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## EDITED BY

Izhar Ali,  
Guangxi University, China

## REVIEWED BY

Haijun Sun,  
Nanjing Forestry University, China  
Ronley C. Canatoy,  
Central Mindanao University, Philippines

## \*CORRESPONDENCE

Neng Li  
✉ lineng8657@ sina.com  
Lili He  
✉ llhe0423@163.com

<sup>†</sup>These authors have contributed equally to this work

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# Effects of biochar combined with nitrification inhibitors on NH<sub>3</sub> and N<sub>2</sub>O emission under different water conditions from vegetable soils

Zhenyuan He<sup>2,3†</sup>, Haohao LÜ<sup>1†</sup>, Yuying Wang<sup>1</sup>, Hangjie Yuan<sup>4</sup>, Yuxue Liu<sup>1</sup>, Neng Li<sup>2\*</sup> and Lili He<sup>1,5\*</sup>

<sup>1</sup>State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Institute of Environment Resource Soil and Fertilizer, Zhejiang Academy of Agricultural Sciences, Hangzhou, China, <sup>2</sup>Bamboo Home Engineering Technology Research Center of National Forestry and Grassland Administration, China National Bamboo Research Center, Hangzhou, China, <sup>3</sup>School of Life Science, Henan University of Science and Technology, Xinxiang, China, <sup>4</sup>Agricultural Technology Extension Center of Hangzhou, Hangzhou, China, <sup>5</sup>Anhui Province Agricultural Waste Fertilizer Utilization and Cultivated Land Quality Improvement Engineering Research Center, Chuzhou, China

Soil nitrogen loss through NH<sub>3</sub> volatilization and N<sub>2</sub>O emissions is a crucial issue in soil ecosystems. In this study, we explored the effects of biochar and the nitrification inhibitor DMPP (dimethyl-phenyl-piperazinium, a nitrification inhibitor) in vegetable soils under 60 and 200% WHC (water holding capacity). Five treatments were set: CK (control), urea (N), urea + biochar (N + C), urea + nitrification inhibitor (N + DMPP), and urea + nitrification inhibitor + biochar (N + C + DMPP). Results found that biochar promoted soil nitrification and ammonia volatilization under both moisture conditions, with higher NH<sub>3</sub> rate accumulation at 200% WHC. DMPP maintained high NH<sub>4</sub><sup>+</sup>-N concentration and increased soil ammonia volatilization, but effectively reduced N<sub>2</sub>O emissions, especially at 200% WHC. The N + C + DMPP treatment further significantly decreased N<sub>2</sub>O cumulative emissions compared to N + DMPP. QPCR results showed that N + C treatment significantly increased AOB (ammonia-oxidizing bacteria) copies compared to N treatment. Applying DMPP alone or with biochar reduced AOB copies by 50.0 and 45.7%, respectively. Soil ammonia-oxidizing archaea (AOA) responded oppositely to DMPP; AOA amounts in N + DMPP and N + C + DMPP treatments increased significantly during the culture. At 60% WHC, the greenhouse effect potential of N + DMPP and N + C + DMPP treatments were 39.0 and 43.2% lower than N, respectively. At 200% WHC, their GWP were decreased by 13.8 and 0.08% compared to N. Adding biochar alone increased the soil's greenhouse potential at both water contents. In conclusion, using nitrification inhibitors alone or in combination with biochar is more effective in reducing the greenhouse effect potential of soil active nitrogen emissions.

## KEYWORDS

biochar, nitrification inhibitor, water contents, active nitrogen gases, *amoA*

# 1 Introduction

Ensuring food security and addressing climate change and environmental degradation are the three major challenges facing countries around the world today. With the large-scale production and application of chemical nitrogen fertilizer, global food production has increased by nearly 150% in the past 50 years, and the average nitrogen (N) fertilizer use has reached  $180 \text{ kg hm}^{-2}$ , 75% higher than the world average (Tilman et al., 2002). At the same time, a large amount of greenhouse gases and reactive nitrogen loss emitted during field food production have promoted the process of global climate warming and environmental deterioration.  $\text{N}_2\text{O}$  emissions are among the most serious environmental consequences of nitrogen fertilizer loss (Wang et al., 2024). Although it exists in trace amounts, its contribution rate to the greenhouse effect reaches 5%, and it has a strong warming potential, which is one of the important greenhouse gases leading to global warming. Studies have shown that about 25–40% of the urea N applied to soil is converted into  $\text{NH}_3$  and volatilized into the atmosphere (Bi et al., 2023).  $\text{NH}_3$  will lead to the acidification of soil and surface water and is the main contributor of haze or gray haze in the air. The control of atmospheric active nitrogen pollutants is an inevitable choice to solve the sustainable development of China's agricultural system, improve the utilization rate of nitrogen fertilizer, and realize the win-win economic and ecological benefits of farmland fertilization.

As a kind of solid material with high carbon content, biochar will interfere with soil N behavior due to its unique properties and interaction with soil components after application (Lehmann et al., 2021; Liu et al., 2024). Due to its well-developed pore structure, large specific surface area, cation exchange capacity and other characteristics, biochar can absorb  $\text{NH}_3$  after input into the soil, and enhance the retention of ammonium ions ( $\text{NH}_4^+$ ) by improving the cation substitution capacity of the soil, and also improve the nitrification rate of the soil, thus affecting the N loss and  $\text{N}_2\text{O}$  emission of the soil (Zhang et al., 2025). Some scholars have found that when the biochar application rate exceeds  $10 \text{ t ha}^{-1}$ , the cost of ecological services like  $\text{N}_2\text{O}$  emission reduction, nitrate leaching reduction, and carbon sequestration is lower than the input cost of biochar (Smith et al., 2019). In order to achieve the best agronomy, environment and economic benefits, it is necessary to prepare lower-cost biochar or to apply high-efficiency biochar mixtures, such as coupled with N fertilizer synergists. Nitrification inhibitors (NIs), such as 2-chloro-6-trichloromethyl pyridine (nitrapyrin, NP), dicyandiamide (DCD), and 3, 4-dimethylpyrazole phosphate (DMPP), are cost-effective N fertilizer synergists that can inhibit the nitrogen nitrification process (Liu et al., 2025). NI can inhibit the emission of  $\text{N}_2\text{O}$  and NO into the atmosphere by nitrification to varying degrees (Bachtsevani et al., 2021). Reports found that there is a strong correlation between  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emission in farmland soil (Martins et al., 2017), and Ma et al. (2020) also found that the total amount of  $\text{NH}_3$  volatilization in soil was significantly negatively correlated with the total amount of  $\text{N}_2\text{O}$  emission ( $r = -0.859$ ,  $p < 0.01$ ). Since NI inhibits nitrification by extending the retention time of ammonium N in soil to varying degrees, it may increase the risk of  $\text{NH}_3$  volatilization emission. Biochar has a large specific surface area, has a good adsorption effect on ammonium N, but it is difficult to inhibit the negatively charged ions  $\text{NO}_2^-$  and  $\text{NO}_3^-$  (Lawrinenko and Laird, 2015). Whether the combined application of the two can play a positive interaction and the related mechanism need to be studied.

