Nitrogen-Induced Changes in Soil Environmental Factors Are More Important Than Nitrification and Denitrification Gene Abundance in Regulating N$_2$O Emissions in Subtropical Forest Soils

Qingyan Qiu$^1$, Abubakari Said Mgelwa$^2$, Shaofei Jin$^{3*}$ and Yalin Hu$^{1*}$

$^1$ Forest Ecology and Stable Isotope Center, College of Forestry, Fujian Agriculture and Forestry University, Fuzhou, China, $^2$ College of Natural Resources Management and Tourism, Mwalimu Julius K. Nyerere University of Agriculture and Technology, Musoma, Tanzania, $^3$ Department of Geography, Minjiang University, Fuzhou, China

Subtropical regions are currently experiencing a dramatic increase in nitrogen (N) deposition; however, the contributions of nitrification and denitrification processes to soil N$_2$O emissions and the underlying mechanisms under increasing N deposition remain unclear. Therefore, a $^{15}$N-tracing laboratory experiment with four N application rates (0, 12.5, 25, and 50 $\mu$g $^{15}$N g$^{-1}$ soil) was conducted to investigate the response of nitrification- and denitrification-derived N$_2$O to N additions in an evergreen broad-leaved forest (BF) and a Pinus forest (PF) in the Wuyi Mountains in southeastern China. Moreover, the abundance of functional genes related to nitrification ($amoA$), denitrification ($nirK$, $nirS$, and $nosZ$), and soil properties were measured to clarify the underlying mechanisms. Results showed that nitrification-derived N$_2$O emissions were generally decreased with increasing N input. However, denitrification-derived N$_2$O emissions were a non-linear response to N additions, with maximum N$_2$O emissions at the middle N application rate. Denitrification-derived N$_2$O was the dominant pathway of N$_2$O production, accounting for 64 to 100% of the total N$_2$O fluxes. Soil NH$_4^+$-N content and pH were the predominant factors in regulating nitrification-derived N$_2$O emissions in BF and PF, respectively. Soil pH and the $nirS$ abundance contributed the most to the variations of denitrification-derived N$_2$O emissions in BF and PF, respectively. Our results suggest that N application has the potential to increase the contribution of denitrification to N$_2$O production in subtropical forest soils. Changes in soil chemical properties induced by N addition are more important than the abundance of nitrification and denitrification functional genes in regulating soil nitrification- and denitrification-derived N$_2$O emissions.

Keywords: nitrification, denitrification, microbial functional genes, N$_2$O emissions, N application
INTRODUCTION

Nitrogen (N) deposition is continuously increasing primarily due to increased anthropogenic fossil fuel combustion, industrialization, and N fertilizer application. Elevated N deposition is expected to not only alter N cycling in forest ecosystems but also enhance N gas loss from the soils (Lu et al., 2011; Fang et al., 2015; Niu et al., 2016; Tang et al., 2018). As one of the primary forms of N gas loss from the soils, nitrous oxide (N2O) is more than 298 times as effective at trapping atmospheric heat as CO2 and is also one of the largest stratospheric ozone-depleting substances (IPCC, 2013). Terrestrial ecosystems contribute ~60% of the global N2O emissions (IPCC, 2013), of which about 15 to 55% are from tropical and subtropical forests that are believed to be the largest N2O terrestrial source (Solomon, 2007; Zhang et al., 2019). Moreover, N deposition in tropical and subtropical areas has been steadily increasing in recent decades (ranging from 30 to 73 kg N ha−1 year−1), and this trend is projected to continue over the coming decades (Zhang et al., 2008; Liu et al., 2013; Zhu et al., 2015).

N2O is produced primarily during the soil microbial processes of nitrification and denitrification, the rates of which are influenced by the inputs of N. Recent studies in subtropical forests demonstrated that the contribution of nitrification-derived N2O to the total N2O emissions was ≤50% and that of denitrification were 53 to 100% under aerobic conditions (Zhang et al., 2011; Zhu et al., 2013a; Han et al., 2018; Tang et al., 2018). In addition, the rates of denitrification have generally increased following N application (Zhu et al., 2013b; Morse et al., 2015; Niu et al., 2016; Han et al., 2018). However, few studies have focused on the response of nitrification-derived N2O emissions to N application, especially in subtropical forests (Han et al., 2018). Therefore, whether the response of nitrification-derived N2O emissions to N application is more pronounced than that of denitrification-derived N2O emissions or whether the contribution of denitrification to N2O production under elevated N input is still dominant over that of nitrification remains unclear. Thus, quantifying the underlying nitrification and denitrification contributions to the N2O production will help us understand the changes in the N transformation process with increasing N input in forest ecosystems.

Soil nitrification and denitrification can be influenced by different soil microbial groups, soil properties, and climate conditions (Levy-Booth et al., 2014; Cheng et al., 2019). Ammonia oxidation is not only the first but also the rate-limiting step of nitrification, which is performed by both ammonia-oxidizing archaea (AOA) and bacteria (AOB) (Chen and Peng, 2020). On the other hand, denitrification is a four-step microbial facilitated reduction process, whereby NO3− is reduced to N2O, NO, N2O, and eventually to N2. The first step for the gas production during denitrification is the reduction of NO2− to NO, which is catalyzed by nitrite reductases encoded by nirK and nirS genes (Levy-Booth et al., 2014; Hu et al., 2015). N2O can be further reduced to N2 by nitrous oxide reductases, which are encoded by nosZ genes (Levy-Booth et al., 2014). While the importance of nitrification and denitrification microorganisms in regulating soil N2O emission has been widely accepted (Chen and Peng, 2020), to what extent the abundance of nitrifier and denitrifier functional gene can be a good predictor of N2O emissions under elevated N inputs remains unclear. Moreover, several recent studies have reported that soil environmental factors (e.g., NH4+, NO3−, pH, TN/TP, and TC/TN) can explain better the changes in soil nitrification and denitrification activities than soil nitrification and denitrification functional genes in fertilized forest soils (Pett-Ridge et al., 2013; Scherbak et al., 2014; Tang et al., 2018, 2019).

Although tropical and subtropical forest soils are the largest N2O terrestrial sources (Solomon, 2007; Soper et al., 2018; Zhang et al., 2019), only a few studies so far in these regions have attempted to directly link the nitrification- and denitrification-derived N2O emissions in fertilized soils to N functional genes (Tang et al., 2018, 2019), and more importantly, to investigate the relative contribution of soil biotic and abiotic factors on the nitrification- and denitrification-derived N2O emissions. Greater insight into the regulation of these processes can help us understand the underlying mechanisms and adopt management practices to lower N2O emissions.

