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The effect of biochar amendment on N-cycling genes in soils: A meta-analysis

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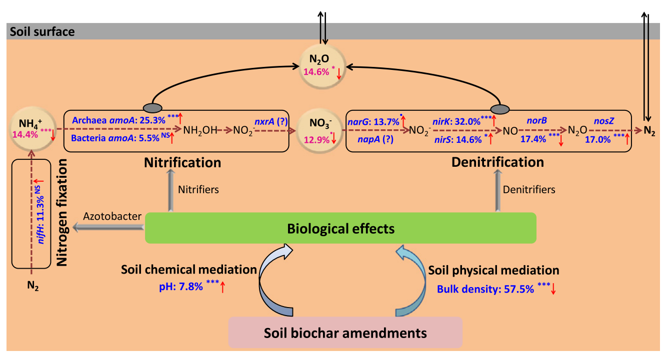
HIGHLIGHTS

- Biochar amendment overall had no significant impact on the abundance of *nifH* and AOB.
- Biochar amendment overall increased the abundance of AOA, *nirK*, *nirS* and *nosZ*.
- Soil pH was an important factor regulating the response of N-cycling genes to biochar addition.
- Biochar amendment overall decreased mineral nitrogen content and inhabited soil N_2O emission.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrogen (N) cycling by soil microbes can be estimated by quantifying the abundance of microbial functional genes (MFG) involved in N-transformation processes. In agro-ecosystems, biochars are regularly applied for increasing soil fertility and stability. In turn, it has been shown that biochar amendment can alter soil N cycling by altering MFG abundance and richness. However, the general patterns and mechanisms of how biochar amendment modifies N-cycling gene abundance have not been synthesized to date. Here, we addressed this knowledge gap by performing a meta-analysis of existing literatures up to 2019. We included five main marker genes involved in N cycling: *nifH*, *amoA*, *nirK*, *nirS* and *nosZ*. We found that biochar addition significantly increased the abundance of ammonia-oxidizing archaea (AOA), *nirK*, *nirS* and *nosZ* by an average of 25.3%, 32.0%, 14.6% and 17.0%, respectively. Particularly, biochar amendment increased the abundances of most N-cycling genes when soil pH changed from very acidic (pH < 5) to acidic (pH: 5.5–6.5). Experimental conditions, cover plants, biochar pyrolysis temperature and fertilizer application were also important factors regulating the response of most N-cycling genes to biochar amendment. Moreover, soil pH significantly correlated with ammonia-oxidizing bacteria (AOB) abundance, while we found that most genes involved in nitrification and denitrification were not significantly correlated with each other across studies. Our results contribute to developing quantitative models of microbially-mediated N-transforming processes in response to biochar addition, and stimulate research on how to use biochar amendment for reducing reactive N gas emissions and enhancing N bioavailability to crop plants in agro-ecosystems.

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1. Introduction

The biogeochemical nitrogen (N) cycling, through fixation, nitrification, and denitrification processes, converts N into multiple chemical forms as it circulates among atmosphere, terrestrial, and marine ecosystems (Thomazo and Papineau, 2013). In agro-ecosystems, bioavailable N is generally a limiting resource, and agricultural soils constantly need to be augmented with external bioavailable N for improving soil fertility and crop yields (Greenwood, 1982), and to cope with the widespread N losses into the environment (Steiner et al., 2010; Hidayat et al., 2012). Soil nitrate leaching leads to eutrophication and groundwater contamination, soil nitrogen oxide release intensifies atmospheric pollution (e.g. acid rain) and global warming (Norton and Stark, 2011; Sutton et al., 2011; Suddick et al., 2013). Therefore, environmental-friendly strategies which incorporate N-cycling processes to control and mitigate N losses from agricultural sources need to be further studied (Stark and Richards, 2008).

The N-transformation processes are largely driven by a diverse range of microorganisms, including ammonia-oxidizing bacteria and archaea, nitrite-reducing bacteria and denitrifying bacteria (Carey et al., 2016; Ouyang et al., 2018), which possess multiple microbial functional genes (MFG). Specifically, the first and rate-limiting step of the nitrification process, ammonia oxidation, is enforced by AOA or AOB, which carry the *amoA* gene encoding for the ammonia monooxygenase (Leininger et al., 2006; Pester et al., 2012). The denitrification process requires the *narG* and *napA* genes, encoding for the subunits of two distinct nitrate reductases: membrane-bound nitrate reductase (NAR) and periplasmic nitrate reductase (NAP), which mediate the reduction of nitrate to nitrite in both denitrification and dissimilatory nitrate reduction to ammonium. Subsequently, nitrite reductase genes (*nirS*/*nirK)* mediate the reduction of NO_2^- to NO, which is the rate-limiting step of denitrification (Braker et al., 2000; Kuypers et al., 2018). The *norB* gene, which encodes for the nitric oxide reductase, is responsible for the reduction of NO to N_2O (Pohlmann et al., 2000). Finally, the *nosZ* gene, which encodes for the N_2O reductase, is responsible for the complete denitrification of N_2O to N_2 (Henry et al., 2006). The abundance and diversity of MFG involved in N-transformation processes are often used to represent the abundance and diversity of N-cycling microbial communities in soils. Thereby, quantification and characterization of MFG involved in the N cycling could offer an approach to directly link microbial groups to soil characteristics and N transformation in terrestrial ecosystems.

Biochar is a carbon-rich product derived from the pyrolysis of different feedstocks, such as crop residues, wood chips, sludge or manure (Liu et al., 2014; Bai et al., 2015; Lan et al., 2019). Biochar has been applied to soils as an optional amendment to improve soil physico-chemical and biological properties, reduce greenhouse gas emissions, sequester carbon, enhance soil fertility and crop yields (Jeffery et al., 2011; Biederman and Harpole, 2013). Moreover, an increasing number of studies show that biochar amendments can alter soil microbial communities (Gul et al., 2015), and N-cycling gene abundance through changing soil physico-chemical properties (Ducey et al., 2013; Zhang et al., 2017b; Yu et al., 2019). For instance, Ducey et al. (2013) found that activated switchgrass-derived biochar increased the abundance of *nifH* and *nirS*, while the abundance of *amoA* and *nosZ* in wheat-planted soil was increased by maize straw-based biochar (Liu et al., 2017). Therefore, the effect of biochar on microbially-mediated N-cycling processes, including nitrification, denitrification and N fixation has been previously investigated in several systems (Ducey et al., 2013; Anderson et al., 2014; Bai et al., 2015; Liu et al., 2017). However, the direction and magnitude of the effect of biochar on N-cycling gene abundance are still poorly understood.

