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High pyrolysis temperature biochars reduce nitrogen availability and nitrous oxide emissions from an acid soil

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Abstract

Biochar–bioenergy coproduction from biomass pyrolysis has the potential to contribute to climate change mitigation. Biochar produced at various pyrolysis temperatures (<600°C) has been widely studied. However, the effect of biochars, produced at high pyrolysis temperature (≥600°C), on soil nitrogen (N) dynamics and nitrous oxide (N₂O) emission is largely unknown. A pot trial was performed to examine the effect of high pyrolysis temperature (600, 700, 850 and 950°C) woody biochars on soil N dynamics, microbial gene abundance and N₂O emissions with (+N) and without N (–N) fertilization from an acid soil. Results showed that all biochar treatments significantly lowered the N₂O emissions in both fertilized and unfertilized regimes. However, the suppressive effect on N₂O emission among different high pyrolysis temperatures was not statistically different. Biochar amendment significantly decreased the concentration of soil NH₄⁺, and lower levels of soil NO₃[–] were observed at the later stage of experiment. Under –N, plant biomass and N uptake were significantly lowered in all biochar treatments. Under +N, biochar addition significantly increased plant biomass, while only the 700°C biochar significantly increased N uptake. This suggests that single application of biochar could limit soil mineral N bioavailability and further decrease plant growth and N uptake in the plant–soil system. Biochar amendments tended to increase nitrous oxide reductase (*nosZ*) gene abundance, but this effect was only significant for biochar produced at 950°C under +N. In conclusion, high pyrolysis temperature biochars can be effectively used to reduce N₂O emission, while increases in *nosZ* gene abundance and decreases in NH₄⁺ and NO₃[–] concentrations in the acid soil are likely to be responsible for the reduction in N₂O emission. Thus, woody biochars as a by-product produced at high pyrolysis temperature have the potential to mitigate soil N₂O emission via modifying N transformation and further affect climate change.

KEYWORDS

Biochar amendment, biomass pyrolysis, greenhouse gas mitigation, microbial gene abundance, mineral nitrogen dynamics, plant nitrogen uptake

1 | INTRODUCTION

Global climate change and the increasing use of fossil fuels have become a severe challenge in the 21st century (Crombie & Mašek, 2015). Mitigating climate change can be achieved by renewable energy sources and reducing greenhouse gas (GHG) emissions (Nelissen, Rütting, Huygens, Ruyschaert, & Boeckx, 2015). Recently, biomass-derived energy (bioenergy) have received increasing attention for the substitution of fossil fuel and global warming (Kirkels & Verbong, 2011). Pyrolysis of biomass is a thermochemical process in an oxygen-depleted environment at temperatures between 300 and 1,000°C (Crombie & Mašek, 2015; Qian, Kumar, Zhang, Bellmer, & Huhnke, 2015). Pyrolysis temperature will greatly affect the yield of biochar, bio-oil and syngas (Laird, Brown, Amonette, & Lehmann, 2009; Lehmann & Joseph, 2012). For the purpose of high bioenergy value, high pyrolysis temperature (600–1,000°C) is generally used to generate more bio-oil and syngas coproducts, but less amount of solid content, biochar (Crombie & Mašek, 2015). It is reported that fast pyrolysis under high temperature yields about 15% biochar and 85% biofuel (bio-oil and syngas; Lehmann & Joseph, 2012; Wright, Brown, & Boateng, 2008). Several studies have reported that biochar as a by-product from biofuel production has the potential to mitigate climate change (e.g., reducing nitrous oxide emission; Case, Mcnamara, Reay, & Whitaker, 2014; Stewart, Zheng, Botte, & Cotrufo, 2013). Nitrous oxide is one of the major greenhouse gases and has a global warming potential about 310 times that of carbon dioxide (CO₂) over a 100-year horizon (Hu, Chen, & Dahlgren, 2016; Lan, Chen, Rezaei Rashti, Yang, & Zhang, 2017). Nitrous oxide (N₂O) is responsible for 8% of global greenhouse gas emissions, while agricultural sources represent about 60% of total anthropogenic N₂O emissions (Harter et al., 2014). The increase in the atmospheric N₂O concentration in the past decades was mainly due to increased N fertilizer inputs (Park et al., 2012). Nitrous oxide is naturally produced in soils through nitrification and denitrification processes (Davidson, Swank, & Perry, 1986), which are significantly influenced by nitrogen (N) fertilization (Ali, Kim, & Inubushi, 2015). The biochar amendment to soil has been recently proposed as a promising strategy to mitigate soil N loss and N₂O emissions from fertilized systems (Cayuela et al., 2013; Van Zwieten et al., 2009).

Many studies have also shown that biochar application improved plant growth and biomass production (Jeffery, Verheijen, Velde, & Bastos, 2011; Major, Rondon, Molina, Riha, & Lehmann, 2010; Saarnio, Heimonen, & Kettunen, 2013). However, Zwieten, Kimber, Morris, Chan, et al., 2010 and Clough, Condrón, Kammann, and Müller (2013) indicated that the incorporation of biochar to soil does not always result in consistent yield increases and plant

responses to biochar amendment, which might vary considerably with biochar's feedstock type and its production pyrolysis temperature. Prendergast-Miller, Duvall, and Sohi (2011) reported no impact of mixed deciduous wood biochar on wheat biomass and N uptake and O'Toole, Knoth de Zarruk, Steffens, and Rasse (2013) also reported wheat straw biochar (500–600°C) had no significant impact on ryegrass biomass. In contrast, a field trial by Lentz and Ippolito (2012) suggested that woody biochars may decrease corn silage yield and N uptake due to lowering N mineralization. Previous studies also found biochar-induced shifts in the composition and functional genes of soil microbial community (Bai et al., 2015; Ducey, Ippolito, Cantrell, Novak, & Lentz, 2013; Harter et al., 2014). However, the link between these shifts and N₂O emissions has not yet been clearly established.

Pyrolysis temperature may affect biochar's physicochemical properties such as pH, bioavailable carbon (C) and N contents, composition of functional groups (e.g., C–O and C–H) and porosity and pore size distribution. This may affect soil and plant nutrient status after biochar incorporation to agroecosystems (Lehmann & Joseph, 2012; Lehmann et al., 2011). Several studies have examined the impact of biochars, with relatively low pyrolysis temperature (<600°C), on soil N availability and N₂O emissions (Case et al., 2015; Cayuela et al., 2013; Van Zwieten et al., 2014). However, the effect of biochars produced at high pyrolysis temperatures (≥600°C which mainly used for biofuel production: bio-oil and syngas) on N₂O emissions has rarely been investigated. Moreover, it has been reported that biochars produced at high pyrolysis temperatures may have a significant mitigation effect on soil N₂O emissions due to their high NO₃⁻ adsorption capacity and the reduction in N substrate availability for N₂O production (Clough et al., 2013).

This study aimed to investigate the effect of high pyrolysis temperature woody biochars on soil N dynamics, the abundance of microbial functional genes, plant biomass and N₂O emissions in an acid soil under conditions with and without N fertilization. Pyrolysis temperature would induce changes in characteristics and chemical composition of biochar. Thus, it was hypothesized that soil amendment of biochars with high pyrolysis temperatures would (a) modify plant N uptake and biomass via changing soil biochemical properties; (b) alter microbial functional gene abundance involved in nitrification and denitrification processes; and (c) suppress N₂O emissions via limiting the bioavailability of soil N sources for soil nitrifiers and denitrifiers.

