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Li, Quan

Elsevier B.V.
2020-02-01

Li, Q, Song, X, Yrjälä, K, Lv, J, Li, Y, Wu, J & Qin, H 2020, 'Biochar mitigates the effect of nitrogen deposition on soil bacterial community composition and enzyme activities in a Torreya grandis orchard', Forest Ecology and Management, vol. 457, 117717. https://doi.org/10.1016/j.foreco.2019.1

http://hdl.handle.net/10138/337220
10.1016/j.foreco.2019.117717

Forest Ecology and Management 457 (2020) 117717

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Biochar mitigates the effect of nitrogen deposition on soil bacterial community composition and enzyme activities in a *Torreya grandis* orchard

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ARTICLE INFO

Keywords:
N deposition
Biochar
Microbial biomass
Soil bacterial community
Enzyme activity

ABSTRACT

Increased reactive N deposition has widespread effects on terrestrial ecosystems, such as biodiversity loss, soil acidification, as well as stimulated plant growth. Empirical studies show that biochar often affects soil quality, crop productivity, soil microbial community composition and enzyme activities. However, the effect of biochar addition on forest soil bacterial community along with enzyme activities under nitrogen (N) deposition and its related mechanisms have not been well studied yet. Therefore, a 2-year field study was conducted to investigate the effects of biochar amendment (0, 20, 40 kg biochar ha^{-1} yr^{-1}) on soil nutrients, enzyme activities, and bacterial community in a *Torreya grandis* orchard under different levels of N deposition (0, 30, 60 kg N ha^{-1} yr^{-1}). N deposition significantly increased soil nutrients availability, such as N, phosphorus (P) and potassium (K), while biochar amendment led to significant increase in soil pH, organic carbon (SOC), total N (TN), total P (TP), available P (AP) and available K (AK). Both N deposition and biochar amendment significantly decreased the soil microbial biomass carbon (MBC), altered soil microbial community and enzyme activities significantly. Biochar addition increased the relative abundance of phylum *Proteobacteria* under different levels of N deposition, but had variable effect on *Acidobacteria* groups. Non-metric multidimensional scaling (NMDS) indicated that biochar amendment can mitigate the effect of N deposition on soil bacterial community composition and enzyme activities. Soil pH and SOC played an important role in shaping soil bacterial community composition, while available AP and AK contents significantly related to the variation of soil enzyme activities. Structure equation modeling (SEM) revealed that N deposition had negative effect on soil enzyme activities while biochar amendment can mitigate this negative effect through increasing AP content. Our result suggests that biochar amendment can mitigate the alteration of soil bacterial community and enzyme activities induced by N deposition, and this mitigation effect was linked to the alteration of soil physicochemical properties, especially the increased AP content. Thus, biochar amendment could be a promising way to develop sustainable forest management under increasing N deposition.

1. Introduction

During the past century, anthropogenic activities such as agricultural N fertilization and fossil fuel consumption have greatly increased the quantity of atmospheric nitrogen (N) deposition (Maaroufi et al., 2015). It is reported that the global N deposition rate has increased three to five-fold over the past century (Denman et al., 2007), and the deposition rate of N is predicted to double by 2050 (Galloway et al., 2004; Phoenix et al., 2011). Increased inputs of anthropogenically derived N have the potential to enhance productivity and carbon (C) sequestration in N-limited ecosystems (Maaroufi et al., 2015). However, soil microorganisms such as bacteria and fungi may not necessarily be limited by the same elements that limit plants (Hobbie, 2005), but could be limited by C, water or phosphorus (P) (Treseder, 2008). Indeed, N deposition or fertilization has been shown to negatively affect microbial growth, alter soil microbial community composition and enzyme activities (Geisseler and Scow, 2014; Jian et al., 2016; Li et al., 2016; Ramirez et al., 2012; Treseder, 2008). Studies based on meta-analysis concluded that negative effects of N addition on soil microbial biomass C (MBC) were widespread in terrestrial ecosystems (Jian et al., 2016; Treseder, 2008). However, the effects of N deposition or fertilization on microbial community composition and specific taxa were inconsistent among various studies. For example, Ramirez et al. (2012) showed that N addition consistently

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https://doi.org/10.1016/j.foreco.2019.117717
Received 18 July 2019; Received in revised form 21 October 2019; Accepted 22 October 2019

altered bacterial community composition, increasing the relative abundance of *Actinobacteria* and *Firmicutes*, and decreasing the relative abundance of *Acidobacteria* and *Verrucomicrobia*, while Freedman et al. (2015) found neither the total nor active forest floor bacterial community was significantly affected by experimental N deposition. Besides from soil microbial community, soil extracellular enzyme activities (EEA) are also sensitive to N deposition. Ramirez et al. (2012) found that N-amended soil consistently had lower activities in a broad suite of extracellular enzymes, while Jian et al. (2016) concluded that N fertilization stimulated hydrolytic EEA but depressed oxidative EEA. These inconsistent results suggest that soil microbial responses to N addition are likely controlled by various mechanisms.

Biochar amendment of soil has been proved to be a promising way to enhance soil C storage and concurrently to increase crop productivity by improving soil physicochemical properties and microhabitat conditions for microorganisms (Chen et al., 2018; Gul et al., 2015; Lehmann et al., 2011). Generally, biochar addition improves soil texture, increases the pH and cation exchange capacity, promotes soil aggregation, and increases the moisture and nutrient retention ability (Nielsen et al., 2014; Zhou et al., 2019). Moreover, the porous surface of biochar serves as a refuge for microbes from predation or abiotic stress (Lehmann et al., 2011). Biochar's character of rich recalcitrant carbon can also provide competitive living species for some specific bacterial taxa with degradation capacity of complex carbon sources, such as some slow growing oligotrophs (Fierer et al., 2007; Sheng and Zhu, 2018). Thus, biochar amendment to soils has recently shown to influence the soil microbial biomass, as well as community structure (Lehmann et al., 2011; Li et al., 2018a,b). Previous studies also revealed variable effects of biochars on soil respiration and EEA (Chen et al., 2018; Gul et al., 2015; Li et al., 2018a,b; Lu et al., 2019). Biochar amendment can both increase or decrease soil respirations. Generally, increase of soil respiration induced by biochar application could be due to the increase of labile soil organic C (SOC) pools and biochar-induced priming of native SOC mineralization (Singh and Cowie, 2014; Reed et al., 2017). In contrast, decrease soil CO_2 emissions induced by biochar application could be explained by the fact that biochar can adsorb organic substances and soil enzymes, thus inhibiting the C-degrading microbial activity (Ameloot et al., 2014; Weng et al., 2017). Lu et al (2019) suggested that the impacts of biochar amendment on the soil greenhouse gas fluxes are greatly dependent on the biochar application rate and time after biochar application. Similar to soil respiration, the influence of biochar on soil EEA depends on the interaction of substrate and enzyme with biochar, and is related to the porosity and surface area of biochar (Gul et al., 2015). Biochar with greater porosity and surface area is expected to reduce EEA due to the functional groups on such biochar would tend to bind substrates and extracellular enzymes (Bailey et al., 2011; Ameloot et al., 2013). Therefore, the impacts of biochar on soil microbial community and EEA are likely to depend strong on factors such as biochar materials, application rates, and measurement time after biochar application (Huang et al., 2018a; Lu et al., 2019).

