Nitrogen Uptake by Plants May Alleviate N Deposition-induced Increase in Soil N₂O Emissions in Subtropical Chinese Fir Plantations

Xiang Zheng

Nanjing Forestry University <https://orcid.org/0000-0002-6504-689X>

Qi Liu

Nanjing Forestry University

Minmin Cao

Nanjing Forestry University

Xiaofang Ji

Chinese Academy of Agricultural Sciences

Jianbing Lu

Nanjing Forestry University

Liu He

Nanjing Forestry University

Lingjuan Liu

Zhejiang Fengyang Mountain-Baishanzu National Nature Reserve

Shenglong Liu

Zhejiang Fengyang Mountain-Baishanzu National Nature Reserve

Jiang Jiang (✉ ecologyjiang@gmail.com)

Nanjing Forestry University <https://orcid.org/0000-0001-5058-8664>

Research

Keywords: Nitrogen enrichment, Nitrous oxide, carbon dioxide, Functional gene abundance, Chinese fir plantations

Posted Date: October 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-956233/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Continuous increasing nitrogen (N) deposition interferes with soil nitrogen cycle of forests, which highly impacts soil N₂O emissions and accelerates global warming. Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is one of the most widely planted species in southern China which locates in the high N deposition area. However, the impact of N deposition on soil N₂O emissions in subtropical Chinese fir plantations and the potential risk of increasing N deposition still remain elusive. Here, we conducted an *in situ* study in a subtropical Chinese fir plantation at Fengyang Mountain Nature Reserve, China, from 2019-2020 with four different levels of N enrichment: control (CK: ambient N deposition), low-N (LN: 50 kg N ha⁻¹ yr⁻¹), medium-N (MN: 100 kg N ha⁻¹ yr⁻¹), and high-N (HN: 200 kg N ha⁻¹ yr⁻¹).

Results

We found that soil N₂O emission rates increased with N enrichment from an average of 5.89 ± 3.66 to 20.11 ± 3.44 $\mu\text{g N m}^{-2} \text{h}^{-1}$. The N enrichment in general showed no significant effect on the abundance of nitrate-reducing bacteria, but it tended to raise the abundance of ammonia oxidizing archaea and bacteria, and to decrease the abundance of N₂O-reducing bacteria, which likely provided the microbial basis for accelerating soil N₂O emissions along with increasing N deposition. However, the relationship of soil N₂O emissions with N input did not match an exponential increase, but it matched a logarithmic increase, illustrating that the risk of increasing N deposition on soil N₂O emissions was attenuated. It is found that N enrichment significantly decreased soil moisture and tended to increase the fir leaf N concentrations and soil CO₂ emission rates. Besides, soil microbial biomass was significantly suppressed by N enrichment during the mid-growing season, while not in end of growing season. These may suggest that N enrichment stimulated plant growth with more N and water uptake, which competed with microorganisms for N and therefore alleviated further increasing N₂O emissions under N enrichment.

Conclusion

This study deepen our understanding of the impacts of increased N deposition on the greenhouse gas (GHG) balance in the Chinese fir plantations, and highlight that plants need to be incorporated as an important explanatory variable when predicting GHG fluxes in the background of global increasing N deposition.

Introduction

Global climate change, driven by the steady increase in atmospheric greenhouse gases (GHGs) emissions, is one of the main threats to the sustainability and stability of ecosystems (L Deng et al., 2020). By 2018, the two principal GHGs nitrous oxide (N₂O) and carbon dioxide (CO₂) increased by 23% and 47%, and reached about 331 ppb and 407 ppm, respectively, as compared to their levels at pre-

industrial time (WMO, 2020). Forest ecosystems contributed 15-55% of the global N₂O emissions with its area accounting for about 30.7% of Earth's total land area (U Nations, 2018). The average emission rate of N₂O from forests to the atmosphere is 3.62 ± 0.16 Tg N year⁻¹, of which 83.9% are from tropical regions (H Q Tian et al., 2016; K R Zhang et al., 2019). Therefore, the mitigation of GHG emissions from forest ecosystems is essential for balancing the GHG concentration in the atmosphere and alleviating global warming.

Aside from the direct application of nitrogen (N) used in intensive agricultural fertilization, atmospheric N deposition is the primary source of forest soil N. N deposition has been increasing as a result of human activities, such as the combustion of fossil fuels and land conversion (S-U Lena and d V Wim, 2018). These activities have accelerated terrestrial N cycling at both the regional and global scales (D N Liu et al., 2019). N deposition has increased the concentrations of soil ammonium-N (NH₄⁺-N), nitrate-N (NO₃⁻-N), plant N, and the leaching of inorganic N (M Lu et al., 2011). These changes directly affect nitrification and denitrification, and thus N₂O emissions. Previous studies have reported that N enrichment increased (E A Davidson et al., 2004; B Koehler et al., 2009), decreased (P A Steudler et al., 2002; P Tian et al., 2018) or had no effect (S J Hall and P A Matson, 2003; A K Müller et al., 2015) on soil N₂O emissions. The discrepancy among these results might be due to the different responses of plant and soil microbes to changes in N availability under different nutrient conditions. For example, E A Davidson et al. (2004) reported that N₂O emissions were weak in N-limited tropical secondary forest, due to the trees taking up extra N. S J Hall and P A Matson (1999) found that N-saturated tropical forests release more nitrogen oxides than N-limited tropical forests due to the suppressed microbial N immobilization. Similarly, N enrichment could either affect CO₂ emissions positively by increasing plant root biomass and microbial activity (L Zhou et al., 2014) or negatively by changing the decomposition of organic matter and energetic costs of N assimilation (J M Craine et al., 2007; I A Janssens et al., 2010). In addition, N₂O and CO₂ emissions from forest soils are also influenced by soil temperature (J Zou et al., 2018), soil moisture (E J Bateman and E M Baggs, 2005), soil pH (Y Wang et al., 2018), C availability (T J Rose et al., 2019), and oxygen availability (K Butterbach-Bahl et al., 2013). The variable response of GHG emissions to N deposition makes it more difficult to understand the potential mechanisms that drive the emissions. Despite a large body of research, it remains unclear how N deposition in forest ecosystems impacts N₂O and CO₂ emissions, especially in subtropical ecosystems and under various environmental conditions.