2 | MATERIALS AND METHODS

2.1 | Soil and biochar

Surface soil (0–10 cm) used for this pot study was collected from a grazing grassland in Park Ridge South,

South-East Queensland (27°43'28" S, 153°1'15" E), Australia. The soil contained 72% sand, 10% silt and 18% clay (sandy loam) with a bulk density of 1.4 g/cm³, which was equivalent to Spodosol in USDA soil classification. The soil collected was air-dried, passed through a 4 mm sieve and properly mixed using an end over end shaker for 24 hr to ensure its homogeneity before carrying out the pot trial. The physicochemical properties of the investigated soil were pH (1:5 soil: water ratio) 3.8, EC (electrical conductivity) 0.1 dS/m, total C 0.84%, total N 0.06%, NH₄⁺-N 15.9 mg/kg, NO₃⁻-N 1.4 mg/kg and DOC (dissolved organic carbon) 56.3 mg/kg. The biochars were produced from pine chips (*Pinus radiata*) at pyrolysis temperatures of 600, 700, 850 and 950°C in a rotary kiln reactor under oxygen-depleted environment. The heating rate was 30°C min⁻¹ and the residence time was 25 min. After residence, biochars were cooled indirectly to below 90°C through indirect water cooling. The main physicochemical properties of the biochars were shown in Table 1. These biochars were then ground and passed through a 2 mm sieve prior to pot trial.

2.2 | Pot trial setup

The chamber units used in this experiment consisted of two identical cylindrical polyethylene chambers (15 cm in diameter and 22 cm in height) as described by Rezaei Rashti, Wang, Chen, Reeves, and Scheer (2017). The lower part was designed as a water-tight pot and the detachable upper part was designed as a gas sampling chamber. The sandy loam soil (3 kg dry weight) was mixed thoroughly with biochars (2.5% w/w dry weight) and N source (urea fertilizer at a rate of 318 mg N per pot, equal to 150 kg N ha⁻¹ based on soil weight) and then gently packed to a bulk density of 1.4 g/cm³ (field bulk density) in a pot. Annual ryegrass (*Lolium rigidum*) was grown (100 seeds per pot) in a controlled temperature environment (22 ± 1°C) for the duration of 90 days. The moisture content of 60% water holding capacity (WHC) was selected as

the desired water status for plant growth and microbial activities and the soil water content was maintained at this level throughout the experiment by adding distilled water to pots according to weight loss (every 2–3 days).

The pot trial was conducted in a completely randomized design using pine biochar with different pyrolysis temperatures. It consisted of 10 treatments namely CK (nil biochar and nil N), CKN (nil biochar plus N), P600 (biochar 600°C), P700 (biochar 700°C), P850 (biochar 850°C), P950 (biochar 950°C), P600N (biochar 600°C plus N), P700N (biochar 700°C plus N), P850N (biochar 850°C plus N) and P950N (biochar 950°C plus N) with six replicates for each treatment. At the end of experiment, ryegrass plants were harvested, dried at 70°C for 48 h and then finely ground prior to chemical analysis. Nondestructive soil samples were taken in three replicates at 7 sampling events on days 1, 7, 14, 21, 42, 63 and 90 of the experiment using polyethylene tubes of 2.5 cm diameter.

2.3 | Soil and plant analysis

Total C and N contents soil and plant samples were determined by dry combustion using Leco TruMac CN analyzer (Leco Corporation, USA). Soil particle size distribution was determined using the hydrometer method as described by Day (1965). Soil pH and electrical conductivity (EC) were determined in 1:5 (w/v) soil/water extracts using a glass electrode. Soil and biochars NH₄⁺-N and NO₃⁻-N contents were determined by 2 M KCl extraction at 1:10 (soil:solution) ratio after shaking on an end over end shaker for 1 hr (Keeney & Nelson, 1982). The biochar samples then recovered for further extraction using hot (95°C) 2 M KCl (Rayment & Lyons, 2011). In brief, the biochar samples (after adding 2 M KCl) were placed into an oven at 95°C for 16 hr before extraction. Both cold and hot 2 M KCl extracts were analyzed for NH₄⁺-N and NO₃⁻-N concentrations using a SmartChem[®]200 Discrete Chemistry Analyser (WESTCO Scientific Instruments Inc.). Microbial biomass C (MBC) and N (MBN) were determined by the

TABLE 1 The main physicochemical properties of the pine biochars produced at high pyrolysis temperatures

Pyrolysis temperature (°C)				pH (1:20 H ₂ O)	EC (μS/cm)	Molar H/C ratio	Ash content (%)	DOC (mg/kg)	DON (mg/kg)	NH ₄ ⁺ -N ^a (mg/kg)	NO ₃ ⁻ -N ^a (mg/kg)	SA ^b (m ² /g)
	TC (%)	TN (%)	C/N ratio									
600	63.7	0.09	724	6.88	82	0.24	1.7	1665	40.6	0.3	0.8	308
700	62.4	0.07	870	8.15	144	0.15	3.5	500	22.1	0.3	1.0	322
850	72.3	0.14	514	8.45	196	0.14	1.6	580	19.5	0.3	1.4	65
950	70.1	0.12	565	8.48	224	0.14	1.5	560	28.6	0.3	2.1	58

Notes. DOC, dissolved organic C; DON, dissolved organic N; EC, electrical conductivity; TC, total carbon content; TN, total nitrogen content.

^aNH₄⁺-N and NO₃⁻-N were determined by the 2 M KCl extraction (cold) followed by hot 2 M KCl extraction, and the combined results from both extractions were presented.

^bSA, Brunauer–Emmett–Teller surface area.

chloroform fumigation–extraction method using an EC factor of 2.64 (Vance, Brookes, & Jenkinson, 1987) and an EN factor of 2.22 (Brookes, Landman, Pruden, & Jenkinson, 1985). Dissolved organic C (DOC) in soil and biochar samples and dissolved organic N (DON) in biochar samples were measured by a TOC_{VCPH/CPN} analyzer (Shimadzu Scientific Instruments, Japan). Ash content of biochar was measured by Proximate Analysis and biochar's elemental H and C were measured by Ultimate Analysis. Biochars' surface area was determined from adsorption isotherms using the Brunauer, Emmett and Teller (BET) equation (Brunauer, Emmett, & Teller, 1938). Results for all analyses were reported on a dry weight basis.

2.4 | Gas sampling and analysis

Gas samples were collected from three replicates of each treatment at days 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 17, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 90 of the experiment, approximately 1 hr after closure of the gas chambers, using a 25-mL gas-tight syringe. The gas samples immediately transferred to pre-evacuated 12-mL glass vials (Labco, UK). The N₂O concentration in gas samples was measured via gas chromatograph (Shimadzu GC-2010 Plus) equipped with an electron capture detector (ECD). The fluxes were calculated from the increases in the N₂O concentration during the sampling time. Linearity tests on gas concentration increased were performed on a subset of sampling occasions during the study for all treatments by taking samples after the closure of chambers every 30 min for 2 hr. Nitrous oxide emissions showed a linear trend over the first hour of the measurement period. The emissions for days without gas sampling were estimated using the arithmetic mean of the measurements on the two closest days (Rezaei Rashti et al., 2015). Cumulative N₂O emissions during the 90-day pot experiment were calculated by linear integration of daily fluxes.

2.5 | DNA extraction and real-time PCR analysis

DNA was extracted from 0.5 g of soil sample using the MoBio Powersoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) as described by Liu et al. (2013). The DNA quality and quantity were determined using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) at 260 nm and the ratios of A₂₆₀/A₂₈₀ ranged from 1.8 to 2.0. The PCR fragments of microbial functional genes were amplified using primers and thermal conditions described in Table S1 and the purified PCR products were cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA). Plasmids, as standard curves used for quantification analysis, were extracted from the positive clones using a Qiagen Miniprep kit (Qiagen,

Germantown, MD, USA). The abundance of investigated microbial genes were *amoA* gene for ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), nitrate reductase gene (*narG*), nitrite reductase gene (*nirS*) and nitrous oxide reductase (*nosZ*). Reactions were carried out on the Mastercycler[®] ep realplex real-time PCR system (Eppendorf, Hamburg, Germany) in triplicate. Melting curves and agarose gel running of PCR products at the end of each quantitative real-time PCR were used to check amplification specificity. No template controls gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was estimated by a 1:10 soil DNA dilution and no inhibition was detected.

