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

KE XU1,2, CHUNMEI WANG1*, XINTONG YANG1

1 College of Environmental Science and Engineering, Beijing Forestry University, Beijing, China, 2 Beijing Solid Waste Treatment Co., Ltd., Beijing Environmental Sanitation Engineering Group Co., Ltd., Beijing, China

* sdwcm@126.com, wangcm@bjfu.edu.cn

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

Introduction

Nitrous oxide (N$_2$O) is not only a potent greenhouse gas whose global warming potential is 298- and 21-fold that of CO$_2$ and CH$_4$, but it also contributes to stratospheric ozone depletion [1]. Emissions of N$_2$O from soil have been identified as the primary source (57%) of total global N$_2$O emissions [2].

Nitrification and denitrification are the two main processes that produce N$_2$O in soils and both can occur simultaneously (Fig 1). N$_2$O is produced by denitrifying bacteria during the reduction of NO$_3^-$ or NO$_2^-$ to N$_2$O and N$_2$, or released as an intermediate product when nitrifying bacteria oxidize NH$_4^+$–N to NO$_3^-$ and NO$_2^-$ [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 N$_2$O emission [4–5, 7–8] (Fig 1). In general, increasing available mineral N in soils leads to enhanced N$_2$O formation and emission via increased nitrification and denitrification rates [9]. Soil N$_2$O emission is also driven by soil temperature and water content [10]. Some previous studies indicated soil N$_2$O emissions were increased under conditions of higher soil water content and soil temperature [10]. The latter may regulate soil N$_2$O emission by influencing N$_2$O-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 N$_2$O in soils.

https://doi.org/10.1371/journal.pone.0189831.g001
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 NH\(_4^+\)-N to NO\(_3^-\)-N deposition, from approximately 5 to 2, from the 1980s to the 2000s, although NH\(_4^+\)-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 N\(_2\)O by disturbing the balance between microbial N mineralization and immobilization, with the consequences for the relative availability of soil NH\(_4^+\)-N and NO\(_3^-\)-N [15] (Fig 1). Most studies report that raising N addition levels could linearly stimulate soil N\(_2\)O emissions [4–5, 16–17]. A meta-analysis of global N addition experiments showed that N additions increased soil N\(_2\)O 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 N\(_2\)O 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 N\(_2\)O emissions [21]. However, some studies indicated that N addition has no significant effect on soil N\(_2\)O emission, which might be attributed to particular N addition threshold level for increased N\(_2\)O emissions [7, 22]. Thornton and Valente [23] found that the increased rate of soil N\(_2\)O 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 N\(_2\)O emission [24]. Furthermore, some studies have shown denitrification to be the main source of soil N\(_2\)O emissions [25–26], whereas other studies reported that nitrification were primarily responsible for soil N\(_2\)O emissions [7, 27–28]. Clearly then, how soil N\(_2\)O emissions respond to additional N appears to be inconsistent.

The NH\(_4^+\)-N/NO\(_3^-\)-N ratio showed a decreasing trend in our study area [29], and so clarifying the response of soil N\(_2\)O emission to different forms and levels of N addition now is necessary. However, several previous studies that stimulated N deposition only considered NH\(_4\)NO\(_3\) [2, 4, 6], while others that did examine N deposition in varied N forms only reported their short-term effects on N\(_2\)O 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 N\(_2\)O emissions in temperate forest soils.

In our study, we report the results of continuous measurements of soil N\(_2\)O 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 N\(_2\)O emission and that this promotion effect likely increased with the N addition level; (2) Applying NO\(_3^-\) and NH\(_4^+\)-N in combination could promote soil N\(_2\)O 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 \(\times\) 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 NH\(_4^+\)−N and NO\(_3^−\)−N showed great variation from month to month in the study area [30], three N-addition forms, namely NaNO\(_3\), (NH\(_4\))\(_2\)SO\(_4\), and NH\(_4\)NO\(_3\), were used to simulate the effects of deposited NH\(_4^+\)−N, NO\(_3^−\)−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 N\(_2\)O emission measurements were performed three times in the first week of each month, from March 2011 to February 2016. Soil N\(_2\)O emissions were measured using a static closed

| Variable      | 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±0.28 | 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.

