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RESEARCH ARTICLE

Effects of biochar particle size and concomitant nitrogen fertilization on soil microbial community structure during the maize seedling stage

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Abstract

Biochar is widely used as a soil amendment, either alone or in association with fertilizer. However, the effects of biochar particle size on the soil microbial community are largely unclear. Biochar was divided into two groups according to diameter sizes: < 1 mm and 2.5–5 mm (labeled as CB1 and CB5, respectively). A pot experiment was established in which maize (*Zea maize L.*) was treated with CB1, CB5, and/or external nitrogen (N, NH_4NO_3). At the end of the seedling period (45 days), analyses of soil microbial community structure and other soil and crop properties were conducted. The biochar (regardless of N addition) enhanced microbial biomass and activity. CB1 had a stronger capacity than CB5 to modify soil microbial community structure by promoting soil microbial groups (e.g., fungi, Gram-negative bacteria), which is likely due to CB1 undergoing a series of more intense processes (e.g., nutrient release, mineralization) than CB5. However, this difference was diminished or disappeared when N was added, mainly due to the masking effect of soil acidification that was induced by N fertilization. Collectively, fine biochar has a stronger effect on soil microbial community than coarse biochar. Particle size only affects soil microbial community structure when biochar is applied alone; it has no effect when biochar is applied in association with chemical N fertilizer, at least during the seedling period. The relationship between particle size and soil microbial community needs to be considered when using biochar for soil amendment.

Keywords Biochar · Particle size · PLFA · Soil amendment

Introduction

The effects of biochar on soil microorganisms have generated wide-reaching concern, since both biochar and microorganisms can regulate soil functions, e.g., nutrient cycling and carbon (C) sequestration (Bamminger et al. 2016; Oliveira et al. 2017; Sheng and Zhu 2018). The soil microbial community is more crucial than any individual microbial species in the process of soil mediation (Raaijmakers and Mazzola 2016). Through regulating soil microbial community structure (Igalavithana et al. 2017), biochar may improve soil nutrient availability and crop productivity (Luo et al. 2017). The response of the soil microbial community to biochar could be used as an indicator of soil function variations in agricultural ecosystems. Thus, elucidating a soil microbial community-biochar paradigm is crucial when using biochar for soil amendment. In agricultural practice, biochars are usually shattered to particles of different diameters before application. However, the effect of biochar particle size on soil microbial community structure remains unclear.

Generally, biochar mediates soil microbial community structure mainly through the alterations of soil chemical properties, such as pH, the C-nitrogen (N) ratio, and cation exchangeable capacity (CEC) (Muhammad et al. 2014; Nielsen et al. 2018; Rutigliano et al. 2014), and physical structures, such as soil aeration and soil aggregates (Busscher et al. 2010;

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Githinji 2014). Additionally, biochar can adsorb organic compounds and provide habitat for microbial groups (Atkinson et al. 2010; Lehmann et al. 2011), which can, in turn, mediate soil microbial community structure. However, these processes may vary with biochar particle size. The particle size of biochar can impact biochar resistance to mineralization (Manyà 2012). Sigua et al. (2014) found that a biochar particle size > 2 mm was more stable than a particle size < 0.42 mm. A similar finding was reported by Zimmerman (2010); biochars < 0.25 mm had a higher mineralization rate than did 0.25 to 2 mm biochars. Ponomarenko and Anderson (2001) reported that the biochar adsorption capacity of soil clays depended on the particle size of the biochar. Particle-size dependent processes in soil-biochar systems may alter soil microbial processes directly or indirectly. Moreover, relative to coarse biochars, fine biochars (such as those < 0.25 mm) can facilitate soil aggregate stability and promote microbial growth. Thus, soil microbial community structure can be modified via occlusion within microaggregates (< 0.25 mm) since biochars promote soil microaggregate formation and stabilization (Brodowski et al. 2006). Additionally, relative to coarse biochars, fine biochars are more easily transported and redistributed to deep soils (Leifeld et al. 2007). The redistribution of fine biochar can alter soil physical environment particularly in clay soils with poor oxidative conditions, and thus alter the distribution of soil microbial groups. Importantly, we previously found that through its mineralization, biochar releases available nutrients (e.g., labile C, mineral N) within a few months of soil application (Zhao et al. 2015c). Such release may be greater for fine biochar particles than for coarse ones.

Consequently, when biochar is used as a soil amendment, its particle size could affect soil microbial community structure. The strength of this effect may vary among microbial groups, although experimental evidence is lacking. For example, fine biochars can promote short-term fungi dominance in the soil microbial community due to the high sensitivity of fungi to soil nutrients (Rifai et al. 2010); organic matter stability and soil quality may subsequently be improved due to the low turnover rate of fungi (Clemmensen et al. 2015). In contrast, coarse biochars may have weaker effects on soil microbial community structure. Biochar application with N fertilizer is presently considered an effective amendment practice. It has been reported that the application of biochar with N fertilizer can alter soil microbial community structure and crop yield (Farrell et al. 2014; Nielsen et al. 2018). Thus, it is possible that the effects of biochar particle size on soil microbial community structure are magnified when N is added.