Microbial nitrification and denitrification are the most predominant sources of N₂O emissions from soil ecosystems (K Butterbach-Bahl et al., 2013). The ammonia oxidation process is the first and rate-limiting step of nitrification, which oxidizes NH₄⁺ or ammonia to nitrite (NO₂⁻). This step is carried out by ammonia-oxidizing bacteria (AOB) or archaea (AOA) and are studied using the ammonium monooxygenase (*amoA*) marker (D J Levy-Booth et al., 2014). In the second step, NO₂⁻ is rapidly oxidized by nitrite oxidizing bacteria to NO₃⁻. Denitrification is the sequential reduction of NO₃⁻ to dinitrogen gas (N₂) through NO₂⁻, nitric oxide (NO), and N₂O. In the process of converting NO₃⁻ to NO or N₂O, two different reductases encoded by the *nirS* or *nirK* genes participate in the catalysis (M M Kuypers et al., 2018), which

have been used in environmental studies as gene markers for denitrifiers (Q Wang et al., 2016). The reduction of N_2O to N_2 is carried out by N_2O reductase encoded by the *nosZ* gene in denitrifying microorganisms (W G Zumft and P M H Kroneck, 2006). The functional genes associated with soil N_2O production and soil N_2O emissions are influenced by soil moisture and temperature (U Szukics et al., 2010), soil pH (C J Carey et al., 2016), and available N concentration (S Hallin et al., 2009). Thus, the abundance of functional genes associated with soil N_2O production is often used for exploring effects of environmental change on nitrification and denitrification processes of microbial communities. Exploring how the functional microbes interact with N deposition in the subtropical forests is helpful for understanding the N_2O emissions in response to N deposition.

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is one of the most widely planted species in southern China based on its fast growth and high timber quality, and this species accounts for 30.4% of artificial timber forest in China (F F Shen et al., 2018). Chinese fir is distributed in the highly developed and high N deposition areas, (Y Lu et al., 2015; J Zhu et al., 2015) likely playing important roles in regulating soil C and N cycling. The response of Chinese fir nutrient absorption, growth, and productivity to nutrient deposition has attracted attention due to the increasing demand for wood (L Li et al., 2019; B Liu et al., 2017; F-C Wang et al., 2019). Nitrogen deposition has been shown to change nutrient acquisition strategies (L Li et al., 2019), soil microbial communities (Q Wang et al., 2018) and soil nutrients status (Q Xia et al., 2021) in Chinese fir stands, and subsequently alter fluxes of soil N_2O and CO_2 . However, studies on the effects of simulated increases in N deposition on soil N_2O and CO_2 emissions from Chinese fir plantations are limited. Here, we conducted an *in situ* experiment to investigate how increased N deposition influences soil N_2O and CO_2 emissions in Chinese fir plantations. We aimed to address the questions: (1) How does increased N deposition affect soil N_2O and CO_2 emissions in Chinese fir plantations? and (2) What is the underlying microbial mechanism driving changes in N_2O emissions under increased N deposition?

Materials And Methods

Site description

Our study was conducted in a subtropical Chinese fir plantation (Table 1) in Fengyang Mountain Nature Reserve, Zhejiang Province, China (28°53'56", 119°10'56" E, 1415 m a.s.l). The nature reserve encompasses an area of 15,171 ha and is characterized by a subtropical humid monsoon climate with an annual mean precipitation of 2400 mm and mean annual temperature of 12.3 °C. Since the establishment of the nature reserve in 1975, the entire study area has been protected from further land use changes or anthropogenic disturbances. Details characterizing the study site and forest stand are listed in Table 1.

In situ experimental design

The local ambient N deposition is estimated to be $34 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. To simulate future climate change scenarios, we set up four different levels of N enrichment (with four replicates for each treatment): a control (CK; ambient N deposition), low-N (LN; $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), medium-N (MN; $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and high-N (HN; $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Each level contained four replications that were randomly assigned to field plots. Sixteen $10 \text{ m} \times 10 \text{ m}$ plots were established with adjacent plots separated by a 10 m wide buffer strip. Nitrogen was added by spraying urea ($\text{CO}(\text{NH}_2)_2$) solution to the forest floor starting in April 2019 for N treatments. Meanwhile, control plots received the same amount of deionized water when N was applied. The N fertilizer was applied every month with an equal split throughout the year.