The impact of biochar addition on N-cycling gene abundance ranges from negative to positive (Ducey et al., 2013; Wang et al., 2015; Zhang et al., 2017b; Lan et al., 2018) and is strongly dependent on the biochar feedstock and the pyrolysis temperature, soil properties and cover plant types (Liu et al., 2014; He et al., 2015; Hagemann et al., 2017; Lan et al., 2019). For instance, at the same application rate such as 8% dry weight, the copy number of *amoA* in soils was higher for dairy manure-derived biochar than those for straw-derived biochar (Liu et al., 2014). As another example of context dependency, the copy number of *nirS* in soil was higher in soils added with 350 °C-produced biochar than those soils added with 500 °C-produced biochar (Liu et al., 2014). Moreover, recent studies have shown that biochar amendment can modify soil pH, in turn affecting the abundance and diversity of N-cycling genes (Hallin et al., 2009; Prosser and Nicol, 2012). For instance, Van Zwieten et al. (2014) suggested that biochar addition to the soil, most likely through an increase in soil pH, increased the abundance of *nosZ* transcripts and hence reduced the emissions of N_2O. Nevertheless, biochar-mediated changes in soil pH usually depend on biochar production, temperature, and ash content of the feedstock (Mukherjee et al., 2011; Slavich et al., 2013). Generally, alkalinity of biochars increases with increasing pyrolysis temperature (Yuan et al., 2011). Lan et al. (2018) found that, under nil nitrogen fertilization, the pH value of acidic soils was more enhenced by 700 °C-produced biochar than by 600 °C-produced biochar. Besides, soil N fertilization (mineral N VS organic N) and cover plant (no-planting VS planting) often result in distinct changes in N-cycling gene abundances in response to biochar application (Xu et al., 2014; Tan et al., 2018). Therefore, the effect of biochar on soil N-cycling gene abundances seems to be dependent on experimental system including cover plant type, biochar properties (Liu et al., 2014; Tan et al., 2018).

To address such widespread context-dependency in a quantitative manner, we here performed a meta-analysis of 36 studies that, to date, have investigated the effect of biochar amendment on the abundance of N-cycling genes (*amoA*, *narG*, *nirK*, *nirS*, *norB* and *nosZ*) in soils. We aimed to address the following questions: (1) Does biochar amendment change N-cycling gene abundance in soils? (2) What factors control the response of N-cycling gene abundance to biochar amendment? (3) Is there any relationship among different N-cycling gene abundance or between N-cycling gene abundance and soil properties in response to biochar amendment?

2. Material and methods

2.1. Data collection

The dataset was compiled by conducting keyword searches in the ISI Web of Science up to February 2019, using combinations of relevant terms ("biochar", "Nitrogen fixation or *nifH*", "ammonia-oxidizing, *amoA*, AOA, or AOB", "nitrite reducing bacteria, *nirS,* or *nirK*", "nitrous oxide-reducing bacteria or *nosZ*", "nitrogen, NH_4^+, or NO_3^-, or N_2O"). Additional searches using the same keywords were conducted in the Google Scholar (Google, Mountain View, CA, USA) and China National Knowledge Infrastructure (CNKI, Beijing, China). Studies were vetted using the following inclusion criteria: (1) the study included soils that were subjected to at least two treatments: biochar amendment and control treatment (without biochar amendment); (2) the study included a quantitative measurement of at least one functional gene involved in N cycling (e.g. *nifH*, *amoA*, *nirS*/*nirK*, *nosZ* gene); (3) the study reported at least one parameter of soil nitrogen composition (e.g. soil nitrate nitrogen, ammonium nitrogen or N_2O emission); (4) the study included means, standard errors (SE) or standard deviations (SD), and at least three independent replicates of each treatment were reported or could be calculated. As agro-systems rely on fertilization to grant crop production, we also included fertilized plots (NPK, but no biochar) as controls, which were compared to the fertilized plots with biochar addition as treatment (NPK, with biochar). In total, the searches yielded 36 papers published between 2013 and 2019 that met the criteria (See Appendix S1).

In order to take full advantage of the published results, multiple outcomes were included in our analyses when data were reported from several independent experiments (i.e. experiments tested on different plant species or treatments with different feedstock, pyrolysis temperature, application rate of biochar). However, only one measurement from each experimental replicate was included to maximize independence among measurements. For instance, in studies where *amoA* gene abundances were measured multiple times from the same experimental unit, we restricted our analyses to the latest time point. In addition, when multiple soil depths were assessed, we used only the shallowest depth. When available, we also included data that measured biochar effect on soil physico-chemical properties (e.g. pH, bulk density) and other N-cycling pathway genes (e.g. *narG, napA and norB*) besides the five main N-cycling marker genes in same selected publication (Appendix S1). If SE was reported, we transformed it to SD, using formula SD = SE∗sqrt (n). If data were presented in graphical form, we extracted data points using the getdata software (http://www.getdata-graph-digitizer.com).

In addition, to examine the overall effect of biochar amendment on the N-cycling gene abundance, a major goal of this meta-analysis was to determine whether particular experimental approaches or systems modify how the N-cycling gene abundance respond to biochar amendment. Therefore, when possible, categorical variables were collected from each study to analyse the variability among N-cycling gene abundance in response to biochar addition. The categorical variables were: (1) experimental condition (incubation, pot or field); (2) cover plant (wild plant species, crop species, tree, no-plant); (3) soil pH (very acidic: <5.5, acidic: 5.5–6.5, neutral: 6.5–7.5, alkaline: >7.5) (Gao et al., 2019); (4) biochar feedstock (wood, sludge, manure, crop residue, biowaste); (5) biochar pyrolysis temperature (<400, 400–500, >500 °C), (6) biochar application rate (10 t ha^{-1}, 10–20 t ha^{-1}, 20–40 t ha^{-1}, 40 t ha^{-1}) (Gao et al., 2019), (7) fertilizer application (organic fertilizer, inorganic fertilizer, no-fertilizer).