To evaluate the changes in soil N2O emissions resulting from nitrification and denitrification processes under N application in subtropical forests and clarify the underlying mechanisms, we carried out a 15N-NO3− and 15N-NH4+ labeling experiment under laboratory conditions. Mineral soils were collected from two subtropical forests, an evergreen broad-leaved forest (BF), and a Pinus forest (PF), which are located in the Wuyi Mountains in southeastern China. The evergreen BF was chosen because it is a product of the monsoon climate and it represents the climax vegetation type of subtropical regions (Zhou et al., 2013). Pinus forest was selected because of its strong contribution to afforestation purposes since the 1980s. It is worth highlighting that the selected pine species have a strong ability to adapt to poor soil conditions in the hilly red soil region of south China, which faced moderate to intense soil and water loss challenges in the 1980s. The forests have been widely planted since that time and account for about 30.5% of the subtropical forests in south China (Cao et al., 2015). Many previous studies have demonstrated that broadleaf/deciduous species produce more N2O than conifers due to a large difference in the production and reduction rates of N2O under the broadleaf/deciduous species (Zhang et al., 2008; Qin et al., 2019). However, some studies reported that coniferous stands emit more N2O than the adjacent deciduous stands that were affected by N deposition. Therefore, the role of different forest types (or tree species) in regulating N2O emissions remains unclear.

This study aimed to (1) investigate the responses of nitrification- and denitrification-derived N2O emissions to elevated N inputs, (2) clarify the dominant factors that control nitrification- and denitrification-derived N2O emissions, and (3) compare the differences in nitrification- and denitrification-derived N2O emissions between the BF and PF. We hypothesized that (1) nitrification- and denitrification-derived N2O emissions would be enhanced with the increased N application and that denitrification may contribute more to the increase in N2O emissions than nitrification, (2) the abundances of functional genes could be more important than soil environmental factors in regulating nitrification- and denitrification-derived N2O emissions, and (3) the nitrification- and denitrification-derived N2O emissions would be greater in the BF than the PF.
MATERIALS AND METHODS

Study Site Descriptions and Soil Sampling Procedures

The experimental soil samples were collected at the Wuyishan National Nature Reserve (WNWNR), located in Fujian Province (27°33′–27°54′N, 117°27′–117°51′E) in southern China. The climate of the WNNR (area: 56,527 ha) is humid monsoon, which is mainly characterized by the mean annual precipitation and temperature of 2,000 mm and 15°C, respectively, as well as 83.5% relative humidity and 100 fog days year−1 (Xu et al., 2010; Wang et al., 2013). The WNNR has two typical vegetation types, that is, evergreen BF and pine forest (PF). The BF and PF are located at about 550 m a.s.l. and 1,100 m a.s.l., respectively.

While Castanopsis carlesii Hay and Castanopsis eyrie Tutch are the dominant species in the BF, Pinus taiwanensis Hayata is the dominant species in the PF (Xu et al., 2010; Lyu et al., 2019). Background atmospheric N deposition in the WNNR is about 8 kg N ha−1 year−1 (Xu et al., 2016; Gurmesa et al., 2022).

In April 2018, three 20 m × 20 m plots without N addition (plots were about 10 m away from each other) were randomly established in each vegetation type, and soil samples were collected with a soil core sampler (8 cm in diameter) from the surface soil layer (0–20 cm) after litter removal. Ten soil cores were randomly collected from each plot and there were a total of 30 soil cores collected in each forest stand. Soil samples from the same forest type were thoroughly mixed to form a composite sample. All soil samples were sieved through a 2-mm mesh sieve and were then divided into two sub-samples. The first sub-sample was maintained at 4°C for 1 week before being used for the incubation experiment. The second sub-sample was air-dried for the measurements of soil chemical properties. The soil at both sites is classified as yellow-red soil based on the Chinese Soil Classification System, equivalent to an Ultisol according to the United States Department of Agriculture Soil Taxonomy classification system (Lyu et al., 2019). Soil chemical properties were shown in Table 1. The concentrations of soil SOC, TN, NH4+-N, and NO3−-N in the PF were significantly higher than those in the BF, whereas soil pH and δ15N were lower in the PF and no significant difference was found in the soil C/N ratio in these two forest soils (Table 1).

15N-Tracing Experiment and Gas Sampling Procedures

This study used a completely randomized factorial design experiment that consisted of four N application rate treatments (0, 12.5, 25, and 50 µg 15N g−1 soil equivalent to 0, 16, 32, and 64 kg N ha−1 year−1). We applied N as 15NH4NO3 (99.10 atom %) and NH415NO3 (99.21 atom %). The rates of N application were in the range of those of N deposition (10 to 73 kg N ha−1 year−1) in subtropical forests (Zhang et al., 2011, 2019). For each forest soil type, 120 glass jars (500 mL) were prepared and divided into two groups: one group comprised of 60 glass jars containing 15NH4NO3 (4 N addition rates × 3 replicates per N application rate × 5 destructive soil sampling) and the other group consisted of 60 glass jars containing NH415NO3. A total of 30 g (oven-dry basis) of fresh soil was weighed into each glass jar and pre-incubated for 7 days at 25°C to allow the recovery of soil microbial activities. Then, 15NH4NO3 or NH415NO3 was dissolved in 4 mL deionized water and added to the soil at a rate of 0, 12.5, 25, and 50 µg 15NH4+-N g−1 soil or 15NO3−-N g−1 soil. Soil without N addition was regarded as a control and was added with the same volume of deionized water to ensure its similarity in soil moisture content with the N-added soil. The soils were then incubated for 15 days at 25°C and their moisture content was maintained at 60% water-holding capacity throughout the entire experiment. Soils were corrected for water loss by weighing each glass jar plus soil every week and deionized water was added as required to maintain constant soil moisture. During gas sampling, three glass jars at each level of N treatment were randomly selected. The glass jars were closed with the gas-tight rubber lids, and gas sampling tubes were fixed into the middle of the rubber lids to enable the collection of the gas samples. Gas samples (20 mL) were collected from each glass jar with a 3-way stopcocks syringe on days 1, 2, 4, 9, and 15 after N application. Five millimeters were taken from each gas sample to determine the N2O concentration (Shimadzu, GC-2014C, Japan) and the remaining 15 mL were used to determine the N2O isotopic composition (IRMS, Isoprime 100, United Kingdom).

Before each gas sampling, the glass jars were opened and flushed with ambient air for approximately 30 min and then resealed with stoppers for 30 min.