Soil water status is an extremely important factor affecting  $\text{N}_2\text{O}$  emission, and it can regulate the effective utilization rate of  $\text{O}_2$ , thus affecting the nitrification and denitrification processes in soil (Liu et al., 2022). It was reported that the  $\text{N}_2\text{O}$  emission of soil decreased significantly under 100% WFPS and 125% WFPS (Wang et al., 2023). Soil moisture state also affects ammonia volatilization loss. Yang et al. (2019) found that the increase of soil water content may increase ammonia volatilization loss through accelerating urea hydrolysis and promoting microbial metabolism. However, the effects of biochar combined with nitrification inhibitors on soil  $\text{N}_2\text{O}$  emission and ammonia volatilization loss under different water content and the mechanism of interaction with related microorganisms are still lacking. The objective of this study was to clarify the specific effects of biochar and DMPP alone and in combination on  $\text{NH}_3$  volatilization and  $\text{N}_2\text{O}$  emissions in soil, and determine whether the use of biochar and DMPP can help reduce greenhouse gas emissions and mitigate the greenhouse effect under different moisture conditions and probe into the relevant microbiological mechanisms through indoor control simulation experiment (constant temperature and light conditions).

## 2 Materials and methods

### 2.1 Materials

The soil used for testing was collected from Xucun Town, Haining City, Zhejiang Province ( $30^\circ 31' \text{N}$ ,  $120^\circ 19' \text{E}$ ). The topsoil (0–20 cm) was collected in April 2021, and residual gravel, crop roots, and debris in the fresh soil were removed. After natural air drying, the soil was screened using 10 meshes. The raw material of biochar is made from rice straw, which is pyrolyzed with a heating rate of  $5^\circ\text{C min}^{-1}$  and final temperature of  $500^\circ\text{C}$  in carbonization furnace for 3 h. The basic physical and chemical properties are as follows: total carbon  $610 \text{ g kg}^{-1}$ , total nitrogen  $14.3 \text{ g kg}^{-1}$ , soil cation exchange capacity (CEC)  $18.9 \text{ cmol kg}^{-1}$ , pH 9.20. The nitrogen fertilizer was urea, which was produced by Sinopharm Group Co., Ltd., with 46% nitrogen content. Nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) was selected for analytical purity and was produced by Macklin Biotechnology Company.

### 2.2 Methods

The soil  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emissions were studied using aerobic incubation experiments. Biochar, DMPP, or a combination of biochar and DMPP was added to urea, and two water conditions were set: 60% WHC (maximum field water capacity) and 200% WHC. Five treatments for each water condition: CK (control), urea (N), urea + biochar (N + C), urea + nitrification inhibitor (N + DMPP), urea + nitrification inhibitor + biochar (N + C + DMPP), each treatment was set up with three replicates, where the urea addition amount was  $0.3 \text{ g N kg}^{-1}$ , and the biochar addition amount was 2% of the soil mass; biochar was passed through a 1 mm sieve prior to the incubation experiment. The amount of DMPP added was recognized as the optimal dosage of 1% of the amount of urea nitrogen. Fresh soil equivalent to 50-g air-dried soil and the corresponding biochar rate were placed in 500 mL conical flasks and shaken thoroughly to ensure the mixtures uniform and then incubated at  $30^\circ\text{C}$  in the dark for 42 days after 7-day pre-incubation (Duan et al., 2017). The flasks were covered with a polyethylene film

and then punctured with small holes to maintain aerobic conditions. Deionized water was added every 2–3 days to compensate for water loss at least 1 day before sampling to avoid gas emission.

### 2.3 Collection and determination of N<sub>2</sub>O emission, NH<sub>3</sub> emission and soil DNA

N<sub>2</sub>O gas was collected at 1, 2, 5, 7, 9, 11, 14, 17, 21, 23, 27, 29, 37, and 42-day intervals using a gas-tight syringe. Prior to gas sampling, the headspace air within the flasks was meticulously purged with ambient air for 15 min at a flow rate of 200 mL per minute. Immediately afterward, the flasks were sealed with silicone rubber stoppers (NQ-704 silicone adhesive sealant) equipped with butyl rubber septa and then incubated for 5 h. For each measurement, gas samples were extracted from the flasks via a three-way stopcock using a 25-mL air-tight syringe. Following sample withdrawal, the flasks were purged with ambient air and left open (He et al., 2016). The concentration of the N<sub>2</sub>O was immediately determined by a gas chromatograph (Agilent 7890) and detected by ECD. The carrier gas employed was a 5% argon-methane mixture, flowing at a rate of 40 mL per minute, and the column temperature was maintained at 40°C. Compressed air was utilized as the standard gas, with a designated value of 313 ppbv. The concentrations of N<sub>2</sub>O were determined by comparing the peak areas of the samples with those of the reference gases supplied by Hangzhou Jingong Special Gas Factory. (calculated by Equations (1) and (2)).

NH<sub>3</sub> gas was collected at 1, 2, 3, 4, 6, 8, 10, and 14-day intervals. The NH<sub>3</sub> flux was measured by a Dräger flow-through chamber technique (Kelsch et al., 2024). Before gas sampling, the flasks were removed from the incubator and uncovered to conduct gas exchange between the inside and outside of the incubation flasks for 30 min (the NH<sub>3</sub> concentration in the headspace reached equilibrium with the ambient air during this period via pre-incubation). The flasks were then sealed with silicone rubber stoppers with one inlet hole equipped with an air check valve to avoid pressure changes, which could affect the NH<sub>3</sub> flux measurements, and an outlet hole equipped with a three-way valve connected to a Dräger tube. The flasks were then immediately capped and incubated for 24 h. The NH<sub>3</sub> concentration of each sample was measured using a Dräger-Tube Sensor (Drägerwerk AG, Lübeck, Germany), which contained a solid-phase acid compound and bromophenol blue pH indicator and was calibrated by the manufacturer. A Dräger tube was inserted into the corresponding Dräger Gas Detector hand pump (semicontinuous suction characteristics). Ambient air was sucked into 100-mL through flasks using a hand pump. The NH<sub>3</sub> concentration was immediately displayed as the total length of the color change. (calculated by Equations (3) and (4)).

Subsamples were destructively sampled at 0, 7, 14, 21, 28, 35, and 42-day intervals, respectively, which were treated with 2 mol L<sup>-1</sup> KCl solution to extract NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> and measure soil pH.

The subsamples were destructively sampled at the 1, 7, and 42-day intervals for and DNA extraction which were immediately stored at 20°C. Total soil genomic DNA was extracted from each soil sample using a PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, United States), following the manufacturer's instructions. Subsequently, the quantification of functional genes (AOA, AOB, and Urec) was performed by real-time quantitative PCR to obtain a view of the specific enzymes for NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> production and emission (Applied Biosystems, Foster City, CA). A standard curve was constructed using plasmid DNA from one representative clone containing each target gene.