2.2. Meta-analysis

Biochar-mediated effect sizes on the N-cycling gene abundance were calculated using the natural logarithm of the response ratio (lnR) (Hedges et al., 1999) of the mean responses in the presence of biochar (T) divided by the response in the absence of biochar (C) such that lnR = ln(T/C). For interpretation of the results, mean effects and confidence intervals were back-transformed, using the formula: (EXP(lnR) − 1) × 100 and reported as the percentage changes between control and biochar additions. Therefore, effect sizes with positive value indicate that biochar amendment increases N-cycling gene abundance. The variance of lnR was calculated as:

$$v_i = \frac{S_T{}^2}{n_T X_T{}^2} + \frac{S_C{}^2}{n_C X_C{}^2}$$

The meta-analysis was performed with the “metaphor” package (Viechtbauer, 2010) in R (R Development Core Team, 2015). First, we estimated the overall effect of biochar amendment on the N-cycling gene abundance and soil physico-chemical properties. As the true effects almost certainly varied across trials due to the non-identical experimental systems, we tested the overall effect size using a random-effects model rather than a fixed-model, which assumes the same value or true effect for all trials. In this analysis, individual effect sizes were weighted by the reciprocal of the sum of the variance between-study and sampling variance within study. The restricted maximum likelihood method (REML) was used to estimate between-study variance. The mean effect size was considered as significantly different from zero if its 95% confidence intervals (CIs) did not include zero (Koricheva et al., 2013)

We assessed potential publication bias in the overall database, using funnel plot and the “trim and fill” method (Jennions et al., 2013). In order to assess the robustness of the observed overall effects of biochar amendment on the N-cycling gene abundance, fail-safe numbers (Nfs) were calculated, using Rosenberg's weighted method ($\alpha = 0.05$) (Rosenberg, 2005). Rosenberg's Nfs indicates how many studies reporting zero effect size would need to be added to the meta-analysis to render the observed effect non-significantly different from zero (Rosenberg, 2005), and if Nfs > 5 × n + 10, the result is considered robust despite the possibility for publication bias (Jennions et al., 2013).

Finally, we performed meta-regressions to explore how multiple moderator variables could affect the biochar-mediated effect size on the N-cycling gene abundance. Meta-regressions are more effective than standard meta-analytic techniques at examining the impact of moderator variables for studying effect sizes (Benton, 2014). We used a mixed-effects model to estimate the effect of each moderator (experimental condition, cover plant, biochar feedstock, biochar pyrolysis temperature, biochar application rate, fertilizer application) on the magnitude of biochar effect. This model assumes that differences among studies within a group are due to random variation, whereas variation between groups is fixed. With this model, the between-group homogeneity (Q_b) was used to estimate the significance of each categorical moderator (Koricheva et al., 2013). If the Q_b was significant, we inferred that the mean effect size differed between moderator levels, and two moderator levels were considered to be significantly different from one another if their 95% CIs did not overlap.

3. Results

3.1. Overall effect of biochar on soil physico-chemical properties and N-cycling gene abundance

When combined across all observations, biochar amendment led to significant changes in soil physico-chemical properties: overall increased soil pH by 7.8% (n = 136, Nfs = 348,739), and decreased soil bulk density by 57.5% (n = 12, Nfs = 542) (Fig. 1). For soil mineral N transformation, biochar amendment decreased soil NH_4^+ by 14.4% (n = 124, Nfs = 12,375) and soil NO_3^- by 12.9% (n = 117, Nfs = 19,611) (Fig. 1).

Moreover, biochar amendment had no significant effect on the abundance of *nifH* and ammonia-oxidizing bacteria (AOB) *amoA* (Fig. 1), but significantly increased the abundance of ammonia-oxidizing archaea (AOA) *amoA* by 25.3% (n = 125, Nfs = 2392), *nirK* by 32.0% (n = 76, Nfs = 6906), *nirS* by 14.6% (n = 111, Nfs = 384) and *nosZ* by 17.0% (n = 99, Nfs = 6477) (Fig. 1). The ‘trim and fill’ method detected none missing studies on right and left sides for the mean effect size of biochar amendment on the abundance of AOA, AOB, *nirS*/*nirK* and *nosZ*, suggesting that there was no significant publication bias. Biochar-mediated expression of *amoA* abundances in AOA (n = 125) were 4.6 times more of that in AOB (n = 144) across all studies. Additionally, biochar amendment increased the *narG* abundance by 13.7% (n = 35, Nfs = 192) but decreased *norB* abundance by 17.4% (n = 3, Nfs = 118). Ultimately, biochar amendment inhibited cumulative N_2O emission by 14.6% (n = 79, Nfs = 5597).

3.2. Impact of moderating variables on soil N-fixation gene abundance

The abundance of *nifH* in response to biochar amendment was dependent on experimental condition (p = 0.001), cover plant (p = 0.002), biochar pyrolysis temperature (p < 0.001) (Fig. 2). More specifically, biochar amendment notably increased *nifH* abundance by 44.5% with no plant cover under incubation experimental condition, and by 88.0% when biochar was produced under low pyrolysis temperature (<400 °C) (Fig. 2). However, biochar amendment significantly decreased *nifH* abundance by 36.0% with potting wild plant species (Fig. 2).

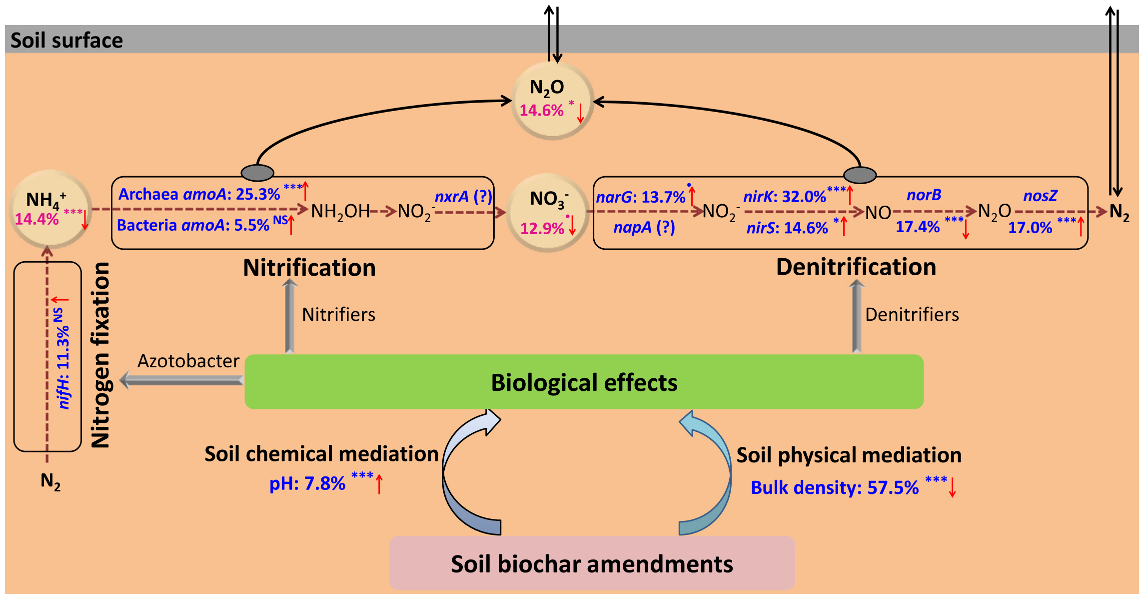

Fig. 1. The magnitude of biochar effect on the key regulation processes of soil N cycling (• $p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS non-significance), "↑" and "↓" mean biochar-mediated promoting and inhibiting effects on N cycling genes, respectively. *nifH* (encoding nitrogenase; key enzyme for N fixation), *amoA* (encoding ammonia monooxygenase; key enzyme for nitrification), *nxrA* (encoding nitrite oxidoreductase, enzyme for nitrification), *NarG/NapA* (encoding nitrate reductase; enzyme for denitrification), *nirK/nirS* (encoding nitrite reductase; key enzyme for denitrification), *norB* (encoding nitric oxide reductase; enzyme for denitrification) and *nosZ* (encoding nitrous oxide reductase; key enzyme for denitrification).