In the hilly red soil region of southern China, cropland soil has high clay content, and the main crop is maize (*Zea maize L.*). A man-made forest of *Cunninghamia lanceolata* is widely distributed across the region, and the sawdust of this tree is widely used as a feedstock for biochar across the region. This study aimed to examine the effects of biochar particle size, with and without N fertilizer, on soil microbial community structure during the maize seedling stage. To do so, a well-controlled pot experiment was conducted with typical soil for the region as the matrix, which was treated with N fertilizer and biochar of two different particle sizes made from a local feedstock. We hypothesized that (i) fine biochars have stronger effects on soil microbial community structure than does coarse biochar, and (ii) these effects are enhanced under N addition.

Materials and methods

Soil and biochar

A typical soil (Plinthudult, 0–20 cm) of the hilly red soil region was collected from Zhejiang Province (27° 02′–31° 11′ N, 118° 01′–123° 25′ E), where the average annual temperature is 15–19 °C and the mean annual precipitation is 1300 mm. Biochar from the trunk of *C. lanceolata* was pyrolyzed (and labeled CB). The preparation and properties of the biochar are detailed in our previous study (Zhao et al. 2015b). Briefly, after being air-dried and shattered, the trunk was pyrolyzed in a muffle furnace at a heating rate of 20 °C min^{-1} and a maximum temperature of 450 °C for 1 h. The soil and biochar properties are shown in Table 1, including soil pH, soil organic C (SOC), total N (TN), cation exchange capacity (CEC), and soil texture; biochar elements C, N, hydrogen (H), and oxygen (O); pH of the biochar; dissolved organic C (DOC); total soluble N (TSN); volatile matter; and ash and specific surface area. The pH levels of the CB and soil are comparable, which can minimize the potential influence of acidity on the results.

Pot experiment with maize

Two CB particle groups, one with a diameter size of < 1 mm and the other with a diameter size of 2.5–5 mm, were prepared (labeled CB1 and CB5, respectively); these diameters were chosen since most biochar particles applied to cropland in this region are < 5 mm. Two kilograms of air-dried soil samples (sieved at 2 mm) were left untreated or were treated with external N (NH_4NO_3) and/or CB. The N addition rate was 0.15 g kg^{-1}, and the addition rate of biochar (both CB1 and CB5) was 2% (wt:wt.). These addition rates were chosen according to local agricultural management.

According to the two-way factorial design with biochar (three levels) and N (two levels) as independent variables, six treatments with three replicates each were established, i.e., SC1 (soil+CB1), SC5 (soil+CB5), SC1N (soil+CB1+N), SC5N (soil+CB5+N), CKN (soil+N), and CK (soil only).

Table 1 Properties of the tested soil and biochar CB (made from *C. lanceolata* trunk) (Zhao et al. 2015b)

Items	Parameters [a]	Values
Soil	pH	4.84
	SOC (g kg^{-1})	4.20
	TN (g kg^{-1})	0.28
	CEC (cmol kg^{-1})	10.93
	Sand (2–0.05 mm, %)	21.7
	Silt (0.05–0.002 mm, %)	37.0
	Clay (< 0.002 mm, %)	41.3
CB	Element C (mg kg^{-1})	712.1
	Element H (mg kg^{-1})	24.7
	Element N (mg kg^{-1})	3.2
	Element O (mg kg^{-1})	260.0
	O:C	0.37
	H:C ($\times 10^{-2}$)	3.47
	DOC (g kg^{-1})	1.88
	TSN (mg kg^{-1})	45.92
	pH	4.88
	Volatile matter (mg kg^{-1})	9.7
	Ash (mg kg^{-1})	10.3
	Specific surface area (m^2 g^{-1})	145.5

[a] SOC, soil organic C; TN, total N; CEC, cation exchange capacity; DOC, dissolved organic C; and TSN, total soluble N

After the soils were placed into plastic pots and packed to approximate 1.2 g cm^{-3} (based on the field condition), the soils of all treatments were adjusted to 60% water-holding capacity. Three maize seeds were sowed at a depth of ~ 5 cm in each pot, and only the highest seedling after germination was retained.

The treatment pots were maintained for 45 days in a greenhouse, during which soil water content was adjusted to maintain a constant level by weight every 2 days. On the 45th day, the plants (both above- and below-ground portions) were harvested. The soils were subsampled, freeze-dried (− 80 °C), and then immediately analyzed for phospholipid fatty acids (PLFA). A portion of each soil sample was stored at 4 °C for measurements of other microbial properties, and the remaining soil was air-dried.

Measurements of soil and seedling-biomass characteristics

Maize shoot and root biomass were determined with an oven-drying method (70 °C, 48 h), and the ratio of shoot biomass to root biomass (S/R) was calculated. Each air-dried soil sample was mixed with deionized water (1:2.5 by wt:vol.) and stirred for 1 min using a magnetic stirrer. After being equilibrated for 30 min, the pH of the mixture was measured with a digital pH meter (FE28-standard, Mettler-Toledo Inc., Switzerland). After being mixed with 2 M KCl (1:5 by wt:vol.), the moist soil sample was shaken (1 h,180 rpm). The mixture was filtered, and nitrate N (NN) and ammonium N (AN) measurements were made on a flow injection analyzer (SA-4000, Skalar Co., Netherlands) (Salazar et al. 2014). Soil CEC was analyzed with the method of ammonium-acetate compulsory displacement (Lu 2000).

