



## RESEARCH REVIEW

# Effects of biochar application on soil nitrogen transformation, microbial functional genes, enzyme activity, and plant nitrogen uptake: A meta-analysis of field studies

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## Abstract

Biochar application can influence soil nitrogen (N) cycle through biological and abiotic processes. However, studies on comprehensive examination of the effects of biochar application on microbially mediated N-cycling processes (N mineralization, nitrification, denitrification, and fixation) and soil N fate (i.e., plant N uptake, soil N<sub>2</sub>O emission, and N leaching) are warranted. Therefore, the aim of this study was to examine the effects of biochar application on soil N transformation, microbial functional gene abundance, enzyme activity, and plant N uptake. To achieve the objective of this study, a meta-analysis involving 131 peer-reviewed field experiments was conducted. Results showed that field application of biochar significantly enhanced soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> content, N mineralization, nitrification, N<sub>2</sub> fixation, and plant N uptake by 5.3%, 3.7%, 15.3%, 48.5%, 14.7%, and 18.3%, respectively, but reduced N<sub>2</sub>O emissions and N leaching by 14.9% and 10.9%, respectively. Biochar application also increased the abundance of soil denitrifying/nitrifying genes (*amoA*, *narG*, *nirS/nirK+S*, and *nosZ*), proportion of N<sub>2</sub> fixation bacteria, and N-acetyl-glucosaminidase activity by 18.6%–87.6%. Soil NO<sub>3</sub><sup>-</sup> content was positively correlated with *AOA-amoA* abundance, and soil N<sub>2</sub>O emission was positively correlated with the relative abundance of genes (e.g., *amoA*, *narG*, and *nirS/nirK*) involved in N<sub>2</sub>O production. Furthermore, long-term biochar application tended to increase *AOB-amoA* and *nirK+S* abundance, especially soil N<sub>2</sub>O emission and N leaching. Overall, the findings of this study indicated that

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biochar application accelerated microbially mediated N-cycling processes under field conditions, thereby enhancing soil N availability and plant productivity. However, long-term biochar application may increase N losses. Therefore, future studies should be conducted to examine the effect of long-term biochar application on the soil N cycle and the underlying microbial mechanisms.

#### KEYWORDS

biochar, functional genes, meta-analysis, soil enzyme activities, soil N cycle

## 1 | INTRODUCTION

Nitrogen (N) is one of the most critical nutrients for plant growth and food production, and the use of N fertilizers has remained a key strategy in agriculture (Zamanian et al., 2018; Zhang et al., 2015). However, excessive use of N fertilizers may aggravate the negative environmental footprint of agriculture by contributing to atmospheric greenhouse gas emissions, pollution of underground water, and eutrophication of surface water (Abdalla et al., 2019; Coskun et al., 2017). Recently, the application of biochar, a recalcitrant carbonaceous biomass product generated via pyrolysis, to agricultural soils has attracted considerable attention as a promising solution for reducing soil N losses and for enhancing the efficiency of N use (Lehmann et al., 2011; Liu et al., 2019). Several meta-analyses have been conducted and have confirmed that biochar application can effectively enhance plant productivity and food production as well as reduce N losses, such as those observed with N leaching and N<sub>2</sub>O emission (Borchard et al., 2018; Cayuela et al., 2014; Gao et al., 2019; He et al., 2017; Liu et al., 2018; Nguyen et al., 2017). Soil N transformation and cycle, to a substantial extent, is regulated by soil microorganisms. However, the effects and microbial mechanisms of biochar application on soil N processes remains to be examined.

Soil microorganisms regulate soil N transformation and cycle, primarily through the expression of their functional genes and the activities of their extracellular enzymes (Borchard et al., 2018; Liu et al., 2018). N-cycling enzymes in soil microbes regulate inorganic N availability via mineralization and hydrolysis (Gul & Whalen, 2016; Zhang, Xiang, et al., 2019). Ammonia-oxidizing genes (*amoA*) in ammonia-oxidizing microorganisms promote ammonia oxidation and N<sub>2</sub>O production during nitrification (Francis et al., 2007). During denitrification, nitrite reductase genes (*nirG*, *nirS*, and *nirK*) of denitrifying microorganisms mediate the rate-limiting step of NO<sub>3</sub><sup>-</sup> or

NO<sub>2</sub><sup>-</sup> to NO and N<sub>2</sub>O (Barnard et al., 2005), whereas the nitrous oxide reductase gene (*nosZ*, encoding N<sub>2</sub>O reductase) is responsible for the complete denitrification of N<sub>2</sub>O to N<sub>2</sub> (Braker et al., 2000; Harter et al., 2014). Finally, N<sub>2</sub> can be used by N<sub>2</sub>-fixing microorganisms to facilitate increase in plant N uptake, which is beneficial for improving plant root growth and the abundance of root secretion (the source of soil organic matter; Azeem et al., 2019). Biochar may be used to enhance soil aeration, to increase soil pH, to promote microbial N immobilization, to establish interactions with available soil N, to modify enzyme activities, and to potentially affect nitrifier and denitrifier communities. Numerous case studies have examined the effects of biochar on N mineralization, nitrification, and fixation, as well as the relevant genes and enzymes. However, the findings of such studies are inconclusive. For instance, some studies have reported that biochar addition can increase N-acetyl-glucosaminidase activity and the abundance of *nifH* (N<sub>2</sub> fixation gene), *amoA*, *narG*, *nirS/K*, and *nosZ* within the microbial communities (Anderson et al., 2014; Foster et al., 2016; Lan et al., 2019; Saarnio et al., 2013). In contrast, other studies have not reported a significant effect of biochar addition on the abundance of N-cycling microbial genes (Chen et al., 2015; Edwards et al., 2018), and few studies have reported a decrease in the abundance of *nifH* and N-cycling enzyme activity (Bai et al., 2015; Liang et al., 2014). A meta-analysis of studies reported on the abundance of microbial genes and enzyme activities in response to biochar application can help improve our understanding of soil N-cycling processes in terrestrial ecosystems.

Quantifying the effects of biochar on soil N cycle and the underlying microbial mechanism under field conditions remains a high priority. Under laboratory conditions, biochar application may boost its direct effects, such as absorbing NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and probably N<sub>2</sub>O, because of high application rates and homogenous biochar incorporation, and the results may be different from those

observed under field conditions (Cayuela et al., 2014; Nguyen et al., 2017). The effect of biochar addition in the field are subject to extended weathering, variable climatic conditions, and inherent spatial and temporal variability in factors that help regulate soil N cycle. Therefore, the findings and mechanisms that are reported under controlled laboratory conditions may not be observed under field conditions (Verhoeven et al., 2017). Indeed, several case studies have observed contrasting changes in soil N availability and a significantly lower mitigation potential of biochar for N<sub>2</sub>O emissions in field and laboratory experiments (Cayuela et al., 2013; Felber et al., 2014). A recent meta-analysis reported highly variable and even opposite responses of microbial N-cycling genes to biochar application in the field compared with results obtained in laboratory experiments. Moreover, the relationships between microbial N-cycling gene abundance and soil N availability or N<sub>2</sub>O emissions remains to be fully understood (Xiao et al., 2019). Although microbial mechanisms and processes may play key roles in regulating soil N cycle in response to biochar application, abiotic processes, such as N absorption and liming, may also affect the soil N cycle (Cayuela et al., 2014; Nguyen et al., 2017). Additionally, accumulating evidence indicates that the direct NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> sorption capacity of biochar in the soil decreases with biochar age, while the nutrient supply and structural characteristics of aging biochar improve soil microbial effects and functions (Martin et al., 2012; Nguyen et al., 2017). Therefore, long-term field experiments provide the opportunity to better evaluate the temporal patterns of microbially mediated N-cycling processes (N mineralization, nitrification, denitrification, and fixation) and soil N fate (i.e., plant N uptake, soil N<sub>2</sub>O emission, and N leaching) in response to biochar application.