### 2.4 Determination items and methods

1. The calculation formula for calculating the soil N<sub>2</sub>O emission rate was as follows:

$$F = \rho \times \Delta C / \Delta t \times 273 / (273 + T) \times V / m \quad (1)$$

where  $F$  is the N<sub>2</sub>O emission rate ( $\mu\text{g N kg}^{-1} \text{ h}^{-1}$ );  $\Delta C/\Delta t$  is the rate of N<sub>2</sub>O concentration in the culture device ( $10^9 \text{ N}_2\text{O-N h}^{-1}$ );  $V$  is the volume of the culture flask ( $\text{m}^3$ );  $T$  stands for culture temperature ( $^{\circ}\text{C}$ );  $m$  is the dry base weight (kg) of the cultured soil.

Cumulative N<sub>2</sub>O emissions from soil are calculated by the following formula:

$$E = \sum_{i+1}^i (f_i + f_{i+1}) / 2 \times (t_i + t_{i+1}) \quad (2)$$

where  $E$  is the cumulative emission of N<sub>2</sub>O ( $\mu\text{g N kg}^{-1}$ );  $f_i$  and  $f_{i+1}$  are the rate of N<sub>2</sub>O emission during culture time  $t_i$  and  $t_{i+1}$ .

2. The calculation formula for calculating the ammonia volatilization rate is as follows:

$$F_N = C \times \frac{M}{V_m} \times \frac{273}{273 + T} \times \frac{V}{m} \times \frac{1}{t} \times k \quad (3)$$

where  $F_N$  is the ammonia volatilization rate ( $\text{mg N kg}^{-1} \text{ day}^{-1}$ );  $C$  is the concentration determined by ammonia detection tube ( $\text{mg L}^{-1}$ );  $M$  is the molar mass of N ( $\text{g mol}^{-1}$ );  $V_m$  is the molar volume of the gas, which is  $22.4 \text{ L mol}^{-1}$  under standard conditions;  $T$  is the culture temperature ( $^{\circ}\text{C}$ );  $V$  is the volume of gas in the culture device ( $\text{mL}$ );  $m$  is soil mass ( $\text{g}$ );  $t$  is time ( $\text{day}$ );  $k$  is the conversion factor, which is the ratio of unit mass to unit volume.

$$E_N (\text{mg N kg}^{-1}) = \int_{i-1}^i F_{\text{NH}_3} \quad (4)$$

Where  $E_N$  indicates the cumulative amount of ammonia volatilization ( $\text{mg N kg}^{-1}$ );  $i$  represents the days of culture ( $\text{day}$ );  $F_{\text{NH}_3}$  stands for ammonia volatilization rate ( $\text{mg N kg}^{-1} \text{ day}^{-1}$ ).

3. Potential greenhouse effect of active soil nitrogen emissions.

$$(\text{GWP}) = \text{direct GWP} (\text{CO}_2 - \text{eq mg kg}^{-1}) + \text{indirect GWP} (\text{CO}_2 - \text{eq mg kg}^{-1}) \quad (5)$$

Among them, the indirect N<sub>2</sub>O emission is calculated as follows: indirect N<sub>2</sub>O = EF × NH<sub>3</sub>-N, EF is the N<sub>2</sub>O emission coefficient of NH<sub>3</sub> volatilization loss, and its value is  $0.01 \text{ mg N}_2\text{O-N mg}^{-1} \text{ N}$ . According to the IPCC (2013) report, approximately 1% (0.2%–5%) of the emitted

$\text{NH}_3\text{-N}$  is deposited on land and converted into  $\text{N}_2\text{O}$ , which is used to estimate the GWP. Its calculation formula is as follows (calculated by Equations (5) and (6)):

$$\text{GWP} \left( \text{CO}_2 - \text{eq mg kg}^{-1} \right) = 298 \times \text{N}_2\text{O} \left( \text{mg N kg}^{-1} \right) \div 28 \times 44 \quad (6)$$

where  $\text{N}_2\text{O-N}$  ( $\text{mg kg}^{-1}$ ) is the cumulative emission of  $\text{N}_2\text{O-N}$  during the culture period.

## 2.5 Data analysis and processing

OriginPro 2019 mapping software and IBM SPASS 22.0, were used to carry out univariate analysis of soil  $\text{NH}_3$  cumulative emissions,  $\text{N}_2\text{O}$  emissions, and soil physicochemical properties in each fertilization period between the treatments. One-way analysis of variance (ANOVA) was used to check for quantitative differences between treatments. Different cases and English letters indicate significant differences among the treatments ( $p < 0.05$ ).

## 3 Results and discussion

### 3.1 Effects of biochar and DMPP on inorganic nitrogen conversion under different water conditions

#### 3.1.1 $\text{NH}_4^+\text{-N}$ content

Figure 1 shows the changes in  $\text{NH}_4^+\text{-N}$  concentration during the incubation period. Urea which applied to the soil decomposes under the action of soil urease. In general, the concentration of  $\text{NH}_4^+\text{-N}$  in the soil showed the similar trend. The peak value of  $\text{NH}_4^+\text{-N}$  in soil under 60% WHC occurred on the 7th day, which was  $333.14 \text{ mg kg}^{-1}$  under N + C + DMPP treatment, and the lowest  $\text{NH}_4^+\text{-N}$  concentration reached  $268.30 \text{ mg kg}^{-1}$  under N + C treatment except CK. Under 200% WHC, the  $\text{NH}_4^+\text{-N}$  concentration of all soil treatments peaked at the 14th day. The highest  $\text{NH}_4^+\text{-N}$  concentration appeared in N + C + DMPP treatment, reaching  $286.71 \text{ mg kg}^{-1}$ , and the lowest appeared in N + C treatment, which was  $95.57 \text{ mg kg}^{-1}$ . Compared with the N treatment, the N + C treatment decreased the  $\text{NH}_4^+\text{-N}$  content, and the application of DMPP significantly increased the  $\text{NH}_4^+\text{-N}$  concentration.

#### 3.1.2 $\text{NO}_3^-\text{-N}$ content

The change in the  $\text{NO}_3^-\text{-N}$  concentration during the incubation period is shown in Figure 1. The figure shows that the concentration of  $\text{NO}_3^-\text{-N}$  increased with time. Under 60% WHC, the  $\text{NO}_3^-\text{-N}$  concentration treated with N and N + C increased rapidly from 7 to 21 days after adding the nitrogen source and then returned to stability, which was significantly higher than that under DMPP treatment. From day 14 to 21, the  $\text{NO}_3^-\text{-N}$  content of each DMPP treatment increased rapidly. The lowest  $\text{NO}_3^-\text{-N}$  concentration in the N + DMPP treatment (except CK) was  $170.18 \text{ mg kg}^{-1}$ . From day 28 to 42, the  $\text{NO}_3^-\text{-N}$  concentration of each treatment remained in the range of  $164.83$  to  $231.8 \text{ mg kg}^{-1}$ . Under 200% WHC, the  $\text{NO}_3^-\text{-N}$  content at 0–14 days in the N + C and N treatments increased rapidly, and the rate of increase with biochar addition was slightly lower. The increase rate of the DMPP treatment was relatively slow and stabilized in the range of

$98.658\text{--}130.956 \text{ mg kg}^{-1}$  after 35 days. The application of DMPP alone or in combination with biochar can effectively slow nitrification, and the inhibitory effect is more significant at 200% WHC.