The impacts of N deposition or biochar amendment on soil microbial community and EEA have been intensively studied in forest, agricultural or grassland ecosystems, only few studies have focused on their combined effects on soil microbial community, especially in the forest plantation soils (Li et al., 2018a,b). Chinese torreya (*Torreya grandis*), a species of Taxaceae family, is an economically important native nut tree species in Southeast China. Recently, the main cultivation area of *T. grandis* is subjected to a high N deposition with an average rate of 30.9 kg N ha^{-1} yr^{-1} (Zhao et al., 2008). In the previous studies, we found that N deposition can decrease soil pH and lead to unbalanced nutrient uptake by *T. grandis* trees, but biochar addition showed a liming effect and can increase nut quality (Zhang et al., 2017, 2019). However, little is known about how biochar and its interaction with N deposition may influence the soil microbial community and enzyme activities. As biochar addition and N deposition generally resulted in contrasting effect on soil physicochemical properties, such as soil pH, cation exchange capacity, and soil organic C quality, we hypothesized that biochar addition to soils could mitigate the effects of N deposition on soil properties, microbial biomass and activity, as well as microbial community composition. In addition, we also hypothesized that the neutralized effect on N deposition by biochar was dose dependent. In this study, we investigated the effects of N deposition and biochar amendment on soil microbial biomass, enzyme activities and soil bacterial community composition in a field experiment over 2 years. Different levels of N deposition (0, 30, 60 kg N ha^{-1} yr^{-1}) and biochar amendment (0, 20, 40 t biochar ha^{-1} yr^{-1}) were applied to evaluate the dose-effect of N and biochar on soil microbial community. Our objectives were (*i*) to verify how soil microbial community responds to different levels of N deposition or biochar application, (*ii*) to determine whether biochar application can mitigate the negative effect of N deposition on soil microbial community, and (iii) to illustrate the main factors that drive the changes in soil enzyme activities and bacterial community composition. This study aimed to better understand the mechanisms of biochar induced changes in soil microbial community and activity under N deposition. This knowledge is important to find an effective biochar application approach to mitigate the negative effects of N deposition on *T. grandis* orchard management in the future.

2. Materials and methods

2.1. Site description

The experimental site is located in Yuqian town, Hangzhou, Zhejiang Province in China (30°14′N, 119°42′E). This area has a subtropical monsoon climate with four distinct seasons and a mean annual precipitation of 1613.9 mm. The mean annual temperature is 15.6 °C, and the mean monthly temperature ranges from 4.5 °C in January to 28.9 °C in July. The soil was clay loam soil classified as Typic Hapludult (IUSS Working Group WRB, 2006) derived from siltstones. The *T. grandis* orchard was established in 2000 with a density of 900 to 1000 trees per hectare. The orchard is fertilized with 58.5 kg N, 58.5 kg P and 58.5 kg K ha^{-1} yr^{-1}, and plowed annually in late October after the harvest.

2.2. Experimental design and soil sampling

According to the local N deposition rate of 30.9 kg ha^{-1} yr^{-1} (Jia et al., 2014) and the widely used method to double and triple the local deposition rate in order to simulate additional N deposition (Li et al., 2019), three levels of N addition were included, such as 0 kg N ha^{-1} yr^{-1} (N0), 30 kg N ha^{-1} yr^{-1} (N1), 60 kg N ha^{-1} yr^{-1} (N2). Within each N level, three levels of biochar were applied, including 0 t biochar ha^{-1} yr^{-1} (C0), 20 t biochar ha^{-1} yr^{-1} (C1), 40 t biochar ha^{-1} yr^{-1} (C2). In total, 9 treatments with different N deposition and biochar application rate were included in this experiment, each with 3 replicates. The experiment was arranged in a completely randomized design. Each treatment was conducted in a 4 × 4 m^2 plot with one *T. grandis* tree in the center. Treatments were separated by buffer zones that were at least 2 m wide.

N was added in the form of ammonium nitrate (NH_4NO_3). From March 2015, NH_4NO_3 solution was evenly sprayed from above the canopy of the *T. grandis* trees with an electric sprayer at the beginning of each month. Control plots received an equal amount of N-free water. Biochar was produced by pyrolysis of wheat straw at 450 °C in a vertical kiln (Sanli New Energy Company, Henan, China), and was ground to pass through a 2 mm sieve. The pH of the biochar was 9.8, surface area 9.7 m^2 g^{-1}, cation exchange capacity (CEC) 189.3 cmol kg^{-1}, total C content 425.3 g kg^{-1}, total N content 5.2 g kg^{-1}, and ash content 18.6%. In March 2015, all the biochar was applied and mixed thoroughly into the top 20 cm of the soil by plowing.

Samples of topsoil (0–20 cm) were collected on a single day in

March 2017. In each plot, five random sampling points were chosen with a minimum distance of 2 m between each other. Five sub-samples were collected with an auger (5 cm in diameter), then manually homogenized immediately to form a composite sample for one plot, and about 10 g of subsoil was immediately frozen in liquid N_2 for DNA extraction. The rest of the composite sample were put into sterilized polyethylene bags and placed on ice to be transported to the laboratory. After removing all visible roots and plant fragments, field-moist soils were divided into two portions. One part of the soil samples were passed through a 2-mm sieve and stored at 4 °C prior to enzyme analysis. Another part was air-dried at room temperature for soil physicochemical analysis.

2.3. Soil physicochemical properties and microbial biomass

SOC was determined by the oil bath-K_2CrO_7 titration method, total N (TN) was analyzed by the Kjeldahl procedure, total phosphorus (TP) was analyzed by molybdenum antimony blue colorimetry, available N (AN) was measured by the hot alkaline permanganate method, available P (AP) was analyzed colorimetrically through molybdenum antimony blue method after the soil was extracted with 1 mol L^{-1} NH_4F solution, and soil available K (AK) was measured by extracting the soil samples with 1 mol L^{-1} NH_4OAc (pH 7.0) and analyzed using the flame photometric method. Soil pH was determined using a glass electrode on a 1:2.5 soil-water suspension after equilibrating for 15 min. All the mentioned soil properties were determined according the protocol described by Lu (1999).

Soil microbial biomass C (MBC) was measured using fumigation-extraction method and the K_2SO_4 extracted C was determined by a TOC-V CPH total organic C analyser (Shimadzu, Japan). Soil MBC was calculated as the difference between the fumigated and un-fumigated extracts using a K_{EC} factor of 0.45 (Brookes et al., 1985; Wu et al., 1990).