The objectives of this study were to determine the effects of biochar application on microbially mediated N-cycling processes (i.e., N mineralization, fixation, and nitrification) under field conditions; to assess the underlying mechanism of microbial regulation of soil N processes and fate in response to biochar application; and to identify the major factors responsible for regulating the effects of biochar on the soil N cycle and relevant microbial properties. The hypotheses formulated for validation were as follows: (1) biochar application would enhance soil N mineralization, nitrification, denitrification, and fixation by stimulating the abundance of N-relevant microbial genes and enzyme activities; (2) the enhanced microbially mediated N-cycling processes under biochar application would increase soil N availability and plant N uptake, but might also result in greater soil N<sub>2</sub>O emission and N leaching; and (3) biochar load, experimental duration, climate, and soil factors could significantly alter the effects of biochar on the soil N cycle and relevant microbial properties.

## 2 | METHODOLOGY

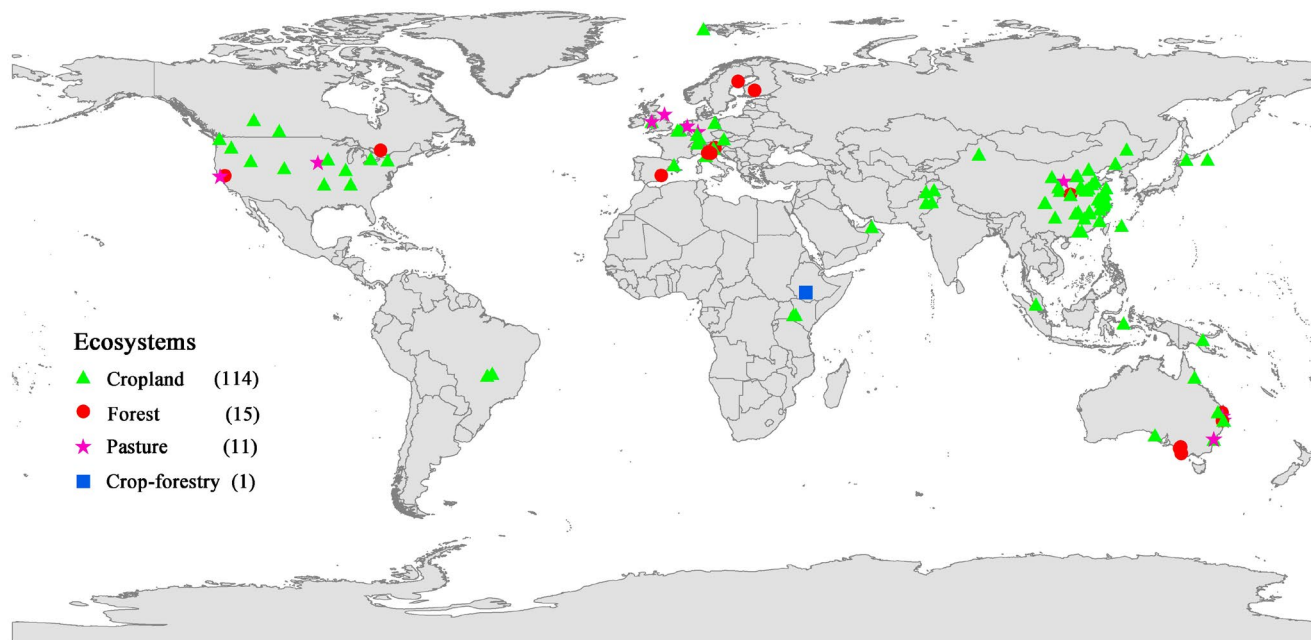
### 2.1 | Data sources and compilation

Published articles available on the Web of Science and Google Scholar were identified using the following search strings: biochar or black carbon; nitrogen, N, nitrate, ammonium, mineral N, or inorganic N; microorganism, microbial, gene, or enzyme; and soil. Data sets in the articles were selected for meta-analysis if they satisfied the following criteria: (1) the experiments were conducted in the field; (2) biochar was produced by anaerobically pyrolyzing organic materials and conformed to the standardized definition put forth by the International Biochar Initiative, but post-physicochemically modified biochar was not considered; (3) at least three replicates were considered for each treatment; (4) the control and biochar treatments were subjected to the same management practices, including tillage, irrigation, and fertilization; and (5) the original data on the relative index of soil microbes (genes) and enzymes on the soil N cycle were extractable from the manuscript (tables and/or figures), including the mean and standard deviation (SD) or standard error (SE). If SE was reported, it was converted to SD using the following equation:  $SD = SE \times \sqrt{n}$  (where  $n$  denotes the replicate number). Approximately 16.0% (21 out of 131) of the observations failed to report any information on the variance (SD or SE), but 1/10th value of the mean could be used to assign the SD in such cases (Luo et al., 2006; Zhang et al., 2018); (6) the biochar application rates were clearly reported; and (7) data on each soil N-cycling enzyme activity were obtained using the same measurement method, that is, the measurement of N-acetyl- $\beta$ -glucosaminidase and leucine-aminopeptidase activities by adopting fluorimetric microplate method (Nannipieri et al., 2018), and measurement of urease activity using the method proposed by Tabatabai and Bremner (1972). A total of 131 peer-reviewed field experiment data sets were selected for subsequent meta-analysis. The relevant data sets are included in the Materials S1.

This study focused on the evaluation of the effects of biochar application on soil N cycle and related microbial abundance and enzyme activities under field conditions. Parameters examined included soil N-cycling microbes, functional genes and enzymes (Table 1), soil N pools (total N [TN], dissolved organic N, microbial biomass N [MBN], NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup>), N transformations (nitrification and denitrification rates), N fixation (biological N<sub>2</sub> fixation, plant N uptake), and N losses (NH<sub>3</sub> volatilization, N<sub>2</sub>O emission, and N leaching). Data set for biochar characteristics included biochar feedstock type, pH, pyrolysis temperature (°C), C/N ratio, ash content (%), total N content (g kg<sup>-1</sup>), cation exchange capacity (cmol kg<sup>-1</sup>), and

**TABLE 1** Description of soil main nitrogen (N) cycle-associated microorganisms (including the N-cycling microbes and functional genes) and enzymes investigated in this study