Measurement of soil N_2O and CO_2 emissions

The fluxes of N_2O and CO_2 were measured using a closed opaque static chamber (diameter $\times$ height = $80 \text{ cm} \times 21 \text{ cm}$), which consisted of a round base collar and a removable top. The top sampling chamber was covered with tinfoil to minimize temperature changes within the chamber headspace during sampling (X Zheng et al., 2020). The round base was permanently inserted 7 cm deep into the soil in April 2019. The removable top and the round base were used to form a sealed chamber headspace which is sealed with water during gas sampling to ensure air tightness. Four gas samples were taken from the chamber at 0, 10, 20 and 30 min after the bases were covered with the chambers using a 60 mL plastic syringe, the contents of which were transferred immediately into a pre-evacuated 100 mL aluminum foil gas bag. N_2O and CO_2 concentrations of the gas samples were analyzed by gas chromatography (Agilent 7890A, Santa Clara, CA, USA) within 48 h after sampling. The fluxes from the soil were calculated using the equation described by J Zhang et al. (2021a), which was based on the linear regression slope of the gas concentration during the time of chamber closure. Soil N_2O and CO_2 emissions were sampled between 10:00–12:00 am (GMT + 8) on each sampling day. Air and soil temperatures were monitored while the gas samples were collected. The sampling frequency was twice per month. N_2O and CO_2 fluxes were sampled semimonthly from mid-May 2020 to early November 2020 (sampling was started after 13 months of N enrichment), which were equivalent to measurements at about 10 days and 24 days after N enrichment during the application of fertilizer.

Soil sampling and analysis

Topsoil (0–20 cm soil) samples were collected along with every gas sample during the study, and the soil collected from the last gas sample was used for the microbial functional gene analysis (bagged on dry ice and stored at -80°C). Four soil cores were randomly collected from each plot and mixed into one composite sample, and then frozen before being passed through a 2 mm sieve. Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were extracted by 2 mol L^{-1} KCl solution and followed by colorimetric analysis (B L Deng et al., 2019; Y W Hu et al., 2017). Soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation extraction method. MBC and MBN are calculated as the difference between the organic C and total N concentrations of fumigated and nonfumigated samples, and the concentration of MBC and MBN of unrecovered biomass was corrected by a k factor of 0.45 (P C Brookes et al., 1985; E

D Vance et al., 1987). We measured the contents of MBC and MBN in soil at the middle (early-August) and end (early-November) of the growing season. Gravimetric soil water content was determined by drying in an oven at 105 °C until the soil reached a constant weight. The pH of soil samples collected in November 2020 were measured at a soil-to-water ratio of 1:2.5 (v/v). We also collected fresh leaves in November 2020, and we determined the concentrations of total C and total N with an elemental autoanalyzer (Vario MAX CN, Elementar Analysensysteme, GmbH, Langenselbold, Germany).

Quantification of microbial functional genes

Total DNA was extracted from 0.5 g soil with FastDNA SPIN Kit for soils (BIO 178 101, Qbiogene, Carlsbad, CA, USA), following the manufacturer's protocol. We used 0.8% agarose gel electrophoresis to detect the quality and integrity of the extracted DNA. The quantification of functional marker genes AOA, AOB, *nirK*, *nirS*, and *nosZ* was performed by real-time quantitative PCR using a CFX96 Optical Real-Time Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Information regarding the gene-specific primers and thermal conditions can be found in our previous study X Zheng et al. (2020). Each reaction mixture (25 µl) consisted of 12.5 µl 1 × SYBR Premix Ex Taq (Takara, Tokyo, Japan), 0.25 µl of each primer (10 mM), and 1 µl of template DNA (1-10 ng). Standard curves were prepared using a serial dilution of known copy number plasmid DNA from one representative clone containing each target gene.

Statistical analyses

We used mixed model analysis of variance (ANOVA) to examine the dependence of soil N₂O and CO₂ emission rates, soil temperature, soil moisture, NH₄⁺-N, and NO₃⁻-N on N addition with time as a random effect. We performed pairwise correlation analyses between soil N₂O and CO₂ emissions among soil abiotic factors (soil temperature and moisture, and NH₄⁺-N and NO₃⁻-N) and biotic factors (MBC and MBN) to examine their relationships. One-way ANOVA analysis was performed to determine the dependence of pH, MBC, MBN, fresh leaf C and N, and microbial functional genes associated with soil N₂O emissions on N addition. We used post-hoc tests (Tukey's HSD) to examine differences among treatment levels with significant results. All statistical analyses were performed in R 4.0.3 (R Core Team, 2020).

Results

Effects of N enrichment on soil characteristics and N₂O and CO₂ emissions

During the experimental period from May to November, the mean air temperature (7.48 to 23.32 °C) and soil temperature (9.75 to 20.07 °C) was 18.08 °C and 15.83°C, respectively (Fig. 1a, 1b). N enrichment significantly increased soil NH₄⁺ and NO₃⁻ concentrations (Figs. 1d, 2a and 2b), but it decreased soil moisture (Figs. 1c and 2c) and soil pH (Fig. 2d). For example, soil moisture decreased from 85% in the controls to 70.9% in the high-N plots (Fig. 2c). N₂O emission rates increased logarithmically with N enrichment from an average of 5.89 ± 3.66 to 20.11 ± 3.44 µg N m⁻² h⁻¹ (Fig. 3a). Only high-N

enrichment significantly increased soil N₂O emission rates, compare with control (Fig. 3a). CO₂ flux showed increasing trends with elevated N enrichment from 122.15 ± 14.86 to 218.29 ± 32.43 mg N m⁻² h⁻¹, but the effect of N enrichment was not statistically significant (Fig. 3b).

