

Biochar amendment affects soil nitrogen transformation and nitrous oxide emission: A ^{15}N tracer study

Jie ZHANG¹, Ping HE², Liang JIN^{3,4}, Dan WEI³, Xinpeng XU², Shicheng ZHAO², Wei ZHOU² and Shaojun QIU^{2,*}

¹Shandong Provincial Key Laboratory of Water and Soil Conservation and Environmental Protection, College of Resources and Environment, Linyi University, Shandong 276000 (China)

²State Key Laboratory of Efficient Utilization of Arable Land in China, Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture and Rural Affairs, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081 (China)

³Institute of Plant Nutrient and Resources, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097 (China)

⁴Institute of Soils, Fertilizers and Environmental Resources, Heilongjiang Academy of Agricultural Sciences, Harbin 150086 (China)

(Received August 20, 2024; revised November 10, 2024; accepted December 27, 2024)

ABSTRACT

Biochar amendment is considered a potential strategy to improve soil fertility and mitigate climate change, yet its specific effects on the fate of fertilizer-derived and native soil nitrogen remain unclear. To elucidate the effect of biochar amendment on soil nitrogen (N) transformation and nitrous oxide (N_2O) emission, a 56-d soil incubation experiment was conducted using ^{15}N isotope tracing to monitor the fate of N. Three treatments were set up: i) no addition of N or biochar control (CK), ii) addition of ^{15}N -labeled ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) (NS), and iii) combined addition of ^{15}N -labeled $(\text{NH}_4)_2\text{SO}_4$ and biochar (NS+BC). The ^{15}N abundances in soil ammonium (NH_4^+), nitrate (NO_3^-), microbial biomass N (MBN), particulate organic N (PON), mineral-associated total N (MTN), and N_2O were measured. Compared with NS, endogenous soil N-derived NH_4^+ -N and N_2O decreased significantly by 34.4%–67.6% and 18.3%–51.2%, respectively, and exogenous N-derived NH_4^+ -N, MTN, and N_2O decreased significantly by 49.6%–81.4%, 15.0%–47.0%, and 42.3%–76.5%, respectively, in NS+BC during the first 14 d. Biochar amendment significantly increased endogenous and exogenous soil N retention in MBN by 16.7%–40.1% and over 200 times, respectively, during the first 21 d. The endogenous and exogenous soil N retention in PON increased by 12.5%–27.0% and 18.2%–78.5%, respectively, over the whole incubation period. For MTN, the endogenous and exogenous soil N retention increased by 3.9%–6.1% and 24.9%–30.6%, respectively, from days 21 to 56. The contribution of endogenous N to the total N_2O emission was > 80% across treatments. These results show that biochar amendment can mitigate soil N_2O emission and promote inorganic N translocation into MBN, PON, and MTN in soil.

Key Words: endogenous N, exogenous N, microbial biomass N, mineral-associated total N, ^{15}N isotope tracing, particulate organic N, soil N pool

Citation: Zhang J, He P, Jin L, Wei D, Xu X P, Zhao S C, Zhou W, Qiu S J. 2026. Biochar amendment affects soil nitrogen transformation and nitrous oxide emission: A ^{15}N tracer study. *Pedosphere*. 36(2): 595–602.

INTRODUCTION

Nitrogen (N) is an essential limiting nutrient in agroecosystems and plays an important role in the environment (Erisman, 2021; Maaz *et al.*, 2021). The combined application of chemical fertilizer with biochar has been used to improve soil fertility and mitigate N loss in recent years (Ding *et al.*, 2016; Liu *et al.*, 2018; Craswell *et al.*, 2021). Biochar is a recalcitrant carbon (C)-rich substance that is produced by thermal degradation of waste biomass under anaerobic conditions (Tan *et al.*, 2017). Previous research has indicated that biochar amendment could alter N transformation mainly by regulating soil physical and biological properties (Sashidhar *et al.*, 2020). Currently, the magnitude and direction of this effect are not clear because there is a lack of consensus among previous reports (Bai *et al.*, 2015; Borchard *et al.*, 2019). Therefore, it is essential to quantify the fate of soil

N to improve our understanding of the effect of biochar amendment on soil fertility.

In terrestrial ecosystems, soil inorganic N, ammonium-N (NH_4^+ -N) plus nitrate-N (NO_3^- -N), can be directly assimilated by soil microbes and plants, or it can enter the soil N pool and be retained in soil *via* biotic and abiotic pathways (Qiu *et al.*, 2016). The soil N pool is mainly made up of microbial biomass N (MBN), particulate organic N (PON), and mineral-associated total N (MTN) (Zhang *et al.*, 2022). Although MBN only comprises a small portion (1%–5%) of the total soil N, it can act as a buffer to maintain the soil N supply. When the soil inorganic N content is excessive, microbial immobilization of inorganic N can effectively prevent N loss, whereas remineralization can increase the soil inorganic N supply (Sugihara *et al.*, 2010; Singh and Gupta, 2018). The N stored in the soil macroparticles ($\geq 53 \mu\text{m}$) separated by wet sieving is referred to as PON, and the remaining N contained in soil microparticles is referred to as

*Corresponding author. E-mail: qiushaojun@caas.cn.

MTN (Bai *et al.*, 2020; Zhang *et al.*, 2022). The PON is easy to decompose and regarded as an accessible N source for soil microbes, whereas MTN is generally harder to decompose and could persist over longer periods in soil (He *et al.*, 2015). Previous research has shown that biochar amendment affects soil biotic and abiotic properties (Lehmann *et al.*, 2011; Palansooriya *et al.*, 2019), which in turn affects the translocation of both exogenous and endogenous N among the N fractions mentioned above. Currently, it is not known if biochar application affects all N fractions to the same extent.