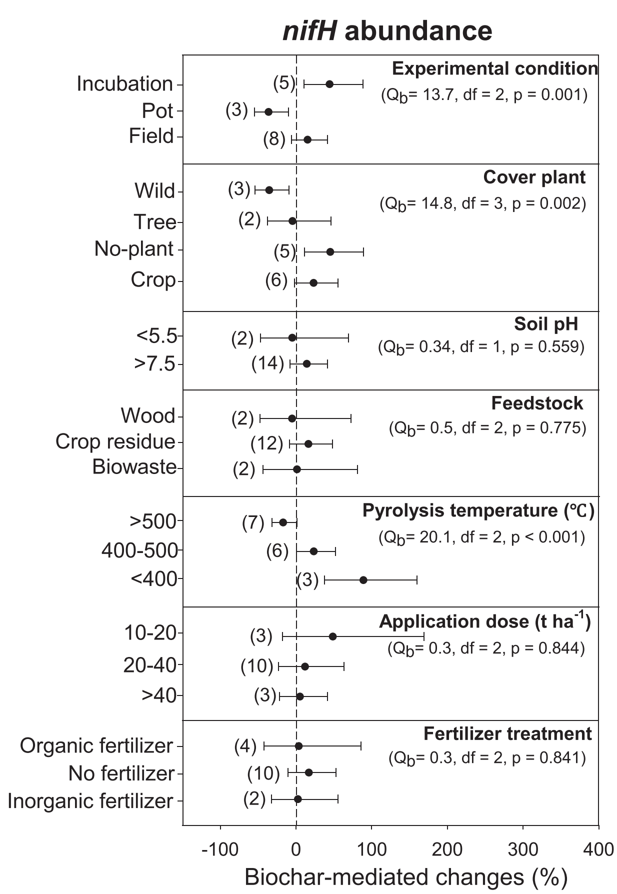

Fig. 2. Average magnitude of biochar-mediated changes in the abundance of *nifH* gene. Error bars denote 95% bias-corrected confidence intervals (CIs). Sample sizes are shown in brackets. The individual effect is significant if the 95% CI does not include zero.

3.3. Impact of moderating variables on soil N-nitrification gene abundance

Biochar-mediated effect on AOA abundance was significantly influenced by experimental condition ($p = 0.005$) and soil pH ($p < 0.001$) (Fig. 3a). Particularly, biochar amendment overall increased the abundance of archaea *amoA* by 67.3% under pot experimental conditions and by 66.7% in acid soils (pH: 5.5–6.5) (Fig. 3a). Moreover, biochar-induced significant increase in AOA abundance was found in soils with potted crop plant species (by 21.2%), without covering plants (by 30.2%), with crop residue-based biochar (by 21.8%), with wood based biochar (by 56.1%), or without fertilization (by 20.3%) or with organic N fertilization (by 39.1%).

AOB abundance in response to biochar amendment was strongly influenced by all categorical variables ($p < 0.01$, Fig. 3b). More specifically, biochar amendment significantly increased AOB by 35.5% and 159.7% with planting crop and wild plant species, respectively, but decreased AOB by 30.5% without covering plants (Fig. 3b). Biochar amendment led to 65.3% increase in the AOB abundance with the application rate <10 t ha^{-1} (Fig. 3b). Moreover, biochar amendment significantly increased AOB abundance by 96.2% only under inorganic N fertilization (Fig. 3b). Interestingly, biochar amendment significantly increased AOB abundance in the very acidic soils (pH: <5.5) but decreased AOB abundance in the neutral soils (pH: 6.5–7.5). Wood-based biochar notably increased AOB abundance by 140.6% but the manure-based biochar decreased AOB abundance by 80.7% (Fig. 3b). High temperature (>500 °C)-produced biochar notably increased AOB abundance by 82.4% but the low temperature (<400 °C)-produced biochar notably decreased AOB abundance by 46.9% (Fig. 3b).

3.4. Impact of moderating variables on soil N-denitrification gene abundance

Biochar-mediated effect on *nirK* abundance was strongly influenced by soil pH ($p < 0.001$), biochar pyrolysis temperature ($p = 0.004$) and fertilizer application ($p = 0.001$) (Fig. 4a). Particularly, medium temperature (400–500 °C)-treated biochars increased *nirK* abundance by 53.3%, and biochar amendment led to a 80.4% and 58.9% increase in *nirK* abundance under inorganic and organic fertilizer application,