Index	Function	Abbreviation
Soil nitrifying microorganisms (Edwards et al., 2018; Lan et al., 2019)		
Archaeal ammonia oxidizers ( <i>amoA</i> )	Oxidation of ammonia to $\text{NO}_3^-$	<i>AOA</i>
Bacterial ammonia oxidizers ( <i>amoA</i> )	Oxidation of ammonia to $\text{NO}_3^-$	<i>AOB</i>
Nitrifying bacteria	Oxidation of nitrite to nitrate	<i>NB</i>
Soil denitrifying microorganisms (Bai et al., 2015; Lan et al., 2019)		
Nitrate reductase gene	Reduction of nitrate to nitrite	<i>narG</i>
Nitrite reductase genes ( <i>nirK/S</i> )	Reduction of nitrite to NO	<i>nirK+S</i>
Nitrous oxide reductase gene	Reduction of $\text{N}_2\text{O}$ to $\text{N}_2$	<i>nosZ</i>
Denitrifying bacteria	Reduction of $\text{NO}_3^-$ to $\text{NO}_2^-$ , $\text{N}_2\text{O}$ or $\text{N}_2$	<i>DB</i>
Soil $\text{N}_2$ -fixing microorganisms (Bai et al., 2015; Chen et al., 2015)		
$\text{N}_2$ fixation gene	Control $\text{N}_2$ fixation yielding compound N	<i>nifH</i>
$\text{N}_2$ fixation bacteria	Fixation of $\text{N}_2$ to compound N	<i>NFB</i>
Soil N-cycling enzymes (Castaldi et al., 2011; Zhang, Jing, et al., 2019)		
N-Acetyl- $\beta$ -glucosaminidase	Hydrolysis of chitooligosaccharides	<i>NAG</i>
Leucine-aminopeptidase	Cleaving of peptide bonds in proteins	<i>LAP</i>
Urease	Hydrolysis of urea	<i>URE</i>
Denitrification enzyme	Reducing of nitrate yielding nitrite	<i>DE</i>

**FIGURE 1** Global distribution of the data sampling sites for soil N-cycling parameters of field experiments used in the meta-analysis. Ecosystems include forest, cropland, pasture, and crop-forestry (a mixture of crop farming and agri-forestry system)

specific surface area (SSA;  $\text{m}^2 \text{g}^{-1}$ ). Data sets for soil properties included those pertaining to soil texture, pH, clay content (%), soil organic C (SOC;  $\text{g kg}^{-1}$ ), and soil total N (STN;  $\text{g kg}^{-1}$ ). Other experimental parameters examined included biochar load/application rate ( $\text{t ha}^{-1}$ ), residence time of biochar in soil (experimental duration; year), and

fertilizer addition. The spatial distribution of matched studies, including different ecosystem types, is shown in Figure 1. Data on climate variables, including mean annual precipitation (MAP) and mean annual temperature (MAT) were obtained from the original and cited papers or were extracted from the database available at <http://>



www.worldclim.org/ using the location information if not directly reported.

## 2.2 | Data acquisition and analysis

The raw data were obtained numerically from text and tables or were extracted from the figures in the original papers using the Get-Data Graph Digitizer 2.26 software. Data sets on the effect of biochar addition at different sampling times (or at the uppermost soil layer) were selected (Zhang et al., 2018). SE and pH (CaCl<sub>2</sub>/KCl) values were converted to SD and pH (H<sub>2</sub>O) values, respectively (Nguyen et al., 2017). To maximize comparability, the explanatory variable data were categorized into different groups. Biochars were classified based on the following characteristics: feedstock materials, including wood (bamboo wood and wood residues), herbs (green waste, straw, grass, and corn stover), lignocellulosic waste (peanut, nut, oat hull, walnut shells, papermill residue, corn cobs, rice husk, and bagasse; Nguyen et al., 2017; Zhang et al., 2018); pyrolysis temperatures (low ≤400°C, medium 400–600°C, and high ≥600°C; Nguyen et al., 2017); biochar loads (<10, 10–20, 21–40, 41–80, and >80 t ha<sup>-1</sup>; Liu et al., 2018; Omondi et al., 2016); and the absence or presence of fertilizer. Experimental durations were categorized into <1, 1–3 years (including 1 year), and ≥3 years (Zhang, Xiang, et al., 2019). Soil textures were categorized into the following three groups: fine (clay, clay loam, silt clay loam, and silt clay), medium (loam, silt loam, loamy silt, and silt), and coarse (sandy loam, sandy clay loam, sandy silt, loamy sand, and sand) textured soils (Cayuela et al., 2014; Omondi et al., 2016). Soils were grouped according to pH values: ≤5, 5 < pH ≤ 6.5, 6.5 < pH ≤ 7.5, and >7.5. Soils were grouped according to STN values: <1, 1 ≤ STN ≤ 2, and >2 g kg<sup>-1</sup> (Borchard et al., 2018). Ecosystem types were grouped as forest, cropland, and pasture (Zhang et al., 2018).

The effect of biochar application was estimated for each observation and was expressed as the natural logarithm transformed of the response ratio (*R*) using the below-mentioned formula (Luo et al., 2006; Rosenberg et al., 2000):

$$R = \ln \left( \frac{X_t}{X_c} \right), \quad (1)$$

where *X<sub>t</sub>* and *X<sub>c</sub>* represent the results of the biochar and control treatments, respectively. The variance (*v<sub>R</sub>*) associated with the response ratio is calculated by using the following formula:

$$v_R = \frac{S_c^2}{n_c X_c^2} + \frac{S_t^2}{n_t X_t^2}, \quad (2)$$

where *S<sub>t</sub>* and *S<sub>c</sub>* represent the standard deviation values of the biochar and control treatments, respectively, and *n<sub>t</sub>* and *n<sub>c</sub>* denote the replicate number of the biochar and control treatments, respectively (Hedges et al., 1999). The effect sizes of the abovementioned categorized groups have been calculated using a categorical random effects model, in which the effect size of each observation is weighted by the inverse of the variance (Adams et al., 1997).

Mean effect sizes of each category and the 95% confidence intervals (CIs) generated via bootstrapping (999 iterations) were calculated using the MetaWin 2.1 software (Rosenberg et al., 2000). The effect sizes were converted to percentage change (*P<sub>c</sub>*; Jian et al., 2016) using the equation provided below:

$$P_c = 100 \% [\exp(R_+) - 1]. \quad (3)$$

The mean effect sizes were considered significantly different from zero if the 95% CIs did not exhibit overlapping with zero and were considered significantly different from each other if their 95% CIs did not show an overlap. The mean of all effect sizes combined was calculated for related soil microbial abundance and enzyme activity to soil N cycle under biochar addition.

In the meta-analysis discussed herein, fail-safe number and funnel plot were considered to elucidate the publication bias and the robustness of the data (Nguyen et al., 2017; Rosenthal & Rosnow, 1984; Rothstein et al., 2006), which were then compared using the formula  $5n + 1$  (*n* denotes the number of cases; Table S1; Figure S1). To evaluate the effects of biochar addition on soil N processes and N-cycling microbial properties among different categories, the heterogeneities (*Q<sub>B</sub>*) were calculated to examine the heterogeneity between groups across all data sets for a given response variable (Table S2). Significant difference between groups (*p* < 0.05) was determined using Chi-square test (Liu et al., 2018; Zhang et al., 2018; Zhang, Xiang, et al., 2019).

## 3 | RESULTS

### 3.1 | Effects of biochar application on soil N cycle under field conditions

Biochar application significantly increased soil TN, MBN, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> levels by 11.1%, 13.2%, 5.3%, and 3.7%, respectively (Table 2). Similarly, biochar application significantly increased N mineralization and nitrification rates by 15.3% and 48.5%, respectively (Figure 2a), but did not significantly affect denitrification rate (Figure 2a). Additionally, biochar application significantly enhanced plant N uptake and biological N<sub>2</sub>

fixation by 18.3% and 14.7%, respectively (Figure 2b). Regarding N losses, biochar application significantly reduced N<sub>2</sub>O emission and N leaching by 14.9% and 10.9%, respectively (Figure 2c).