After being fumigated with alcohol-free chloroform (24 h, 25 °C), the moist soil sample was extracted with a 0.5 M K_2SO_4 solution (30 min, 180 rpm) at the ratio of 1:4 (wt:vol.). The same procedure was conducted for the unfumigated soil sample. Both extracts from the fumigated and unfumigated mixtures were determined for soluble organic C and total N with a vario TOC analyzer (Elementar Co., Germany). Soil microbial biomass C (MBC) and N (MBN) were calculated according to Eqs. 1 and 2, respectively (Brookes et al. 1985; Vance et al. 1987):

$$MBC = EC \times 2.64 \quad (1)$$

$$MBN = EN \times 1.85 \quad (2)$$

where EC and EN are the differences between the organic C and soluble total N extracted from fumigated and unfumigated samples, respectively; 2.64 and 1.85 are dimensionless constants.

Soil basal respiration (SBR) was measured according to the literature (Li et al. 2016). The moist soil samples (equivalent to 30 g dw) were collected into 1-L Erlenmeyer flasks and adjusted to 60% water-holding capacity. In each flask, a small beaker filled with 15 mL NaOH (1 M) was placed over the soil surface to trap CO_2 released from the soil. Then, the flasks were incubated at 25 °C for 24 h in the dark. After the incubation, the NaOH solution in the small beaker was mixed with $BaCl_2$; the mixture was then titrated with 0.1 M HCl. A NaOH solution without soil was also incubated and titrated as a control.

Following the procedures of Luo et al. (2016), PLFA analysis was conducted. The phospholipids were extracted with a mixture of chloroform, citric acid, and methanol eluted by methanol and separated on a silica column. The fatty acid methyl esters (FAMEs) were formed when the separated phospholipids were methylated with a KOH methanolysis solution. Using methyl nonadecanoate 19:0 as an internal standard, the FAMEs were detected via a capillary gas chromatography system (Agilent 6850 Series, Agilent Technologies Inc., USA). The FAMEs were identified on a MIDI system (MIDI Inc., USA) and then, the PLFA amounts were calculated. The PLFAs were eliminated if they appeared in only one sample or their contributions were less than 1% in all samples (Zhong et al. 2010). Individual PLFAs were used to identify broad taxonomic groups in the microbial community. Based on the description in the literature (Zelles 1997),

actinomycetes (ACT) were characterized by fatty acids with a methyl branch on the 10th C; Gram-negative bacteria (GN) were identified by monounsaturated and cyclopropyl fatty acids; and Gram-positive bacteria (GP) were indicated by branched saturated fatty acids. Fungi were characterized by 18:2ω6c (Liu et al. 2018). Total bacteria were calculated as the sum of GP and GN.

Statistics

Two-way ANOVA was performed using SPSS v18.0 (SPSS Inc., USA) to test for differences in PLFA groups and other soil properties among the treatments at the end of the pot experiment. Principal component analysis (PCA) and redundancy analysis (RDA) were conducted to characterize microbial distributions and the relationships between environmental variables and soil microbial community structure, respectively, using Canoco5 (Microcomputer Power, USA). Monte Carlo analysis (on log-transformed, centered, and standardized data) was performed to test the effects of the RDA axis on soil microbial community structure and to test for environmental variable contributions ($P < 0.05$) to PLFA-data variances.

Results

Soil and seedling-biomass characteristics at the end of the pot experiment

According to the two-way ANOVA, the variance in MBC ($F_{2,12} = 110.00$, $P < 0.001$) was mainly regulated by CB addition, whereas pH was mainly influenced by external N (Table 2). Most parameters (e.g., SBR, AN, NN, and CEC) were affected by both external N and CB and their interactions. SC1 and SC5 had higher levels of MBC, MBN, and SBR than CK; a similar trend was also found when N was added (SC1N and SC5N vs. CKN, Fig. 1). However, the differences in MBC, MBN, and MBC/MBN between SC1 and SC5 and between SC1N and SC5N were not significant ($P > 0.05$). CEC increased following biochar addition (whether alone or in association with external N), and this pattern was more pronounced in the CB1 treatments than in the CB5 treatments (SC1 vs. SC5 and SC1N vs. SC5N). Similar trends were found for both AN and NN in the soil-biochar treatments (SC1 vs. SC5) but not in the soil-biochar-N treatments (SC1N vs. SC5N). The pH was lower following external N addition.