Table 1. Soil properties of the sampling area.

https://doi.org/10.1371/journal.pone.0189831.t001
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 N$_2$O concentration analysis. Gas samples were analyzed within 6 h in a gas chromatograph (Agilent 7890A, Agilent Technologies Inc., Palo Alto, CA, USA) [33].

Soil N$_2$O emissions were calculated as follows [33]:

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

(1)

where, $F_{N_2O}$ refers to N$_2$O emission ($\mu$g m$^{-2}$ h$^{-1}$); $D$ refers to the gas density of the chamber (mol m$^{-3}$); $D = WP/RT$; $W$ refers to the molar mass of N$_2$O (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 (J mol$^{-1}$ 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 N$_2$O emissions were calculated by interpolating the N$_2$O emissions measured between sampling periods [34]. Cumulative N$_2$O emissions were calculated spanning the time period from March to February next year as follows [35]:

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

(2)

where, $F$ is the N$_2$O emissions ($\mu$g m$^{-2}$ 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 N$_2$O 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 NH$_4^+$–N and NO$_3^-$–N content analyses, while the remaining portion was air-dried for pH measurement. Soil NO$_3^-$–N and NH$_4^+$–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:

\[
WFPS = \left( \frac{WC \times BD}{100} \right) \times \frac{100}{(1 - BD/2.65)}
\]  

(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 \(\text{N}_2\text{O}\) emissions, annual cumulative \(\text{N}_2\text{O}\) emissions, ST, WFPS, and inorganic N concentrations. We examined the differences in annual \(\text{N}_2\text{O}\) 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 \(\text{N}_2\text{O}\) emissions and environmental variables. Means and standard deviations of \(\text{N}_2\text{O}\) emissions were calculated, and the plot values represented means (\(n = 3\)) ± standard error (SE).

**Results**

**Soil \(\text{N}_2\text{O}\) emissions under N addition**

During the 5-year experimental period, the temperate forest soil was a net source of \(\text{N}_2\text{O}\). Soil \(\text{N}_2\text{O}\) 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 \(\text{N}_2\text{O}\) emissions was concentrated in August of each year (Fig 2). Soil \(\text{N}_2\text{O}\) 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 \(\text{N}_2\text{O}\) 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 \(\text{N}_2\text{O}\) emissions (\(P < 0.01\), Table 2). As for the two N-level addition treatments, the N-addition treatments significantly increased soil \(\text{N}_2\text{O}\) emissions, and this promotion effect was enhanced as the N-addition levels increased (Table 2, Fig 2). Soil \(\text{N}_2\text{O}\) emissions ranged from 1.30 \(\mu\)g m\(^{-2}\) h\(^{-1}\) to 34.44 \(\mu\)g m\(^{-2}\) h\(^{-1}\), with an average value of 11.55 \(\mu\)g m\(^{-2}\) h\(^{-1}\) in the control plots (Fig 2). Compared to the control, the average \(\text{N}_2\text{O}\) 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 \(\mu\)g m\(^{-2}\) h\(^{-1}\) in the L-NH\(_4\)NO\(_3\) and H-NH\(_4\)NO\(_3\) 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 \(\text{N}_2\text{O}\) emissions were significantly increased by NH\(_4\)NO\(_3\), (NH\(_4\))\(_2\)SO\(_4\), and NaNO\(_3\) additions in the order of NH\(_4\)NO\(_3\) > (NH\(_4\))\(_2\)SO\(_4\) > NaNO\(_3\) > control for the same level of N addition (Fig 2, Table 2). Compared to the control, the average \(\text{N}_2\text{O}\) emissions in the NH\(_4\)NO\(_3\), (NH\(_4\))\(_2\)SO\(_4\), and NaNO\(_3\) 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 N$_2$O emissions ($P > 0.05$, Table 2).