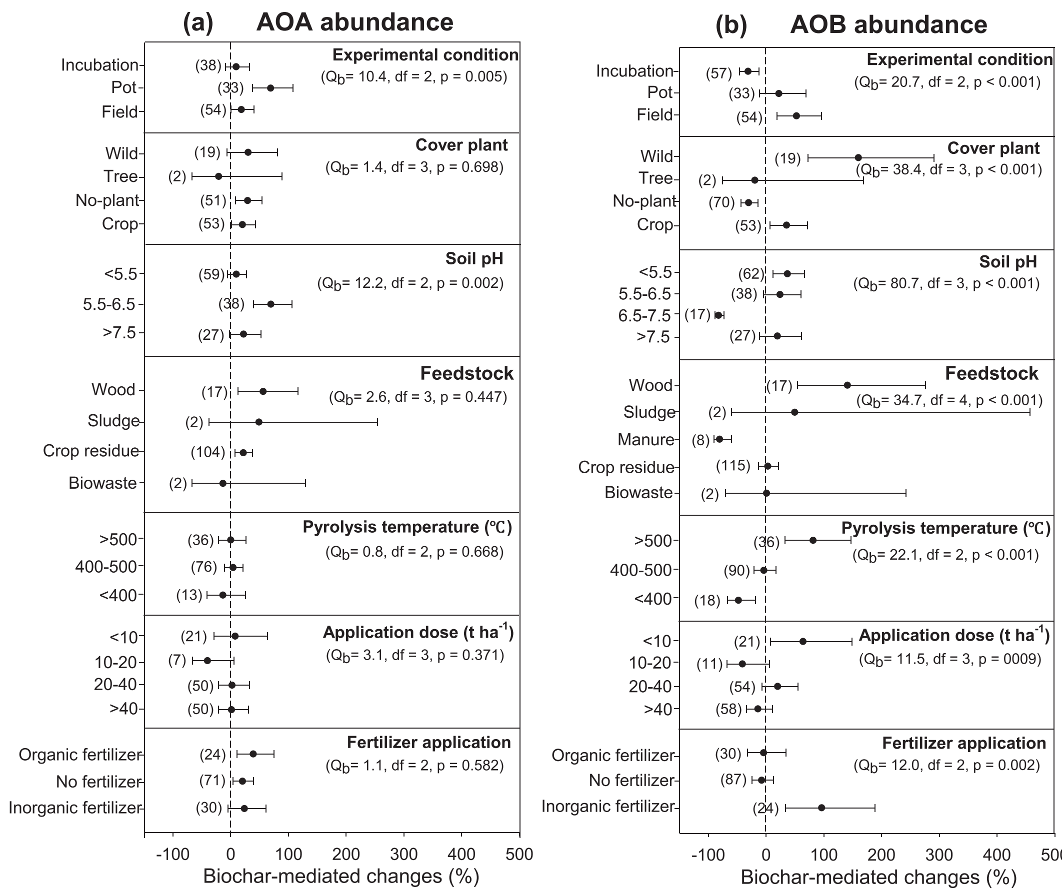

Fig. 3. Average magnitude of biochar-mediated changes in the *amoA gene* abundance of ammonia-oxidizing archaea (AOA) (a) and ammonia-oxidizing bacteria (AOB) (b). Error bars denote 95% bias-corrected confidence intervals (CIs). Sample sizes are shown in brackets. The individual effect is significant if the 95% CI does not include zero.

respectively (Fig. 4a). Biochar amendment also notably increased *nirK* abundance by 34.0% and 60.2% under incubation and pot experimental condition, respectively. Biochar amendment significantly increased *nirK* abundance by 46.9% and 24.5% when soil was with no cover plants and with covering crop plant species, respectively. Crop residue-based biochar significantly increased *nirK* abundance by 35.4%. Additionally, biochar amendment significantly increased *nirK* abundance when the application rate of biochar over 10 t ha^{-1} ($p < 0.005$, Fig. 4a).

There was no significant difference in biochar-mediated effect on *nirS* abundance across most of the categorical variables except for soil pH (Fig. 4b). Particularly, biochar amendment increased *nirS* abundance by 25.6% in acidic soils (pH: 5.5–6.5) (Fig. 4b). Besides, biochar amendment significantly increased *nirS* abundance by 28.2% under field experimental condition, by 60.6% with planting wild plant species, by 11.2% when the biochar pyrolysis temperature was over 500 °C, by 22.2% with biochar application dose at 20–40 t ha^{-1}, and by 56.0% under inorganic fertilization (Fig. 4b).

Similarly, biochar-mediated effect on *nosZ* abundance did not significantly vary among all categorical variables. Within each categorical variable, the *nosZ* abundance in response to biochar amendment was increased in field (by 23.7%) and pot (by 24.3%) experiment studies (Fig. 4c). In particular, biochar stimulated *nosZ* abundance by 29.9% and 23.9% with planting wild plants (grass) and crops, respectively (Fig. 4c). Interestingly, biochar amendment notably increased *nosZ* abundance by 16.9% and 19.4% in the studies where the biochar feedstock was crop residue and the pyrolysis temperature was 400–500 °C, respectively. Biochar addition with the application rate of low 10 and over 40 t ha^{-1} notably increased *nosZ* abundance by 29.8% and 16.6%. Additionally, biochar amendment significantly increased *nosZ* abundance by 14.5% and 21.7% in studies where non-fertilizer and inorganic fertilizers were applied, respectively (Fig. 4c).

3.5. Correlations between soil properties and N-cycling gene abundance driven by biochars

We found that soil pH was significantly correlated with AOB (Table 1). Soil bulk density and NH_4^+ content were negatively correlated with *nirK* abundance (Table 1). Soil NO_3^- content was significantly negatively correlated with *nifH* abundance (Table 1). Soil N_2O emission was negatively correlated with AOA abundance but positively correlated with *nirS* abundance (Table 1). Moreover, the *nirK* abundance was positively and significantly correlated with *nifH* abundance (Table 1). The *nirS* abundance was positively and significantly correlated with AOB and *nirK* abundance (Table 1).

4. Discussion

The abundance of microbial functional gene (MFG) involved in N transformation is often used to represent the abundance and diversity of microbial communities responsible for the different N-cycling processes. We here addressed the effect of biochar amendment on the abundance of MFG involved in N-fixation (Zhang et al., 2017b; Zhou et al., 2018), nitrification (He et al., 2015) and denitrification (Ducey et al., 2013). We found that biochar amendment overall had no notable effect on the abundance of MFG involved in N-fixation, while biochar significantly increased the abundance of most MFG involved in nitrification and denitrification. We further analysed several influencing factors altering the direction and magnitude of N-cycling gene abundance in