2.4. Soil enzyme activity assay

Soil enzyme activities associated with soil C and N turnover were determined. Catalase activity (EC 1.11.1.6) was determined using $KMnO_4$ as the substrate and incubation at 37 °C for 24 h (Jiang et al., 2009). Cellulase activity (EC 3.2.1.4) was determined using nitrosalicylic acid colorimetry as described by Alef and Nannipieri (1995). The amount of glucose released over 72 h was assayed colorimetrically at 540 nm. β-Fructofuranosidase activity (EC 3.2.1.26) was determined from the quantity (μmol) of glucose formed in 1 g soil at 37 °C after 24 h (Frankeberger and Johanson, 1983). Urease activity (EC 3.5.1.5) was determined using urea as the substrate and incubation at 18 °C for 2 h (Kandeler and Gerber, 1988). The NH_4^+ concentration was determined with a Flow Injection Analyzer (Skalar). The activities of nitrate reductase (EC 1.6.6.1) and nitrite reductase (EC 1.7.2.1) were assayed using KNO_3 and $NaNO_2$ as substrates, respectively, as described by Daniel and Curran (1981).

2.5. Soil DNA extraction and high-throughput sequencing

DNA was extracted from 0.25 g soil using an Ezup Column Soil DNA Purification Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. Nucleotide-free water was used as a blank. DNA was eluted with 50 μL elution buffer, quantified by NanoDrop ND-1000 (Thermo Scientific, USA), and stored at −80 °C.

Primer set 338F and 806R targeting V3-V4 regions were used to amplify the bacterial 16S rRNA gene (Caporaso et al., 2010). The 5′ ends of the primers were tagged with specific barcodes for Illumina sequencing. Polymerase chain reaction (PCR) amplification was performed in a 25 μL reaction mixture containing 25 ng template DNA, 12.5 μL PCR Premix (New England Biolabs, MA, USA), 2.5 μL of each primer (1 μM), and PCR-grade water to adjust the volume. PCR reactions were carried out in triplicate and were performed on a S1000 Thermal Cycler (Bio-Rad) according to the previously published protocols (Qin et al., 2017). Three replicate PCR products of the same sample were pooled prior to be purified with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified using Qubit assay (Invitrogen, USA). Amplicons were pooled for sequencing, and the size and quantity of the amplicon libraries were determined with Agilent 2100 Bioanalyzer (Agilent, USA) and Library Quantification Kit for Illumina (Kapa Biosystems, Woburn, MA, USA), respectively. The PhiX control library (V3) (Illumina) was combined with the amplicon library (expected at 30%).

Samples were sequenced using PE250 Illumina MiSeq platform according to standard protocols. Paired-end reads were assigned to samples based on the unique barcodes and truncated by cutting off the barcode and primer sequences. Paired-end reads were merged using FLASH, and quality filtering was done using fqtrim v0.94. Chimeric sequences were filtered and sequences were assigned into operational taxonomic units (OTUs) at ≥97% similarity using Vsearch v2.3.4. Taxonomic characterization of the representative sequences of bacterial OTUs was performed using the SILVA 16S rRNA database. Community alpha diversity indices including phylogenetic diversity and chao1 were generated based on the obtained OTUs using QIIME v1.8.0.

2.6. Statistical analysis

Data were analyzed using General Linear Model (GLM), with N deposition and biochar application as the two main factors affecting soil properties, bacterial diversity indices, and enzyme activities. The relationships between soil characteristics, bacterial diversity and enzyme activities were tested with the Pearson correlation. Changes in the structures of soil microbial community and enzymatic activity were visualized using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance matrix. To assess how N deposition and/or biochar application influence bacterial community composition and enzyme activity, a permutational multivariate analysis of variance (PERMANOVA) was carried out using the adonis function in the package "vegan" in R. Soil physicochemical properties were fitted with 'envfit' onto the NMDS ordination use vegan package. The significance of these environmental variables was tested based on 999 permutations. A structural equation model (SEM) was constructed to investigate relationships among N deposition, biochar amendment, and other soil factors anticipated to affect soil bacterial community and enzyme activities. Soil bacterial community and enzyme activities were represented by the first principal component (PC1). The model was constructed in AMOS 18.0 software. The fitness of the model to the data was tested using the maximum likelihood (χ^2) goodness-of-fit test, goodness-of-fit index (GFI) and root-mean-square error of approximation (RMSEA).

3. Results

3.1. Soil physicochemical properties and microbial biomass

Nitrogen deposition and biochar amendment have important impacts on soil physicochemical properties (Table 1). Higher amount of N deposition significantly increased soil AN, AP and AK, but have no obvious effect on soil pH, SOC, TN and TP. Biochar amendment significantly increased soil pH, SOC, TN, TP, as well as AP and AK contents, except for soil AN content. The interaction effects of biochar amendment and N deposition was found significant for TN content only (Table 1). Both N deposition and biochar amendment significantly decreased soil microbial biomass. However, no significant difference was found between the two N deposition or biochar amendment levels (Fig. 1).

Table 1
Soil physicochemical properties under different levels of N deposition and/or biochar amendment.

Treatments		pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
C0	N0	6.02 ± 0.14	26.70 ± 4.20	1.93 ± 0.34	0.90 ± 0.03	25.20 ± 9.44	172.34 ± 30.15	158.67 ± 20.37
	N1	6.09 ± 0.34	29.77 ± 3.38	2.60 ± 0.24	0.91 ± 0.05	32.90 ± 6.59	176.06 ± 12.80	139.67 ± 4.03
	N2	5.48 ± 0.33	28.00 ± 1.35	2.40 ± 0.08	0.84 ± 0.06	29.87 ± 4.99	195.16 ± 11.55	149.33 ± 31.85
C1	N0	6.55 ± 0.28	30.70 ± 2.64	3.13 ± 0.29	1.08 ± 0.12	27.97 ± 2.88	188.26 ± 2.71	168.67 ± 13.91
	N1	7.17 ± 0.64	33.83 ± 3.03	2.37 ± 0.31	1.12 ± 0.04	26.83 ± 5.95	251.41 ± 32.06	151.33 ± 9.57
	N2	6.96 ± 0.25	36.37 ± 1.94	2.80 ± 0.08	1.00 ± 0.06	32.67 ± 5.95	236.55 ± 10.82	220.33 ± 19.01
C2	N0	7.02 ± 0.44	34.20 ± 2.10	2.87 ± 0.05	1.27 ± 0.08	23.10 ± 4.68	189.85 ± 30.93	193.67 ± 6.13
	N1	7.40 ± 0.33	42.27 ± 6.65	2.27 ± 0.34	1.16 ± 0.17	38.03 ± 2.93	221.69 ± 30.27	189.33 ± 19.70
	N2	7.83 ± 0.29	36.77 ± 4.60	3.00 ± 0.62	1.17 ± 0.09	38.03 ± 4.65	216.92 ± 13.48	224.33 ± 27.92
P value	C	< 0.01	< 0.01	< 0.05	< 0.01	0.42	< 0.01	< 0.01
	N	0.25	0.10	0.21	0.24	< 0.05	< 0.05	< 0.01
	C × N	0.12	0.58	< 0.05	0.72	0.323	0.47	0.19

Data are means ± S.E., $n = 3$. Significant differences among different levels of N deposition or biochar amendment were analyzed using the two-way ANOVA with N deposition (N) and biochar amendment (C) as main effects. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; AP, available phosphorus; AK, available potassium. C0, C1, and C2 indicating treatments amended with 0 kg ha^{-1} yr^{-1}, 20 kg ha^{-1} yr^{-1}, and 40 kg ha^{-1} yr^{-1} biochar, respectively. N0, N1, and N2 indicating treatments with 0 kg ha^{-1} yr^{-1}, 30 kg ha^{-1} yr^{-1}, and 60 kg ha^{-1} yr^{-1} N application rate, respectively.