2.6 | Statistical analysis

Statistical analysis was conducted using Statistix 8.0 software package and figures were produced using Sigmaplot 11.0 (Systat Software, Inc). Before analysis, normality of all data was checked, and the data were log-transformed and centered when needed. Three-way repeated measures ANOVA was conducted to examine the interaction of biochar type (pyrolysis temperature), N fertilization treatment and sampling time on soil biochemical parameters and N₂O fluxes. Two-way repeated measures ANOVA was performed to examine the interaction of biochar type and sampling time on soil biochemical parameters and N₂O fluxes, with or without N fertilization and to examine the effects of biochar type and N fertilization treatment and their interaction on plant biomass, plant N uptake, soil total C and N at the end of experiment. One-way ANOVA was also used to evaluate the 90-day cumulative N₂O emission between different biochar treatments for each of the N fertilization regimes. When the significance was different ($p < 0.05$), multiple comparisons (all-pairwise comparisons) were carried out using least significance difference (LSD) test. The canonical correspondence analysis (CCA) was used to examine the relationship between soil biochemical properties and microbial functional gene abundance. The principal component analysis (PCA) was also used to determine the key drivers of N₂O emissions (CANOCO 4.5 for Windows, Microcomputer Power, USA). The significance of the ordination axes was calculated by the Monte Carlo permutation test ($n = 499$).

3 | RESULTS

3.1 | Soil chemical and microbial properties as affected by biochar amendment

Soil pH and EC were significantly ($p < 0.001$) affected by biochar type (pyrolysis temperature), N fertilization, sampling time and their interactions (Table S2–S4). In the –N treatments, all biochar treatments significantly ($p < 0.05$)

increased soil pH compared to the control with the highest pH being observed in the P950 treatment, while in the +N treatments, soil pH generally decreased over time for all the treatments (Figure 1a,b). The soil pH values in biochar treatments with higher pyrolysis temperatures (850 and 950°C) were significantly higher than the other treatments regardless of N fertilization (Figure 1a,b). Soil EC values in all biochar-amended treatments were consistently lower than the control regardless of N fertilization (Figure 1c,d). In the -N treatments, the effect of biochars pyrolysis temperature on soil EC values was not significant during the experimental period. However, in the +N treatments, the P600N treatment showed significantly higher EC values than the P850N treatment, whereas the differences in soil EC values of P600N, P700N and P950N treatments were not statistically significant.

Concentrations of soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were significantly ($p < 0.05$) affected by biochar type, N fertilization and sampling time (Table S2–S4). In the -N treatments, the overall effect of high pyrolysis temperature biochars on soil $\text{NH}_4^+\text{-N}$ concentration was not significant (Figure 2a), while in the +N treatments, the concentration of soil $\text{NH}_4^+\text{-N}$ in biochar-amended treatments was lower than the CKN treatment (Figure 2b). However, the P600N treatment showed significantly higher $\text{NH}_4^+\text{-N}$ concentration in comparison with other biochar-amended treatments

(Figure 2b). The initial concentrations of soil $\text{NO}_3^-\text{-N}$ were low (0.2–3.3 mg/kg) in both -N and +N treatments (Figure 2c,d). In the -N treatments, the concentration of soil $\text{NO}_3^-\text{-N}$ generally increased over time, particularly in the CK treatment, with a sharp increase after the initial 4 weeks (Figure 2c). However, by the end of experiment, in the -N treatments, soil $\text{NO}_3^-\text{-N}$ concentration in the CK treatment (13.6 mg/kg) was significantly higher than all biochar-amended treatments (Figure 2c). In the +N treatments, soil $\text{NO}_3^-\text{-N}$ concentration sharply increased in the first 4 weeks, but slightly decreased thereafter (Figure 2d). The $\text{NO}_3^-\text{-N}$ concentration in the CKN treatment was higher than that in all biochar treatments after the initial four weeks of the experiment. Overall, the $\text{NO}_3^-\text{-N}$ concentration in the P700N treatment was higher than all other biochar-amended treatments at days 28 and 42. (Figure 2d).

Soil MBC, rather than MBN, was significantly ($p < 0.05$) affected by biochar type, N fertilization, sampling time and their interactions (Table S2–S4). Overall, soil MBC in all treatments decreased with time, especially in the first two weeks of the experiment, although there were some fluctuations (Figure 3a,b). The effect of high pyrolysis temperature biochars on soil MBC content was not statistically significant in both -N and +N treatments. Soil MBC in the CK and CKN treatments were

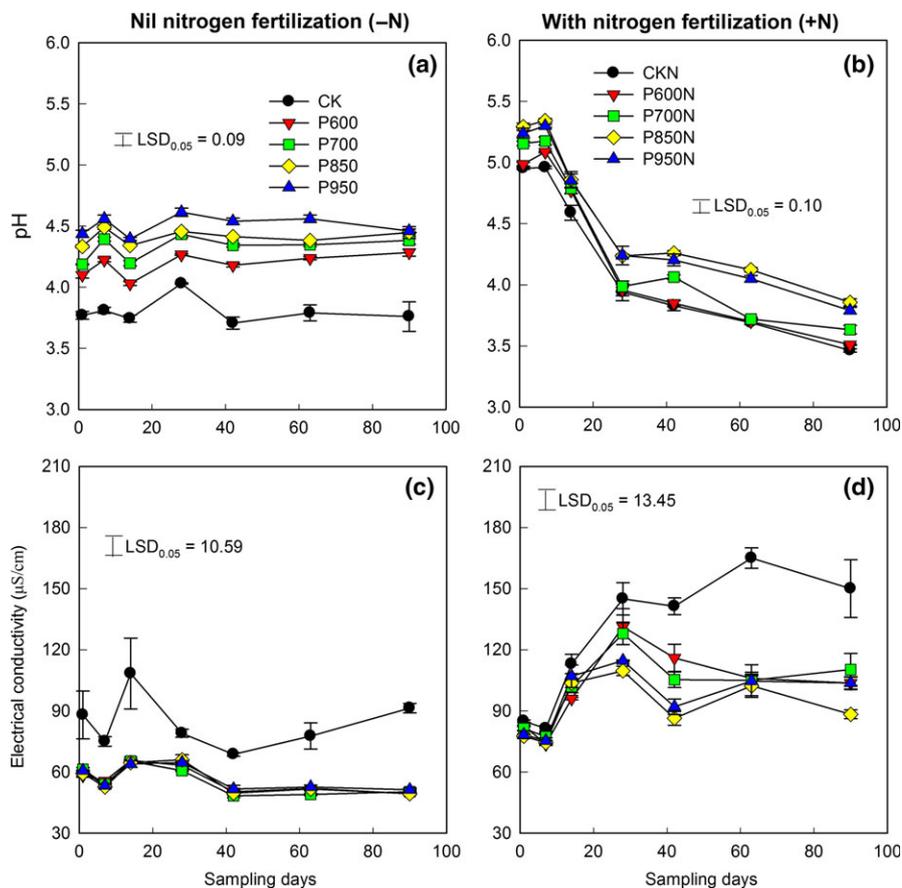


FIGURE 1 Dynamics of soil pH and EC in the -N (a, c) and +N (b, d) treatments, amended with 2.5% biochar compared with the control (CK) under 60% WHC, over the 90-day pot trial. Vertical bars represent standard error ($n = 3$). P600, pine biochar produced at 600°C; P700, pine biochar produced at 700°C; P850, pine biochar produced at 850°C; P950, pine biochar produced at 950°C. CKN, control plus N fertilizer; P600N, pine biochar produced at 600°C plus N fertilizer; P700N, pine biochar produced at 700°C plus N fertilizer; P850N, pine biochar produced at 850°C plus N fertilizer; P950N, pine biochar produced at 950°C plus N fertilizer