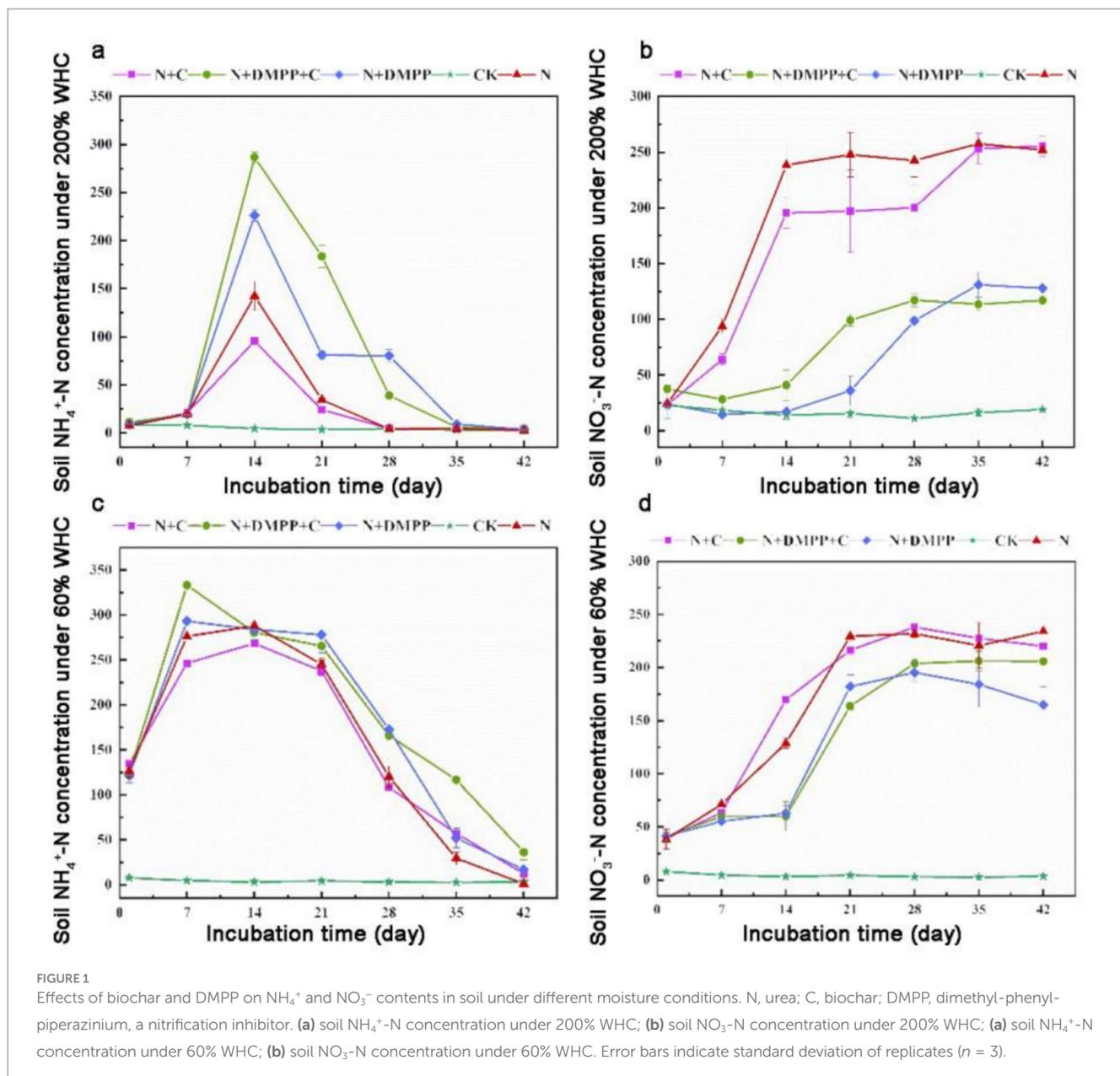
The primary factors influencing soil nitrification encompass soil moisture, aeration conditions, the *amoA* gene abundance of AOA and AOB, soil pH, and the content of soil organic matter (Ren et al., 2023). In this study, we found that the N + C treatment could reduce the  $\text{NH}_4^+\text{-N}$  concentration in the soil under 60% WHC culture, increase the nitrification rate constant, indicating that the addition of straw biochar could improve the nitrification of the soil. Biochar effectively improved the soil aeration status, which was conducive to the accelerated growth and reproduction of AOB, and increased their activity, which was conducive to nitrification (Hu et al., 2024). Biochar could increase acid soil pH value and weak the decrease in soil pH, which can directly or indirectly affect the nitrification of soil (Aurangzeib et al., 2024).

### 3.2 Changes in AOA and AOB gene abundance of biochar and DMPP under different water content

As shown in Figure 2, under 60% WHC conditions, there were significant differences in the copy number of *amoA* gene in AOB between the treatments. Compared to the N treatment, the copy number of *amoA* gene in AOB significantly increased in the N + C treatment on day 1 and 7. The N + DMPP and N + C + DMPP treatments had a significant inhibitory effect on the number of AOB, especially on day 1, when the inhibition rates were as high as 50.0 and 45.7%, respectively, compared with the N treatment. The number of AOB treated with N + DMPP on day 1 and 42 was not different from that in the CK treatment. The response of AOA to DMPP was opposite to that of AOB, and the amount of AOA under N + DMPP and N + C + DMPP treatments increased significantly during the entire culture period, especially on the 42 days.

Under 200% WHC conditions, compared with the N treatment, the N + C treatment increased *amoA* gene copy number of AOB by 51.53%, especially on the 7th day. N + DMPP inhibited the growth and reproduction of AOB. The response of soil AOA to nitrification inhibitors differs from that of AOB. During the entire incubation period, the growth and reproduction of AOA treated with DMPP were significantly promoted.

N and nitrification inhibitors play crucial roles in the soil ecosystem and have complex and significant impacts on the gene abundances AOA and AOB. It has been found that with the increase in the application rate of nitrogen fertilizers, the gene abundance of AOB shows an upward trend in the short term, which is because AOB has a strong affinity for higher-concentration  $\text{NH}_4^+\text{-N}$ , and can rapidly utilize these additional nitrogen sources for metabolism and proliferation (Ren et al., 2023). While, AOA often has stronger adaptability in acidic environments (Wu et al., 2020). DMPP can interfere with the key metabolic pathways of AOB, hinders their utilization of ammonium nitrogen, and thus affects their growth and reproduction. In contrast, due to the differences in metabolic pathways and ecological niches between AOA and AOB, AOA responds relatively weakly to DMPP (Liu et al., 2023). As a carbon-rich solid material, biochar, after entering the soil, exerts multi-dimensional impacts on the gene abundances of AOA and AOB by virtue of its unique physical and chemical properties. As a carbon-rich solid material, biochar, after entering the soil, exerts multi-dimensional impacts on the gene abundances of AOA and AOB by virtue of its unique physical and