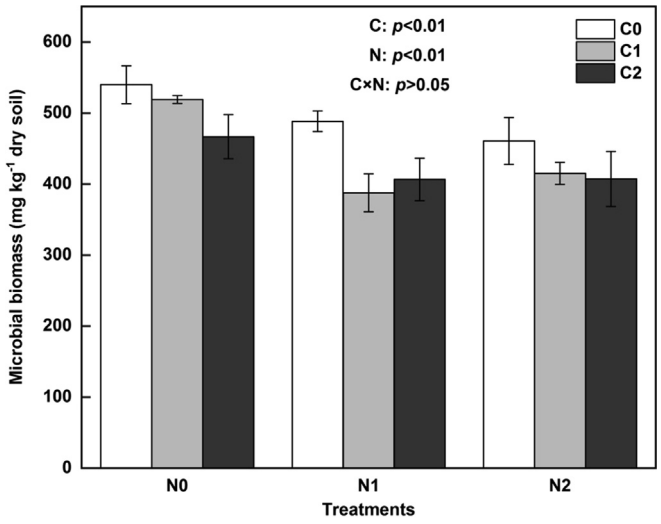

Fig. 1. Soil microbial biomass under different levels of N deposition and/or biochar amendment. The error bars represent the standard error ($n = 3$). Significant differences among different levels of N deposition or biochar amendment were analyzed using the two-way ANOVA with N deposition (N) and biochar amendment (C) as main effects.

3.2. Soil microbial community composition

According to NMDS analysis, both N deposition and biochar amendment changed the soil bacterial community to great extent with soil pH and SOC content as the factors which had significant impacts on bacterial community compositions. Soil AK content also had marginal impact on bacterial community composition (Fig. 2a). In comparison with the N0C0, application of biochar significantly changed soil bacterial community. Moreover, treatments amended with different amount of biochar, for example, N0C1 and N0C2, had significant difference between each other (Fig. 2b). Similarly, N deposition also changed soil bacterial community composition greatly, and treatments with different N deposition level (N1C0 and N2C0) were distinct from each other (Fig. 2b). Biochar amendment can mitigate the change of soil bacterial community composition induced by N deposition. At both N1 and N2 deposition levels, treatments with higher amount of biochar amendment (i.e. N1C2 and N2C2) had similar bacterial community composition with the N0C0 control, respectively (Fig. 2c, d).

Lower level of N deposition significantly decreased the relative abundance of *Alphaproteobacteria* (Fig. 3a), while higher level of N deposition decreased *Beta-* and *Deltaproteobacteria* but increased *Acidobacteria*_Gp2 abundance (Fig. 3b). Lower amount of biochar amendment (C1) decreased *Acidobacteria*_Gp3 abundance at N1 deposition level, while higher amount of biochar amendment (C2) also decreased *Acidobacteria*_Gp2 but increased *Acidobacteria*_Gp6 abundance (Fig. 3a). At N2 deposition level, lower amount of biochar amendment decreased the relative abundance of unclassified *Chloroflexi*, *Deltaproteobacteria*, and *Acidobacteria*_Gp3 (Fig. 3b). However, no difference of any bacterial taxa was found between N2C2 treatment and the N0C0 control.

3.3. Soil enzyme activities

Biochar amendment decreased soil cellulase, β-fructofuranosidase, nitrate and nitrite reductase activities, while low amount of biochar amendment increased soil catalase and urease activities (Fig. 4). N deposition increased soil cellulase and nitrate reductase activities but decreased urease activity (Fig. 4b, d, e). Lower amount of N deposition significantly increased soil catalase and β-fructofuranosidase activities, but higher amount of N deposition decreased or had no effect on them (Fig. 4a, c). However, N deposition had no effect on soil nitrite reductase activity (Fig. 4f). Biochar amendment and N deposition had significant interaction effects on all the soil enzyme activities tested in this study (Fig. 4).

All the soil enzyme activities were significantly affected by both N deposition and biochar amendment ($P < 0.01$, Fig. 5). Two-way PERMANOVA results indicated that N deposition and biochar amendment had significant interaction effect on soil enzyme activities ($P < 0.01$). The variation of soil enzyme activities was significantly related with soil AP and AK contents (Fig. 5a). Among treatments without any N deposition, different biochar amendment rates can lead to distinct soil enzyme activities (Fig. 5b). N deposition also changed soil enzyme activities significantly when there was no biochar amendment ($P < 0.05$). However, no significant difference was found between the two treatments with different N deposition rates (Fig. 5b). At both N1 and N2 deposition levels, biochar amendment, especially the higher amount of biochar amendment can mitigate the changes of soil enzyme activities. No significant differences were found between N0C0 and N1C2, as well as N2C2 treatment (Fig. 5c, d).

3.4. Path analysis

To further characterize the differentiated effects of the N deposition and biochar amendment on soil physicochemical properties, soil bacterial community composition and enzyme activities, structural equation model (SEM) was constructed (Fig. 6). We observed a χ^2 of 24.450