### 3.2 | Effects of biochar application on soil microbial abundance and enzyme activity

Regarding N mineralization, biochar application significantly enhanced N-cycling enzyme levels and N-acetyl- $\beta$ -glucosaminidase activities by 20.9% and 87.6%, respectively (Figure 3a). Regarding nitrification and denitrification, biochar application obviously increased the abundance of soil *amoA* and nitrifying bacteria by 22.7%

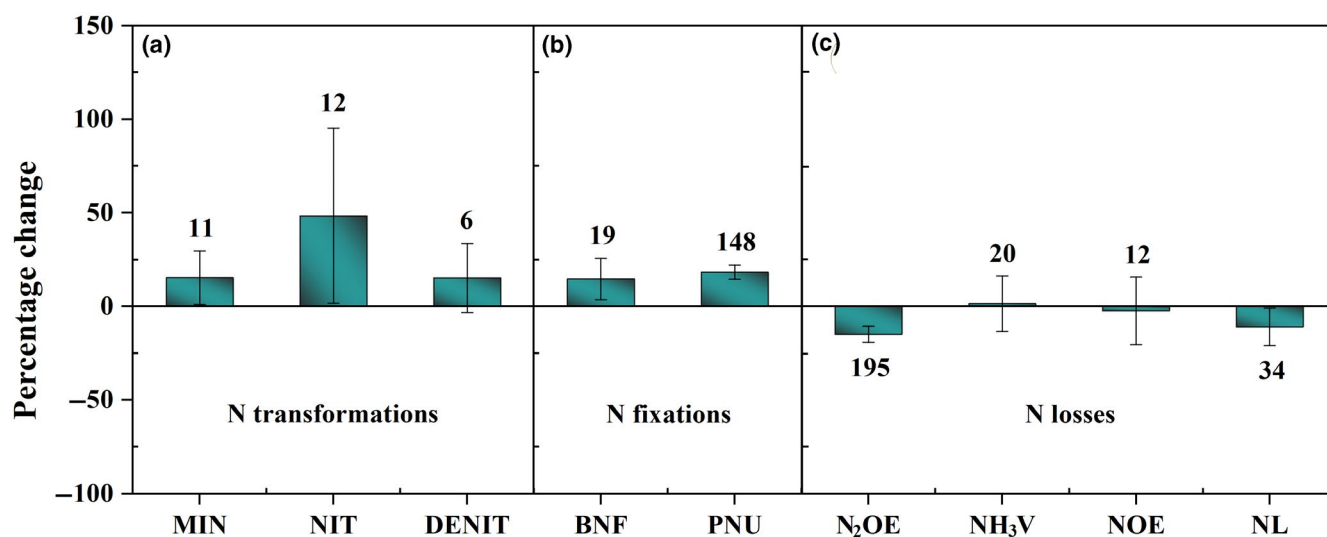
and 82.7%, respectively. Moreover, biochar application greatly increased the abundance of denitrification bacteria, with 57.1%, 19.2%, 23.7%, 36.8%, and 18.2% increase in the copy numbers of *narG*, *nirK+S*, *nirS*, and *nosZ*, respectively (Figure 3b). Regarding N<sub>2</sub> fixation, biochar application greatly increased N<sub>2</sub>-fixing bacteria abundance by 45.7%, but did not greatly affect the *nifH* copy number (Figure 3c).

### 3.3 | Effects of major control factors on soil microbial properties and N cycle

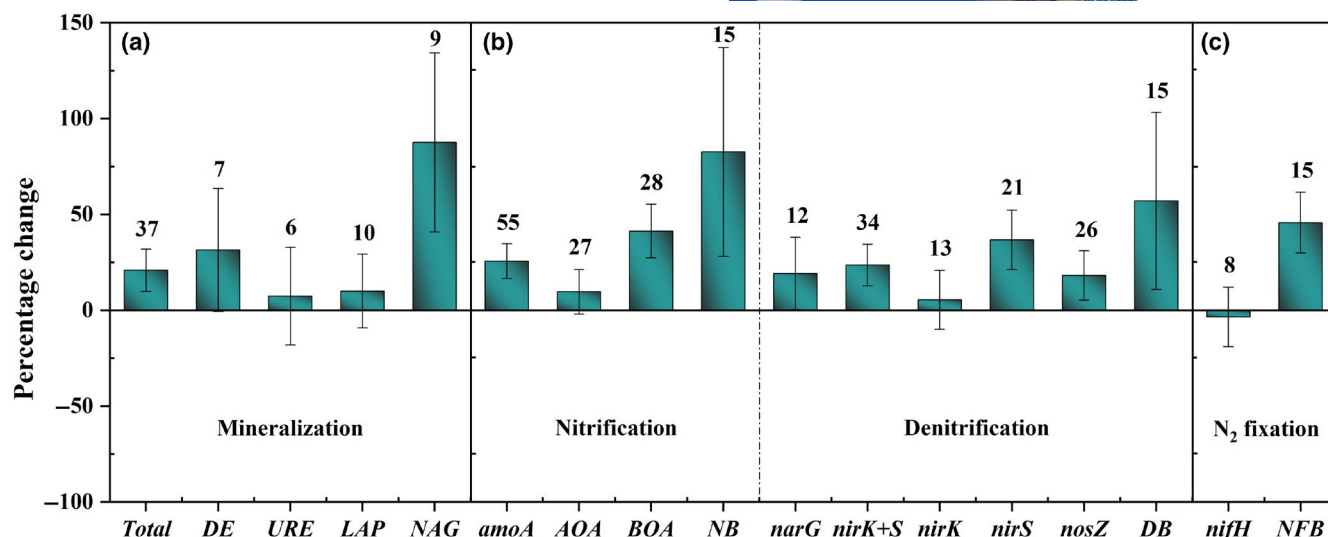
Biochar application the greatest improved the abundances of *amoA*, *nirK+S*, and *nosZ* in soil with pH  $\leq 5$  or pasture soil (Figure 4). The *amoA*, *nirK+S*, and *nosZ* contents were also the most significantly increased when the load and duration of biochar applications were 21–40 t ha<sup>-1</sup> and 1–3 years, respectively (Figure 4; Figure S2a). In contrast, biochar application reduced *amoA* abundance in most forest soils (Figure 4a; Table S2). Soil *amoA* abundance was significantly increased by the addition of biochar with high pH values (range: 9–10) and biochars produced using herb or low pyrolysis temperature (Figure 4a). Compared with other biochar types, application of biochar (pH < 8) produced using herb feedstock under low pyrolysis temperature enhanced *nirK+S* abundance in the most considerable manner, whereas application of biochar (pH range: 9–10) produced using lignocellulosic feedstock under conditions of subjection to medium pyrolysis temperature increased *nosZ* abundance in the most considerable

**TABLE 2** Effects of biochar application on soil nitrogen (N) pools, including total N (TN), microbial biomass N (MBN), total organic N (TON), total dissolved N (TDN), dissolved organic N (DON), and soil NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> contents