Quantification of the Contributions of Nitrification and Denitrification to N2O Emissions

The fractions of N2O derived from denitrification (FD) and nitrification (FN) processes were calculated using the following equations from Stevens et al. (1997):

\[
FD = \frac{(a_m - a_n)}{(a_d - a_n)}
\]

(1)

\[
FN = 1 - FD
\]

(2)

where \(a_m\) is the 15N atom % of N2O, \(a_n\) is the 15N atom % of N2O from the nitrification process (assumed to be equivalent to 15N

| TABLE 1 | Soil chemical properties in the BF and PF. |
|---------|-----------------------------------------|
| Forest type | SOC (%) | TN (%) | C/N | δ15N (%) | pH | NH4+-N (mg kg−1) | NO3−-N (mg kg−1) |
| BF | 4.10 ± 0.07b | 0.21 ± 0.01b | 19.80 ± 0.47a | 10.84 ± 1.24a | 4.80 ± 0.03a | 91.89 ± 1.13b | 9.40 ± 0.06b |
| PF | 5.67 ± 0.20a | 0.27 ± 0.01a | 20.63 ± 0.12a | 9.87 ± 1.86b | 4.72 ± 0.1b | 126.66 ± 3.07a | 15.37 ± 0.56a |

BF and PF indicate the broad-leaved forest and Pinus forest, respectively. Values are means ± standard error, n = 3. Different lowercase letters in the same column represent the difference was significant at P < 0.05.
atom % of the soil NH$_4^+$), and $a_4$ is the 15N atom % of N$_2$O from denitrification (assumed to be equivalent to 15N atom % of the soil NO$_3^-$).

The atom percent of 15N is defined as 15N atom %

\[
\text{15N atom %} = \frac{15N}{(15N + 14N)} \times 100\%
\]  

Denitrification-derived N$_2$O flux = $FD \times N_2$O flux

Nitrification-derived N$_2$O flux = $FN \times N_2$O flux

Soil Inorganic N, Net Nitrification Rate, Dissolved Organic Carbon, Dissolved Organic Nitrogen, and pH Measurements

Soil samples were also collected after gas sampling. Five times destructive soil sampling was used for the analysis of the soil NH$_4^+$ and NO$_3^-$ contents, as well as the 15N values of soil NH$_4^+$ and NO$_3^-$. Dissolved organic carbon (DOC) and nitrogen (DON) contents and soil pH were also determined at the end of the experiment. Soil NH$_4^+$ and NO$_3^-$ contents were extracted using 2 mol L$^{-1}$ KCl for 1 h and then the extracted solutions were divided into two parts. One part was reacted with sodium salicylate (for NH$_4^+$ solution) and hydrazine sulfate solution (for NO$_3^-$ solution) and measured, respectively, at 660 and 550 nm using a discrete wet chemistry analyzer (SmartChem 200, AMS Alliance, Italy) to determine the concentrations of soil NH$_4^+$-N and NO$_3^-$-N. The other part was used for 15N measurements by distillation using MgO and Devarda’s alloy (Zhang et al., 2011). The DOC in the soil was extracted with 2 mol L$^{-1}$ KCl at 1:5 w/v and measured using a TOC-TN analyzer (Shimadzu TOC, Japan). The soil DON concentration was determined by calculating the difference between soil total dissolved nitrogen (2 mol L$^{-1}$ KCl extracted at 1:5 w/v) and soil mineral nitrogen (Jones and Willett, 2006). Soil pH was measured at a 1:2.5 soil to solution ratio using a pH detector (PHS-3C, Sheng Ci, Shanghai, China).

Quantification of the amoA, nirK, nirS, and nosZ Gene Abundances

At the end of the incubation experiment, soil samples for molecular analyses were immediately stored at −80°C before the aforementioned analysis. DNA was extracted from 0.3 g of frozen soils using a Mo Bio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, United States) according to the manufacturer’s protocol. The concentration and purity of the extracted DNA were determined with a spectrophotometer at 260 nm (NanoDrop Technologies, United States). All extracted soil DNA samples were stored at −80°C before analysis. Quantitative polymerase chain reaction (qPCR) was used to estimate the abundance of nitrification and denitrification functional genes (amoA, nirK, nirS, and nosZ) using a real-time PCR detection system. The PCR primers used in this study are listed in Supplementary Table 1. The 10-fold serially diluted plasmids carrying each target gene were subjected to real-time PCR assays in triplicate to generate a standard curve. The qPCR efficiencies for AOA-amoA, AOB-amoA, nirK, nirS, and nosZ were 0.85, 0.94, 0.96, 0.91, and 0.87, respectively. The corresponding determination coefficients of the standard curve for AOA-amoA, AOB-amoA, nirK, nirS, and nosZ were 0.998, 0.993, 0.990, 0.999, and 0.996, respectively.

Statistical Analyses

The gene copy numbers were log-transformed to meet the homogeneity of variance requirements. Three-way ANOVA was used to examine the effects of forest type, N application rate, sampling date, and their interactions on soil N$_2$O flux, nitrification-, and denitrification-derived N$_2$O emissions, as well as on soil NH$_4^+$-N, NO$_3^-$-N and their ratio (NH$_4^+$-N/NO$_3^-$-N). Two-way ANOVA with Duncan’s test was performed to determine the effects of forest type, N application rate, and their interactions on the soil properties (soil pH, DOC, and DON) and the abundances of soil nitrification and denitrification functional genes (amoA, nirK, nirS, and nosZ). All of the above statistical analyses were performed using SPSS 16.0 (SPSS Inc., Champaign, IL, United States).

To estimate the direct and indirect effects of soil biotic and abiotic factors on soil nitrification- and denitrification-derived N$_2$O emissions in the BF and MF, structural equation models (SEM) were performed based on our knowledge of soil biotic and abiotic properties in relation to soil nitrification- and denitrification-derived N$_2$O. Before the SEM analyses, the units of the predictor and dependent parameters were adjusted to obtain comparable parameter variances according to the standardized method. The quality of the SEM model was assessed by using a low $\chi^2$ value and $P > 0.05$, the root-mean-square error of approximation (RMSEA, the RMSEA $\leq 0.08$ indicates a model fit), and the model fit index (GFI $> 0.90$). All SEM analyses were using Amos 17.0 (IBM, SPSS, NY, United States).

RESULTS

Effects of N Addition on Total and Nitrification- and Denitrification-Derived N$_2$O Emissions

Soil N$_2$O fluxes decreased with increasing incubation period (Figure 1) and were significantly affected by forest type, N application rate, sampling date, and their interactions (Table 2). The N$_2$O flux values in the BF ranged from 0.05 to 3.85 µg N kg$^{-1}$ day$^{-1}$ throughout the entire incubation period (Figure 1), with the average fluxes across all the treatments being about 2.4 times greater than those in the PF (Figure 1). Non-linear responses of the soil N$_2$O fluxes to N addition were observed in both the BF and the PF. Specifically, the highest amount of soil N$_2$O emissions was noted at low and moderate N applications in the BF and PF, respectively (Figure 2A).