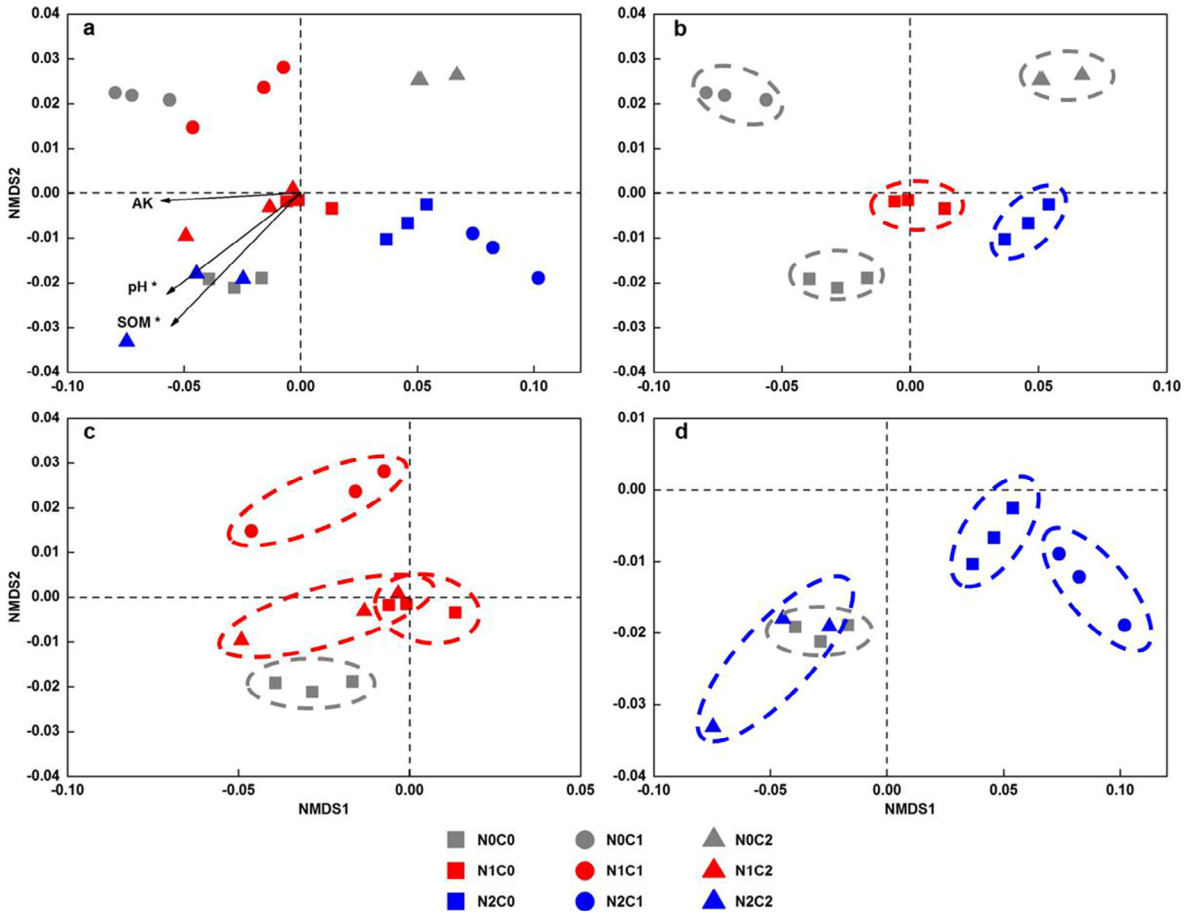

Fig. 2. Soil bacterial community compositions under different levels of N deposition and/or biochar amendment revealed by nonmetric multidimensional scaling (NMDS). The arrows indicated the soil parameters that had significant impact on bacterial communities (*, $P < 0.05$). SOC, soil organic carbon; AK, available K.

for this model (df = 16, $P = 0.08$). Failure to reject the null hypothesis ($P > 0.05$) indicates that the model was a good fit. We also calculated a GFI of 0.843 and a RMSEA < 0.001. Results showed that biochar had important direct effects on soil pH (0.799), SOC (0.662) and available P (0.338), while N deposition only had important direct effects on soil available N (0.437) and P (0.338). Considering the total effects, soil available P positively regulated soil enzyme activities (0.505) while soil pH alone negatively regulated (-0.385) them (Table S1). Biochar amendment had larger indirect effect than N deposition on both soil bacterial community and enzyme activities (Table S1). Overall, biochar amendment, as well as soil pH and SOC had greater total effects on soil bacterial community than N deposition. Moreover, biochar amendment and soil available P had important positive effect while soil available N had great negative effect on soil enzyme activities (Fig. 6; Table S1).

4. Discussion

4.1. Effects of N deposition

The effect of N deposition or fertilization on soil microbial community composition and biomass has been well recognized (Ai et al., 2018; Freedman et al., 2015; Jian et al., 2016; Li et al., 2016; Treseder, 2008). Negative effects of N addition on soil microbial biomass were widespread in terrestrial ecosystems (Treseder, 2008). Previous studies indicated that N fertilization reduced microbial respiration by 8%-11% (Liu and Greaver, 2010; Ramirez et al., 2010; Treseder, 2008) and MBC by 15%-35% (Boot et al., 2016; Liu and Greaver, 2010; Ramirez et al., 2010; Treseder, 2008). Based on 65 published studies, Jian et al. (2016) concluded that N fertilization inhibited MBC by 9.5%. Our findings of decreased MBC following N amendments (11.5%–15.9% less in N deposition vs. N0 plots) are in line with these previous results in other ecosystems (Fig. 1). Increased acidification and solubility of aluminum which is toxic to soil microorganisms could be possible mechanisms for decreased microbial biomass following N deposition (Geisseler and Scow, 2014; Vitousek et al., 1997). However, N deposition in the present study had no significant effect on soil pH (Table 1). Therefore, the possible reason could be due to the increased osmotic potential in soil solution owing to the N deposition, which could result in toxicity for specific microorganisms (Treseder, 2008). The copiotrophic hypothesis suggested that N addition reduced the relative abundance of oligotrophic taxa as the relieved N limitation allows them to be outcompeted by more copiotrophic taxa (Ai et al., 2018; Ramirez et al., 2012). In the present study, N deposition significantly increased soil available N, P and K contents (Table 1), which may increase osmotic potential in soil solution and inhibit the slow growing oligotrophs altering microbial community composition.

According to the NMDS ordination plot, our results observed the shifts of soil microbial community composition caused by the two levels of N deposition (Fig. 2b). The results are in accordance with Ramirez et al. (2012) who reported that N addition consistently altered bacterial community composition. Previous studies indicated that the shifts of soil microbial community composition could be due to the increase and decrease of specific bacterial groups (Ai et al., 2018; Ramirez et al., 2012). In the present study, the main effects of N deposition on microbial community composition were reductions in *Alpha-*, *Beta-*, and *Deltaproteobacteria*, and increase in *Acidobacteria* Gp2 (Fig. 3). Our results were not consistent with the previous studies which indicated that *Acidobacteria* was generally classified as slow-growing oligotrophs and