Interannual soil cumulative N$_2$O 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 N$_2$O emissions (Table 2). In the

![Graph showing variations of soil N$_2$O 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).](https://doi.org/10.1371/journal.pone.0189831.g002)
control plot, cumulative N\textsubscript{2}O 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 N\textsubscript{2}O 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 NH\textsubscript{4}NO\textsubscript{3}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, and NaNO\textsubscript{3} additions in the order of NH\textsubscript{4}NO\textsubscript{3} > (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} > NaNO\textsubscript{3} > control (P < 0.05), but no significant differences were found between NaNO\textsubscript{3} and control plots in the first year (Table 3, P > 0.05).

**Environmental variables and their correlation with N\textsubscript{2}O 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%.

Soil temperature (ST) at the 5-cm depth fluctuated greatly, following changes in air temperatures in wet seasons (May to September) and lower in dry seasons (November to February). 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%

Table 3. Cumulative N\textsubscript{2}O emission (kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) from different N addition treatments plots.

| Treatments | 2011 | 2012 | 2013 | 2014 | 2015 |
|------------|------|------|------|------|------|
| Control    | 0.98±0.02\textsubscript{a(a)} | 1.01±0.09\textsubscript{a(a)} | 1.08±0.08\textsubscript{a(a)} | 1.04±0.15\textsubscript{a(a)} | 0.97±0.14\textsubscript{a(a)} |
| L-NH\textsubscript{4}NO\textsubscript{3} | 2.52±0.03\textsubscript{a(a)} | 4.28±0.34\textsubscript{a(d)} | 5.32±0.43\textsubscript{a(d)} | 3.18±0.10\textsubscript{c(c)} | 2.88±0.04\textsubscript{b(b)} |
| H-NH\textsubscript{4}NO\textsubscript{3} | 4.73±0.04\textsubscript{a(a)} | 5.82±0.75\textsubscript{a(b)} | 7.52±0.77\textsubscript{f(c)} | 5.22±0.46\textsubscript{b(b)} | 4.59±0.74\textsubscript{d(d)} |
| L-(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} | 1.69±0.15\textsubscript{b(b)} | 3.16±0.16\textsubscript{b(b)} | 3.84±0.54\textsubscript{c(c)} | 2.75±0.30\textsubscript{c(b)} | 2.86±0.23\textsubscript{b(b)} |
| H-(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} | 3.41±0.00\textsubscript{a(a)} | 5.25±0.24\textsubscript{a(c)} | 6.21±0.25\textsubscript{a(d)} | 4.13±0.48\textsubscript{d(d)} | 3.71±0.28\textsubscript{b(b)} |
| L-NaNO\textsubscript{3} | 1.15±0.18\textsubscript{a(a)} | 1.89±0.20\textsubscript{a(b)} | 2.57±0.23\textsubscript{c(b)} | 2.65±0.25\textsubscript{b(b)} | 2.63±0.32\textsubscript{d(c)} |
| H-NaNO\textsubscript{3} | 2.31±0.17\textsubscript{c(c)} | 3.30±0.05\textsubscript{b(b)} | 4.97±0.73\textsubscript{d(c)} | 3.95±0.28\textsubscript{d(c)} | 3.67±0.69\textsubscript{c(b)} |

L: 50 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}; H: 150 kg N ha\textsuperscript{-1} yr\textsuperscript{-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.
Soil NH$_4^+$–N and NO$_3^-$–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 NO$_3^-$–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 NO$_3^-$–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 NH$_4^+$–N significantly accumulated in the N addition plots and its concentration ranged from 2.32 to 6.74 mg kg$^{-1}$. The accumulation of NH$_4^+$–N caused by N addition was less than that of NO$_3^-$–N in soil. Soil NH$_4^+$–N concentration was significantly influenced by the