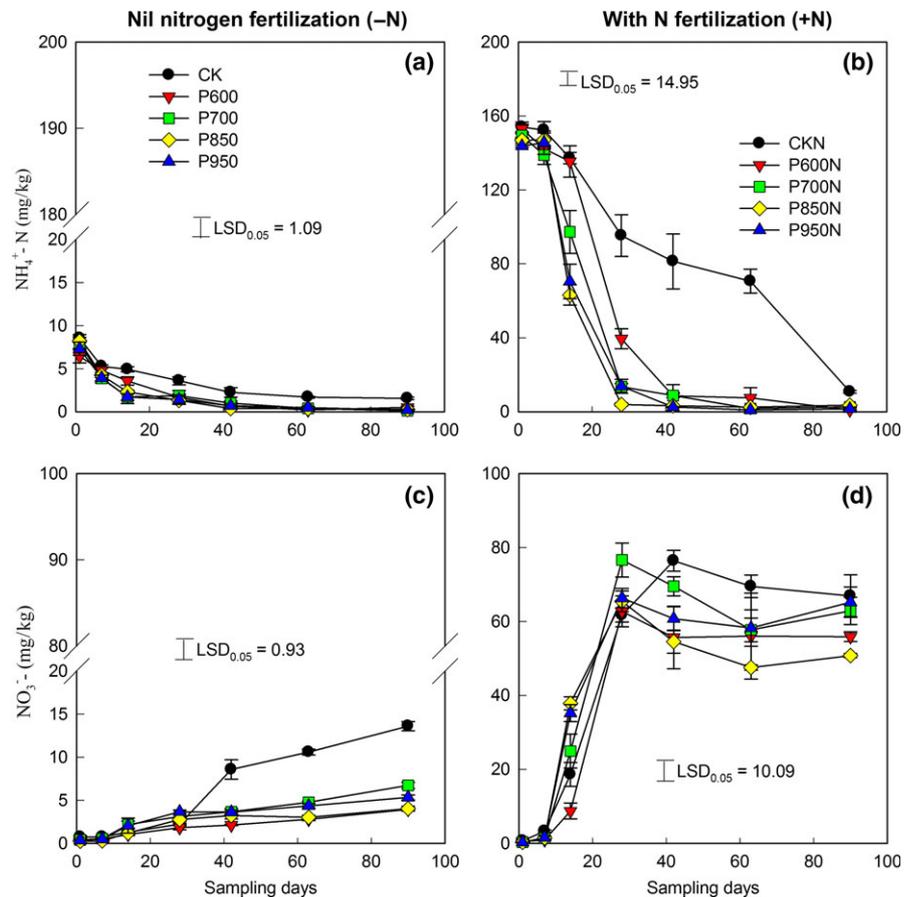


FIGURE 2 Dynamics of soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the $-N$ (a, c) and $+N$ (b, d) treatments, amended with 2.5% biochar compared with the control (CK) under 60% WHC, over the 90-day pot trial. Vertical bars represent standard error ($n = 3$). See Figure 1 for abbreviations of biochar treatments

significantly ($p < 0.05$) lower than all biochar-amended treatments by the end of experiment. The average concentration of MBN in biochar-amended treatments was higher than CK in the $-N$ treatments (Figure 3c). Under the $-N$ condition, soil MBN concentration in the P850 treatment tends to be higher than other biochar-amended treatments, but these differences were not statistically significant (Figure 3c). However, in the $+N$ treatments, the concentration of MBN generally decreased over time in all of treatments, although some large variations were observed (Figure 3d). In addition, the effect of high pyrolysis temperature biochars on soil MBN concentration was not statistically significant in both $-N$ and $+N$ treatments.

Soil DOC was significantly ($p < 0.05$) influenced by biochar, N fertilizer and sampling time and their interactions and two-way repeated measures ANOVA also showed that soil DOC was significantly ($p < 0.05$) influenced by biochar, sampling time and their interactions in both $-N$ and $+N$ treatments (Table S2–S4). The concentration of soil DOC showed similar trends during the experiment for all the treatments, namely the concentration of soil DOC was radically decreased in the first week and thereafter generally increased over time (Figure 4). In the $-N$ treatments, the DOC concentration in P600 was significantly ($p < 0.05$) higher than other biochar treatments, while had no significant difference with CK treatment. The

P950 treatment showed the lowest DOC concentration among the investigated treatments throughout the experiment (Figure 4a). In the $+N$ treatments, soil DOC concentration in P600N treatment was significantly ($p < 0.05$) higher than other biochar-amended treatments, by the end of experiment, while the soil DOC in all biochar treatments was significantly ($p < 0.05$) lower than the CKN treatment (Figure 4b).

3.2 | Nitrous oxide fluxes

Soil N_2O fluxes were significantly ($p < 0.05$) affected by biochar type, N fertilizer, sampling time and their interactions (Table S5). In the $-N$ treatments, the N_2O flux in the CK treatment was peaked at day 3, while in the biochar treatments the N_2O fluxes were peaked at day 21 (Figure 5a). The daily N_2O fluxes in the CK treatment were generally higher than biochar treatments, while the effect of high pyrolysis temperature biochars on N_2O fluxes was not statistically significant. In the $+N$ treatments, there were no significant differences between the control and biochar-amended treatments in the first 3 weeks, whereas the CKN treatment had significantly higher daily N_2O fluxes than all biochar treatments from day 21 till the end of experiment (Figure 5b). The effect of high pyrolysis temperature biochars on daily N_2O fluxes was not

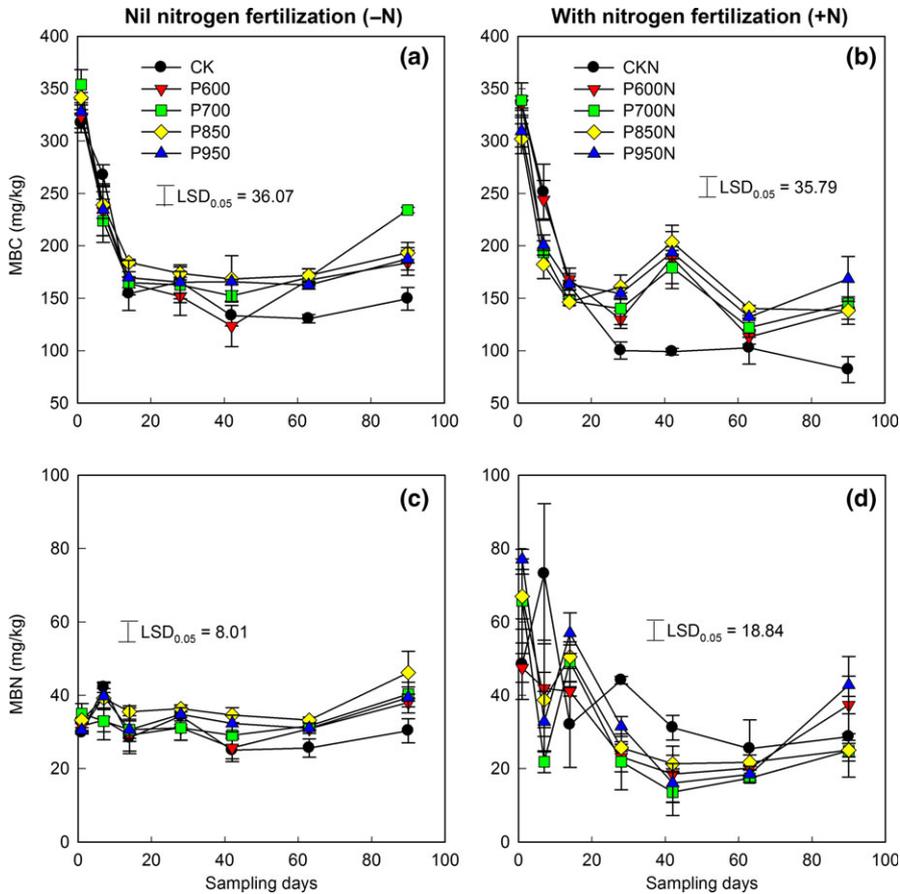


FIGURE 3 Dynamics of soil MBC and MBN in the -N (a, c) and +N (b, d) treatments, amended with 2.5% biochar compared with the control (CK) under 60% WHC, over the 90-day pot trial. Vertical bars represent standard error ($n = 3$). See Figure 1 for abbreviations of biochar treatments