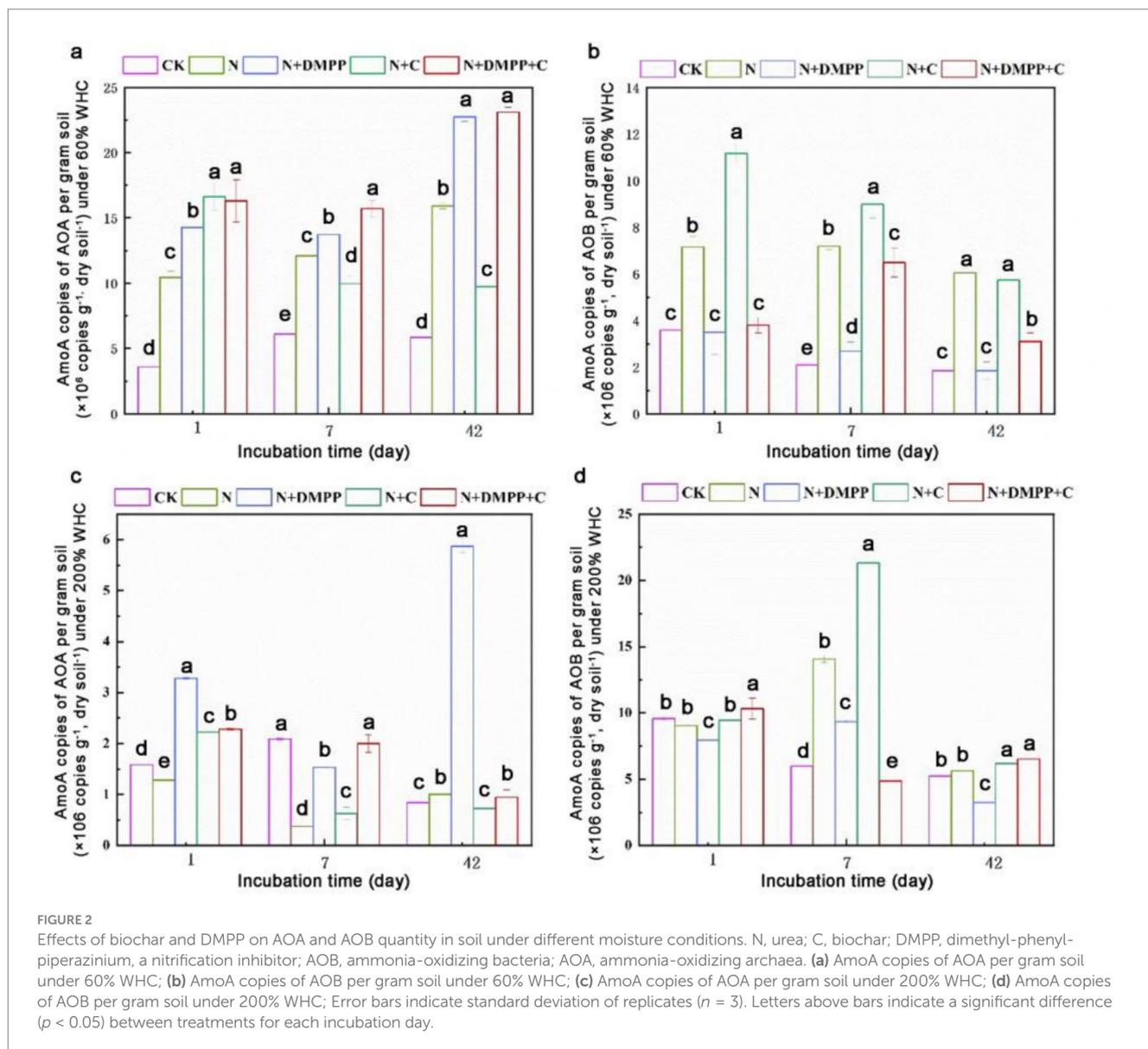
chemical properties. Biochar could improve soil aeration and WHC, which are conducive to the growth of aerobic AOB (Fang et al., 2021); while, AOA usually has certain advantages in acidic environments. Alkaline biochar may inhibit the growth of some AOA. Biochars prepared from different raw materials, as well as different soil types and environmental conditions, will all lead to differences in the influencing results, which still await further in-depth research.

### 3.3 Effects of biochar and DMPP on soil $\text{NH}_3$ emission rate and cumulative emission under different moisture

The  $\text{NH}_3$  emission rates under the two WHCS after the application of nitrogen sources are shown in Figure 3. The variation trend of the  $\text{NH}_3$  emission was essentially the same, showing an increase to the peak value and then a gradual decrease until no  $\text{NH}_3$  was detected.

Under 60% WHC water conditions, after adding N fertilizer for 3–5 days, the  $\text{NH}_3$  rate reached the peak, among which  $17.93 \text{ mg N kg}^{-1}$  in N treatment, and  $12.67 \text{ mg N kg}^{-1}$  in N + C treatment. The peak emission rates of N + DMPP and N + DMPP + C treatments were  $20.3$  and  $20.41 \text{ mg N kg}^{-1}$ , respectively. Under 200% WHC water condition, all treatments reached the peak on the 4th day, among which the peak value was  $24.3, 25.2, 27.17, 29.5 \text{ mg N kg}^{-1}$  in treatment of N, N + C, N + DMPP, N + DMPP + C, respectively.

As can be seen from Figure 3, the addition of biochar, DMPP, or their combination had a significant effect on the cumulative loss of soil ammonia. Under 60% WHC water conditions, no ammonia was detected in the CK group (without N source added),  $\text{NH}_3$  loss was significantly increased under biochar and DMPP treatments. The maximum  $\text{NH}_3$  emissions appeared in the N + DMPP + C treatment which was  $86 \text{ mg N kg}^{-1}$  and N + DMPP treatment, which was  $85.77 \text{ mg N kg}^{-1}$ . Compared with N treatment,  $\text{NH}_3$  loss in N + C was increased by 17.51%, which was  $49.67 \text{ mg N kg}^{-1}$ . When the water



condition was 200% WHC, biochar and DMPP promoted the cumulative loss of ammonia under flooded conditions.

The above results show that the addition of biochar, a nitrification inhibitor, or their combination increased the cumulative ammonia volatilization in soil under the two moisture conditions, and the effect of DMPP on increasing ammonia volatilization was more significant under 60% WHC conditions. The cumulative volatilization of ammonia at 200% WHC was generally higher than that at 60% WHC, and the nitrification inhibitor DMPP alone tended to promote ammonia volatilization in different land-use types (He et al., 2018). The direct addition of biochar to soil inevitably affects the nitrogen cycle. The results of this study showed that biochar addition increased the cumulative  $\text{NH}_3$  volatilization under the two water conditions, which is consistent with the results of the study of Zhao et al. (2018), who found that total ammonia volatilization increased by approximately 102% after 10 seasons of biochar addition in a pot experiment of wheat/millet rotation. Previous studies have found that biochar has a large specific surface area and abundant oxygen-containing functional groups, which has a good adsorption effect on  $\text{NH}_3$  in dryland soil and can reduce the loss of soil ammonia volatilization (Zheng et al.,

2018). The main reason that short-term biochar input significantly increased soil  $\text{NH}_3$  emissions may be that biochar can significantly increase soil pH and improve soil permeability (Castaldi et al., 2011).