Nitrous oxide (N₂O) is released into the atmosphere as a byproduct during N transformation among the different soil N fractions. Release of N₂O increases the risk of global warming. Biochar amendment has been proposed as a way to decrease N₂O emission by changing soil physicochemical properties and affecting microbial activity (Butterbach-Bahl *et al.*, 2013; Borchard *et al.*, 2019; Dong *et al.*, 2020). However, results about the effects of chemical fertilizer and biochar on soil N₂O emission are still inconsistent (Verhoeven *et al.*, 2017; Liu *et al.*, 2018). For instance, some studies have concluded that biochar amendment could mitigate soil N₂O emission by at least 12% (Cayuela *et al.*, 2013, 2015; Feng and Zhu, 2017), whereas other studies reported that combining chemical fertilizer with biochar increases soil N₂O emission (Clough *et al.*, 2010; Shen *et al.*, 2014). Both exogenous and endogenous N are sources of N₂O (Alam *et al.*, 2019), and it is unclear whether biochar application has the same effect on both sources. To facilitate accurate evaluation of N₂O emission and develop specific mitigation strategies, it is necessary to distinguish and quantify N₂O derived from different N sources (Xu *et al.*, 2023).

In this study, we conducted a soil incubation experiment using ¹⁵N isotope tracing to explore soil N transformation and N₂O emission. The distributions of exogenous and endogenous N in the soil N pool and N₂O emission were investigated under N fertilization with and without biochar amendment. Furthermore, the dynamic responses of N derived from different sources and N₂O emission after biochar application were evaluated. We hypothesized that biochar amendment would promote exogenous N translocation into soil organic N pools and decrease N₂O emission from both exogenous and endogenous N.

MATERIALS AND METHODS

Soil and biochar

The soil used in this study was collected from the 0–20 cm layer of an unfertilized maize field (45°50' N, 126°51' E) after maize harvest at the experimental station of Heilongjiang Academy of Agricultural Sciences near Harbin, Heilongjiang Province, China. The soil is an Udic Mollisol (Soil Survey

Staff, 2014). After thoroughly mixed and passed through a 2-mm sieve, a portion of the soil sample was air-dried and analyzed for its physicochemical properties (Table I), and the remainder was stored at 4 °C before use.

TABLE I

Basic physicochemical properties of the soil used in this study

Property	Unit	Value
Sand	g kg ⁻¹	274
Silt	g kg ⁻¹	480
Clay	g kg ⁻¹	246
Organic C	g kg ⁻¹	19.4
Total N	g kg ⁻¹	1.8
Olsen-P	mg kg ⁻¹	9.6
Available K	mg kg ⁻¹	155.9
NH ₄ ⁺ -N	mg kg ⁻¹	1.0
NO ₃ ⁻ -N	mg kg ⁻¹	21.8
pH		6.9

The biochar used in this study was prepared by pyrolyzing maize straw at 500 °C for 20 min in a continuous reactor. The obtained biochar was ground using a metal mortar and pestle, passed through a 1-mm sieve, and thoroughly homogenized. The biochar had a pH of 9.4 and contained 10.3 g kg⁻¹ N, 1.88 g kg⁻¹ phosphorus, and 10.9 g kg⁻¹ potassium. The ¹⁵N natural abundance of the biochar was 3.7‰, and the C/N ratio was 49.3.

Experimental design

Before the experiment, fresh soil with 40% water-filled pore space was pre-incubated at 20 °C in the dark for 1 week. In the soil incubation experiment, three treatments were established as follows: i) no addition of N or biochar control (CK), ii) addition of ¹⁵N-labeled (20.2 atom%) (NH₄)₂SO₄ (NS), and iii) combined addition of ¹⁵N-labeled (20.2 atom%) (NH₄)₂SO₄ and biochar (NS+BC). At the start of the experiment, 220 g pre-incubated soil (*i.e.*, 187 g dry soil) was put into each 1-L glass bottle (a total of 72). For the NS and NS+BC treatments, ¹⁵N-labeled (NH₄)₂SO₄ was added at 70 mg N kg⁻¹ dry soil and mixed thoroughly with the soil. For the NS+BC treatment, biochar was added at 7 g kg⁻¹ dry soil, corresponding to a biochar/soil mass ratio of 1:150 (weight:weight) in the plow layer (0–20 cm). Then, all bottles were sealed with rubber stoppers and incubated in the dark at 20 °C. Each day, the bottles were unsealed (by removing the stoppers) and allowed to vent for 15 min to ensure aerobic conditions. During the whole 56-d incubation period, soil water-filled pore space was kept at approximately 45% based on the weighing method by adding distilled water as described by Bai *et al.* (2020). At days 1, 3, 7, 14, 21, 28, 42, and 56, three bottles were retrieved from each treatment for soil analyses, and gas samples (15 and 30 mL) were collected from the headspaces of three bottles of each treatment 6 h after the 15-min venting using syringes with stopcocks.

Laboratory analysis

To determine soil NO_3^- -N and NH_4^+ -N contents, 20 g fresh soil was extracted with 1 mol L^{-1} KCl solution, shaken for 60 min on a reciprocal shaker, and then filtered. The filtrate was analyzed using a continuous-flow analyzer (Bran Luebbe GmbH, Germany). Before determining the ^{15}N abundance of extracted inorganic N, the NH_4^+ -N was oxidized to N_2 using NaBrO, and the NO_3^- -N was reduced to N_2O using Devarda's alloy (Hauck *et al.*, 1994; Laughlin *et al.*, 1997). Then, ^{15}N abundance was determined by mass spectrometry (Hydra 20-22, Sercon Ltd., UK).

Soil MBN was determined using the chloroform fumigation-extraction method (Vance *et al.*, 1987). First, 25 g fresh soil was fumigated with CHCl_3 at 25 °C for 24 h. Another 25 g fresh soil was not fumigated. Both soil samples were extracted with 0.5 mol L^{-1} K_2SO_4 on a shaker (220 r min^{-1} , 0.5 h) and filtered. The Kjeldahl method was used to quantify the K_2SO_4 -extractable N (Cabrera and Beare, 1993). The total ^{15}N abundances of the extracts were measured using a mass spectrometer. Soil MBN (mg kg^{-1}) was calculated as:

$$\text{MBN} = (\text{N}_f - \text{N}_{\text{unf}}) / K \quad (1)$$

where N_f and N_{unf} are the total N contents of the fumigated and unfumigated soils, respectively (mg kg^{-1}), and K is a conversion factor taking the value of 0.57 (Jenkinson, 1988).