N effects on microbial biomass and functional genes associated with soil N₂O emissions

Over the study period, N enrichment significantly decreased soil MBC and MBN in the mid-growing season but it had no effect during the end of the growing season (Figs. 4a and 4c). The microbial biomass at the end of the growing season was significantly higher than that in the mid-growing season (Figs. 4a and 4c). Fresh leaf N concentration tended to increase with N enrichment (Fig. 4b), while the concentration of leaf C remained consistent (Fig. 4d). The N enrichment significantly increased the abundance of AOA and AOB (Figs. 5a and 5b), and significantly decreased the abundance of *nosZ* (Fig. 5e) but it had no effect on the abundance of *nirS* or *nirK* (Figs. 5c and 5d).

Relationships between environmental factors and soil N₂O and CO₂ emissions

Among the environmental factors we measured, soil N₂O emission rates were negatively correlated with MBC and MBN, but there were no relationships with soil temperature, soil moisture, air temperature, NH₄⁺-N, or NO₃⁻-N. Aside from MBC and soil moisture, the other environmental factors, including soil temperature, air temperature, NH₄⁺-N, and NO₃⁻-N, were not correlated with soil CO₂ emission rates. We found a significant negative relationship between NH₄⁺-N and MBN, and between NH₄⁺-N and MBC (Figs. 6a and 6b). Soil NO₃⁻-N was positively correlated with MBC (Fig. 6a).

Discussion

N enrichment-induced N₂O emissions driven by increased nitrification

In our study, N enrichment increased the abundance of the AOA and AOB genes (Figs. 5a and 5b) but did not affect *nirK* or *nirS* (Figs. 5c and 5d), which indicated that N-stimulated N₂O emissions may be driven by increased nitrification. The relative abundance of functional genes associated with soil N₂O production are regulated by soil N availability and pH (S Hallin et al., 2009). It has been found that AOB can tolerate high NH₄⁺ concentrations and high pH conditions more than AOA due to the difficulty of the ammonia monooxygenase of AOB to absorb NH₄⁺ in low pH soil (C J Carey et al., 2016). For example, H J Di et al. (2010) found that the AOB and AOA preferred different soil N conditions under N enrichment for growth. However, we found that N enrichment also enhanced the abundance of AOA, and the number of AOA gene copies were approximately 185 to 1900-fold more abundant than AOB (Figs. 5a and 5b), which may have been due to lower pH and N-limitation in the Chinese fir plantation. Indeed, AOA is normally reported as being dominant over AOB in acidic soils (H Hu et al., 2014; S Leininger et al., 2006; J-P Shen et al., 2012), and thus AOA could play a more important role than AOB in soil N₂O production of nitrification (M-Y Jung et al., 2014; M Stieglmeier et al., 2014). As far as our study is concerned, it remains

unclear which populations of ammonia-oxidizers (AOA and AOB) are primarily responsible for nitrification in forest soils, or which contributed more N_2O in response to N enrichment.

Nitrogen enrichment decreased the number of *nosZ* gene copies (Fig. 5e), but did not affect the abundance of *nirK* or *nirS* (Figs. 5c and 5d). N_2O reductase encoded by the *nosZ* gene catalyzes the reduction of N_2O to N_2 , which is the main way N_2O is reduced (M M Kuypers et al., 2018). Nitrogen-enrichment induced soil acidification may cause N_2O reductase to malfunction during denitrification, leading to higher $\text{N}_2\text{O}/\text{N}_2$ ratios (L Bergaust et al., 2010; N Raut et al., 2012), a process that was supported by our results.

We found that soil microbial biomass was significantly negatively correlated with $\text{NH}_4^+\text{-N}$, and that soil $\text{NO}_3^-\text{-N}$ was positively correlated with MBC (Figs. 6a and 6b). The content of $\text{NO}_3^-\text{-N}$ in the high-N and mid-N treatments was significantly higher than that in low-N, but there was no difference in $\text{NH}_4^+\text{-N}$ among the three treatments. Our results suggested that nitrification may be the main source of N_2O emissions, which caused an accumulation of nitrate in the soil. Soil moisture is crucial for gas diffusion and plays a dominant role in controlling nitrification and denitrification, which in turn affects N_2O emissions (K Butterbach-Bahl et al., 2013). Although our soil had high moisture (72.6% to 97.4%), and the soil bulk density was low (0.76 ± 0.10), N enrichment further reduced soil moisture and created conditions favorable for nitrification, which therefore likely facilitated N_2O emissions from nitrification. Soil N_2O -associated functional genes (AOB, AOA, *nirK*, *nirS*, and *nosZ*), microorganisms (MBC and MBN), N_2O substrates ($\text{NH}_4^+\text{-N}$ and soil $\text{NO}_3^-\text{-N}$), and environmental factors (soil moisture and pH) indicated that the increase in N_2O emissions caused by N enrichment may come from microbial nitrification.

Plant and soil microbe may alleviate N deposition-induced increase in soil N_2O emissions

With respect to the abundance of N_2O emissions related functional genes, the N enrichment in general showed no effect on the abundance of nitrate-reducing bacteria, but it raised the abundance of ammonia oxidizing archaea and bacteria, and decreased the abundance of N_2O -reducing bacteria, providing the microbial basis for accelerating soil N_2O emissions along with increasing N deposition (Fig. 5). Therefore, we speculate that the relationship between N_2O emission and N input may be exponential growth. However, in contrary to our speculation, we found that the soil N_2O emissions increased logarithmically with N input (Fig. 3a), illustrating that the risk of increasing N deposition on soil N_2O emissions was attenuated.