DMPP increases ammonia volatilization which delays the nitrification reaction of  $\text{NH}_4^+$ -N, resulting in a higher concentration of  $\text{NH}_4^+$ -N in soil and surface water, thereby promoting ammonia loss (Liu et al., 2025). In this study, the cumulative volatilization of ammonia in the soil was increased by adding DMPP or combining with biochar under two WHCs condition, and the cumulative  $\text{NH}_3$  loss of the N + C + DMPP treatment was found to be higher than that of the biochar and DMPP treatments alone.

### 3.4 Effects of biochar and DMPP on soil $\text{N}_2\text{O}$ emission rate and cumulative emission under different moisture conditions

As can be seen from Figure 4, under 60% WHC condition, peak  $\text{N}_2\text{O}$  emission occurred in N + C treatment on the 5th day, which

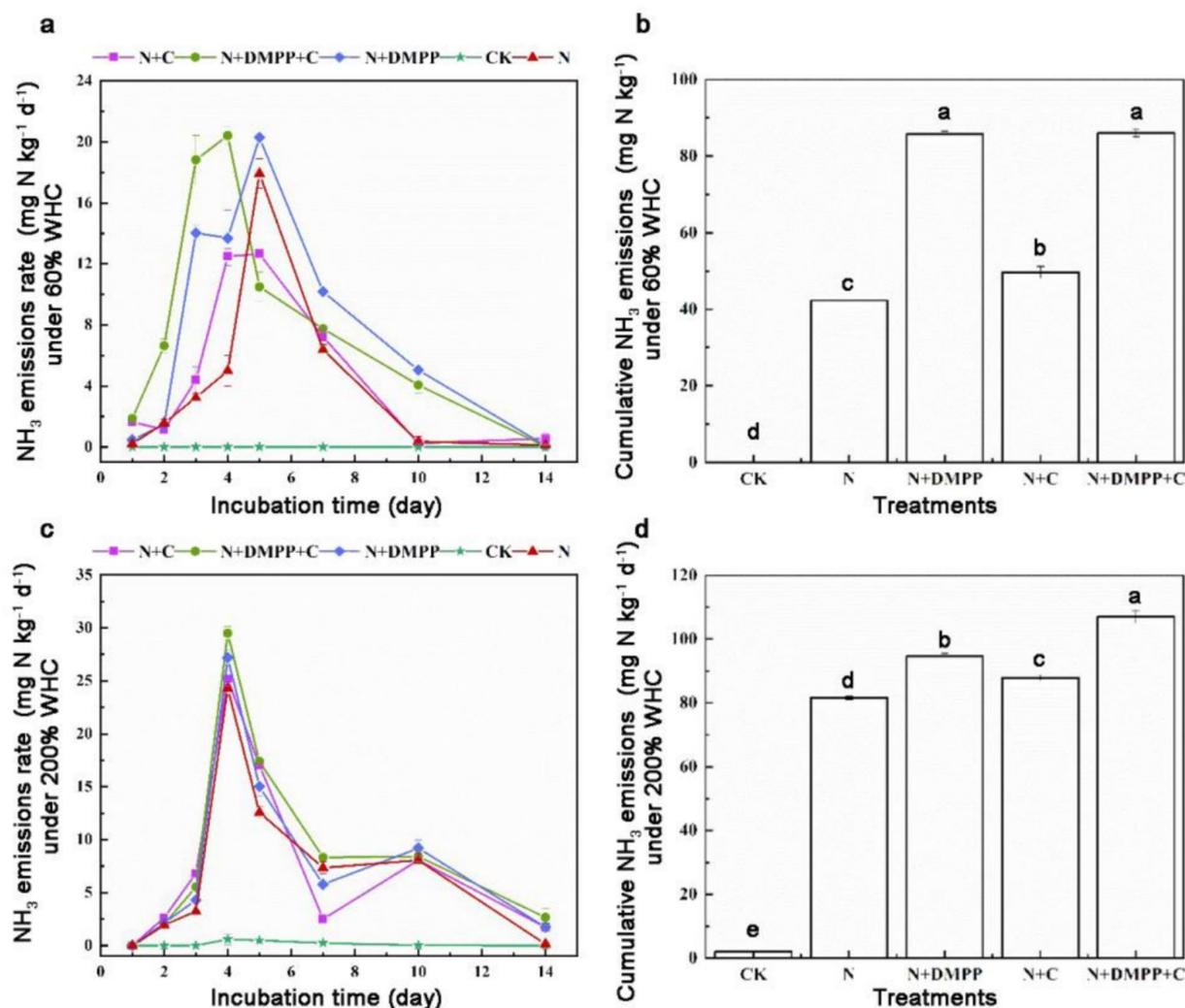


FIGURE 3

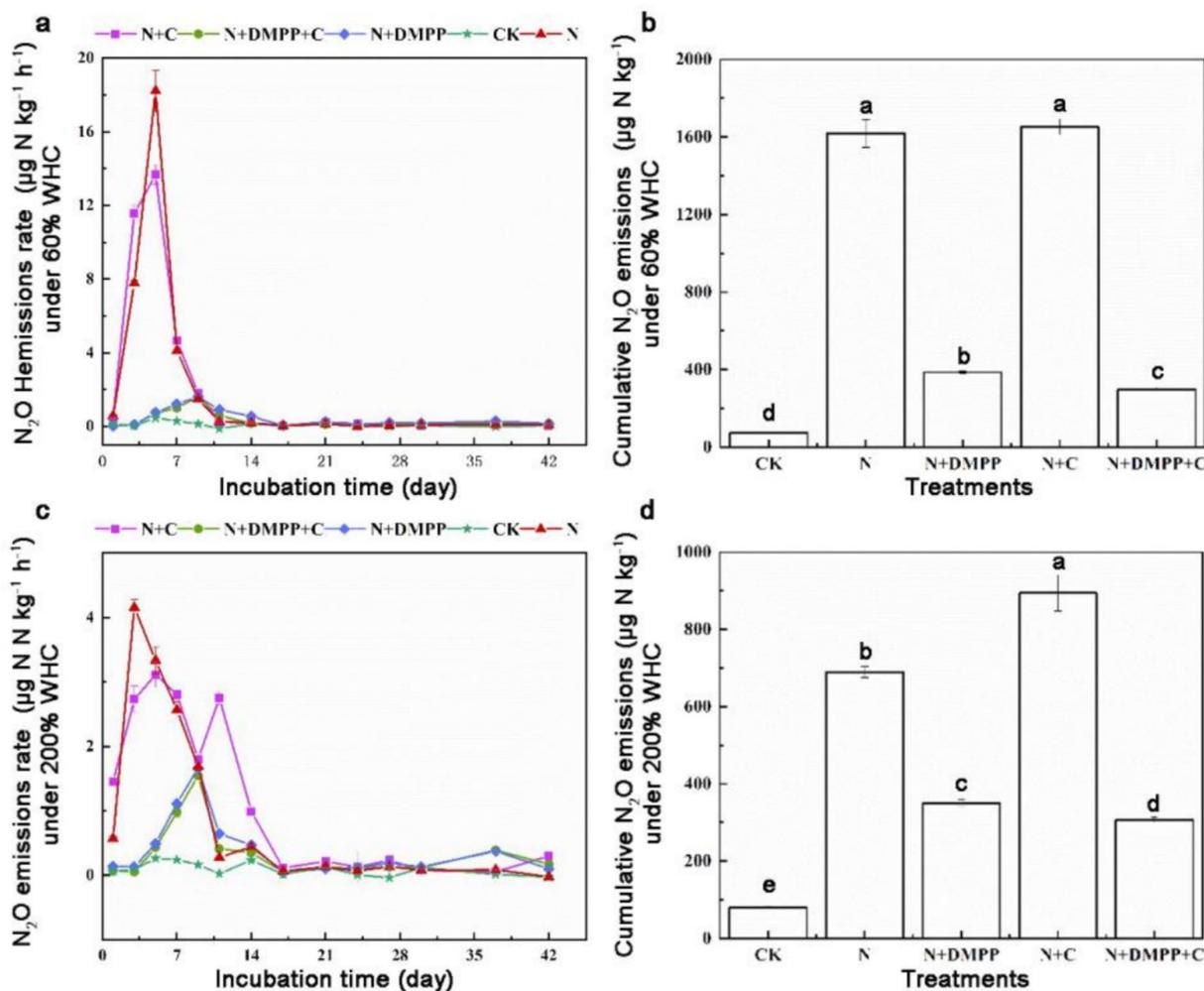
Effects of single or combined application of biochar and DMPP on  $\text{NH}_3$  emission rate and cumulative emission in soil under different moisture conditions. N, urea; C, biochar; DMPP, dimethyl-phenyl-piperazinium, a nitrification inhibitor. (a)  $\text{NH}_3$  emissions rate under 60% WHC; (b) cumulative  $\text{NH}_3$  emissions under 60% WHC; (c)  $\text{NH}_3$  emissions rate under 200% WHC; (d) cumulative  $\text{NH}_3$  emissions under 200% WHC. Error bars indicate standard deviation of replicates ( $n = 3$ ). Letters above bars indicate a significant difference ( $p < 0.05$ ) between treatments.