Particulate organic matter and mineral-associated organic matter were separated using established methods (Bronson *et al.*, 2004). Dry soil (< 2 mm) of 30 g was weighed into a flask containing 120 mL 5% sodium hexametaphosphate solution, and the flask was shaken for 60 min. The resulting slurry was sieved through a 53- μm sieve. The < 53 μm and ≥ 53 μm soil fractions were collected in different drying pans and oven-dried at 60 °C. The N in the particulate (≥ 53 μm) and mineral-associated (< 53 μm) organic matter were regarded as PON and MTN, respectively. They were quantified using an elemental analyzer (Elementar, Germany). Furthermore, the ^{15}N abundances of PON and MTN were determined by mass spectrometry.

The 15-mL gas sample of each treatment was used to determine the concentration of N_2O by gas chromatography, whereas the 30-mL gas sample was used to analyze the ^{15}N abundance of N_2O using a gas chromatograph with an isotope ratio mass spectrometer (Thermo Fisher Scientific, USA). The emission of N_2O -N (E , mg kg^{-1}) was calculated using the following equation (Bai *et al.*, 2020):

$$E = \frac{28VP(\rho_s - \rho_n)}{TRw} \quad (2)$$

where ρ_s and ρ_n are the N_2O concentrations of gas sample and natural air, respectively ($\mu\text{L L}^{-1}$), V is the headspace

volume of the glass bottle (*i.e.*, 0.81 L), P is the standard atmospheric pressure (*i.e.*, 101.3 kPa), T is the absolute temperature (*i.e.*, 293 K), R is the universal gas constant (*i.e.*, 8.314 L kPa $\text{K}^{-1} \text{mol}^{-1}$), 28 is the molar mass of N in N_2O (g mol^{-1}), and w is the air-dried soil weight (g).

The exogenous and endogenous N-derived soil N fractions (*e.g.*, NO_3^- -N, NH_4^+ -N, MBN, PON, and MTN) or N_2O -N emission in NS or NS+BC were calculated using the following equations:

$$\text{N}_{\text{df_ex}} = \frac{\text{N}_T(A_T - A_{\text{CK}})}{A_f - A_{\text{CK}}} \quad (3)$$

$$\text{N}_{\text{df_en}} = \text{N}_T - \text{N}_{\text{df_ex}} \quad (4)$$

where $\text{N}_{\text{df_ex}}$ and $\text{N}_{\text{df_en}}$ are the contents of soil fractions (*e.g.*, NO_3^- -N, NH_4^+ -N, MBN, PON, and MTN) or emissions of N_2O -N derived from exogenous and endogenous N, respectively, in NS or NS+BC (mg kg^{-1}), N_T is the content of the corresponding soil fraction or emission of N_2O -N in NS or NS+BC (mg kg^{-1}), and A_T , A_{CK} , and A_f are the ^{15}N excess abundances in NS or NS+BC, CK, and the ^{15}N -labeled $(\text{NH}_4)_2\text{SO}_4$, respectively.

Statistical analysis

Data analysis was carried out using the SPSS 17.0 software. The level of statistical significance was set at 0.05. Student's *t*-test was used to compare the differences between treatments. To calculate the cumulative N_2O emission during the 56-d incubation, linear interpolation was used to estimate the gas emission values for the missing days.

RESULTS

Soil N fractions

Compared with CK, both NS and NS+BC significantly increased endogenous N-derived NO_3^- -N and inorganic N over the whole incubation period and NH_4^+ -N during the first 14 d (Fig. 1). Compared with NS, NS+BC significantly decreased the contents of endogenous N-derived soil NH_4^+ -N and inorganic N by 34.4%–67.6% and 8.1%–18.2%, respectively, during the first 7 d, significantly decreased the contents of exogenous N-derived soil NH_4^+ -N and inorganic N by 49.6%–81.4% and 8.5%–38.2%, respectively, during the first 14 d, and significantly increased exogenous N-derived soil NO_3^- -N by 15.1%–204.9% during the first 7 d.

After $(\text{NH}_4)_2\text{SO}_4$ application, the content of MBN derived from endogenous N increased significantly by 18.6%–86.9% over the whole experimental period (Fig. 2). Compared with NS, NS+BC significantly increased the contents of endogenous and exogenous N-derived MBN by 16.7%–40.1% and more than 200 times, respectively, during the first 21 d.