Soil microbial groups at the end of the pot experiment

Most microbial groups (fungi, GN, GP, and bacteria) and GP/GN were affected by both external N and CB and their interactions (Table 2). However, F/B was mainly regulated by N addition. In the soil-biochar treatments, the abundances of most microbial groups were greater in CB1 and CB5 than in CK (Fig. 2). Fungi, GN, GP, and bacteria abundances were significantly higher in SC1 than in SC5, but no difference in the abundances of these groups was found between SC1N and SC5N. Corresponding differences were also apparent in the PCA where SC1 was separated distinctly from SC5 and CK by PCA2 (Fig. 3). A total of 89.2% of the variance in microbial abundance was explained by the two axes. Bacteria and GP abundance had strong negative loadings on PCA1, while fungi had a strong positive loading on PCA2. The GP/GN levels were significantly lower in SC1 than in SC5, whereas no significant difference in GP/GN was observed between SC1N and SC5N.

Table 2 Statistic parameters of N and CB effects on soil chemical and microbial parameters based on a two-way ANOVA

Parameters [a]	N		CB		N×CB	
	F	*P*	*F*	*P*	*F*	*P*
MBC	1.70	0.217	110.00	0.000	0.55	0.591
MBN	0.87	0.371	121.74	0.000	16.67	0.000
MBC/MBN	0.29	0.603	3.82	0.052	34.01	0.000
SBR	46.07	0.000	174.66	0.000	10.66	0.002
AN	885.06	0.000	21.51	0.000	12.51	0.001
NN	523.17	0.000	16.86	0.000	6.17	0.014
pH	1457.49	0.000	1.68	0.227	0.69	0.520
CEC	25.51	0.000	232.15	0.000	18.73	0.000
Shoot	42.37	0.000	14.39	0.001	3.58	0.060
Root	75.00	0.000	22.58	0.000	1.00	0.397
S/R	1.97	0.186	17.99	0.000	5.66	0.019
ACT	10.49	0.007	1.33	0.301	4.08	0.045
Fungi	101.62	0.000	68.64	0.000	49.75	0.000
GN	28.68	0.000	50.53	0.000	52.16	0.000
GP	7.46	0.018	52.53	0.000	36.77	0.000
Bacteria	25.41	0.000	64.79	0.000	59.73	0.000
F/B	25.33	0.000	3.56	0.061	3.71	0.056
GP/GN	8.15	0.014	4.30	0.039	4.12	0.044

[a] MBC, microbial biomass C; MBN, microbial biomass N; MBC/MBN, the ratio of MBC to MBN; SBR, soil basal respiration; AN, Ammonium N; NN, Nitrate N; CEC, cation exchange capacity; S/R, the ratio of shoot biomass to root biomass; ACT, actinomycetes; GN, Gram-negative bacteria; GP, Gram-positive bacteria; GP/GN, the ratio of GP to GN; F/B, fungal/bacterial ratio; N, external N; CB, biochar CB; and N×CB, interactions between external N and CB

RDA of soil microbial community structure in the different treatments

A total of 75.7% of the variance in the PLFA data was explained by the first two RDA axes (Fig. 4). Both of the