The application rates of N fertilizer altered not only the total amount of soil N$_2$O fluxes but also the nitrification- and denitrification-derived N$_2$O fluxes (Figures 1, 2). The nitrification- and denitrification-derived N$_2$O emissions were significantly higher in the BF than in the PF (Figure 2B). The nitrification-derived N$_2$O fluxes were in the range of 0.03 to 2.66 µg N kg$^{-1}$ day$^{-1}$ and 0 to 0.45 µg N kg$^{-1}$ day$^{-1}$ in the BF...
and PF, respectively (Figures 1C,D), and these fluxes decreased significantly as N application rates increased in both forests. At high rates of N addition, the decrease in nitrification-derived \( \text{N}_2\text{O} \) fluxes was approximately 84.6% in the BF and 100% in the PF relative to those observed at low N addition rates (Figure 2B). The denitrification-derived \( \text{N}_2\text{O} \) fluxes were in the range of 0.4 to 1.7 \( \mu \)g N kg\(^{-1}\) day\(^{-1}\) and 0.2 to 0.8 \( \mu \)g N kg\(^{-1}\) day\(^{-1}\) in the BF and PF, respectively (Figures 1E,F) and maximum average of denitrification-derived \( \text{N}_2\text{O} \) emissions was observed in middle N application rate in both BF and PF (Figure 2B). Moreover, denitrification was the dominant pathway of soil \( \text{N}_2\text{O} \) production in the present study, accounting for 64 to 90% and 81 to 100% of the total \( \text{N}_2\text{O} \) fluxes in the BF and PF, respectively (Figure 2C).

**Effects of N Addition on Soil Inorganic N, pH, Dissolved Organic Carbon, and Dissolved Organic Nitrogen**

Soil \( \text{NO}_3^- \)-N and \( \text{NH}_4^+ \)-N contents were significantly affected by forest type and N application rate (Table 2). The soil \( \text{NO}_3^- \)-N and \( \text{NH}_4^+ \)-N contents increased from day 1 to day 9 and then decreased significantly on the last day of the incubation experiment (Figure 3). These soil inorganic N contents increased with increasing N application rates, and they were significantly higher in the PF than in the BF (Figures 3A–D). Soil \( \text{NH}_4^+ \)-N contents in the control treatments were, respectively, \( \sim9\) - and \( \sim7\)-fold greater in the BF and PF compared with soil \( \text{NO}_3^- \)-N contents (Figures 3E,F). Moreover, the ratio of soil \( \text{NH}_4^+ \)-N to soil \( \text{NO}_3^- \)-N decreased with increasing N inputs. High N application significantly decreased soil pH in both forests. While high N addition significantly decreased soil DON content in PF, no significant effect of N addition on soil DOC was observed in this forest type (Figure 4).

**Effects of N Addition on the Abundances of Nitrification and Denitrification Functional Genes**

While the abundances of AOA- and AOB-ammoA genes in the BF and PF were not significantly affected by N inputs (Figures 5A–D), those of nirK and nirS genes were increased substantially by N inputs (Figures 5E,F). The increase in abundance of nirK genes

---

**TABLE 2** | \( P \)-values from Repeated-measure ANOVA of the effects of forest type (\( \text{FT} \)), N application rates (\( N \)), sampling date (\( D \)), and their interaction on the \( \text{N}_2\text{O} \) fluxes, nitrification-, and denitrification-derived \( \text{N}_2\text{O} \) emissions, soil \( \text{NO}_3^- \)-N, and \( \text{NH}_4^+ \)-N, \( n = 3 \).

| \( \text{FT} \) | \( N \) | \( D \) | \( \text{FT} * N \) | \( \text{FT} * D \) | \( N * D \) | \( \text{FT} * N * D \) |
|---|---|---|---|---|---|---|
| \( \text{N}_2\text{O} \) fluxes | <0.001 | <0.01 | <0.001 | 0.157 | <0.001 | <0.001 | <0.001 |
| Nitrification-derived \( \text{N}_2\text{O} \) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.274 | <0.001 |
| Denitrification-derived \( \text{N}_2\text{O} \) | <0.001 | <0.001 | <0.001 | 1.23 | <0.001 | <0.001 | 0.05 |
| \( \text{NO}_3^- \)-N | <0.001 | <0.001 | <0.001 | 0.605 | <0.001 | <0.001 | <0.001 |
| \( \text{NH}_4^+ \)-N | <0.001 | <0.001 | <0.001 | 0.554 | <0.001 | <0.001 | <0.01 |
| \( \text{NH}_4^+ \)-N/ \( \text{NO}_3^- \)-N | 0.222 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.01 |
in the PF (12%) was greater than that in the BF (5%), whereas the increase in abundance of nirS genes in the BF (15%) was slightly higher compared with that in the PF (14%). Inputs of N had no significant effect on nosZ gene abundance (Figure 5G).

Controlling Factors of Nitrification- and Denitrification-Derived N₂O Emissions

According to the SEM, soil properties (soil NH₄⁺-N, NO₃⁻-N, DON, and pH) and nitrification and denitrification functional genes (AOA-amoA, AOB-amoA, nirK, nirS, and nosZ) explained 99 and 95% of the variance observed in nitrification-derived N₂O emissions in the BF and PF, respectively (Figures 6A,B). These variables also could explain 55 and 88% of the variance of denitrification-derived N₂O emissions in the BF and PF, respectively (Figures 6C,D). The dominant factor in regulating soil nitrification-derived N₂O emission in the BF and PF was soil NH₄⁺-N (standardized total coefficient = −0.93, P < 0.001) and soil pH (standardized total coefficient = −0.74, P < 0.001), respectively, (Table 3). The dominant factors in regulating soil denitrification-derived N₂O in BF and PF were significantly differently (Figures 6C,D and Table 3). In the BF, it was soil pH. However, in the PF, the dominant regulatory factor was nirS (Table 3).

DISCUSSION

Effects of N Addition on Nitrification- and Denitrification-Derived N₂O Emissions

In the present study, soil N₂O fluxes responded non-linearly to N addition. Low- and middle-level N application rates increased soil N₂O emissions which might be due to N application and increased N availability (Figure 3) for nitrifying and denitrifying microorganisms. However, high N input had no significant effect on soil N₂O emission from BF or even decreased soil N₂O emission from PF (Figure 2B). Similarly, previous studies carried out in a subtropical montane forest also reported that short-term low and medium N addition favored N₂O production but this effect became weaker or even decreased N₂O emissions at high N addition (Han et al., 2018). A possible reason may be that the positive effect of high N application on denitrification-derived N₂O in both forests was partially offset by its negative effect on nitrification-derived N₂O (Figure 2B). Another explanation may be that the consumption of N₂O may be greater than the production of N₂O at high N treatment (Senbayram et al., 2012).