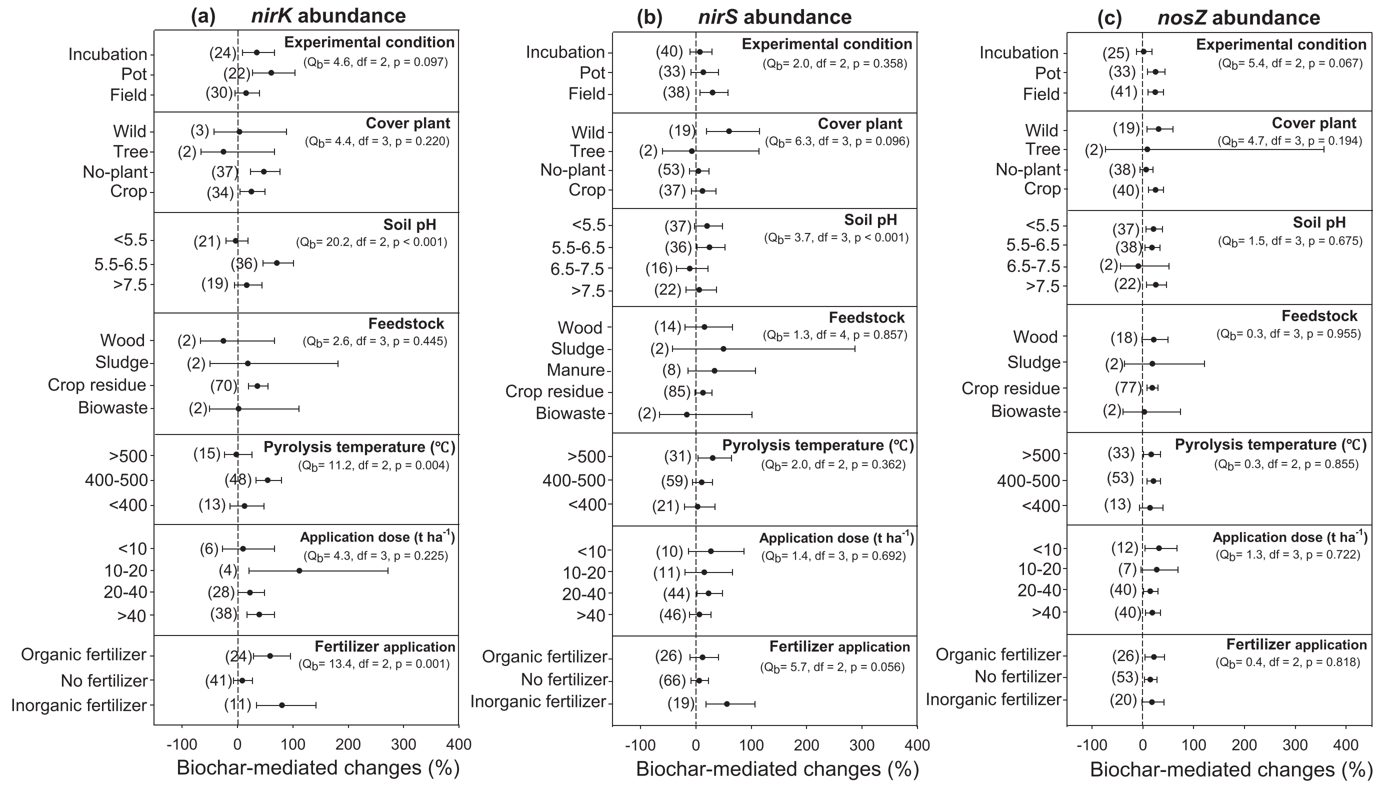

Fig. 4. Average magnitude of biochar-mediated changes in the abundance of *nirK* (a), *nirS* (b) and *nosZ* (c). Error bars denote 95% bias-corrected confidence intervals (CIs). Sample sizes are shown in brackets. The individual effect is significant if the 95% CI does not include zero.

response to biochar amendment, including experimental condition, cropping system, fertilization application, soil and biochar properties.

4.1. Biochar-driven effects on N-fixation gene abundance

Previous studies have shown that biochar can alter MFG abundance related to nitrogen fixation (Ducey et al., 2013; Zhang et al., 2017b; Li et al., 2019). For instance, Ducey et al. (2013) found that the relative abundance of *nifH* was increased three-folds in response to biochar amendment at the application dose of 10% (W/W). However, our meta-analysis results indicated that biochar amendment did not significantly increase *nifH* abundance across all observations. The lack of significant effects of biochar amendment on *nifH* in our meta-analysis was likely driven by the large variability of experimental systems under investigation. Indeed, through heterogeneity analysis, we found that *nifH* abundance in response to biochar amendment strongly depended on the experimental conditions and cover plant used. Specifically, biochar amendment strongly increased *nifH* abundance in soils without covering plants under incubation experiment, but decreased *nifH* abundance in soils with planting wild plant species (e.g. alfalfa) under pot experimental condition, suggesting that biochar amendment stimulates free-living diazotrophs but inhibits symbiotic diazotrophs when legumes are planted. A previous study found that the increased soil pH limits the flourishing of soil diazotrophs (Lin et al., 2018). The decreased *nifH* abundance in presence of plants could thus be explained by the inhibitory effect of biochar on symbiotic N-fixers via increasing soil pH. However, we also found inconsistencies with these findings. For instance, Pereira e Silva et al. (2011) found that soil pH positively correlated with *nifH* gene abundance. Likely, these inconsistent results are partly driven by other experimental factors such as soil nutrient availability (Wang et al., 2017). Inorganic N fertilizers are often applied together with P which has been frequently documented as a limiter of N fixation (Reed et al., 2013), while organic fertilizers carry readily accessible C for microbe growth and have been shown to increase diazotroph abundance and activity (Perez et al., 2014). Here, the studies of biochar-mediated effect on *nifH* abundance in the meta-analysis were under different inorganic and organic fertilizer applications. Therefore, the inconsistent application dose of P, as well as C, within inorganic and organic N

Table 1
Pearson correlation coefficients between effect size (LnR) of soil properties and N-cycling gene abundance in response to biochar amendment. Numbers in bold font with underline indicate significant correlation coefficients at $p < 0.05$. The number of observations is given in parentheses.

	nifH	AOA	AOB	*nirK*	*nirS*	*nosZ*
pH	−0.32 (14)	0.05 (114)	**0.23 (130)**	−0.19 (72)	−0.01 (107)	−0.13 (91)
Bulk density	0.90 (4)	0.37 (12)	0.36 (12)	**−0.90 (5)**	−0.66 (5)	−0.82 (5)
NH_4^+	0.27 (10)	0.15 (111)	−0.11 (116)	**−0.32 (67)**	0.08 (87)	−0.06 (91)
NO_3^-	**−0.78 (13)**	−0.11 (104)	−0.06 (107)	0.01 (69)	−0.04 (88)	−0.04 (92)
N_2O	−0.87 (4)	**−0.28 (56)**	−0.001 (72)	0.20 (29)	**0.41 (63)**	−0.12 (52)
nifH		−0.35 (16)	−0.22 (13)	**0.68 (16)**	−0.16 (16)	0.38 (16)
AOA			0.17 (122)	0.21 (76)	0.10 (92)	0.13 (92)
AOB				−0.08 (73)	**0.26 (105)**	0.18 (89)
nirK					**0.32 (76)**	0.07 (76)
nirS						0.19 (95)

fertilization may also help explain the observed variable effects on *nifH* abundance (Reed et al., 2011; Dynarski and Houlton, 2018). Finally, it is worth noting that these biochar-driven patterns in *nifH* abundance were obtained from <10 observations. Therefore, more studies should be conducted to examine how biochar amendment influences *nifH* gene abundances of symbiotic and free-living diazotrophs in the future.