Compared with CK, NS significantly decreased the con-

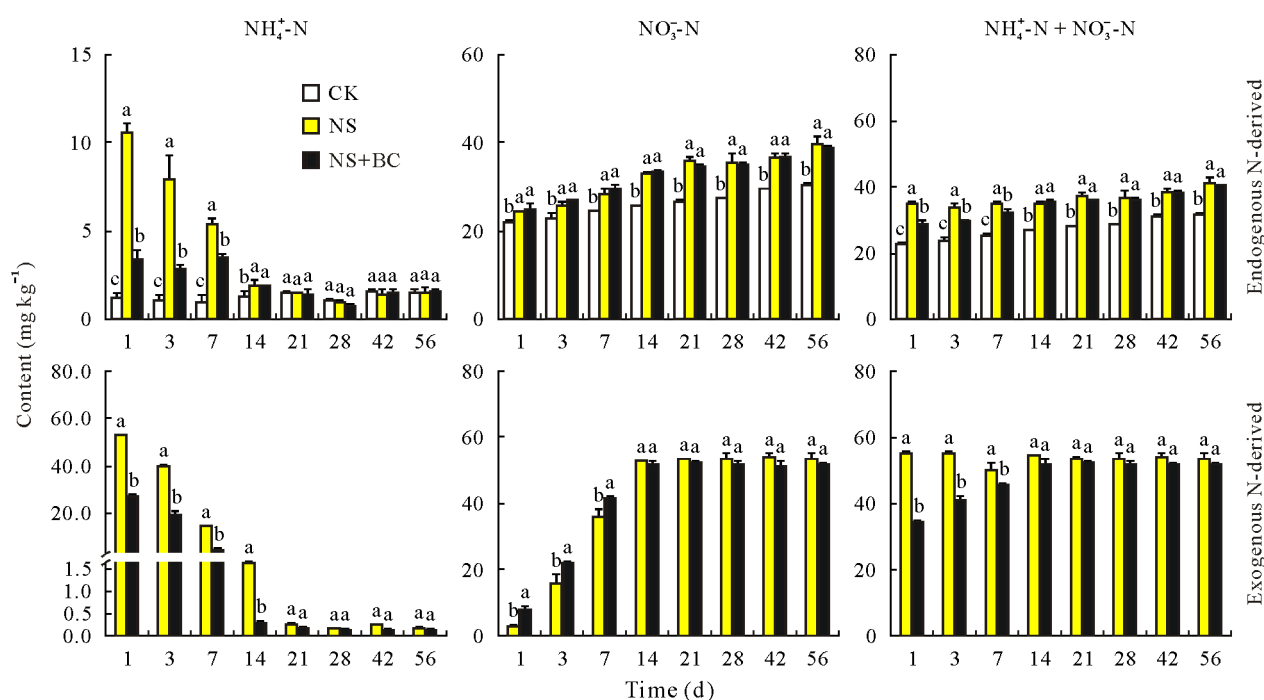


Fig. 1 Contents of endogenous and exogenous N-derived $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ (*i.e.*, inorganic N) in soil of different N fertilizer and biochar application treatments during a 56-d soil incubation experiment. Error bars are standard errors of the means ($n = 3$). Different letters above the bars represent significant differences between treatments for a same time at $P < 0.05$. CK = no addition of N or biochar control; NS = addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$; NS+BC = combined addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$ and maize straw biochar.

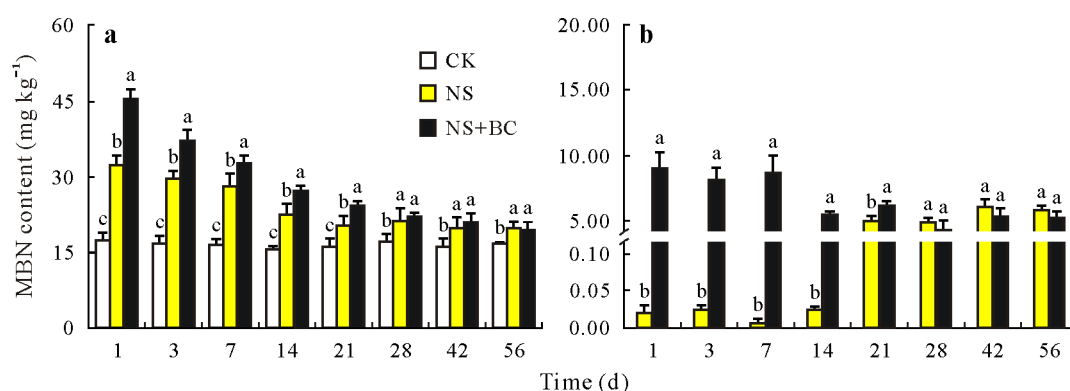


Fig. 2 Contents of endogenous (a) and exogenous (b) N-derived microbial biomass N (MBN) in soil of different N fertilizer and biochar application treatments during a 56-d soil incubation experiment. Error bars are standard errors of the means ($n = 3$). Different letters above the bars represent significant differences between treatments for a same time at $P < 0.05$. CK = no addition of N or biochar control; NS = addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$; NS+BC = combined addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$ and maize straw biochar.

tent of endogenous N-derived PON by 8.7%–15.6% during the 56-d incubation except at day 7 and increased the content of endogenous N-derived MTN by 7.1%–14.1% during the first 14 d (Fig. 3). Compared with NS, NS+BC significantly increased the contents of endogenous and exogenous N-derived PON by 12.5%–27.0% and 18.2%–78.5%, respectively, over the whole incubation period, significantly decreased the content of exogenous N-derived MTN by 15.0%–47.0% during the first 7 d, and significantly increased the contents of endogenous and exogenous N-derived MTN by 3.9%–6.1% and 24.9%–30.6%, respectively, from days 21 to 56.

N₂O emission

Compared with CK, NS treatment significantly increased the emission of endogenous N-derived N_2O by 23.8%–336.2% during the first 42 d (Fig. 4). Compared with NS, NS+BC significantly decreased the emission of endogenous N-derived N_2O by 18.3%–51.2% throughout the incubation period except at day 56 and significantly decreased the emission of exogenous N-derived N_2O by 42.3%–76.5% throughout the incubation period.

The total N_2O emissions were in the order of $\text{NS} > \text{NS+BC} > \text{CK}$ for the whole incubation period (Table II).