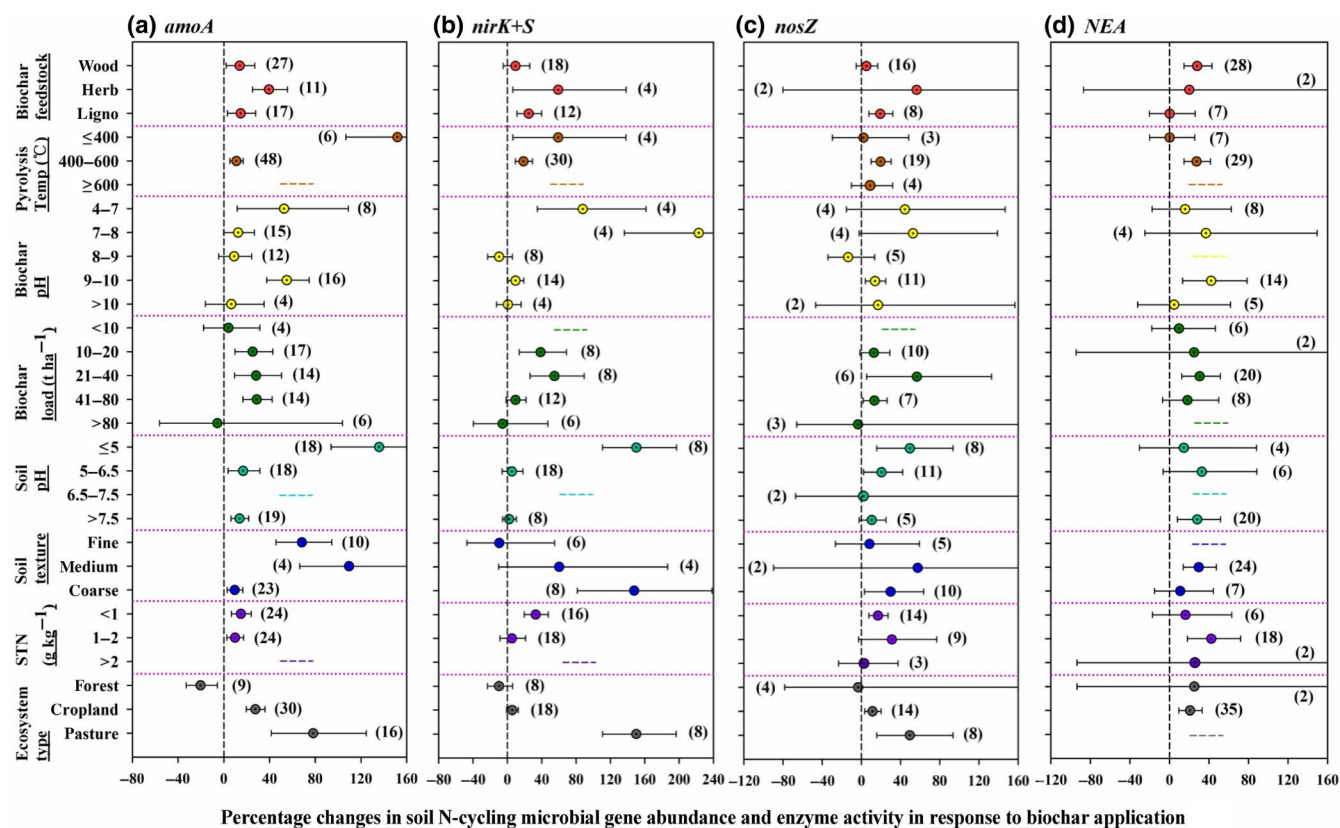
Index	Sample size	Effect size (%)	95% CI (%)
TN	330	11.10	9.43, 12.8
MBN	90	13.17	8.26, 18.3
TON	6	3.22	-16.3, 27.3
TDN	14	-7.60	-16.4, 2.12
DON	20	2.80	-6.07, 12.5
NH <sub>4</sub> <sup>+</sup>	181	5.30	2.49, 8.19
NO <sub>3</sub> <sup>-</sup>	196	3.73	1.08, 6.45



**FIGURE 2** Relative changes of index for major N-cycling processes in biochar-treated soils compared with controls. Bars indicate 95% confidence intervals, and the number of observations is displayed on the upper portion of the bar. (a) N transformations include soil N mineralization ratio (MIN), nitrification ratio (NIT), and denitrification ratio (DENIT); (b) N fixations include biological N<sub>2</sub> fixation (BNF) and plant N uptake (PNU); (c) N losses include soil NH<sub>3</sub> volatilization (NH<sub>3</sub>V), N<sub>2</sub>O (N<sub>2</sub>OE) and NO (NOE) emissions, and soil N leaching (NL)



**FIGURE 3** Means values and 95% confidence intervals of the percentage changes (%) of biochar effects on soil N-cycling (a) enzyme activity in the mineralization process, (b) the proportion of microorganisms and genes in the nitrification and denitrification processes, and (c) microbial and gene abundance in the  $N_2$  fixation. The abbreviations of the soil N-cycling microorganisms, genes, and enzymes are presented in the Table 1. Total represents total soil N-cycling enzyme activities. The sample size of each variable is displayed on the upper portion of the bar



**FIGURE 4** Effects of biochar application on the abundance of soil (a) *amoA*, (b) *nirK+S*, (c) *nosZ*, and (d) *NEA* (N-cycling enzyme activity). Ligno represents lignocellulosic. Bars indicate 95% confidence intervals. Data in brackets at right side of each panel represent the number of observations

manner (Figure 4b,c; Table S2). There was a significant increase in N-cycling enzyme activity in alkaline soils subjected to treatment with biochars (pH range: 9–10; loading

rate: 21–40 t ha<sup>-1</sup>) produced using wood feedstock under conditions of subjection to medium pyrolysis temperature (Figure 4d; Table S2).

Compared with biochar application only, combined biochar and N fertilizer application significantly increased *amoA*, *nirK+S*, and *nosZ* abundance (Table S3). Similarly, combined biochar and fertilizer application resulted in higher soil TN,  $\text{NH}_4^+$ , and MBN levels (Table S3). Particularly, combined biochar and fertilizer application for a period of more than 3 years significantly enhanced soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  levels (Figure S3). However, combined biochar and fertilizer application significantly decreased  $\text{N}_2\text{O}$  emission and N leaching by 17.8% and 36.2%, respectively (Table S3).

### 3.4 | Relationship between soil N cycle, control factors, and microbial properties

In the soil N pools, only  $\text{NO}_3^-$  level showed a significantly positive correlation with AOA (*amoA*) abundance ( $p < 0.01$ ; Table 3). Plant N uptake was not significantly correlated with *amoA*, *nirK+S*, and *nosZ* abundance ( $p > 0.05$ ; Table 3). Regarding N losses,  $\text{N}_2\text{O}$  emission was greatly and positively correlated with *amoA* ( $p < 0.05$ ), *nirK+S*, *nirK*, and *nirS* abundance ( $p < 0.01$ ). Additionally,  $\text{NH}_3$  volatilization was notably and negatively correlated with *amoA* abundance ( $p < 0.01$ ; Table 3).  $\text{N}_2\text{O}$  emission and N leaching were notably and positively correlated with experimental duration ( $p < 0.05$ ), indicating that long-term application of biochar might lead to an increase in  $\text{N}_2\text{O}$  emission and N leaching (Figures 5a,b, and 6).

Based on various conditions encountered in the study, it was observed that *amoA* abundance was significantly and negatively correlated with SOC and STN values ( $p < 0.05$ ; Table S4), whereas AOB (*amoA*) abundance was significantly and positively correlated with experimental duration ( $p < 0.01$ ; Figure 5c). Additionally,

*nirK+S* abundance was significantly and positively correlated with biochar TN ( $p < 0.05$ ) and experimental duration ( $p < 0.01$ ); however, it was negatively correlated with biochar pH ( $p < 0.01$ ) and with soil pH ( $p < 0.05$ ; Figure 5d; Table S4). Furthermore, *nosZ* abundance was significantly and positively correlated with biochar TN ( $p < 0.05$ ), while soil N-cycling enzyme activity was positively correlated with biochar SSA ( $p < 0.05$ ; Table S4).  $\ln R$  values of *amoA*, *nirK+S*, and *nosZ* abundance were greatly and positively correlated with MAP ( $p < 0.05$ ), whereas soil N-cycling enzyme activity was not significantly correlated with MAP and MAT ( $p > 0.05$ ; Figure S4).