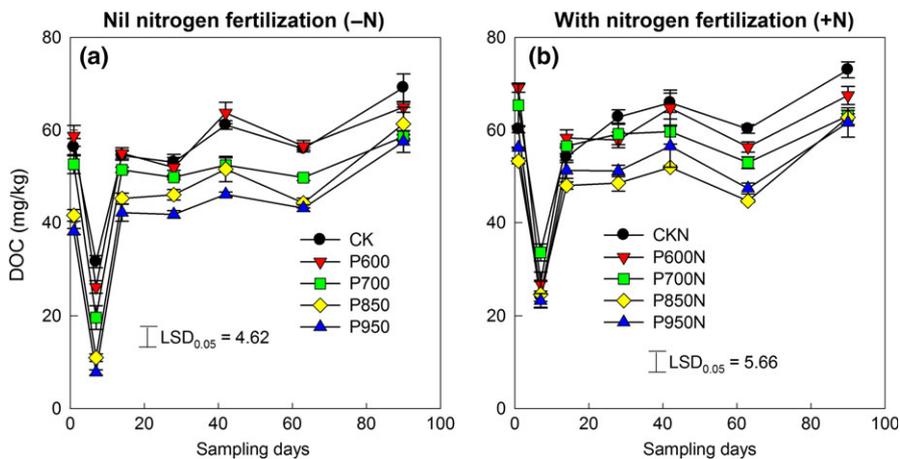


FIGURE 4 Dynamics of soil DOC in the -N (a) and +N (b) treatments, amended with 2.5% biochar compared with the control (CK) under 60% WHC, over the 90-day pot trial. Vertical bars represent standard error ($n = 3$). See Figure 1 for abbreviations of biochar treatments

significant except at weeks 4 and 5 of the experiment which P600N treatment had significantly higher N_2O fluxes than other biochar treatments.

In both -N and +N treatments, cumulative N_2O emissions in the biochar-amended treatments were significantly ($p < 0.01$) lower than the related control (CK and CKN) treatment (Figure 5c,d). Biochar amendment to -N and +N treatments significantly ($p < 0.01$) reduced N_2O emissions by 33%–45% and 75%–85%, respectively. The effect of biochars' pyrolysis temperature on cumulative N_2O emissions was not statically significant ($p > 0.05$) in both -N

and +N treatments, although cumulative N_2O emissions in P600N was higher than other biochar treatments.

3.3 | Microbial functional gene abundance

Two-way ANOVA showed a significant ($p < 0.05$) interaction between biochar type and N fertilization for AOB, *narG* and *nosZ* genes while no significant interaction was observed for AOA and *nirS* genes (Table 2). However, N fertilization had significant impact on the abundance of AOA gene. AOA gene abundance in CK (5.04×10^6

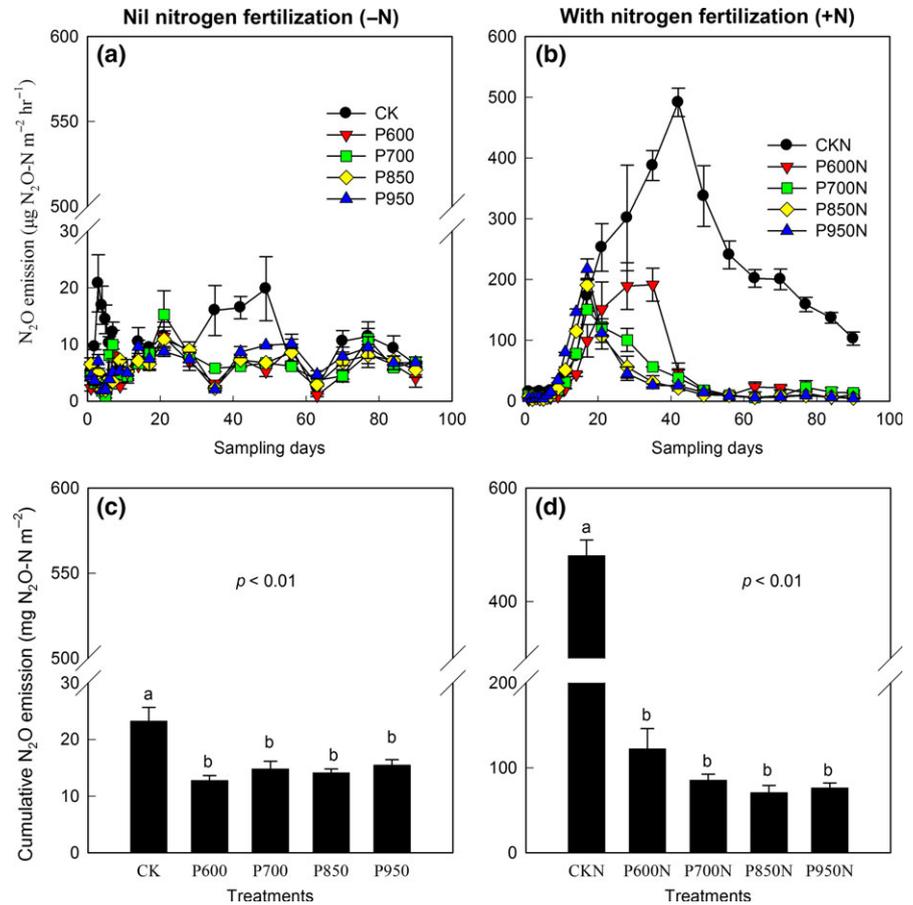


FIGURE 5 Soil N_2O fluxes in the $-N$ (a) and $+N$ (b) treatments and cumulative N_2O emissions in the $-N$ (c) and $+N$ (d) treatments, amended with 2.5% biochar compared with the control (CK) under 60% WHC, over the 90-day pot trial. Vertical bars represent standard error ($n = 3$). See Figure 1 for abbreviations of biochar treatments

TABLE 2 The p values from the two-way ANOVA for the effects of biochar pyrolysis temperature (B) and N fertilization (N) on the abundance of microbial functional genes

Source factor	df	AOA	AOB	<i>narG</i>	<i>nirS</i>	<i>nosZ</i>
Biochar type (B)	4	0.314 ^{ns}	<0.001***	0.02*	0.203 ^{ns}	0.031*
N fertilizer (N)	1	0.009**	<0.001***	0.245 ^{ns}	0.586 ^{ns}	0.027*
B × N	4	0.277 ^{ns}	<0.001***	<0.001***	0.549 ^{ns}	0.014*

Notes. Symbols indicate the p value significance of the term: ns, not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

copies per g dry soil) and P950 (5.20×10^6 copies per g dry soil) was significantly ($p < 0.05$) higher than that of CKN, P600N and P950N while they had no significant difference with other treatments (Table 3). In both $-N$ and $+N$ treatments, no significant difference in AOA was observed among treatments (Table 3). AOB gene abundance in P850N (2.75×10^7 copies per g dry soil) and P950N (2.80×10^7 copies per g dry soil) was significantly ($p < 0.05$) higher than all other treatments. AOB in $+N$ treatments was significantly ($p < 0.05$) higher than those in $-N$ treatments. Under $-N$ treatments, no significant difference in AOB was observed among treatments while under $+N$ treatments, AOB in CKN was the lowest compared to all biochar-amended treatments. The results also indicated that the copy numbers of *narG* gene in the

P950N treatment (2.28×10^7 copies per g dry soil) was significantly ($p < 0.05$) higher than all other treatments, while the effect of high pyrolysis temperature biochars on *narG* gene abundance was not statistically significant except for P950N treatment. In addition, no significant differences were observed among treatments in the abundance of *nirS* gene. The abundance of *nosZ* gene in the P950N treatment (2.29×10^6 copies per g dry soil) was significantly higher than all other treatments except for P600 and P850 treatments. In $-N$ treatments, the effect of high pyrolysis temperature biochars on the abundance of *nosZ* was not significant, while in $+N$ treatments, *nosZ* gene in the CKN was the lowest but with no significant difference with all biochar-amended treatments except for P950N.