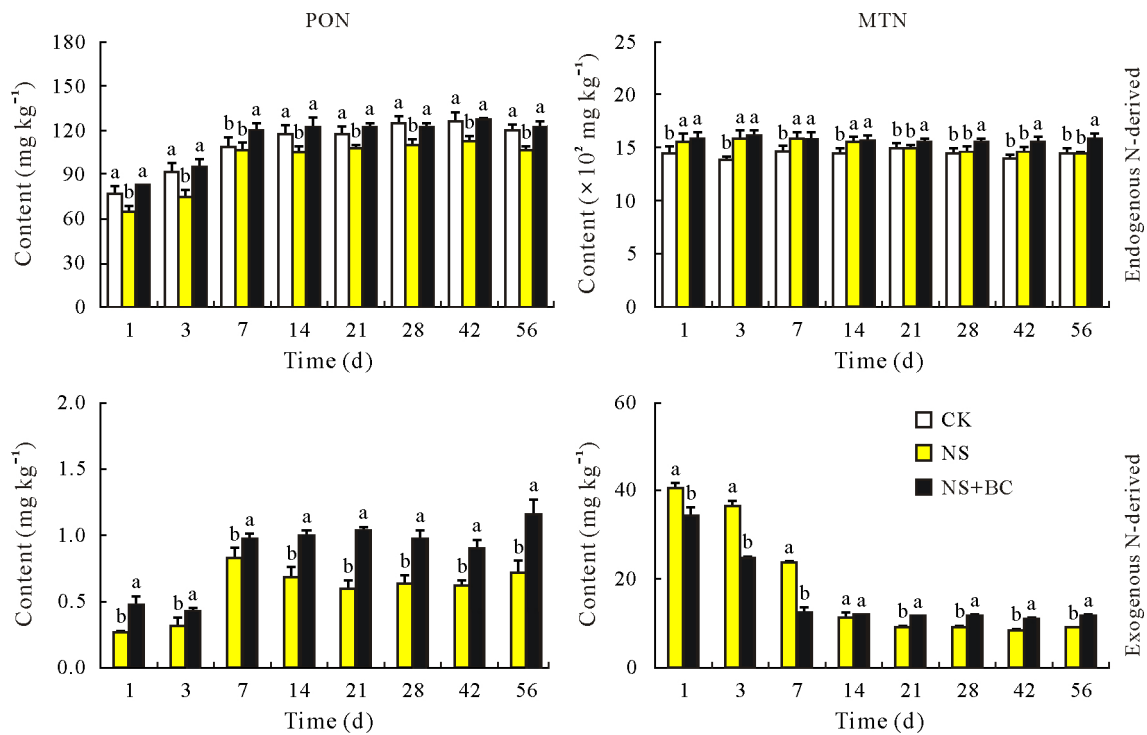


Fig. 3 Contents of endogenous and exogenous N-derived particulate organic N (PON) and mineral-associated total N (MTN) in soil of different N fertilizer and biochar application treatments during a 56-d incubation experiment. Different letters above the columns represent significant differences between treatments for a same time at $P < 0.05$. Error bars are standard errors of the means ($n = 3$). CK = no addition of N or biochar control; NS = addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$; NS+BC = combined addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$ and maize straw biochar.

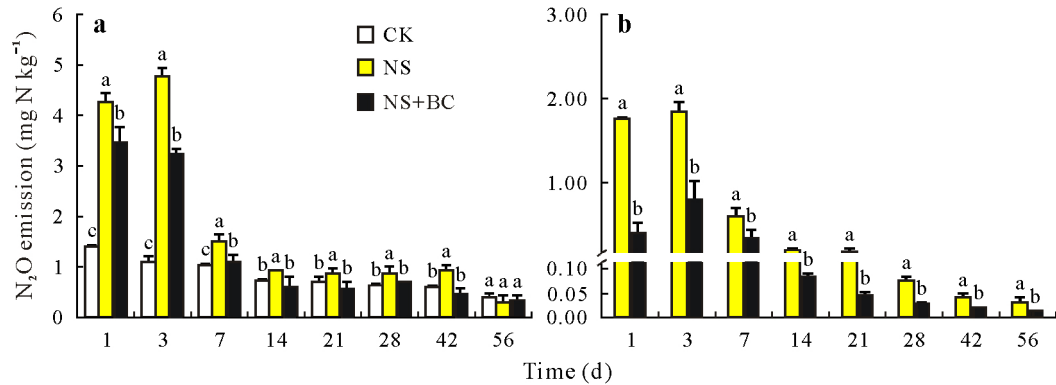


Fig. 4 Emissions of endogenous (a) and exogenous (b) N-derived N_2O in different N fertilizer and biochar application treatments during a 56-d soil incubation experiment. Error bars are standard errors of the means ($n = 3$). Different letters above the bars represent significant differences between treatments for a same time at $P < 0.05$. CK = no addition of N or biochar control; NS = addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$; NS+BC = combined addition of ^{15}N -labeled (20.2 atom%) $(\text{NH}_4)_2\text{SO}_4$ and maize straw biochar.

Compared with NS, the exogenous and endogenous N-derived N_2O emissions from NS+BC decreased significantly by 58.7% and 31.1%, respectively.

DISCUSSION

Soil N pools

As the preferred N source for soil microbes (Wong *et al.*, 2008; Geisseler *et al.*, 2010), the transformation of $\text{NH}_4^+\text{-N}$ is intensified if there are additional available C sources (Fischer and Kuzyakov, 2010; Fischer *et al.*, 2010). Biochar

contains a small fraction of available C and has a large specific surface area, which is beneficial for microbe survival and will enhance microbial N demand in the short term (Keith *et al.*, 2011; Farrell *et al.*, 2013). We found that biochar application significantly decreased the transformation of both exogenous and endogenous N to $\text{NH}_4^+\text{-N}$ (Fig. 1) and promoted the translocation of these N sources into soil organic N (MBN and PON) during the first 21 d (Figs. 1–3). These results supported our hypothesis that biochar application would promote exogenous N translocation into soil organic N pool. Additionally, biochar application decreases soil bulk density

TABLE II

Cumulative emissions of N₂O derived from endogenous (N₂O-en) and exogenous (N₂O-ex) N in different N fertilizer and biochar application treatments at the end of a 56-d soil incubation experiment

Treatment ^{a)}	N ₂ O-en	N ₂ O-ex	Total ^{b)}
	mg N kg ⁻¹		
CK	38.3 ± 1.9 ^{c,d)}		38.3 ± 1.9c
NS	65.4 ± 3.4a	15.9 ± 1.6a	81.3 ± 1.8a
NS+BC	45.1 ± 4.6b	6.6 ± 0.6b	51.7 ± 2.2b

^{a)}CK = no addition of N or biochar control; NS = addition of ¹⁵N-labeled (20.2 atom%) (NH₄)₂SO₄; NS+BC = combined addition of ¹⁵N-labeled (20.2 atom%) (NH₄)₂SO₄ and maize straw biochar.