The IPCC assume a linear relationship between N application rate and N_2O emissions in inventory reporting, however, plenty of studies show a nonlinear relationship under specific soil-climatic conditions. A meta-analysis conducted by I Shcherbak et al. (2014) showed a general trend of exponentially increasing N_2O emissions as N inputs increase to exceed crop needs. Whereas, in N-limited or low N input systems such as the tropical montane rainforest (S J Hall and P A Matson, 2003) or the sub-Saharan Africa (I Shcherbak et al., 2014), the N_2O response to N inputs likely grew significantly slower than linear.

This may suggest that the Chinese fir plantation was N-limited in our study area, which was consistent with [L Li et al. \(2019\)](#).

Under the N-limited system in our study, the competition of available N (NH_4^+ and NO_3^-) by plants uptake may be a major reason for the slower than linear rate of soil N_2O emissions with increasing N input. In this study, N enrichment not only stimulated plant growth, but also increased leaf N content. We also observed that N enrichment tended to increase fresh leaf N concentration, even though these increases were not significant (Fig. 4b). This observation was partially consistent with [M Lu et al. \(2011\)](#), who found that N enrichment substantially increased the N content in leaves based on a meta-analysis. The increase in foliar mass (dilution effect) ([M Jonard et al., 2015](#); [A Schmitz et al., 2019](#)) may be the reason why the leaf N content did not respond to different levels of N enrichment, which was supported indirectly by the decreased soil water content as N enrichment increased. [E A Davidson et al. \(2004\)](#) showed that N enrichment increased tree biomass and foliar N concentration, which resulted in no clear response of N_2O emissions to N enrichment. Taken together, the plant's absorption of available N from the soil effectively reduced the N_2O emissions caused by N enrichment in the N-limited Chinese fir plantation. However, our results are based on short-term nitrogen enrichment treatments, and thus long-term observations of the response of Chinese fir plantation GHGs to N enrichment are necessary to more fully understand the long-term impacts of N enrichment.

The soil moisture decreased markedly with N enrichment in our study (Figs. 1c and 2c), which may indirectly support the competition of plants with microbes to uptake N nutrients together with soil water ([B Koehler et al., 2009](#); [A K Müller et al., 2015](#); [X Zheng et al., 2020](#)). It has been reported that N enrichment can increase plants transpiration while decreasing soil water leaching below the rooting zone ([X Lu et al., 2018](#)). This phenomenon may have resulted from promoted plant growth due the increased availability of N ([J Jiang et al., 2019](#)), which increased the absorption of water by roots and transpiration, and thus decreased soil moisture.

Soil microorganisms are sensitive to changes in the availability of soil N ([C Wang et al., 2018](#)). Previous studies reported that N enrichment could increase microbial biomass by relieving N limitation ([Z Zhou et al., 2017](#)) or inhibit microbial biomass by decreasing soil pH ([K K Treseder, 2008](#)). N enrichment significantly decreased soil pH (Fig. 2d) agreed with [D S Tian and S L Niu \(2015\)](#), who found that soil pH decreased by 0.26 on average globally due to N enrichment and enhanced Al^{3+} concentrations in soils. The lower soil microbial biomass under increased N enrichment observed in our study (Figs. 4a and 4c) may have resulted from enhancements in the content of Al^{3+} due to lower pH, and thus caused Al^{3+} poisoning in microbes ([W D Bowman et al., 2008](#)). Interestingly, we found that the biomass in the middle of the growing season was significantly lower than that in the end of the growing season (Figs. 4a and 4c), which was inconsistent with the view that microorganisms were at the optimal temperature ([J Pietikäinen et al., 2005](#)). Indeed, under N-restricted conditions, the results of the isotopic labeling of N^{15} indicated that plants can in fact successfully compete for N with microbes after N enrichment ([J P Kaye and S C Hart, 1997](#)). This finding indicated that the competition between plants and microorganisms

intensified plant growth in the vigorous growth stage, was alleviated in the slow growth stage, and thus promoted the growth of microorganisms. In addition, soil N₂O emission rates were negatively correlated with soil microbial biomass (MBC and MBN; Tab. 2). [S J Hall and P A Matson \(2003\)](#) found that microbial N immobilization was part of the reason for the low losses of soil N-oxides following N fertilization. Thus, microbial N fixation can mitigate N₂O emissions to some extent, and the mitigation effect is affected by plant and soil pH.

During our observation period, N enrichment increased soil CO₂ emissions, but the differences caused by N enrichment were not statistically significant (Fig. 3b). Soil CO₂ emission rates depended on the divergent responses of autotrophic respiration of plant roots and heterotrophic respiration of microorganisms to N enrichment ([I A Janssens et al., 2010](#); [J Zhang et al., 2021b](#)). Suppressive impacts of N enrichment on heterotrophic respiration have been often reported ([I A Janssens et al., 2010](#); [K S Ramirez et al., 2012](#); [L Zhou et al., 2014](#)), and several drivers have been suggested, including a shift in the microbial community ([M Gallo et al., 2004](#); [K S Ramirez et al., 2012](#)), decreased microbial biomass ([K K Treseder, 2008](#); [C Wang et al., 2018](#)), depressed 'microbial N mining' processes ([J M Craine et al., 2007](#); [K Michel and E Matzner, 2003](#)), and C limitation caused by increased recalcitrant C ([I A Janssens et al., 2010](#)). Our finding that microbial biomass tended to decrease with N enrichment in Chinese fir plantations (Figs. 4a and 4c) were in line with in [F F Shen et al. \(2018\)](#), which suggested that the response of heterotrophic respiration to N enrichment may decrease or remain unchanged rather than increase. Conversely, we observed that N enrichment increased soil CO₂ emissions (Fig. 3b), which indicated that there was a dominant contribution by plant root growth to increased CO₂ emissions, as evidenced by the decreased soil moisture (Fig. 2c).