![Temperature and Water Filled Pore Space](https://doi.org/10.1371/journal.pone.0189831.g003)

**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 (NO$_3^-$ and NH$_4^+$).

|               | ST      | WFPS    | NO$_3^-$ | NH$_4^+$ |
|---------------|---------|---------|----------|----------|
|               | d.f.    | F       | P        | F        | P        | F        | P        | F        | P        |
| 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   | **<0.001** | 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.

https://doi.org/10.1371/journal.pone.0189831.t004
three N forms, which increased NH$_4^+$–N in the order of (NH$_4$)$_2$SO$_4$ > NH$_4$NO$_3$ > NaNO$_3$ and by 57.40%, 36.27%, and 31.84% when compared with the control (2.98 mg kg$^{-1}$), respectively (Fig 4, Fig 5).
The correlation analysis showed that soil N\textsubscript{2}O 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 N\textsubscript{2}O emissions were extremely significantly (\(P < 0.01\)) correlated with ST, WFPS, and soil NH\textsubscript{4}\textsuperscript{+}–N and NO\textsubscript{3}–N (Fig 6).
Discussion

Promotion effects of N addition on soil N\textsubscript{2}O emissions

Our results showed that the temperature plantation in northern China was a source of atmospheric N\textsubscript{2}O under natural conditions. The mean N\textsubscript{2}O emissions value in the control was 11.55 μg N\textsubscript{2}O–N m\textsuperscript{-2} h\textsuperscript{-1}; this rate is comparable to that reported by Butterbach-Bahl et al. [16] who found that the N\textsubscript{2}O emissions in soils of spruce forests in Germany and Ireland ranged from 3.5 to 16.4 μg N m\textsuperscript{-2} h\textsuperscript{-1}. In our study, NaNO\textsubscript{3}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, and NH\textsubscript{4}NO\textsubscript{3} addition at levels of 50 and 150 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} significantly increased soil N\textsubscript{2}O 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-NaNO\textsubscript{3} addition, the increase in soil N\textsubscript{2}O 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 N\textsubscript{2}O 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 N\textsubscript{2}O 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 N\textsubscript{2}O 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 N\textsubscript{2}O emissions from short-term observations.

**Relationships between soil N\textsubscript{2}O emissions and soil properties**

In our study area, the soil concentration of NO\textsubscript{3}\textsuperscript{−}–N was higher than that of NH\textsubscript{4}\textsuperscript{+}–N, and the accumulation of NO\textsubscript{3}\textsuperscript{−}–N caused by N addition was more than soil NH\textsubscript{4}\textsuperscript{+}–N concentration. On the one hand, although the soil NO\textsubscript{3}\textsuperscript{−}–N concentration was directly increased by NO\textsubscript{3}\textsuperscript{−}–N addition, the NH\textsubscript{4}\textsuperscript{+}–N addition could have enhanced the activity of soil nitrifiers and led to the NO\textsubscript{3}\textsuperscript{−}–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 \textsuperscript{15}N tracing method suggest that plants in temperate forest at our site preferred NH\textsubscript{4}\textsuperscript{+}–N, which led to more NH\textsubscript{4}\textsuperscript{+}–N becoming assimilated, such that the accumulation of NH\textsubscript{4}\textsuperscript{+}–N in the soil was relatively little and brief [56].

We found that the soil N\textsubscript{2}O emissions were significantly correlated with concentrations of soil NH\textsubscript{4}\textsuperscript{+}–N and NO\textsubscript{3}\textsuperscript{−}–N, suggesting soil N\textsubscript{2}O emission was dominated by both nitrification and denitrification processes. Since atmospheric N deposition can significantly promote soil N\textsubscript{2}O emission, and exogenous NH\textsubscript{4}\textsuperscript{+}–N and NO\textsubscript{3}\textsuperscript{−}–N inputs into temperate forests may have synergic effects on soil N\textsubscript{2}O 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 \textsuperscript{15}N tracer methods. High ST, together with a relatively high WFPS, tend to promote both nitrification and denitrification processes [57] and consequently, high N\textsubscript{2}O 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 N\textsubscript{2}O 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 N\textsubscript{2}O production [36, 60].