The application of N not only affects total soil N₂O emissions but also the contribution of nitrification and denitrification processes to soil N₂O production. In the present study, the average nitrification-derived N₂O emissions under different N treatments ranged from 0 to 0.37 µg N kg⁻¹ day⁻¹ (Figures 1C,D), which is similar to a previous study in subtropical forests [0.06–0.40 µg N kg⁻¹ day⁻¹, Zhang et al. (2011)]. Over 15 days of incubation, nitrification-derived N₂O contributed 0 to 36% of the total N₂O emissions (Figure 2). This range is within the range (<50%) that has been documented in previous studies carried out in the subtropical forest with similar N application rates (Zhang et al., 2011; Han et al., 2018). In contrast to our first hypothesis, our result showed a decrease in the contribution of the nitrification process to the total N₂O emissions following N application (Figure 3), and the magnitude of decrease was about 80 to 100% across the two forests. Deppe
et al. (2017) also found that N addition can significantly decrease the contribution of nitrification to N$_2$O production in a Haplic Luvisol soil, but the magnitude of decrease (7 to 21%) was not as pronounced as the one observed in the present study. One possible explanation for this phenomenon may be that N application decreased soil pH (Figure 4A), and soil nitrification rates have been demonstrated to be relatively low in acidic soils and decrease with decreasing soil pH (Cheng et al., 2013; Wang et al., 2020).

In contrast to the response of nitrification-derived N$_2$O emissions to N input as explained above, the highest denitrification-derived N$_2$O emissions occurred at the middle N application rate in both BF and PF, and the denitrification process is the dominant pathway for the production of soil N$_2$O emission, accounting for 64 to 100% of the total N$_2$O fluxes (Figure 3C). Previous studies carried out across subtropical forests reported that 53 to 100% of the total N$_2$O emissions were derived from denitrification (Zhang et al., 2011; Zhu et al., 2013a; Han et al., 2018). Moreover, the relative contributions of denitrification to N$_2$O emissions in forest soils were found to increase significantly with increasing N application (Zhu et al., 2013a; Morse et al., 2015; Niu et al., 2016), with the magnitude of increase ranging from 51 to 130% in a subtropical forest (Zhu et al., 2013a). The significantly higher relative contribution of denitrification to total N$_2$O production in the PF compared with the BF in the present study can be partly attributed to the greater decrease in nitrification-derived N$_2$O emissions in the PF (100%) than in the BF (80%).

Moreover, both nitrification- and denitrification-derived N$_2$O emissions were significantly higher in the BF relative to the PF, which indicated that the BF was a more important N$_2$O emission source than the PF. This is consistent with our third hypothesis. A study by Zhang et al. (2008) in southern China also reported that the emissions of N$_2$O from a mature monsoon evergreen BF were significantly higher compared to those from a PF. The authors attributed their observation to the higher soil N availability in the monsoon evergreen BF. However, their explanation cannot be extended to our findings because the BF had significantly lower soil available N content than the PF (Figure 3). Considerable differences in the amount of N$_2$O emissions among the investigated forest soils might be attributed to the differences in tree species, or more specifically, their influences on the soil’s abiotic and/or biotic properties (Butterbach-Bahl et al., 1997; Qin et al., 2019). It has been reported that trees in BF are associated with arbuscular mycorrhizal fungi, which can promote soil microbial communities with higher N cycling potential and activity relative to microbial communities in soils dominated by the trees associated with ectomycorrhizal fungi in the PF (Mushinski et al., 2021).

Overall, our results supported part of our first hypothesis as the denitrification process was the dominant pathway of soil N$_2$O production. However, the denitrification-derived N$_2$O emissions were observed as non-linear responses to N application in both forests. In addition, the nitrification-derived N$_2$O emissions decreased with increasing N application in both BF and PF, which is in contrast with our first hypothesis. An explanation for this phenomenon is presented in section “Controlling Factors Related to Nitrification-Derived N$_2$O Emissions in Forest Soils.”

**Controlling Factors Related to Nitrification-Derived N$_2$O Emissions in Forest Soils**

Soil NH$_4^{+}$-N content and soil pH contributed the most to the variations of nitrification-derived N$_2$O emissions in the BF and

**FIGURE 3** | Dynamics of soil NO$_3^{-}$-N (A,B) and NH$_4^{+}$-N contents (C,D) and soil NH$_4^{+}$-N to NO$_3^{-}$-N ratio (E,F) under different treatments. Values are means ± standard error (n = 3). Different lowercase letters indicate that the soil NO$_3^{-}$-N and NH$_4^{+}$-N contents and their ratios differed significantly among treatments at each sampling time (P < 0.05). C, L, M, and H represent N application rates of 0, 12.5, 25, and 50 µg $^{15}$N g$^{-1}$ soil, respectively. BF and PF represent the broad-leaved forest and Pinus forest, respectively.
hypothesis, our results suggest that N-induced changes in soil environmental factors were more important than the abundance of *amoA* genes in regulating nitrification-derived N\(_2\)O emissions. Similarly, Tang et al. (2019) found that soil environmental factors (especially soil NH\(_4^+\) content) accounted for 50 to 77% of the variation in potential nitrification activities, which was better explained than nitrification functional genes in fertilized soils. In the BF, high soil NH\(_4^+\)-N concentrations not only had a direct negative effect on nitrification-derived N\(_2\)O emission but also had an indirect negative effect via influencing soil pH and the abundance of AOB (Figure 6A). The reasons for high soil NH\(_4^+\)-N contents reducing nitrification-derived N\(_2\)O emission may be as follows: ammonia oxidizers are unable to tolerate high NH\(_4^+\)-N contents (>100 mg N kg\(^{-1}\) soil) in acidic soils (Gubry-Rangin et al., 2010; Carey et al., 2016; Farquharson, 2016; Breuillin-Sessoms et al., 2017; Li et al., 2018; Liu et al., 2018). In the current study, the average soil NH\(_4^+\)-N content in N-amended soils was much higher than not only the aforementioned 100 mg N kg\(^{-1}\) soil but also other soil NH\(_4^+\)-N content averages that have been reported in previous studies in various subtropical fertilized forest soils (ranging from 1 to 50 mg N kg\(^{-1}\) soil) (Han et al., 2018; Tang et al., 2018, 2019; Wang et al., 2018). This indicates the lack of efficient oxidation of NH\(_4^+\) to NO\(_3^-\) during nitrification that has led to lower nitrification-derived N\(_2\)O emissions in N-amended soils (Farquharson, 2016; Wang et al., 2020).