## 4 | DISCUSSION

### 4.1 | The overall effect of biochar application on microbially mediated N-cycling processes

This meta-analysis discussed herein revealed that biochar application significantly increased soil N mineralization, nitrification, and fixation (Figure 2), a finding which was similar to that reported by Xiao et al. (2019). The increase in soil N mineralization, nitrification, and fixation was accompanied by the corresponding increase in the abundance of relevant genes and enzyme activities (Figure 3). The results confirmed the first hypothesis, as conceptualized and illustrated in Figure 6. The increase in biological  $\text{N}_2$  fixation by biochar application was related to an increase in the abundance of  $\text{N}_2$  fixation bacteria rather than *nifH* abundance (Figure 3c; Rondon et al., 2007). A previous study indicated that the presence of functional genes might not necessarily explain microbial functional

**TABLE 3** Pearson correlation coefficients between the response ratios (Equation 1) of soil N-cycling genes (*amoA*, AOA, AOB, *narG*, *nirK+S*, *nirK*, *nirS* and *nosZ*) abundance, N-cycling enzyme activity (NEA), and soil N-cycling of related factors (i.e., soil total nitrogen [N])

Index	<i>amoA</i>	AOA	AOB	<i>narG</i>	<i>nirK+S</i>	<i>nirK</i>	<i>nirS</i>	<i>nosZ</i>	NEA
TN	0.166	0.164	0.236	0.863	0.264	0.314	0.223	0.478	−0.059
DON	0.032	0.035	0.029	0.443	0.214	0.091	0.378	—	0.707
$\text{NH}_4^+$	0.102	−0.081	0.176	0.107	−0.131	−0.125	−0.143	−0.182	0.034
$\text{NO}_3^-$	0.185	0.619 <sup>b</sup>	−0.085	−0.159	0.115	−0.097	0.286	0.331	−0.214
$\text{N}_2\text{OE}$	0.334 <sup>a</sup>	−0.070	0.849 <sup>b</sup>	0.808 <sup>a</sup>	0.885 <sup>b</sup>	0.965 <sup>b</sup>	0.860 <sup>b</sup>	0.363	—
$\text{NH}_3\text{V}$	−0.892 <sup>b</sup>	−0.822	−0.962 <sup>b</sup>	—	—	—	—	—	—
PNU	−0.031	−0.420	0.397	−0.036	−0.089	0.088	−0.072	0.152	—
DENIT	—	—	—	—	0.467	0.438	0.535	−0.437	—

Abbreviations: *amoA*, including AOA and AOB; DENIT, denitrification ratio; DON, dissolved organic nitrogen;  $\text{N}_2\text{OE}$ ,  $\text{N}_2\text{O}$  emissions;  $\text{NH}_3\text{V}$ ,  $\text{NH}_3$  volatilization; *nirK+S*, including *nirK* and *nirS*; PNU, plant N uptake; TN, total nitrogen.

<sup>a</sup>Represent the significance levels of  $p < 0.05$ .

<sup>b</sup>Represent the significance levels of  $< 0.01$ .



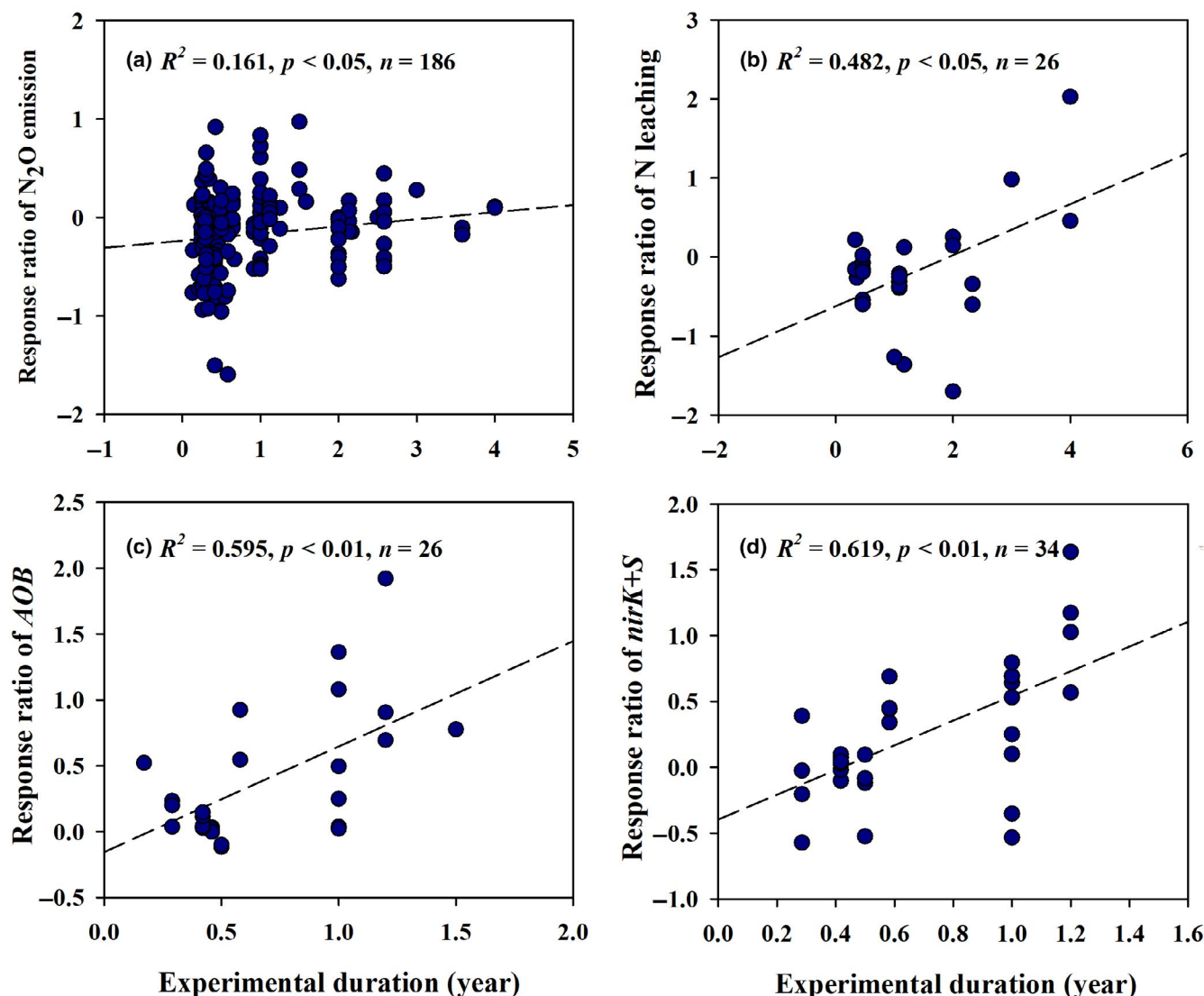


FIGURE 5 Experimental duration (year) is related with (a)  $N_2O$  emission, (b) N leaching, (c) AOB (*amoA* including AOB: Bacterial ammonia oxidizers), and (d) *nirK*+S abundance under biochar treatment conditions

capacity; however, functional gene (*nifH*) abundance may partly explain the nutrient transformations occurring in soil (Bai et al., 2015). Although direct relationships were not established in the present study, our results corroborate the hypothetical mechanism, in which soil N transformation and patterns are primarily subject to regulation by N relevant soil microbial genes and enzymes.