TABLE 3 The abundance (copies per g dry soil) of microbial functional genes (AOA, AOB, *narG*, *nirS* and *nosZ*) at day 42 with (+N) and without N (–N) fertilization from biochar-amended soils. Standard errors are presented in the brackets. Lowercase letters indicate significant differences between treatments at $p < 0.05$

Fertilization	Treatment	AOA	AOB	<i>narG</i>	<i>nirS</i>	<i>nosZ</i>
–N	CK	5.04×10^6 (1.3×10^6) a	0.03×10^7 (8.8×10^4) e	1.37×10^7 (1.5×10^6) b,c	3.58×10^6 (1.5×10^6) a	1.49×10^6 (2.0×10^5) b,c,d,e
	P600	2.32×10^6 (4.6×10^5) a,b,c	0.03×10^7 (3.2×10^4) e	1.42×10^7 (0.4×10^6) b	4.61×10^6 (0.4×10^6) a	1.81×10^6 (0.7×10^5) a,b,c
	P700	4.39×10^6 (1.9×10^6) a,b	0.04×10^7 (1.1×10^5) e	1.07×10^7 (0.4×10^6) b,c	3.63×10^6 (3.9×10^5) a	1.50×10^6 (3.9×10^5) b,c,d,e
	P850	3.99×10^6 (1.2×10^6) a,b,c	0.11×10^7 (2.8×10^5) e	1.17×10^7 (0.3×10^6) b,c	4.02×10^6 (0.3×10^6) a	1.99×10^6 (0.3×10^5) a,b
	P950	5.20×10^6 (1.6×10^6) a	0.05×10^7 (1.2×10^5) e	1.00×10^7 (0.5×10^6) b,c	5.91×10^6 (0.5×10^6) a	1.56×10^6 (2.2×10^5) b,c,d
+N	CKN	1.05×10^6 (4.5×10^4) c	0.43×10^7 (6.7×10^5) d	1.08×10^7 (1.5×10^6) b,c	8.02×10^6 (1.5×10^6) a	0.89×10^6 (2.0×10^5) e
	P600N	1.32×10^6 (2.1×10^5) b,c	0.69×10^7 (1.2×10^6) c	0.92×10^7 (0.7×10^6) c	3.89×10^6 (0.7×10^6) a	1.00×10^6 (1.3×10^5) d,e
	P700N	3.64×10^6 (1.3×10^6) a,b,c	1.40×10^7 (1.2×10^6) b	1.03×10^7 (3.5×10^6) b,c	9.01×10^6 (3.5×10^6) a	1.25×10^6 (2.1×10^5) c,d,e
	P850N	3.65×10^6 (3.1×10^5) a,b,c	2.75×10^7 (1.1×10^6) a	1.36×10^7 (1.1×10^6) b,c	2.94×10^6 (1.1×10^6) a	1.26×10^6 (1.4×10^5) c,d,e
	P950N	1.29×10^6 (1.5×10^5) b,c	2.80×10^7 (4.4×10^5) a	2.28×10^7 (3.3×10^6) a	7.66×10^6 (3.3×10^6) a	2.29×10^6 (5.1×10^5) a

The values in bold indicate the highest copy numbers for individual gene. See Figure 1 for abbreviations of biochar treatments.

3.4 | Plant biomass, N uptake and soil C and N contents

Two-way ANOVA showed significant interactions between biochar type and N fertilization for plant dry matter, plant N content and plant N uptake (Table 4). Plant biomass in P600N, P700N, P850N and P950N was significantly ($p < 0.05$) higher than all other treatments (Table 5). However, there was no significant difference between CK and CKN (Table 5), implying that applied N fertilizer had no major impact on ryegrass growth under nonbiochar amendment conditions. In the –N treatments, biochar amendments had significant ($p < 0.05$) negative impact on plant biomass (Table 5). However, in the +N treatments, all biochar treatments increased plant biomass (17%–39%) compared with the CKN, although the observed differences

were only significant for P700N treatment (Table 5). N fertilization significantly ($p < 0.05$) increased plant N content. Plant N content in the CKN was significantly ($p < 0.05$) greater than all other treatments, while biochar amendments significantly ($p < 0.05$) decreased plant N content compared with the related control (CK and CKN) in –N and +N treatments (Table 5). On the other hand, the effect of high pyrolysis temperature biochars on plant N content was not statistically significant (Table 5). But plant N uptake in the P700N treatment was significantly higher than all other treatments (Table 5). Biochar amendments significantly ($p < 0.05$) increased soil C content, while the application of biochars with higher pyrolysis temperatures (850 and 950°C) resulted in higher soil total C content than biochars with lower pyrolysis temperatures (600 and 700°C; Table 5). Soil total N content in P600N, P700N, P850N and

TABLE 4 The p values from two-way ANOVA for the effects of biochar pyrolysis temperature, N fertilization and their interactions on soil total C and N, ryegrass dry matter and N uptake

Source	Df	Plant dry matter	Plant N content	Plant N uptake	Soil total C	Soil total N
Biochar pyrolysis temperature	4	0.162 ^{ns}	0.000***	0.000***	0.000***	0.003**
N fertilization (N)	1	0.000***	0.000***	0.000***	0.036*	0.000***
B × N	4	0.000***	0.013*	0.000***	0.619 ^{ns}	0.513 ^{ns}

Note.. Symbols indicate the p value significance of the term: ns, not significant.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 5 Effect of different pyrolysis temperatures of biochar on ryegrass biomass and N uptake with (+N) or without N (–N) fertilization

Fertilization	Treatment	Dry biomass (g/pot)	Plant N (%)	Plant N uptake (mg/pot)	Soil total C (g/kg)	Soil total N (g/kg)
–N	CK	1.03 ± 0.02 c	4.64 ± 0.15 c	47.6 ± 0.5 c	8.1 ± 0.3 d	0.59 ± 0.02 c
	P600	0.66 ± 0.01 d	2.70 ± 0.06 d	21.3 ± 0.8 d	29.1 ± 0.2 b,c	0.61 ± 0.01 c
	P700	0.64 ± 0.02 d	2.84 ± 0.04 d	18.2 ± 0.3 d	28.7 ± 0.8 c	0.60 ± 0.02 c
	P850	0.63 ± 0.03 d	2.86 ± 0.19 d	17.8 ± 0.5 d	30.1 ± 1.0 a	0.61 ± 0.03 c
	P950	0.76 ± 0.02 d	2.81 ± 0.05 d	17.7 ± 0.7 d	29.7 ± 0.8 a,b	0.61 ± 0.02 c
+N	CKN	1.07 ± 0.03 c	6.52 ± 0.05 a	69.5 ± 1.5 b	8.1 ± 0.2 d	0.68 ± 0.0 b
	P600N	1.25 ± 0.12 b	5.34 ± 0.09 b	66.8 ± 5.3 b	29.8 ± 0.5 a,b	0.72 ± 0.0 a
	P700N	1.49 ± 0.12 a	5.35 ± 0.06 b	80.0 ± 7.0 a	29.7 ± 0.1 a,b	0.72 ± 0.0 a
	P850N	1.25 ± 0.01 b	5.14 ± 0.02 b	64.1 ± 0.7 b	30.4 ± 0.2 a	0.72 ± 0.0 a
	P950N	1.25 ± 0.05 b	5.37 ± 0.04 b	67.4 ± 3.0 b	30.1 ± 0.9 a	0.72 ± 0.0 a
	LSD _{0.05}	0.178	0.313	9.2	1.02	0.02

The values are the means ± SE for $n = 3$; different lowercase letters in a single column indicate significant difference between treatments at $p < 0.05$. See Figure 1 for the abbreviations of treatments.

P950N was significantly higher than all other treatments (Table 5). In the –N treatments, biochar amendment increased soil total N content, but no significant differences were observed between biochar treatments and control (Table 5). However, in the +N treatments, biochar amendment significantly ($p < 0.05$) increased soil total N content compared with CKN (Table 5). In addition, no significant differences in soil total N content were observed between biochar treatments, at both –N and +N treatments (Table 5).