^{b)}N₂O-en + N₂O-ex.

^{c)}Means ± standard errors (*n* = 3).

^{d)}Different letters within a column indicate significant differences between treatments at *P* < 0.05.

and improves soil aeration (Oguntunde *et al.*, 2008; Major *et al.*, 2010), which enhances NH₄⁺-N oxidation to NO₃⁻-N in the short term (Hale *et al.*, 2023). Thus, biochar application significantly decreased exogenous N-derived NH₄⁺-N and increased exogenous N-derived NO₃⁻-N during the first 7 d. However, there was no significant difference in endogenous N-derived NO₃⁻-N between NS and NS+BC throughout the incubation period. This was attributed to the fact that the content of endogenous N-derived NH₄⁺-N was much lower than that of fertilizer N-derived NH₄⁺-N.

In our research, biochar-N was applied at 72.1 mg kg⁻¹ soil. It has been demonstrated that biochar produced at > 500 °C is highly resistant to decomposition (Lee *et al.*, 2013; Fiorentino *et al.*, 2019). Thus, the contribution of biochar-N to PON should be negligible in NS+BC. However, compared with NS, NS+BC significantly increased the transformation of exogenous and endogenous N to PON throughout the incubation period. This was probably due to two reasons. First, biochar can serve as a nucleus for soil particles, and its microporous structure and large specific surface area are beneficial for N adsorption (Tan *et al.*, 2017), which would contribute to the formation of PON. Second, microbial secretions can function as transient binding agents to form macroaggregates (Du *et al.*, 2017), and higher microbial activities commonly found in biochar application treatments would contribute to the formation of PON. For MTN, previous research has shown that biochar application enhances inorganic N fixation on the surfaces of soil minerals because of the high cation exchange capacity of biochar (Tan *et al.*, 2017; Sokol *et al.*, 2019), which increases the content of MTN. However, our results were different for fertilizer N-derived MTN during the first 7 d. This difference was ascribed to the competition and fast assimilation of N by soil microbes, which would inhibit N fixation on the surfaces of soil minerals. A noteworthy finding was that biochar application did not affect the MTN derived from soil N during the early stages, which was attributed to the very low content of soil inorganic N compared with that of MTN (Fig. 3).

N₂O emission

The cumulative N₂O emission in NS was significantly higher than that in CK (Table II). This was because chemical fertilizer N was an important source of direct soil N₂O emission, and it also enhanced endogenous N-derived N₂O emission (Li *et al.*, 2015; Zhang *et al.*, 2021). Our research demonstrated that the cumulative soil N-derived N₂O emission increased by 70.8% in NS compared with CK. This difference was attributed to two mechanisms. First, exogenous N application has a priming effect on soil organic N mineralization (Xu *et al.*, 2021). Second, soil microbes immobilize exogenous N but not endogenous N, which increases the accessibility of endogenous N for N₂O-producing microbes (Kuzyakov *et al.*, 2000). The average contribution of exogenous N-derived N₂O to the cumulative N₂O emission was 16.9%, which was much lower than that of endogenous N-derived N₂O. Therefore, endogenous N was the main contributor to soil N₂O emission in this research. This result agreed with previous research by Li *et al.* (2013) and Bai *et al.* (2020) on black soil. The background emission value of N₂O from black soil is relatively high. The cumulative emission of N₂O derived from endogenous N in CK of the present study was 38.3 mg N kg⁻¹, which was much higher than the cumulative emission of N₂O derived from exogenous N in NS and NS+BC.

Compared with NS, NS+BC significantly mitigated N₂O emission from both endogenous and exogenous N sources (Fig. 4, Table II), which was consistent with the results of other studies (Verhoeven *et al.*, 2017; Borchard *et al.*, 2019). There are several possible reasons for the lower N₂O emission in NS+BC. First, biochar application can decrease the bioavailability of soil inorganic N, and the non-electrostatic adsorption of NO₃⁻-N and/or NH₄⁺-N on biochar will decrease the availability of endogenous N for nitrification and denitrification (Nelissen *et al.*, 2014; Saarela *et al.*, 2020; Begum *et al.*, 2021). Second, a fraction of the labile organic C source contained in biochar plays an essential role in reducing soil N₂O emission. This labile organic C could be rapidly assimilated by soil microbes for their growth and N immobilization (Fig. 2), which could also reduce the availability of endogenous N for nitrification and denitrification. Third, previous research using meta-analysis has shown that biochar can act as a redox catalyst to facilitate electron translocation to denitrifiers, which would enhance the reduction of N₂O.

CONCLUSIONS

Compared with N chemical fertilizer application alone, biochar amendment significantly decreased the bioavailability of both exogenous and endogenous N-derived inorganic N and promoted their storage in organic forms (*i.e.*, MBN,

PON, and MTN) in soil. Additionally, endogenous N was the main factor contributing to cumulative N₂O emission. Chemical fertilizer application greatly increased soil N₂O emission mainly by enhancing endogenous N-derived N₂O emission. Biochar amendment significantly mitigated endogenous and exogenous N-derived N₂O emission. Overall, biochar amendment could decrease the bioavailability of soil inorganic N and N₂O emission and promote N retention in organic forms in soil.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENT

This study was supported by the Innovation Program of Chinese Academy of Agricultural Sciences (No. CAAS-CSAL-202302) and the Project of Young Innovation Team in the Universities of Shandong Province, China (No. 2023KJ218).