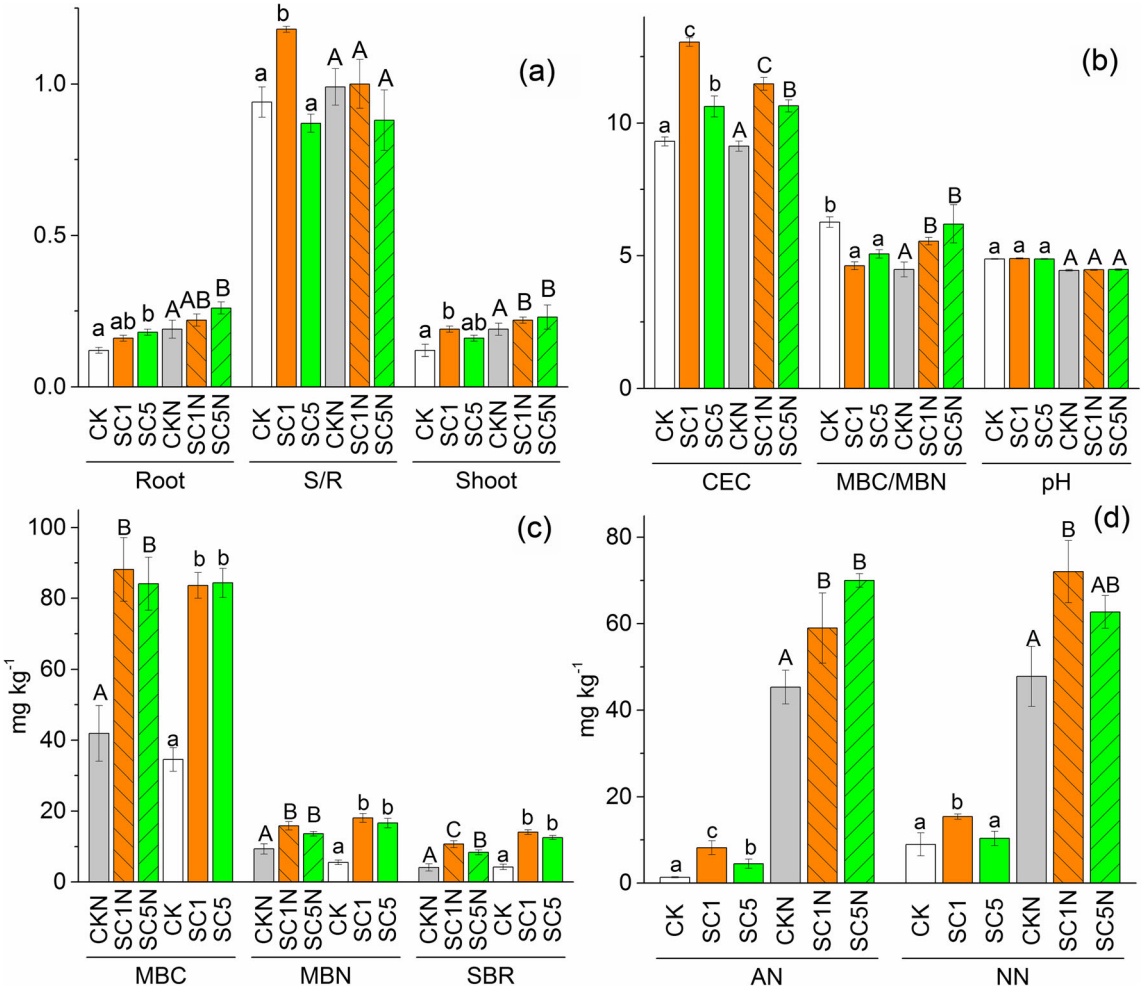

Fig. 1 Soil properties and maize biomass across soil biochar and soil-biochar-N treatments. MBC, microbial biomass C; MBN, microbial biomass N; MBC/MBN, the ratio of MBC to MBN; AN, Ammonium N; NN, Nitrate N; SBR, soil basal respiration; CEC, cation exchange capacity; and S/R, the ratio of shoot biomass to root biomass. The units of root and shoot in Fig. 1a are g; the unit of CEC in Fig. 1b is cmol kg^{-1}.SC1, soil+CB1; SC5, soil+CB5; SC1N, soil+CB1+N; SC5N, soil+CB5+N; CKN, soil+N; and CK, soil only. CB1 and CB5 are biochar CB particles with diameter sizes of < 1 and 2.5–5 mm, respectively. Each value is the mean of 3 replicates with standard deviation in the bracket. The values marked with the same lowercase letters across soil-biochar treatments or those with the same uppercase letters across soil-biochar-N treatments are not significantly different ($P > 0.05$).

axes were significant according to the Monte Carlo permutation test (Table 3), indicating that the selected factors had a comprehensive influence on the PLFA-data distribution. Root (37.7%), CEC (34.4%), MBC (11.1%), and pH (5.1%) were the top 4 contributors to the PLFA-data variance, of which root, MBC, and pH were highly significantly ($P < 0.01$) correlated with RDA1, and CEC was highly significantly correlated with RDA2. SC1, SC5, and CK were separated from one another only for the soil-biochar treatments. SC1 centered on the area with a high CEC level, whereas CK was positioned in the area of high pH and low inorganic N and root biomass levels. Both SC1N and SC5N were distributed along a gradient with higher levels of inorganic N and root biomass. GN were positively correlated with CEC; GP were positively correlated with root biomass, AN, and NN and was negatively correlated with pH. Bacteria were positively correlated with MBC and MBN.

Discussion

Changes in soil microbial community structure after biochar and external N additions

Whether alone or in association with external N, CB addition enhanced microbial biomass and activity during the maize seedling period, which is consistent with previous reports (Yuan et al. 2017). The enhancement is largely due to the elevated nutrients (e.g., CEC, TSN, DOC, and ash) provided by CB (Kolb et al. 2009). This enhancement can promote microbial degradation and increase the contribution of