3.5 | Relationships between soil biochemical parameters and the abundance of microbial functional genes

Canonical correspondence analysis (CCA) was used to identify the main soil biochemical parameters that regulate the patterns of soil microbial functional gene abundance. CCA axes 1 and 2 accounted for over 97% of the overall variances. Biochemical parameters and gene abundances correlations for the first two axes were 0.97 and 0.98, respectively, indicating that the abundance of denitrification genes was highly affected by soil biochemical factors (Figure 6). The soil EC and NO_3^- concentrations accounted for the largest proportion of variance in the abundance of microbial functional genes, followed by soil MBC, MBN, DOC, TDN and NH_4^+ concentrations, respectively. The soil EC and NO_3^- concentrations were positively, and MBN content was negatively correlated with the abundance of soil AOB. In addition, soil pH and MBN contents were positively, and EC, TDN and NH_4^+ concentrations were negatively correlated with the abundance of soil AOA. The results also indicated that the abundance of *narG*, *nirS* and *nosZ* were clustered close to the center of the plot, so the investigated biochemical parameters had a weak or no correlation with the abundance of these genes.

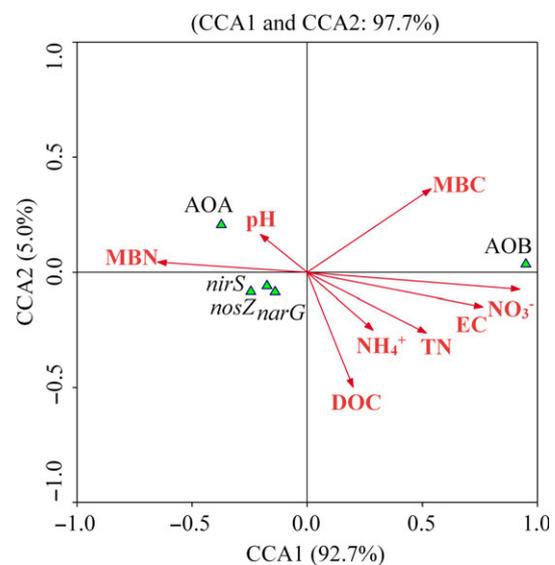


FIGURE 6 Biplot of canonical correspondence analysis (CCA) of the relationships between soil biochemical parameters and microbial functional gene abundances. The green triangles indicate the different functional groups, while the red arrows and their lengths indicate the direction and extent of effects of different biochemical parameters. The first two dimensions CCA1 and CCA2 represent the relationship between gene abundances and biochemical factors. The CCA1 and CCA2 represent 92.7% and 5.0% of the variation for the entire data. TDN refers to total dissolved nitrogen

3.6 | Principal component analysis (PCA) of N_2O fluxes, soil biochemical parameters and the abundance of functional genes

The PCA was carried out to distinguish the effect of different treatments on soil biochemical parameters and N_2O fluxes as well as determining the key drivers of N_2O

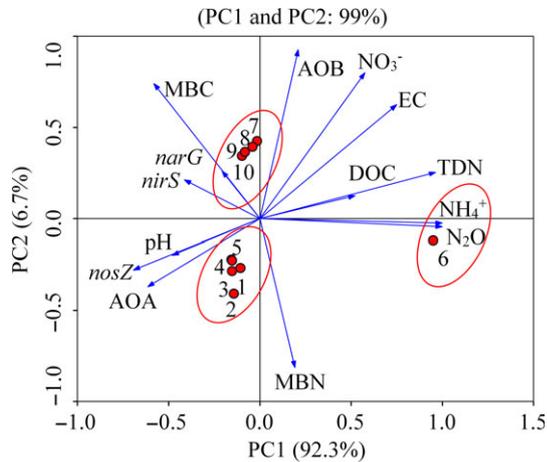


FIGURE 7 Ordination plot of principal component analysis (PCA) of loading values of all biochemical parameters including N_2O flux and separation of different treatments over the entire 90-day pot trial. Numbers in parentheses are percentage variance by each principal component (PC). The red circles represent soil and gas samples from 10 treatments. 1, CK; 2, P600; 3, P700; 4, P850; 5, P950; 6, CKN; 7, P600N; 8, P700N; 9, P850N; 10, P950N. See Figure 1 for abbreviations of biochar treatments

emissions. The first two ordination axes (PC1 and PC2) explained 99% of the variance (Figure 7). All treatments with nil N fertilization were grouped in a separate cluster from the N fertilized treatments. In the +N treatments, all biochar-amended treatments were in the same cluster and clearly separated from CKN treatment, with higher ordinate scores along PC1. Soil NH_4^+ , EC, TDN and N_2O emission demonstrated high positive loading values (≥ 0.7) to PC1, while *nosZ* gene showed high negative loading value (≤ -0.7). The above variables together contributed 68.3% to PC1. The abundance of AOB gene and NO_3^- concentration showed high positive loading values to PC2, while MBN showed high negative value. The above factors together contributed 60.7% to PC2. In addition, soil NH_4^+ and total dissolved N (TDN) were positively, and *nosZ* abundance was negatively correlated with N_2O emissions.

4 | DISCUSSION

4.1 | Effect of biochar on soil N dynamics, plant biomass and N uptake

Biochar amendments in this study significantly decreased soil NH_4^+ concentration and lowered the levels of soil NO_3^- at the later stage of the experiment (Figure 2c,d). This suggests that the application of biochar could limit mineral N availability by either increasing the mineral N fixation or facilitating N loss through non- N_2O emissions. Previous studies also reported that biochar addition to soils would significantly alter N cycling by decreasing mineral

N availability through direct adsorption or microbial immobilization (Clough & Condron, 2010; Lehmann et al., 2003). The findings of this study also indicated that biochar amendments had no significant impact on soil NH_4^+ and NO_3^- concentrations in the first days of the experiment, but over time (two weeks for NH_4^+ -N and four weeks for NO_3^- -N) biochar amendments reduced the concentration of soil mineral N (Figure 2c,d). In our study, C/N ratio in these high pyrolysis temperature biochars was extremely high (>500 ; Table 1). Also, obvious low input of labile N relative to the input of labile C (e.g., DOC) as evidenced by biochars' characteristics (Table 1) further supported the adsorption and microbial immobilization of mineral N to biochar surfaces. Thus, our study showed that the impact of biochar on the bioavailability of mineral N might be time-dependent because microorganisms would gradually utilize C to support microbial growth and consequently resulted in N immobilization. The increase in soil pH after biochar amendment might also cause a priming effect; thereby increasing soil native dissolved organic C.

Biochar amendments have been previously shown to have either positive, or negative or no effect on plant growth and crop productivity (Chan, Zwieten, Meszaros, Downie, & Joseph, 2007; Haider, Steffens, Moser, Müller, & Kammann, 2017; Jaiswal, Elad, Graber, & Frenkel, 2014; Nguyen, Scheer, Rowlings, & Grace, 2016). However, the findings of this experiment showed that biochar amendments significantly reduced plant biomass in the absence of N fertilizer, whereas combined application of biochar and N fertilizer increased plant biomass (Table 5). Considering the low N contents and high C/N ratios (>500) of the applied biochars, ryegrass growth in biochar-amended soil at -N treatments was likely to be limited by the N supply (e.g., low NH_4^+ and NO_3^-) in the N-deficient environment. This is in agreement with the findings of (Asai et al., 2009; Kammann et al., 2015) which reported a decrease in yield following pure application of biochar, without N fertilization, to an N-deficient soil. However, the increase in plant biomass in the presence of N fertilizer indicated that biochar application had positive interaction with additional N supply, which might indirectly improve soil growing condition and consequently enhancing plant growth. Our study showed that without biochar amendment conditions, N fertilization significantly increased plant N uptake, but not plant biomass (Table 5). This could be attributed to ryegrass absorbing more N in this N-deficient soil as a result of significant plant N content from CKN relative to CK, and N input might not be a limiting factor for the ryegrass growth under nonbiochar amendment conditions. However, Biochar application significantly decreased plant N content in the unfertilized and fertilized treatments, which is most likely attributed to lower mineral N (NH_4^+ and NO_3^-) bioavailability for plant uptake in the

biochar-amended treatments (Figure 2c, d). The decrease in plant N uptake observed at the biochar-amended treatments, in the absence of N fertilizer, was in contrast with previous studies which reported that biochar application increased N uptake in maize cropping system with nil N fertilization (Nguyen et al., 2016; Zhang et al., 2012). This was possibly related to the difference in biochar feedstock and application rate as well as investigated soil type (e.g., N content). However, in the presence of N fertilizer, plant N uptake was increased by the biochar amendment, with only P700N treatment showing a significant difference from the control. The higher plant N uptake in the P700N treatment compared with other N fertilizer treatments may be related to its higher biomass production, which was likely to result from the improvement of soil conditions, for plant growth. Biochar's structural investigation is warranted on how the biochar produced at high pyrolysis temperature would affect the soil properties and plant growth (Joseph, Kammann, & Shepherd, 2018).