reached to  $18.22 \mu\text{g kg}^{-1}$ . The emission peaks of N + DMPP + C and N + DMPP treatments appeared on the 9th day, only  $1.70$  and  $1.60 \mu\text{g kg}^{-1}$ , respectively. DMPP, alone or in combination with biochar, can delay the peak time of  $\text{N}_2\text{O}$  and reduce emission rate. Under 200% WHC condition, the cumulative emissions of  $\text{N}_2\text{O}$  in all treatments ranged from  $0.23$  to  $4.16 \mu\text{g kg}^{-1}$  (Figure 4). The peaks of  $\text{N}_2\text{O}$  emissions in N + C, N + DMPP and N + DMPP + C treatments were  $3.11$ ,  $1.67$ , and  $1.54 \mu\text{g kg}^{-1}$ , respectively, which were reduced by 25.2, 59.9, and 63%, respectively, compared with that in N treatment.

Under 60% WHC, the cumulative emissions of  $\text{N}_2\text{O}$  were  $1617.27 \mu\text{g kg}^{-1}$  under N treatment and  $1651.39 \mu\text{g kg}^{-1}$  under N + C treatment. DMPP alone and combination with BC significantly reduced cumulative emissions of  $\text{N}_2\text{O}$  by 76.1 and 81.5% compared with N treatment, and the mixed application of biochar had a better effect on soil reduction. Under 200% WHC condition,  $\text{N}_2\text{O}$  cumulative emissions under N treatment were  $689.40 \mu\text{g kg}^{-1}$ , and those were

$893.9 \mu\text{g kg}^{-1}$  under N + C treatment,  $350.16 \mu\text{g kg}^{-1}$  under N + DMPP treatment and  $307.06 \mu\text{g kg}^{-1}$  under N + DMPP + C treatment, which were decreased by 49.2 and 55.5% when compared with N treatment, respectively.

The results showed that under 200% WHC, the N and N + C treatments decreased the accumulation of  $\text{N}_2\text{O}$  by 57.4 and 45.9%, respectively, when compared with the 60% WHC treatment. An increase in water content could reduce the accumulation of  $\text{N}_2\text{O}$  under conventional fertilization and biochar treatments. DMPP showed a better effect in reducing  $\text{N}_2\text{O}$  emissions under both water conditions, and the effect of DMPP combined with biochar was better. As one of the most important greenhouse gases,  $\text{N}_2\text{O}$  has a significant impact on global climate and environmental change. The  $\text{N}_2\text{O}$  emission from farmland soils are generally produced through nitrification and denitrification by nitrifying microorganisms (Wu et al., 2024). In this experiment, the conversion process of  $\text{NH}_4^+$  in soil under the 60% WHC conditions



**FIGURE 4**  
Effects of single or combined application of biochar and DMPP on N<sub>2</sub>O emission rate and cumulative emission in soil under different moisture conditions. N, urea; C, biochar; DMPP, dimethyl-phenyl-piperazinium, a nitrification inhibitor. (a) N<sub>2</sub>O emissions rate under 60% WHC; (b) cumulative N<sub>2</sub>O emissions under 60% WHC; (c) N<sub>2</sub>O emissions rate under 200% WHC; (d) cumulative N<sub>2</sub>O emissions under 200% WHC. Error bars indicate standard deviation of replicates (*n* = 3). Letters above bars indicate a significant difference (*p* < 0.05) between treatments.

was carried out in aerobic culture, so the emission of N<sub>2</sub>O mainly came from the nitrification process of soil; under 200% WHC conditions, due to anaerobic waterlogging, denitrification was the main way to produce N<sub>2</sub>O under anaerobic or anoxic conditions. When the water content was low, biochar promoted the nitrification process of the soil, whereas when the water content was high, it led to the deterioration of soil ventilation and promoted denitrification. However, studies have also shown that when the water content is high, the anaerobic environment is conducive to the growth and reproduction of denitrifying microorganisms, promoting the denitrification of soil, increasing the emission of N<sub>2</sub>O. Alternatively, the higher ash content of biochar leads to an increase in soil N<sub>2</sub>O emissions.

The monitoring data in the wheat-maize rotation system in calcareous soil showed that the nitrification inhibitor DMPP can also effectively reduce soil N<sub>2</sub>O emissions, which is the same trend as the conclusion of this study (Zhao et al., 2017). Under the two water conditions, compared with the single application

of DMPP, the cumulative emissions of N<sub>2</sub>O under the combined application of biochar and DMPP were further reduced to a significant level, indicating that DMPP could weaken the excitation effect of biochar addition on the nitrification process, thus further contributing to emission reduction. Some studies have also shown that the combined application of biochar and DMPP has N<sub>2</sub>O effect emissions in vegetable ecosystems, which may be related to the properties of the biochar, soil type, N<sub>2</sub>O, pH, moisture, texture, organic matter content, and other factors (Li et al., 2023).