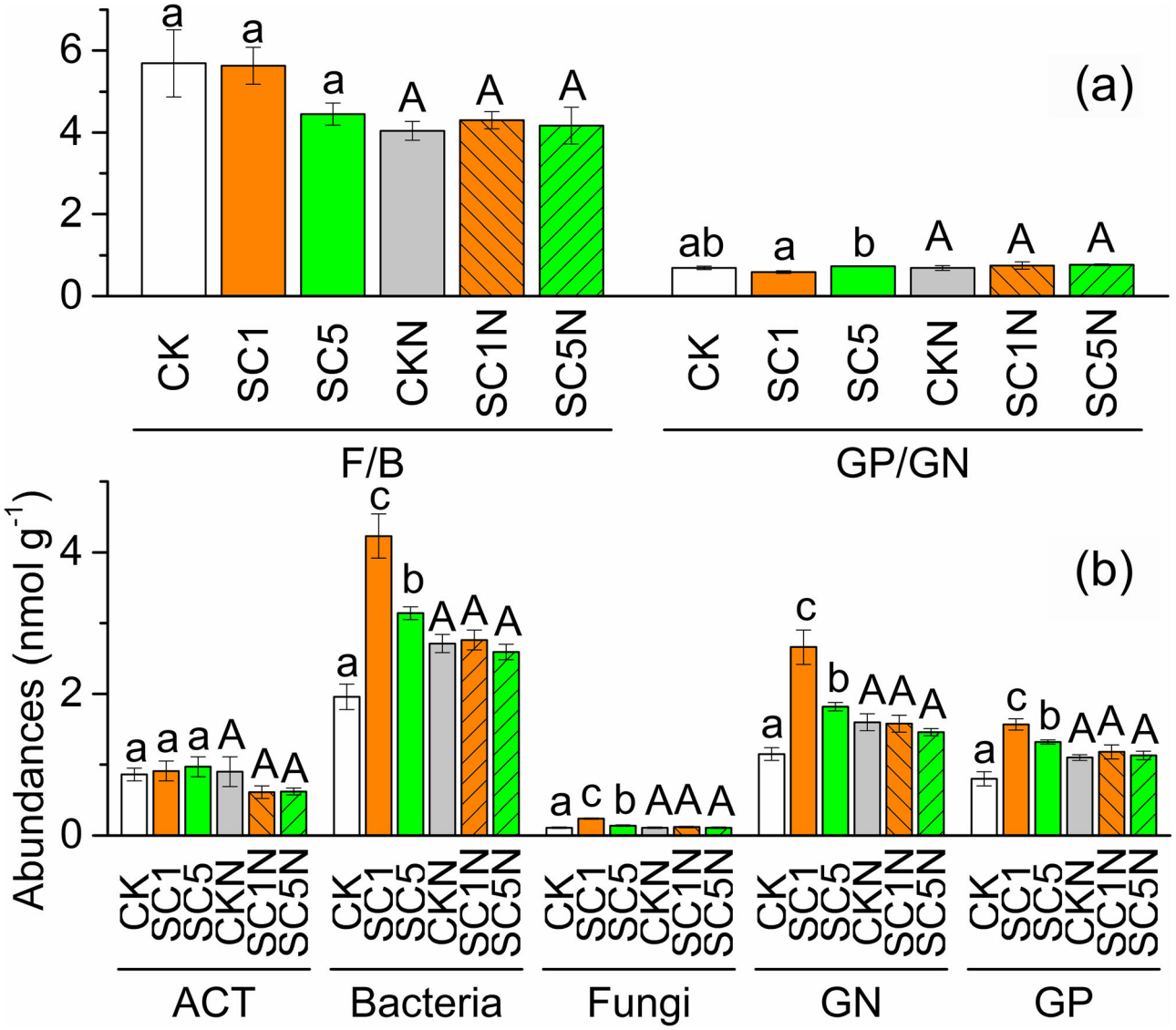

Fig. 2 The PLFA-group abundances and related parameters across soil biochar and soil-biochar-N treatments. ACT, actinomycetes; GN, Gram-negative bacteria; GP, Gram-positive bacteria; GP/GN, the ratio of GP to GN; and F/B, the ratio of fungi to bacterium. The F/B values in Fig. 2a have been transformed by $\times 10^2$.

microbial biomass to SOC, as reflected by the lower levels of MBC/MBN in the soil-biochar treatments than in CK (Khan et al. 2016). However, the addition of N decreased soil microbial activity rather than MBC and MBN (as evident from the comparisons of SC1 vs. SC1N and SC5 vs. SC5N). Most likely, external N decreases pH level and weakens the effect of biochar on soil microorganisms, since soil acidity is crucial to microbial processes (Jeanbille et al. 2016). This phenomenon acts as the "bet-hedging strategy" (Liu et al. 2018).

Soil microbial community structure appears to be regulated by CB alone and not by the association of biochar with external N. GN prefer sufficient labile C and nutrients, whereas GP prefer low-quality (relative high C/N ratios) organic materials (Bray et al. 2012). However, enriched GP in the soil microbial community have been found in soil-biochar-N treatments despite mineral N addition (as also indicated in the present study by the GP/GN distribution). A possible reason for this observation is that relative to GN, GP have thicker cell walls, allowing them to be largely protected from the exacerbation of soil acidification caused by the added N (Hammesfahr et al. 2008). Such protection was reflected in the negative correlations between GP and pH and by the positive correlations between GP and AN and NN (Fig. 4). Thus, soil acidification is very likely the main driver of soil microbial community structure mediation in soil-biochar-N treatments. On the other hand, fungi should remain stable in the soil microbial community, whether in soil-biochar treatments or soil-biochar-N treatments, since they have a wider range of pH values for optimal growth than do bacteria (Rousk et al. 2010). However, in the present study, fungi dominated the changes