**Conclusions**

This study emphasizes the effects of different N forms and levels on N\textsubscript{2}O emissions from a temperate forest over 5-year experimental period. We found that the accumulation of soil NO\textsubscript{3}\textsuperscript{−}–N was significantly higher than that of soil NH\textsubscript{4}\textsuperscript{+}–N due to N addition. N addition initially promoted soil N\textsubscript{2}O emission yet this promoting effect, although it existed, weakened in
the following years. High level N addition had a stronger promotion effect upon soil N\textsubscript{2}O emission than did the low level N addition. Meanwhile, the combined application of NH\textsubscript{4}\textsuperscript{+}–N and NO\textsubscript{3}\textsuperscript{−}–N promotes N\textsubscript{2}O emissions more than their single applications, and NH\textsubscript{4}\textsuperscript{+}–N addition had a stronger promotion effect for soil N\textsubscript{2}O emission than did the NO\textsubscript{3}–N addition. In addition, WFPS, ST, soil NH\textsubscript{4}\textsuperscript{+}–N, and NO\textsubscript{3}–N were all positively related to the N\textsubscript{2}O emissions. In the future, the long-term observation of soil N\textsubscript{2}O emissions, and the measurement of microbial functional groups using \textsuperscript{15}N tracer methods, will be necessary to clarify the mechanisms responsible for the soil N\textsubscript{2}O emissions.

Supporting information

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

Acknowledgments

We are grateful for the comments and criticisms of the anonymous reviewers.

Author Contributions

Conceptualization: Chunmei Wang.
Data curation: Ke Xu, Xintong Yang.
Formal analysis: Ke Xu, Xintong Yang.
Funding acquisition: Chunmei Wang.
Investigation: Ke Xu.
Methodology: Ke Xu, Xintong Yang.
Project administration: Ke Xu, Chunmei Wang.
Resources: Ke Xu, Chunmei Wang.
Software: Ke Xu, Xintong Yang.
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 N\textsubscript{2}O 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 N\textsubscript{2}O 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 CH$_4$ uptake and increases N$_2$O 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, Wewei D, et al. Pulse increase of soil N$_2$O emission in response to N addition in a temperate forest on Mt Chilang, 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 semi-arid temperate steppe in Inner Mongolia: effects of mineral nitrogen fertilizer levels and forms. Plant Soil. 2011; 342(1–2):345–357.

8. Rafique R, Hennessey 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 N$_2$O 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. Bijoor NS, Czimczik CI, Pataki DE, Billings SA. Effects of temperature and fertilization on nitrogen cycling and community composition of an urban lawn. Glob Chang Biol. 2006; 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 Chang Biol. 2008; 14(6):1305–1318.

13. Liu X, Zhang Y, Han W, Tang A, Shen J, Cui Z, Vlousek 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. Atoms 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 CH$_4$ 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. 2008; 12(1):33–45.

22. Malhi SS, Lekme K, Wang ZH, Chhabra BS. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. Soil Tillage Res. 2008; 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, Jettenas OJ, et al. Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. Forest Ecol Manag. 1998; 101(1–3):37–95.

25. Chen GC, Tam NFY, Ye Y. Spatial and seasonal variations of atmospheric N$_2$O and CO$_2$ 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.
30. Jiang C, Yu G, Fang H, Cao G, Li Y. Short-term effect of increasing nitrogen deposition on CO

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 simulated 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, Xuan X, Jiang Y. 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, Erismann 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. Agr Ecosyst Environ. 2006; 113(1–4):370–377.

49. Aber J, McDowell W, Nadelhoffe K, Magill A, Bernstson 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, Säwström 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 Envin 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.