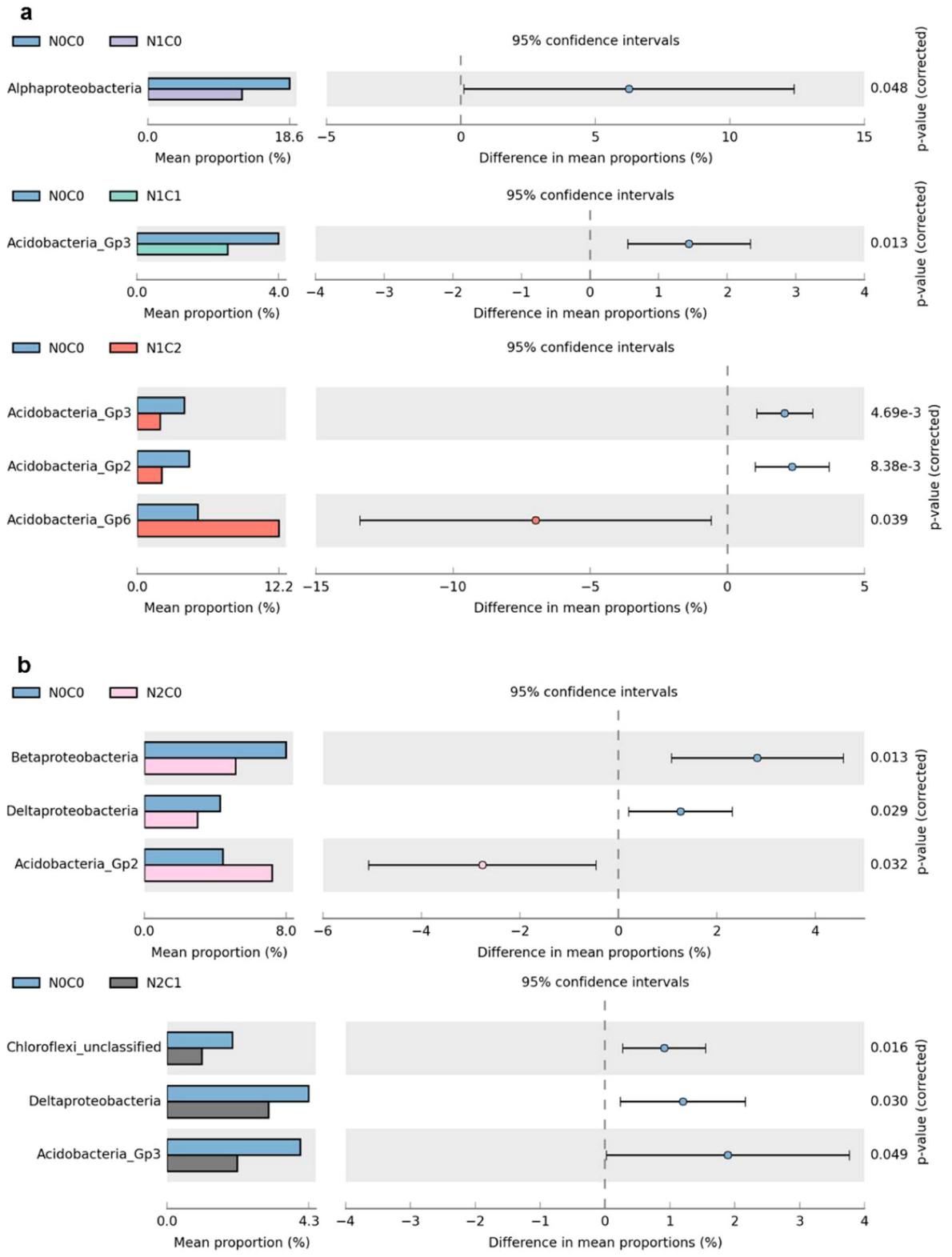

Fig. 3. Changes of key bacterial taxa in response to N deposition and/or biochar amendment according to the response ratio method at a 95% confidence interval.

thrives in soils with low available nutrients (Fierer et al., 2007; Ramirez et al., 2012). However, results of an experimental N deposition in Chinese Fir (*Cunninghamia lanceolata*) plantation indicated that nitrate input increased the relative abundance of Gp2 (Liu et al., 2017). Swathi et al. (2013) reported that Gp2 was not significantly influenced by N application. Hence, the microbial community has complex relationships with nitrogen dose and form, and could be inconsistent among various studies.

Likewise, soil enzyme activities can be highly sensitive to increased N deposition, especially those enzymes that degrade complex C compounds (Ai et al., 2018; Geisseler and Scow, 2014; Ramirez et al., 2012). Our results showed changed soil enzyme activities under N deposition (Fig. 4; Fig. 5b). Jian et al. (2016) concluded that N fertilization stimulated hydrolytic extracellular enzyme activities but depressed

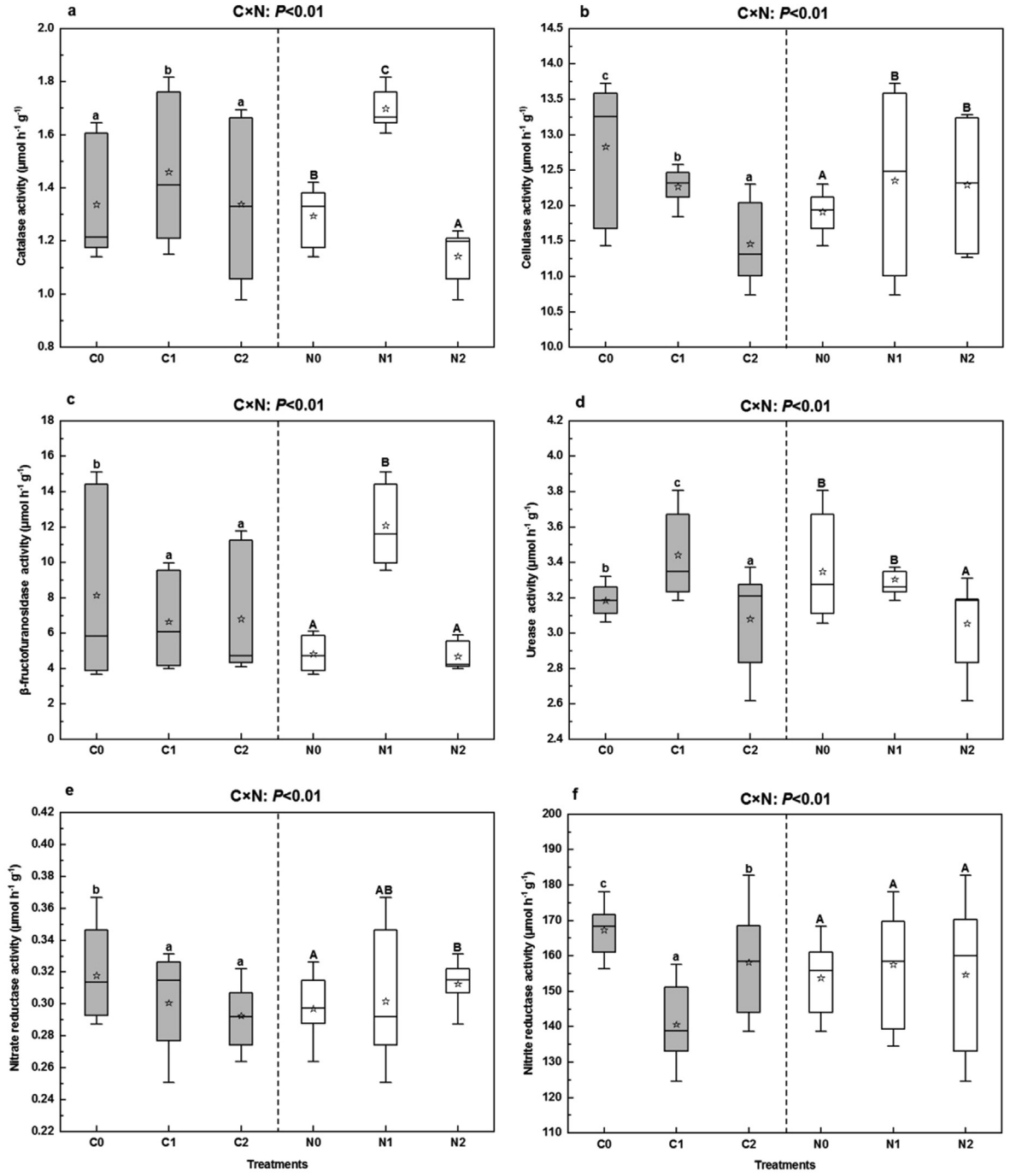

Fig. 4. Soil enzyme activities under different levels of N deposition or biochar amendment. Box plots display the first (25%) and third (75%) quartiles, the median and the maximum and minimum observed values within each data set. Each data set represents 9 replicates. Different letters indicate significant differences among different levels of N deposition or biochar amendment ($P < 0.05$).

oxidative enzyme activities. The present results proved that the activity of cellulose which is associated with hydrolytic C acquisition increased significantly after N deposition (Fig. 4b). Sufficient N supply appears to sustain soil microbes to produce more extracellular enzymes associated with hydrolytic C-acquisition (Jian et al., 2016). However, the urease activity decreased after N deposition, especially in the N2 level of deposition (Fig. 4d). According to the principles of enzyme stoichiometry, C degrading enzyme activity may be enhanced while N degrading enzyme activities are reduced under N amendments (Sinsabaugh et al., 2009). This is because a large quantity of available N in soil could have substantially relieved N limitations for microbes and caused more conservative production of N-associated enzymes (Sinsabaugh et al., 2008). N deposition also increased soil nitrate and nitrite reductase activities, especially for nitrate reductase (Fig. 4e). The accelerated nitrate and nitrite reductase activities may be attributed to the increase of nitrogen content, which could act as the substrate of denitrification.