REFERENCES

- Alam M A, Khan M I, Cho S R, Lim J Y, Song H J, Kim P J, Das S. 2019. Source partitioning and emission factor of nitrous oxide during warm and cold cropping seasons from an upland soil in South Korea. *Sci Total Environ.* **662**: 591–599.
- Bai J S, Qiu S J, Jin L, Wei D, Xu X P, Zhao S C, He P, Wang L G, Christie P, Zhou W. 2020. Quantifying soil N pools and N₂O emissions after application of chemical fertilizer and straw to a typical chernozem soil. *Biol Fertil Soils.* **56**: 319–329.
- Bai S H, Reverchon F, Xu C Y, Xu Z H, Blumfield T J, Zhao H T, Van Zwieten L, Wallace H M. 2015. Wood biochar increases nitrogen retention in field settings mainly through abiotic processes. *Soil Biol Biochem.* **90**: 232–240.
- Begum S A, Golam Hyder A H M, Hicklen Q, Crocker T, Oni B. 2021. Adsorption characteristics of ammonium onto biochar from an aqueous solution. *AQUA-Water Infrastruct Ecosystems Soc.* **70**: 113–122.
- Borchard N, Schirrmann M, Cayuela M L, Kammann C, Wrage-Mönnig N, Estavillo J M, Fuertes-Mendizábal T, Sigua G, Spokas K, Ippolito J A, Novak J. 2019. Biochar, soil and land-use interactions that reduce nitrate leaching and N₂O emissions: A meta-analysis. *Sci Total Environ.* **651**: 2354–2364.
- Bronson K F, Zobeck T M, Chua T T, Acosta-Martinez V, van Pelt R S, Booker J D. 2004. Carbon and nitrogen pools of southern high plains cropland and grassland soils. *Soil Sci Soc Am J.* **68**: 1695–1704.
- 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 Roy Soc B: Biol Sci.* **368**: 20130122.
- Cabrera M L, Beare M H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Sci Soc Am J.* **57**: 1007–1012.
- Cayuela M L, Jeffery S, van Zwieten L. 2015. The molar H:C_{org} ratio of biochar is a key factor in mitigating N₂O emissions from soil. *Agric Ecosyst Environ.* **202**: 135–138.
- Cayuela M L, Sánchez-Monedero M A, Roig A, Hanley K, Enders A, Lehmann J. 2013. Biochar and denitrification in soils: When, how much and why does biochar reduce N₂O emissions? *Sci Rep.* **3**: 1732.
- Clough T J, Bertram J E, Ray J L, Condron LM, O'Callaghan M, Sherlock R R, Wells N S. 2010. Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. *Soil Sci Soc Am J.* **74**: 852–860.
- Craswell E T, Chalk P M, Kaudal B B. 2021. Role of ¹⁵N in tracing biologically driven nitrogen dynamics in soils amended with biochar: A review. *Soil Biol Biochem.* **162**: 108416.
- Ding Y, Liu Y G, Liu S B, Li Z W, Tan X F, Huang X X, Zeng G M, Zhou L, Zheng B H. 2016. Biochar to improve soil fertility. A review. *Agron Sustain Dev.* **36**: 36.
- Dong W X, Walkiewicz A, Bieganowski A, Oenema O, Nosalewicz M, He C H, Zhang Y M, Hu C S. 2020. Biochar promotes the reduction of N₂O to N₂ and concurrently suppresses the production of N₂O in calcareous soil. *Geoderma.* **362**: 114091.
- Du Z L, Zhao J K, Wang Y D, Zhang Q Z. 2017. Biochar addition drives soil aggregation and carbon sequestration in aggregate fractions from an intensive agricultural system. *J Soil Sediment.* **17**: 581–589.
- Erisman J W. 2021. How ammonia feeds and pollutes the world. *Science.* **374**: 685–686.
- Farrell M, Kuhn T K, Macdonald L M, Maddern T M, Murphy D V, Hall P A, Singh B P, Baumann K, Krull E S, Baldock J A. 2013. Microbial utilisation of biochar-derived carbon. *Sci Total Environ.* **465**: 288–297.
- Feng Z J, Zhu L Z. 2017. Impact of biochar on soil N₂O emissions under different biochar-carbon/fertilizer-nitrogen ratios at a constant moisture condition on a silt loam soil. *Sci Total Environ.* **584-585**: 776–782.
- Florentino N, Sánchez-Monedero M A, Lehmann J, Enders A, Fagnano M, Cayuela M L. 2019. Interactive priming of soil N transformations from combining biochar and urea inputs: A ¹⁵N isotope tracer study. *Soil Biol Biochem.* **131**: 166–175.
- Fischer H, Ingwersen J, Kuzyakov Y. 2010. Microbial uptake of low-molecular weight organic substances out-competes sorption in soil. *Eur J Soil Sci.* **61**: 504–513.
- Fischer H, Kuzyakov Y. 2010. Sorption, microbial uptake and decomposition of acetate in soil: Transformations revealed by position-specific ¹⁴C labeling. *Soil Biol Biochem.* **42**: 186–192.
- Geisseler D, Horwath W R, Joergensen R G, Ludwig B. 2010. Pathways of nitrogen utilization by soil microorganisms—A review. *Soil Biol Biochem.* **42**: 2058–2067.
- Hale L, Hendratna A, Scott N, Gao S D. 2023. Biochar enhancement of nitrification processes varies with soil conditions. *Sci Total Environ.* **887**: 164146.
- Hauck R D, Meisinger J J, Mulvaney R L. 1994. Practical considerations in the use of nitrogen tracers in agricultural and environmental research. In Weaver R W, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds.) *Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties*. Soil Science Society of America, Madison. pp. 907–950.
- He Y T, Zhang W J, Xu M G, Tong X G, Sun F X, Wang J Z, Huang S M, Zhu P, He X H. 2015. Long-term combined chemical and manure fertilizations increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. *Sci Total Environ.* **532**: 635–644.
- Jenkinson D S. 1988. Determination of microbial biomass carbon and nitrogen in soil. In Wilson J R (ed.) *Advances in Nitrogen Cycling in Agricultural Systems*. CAB International, Wallingford. pp. 368–386.
- Keith A, Singh B, Singh B P. 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. *Environ Sci Technol.* **45**: 9611–9618.
- Kuzyakov Y, Friedel J K, Stahr K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biol Biochem.* **32**: 1485–1498.
- Laughlin R J, Stevens R J, Zhuo S. 1997. Determining nitrogen-15 in ammonium by producing nitrous oxide. *Soil Sci Soc Am J.* **61**: 462–465.
- Lee Y, Park J, Ryu C, Gang K S, Yang W, Park Y K, Jung J, Hyun S. 2013. Comparison of biochar properties from biomass residues produced by slow pyrolysis at 500 °C. *Bioresour Technol.* **148**: 196–201.