The increase in soil N mineralization, nitrification, denitrification, and fixation in response to biochar application may present with significant implications for soil N pools (Zhou et al., 2017). For instance, N mineralization can promote N transformation from organic to inorganic forms (e.g.,  $NH_4^+$  and  $NO_3^-$ ), while nitrification can further catalyze the conversion of  $NH_4^+$  to  $NO_3^-$  (Li et al., 2020; Nguyen et al., 2017).  $N_2$  may also be converted to  $NH_4^+$  via biological fixation (Azeem et al., 2019). Therefore, the increase in N mineralization,

nitrification, and biological  $N_2$  fixation might have contributed to the higher N availability in the present study, which confirmed the second hypothesis. The positive correlation observed between  $NO_3^-$  content and AOA-*amoA* abundance (Table 3) suggested that the AOA-dominant nitrification process was likely responsible for the dynamics of  $NO_3^-$  regulation in the soil. However, the increased N availability showed in the present study contradicted those reported in a previous meta-analysis, in which biochar application was observed to decrease soil N availability with an increase in *amoA* abundance (Liu et al., 2018, 2019; Xiao et al., 2019). Biochar and N fertilizers are usually applied concurrently in the field, and this stimulates natural soil organic matter mineralization and increases  $NO_3^-$  and  $NH_4^+$  production (Clough et al., 2013; Nguyen et al., 2017). Under field conditions, the pores and surface area of the applied biochar can

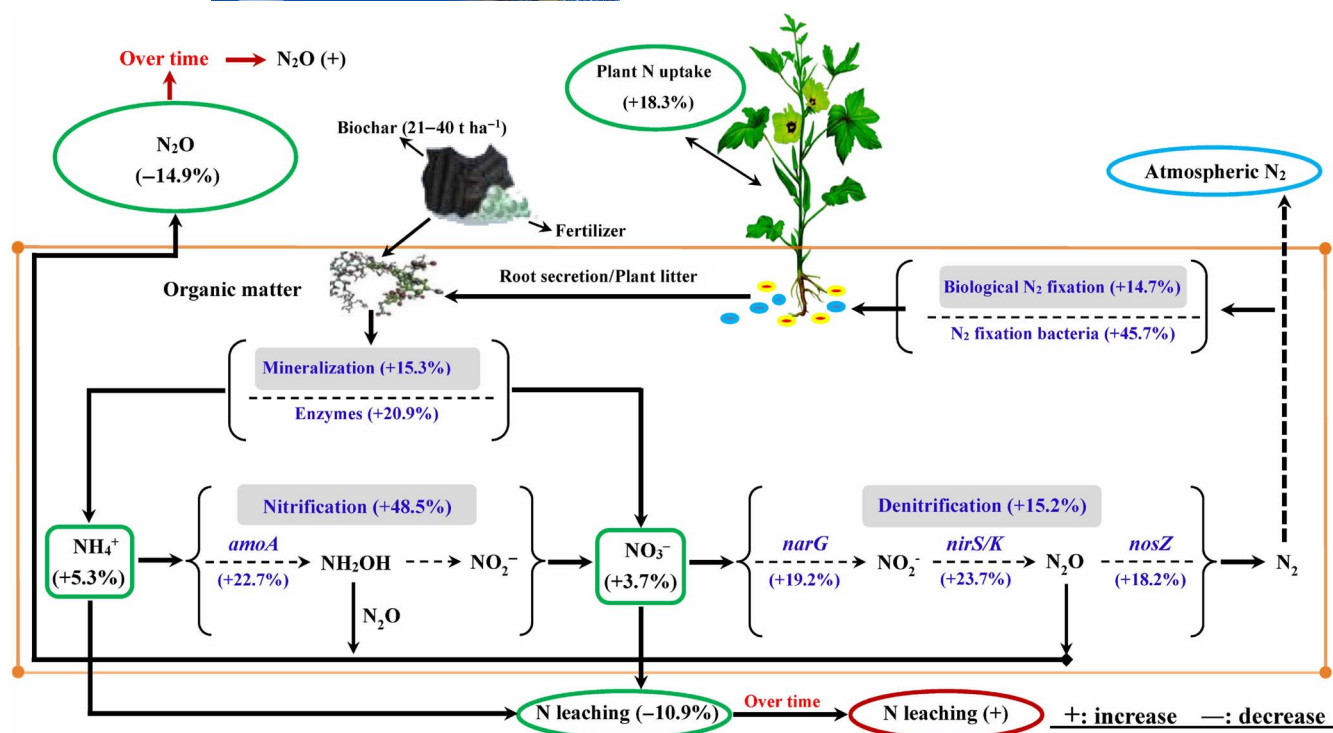


FIGURE 6 Summary of the potential mechanisms of related microbes or microbial genes, and enzyme activity on soil N-cycling processes in response to biochar application. Data represent the percentage change of the corresponding items induced by biochar application

be occupied by soil particles and organic matter (Zhang et al., 2018). This effect subsequently reduces the surface functional groups of biochar responsible for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  adsorption, and thus increasing soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  levels (Zhang et al., 2018; Zhou et al., 2017). The N compounds adsorbed on biochar surface can be desorbed over time and may be rendered bioavailable (Taghizadeh-Toosi et al., 2012).

Using laboratory data, previous meta-analyses showed that biochar application exerted significantly positive effects on N mineralization and fixation. However, compared with field trials, application loads are usually markedly higher and biochar-soil incorporation is reportedly more uniform in lab experiments; these aspects may be responsible for the results obtained in laboratory studies, which are different from those obtained in field studies, thus highlighting the importance of conducting field studies. For instance, the higher surface negatively charged functional groups (carboxyl and phenolic hydroxyl) and the establishment of electrostatic bridge-bonding with divalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) of biochar can aid the absorption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in laboratory experiments (Gai et al., 2014; Nguyen et al., 2017). Nitrogen is the most limiting nutrient for plant growth and food production (Zamanian et al., 2018). Increased soil N availability stimulates plant growth and increases plant N uptake (Figures 2a and 6). Therefore, the findings of this study showed that biochar

applications in field soils could effectively enhance plant productivity and food production (Liu et al., 2013), thus confirming the findings of laboratory experiments.

Theoretically, the increase in soil microbially mediated N-cycling in response to biochar application observed in the present study should increase soil N losses. Considering that nitrate demonstrates a strong electrostatic repulsion with most soil particles, the increased  $\text{NO}_3^-$  levels observed under biochar application may accelerate N losses via nitrate leaching (Gao et al., 2019; Lopez-Aizpun et al., 2020). Moreover, biochar-enhanced nitrification rate can aid the increased production of  $\text{N}_2\text{O}$ , resulting in greater  $\text{N}_2\text{O}$  losses (Cayuela et al., 2014). However, the results of the present study showed that biochar application decreased both N leaching and  $\text{N}_2\text{O}$  emission (Figure 5). Previous studies attributed the reduced N leaching to the direct adsorption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (e.g., Liu et al., 2019); however, soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  levels should decrease rather than increase N availability (Figure 1). Therefore, the biochar adsorption mechanism cannot be considered to completely explain the phenomenon of reduction in N leaching reported in the present study and may not be applicable under field conditions. Biochar application can increase soil water holding capacity due to its large SSA and high porosity, thereby reducing soil water percolation and N leaching (Novak et al., 2012). In the present study, biochar application significantly reduced soil water leaching by

19.2%–52.3% (Güereña et al., 2013; Li et al., 2017), compared with that observed in the control treatment.