Soil NH\(_4^+\)-N content also had a direct significant effect on nitrification-derived N\(_2\)O emission in the BF, but its total effect was offset by its indirect effect on soil pH (Figure 6B). This resulted in soil pH playing a more important role in regulating nitrification-derived N\(_2\)O emissions in the PF (Table 3). Previous studies have reported that soil with a lower pH support less microbial diversity and lower N-cycle potential (Cheng et al., 2019; Mushinski et al., 2021). A meta-analysis reports that N addition dramatically decreases the ratios of fungi to bacteria and microbial C to N on an average by 10 and 8%, respectively (Zhou et al., 2017). In addition, the nitrification rate is generally decreased with decreasing soil pH, and its rate can be inhibited in soil with a pH lower than 5 (Zhao et al., 2018; Cheng et al., 2019). This could explain why the nitrification-derived N\(_2\)O emission at high N addition in the PF is close to zero (Figure 2B). Overall, in terms of nitrification-derived N\(_2\)O emissions, changes in soil environmental factors (e.g., soil NH\(_4^+\)-N and pH) induced by N application play more important roles than changes in the abundance of AOA and AOB in controlling its emission.

**Controlling Factors Related to Denitrification-Derived N\(_2\)O Emissions in Forest Soils**

Soil pH is the most important controller of denitrification-derived N\(_2\)O emission in the BF (Figure 6C and Table 3), and high N application significantly decreased soil pH (Figure 4). The reasons for soil pH regulating denitrification-derived N\(_2\)O emissions in BF may be as follows. Soil pH can exert a direct and an indirect effect on biological properties (e.g., reductase activities, the abundance, and expression of functional genes,
FIGURE 5 | Abundances of nitrification (A–D) and denitrification (E,F) functional genes under different treatments. Values are means ± standard error (n = 3). Different lowercase letters indicate that the functional gene abundance differed significantly among treatments (P < 0.05). C, L, M, and H represent N application rates of 0, 12.5, 25, and 50 µg ¹⁵N g⁻¹ soil, respectively. BF and PF represent the broad-leaved forest and Pinus forest, respectively. F, N, and F × N represent the effects of forest type, N application, and their interactions on these indicators. ** represent the effects of forest type or N application or their interactions on these indicators were significant at P < 0.05 and P < 0.01, respectively. ns indicate their effects were not significant at P < 0.05.
Structural equation modeling (SEM) analysis of the effects of soil biotic and abiotic properties on soil nitrification-derived N$_2$O emissions in BF (A), PF (B), and on soil denitrification-derived N$_2$O emissions in BF (C) and PF (D), respectively. Continued and dashed arrows indicated positive and negative relationships in a fitted SEM, respectively. Arrow line thickness indicates the strength of the causal relationship. Numbers adjacent to arrows represented standardized path coefficients of the relationships (*P < 0.05, **P < 0.01, ***P < 0.01). Double-headed arrows represented covariance between variables. The total variation explained by the model is indicated by $R^2$.

**TABLE 3** Standardized total effects of each explanatory variable on nitrification- and denitrification-derived N$_2$O emissions.

| Explanatory variable | Nitrification-derived N$_2$O emissions | Denitrification-derived N$_2$O emissions |
|----------------------|---------------------------------------|----------------------------------------|
| BF                   |                                       |                                        |
| NH$_4^+$-N           | -0.93                                 |                                        |
| AOB                  | 0.34                                  |                                        |
| DON                  | -0.26                                 |                                        |
| pH                   | 0.22                                  |                                        |
| PF                   |                                       |                                        |
| pH                   | -0.74                                 |                                        |
| NH$_4^+$-N           | -0.54                                 |                                        |
| DON                  | 0.41                                  |                                        |
| AOB                  | 0.15                                  |                                        |
| pH                   |                                       | -0.85                                  |
| nosZ                 | 0.36                                  |                                        |
| nirK                 | -0.34                                 |                                        |
| NO$_3^-$-N           | -0.25                                 |                                        |
| nirS                 | 0.86                                  |                                        |
| nosZ                 | 0.35                                  |                                        |
| pH                   | 0.33                                  |                                        |

The reductases for nitrate, nitrite, and nitric oxide are more active at pH <7 (Gillam et al., 2008), while the N$_2$O reductase activities are inhibited in soil with low pH (Levy-Booth et al., 2014). A review covering 50 years of research on the effects of soil pH on denitrification also reports that there is a consistent negative relationship between denitrification-derived N$_2$O production and soil pH (Šimek and Cooper, 2002). A close relationship between soil pH and denitrification-derived N$_2$O in the BF suggested that soil pH is a strong predictor of denitrifier activity in the BF. In the PF, the abundance of nirS is the dominant factor controlling denitrification-derived N$_2$O emissions. Previous studies have also reported that the abundance of denitrification genes correlated with nirS gene abundance (Morales et al., 2010).

The dominant factors in regulating soil denitrification-derived N$_2$O emission were different in these two forests. This may be because differences in the tree species lead to variations in soil environment and the community composition of functional microbes, and changes in these variations induced the differences...
in the nitrification- and denitrification-derived N$_2$O emissions in these acidic forest soils (Barberán et al., 2015; Soper et al., 2018). Moreover, regardless of nitrification- or denitrification-derived N$_2$O emissions, soil environmental factors are more important than nitrifier and denitrifier abundance in regulating soil N$_2$O emissions in both forests (except denitrification-derived N$_2$O emission in PF). Previous studies also demonstrate that tree species-induced changes in community composition and environmental factors play more important roles in regulating soil N$_2$O emissions than the abundance of nitrifier/denitrifier functional genes (Qin et al., 2019). In the present study, we still do not know how the species diversity or the nature of individual species affect nitrification- and denitrification-derived N$_2$O production in the field, even though several studies have investigated N$_2$O emission from plantation forest soils (Arai et al., 2008; Wang et al., 2014; Kou-Giesbrecht and Menge, 2021). Further study needs to focus on the role of the identities of specific species in the production processes of N$_2$O under different N inputs.