Conclusions

Elevated N enrichment increased N₂O emissions following a logarithmic trend in N-limited Chinese fir plantations. Nitrogen enrichment promoted plant N immobilization by increasing plant growth and leaf N concentration, which therefore dampened the increased N₂O emissions caused by N enrichment. N enrichment enhanced the abundance of AOA and AOB, which may indicate that nitrification is the main source of N-stimulated N₂O emissions. Considering the increased deposition of N in Chinese fir plantations due to human activity, our findings are of great importance for forest management. However, long-term studies are needed to more fully explore the threshold and the cumulative effects of increased N deposition on GHG fluxes in Chinese fir plantations.

Declarations

Acknowledgments

We are grateful to the Zhejiang Fengyang Mountain-Baishanzu National Nature Reserve for the venues supplement and logistical support during our research.

Authors' contributions

Xiang Zheng and Jiang Jiang designed research, Qi Liu revised and perfected the design of the experiments. Xiang Zheng and Minmin Cao performed research, collected and analyzed data; All authors discussed the results and revised the manuscript. The author(s) read and approved the final manuscript.

Funding

This study was supported by the National Key R&D Program of China (2017YFC0505502), the National Natural Science Foundation of China (32071612, 41701225, 31901196), and the Natural Science Foundation of Jiangsu Province (BK20170920, BK20190746).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Bateman E J, Baggs E M (2005) Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biol. Fertility Soils*, 41, 379-388.
- Bergaust L, Mao Y, Bakken L R, Frostegård A (2010) Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrous oxide reductase in *Paracoccus denitrificans*. *Appl. Environ. Microbiol.*, 76, 6387-96.
- Bowman W D, Cleveland C C, Halada L, Hreško J, Baron J S (2008) Negative impact of nitrogen deposition on soil buffering capacity. *Nat. Geosci.*, 1, 767-770.
- Brookes P C, Landman A, Pruden G, Jenkinson D S (1985) Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.*, 17, 837-842.

- Butterbach-Bahl K, Baggs E M, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos. Trans. R. Soc. B-Biol. Sci.*, 368, 20130122.
- Carey C J, Dove N C, Beman J M, Hart S C, Aronson E L (2016) Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biol. Biochem.*, 99, 158-166.
- Craine J M, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. *Ecology*, 88, 2105-2113.
- Davidson E A et al. (2004) Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecol. Appl.*, 14, 150-163.
- Deng B L et al. (2019) Effects of biochar and dicyandiamide combination on nitrous oxide emissions from *Camellia oleifera* field soil. *Environ. Sci. Pollut. Res. Int.*, 26, 4070-4077.
- Deng L et al. (2020) Soil GHG fluxes are altered by N deposition: New data indicate lower N stimulation of the N_2O flux and greater stimulation of the calculated C pools. *Glob. Chang. Biol.*, 26, 2613-2629.
- Di H J et al. (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiol. Ecol.*, 72, 386-394.
- Gallo M, Amonette R, Lauber C, Sinsabaugh R L, Zak D R (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microb. Ecol.*, 48, 218-229.
- Hall S J, Matson P A (1999) Nitrogen oxide emissions after nitrogen additions in tropical forests. *Nature*, 400, 152-155.
- Hall S J, Matson P A (2003) Nutrient status of tropical rain forests influences soil N dynamics after N additions. *Ecol. Monogr.*, 73, 107-129.
- Hallin S, Jones C M, Schlöter M, Philippot L (2009) Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME journal*, 3, 597-605.
- Hu H, Xu Z, He J-Z (2014) Ammonia-oxidizing archaea play a predominant role in acid soil nitrification. *Adv Agron*, 125, 261-302.
- Hu Y W et al. (2017) The non-additive effects of temperature and nitrogen deposition on CO_2 emissions, nitrification, and nitrogen mineralization in soils mixed with termite nests. *Catena*, 154, 12-20.
- Janssens I A et al. (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci.*, 3, 315-322.

- Jiang J et al. (2019) Interactive effects of nitrogen and phosphorus additions on plant growth vary with ecosystem type. *Plant Soil*, 440, 523-537.
- Jonard M et al. (2015) Tree mineral nutrition is deteriorating in Europe. *Glob. Chang. Biol.*, 21, 418-430.
- Jung M-Y et al. (2014) Isotopic signatures of N₂O produced by ammonia-oxidizing archaea from soils. *The ISME journal*, 8, 1115-1125.
- Kaye J P, Hart S C (1997) Competition for nitrogen between plants and soil microorganisms. *Trends Ecol. Evol.*, 12, 139-143.
- Koehler B, Corre M D, Veldkamp E, Wullaert H, Wright S J (2009) Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Glob. Chang. Biol.*, 15, 2049-2066.
- Kuypers M M, Marchant H K, Kartal B (2018) The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.*, 16, 263-276.
- Leininger S et al. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 442, 806-9.
- Lena S-U, Wim d V (2018) Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Glob. Chang. Biol.*, 24, e416-e431.
- Levy-Booth D J, Prescott C E, Grayston S J (2014) Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biol. Biochem.*, 75, 11-25.
- Li L et al. (2019) Different responses of absorptive roots and arbuscular mycorrhizal fungi to fertilization provide diverse nutrient acquisition strategies in Chinese fir. *For. Ecol. Manage.*, 433, 64-72.
- Liu B et al. (2017) Excessive accumulation of Chinese fir litter inhibits its own seedling emergence and early growth—a greenhouse perspective. *Forests*, 8, 341.
- Liu D N, Guo X D, Xiao B W (2019) What causes growth of global greenhouse gas emissions? Evidence from 40 countries. *Sci. Total Environ.*, 661, 750-766.
- Lu M et al. (2011) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol.*, 189, 1040-1050.
- Lu X et al. (2018) Plant acclimation to long-term high nitrogen deposition in an N-rich tropical forest. *Proc. Natl. Acad. Sci. U. S. A.*, 115, 5187-5192.
- Lu Y, Coops N, Wang T, Wang G (2015) A Process-Based Approach to Estimate Chinese Fir (*Cunninghamia lanceolata*) Distribution and Productivity in Southern China under Climate Change. *Forests*, 6, 360-379.