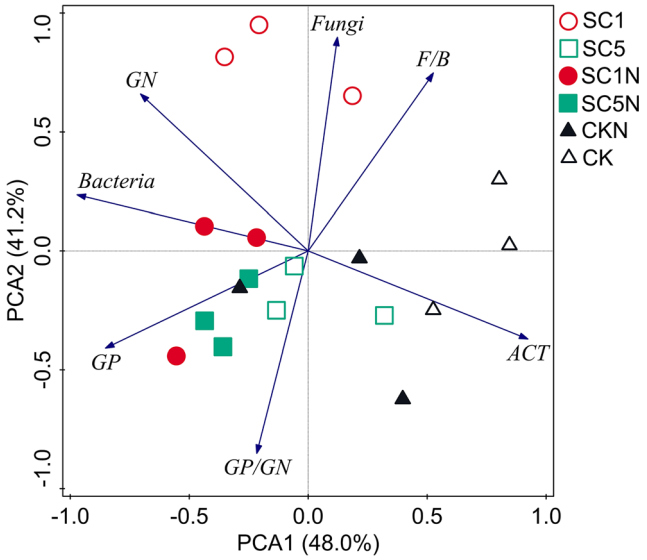

Fig. 3 PCA of soil microbial groups in different treatments

in soil microbial community structure in the soil-biochar treatments, especially in SC1 (Fig. 3). In this infertile soil, fungi are sensitive to nutrients, and excessive N could decrease C and nutrient availability via improving soil solution osmotic potential (Rifai et al. 2010) or reacting with soil C to generate recalcitrant compounds (e.g., melanoidins, polymers) (Treseder 2008). This can be confirmed by the external N effect on CEC in soil-biochar-N treatments (Table 2 and Fig. 1). Thus, the primary factor driving soil microbial community structure mediation in soil-biochar treatments is soil-nutrient status, which is different from the conclusion for the soil-biochar-N treatments. Notably, external N can stimulate the shoot and root biomasses, which can be enhanced by concomitant CB (as evidenced by the comparisons of soil-biochar-N treatments vs. CKN). These findings indicate that N fertilizer

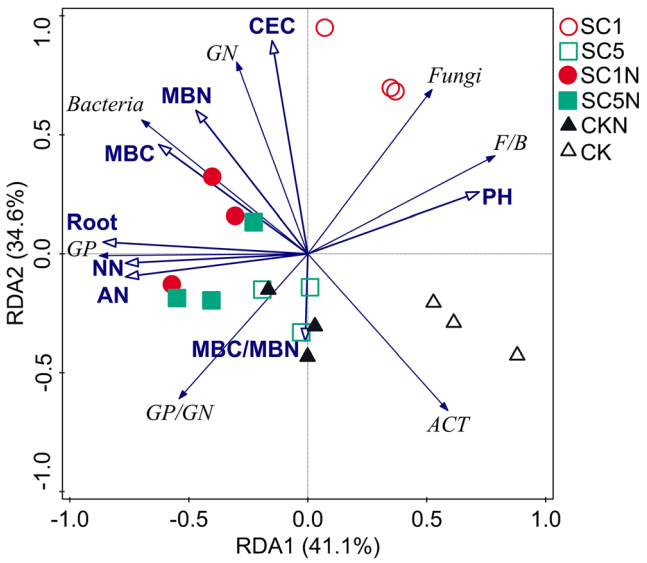

Fig. 4 RDA of soil microbial groups in different treatments

dominates maize seedling production regardless of the short-term changes in soil microbial community structure.

As expected, the changes in soil acidity are mainly affected by external N but not by CB (Table 2), indicating that soil pH was the dominant driver of soil microbial community structure mediation in the soil-biochar-N treatments, whereas soil nutrients played this role in the soil-biochar-treatments. This finding is consistent with the previous work (Ma et al. 2016). Theoretically, following CB addition, soil microbial community structure is mainly modified by nutrient-sensitive microbial groups, suggesting that soil quality could be gradually amended by the addition of biochar alone.

Effect of biochar particle size on soil microbial community structure

Fine biochar CB1 had stronger effects on soil microbial community structure (e.g., fungi, GN) than did CB5 (Fig. 2 and Fig. 3), which supports our hypothesis (i). Soil nutrients were the main driver of the shifts in soil microbial community structure in the soil-biochar treatments ("Changes in soil microbial community structure after biochar and external N additions" section); compared with CB5, CB1 had a greater capacity to improve nutrient availability (e.g., CEC, AN, NN). Therefore, GN and fungi dominated the soil microbial community structure modification in SC1 due to their rapid responses to nutrients (Bray et al. 2012; Kramer and Gleixner 2008). The short-term nutrient release observed in the present study is consistent with our previous findings (Zhao et al. 2015a; Zhao et al. 2015c), and such short-term release can be achieved within a few weeks via the surface oxidization of biochar (Peng et al. 2011). When coarse biochars are smashed into finer ones, some nonlabile C may be degraded and released to the soil (Bird et al. 1999). Thus, CB1 might be more susceptible to mineralization and supply more nutrients than CB5 in the short term; this interpretation is supported by the higher levels of CEC, AN, and NN in SC1 than in SC5.