Some studies have indicated that increase in denitrification rate (increased abundance of  $\text{N}_2\text{O}$ -reducing bacteria in certain cases) can promote the conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$ , thus reducing  $\text{N}_2\text{O}$  emission (Ameloot et al., 2016; Xiao et al., 2019). However, the present study indicated that biochar application increased *narG* and *nirS/K* abundance (the presteps of  $\text{N}_2\text{O}$  production) compared with *nosZ* abundance ( $\text{N}_2\text{O}$  consumption; Figure 3b). Moreover, soil  $\text{N}_2\text{O}$  emissions were positively correlated with *amoA*, *narG*, and *nirS/K* abundance, but did not exhibit correlation with *nosZ*, under the biochar application conditions (Table 3), and this finding was similar to the recent study (Xiao et al., 2019). Several abiotic processes via the direct effects of biochar application also contributed to the net reduction in  $\text{N}_2\text{O}$  emissions observed in this study (Figure 6; He et al., 2017; Verhoeven et al., 2017). Although the abiotic reactions of  $\text{N}_2\text{O}$  formation and consumption in soil have been rarely investigated, the potential role of biochar as a redox catalyst should not be neglected. Cayuela et al. (2013) have demonstrated that biochar does not induce abiotic  $\text{NO}_3^-$  reduction to  $\text{N}_2\text{O}$  through catalytic reactions. However, Cornelissen et al. (2013) showed that anhydrous biochars could directly adsorb  $\text{N}_2\text{O}$ , which could be considered as a plausible mechanism for  $\text{N}_2\text{O}$  emission reduction. The functions of biochar as an electronic conductor and electron shuttle can facilitate electron transport to soil denitrifying microorganisms (Cayuela et al., 2013; Sun et al., 2017). The hydrophilic property of biochar and its combination with soil microaggregates can also confer protection to soil microsites against exposure to oxygen, thus enabling the establishment of further reduced conditions favorable for  $\text{N}_2\text{O}$  conversion to  $\text{N}_2$  (Figure 6; Lehmann et al., 2005). Additionally, the depths of the biochar surface layer (i.e., 10–20 cm) should not affect field  $\text{N}_2\text{O}$  production (Van Groenigen et al., 2005). Nevertheless,  $\text{N}_2\text{O}$  can be adsorbed by biochar and/or may be reduced to  $\text{N}_2$  when it moves up through the soil-biochar profile (Clough et al., 2005; Quin et al., 2015).

## 4.2 | Effect of biochar application on microbially mediated N-cycling under different conditions

Unlike the contrasting results reported in previous studies, the present study showed similar temporal patterns in soil microbial mediated N-cycling and soil N losses in response to biochar application (Figure 5). In the present study, soil N availability increased with increase in biochar application duration, which might be attributable to a decrease in biochar sorption capacity for N (Figure S3;

Martin et al., 2012). The higher inorganic N content in the soil provides more N substrates for microorganisms, and hence affects nitrification and denitrification (Barnard et al., 2005). Similarly, there was an increase in *AOB-amoA* and *nirK+S* abundance with increase in biochar application duration (Figure 5c,d), indicating that biochar could further enhance soil nitrification and denitrification. The increase in soil microbially mediated N-cycling and the decrease in biochar absorbing effect over time can potentially increase soil N loss. The results suggested that biochar application increased N leaching and resulted in the exertion of a negligible effect on  $\text{N}_2\text{O}$  emissions in long-term experiments (Figures 5a,b, and 6). These findings suggest that the increase in microbially mediated N-cycling in response to biochar application may be observed in a more dominant manner in processes regulating the soil N cycle under field conditions. Moreover, long-term application of biochar may increase N loss under field conditions (Figure 6). Therefore, under field conditions, meticulous management of biochar application over time may exhibit more profound implications for climate change mitigation.

Soil pH, content of soil moisture, and  $\text{O}_2$  are the main variables affecting soil N-relevant microbial gene abundance (Gul & Whalen, 2016). Soil microorganisms often perform well in a moderate pH range and under conditions of appropriate levels of moisture and  $\text{O}_2$  (Gul et al., 2015). Previous reviews have shown that biochar application can be deemed a novel strategy to increase soil pH, supply base cations, and may consequently alter the soil microbial N cycle (Castaldi et al., 2011; Zhu et al., 2017). In the present study, biochar application significantly increased gene abundance in soils with  $\text{pH} \leq 5$  compared with that in soils of other pH values (Figure 4). Furthermore, a significantly positive relationship was observed between denitrifying gene abundance (*nirK+S* and *nosZ*) and MAP under biochar application conditions (Figure S4). Anaerobic conditions created via higher precipitation can help promote higher microbial abundance (i.e., *nirK* and *nosZ*) and denitrification (Anderson et al., 2014; Felber et al., 2014; Liu et al., 2018). Moreover, high SSA and a substantial proportion of small pores of biochar can help retain more soil moisture and may reduce  $\text{O}_2$  concentration (especially in sandy soils), thus promoting the growth of denitrifying microbes and increasing related gene abundance (Lehmann et al., 2011; Zhang, Jing, et al., 2019). However, the above-mentioned influencing factors do not play key roles in regulating the activities of N-cycling enzymes in response to biochar application under field conditions (Figure 4; Figure S4).

Furthermore, the present study showed that there was a significant increase in enzyme activity and the abundance of soil N-cycling genes, with the highest values

obtained for soils treated with biochar load of 21–40 t ha<sup>-1</sup> (Figure 4). High loads may enhance the capacity of biochar to absorb more N in the soil, subsequently limiting microbial N transformation and cycling (Gul & Whalen, 2016; Nguyen et al., 2017). Compared with natural ecosystems, biochar application significantly increased the enzyme activity and gene abundance in cropland and pasture, phenomena which might be attributed to fertilizers used in agriculture (Liu et al., 2018). The use of N fertilizers has remained a critical strategy in agriculture, and may provide more N substrates, thus increasing the abundance of N-cycling genes and enzyme activity (Ouyang et al., 2018). Moreover, compared with biochar application only, combined fertilizer and biochar application significantly increased the abundance of N-cycling genes and soil N cycle (Table S3).

Overall, the findings of present study showed that the effect of biochar application on soil N cycle and relevant microbial properties was dependent on biochar load, experimental duration, climate, and soil factors. Nevertheless, it should be noted that extremely few field studies and limited data acquired from Canada, Africa, and Russia were used in the present study. Therefore, it is necessary to conduct more extended field experiments to examine the different factors and long-term effects of biochar application on the soil N cycle and the underlying mechanisms.

## 5 | CONCLUSIONS

The findings of this study showed that biochar application accelerated microbially mediated N-cycling processes (mineralization, nitrification, denitrification, fixation, and relevant microbial gene abundances and enzyme activities), which enhanced soil N availability and plant N uptake. Despite contrasting results obtained on the effects of biochar application on microbially mediated N-cycling processes and soil N losses, similar temporal patterns were observed in the present study. Particularly, there was a decrease in biochar N adsorption with increase in treatment duration, which might pose potential ecological risk in the long run. Moreover, biochar application (load of 21–40 t ha<sup>-1</sup>) to acidic and fertilized soils, particularly in high precipitation regions, was favorable for enhancing N-relevant microbial gene abundance and enzyme activity. The information obtained from the present study is valuable for global N management.

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## CONFLICT OF INTEREST

All 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.


## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of the article at the publisher's website.

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