- Müller A K, Matson A L, Corre M D, Veldkamp E (2015) Soil N₂O fluxes along an elevation gradient of tropical montane forests under experimental nitrogen and phosphorus addition. *Front. Earth Sci.*, 3, 66.
- Michel K, Matzner E (2003) Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. *Biol. Fertility Soils*, 38, 102-109.
- Nations U (2018) The Sustainable Development Goals Report 2018, New York.
- Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiol. Ecol.*, 52, 49-58.
- Ramirez K S, Craine J M, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob. Chang. Biol.*, 18, 1918-1927.
- Raut N, Dörsch P, Sitaula B K, Bakken L R (2012) Soil acidification by intensified crop production in South Asia results in higher N₂O/(N₂+N₂O) product ratios of denitrification. *Soil Biol. Biochem.*, 55, 104-112.
- Rose T J, Kearney L J, Morris S, Van Zwieten L, Erler D V (2019) Pinto peanut cover crop nitrogen contributions and potential to mitigate nitrous oxide emissions in subtropical coffee plantations. *Sci. Total Environ.*, 656, 108-117.
- Schmitz A et al. (2019) Responses of forest ecosystems in Europe to decreasing nitrogen deposition. *Environ. Pollut.*, 244, 980-994.
- Shcherbak I, Millar N, 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, 9199-9204.
- Shen F F et al. (2018) Soil N/P and C/P ratio regulate the responses of soil microbial community composition and enzyme activities in a long-term nitrogen loaded Chinese fir forest. *Plant Soil*, 436, 91-107.
- Shen J-P, Zhang L-M, Di H J, He J-Z, 2012. A review of ammonia-oxidizing bacteria and archaea in Chinese soils, *Front Microbiol*, pp. 296.
- Steudler P A et al. (2002) Trace gas responses of tropical forest and pasture soils to N and P fertilization. *Global Biogeochem. Cycles*, 16, 1023.
- Stieglmeier M et al. (2014) Aerobic nitrous oxide production through N-nitrosating hybrid formation in ammonia-oxidizing archaea. *The ISME journal*, 8, 1135-1146.
- Szükics U et al. (2010) Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiol. Ecol.*, 72, 395-406.

- Tian D S, Niu S L (2015) A global analysis of soil acidification caused by nitrogen addition. *Environ. Res. Lett.*, 10, 024019.
- Tian H Q et al. (2016) The terrestrial biosphere as a net source of greenhouse gases to the atmosphere. *Nature*, 531, 225-228.
- Tian P, Zhang J B, Cai Z C, Jin G Z (2018) Different response of CO₂ and N₂O fluxes to N deposition with seasons in a temperate forest in northeastern China. *J. Soils Sed.*, 18, 1821-1831.
- Treseder K K (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.*, 11, 1111-1120.
- Trivedi P, Anderson I C, Singh B K (2013) Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends Microbiol.*, 21, 641-651.
- Vance E D, Brookes P C, Jenkinson D S (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.*, 19, 703-707.
- Wang C, Liu D, Bai E (2018) Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol. Biochem.*, 120, 126-133.
- Wang F-C et al. (2019) Effects of nutrient addition on foliar phosphorus fractions and their resorption in different-aged leaves of Chinese fir in subtropical China. *Plant Soil*, 443, 41-54.
- Wang Q et al. (2016) Effects of the nitrification inhibitor dicyandiamide (DCD) on N₂O emissions and the abundance of nitrifiers and denitrifiers in two contrasting agricultural soils. *J. Soils Sed.*, 17, 1635-1643.
- Wang Q et al. (2018) Influence of nitrogen and phosphorus additions on N₂-fixation activity, abundance, and composition of diazotrophic communities in a Chinese fir plantation. *Sci. Total Environ.*, 619, 1530-1537.
- Wang Y et al. (2018) Soil pH as the chief modifier for regional nitrous oxide emissions: New evidence and implications for global estimates and mitigation. *Glob. Chang. Biol.*, 24, e617-e626.
- WMO (2020) Statement on the state of the global climate in 2019, Geneva Switzerland.
- Xia Q et al. (2021) Increase of soil nitrogen availability and recycling with stand age of Chinese-fir plantations. *For. Ecol. Manage.*, 480, 118643.
- Zhang J et al. (2021a) Management scheme influence and nitrogen addition effects on soil CO₂, CH₄, and N₂O fluxes in a Moso bamboo plantation. *For Ecosyst*, 8, 6.
- Zhang J et al. (2021b) Different responses of soil respiration and its components to nitrogen and phosphorus addition in a subtropical secondary forest. *For Ecosyst*, 8, 37.

Zhang K R et al. (2019) Spatial and temporal variations of N₂O emissions from global forest and grassland ecosystems. *Agric. For. Meteorol.*, 266-267, 129-139.