### 3.5 Effects of biochar and DMPP on the greenhouse potential of N<sub>2</sub>O under different moisture conditions

It can be seen from Figure 5 that under 60% WHC water condition, the greenhouse potential (GWP) of N + C treatment

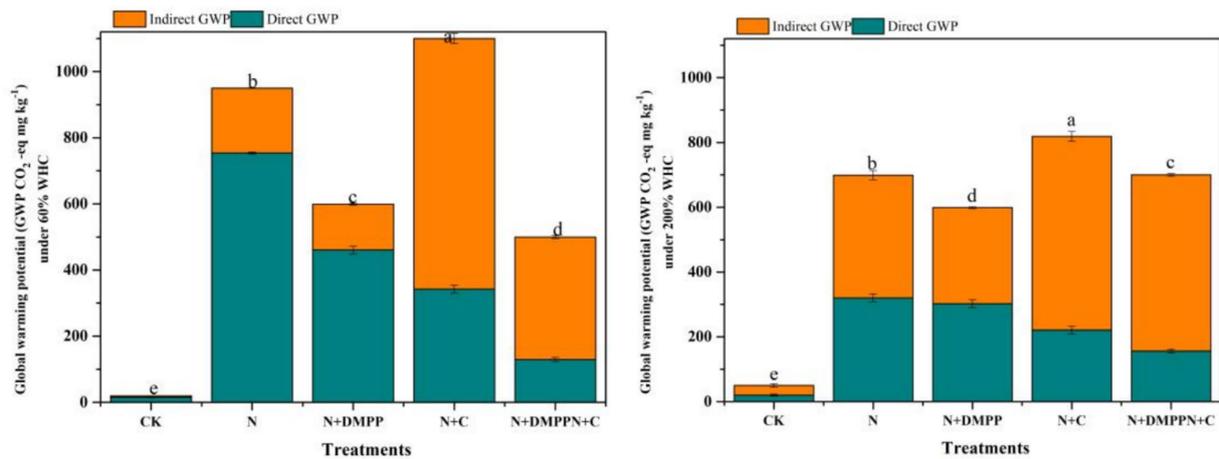


FIGURE 5

Effects of biochar and DMPP alone or in combination on global warming potentials (GWP). N, urea; C, biochar; DMPP, dimethyl-phenyl-piperazinium, a nitrification inhibitor. Error bars indicate standard deviation of replicates ( $n = 3$ ). Letters above bars indicate a significant difference ( $p < 0.05$ ) between treatments.

is 5.3% higher than that of N treatment. The GWP of the N + DMPP + C and N + DMPP treatments were significantly lower than that of the N treatment, with reduction rates of 43.2 and 39.0%, respectively. For the N, N + C, N + DMPP, and N + DMPP + C treatments, the contribution rates of direct N<sub>2</sub>O emissions to GWP were 79.3, 76.9, 31.1, and 25.8%, respectively, and the contribution rates of indirect N<sub>2</sub>O emissions (NH<sub>3</sub> volatilization and resedimentation) to GWP were 20.7, 23.1, 70.0, and 74%, respectively. Direct N<sub>2</sub>O emissions from soil under the N and N + C treatments accounted for the main position of GWP, and indirect N<sub>2</sub>O emissions caused by NH<sub>3</sub> volatilization in the two treatments with DMPP were the main greenhouse effect potential.

Under 200% WHC, the GWP of the N + C treatment was still the highest, 17.8% higher than that of the N treatment. Compared with the N treatment, the GWP of the N + DMPP + C and N + DMPP treatments were significantly reduced by 8.5 and 13.8%, respectively. For the N, N + C, N + DMPP, and N + DMPP + C treatments, the contribution rates of N<sub>2</sub>O direct emissions to the GWP were 45.8, 50.4, 27.0, and 22.3%, respectively, and the contribution rates of NH<sub>3</sub> to the GWP were 54.3, 49.6, 73, and 77.7%, respectively. In conclusion, the indirect emission of N<sub>2</sub>O caused by NH<sub>3</sub> volatilization in soil with nitrification inhibitors occupied a dominant position in the greenhouse gas warming potential of the soil. The GWP of the N + DMPP and N + C + DMPP treatments were 13.8 and 8.5% lower than that of N. Du et al. (2019) showed that the contribution ratio of N<sub>2</sub>O to the GWP was only 5.25% on average. In this study, the direct N<sub>2</sub>O emission of soil under N and N + C treatment was 76.9–79.3% under 60% WHC, accounting for the main position of soil greenhouse gas warming potential. Approximately 1% (0.2–5%) of the discharged NH<sub>3</sub>-N is deposited on land and converted into N<sub>2</sub>O. When considering the indirect emission of N<sub>2</sub>O related to NH<sub>3</sub> volatilization in the current study, the contribution rates of NH<sub>3</sub> to GWP in the soils treated with N + DMPP and N + DMPP + C were 70.0 and 74%, respectively. The indirect emission of

N<sub>2</sub>O caused by ammonia volatilization occupies a dominant position in soil GHG warming potential. Many studies have shown that biochar application can effectively reduce GWP (Yan et al., 2020) in the first year, which differs from the conclusions of this study. The main reason for this is that the N + C treatment in this experiment promoted both N<sub>2</sub>O emission and ammonia volatilization, resulting in increased emissions of active nitrogen gas, thus increasing the greenhouse effect potential of direct and indirect emissions of N<sub>2</sub>O. Under 200% WHC conditions, N<sub>2</sub>O emissions from GWP treated with N, N + C and N<sub>2</sub>O indirect emission caused by ammonia volatilization occupied the same position, and N<sub>2</sub>O indirect emission caused by N + DMPP and N + DMPP + C treated with ammonia volatilization still occupied the dominant position of soil GWP. However, this study did not consider the warming potentials of the CO<sub>2</sub> and CH<sub>4</sub> gases. Future studies should comprehensively consider the greenhouse effect potential of active nitrogen gases, CO<sub>2</sub> and CH<sub>4</sub>, and the long-term combined application of biochar and inhibitors to reduce the warming potential of active nitrogen gases in farmland.

## 4 Conclusion

The addition of biochar promoted nitrification and ammonia volatilization in soil under both types of water, and the loss of ammonia volatilization was more significant under flooding conditions. Under both water conditions, DMPP alone significantly enhanced the mitigation of N<sub>2</sub>O emissions. While, the cumulative emissions of N<sub>2</sub>O were further reduced under the combined application of biochar and DMPP and reached a significant level. The addition of biochar alone increased the greenhouse potential of the soil under both 60 and 200% WHC conditions, while nitrification inhibitors alone or in combination with biochar can significantly reduce the greenhouse effect potential. To maximize N<sub>2</sub>O mitigation and reduce GWP, biochar and DMPP should be applied together, particularly in soils with moderate moisture levels

(e.g., 60% WHC) and regular monitoring of AOB and AOA populations is recommended to assess the long-term effects of DMPP and biochar on soil nitrification processes and greenhouse gas emissions.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

HY: Writing – review & editing, Data curation, Formal analysis, Software, Visualization, Writing – original draft. LH: Data curation, Formal analysis, Writing – original draft, Validation. WY: Data curation, Formal analysis, Validation, Writing – original draft. YJ: Formal analysis, Writing – original draft. LX: Writing – review & editing. LN: Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. HL: Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing, Validation, Visualization.

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## 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.

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