The possible underlying mechanisms are as follows: (a) fine biochars can mineralize to greater extents in soils than can coarse biochars due to their greater intrasurface accessibility (Zimmerman 2010); (b) fine biochars may undergo greater surface-negative-charge than coarse biochars, which promotes the release of CEC and other nutrients (Liang et al. 2006), as shown by the CEC distribution (Fig. 1); (c) after being fully mixed with soils, fine biochars are more available than are coarse ones to interact with plant roots and can stimulate the related rhizosphere microbiome within a short time frame (de Vries et al. 2006; Kolton et al. 2017), since most microorganisms are enriched in the rhizosphere (root contribution, Table 3); and (d) it can be easier for fine biochars than for coarse ones to adsorb organic materials and form organo-mineral complexes due to their higher accessibility, which can alter microbial distributions. Physical mechanisms may also contribute to

Table 3 Monte Carlo permutation test on the first two RDA axes, environmental variable contributions to the axes, and correlation coefficients between environmental variables and the axes

Parameters [a]	Axis1	Axis2	Environmental variable contributions	
			Contributions (%)	*P* values
Monte Carlo permutation test				
P values	0.018	0.026		
Environmental variables				
Root	−0.860**	0.049	37.7	0.002
CEC	−0.150	0.895**	34.4	< 0.001
MBC	−0.627**	0.459	11.1	0.008
pH	0.720**	0.259	5.1	< 0.001
MBC/MBN	−0.011	−0.368	3.6	< 0.001
MBN	−0.470*	0.603**	5.5	0.090
AN	−0.766**	−0.097	1.5	< 0.001
NN	−0.767**	−0.039	1.2	0.724

[a] Variables that had very little contributions (< 1%) to PLFA variances were not listed here. Single asterisk, significantly correlated at 0.05 level; double asterisks, significantly correlated at 0.01 level

the difference in soil microbial community structure between SC1 and SC5. For instance, fine biochars can be mixed well with soils and attract heat, supplying more opportunities for microbial colonization (Gul et al. 2015). Thus, soil structure characteristics (e.g., water-holding capacity, aeration conditions) can be more easily altered by fine biochars than by coarse ones (Gomez et al. 2014; Pokharel et al. 2018). Alterations in these characteristics could potentially impact soil microbial community composition, since soil chemical characteristics are closely related to the soil environment (e.g., aeration conditions) in loamy clay-like soil (Zhao et al. 2015d).

Consequently, CB1 can benefit soil amendment and C sequestration via mediating soil microbial community structure since fungi have a lower turnover rate (Clemmensen et al. 2015). This possibility is supported by the higher S/R levels in SC1 than in SC5, indicating an increase in the resource supply with CB1 addition (Lehmann et al. 2011). In addition to having high mobility, fine biochars can be transported along with soil profiles via mixing by animals (e.g., earthworms) (Eckmeier et al. 2007). Such transport can enhance soil aggregate formation and soil structure amelioration (Wang et al. 2017) and, thus, could benefit soil microbial community modification and improve soil quality and crop production. Therefore, we speculate that in contrast to coarse biochars, fine biochars can reform an agricultural ecosystem in the long term. However, support for hypothesis (ii) was not obtained; that is, the particle-size effect on soil microbial community structure mediation was not evident when N was added. This finding suggests that the soil-acidification effect ("Changes in soil microbial community structure after biochar and external N additions" section) outweighs the particle-size effect. The effect of acidification was reflected in the nonsignificant difference in S/R between SC1N and SC5N, further supporting the conclusion that among the amendments, N fertilizer had the greatest effect on maize production at the seedling stage. Thus, when considering the use of biochar in association with chemical N fertilizer as a soil amendment, the fact that soil acidity has considerable effects on soil ecological processes regardless of particle size, at least at the maize seedling stage, should be considered.

The effect of particle size may vary with biochar type, as biochar characteristics are strongly influenced by the raw material and pyrolysis conditions (Sanroman et al. 2017). Soil type and biochar application rate may also be important due to their influences on soil chemical and biological properties (Chen et al. 2013; Gomez et al. 2014; Wu et al. 2018). In the long term, the effect of particle size on soil microbial community structure can be expected to vary continuously in soils, since biochars continue to oxidize/mineralize and alter soil physicochemical properties over time (Yuan et al. 2017). Furthermore, the whole maize growth period should be considered when assessing the relationship between biochar particle size and soil microbial community structure. Nevertheless, this study presents experimental evidence of the biochar modification of soil microbial community structure and the influence of particle size at the maize seedling stage. Regardless of whether biochars are applied in association with N fertilizer, their particle sizes should be taken into account when using biochar as a soil amendment in agricultural practices.

Conclusions

During the maize seedling period, fine biochar CB1 has a stronger capacity than CB5 to modify soil microbial community structure by promoting soil microbial groups (e.g., fungi,

GN); this stronger capacity is because CB1 undergoes a series of more intense processes (e.g., nutrient release, mineralization) than does CB5. However, this difference diminishes or even disappears when external N is added because of the impact of soil acidification induced by the N. Thus, for this type of biochar, particle size affects soil microbial community structure only when biochar is applied alone, not when it is applied in association with chemical N fertilizer, at least during the seedling period. This study presents direct empirical evidence for the relevance of particle size when using biochar to amend soils. The effect of particle size should be considered when using biochar for soil amendment in agricultural practice, especially in clay soils.

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