**CONCLUSION**

Our results demonstrated clearly that nitrification-derived N$_2$O emissions decreased with N application rates, while denitrification-derived N$_2$O emissions were non-linear responses to N addition. The total soil N$_2$O emissions, nitrification-, and denitrification-derived N$_2$O emissions were significantly higher in the broadleaf forest than in the conifers forest. Changes in soil environmental factors (i.e., pH and soil NH$_4^+$-N content) induced by N addition are more important than the abundance of nitrification and denitrification functional genes in regulating the production process of soil N$_2$O. The incorporation of soil nitrogen substrates, soil pH, and forest type into the nitrogen cycling model will more precisely predict the global N$_2$O from soil under elevated N deposition.

**REFERENCES**

Arai, S., Ishizuka, S., Ohta, S., Ansori, S., Tokuchi, N., Tanaka, N., et al. (2008). Potential N$_2$O emissions from leguminous tree plantation soils in the humid tropics. *Glob. Biogeochem. Cycles* 22:GB2028. doi: 10.1029/2007GB002965

Barberán, A., McGuire, K. L., Wolf, J. A., Jones, F. A., Wright, S. J., Turner, B. L., et al. (2015). Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecol. Lett.* 18, 1397–1405. doi: 10.1111/ele.12536

Breuillin-Sessoms, F., Venterea, R. T., Sadowsky, M. J., Coulter, J. A., Clough, T. I., and Wang, P. (2017). Nitrification gene ratio and free ammonia explain nitrite and nitrous oxide production in urea-amended soils. *Soil Biol. Biochem.* 111, 143–153. doi: 10.1016/j.soilbio.2017.04.007

Butterbach-Bahl, K., Gasche, R., Breuer, L., and Papen, H. (1997). Fluxes of NO and N$_2$O from temperate forest soils: impact of forest type, N deposition and of timing on the NO and N$_2$O emissions. *Nutr. Cycl. Agroecosyst.* 48, 79–90. doi: 10.1023/A:1007955210170

Cao, L., Liang, Y., Wang, Y., and Lu, H. (2015). Runoff and soil loss from Pinus massoniana forest in southern China after simulated rainfall. *Catena* 129, 1–8. doi: 10.1016/j.catena.2015.02.009

Carey, C. J., Dove, N. C., Beman, J. M., Hart, S. C., and Aronson, E. L. (2016). Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biol. Biochem.* 99, 158–166. doi: 10.1016/j.soilbio.2016.05.014

Chen, W. B., and Peng, S. L. (2020). Land-use legacy effects shape microbial contribution to N$_2$O production in three tropical forests. *Geoderma* 358, 113979. doi: 10.1016/j.geoderma.2019.113979

Cheng, Y., Wang, J., Chang, S. X., Cai, Z., Müller, C., and Zhang, J. (2019). Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: a review. *Environ. Pollut.* 244, 608–616. doi: 10.1016/j.envpol.2018.10.054

Cheng, Y., Wang, J., Mary, B., Zhang, J. B., Cai, Z. C., and Chang, S. X. (2013). Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. *Soil Biol. Biochem.* 57, 848–857. doi: 10.1016/j.soilbio.2012.08.021

Deppe, M., Well, R., Giesemann, A., Spott, O., and Flessa, H. (2017). Soil N$_2$O fluxes and related processes in laboratory incubations simulating ammonium fertilizer depots. *Soil Biol. Biochem.* 104, 68–80. doi: 10.1016/j.soilbio.2016.10.005

Fang, Y., Koba, K., Makabe, A., Takahashi, C., Zhu, W., Hayashi, T., et al. (2015). Microbial denitrification dominates nitrate losses from forest ecosystems. *Proc. Natl. Acad. Sci. U.S.A.* 112, 1470. doi: 10.1073/pnas.1416776112

Farquharson, R. (2016). Nitrification rates and associated nitrous oxide emissions from agricultural soils – a synopsis. *Soil Res.* 54, 469–480. doi: 10.1071/SR15304

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**AUTHOR CONTRIBUTIONS**

QQ: conceptualization, methodology, software, investigation, supervision, validation, formal analysis, writing–original draft, writing–reviewing and editing, visualization, data curation, and resources. AM: conceptualization, methodology, software, validation, formal analysis, writing–original draft, writing–reviewing and editing, visualization, and supervision. YH: conceptualization, methodology, software, validation, formal analysis, writing–original draft, writing–reviewing and editing, visualization, and supervision. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was funded by the National Natural Science Foundation of China (Grant Number 41703066), the Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, Chinese Academy of Sciences (Grant Number Y821161001-DE2018025), the Forestry Peak Discipline Construction Project, and the star-up founding of Minjiang University (32304307).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.950367/full#supplementary-material
Gillam, K., Zebarth, B., and Burton, D. (2008). Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. Can. J. Soil Sci. 88, 133–143. doi: 10.4141/CJS06005

Gubry-Rangin, C., Nicol, G. W., and Prosser, J. I. (2010). Archaea rather than bacteria control nitrification in two agricultural acidic soils. FEMS Microbiol. Ecol. 74, 566–574. doi: 10.1111/j.1574-6941.2010.00971.x

Guermes, G. A., Wang, A., Li, S., Peng, S., de Vries, W., Gundersen, P., et al. (2022). Retention of deposited ammonium and nitrate and its impact on the global forest carbon sink. Nat. Commun. 13, 1–9. doi: 10.1038/s41467-022-28345-1

Han, X., Shen, W., Zhang, J., and Müller, C. (2018). Microbial adaptation to long-term N supply prevents large responses in N dynamics and N losses of a subtropical forest. Soil. Total Environ. 626, 1175–1187. doi: 10.1016/j.soilenv.2018.01.132

Han, X., Xu, C., Nie, Y., He, J., Wang, W., Deng, Q., et al. (2019). Seasonal variations in N2O emissions in a subtropical forest with exogenous nitrogen enrichment are predominately influenced by the abundances of soil nitrifiers and denitrifiers. J. Geophys. Res. Biogeosci. 124, 3635–3651. doi: 10.1029/2019GC005477

Hu, H. W., Chen, D., and He, J. Z. (2015). Microbial regulation of post-harvest nitrous oxide formation: understanding the biological pathways for prediction of emission rates. FEMS Microbiol. Rev. 39, 729–749. doi: 10.1093/femsec/ ufv021

IPCC (2013). “climate change 2013: the physical science basis,” in Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. (Cambridge: Cambridge University Press), 1535.