Zheng X et al. (2020) Litter removal enhances soil N₂O emissions: Implications for management of leaf-harvesting *Cinnamomum camphora* plantations. *For. Ecol. Manage.*, 466, 118121.

Zhou L et al. (2014) Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Glob. Chang. Biol.*, 20, 2332-2343.

Zhou Z, Wang C, Zheng M, Jiang L, Luo Y (2017) Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. *Soil Biol. Biochem.*, 115, 433-441.

Zhu J 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.

Zou J, Tobin B, Luo Y, Osborne B (2018) Differential responses of soil CO₂ and N₂O fluxes to experimental warming. *Agric. For. Meteorol.*, 259, 11-22.

Zumft W G, Kroneck P M H (2006) Respiratory transformation of nitrous oxide (N₂O) to dinitrogen by Bacteria and Archaea. *Adv. Microb. Physiol.*, 52, 107-227.

Tables

Table 1 Characteristics of soil and forest stand in the studied area. Means ± SE (standard error). TN, total nitrogen; TC, total carbon; NH₄⁺-N, ammonium nitrogen; NO₃⁻-N, nitrate nitrogen; TP, total phosphorus; DBH, diameter at breast height.

Soil		Forest stand	
TN (g kg ⁻¹)	4.11±0.03	Age (year)	39
TC (g kg ⁻¹)	58.73±0.37	Litter (kg m ⁻²)	0.19±0.06
NH ₄ ⁺ -N (mg kg ⁻¹)	15.71±1.14	Mean DBH (cm)	21.75±0.35
NO ₃ ⁻ -N (mg kg ⁻¹)	14.66±0.87	Mean High (m)	13.20±0.30
TP (g kg ⁻¹)	0.3±0.05	Stem density (tree ha ⁻¹)	2094±120
Bulk density (g cm ⁻³)	0.76±0.10	Canopy	0.83

Table 2 Pairwise correlation between soil N₂O and CO₂ emission rates and soil environmental factors, soil available nitrogen (NH₄⁺-N, NO₃⁻-N), soil microbial biomass (MBC, MBN), and air temperature. Significant results are shown in bold.

Factor		Soil temperature	Soil moisture	Air temperature	NH ₄ ⁺ -N	NO ₃ ⁻ -N	MBC	MBN
N ₂ O	Coefficient	0.178	-0.167	0.145	0.094	0.129	-0.639	-0.472
	P values	0.07	0.098	0.139	0.399	0.224	0.004	0.048
CO ₂	Coefficient	0.068	0.194	0.109	0.042	0.108	-0.512	-0.444
	P values	0.486	0.043	0.262	0.705	0.310	0.025	0.057

Figures

Figure 1

Dynamics of (a) air temperature (T_{air} , °C) and precipitation (mm), (b) soil temperature (°C), (c) soil mass water content (%), and (d) ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) as affected by our in situ simulated increases in N deposition. P values of mixed model ANOVA are shown. CK: control; LN: low-N; MN: medium-N; HN: high-N; NS: not significant; Time: studied time in days as random effects.

Figure 2

Soil (a) ammonium nitrogen ($\text{NH}_4^+\text{-N}$) concentrations, (b) nitrate nitrogen ($\text{NO}_3^-\text{-N}$) concentrations, (c) soil mass water content, and (d) pH (means $\pm$ se) as affected by our in situ simulated increases in N deposition. CK: control; LN: low-N; MN: medium-N; HN: high-N. Bars connected by the same letter are not significantly different in post-hoc tests at $\alpha = 0.05$.

Figure 3

Dynamics of soil (a) N₂O and (b) CO₂ emission rates as affected by our in situ simulated increases in N deposition and relationship between the N₂O emission rates and N input rate (a). P values of mixed model ANOVA are shown. CK: control (ambient N deposition, 34 kg N ha⁻¹ yr⁻¹); LN: low-N (50+34 kg N ha⁻¹ yr⁻¹); MN: medium-N (100+34 kg N ha⁻¹ yr⁻¹); HN: high-N (200+34 kg N ha⁻¹ yr⁻¹); NS: not significant; Time: studied time in days as random effects. Means± se of bars connected by the same letter are not significantly different in post-hoc tests at $\alpha=0.05$.

Figure 4

(a) Soil microbial biomass N, (b) fresh leaf N concentration, (c) soil microbial biomass C, and (d) fresh leaf C concentration (means $\pm$ se) as affected by our in situ simulated increases in N deposition. Different capital letters indicate significant differences among the simulated N enrichment treatment; significant differences among growing seasons are indicated by different small letters based on post-hoc tests at $\alpha=0.05$. CK: control; LN: low-N; MN: medium-N; HN: high-N.

Figure 5

Functional gene number (means $\pm$ se) associated with soil N₂O emissions as affected by simulated N enrichment treatment. Different capital letters indicate significantly different between N treatments at $P < 0.05$. AOA, archaeal amoA; AOB, bacterial amoA. CK: control; LN: low-N; MN: medium-N; HN: high-N.

Figure 6

Relationship between the (a) soil microbial biomass C (MBC) and that of ammonium (NH_4^+), nitrate (NO_3^-), and as a function between the (b) soil microbial biomass N (MBN) and ammonium (NH_4^+). Line is the best-fit regression, where $\text{MBC} \sim \text{NH}_4^+$ and $\text{MBN} \sim \text{NH}_4^+$ are the black solid line and $\text{MBC} \sim \text{NO}_3^-$ is the gray dashed line. Each symbol represents one observation: black circles = NH_4^+ , gray triangles = NO_3^- . NS = non-significant ($P > 0.05$).