil Biol Biochem*. 2013; 57(3):577–586.
27. Drastig K. Effects of nitrogen fertilization and irrigation on NO emissions from a sandy soil in Germany. *Arch Agro Soil Sci*. 2015; 61(5):1–12.

28. Wang L, Cai Z. Nitrous oxide production at different soil moisture contents in an arable soil in China. *Soil Sci Plant Nutr*. 2008; 54(5):786–793.
29. Liu X, Ju X, Zhang Y, He C, Kopsch J, Zhang F. Nitrogen deposition in agroecosystems in the Beijing area. *Agri Ecosys Environ*. 2006; 113(1–4):370–377.
30. Jiang C, Yu G, Fang H, Cao G, Li Y. Short-term effect of increasing nitrogen deposition on CO₂, CH₄ and N₂O fluxes in an alpine meadow on the Qinghai–Tibetan Plateau, China. *Atmos Environ*. 2010; 44(24):2920–2926.
31. Gao Y, Chen H, Zeng X. Effects of nitrogen and sulfur deposition on CH₄ and CO₂ fluxes in high-altitude peatland soil under different water tables in the Tibetan Plateau. *Soil Sci. Plant Nutr*. 2014; 60(3):404–410.
32. Wang C, Yang X, Xu K. Effect of chronic nitrogen fertilization on soil CO₂ flux in a temperate forest in North China: a 5-year nitrogen addition experiment. *J Soil Sediment*. Forthcoming 2017. <https://doi.org/10.1007/s11368-017-1760-y>
33. Zheng X, Mei B, Wang Y, Xie B, Wang Y, Dong H, et al. Quantification of N₂O fluxes from soil–plant systems may be biased by the applied gas chromatograph methodology. *Plant Soil*. 2008; 311(1–2):211–234.
34. Dong Y, Zhang S, Qi Y, Chen Z, Geng Y. Fluxes of CO₂, N₂O and CH₄ from a typical temperate grassland in Inner Mongolia and its daily variation. *Chinese Science Bull*. 2000; 45(17):1590–1594.
35. Yang X, Wang C, Xu K. Response of soil CH₄ fluxes to stimulated nitrogen deposition in a temperate deciduous forest in northern China: A 5-year nitrogen addition experiment. *Eur J Soil Biol*. 2017; 82:43–49.
36. Xu K, Wang C, Zhang Y, Yang X, Liu W. Effect of simulated atmospheric nitrogen deposition on soil microbial community structure in temperate forest. *Chinese J Ecol*. 2016; 35(10):2676–2683.
37. Bai E, Li W, Li S, et al. Pulse increase of soil N₂O emission in response to N addition in a temperate forest on Mt Changbai, northeast China. *PLoS ONE*. 2014; 9(7):e102765. <https://doi.org/10.1371/journal.pone.0102765> PMID: 25079363
38. Dang XS, Cheng SL, Fang HJ, Yu GR, Han SJ, Zhang JH, Wang M, Wang YS, Xu MJ, Li LS, Wang L. The controlling factors and coupling of soil CO₂, CH₄ and N₂O fluxes in a temperate needle-broad-leaved mixed forest. *Acta Ecologica Sinica*. 2015; 35(19):6530–6540.
39. Templer PH, Pinder RW, Goodale CL. Effects of nitrogen deposition on greenhouse-gas fluxes for forests and grasslands of North America. *Front Ecol Environ*. 2012; 10(10):547–553.
40. Zhang L, Song C, Wang D, Wang Y. Effects of exogenous nitrogen on freshwater marsh plant growth and N₂O fluxes in Sanjiang Plain, Northeast China. *Atmo Environ*. 2007; 41(5):1080–1090.
41. Malhi SS, Harapiak JT, Nyborg M, Gill KS, Monreal CM, Gregorich EG. Total and light fraction organic C in a thin Black Chernozemic grassland soil as affected by 27 annual applications of six rates of fertilizer N. *Nutr Cycl Agroecosys*. 2003; 66(1):33–41.
42. Kim DG, Hernandez–Ramirez G, Giltrap D. Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis. *Agr Ecosyst Environ*. 2013; 168(2908):53–65.