4.2. Effects of biochar amendment

Numerous studies have revealed that biochar amendment can influence soil microbial biomass, community composition, as well as enzyme activities, due to the changes in soil physicochemical properties and microhabitat conditions for microorganisms (Chen et al., 2019; Gul et al., 2015; Lehmann et al., 2011; Li et al., 2018a,b). Response of soil

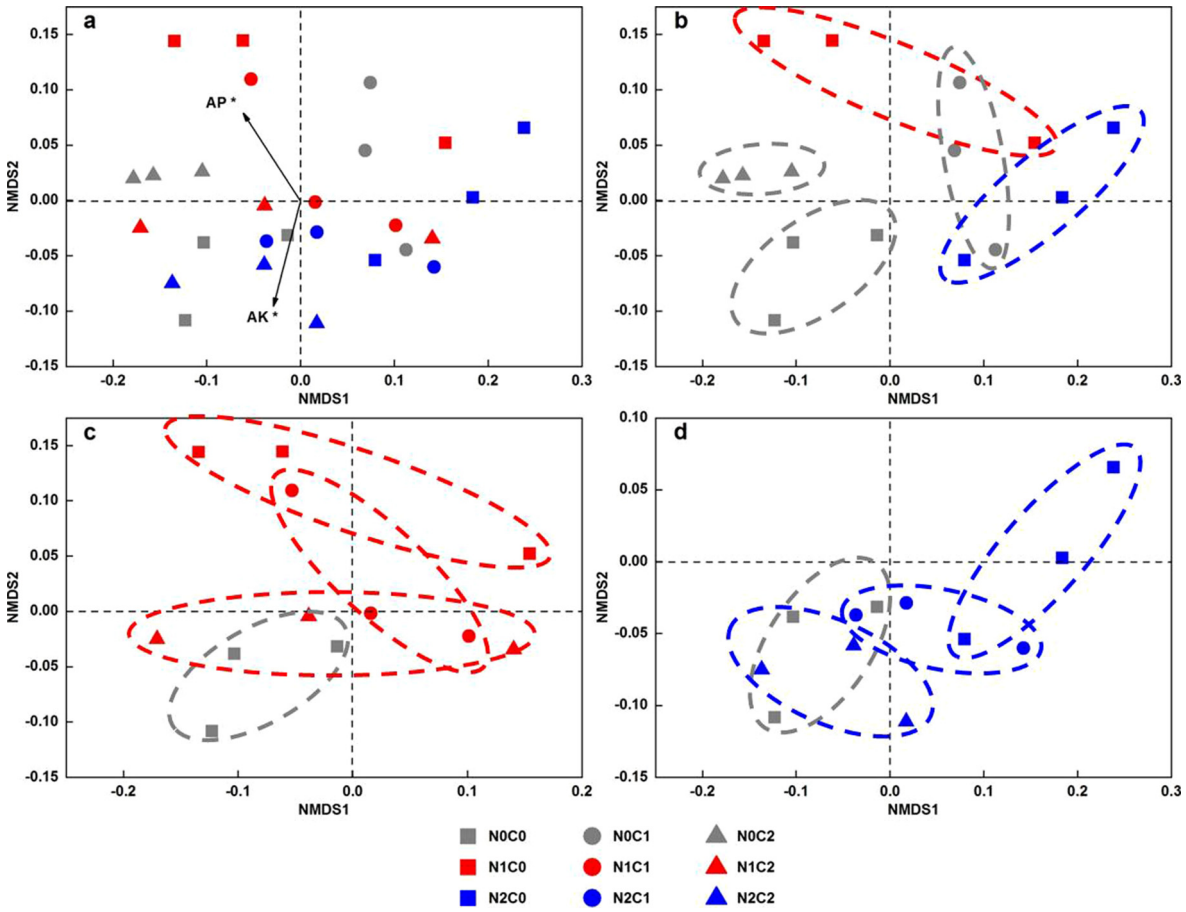

Fig. 5. Soil enzyme activities under different levels of N deposition and/or biochar amendment revealed by nonmetric multidimensional scaling (NMDS). The arrows indicated the soil parameters that had significant impact on bacterial communities (*, $P < 0.05$). AP and AK indicate available P and K, respectively.

MBC to biochar amendment are inconsistent in many studies, depending on the derived materials, pyrolyzed temperature, and soil textures, etc (Gul et al., 2015). Some studies showed greater MBC in soils amended with biochars (Ameloot et al., 2013; Chen et al., 2019; Wang et al., 2015), while others showed no changes in MBC when slow pyrolyzed (470–500 °C) wood derived biochars were applied at rates of 2–20% by mass during short term experiment (Mitchell et al., 2015; Prayogo et al., 2014). In our study, significant decrease in soil MBC was observed under slow pyrolyzed (450 °C) biochar derived from wheat straw. Dempster et al. (2012) also reported 28% reduction ($P < 0.05$) in MBC in response to the amendment of slow pyrolyzed *Eucalyptus* wood biochar produced at 600 °C. It could be due to that biochar can attract and retain water and nutrients from the soil solution and make them inaccessible to microorganisms, and then this could leave microorganisms nutrient-impoverished for a period (Gul et al., 2015).