Jones, D. L., and Willett, V. B. (2006). Experimental evaluation of methods to quantitatively dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Bio. Biochem. 38, 991–999. doi: 10.1016/j.soilbio.2005.08.012

Kou-Giesbrecht, S., and Menge, D. N. (2021). Nitrogen-fixing trees increase soil nitrous oxide emissions: a meta-analysis. Ecology 102(6):3415. doi: 10.1002/ecy.3415

Levy-Booth, D. J., Prescott, C. E., and Grayston, S. J. (2014). Microbial functional gene pools, transcription and kinetics of NO, N2O and N2 production as genes involved in nitrogen fixation, nitrification and denitrification in forest soils. FEMS Microbiol. Rev. 39, 345–354. doi: 10.1016/j.femsre.2013.06.006

Li, D., Zhang, X., Green, S. M., Dungait, J. A. J., Wen, X., Tang, Y., et al. (2018). N2O emission and the N2O/N2O+N2 product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agric. Ecosystem. Environ. 147, 4–12. doi: 10.1016/j.agee.2011.06.022

Shcherbak, I., Millar, N., and Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. Proc. Natl. Acad. Sci. U.S.A. 111, 9199. doi: 10.1073/pnas.1322434111

Slamek, M., and Cooper, J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. Eur. J. Soil Sci. 53, 345–354. doi: 10.1046/j.1365-2389.2002.00461.x

Solomon, S. (2007). Climate Change 2007 – The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge: Cambridge University Press.

Soper, F. M., Sullivan, B. W., Nasto, M. K., Osborne, B. B., Bru, D., Balzotti, C. S., et al. (2018). Remotely sensed canopy nitrogen correlates with nitrous oxide emissions in a lowland tropical rainforest. Ecology 99, 2080–2089. doi: 10.1002/ecy.2434

Stevens, R., Laughlin, R., Burns, L., Arah, J., and Hood, R. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Bio. Soil. Ecol. 29, 139–151. doi: 10.1016/S0038-0717(96)00303-3

Tang, W., Chen, D., Phillips, O. L., Liu, X., Zhou, Z., Li, Y., et al. (2018). Effects of long-term increased N deposition on tropical montane forest soil N2 and N2O emissions. Soil Bio. Soil. Ecol. 126, 194–203. doi: 10.1016/j.soilbio.2018.08.027

Tang, Y., Yu, G., Zhang, X., Wang, Q., Tian, D., Tian, J., et al. (2019). Environmental variables better explain changes in potential nitrification and denitrification activities than microbial properties in fertilized forest soils. Sci. Total. Environ. 647, 653–662. doi: 10.1016/j.scitotenv.2018.07.437

Wang, G., Zhou, Y., Xu, X., Ruan, H., and Wang, J. (2013). Temperature sensitivity of soil organic carbon mineralization along an elevation gradient in the Wuyi Mountains, China. PLoS One 8:e53914. doi: 10.1371/journal.pone.0053914

Wang, H., Liu, S., Zhang, X., Mao, Q., Li, X., You, Y., et al. (2018). Nitrogen addition reduces soil bacterial richness, while phosphorus addition alters community composition in an old-growth N-rich tropical forest in southern China. Soil. Bio. Soil. Ecol. 127, 22–30. doi: 10.1016/j.soilbio.2018.08.022

Wang, J., Tu, X. S., Zhang, H. M., Cui, J. Y., Ni, K., Chen, J. L., et al. (2020). Effects of ammonium-based nitrogen addition on soil nitrification and nitrous gas emissions depend on fertilizer-induced changes in pH in a tea plantation soil. Sci. Total. Environ. 747,8. doi: 10.1016/j.scitotenv.2020.141340

Wang, Y., Cheng, S., Fang, H., Yu, G., Xu, M., Dang, X., et al. (2014). Simulated nitrogen deposition reduces CH4 uptake and increases N2O emission from a subtropical plantation forest soil in southern China. PLoS One 9:e93571. doi: 10.1371/journal.pone.0093571

Xu, W., Li, W., Chen, S., Yang, D., Zhang, Y., Xue, J., et al. (2016). Quantification of nitrogen dry and wet deposition in Fujian tobacco planting area. Acta. Ecol. Sin. 36, 424–432. doi: 10.1615/chines.2016.09.007

Xu, X., Zhou, Y., Ruan, H., Luo, Y., and Wang, J. (2010). Temperature sensitivity increases with soil organic carbon recalcitrance along an elevational gradient in the Wuyi Mountains, China. Soil. Bio. Soil. Ecol. 42, 1811–1815. doi: 10.1016/j.soilbio.2010.06.021

Zhang, J., Cai, Z., and Zhu, T. (2011). N2O production pathways in the subtropical plantation forest soil in southern China. Atmos. Environ. 45, 459–467. doi: 10.1016/j.atmosenv.2010.09.026

Zhang, K., Zhuo, Q., Liu, J., Wang, M., Zhou, X., Li, M., et al. (2019). Spatial and temporal variations of N2O emissions from global forest and grassland ecosystems.
ecosystems. *Agric. For. Meteorol.* **266-267**, 129–139. doi: 10.1016/j.agrformet.2018.12.011

Zhang, W., Mo, J., Yu, G., Fang, Y., Li, D., Lu, X., et al. (2008). Emissions of nitrous oxide from three tropical forests in Southern China in response to simulated nitrogen deposition. *Plant Soil* **306**, 221–236. doi: 10.1007/s11104-008-9578-7

Zhao, W., Zhang, J., Müller, C., and Cai, Z. (2018). Effects of pH and mineralisation on nitrification in a subtropical acid forest soil. *Soil Res.* **56**, 275–283. doi: 10.1071/SR17087

Zhou, G., Peng, C., Li, Y., Liu, S., Zhang, Q., Tang, X., et al. (2013). A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broad-leaved forest in Southern China. *Glob. Change Biol.* **19**, 1197–1210. doi: 10.1111/gcb.12128

Zhou, Z., Wang, C., Zheng, M., Jiang, L., and Luo, Y. (2017). Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biol. Biochem.* **115**, 433–441. doi: 10.1016/j.soilbio.2017.09.015

Zhu, J., He, N., Wang, Q., Yuan, G., Wen, D., Yu, G., et al. (2015). The composition, spatial patterns, and influencing factors of atmospheric wet nitrogen deposition in Chinese terrestrial ecosystems. *Sci. Total Environ.* **511**, 777–785. doi: 10.1016/j.scitotenv.2014.12.038

Zhu, J., Mulder, J., Bakken, L., and Dörsch, P. (2013a). The importance of denitrification for N2O emissions from an N-saturated forest in SW China: results from in situ 15N labeling experiments. *Biogeochemistry* **116**, 103–117. doi: 10.1007/s10533-013-9883-8

Zhu, J., Mulder, J., Solheimsid, S. O., and Dörsch, P. (2013b). Functional traits of denitrification in a subtropical forest catchment in China with high atmogenic N deposition. *Soil Biol. Biochem.* **57**, 577–586. doi: 10.1016/j.soilbio.2012.09.017

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Qiu, Mgelwa, Jin and Hu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.