- Lehmann J, Rillig M C, Thies J, Masiello C A, Hockaday W C, Crowley D. 2011. Biochar effects on soil biota—A review. *Soil Biol Biochem.* **43**: 1812–1836.
- Li B, Fan C H, Zhang H, Chen Z Z, Sun L Y, Xiong Z Q. 2015. Combined effects of nitrogen fertilization and biochar on the net global warming potential, greenhouse gas intensity and net ecosystem economic budget in intensive vegetable agriculture in southeastern China. *Atmos Environ.* **100**: 10–19.
- Li L J, Han X Z, You M Y, Horwath W R. 2013. Nitrous oxide emissions from Mollisols as affected by long-term applications of organic amendments and chemical fertilizers. *Sci Total Environ.* **452–453**: 302–308.
- Liu Q, Zhang Y H, Liu B J, Amonette J E, Lin Z B, Liu G, Ambus P, Xie Z B. 2018. How does biochar influence soil N cycle? A meta-analysis. *Plant Soil.* **426**: 211–225.
- Maaz T M, Sapkota T B, Eagle A J, Kantar M B, Bruulsema T W, Majumdar K. 2021. Meta-analysis of yield and nitrous oxide outcomes for nitrogen management in agriculture. *Global Change Biol.* **27**: 2343–2360.
- Major J, Rondon M, Molina D, Riha S J, Lehmann J. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna Oxisol. *Plant Soil.* **333**: 117–128.
- Nelissen V, Saha B K, Ruyschaert G, Boeckx P. 2014. Effect of different biochar and fertilizer types on N₂O and NO emissions. *Soil Biol Biochem.* **70**: 244–255.
- Oguntunde P G, Abiodun B J, Ajayi A E, van de Giesen N. 2008. Effects of charcoal production on soil physical properties in Ghana. *J Plant Nutr. Soil Sci.* **171**: 591–596.
- Palansooriya K N, Wong J T F, Hashimoto Y, Huang L B, Rinklebe J, Chang S X, Bolan N, Wang H L, Ok Y S. 2019. Response of microbial communities to biochar-amended soils: A critical review. *Biochar.* **1**: 3–22.
- Qiu S J, Gao H J, Zhu P, Hou Y P, Zhao S C, Rong X M, Zhang Y P, He P, Christie P, Zhou W. 2016. Changes in soil carbon and nitrogen pools in a Mollisol after long-term fallow or application of chemical fertilizers, straw or manures. *Soil Till Res.* **163**: 255–265.
- Saarela T, Lafdani E K, Laurén A, Pumpanen J, Palviainen M. 2020. Biochar as adsorbent in purification of clear-cut forest runoff water: Adsorption rate and adsorption capacity. *Biochar.* **2**: 227–237.
- Sashidhar P, Kochar M, Singh B, Gupta M, Cahill D, Adholeya A, Dubey M. 2020. Biochar for delivery of agri-inputs: Current status and future perspectives. *Sci Total Environ.* **703**: 134892.
- Shen J L, Tang H, Liu J Y, Wang C, Li Y, Ge T D, Jones D L, Wu J S. 2014. Contrasting effects of straw and straw-derived biochar amendments on greenhouse gas emissions within double rice cropping systems. *Agric Ecosyst Environ.* **188**: 264–274.
- Singh A S, Gupta V K. 2018. Soil microbial biomass: A key soil driver in management of ecosystem functioning. *Sci Total Environ.* **634**: 497–500.
- Soil Survey Staff. 2014. Keys to Soil Taxonomy. 12th Edn. USDA-Natural Resources Conservation Service, Washington.
- Sokol N W, Sanderman J, Bradford M A. 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. *Global Chang Biol.* **25**: 12–24.
- Sugihara S, Funakawa S, Kilasara M, Kosaki T. 2010. Dynamics of microbial biomass nitrogen in relation to plant nitrogen uptake during the crop growth period in a dry tropical cropland in Tanzania. *Soil Sci Plant Nutr.* **56**: 105–114.
- Tan Z X, Lin C S K, Ji X Y, Rainey T J. 2017. Returning biochar to fields: A review. *Appl Soil Ecol.* **116**: 1–11.
- 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.
- Verhoeven E, Pereira E, Decock C, Suddick E, Angst T, Six J. 2017. Toward a better assessment of biochar-nitrous oxide mitigation potential at the field scale. *J Environ Qual.* **46**: 237–246.
- Wong K H, Hynes M J, Davis M A. 2008. Recent advances in nitrogen regulation: A comparison between *Saccharomyces cerevisiae* and filamentous fungi. *Eukaryotic Cell.* **7**: 917–925.
- Xu C, Han X, Zhuge Y P, Xiao G M, Ni B, Xu X C, Meng F Q. 2021. Crop straw incorporation alleviates overall fertilizer-N losses and mitigates N₂O emissions per unit applied N from intensively farmed soils: An in situ ¹⁵N tracing study. *Sci Total Environ.* **764**: 142884.
- Xu C, Zhu H S, Wang J, Ji C, Liu Y B, Chen D Y, Zhang H, Wang J D, Zhang Y C. 2023. Fertilizer N triggers native soil N-derived N₂O emissions by priming gross N mineralization. *Soil Biol Biochem.* **178**: 108961.
- Zhang J, Guo Y J, Han J, Ji Y Z, Zhang L J. 2021. Greenhouse gas emissions and net global warming potential of vineyards under different fertilizer and water managements in North China. *Agric Water Manage.* **243**: 106521.
- Zhang J, Zhang L J, Qiu S J. 2022. Biochar amendment benefits ¹⁵N fertilizer retention and rhizosphere N enrichment in a maize-soil system. *Geoderma.* **412**: 115713.