Biochars are frequently reported to result in a shift in the bacterial and fungal community structure. Our results are consistent with previous studies that biochar affects the soil microbial biomass and activity, changes the soil bacterial community structure (Gul et al., 2015; Huang et al., 2018a; Mackie et al., 2015). Studies showed considerable changes in soil microbial community composition towards gram-positive bacteria both in laboratory incubation and a rice paddy field trial (Chen et al., 2019; Jiang et al., 2016). In our study, we found that biochar amendment not only changed soil microbial community

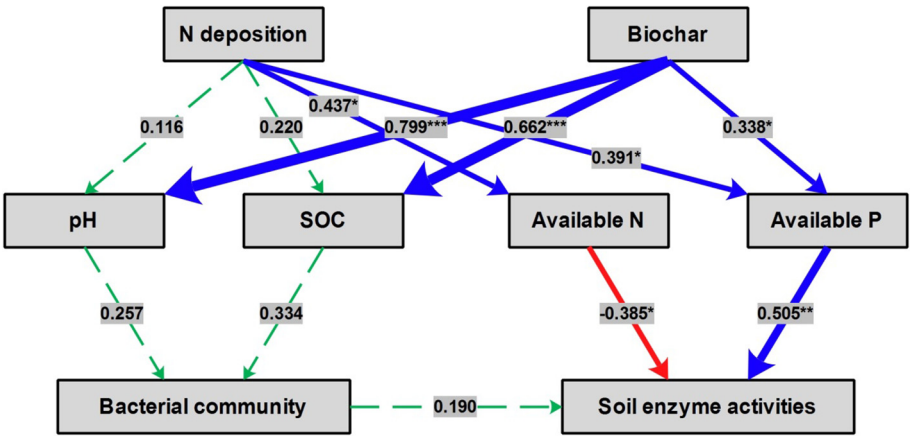

Fig. 6. Structure equation modeling revealed the effects of N deposition and biochar amendment on soil bacterial community and enzyme activities. Path coefficients are calculated after 1000 bootstraps and reflected in the width of the arrow, with blue and red indicating positive and negative effects, respectively. Dashed green arrows show that coefficients did not differ significantly from 0 ($P > 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2b) but also mitigated the influence of N deposition on microbial community composition (Fig. 2c, d). Biochar addition can increase soil pH, cation exchange capacity, and the moisture and nutrient retention ability, hence indirectly enhances microbial activity and functions (Huang et al., 2018b; Nielsen et al., 2014; Xu et al., 2016). At the N1 deposition level, biochar amendment mainly affected the relative abundance of *Acidobacteria* when compared to N0C0 treatment (Fig. 3a). Sheng and Zhu (2018) found that *Acidobacteria* abundance increased following biochar addition, which probably because of their capacity for using recalcitrant carbon sources existing in biochar. However, we found biochar amendment at N1 deposition level decreased the relative abundance of *Acidobacteria* Gp2 and Gp3 but increased Gp6 (Fig. 3a). Though *Acidobacteria* has been shown to be sensitive to pH shifts, its subgroups show variable response to acidity (Whitman et al., 2016). *Acidobacteria* Gp1, 2, and 3 have shown to increase at lower pH, whereas Gp4, 5, 6, 7, and 17 have increased at higher pH (Bartram et al., 2014; Rousk et al., 2010). At the N2 deposition level, lower amount of biochar amendment (C1) decreased *Chloroflexi* and *Acidobacteria* Gp2 abundance, but no difference of any bacterial taxa was found between N2C2 and N0C0 (Fig. 3b). The mitigation of negative effect from high N deposition level by high dose of biochar could be due to the neutralization of soil pH, increased nutrients availability, and improved plant growth. We have found that biochar application can enhance soil fertility and alleviate unbalanced nutrient uptake caused by N deposition in *Torreya grandis* trees (Zhang et al., 2017, 2019).

Biochar with greater porosity and surface area has generally been found to reduce the soil enzymatic activities, since such biochar would tend to bind substrates and extracellular enzymes, thus interfering with the rate of substrate diffusion to the active site of enzyme catalysis (Gul et al., 2015; Lehmann et al., 2011; Nannipieri et al., 2012). We found initial increase and thereafter decrease in catalase and urease activities with increasing biochar amendment rate (Fig. 4a, d). Ameloot et al. (2013) reported a 47% reduction in dehydrogenase activity with biochar produced at 700 °C during a 117 days laboratory study. However, several studies found a series of enzymes related to N utilization, like urease and L-leucine aminopeptidase, increased with the increase of biochar addition rate (Bailey et al., 2011; Wang et al., 2015). It is presumably that biochar application rate is an important factor control the activity of N cycling enzymes. Moreover, decreased activities of soil enzymes involved in C cycling and denitrification was observed with the increase of biochar amendment rate (Fig. 4). This decrease most likely due to sorption or blocking of either enzyme or substrate, presumably caused by excessive biochar porosity and reactive surface area (Jindo et al., 2012).

4.3. Impact of soil environmental changes on bacterial community compositions and enzyme activities

Soil microbial community abundance and structure have been widely used as indicators of soil quality because of their sensitivity to environmental change (Marschner et al., 2003; Qin et al., 2014, 2017). N deposition and biochar amendment to soils have been proved to affect microbial community composition and functions, which were related to the changed soil physico-chemical properties. In this study, NMDS showed that the variation of bacterial community composition was significantly related to soil pH and SOC (Fig. 2a), while enzyme activities was related to soil available P and K contents (Fig. 5a). Our results are in line with previous studies that pH is the major determining factor in microbial community structure (Ai et al., 2018; Qin et al., 2014; Rousk et al., 2010). However, soil pH is not the only factor affecting bacterial community. Many studies have shown that changes of soil microbial biomass and community composition were closely related to the soil C content (Ai et al., 2012; Bowles et al., 2014), which is consistent with our observations. Besides soil pH and organic C, the stoichiometric variations of C, N and P also play an important role in microbial community succession (Li et al., 2017). We found that biochar amendment leads to increased soil available P and K contents (Table 1), which could probably alleviate the P and K limitation for microorganisms in this N enrich environment. Our previous studies also indicated that biochar application enhanced uptake of P, decreased foliar N:P ratios, and alleviated P limitation of *Torreya grandis* tree growth induced by N addition (Zhang et al., 2019). However, the effects of AP and AK on microbial community were not as notable as on soil enzyme activities, this is due to the extracellular enzymes synthesized by soil microbes as the rate-limiting step for microbial metabolism (Jones et al., 2009), and the enzyme stoichiometric ratios directly reflect the ability of microorganisms to use nutrients (Jones et al., 2009; Moorhead and Sinsabaugh, 2006; Xu et al., 2017). Our SEM results also showed that alteration in soil AN and AP contents were important for regulating soil enzyme activities, with soil enzyme activities negatively respond to AN but positively respond to AP contents (Fig. 6). The findings further confirmed that N deposition has a negative effect on soil enzyme activities through changing the stoichiometric ratios of soil nutrients, while biochar amendment could alleviate P limitation through increasing P availability.

5. Conclusions

The decreased soil MBC and altered soil bacterial community composition we found in response to N deposition were typical for many agricultural and forest ecosystems. Biochar amendment alone also had negative effect on soil MBC, and altered soil microbial community composition. Biochar amendment can mitigate the shifts of both bacterial community composition and soil enzyme activities induced by the two levels of N deposition. N deposition negatively regulated soil enzyme activities mainly through increasing AN content, while biochar amendment can mitigate the negative effect probably through increasing soil AP content and improved the stoichiometric ratio of available nutrients for soil enzymes. Though the influence of biochar on soil microbial community composition and enzyme activities is highly complex, our results provide solid evidence that biochar amendment may have great potential in mitigating the negative effects of increasing N deposition in the coming decades, and is highly relevant for the development of sustainable forest management.

Declaration of Competing Interest

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

Acknowledgement

The research was financially supported by Natural Science Foundation of China (Grant Nos. 31570616 and 31470529). We thank Dr. Scott X. Chang and Dr. Yanjiang Cai for their assistance in manuscript preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.117717.

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