4.2. Biochar-driven effects on nitrification gene abundance

Ammonia oxidation is the first and rate-limiting step of nitrification in the N cycling. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) carrying *amoA* gene are an abundant and ubiquitously distributed group of soil microorganisms and contribute significantly to ammonia oxidation process in various environments (Schauss et al., 2009; Schleper, 2010). We found that biochar amendment increased the AOA abundance across all studies, and the AOB abundance in response to biochar was strongly influenced by experimental conditions and biochar properties. Previous studies have shown that biochar amendment could improve soil aeration (Zhang et al., 2010) and increase soil pH (Lin et al., 2017), and these two factors are usually related to the abundance of ammonia oxidizers (e.g. AOA, AOB) (French et al., 2012; Zhalnina et al., 2015). For instance, wheat-derived biochar greatly stimulated AOB abundance with the increased soil pH in the presence of soybeans (Zhang et al., 2017a). Soil pH has also been indicated as one important factor driving niche specialisation and differentiation between AOA and AOB (Prosser and Nicol, 2012). Nicol et al. (2008) found that the *amoA* gene copy numbers in AOA decreased, while *amoA* gene copy numbers in AOB increased with pH ranging from 4.9 to 6.9. Therefore, changes in soil pH, mediated by biochar amendment, might be another important environmental force driving the difference between AOA and AOB abundance, and the variability of nitrification process in soils (Prosser and Nicol, 2012; Li et al., 2015; Gubry-Rangin et al., 2017).

We also found that AOA may contribute more than AOB to the enhanced nitrification in response to biochar amendment across all observations. However, after re-analysing only the investigated experiments that measured AOA and AOB simultaneously ($n = 122$) in the biochar-amended agricultural soils, we found that the effect size of biochar amendment on AOB abundance (lnR: 35.3%, CIs: 19.8%–52.7%) was over 1.3 times higher than that of AOA abundance (lnR: 26.0%, CIs: 12.2%–41.4%), suggesting that AOB were much more responsive than AOA to biochar amendment in same manipulative conditions. Similar patterns were found under other agriculture practices such as N fertilization (Carey et al., 2016; Ouyang et al., 2018). Ouyang et al. (2017), using Michaelis-Menten kinetic rate equations, found that the maximum activity (Vmax) and half saturation constant (Km) of AOB were 10–20 times and 15–40 times higher, respectively, than those of AOA in an agricultural soil treated with inorganic fertilizers. That said, with the current information, we cannot rule out that the differences in ammonia oxidation kinetics in response to fertilization could also explain the much greater response of AOB than AOA to biochar amendment. Taken together, the implications of these findings are that AOB is a more responsive and controllable target group for N management to reduce N loss and improve N use efficiency than AOA in biochar-amended agricultural soils.

Moreover, the abundance of AOA and AOB in response to biochar amendment was influenced by experimental conditions and biochar properties such as fertilizer application and biochar feedstock. Particularly, biochar amendment accompanied with organic fertilizers led to an obvious increase in AOA abundance, suggesting that the application of organic N fertilizers often had a much stronger synergistic facilitation effect on AOA abundance than those with the application of inorganic N fertilizers. Organic fertilizers usually supply organic carbon and nutrients to soils, which support the growth of microbial populations. Soil N-cycling microbial populations should also increase alongside with an overall increase in soil microbial biomass under organic N fertilization, especially for nitrifiers, such as AOA and AOB (Ouyang et al., 2018). However, the lack of significant enhancing effect of biochar on AOB abundance could be due to the variation caused by categorical variables such as cover plants, soil pH, and biochar feedstock. Here, we found that biochar amendment increased AOB abundance in the very acidic soils but decreased it in the near-neutral soils. Moreover, we found that wood-driven and high pyrolysis temperature-produced biochar increased AOB abundance, but manure-driven and low pyrolysis temperature-produced biochar decreased AOB abundance. Such effects could be explained by the fact that wood-based biochar addition results in increased numbers of soil macropores and improved soil aeration (Lei and Zhang, 2013), thus providing a more favourable environment for microbes, than manure-based biochar addition (Vinther et al., 1999; Zhang et al., 2010). In summary, our results related to microbial functional genes involved in nitrification process indicate that biochar amendment may be a desirable and effective practice to promote nitrification in agro-ecosystems, especially in acidic soils (Teutscherova et al., 2017), as it stimulated the growth of both AOA and AOB.

4.3. Biochar-driven effects on denitrification gene abundance

Denitrification is the stepwise reduction from NO_3^- to NO_2^-, NO, N_2O and ultimately to N_2, which is an heterotrophic bacteria and fungi redox-mediated process that mainly occurs in anaerobic conditions (Wrage et al., 2001). Specifically, the denitrification pathway from NO_3^- to NO_2^- is catalyzed by a series of reductases encoded by genes (e.g. *narG*, and *napA*) that can be used as proxies in determining the potential for nitrate reduction processes within environments (Philippot and Hallin, 2005). In this study, we found that biochar amendment enhanced *narG* abundance, while the literature still lacks evidence for how biochar amendment affects *napA* gene expression. On the other hand, given that both *nirK* and *nirS* are essential genes in the reduction of NO_2^-, and are regarded as target genes for measuring soil denitrification (Braker et al., 2000; Kuypers et al., 2018), most of the current biochar studies have focused on how the *nirS* and *nirK* gene mediate the stepwise reduction from NO_2^- to NO. Here, we found that biochar addition caused a significant increase in the abundance of both *nirK* and *nirS*, and their abundance was negatively correlated with soil bulk density. We hypothesize that this correlation is driven by the biochar-mediated improvement of soil aeration, and subsequent decrease in soil bulk density, ultimately stimulating the growth and diversity of denitrifiers (Zhang et al., 2010; Gul et al., 2015; Liu et al., 2017). However, the biochar-mediated effect on *nirK* was influenced by the concomitance of several external or internal factors. For instance, the increase of *nirK* abundance at medium temperature (400–500 °C)-produced biochar, is accompanied with the soil pH ranging from 5.5 to 6.5, suggesting that the biochar amendments stimulate the abundance of *nirK* genes through enhancing amelioration effects on soil pH. While variation in pyrolysis temperature of biochars can modify soil pH values. Specifically, wheat straw-derived biochar produced at low temperature (200 °C) was acidic, whereas, at high temperature (600 °C), it was alkaline (Zhang et al., 2015). Therefore, the increase in *nirK* gene abundance in response to biochar amendment is highly context-dependent. In this study, we also found that only the crop residue-based biochar amendment resulted in increased abundance of the denitrification genes, particularly *nirK* and *nosZ.* Since the labile organic C is greater in biochar made from feedstocks including crop residues and manure that are high in carbohydrates, but is relatively low in lignin rich wood biochar (Downie et al., 2009). These abundant labile organic molecules in crop residue-driven biochars may increase the turnover of C and N in microbial biomass, in turn stimulating microbial growth and denitrification enzyme activity (Anderson et al., 2018; Tao et al., 2018). Moreover, biochar-mediated increase in the abundance of *nirK* was more pronounced under organic or inorganic N fertilization than without fertilizer application. A previous research has shown that inorganic and organic fertilizers increased the abundance of nitrifying and denitrifying genes (e.g. bacterial *amoA*, *nirK* and *nosZ*) via promoting soil P availability (Sun et al., 2015). Therefore, the improvement of soil nutrient availability by fertilization could further synergistically promote the biochar-mediated increase in the abundance of N-cycling genes.