4.2 | Effect of biochar on microbial functional gene abundance

A few studies have been carried out recently regarding the effects of biochar application on soil microbial community and the abundance of microbial functional genes (Harter et al., 2014; Van Zwieten et al., 2014; Xu et al., 2014). However, the mechanisms behind these complicated processes are still not clear. In this experiment, the effect of biochar on the abundance of N related microbial functional genes varied considerably with high pyrolysis temperature biochars and N fertilization. In the $-N$ treatments, biochar amendment did not show a significant impact on N related functional genes, which is consistent with the findings of Anderson, Hamonts, Clough, and Condon (2014) who reported that biochar did not influence soil N transformations or microbial community structure under ruminant urine patches. However, in the $+N$ treatments, high pyrolysis temperature biochars significantly affected the abundance of AOB, *narG* and *nosZ* genes. A significant increase in AOB abundance, in the P850N and P950N treatments, relative to the CKN was observed in the presence of N fertilizer. This could be attributed to higher intrinsic pH, total N and concentration of NO_3^- in these two biochars compared to the biochars produced at lower pyrolysis temperatures (P600N and P700N; Table 1). It might also be related to lower surface areas of these two high pyrolysis temperature biochars (P850N and P950N), which would lead to the lower NH_4^+ adsorption, contributing to higher available NH_4^+ and abundance of AOB. However, biochar amendments had no significant impact on the abundance of AOA, which is in accordance with previous studies (Ducey et al., 2013; Harter et al., 2014). It is

recognized that ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) play a vital role in the ammonia oxidation process in various environments (Chen, Zhu, Xia, Shen, & He, 2008; Francis, Beman, & Kuypers, 2007). The AOB relies on the oxidation of NH_4^+-N for energy production and growth (Arp, Chain, & Klotz, 2007), which could be responsible for lower concentration of NH_4^+ in the biochar treatments in the presence of N fertilizer. Biochar addition also increased *nosZ* gene abundance in both unfertilized and fertilized treatments, although only P950N showed a significant difference from CKN in the $+N$ treatments. Previous studies also reported that biochar amendment could enhance the abundance of *nosZ* gene (Ducey et al., 2013; Harter et al., 2014). Therefore, it is likely that the application of biochar to soil potentially enhanced the abundance of *nosZ* and might further affected denitrification processes (e.g., N_2O to N_2) in biochar-amended soils.

4.3 | Effect of biochar on N_2O emission

Previous studies have shown that biochars with different characteristics may affect N_2O emission differently (Ameloot et al., 2013; Spokas & Reicosky, 2009; Stewart et al., 2013). In this study, biochar amendments significantly reduced soil N_2O emissions in both $-N$ and $+N$ treatments, while the magnitude of N_2O reduction was higher in $+N$ treatments (75%–85%), in comparison with $-N$ treatments (33%–45%). This is in accordance with previous studies, which reported that biochar amendments were able to suppress soil N_2O emission from soils (Agegnehu, Bass, Nelson, & Bird, 2016; Case et al., 2015). Cayuela, Jeffery, and Zwieten (2015) found that the molar H/C ratio of biochar was a key factor in mitigating N_2O emission through meta-analysis, particularly for biochar with molar H/C ratio of <0.3 which was more effective in lowering N_2O emission than those with molar H/C ratio >0.5 . In our study, the molar H/C ratio of four different pyrolysis temperature biochars was <0.3 (Table 1), which indirectly confirmed that the molar H/C ratio was a key driver responsible for N_2O reduction from biochar-amended soils. However, biochars' effect on N_2O emissions did not significantly vary with pyrolysis temperature in the current study. This is in contrast with previous studies which reported a significant effect of pyrolysis temperature on N_2O emissions with less emissions at higher pyrolysis temperatures ($>600^\circ\text{C}$) compared to lower pyrolysis temperatures ($<500^\circ\text{C}$; Ameloot et al., 2013; Nelissen, Saha, Ruysschaert, & Boeckx, 2014). The reason for these discrepancies could be attributed to the very high biochar pyrolysis temperature and very low initial N substrate of the applied biochars in the current experiment, which resulted in a nonsignificant impact of pyrolysis temperature on N_2O emissions.

Different abiotic and biotic mechanisms have been suggested to be responsible for N₂O emissions (Harter et al., 2014; Lan et al., 2017; Quin, Joseph, & Husson, 2015). The principal component analysis (PCA) showed that NH₄⁺ and total dissolved N (TDN) contents had significant and positive correlated with N₂O emissions, while *nosZ* abundance had negative correlation with N₂O emissions in the peak of daily N₂O fluxes at day 42. Furthermore, these three variables together with daily N₂O fluxes contributed 58.6% to PC1. This indicates that a combination of both chemical and microbial factors such as NH₄⁺ and total dissolved N concentrations as well as *nosZ* gene abundance were the key drivers of N₂O emissions in this study. It has also been reported that the availability of NH₄⁺ and NO₃⁻ would decrease after biochar addition, probably due to the increase in N sorption by large surface areas of biochar and enhancing microbial immobilization (Singh, Hatton, Singh, Cowie, & Kathuria, 2010; Zwieten, Kimber, Morris, Downie, et al., 2010). This may lead to a decrease in N availability for nitrification and denitrification processes and consequently reduction in N₂O emission. Andersen and Petersen (2009) and Rezaei Rashti et al. (2016) reported that immobilization of N typically occurred rapidly after incorporation of organic residues (e.g., biochar) with high C/N ratios. Lehmann et al. (2003) also suggested that the application of high C/N ratio biochars could increase the NO₃⁻ immobilization by microorganisms and consequently reduce NO₃⁻ concentration in comparison with untreated soils. The use of high pyrolysis temperature biochars (600–950°C) in this study, with extremely high C:N ratios (> 500), indirectly confirmed that biochar amendments have a great potential to immobilize soil mineral N (NH₄⁺ and NO₃⁻) and further reduce N₂O emissions. The result of a 123-day incubation experiment by Zheng, Stewart, and Cotrufo (2012), using wood biochar, indicated that cumulative N₂O emissions were remarkably well explained by an increasing exponential relationship with soil total N contents across two temperate soils. Zwieten, Kimber, Morris, Downie, et al., 2010 also found that a decrease in NH₄⁺ and NO₃⁻ concentrations following green waste biochar application was likely to be responsible for the reduction in N₂O emissions. Therefore, it can be concluded that the reduction in N₂O emissions from biochar-amended soil, in the current experiment, might be due to the adsorption of NH₄⁺ by biochar with a consistent decline in soil NH₄⁺ concentrations.

Recent studies have also reported that biochar application may significantly increase *nosZ* gene abundance, which suggests that *nosZ* gene is likely to be one of the key factors responsible for N₂O reduction (Van Zwieten et al., 2014; Xu et al., 2014). In the current study, biochar application enhanced *nosZ* gene abundance, although the increasing effect was only significant in higher pyrolysis

temperatures (e.g., 950°C in +N treatment). The N₂O reductase gene (*nosZ*) dominates the process that converts N₂O to dinitrogen (N₂) gas (Harter et al., 2014). Thus, the increase in *nosZ* gene abundance would result in lower N₂O emissions. Although soil microbial analysis only occurred at the N₂O emission peak (day 42), which limits the explanation of treatments N₂O emission during the whole 90-day pot experiment, this sampling time could represent the larger variance over the whole experiment and the findings could partially explain the lower cumulative N₂O emission from biochar-amended soils. However, further investigations are required with more frequent sampling and a wide range of soil types to confirm the relationship between soil denitrifying functional genes and N₂O emissions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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