43. Kuzyakov Y, Xu X. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytol*. 2013; 198(3):656–669. <https://doi.org/10.1111/nph.12235> PMID: 23521345
44. Zhang Y, Wang C M, Xu K, Yang XT. Effect of simulated nitrogen deposition on soil enzyme activities in a temperate forest. *Acta Ecologica Sinica*. 2017; 37(6): 1956–1965.
45. Hefting MM, Bobbink R, de Caluwe H. Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. *J Environ Qual*. 2003; 32(4):1194–1203. PMID: 12931872
46. Davidson EA. Sources of nitric oxide and nitrous oxide following wetting of dry soil. *Soil Sci Soc Am J*. 1992; 56(1):95–102.
47. Liu X, Zhang Y, Han W, Tang A, Shen J, Cui Z, Vitousek P, Erisman JW, Goulding K, Christie P, Fangmeier A, Zhang F. Enhanced nitrogen deposition over China. *Nature*. 2013; 494(7438):459–462. <https://doi.org/10.1038/nature11917> PMID: 23426264
48. Liu X, Ju X, Zhang Y, He C, Kopsch J, Fusuo Z. Nitrogen deposition in agroecosystems in the Beijing area. *Agri Ecosyst Environ*. 2006; 113(1–4):370–377.
49. Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I. Nitrogen saturation in temperate forest ecosystems—hypotheses revisited. *Bioscience*. 1998; 48(11):921–934.
50. Chen H, Li D, Gurmesa GA, Yu G, Li L, Zhang W, Fang H, Mo J. Effects of nitrogen deposition on carbon cycle in terrestrial ecosystems of China: A meta-analysis. *Environ Pollut*. 2015; 206:352–360. <https://doi.org/10.1016/j.envpol.2015.07.033> PMID: 26232918

51. Liu D, Song C. Effects of inorganic nitrogen and phosphorus enrichment on the emission of N₂O from a freshwater marsh soil in Northeast China. *Environ Earth Sci*. 2010; 60(4):799–807.
52. Stapleton LM, Crout NMJ, Sävström C, Marshall WA, Poulton PR, Tye AM, et al. Microbial carbon dynamics in nitrogen amended Arctic tundra soil: Measurement and model testing. *Soil Biol Biochem*. 2005; 37(11):2088–2098.
53. Liu D, Song C. Effects of phosphorus enrichment on mineralization of organic carbon and contents of dissolved carbon in a freshwater marsh soil. *Chi Environ Sci*. 2008; 28(9):769–774.
54. Fang H, Yu G, Cheng S, Zhu T, Zheng J, Mo J, Yan J, Luo Y. Nitrogen-15 signals of leaf-litter-soil continuum as a possible indicator of ecosystem nitrogen saturation by forest succession and N loads. *Biogeochemistry*. 2011; 102(1–3):251–263.
55. Wang Y, Cheng S, Fang H, Yu G, Xu M, et al. Simulated nitrogen deposition reduces CH₄ Uptake and Increases N₂O Emission from a Subtropical Plantation Forest Soil in Southern China. *PLoS ONE*. 2014; 9(4):e93571. <https://doi.org/10.1371/journal.pone.0093571> PMID: 24714387
56. Sheng W, Yu G, Fang H, et al. Sinks for inorganic nitrogen deposition in forest ecosystems with low and high nitrogen deposition in China. *PLoS ONE*. 2014; 9(2):e89322. <https://doi.org/10.1371/journal.pone.0089322> PMID: 24586688
57. Xu Y, Xu Z, Cai Z, Reverchon F. Review of denitrification in tropical and subtropical soils of terrestrial ecosystems. *J Soil Sediment*. 2103; 13(4):699–710.
58. Groffman PM. Nitrification and denitrification in soil: A comparison of enzyme assay, incubation and enumeration methods. *Plant Soil*. 1987; 97(3):445–450.
59. Li K, Gong Y, Song W, Lv J, Chang Y, Hu Y, et al. No significant nitrous oxide emissions during spring thaw under grazing and nitrogen addition in an alpine grassland. *Glob Change Biol*. 2012; 18(8):2546–2554.
60. Buckeridge KM, Cen YP, Layzell DB, Grogan P. Soil biogeochemistry during the early spring in low arctic mesic tundra and the impacts of deepened snow and enhanced nitrogen availability. *Biogeochemistry*. 2010; 99(1–3):127–141.