We also found that biochar amendment could influence the abundance of *nosZ* depending on the experimental conditions, such site of planting, feedstock and pyrolysis temperature. Along these lines, several studies have shown that biochar amendment can change the abundance of denitrifiers, especially of N_2O reducing microorganisms possessing a typical *nosZ* gene (Liu et al., 2017; Tan et al., 2018; Zhou et al., 2018). However, the mechanisms underlining such microbial changes still need to be fully addressed (Harter et al., 2016). What we know, however, is that individual N_2O-reducing taxa can differ considerably in N_2O reduction activity. Therefore, biochar-mediated taxonomic shifts among typical and atypical *nosZ* gene carrying N_2O-reducing microbial communities might affect N_2O reduction rates and net soil N_2O emission (Cavigelli and Robertson, 2001; Tago et al., 2011).

4.4. Linkages between N-cycling gene abundance and soil N_2O emission

Although several independent studies found positive correlations among the abundance of N-cycling genes (Liu et al., 2019; Sun et al., 2019), most genes involved in nitrification and denitrification were not significantly correlated with each other in the present meta-analysis, except for the correlation between *nirS* and AOB/*nirK*. Such inconsistencies could be explained for some groups of genes. For instance, we found that the abundance of AOA or AOB was strongly influenced by the experimental system and soil pH. Therefore, the biochar-mediated inconsistent changes in AOA or AOB would favour non-significant correlations between AOA or AOB and most other genes involved in nitrification and denitrification. On the other hand, the lack of correlation between nitrifier and denitrifier gene abundances can be attributed to these groups having different life strategies (e.g. autotrophic versus heterotrophic; aerobic versus anaerobic) and therefore responding to different mechanisms controlling changes in population dynamics (Ruiz-Rueda et al., 2009; Szukics et al., 2010; Kastl et al., 2015).

Interestingly, we found a significant positive correlation between *nirK*/*nirS* abundance and N_2O emission. Because it was shown that denitrifers harboring *nirS* and *nirK* genes lack the genetic capacity to reduce N_2O (Philippot et al., 2011), they could be considered as the major contributors to N_2O production during denitrification process. However, our meta-analysis results showed that biochar amendment ultimately decreased soil N_2O emissions by 14.6% overall. This is in line with earlier studies showing that the production of N_2O generally decreases in soils with biochar amendment (Xu et al., 2014; Hagemann et al., 2017). Since we observed a negative correlation between *nosZ* abundance and N_2O emission, we could speculate that the reduction of soil N_2O emission in response to biochar amendment is related to the abundance of *nosZ* in soils (Van Zwieten et al., 2014).

Moreover, given that the nitrification and denitrification processes involve oxidation and reduction (redox) reactions (Wrage et al., 2001), the production or consumption of N_2O is tightly linked to the soil redox potential (Eh) (DeAngelis et al., 2010). In this regard, since practically all biochars contain electroactive functional groups (e.g. quinone/hydroquinone) and redox active minerals (e.g. Fe or Mn oxides), biochars can serve as electron acceptors or donors during the redox processes (Graber et al., 2014; Klupfel et al., 2014; Joseph et al., 2015), and thus favour the transport of electrons to microorganisms (Kappler et al., 2014). However, the biochar-mediated changes in soil redox reactions are dependent on pyrolysis temperature, feedstock and soil type. For instance, biochars produced below 550 °C are complex semiconductors that can both accept and donate electrons and can thus act as either (or both) a source or sink of charge in soils (Joseph et al., 2013). While biochars derived from wastewater sludge, grass, pig manure, wheat straw under same pyrolysis temperature (550 °C) had 2.209, 0.152, 0.696, 0.074 g/kg Fe (w/w), respectively, (Zhao et al., 2013). Thus, variation in iron minerals in different biochars could catalyse a range of redox reactions associated with N-cycling, nitrification and denitrification (Van Zwieten et al., 2015). Particularly, biochars with a high content of Fe oxide nanoparticles at the surfaces of pores could significantly increase the rate of reduction of NO_3^- and NO_2^- by lowering the free energy required for the process (Van Zwieten et al., 2015). Moreover, in paddy soil system where flooding and drying cycles occur, biochar amendment reduces soil Eh and Fe availability, which in turn can affect N-cycling gene expressions (Wang et al., 2018). Unfortunately, the biochar-driven soil Eh changes in soil N-cycling gene abundance is still rarely studied. We were able to only retrieve one study of biochar-mediated effect on N-cycling gene where soil Eh was tested simultaneously in this meta-analysis (Wang et al., 2018). Thus, future research on N_2O emissions in response to biochar amendment should further also focus on the effects of biochar-mediate soil Eh changes during the denitrification processes.

5. Conclusions

The present meta-analysis investigated changes in the abundance of soil microbial functional gene associated with the N cycling and soil physico-chemical properties following biochar amendment. We found that biochar amendment overall had no significant impact on the abundance of nitrogen fixation gene (*nifH*) and AOB, but significantly increased the abundance of AOA and denitrification gene (*nirK*, *nirS* and *nosZ*). Experimental conditions, soil pH, cover plants, biochar pyrolysis temperatures and feedstock, and fertilizer application were major factors regulating the responses of N-cycling genes to biochar amendment. However, we also identified several venues of future research for better comprehending the effect of biochar on N cycling. Specifically, we advocate the implementation of Eh measurements, the corresponding nitrification and denitrification enzyme activities by kinetic analysis and the functional roles of nitrifying and denitrifying microbial community composition structure on N-transformation processes in response to biochar amendment. Taken together, the comprehensive quantification research of N-cycling gene responses to biochar amendment will help develop more accurate models of functional microbial populations and N cycling and improve strategies for controlling N losses in agro-ecosystems.

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