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Microbial functional diversity responses to 2 years since biochar application in silt-loam soils on the Loess Plateau

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ABSTRACT

The structure and function of soil microbial communities have been widely used as indicators of soil quality and fertility. The effect of biochar application on carbon sequestration has been studied, but the effect on soil microbial functional diversity has received little attention. We evaluated effects of biochar application on the functional diversities of microbes in a loam soil. The effects of biochar on microbial activities and related processes in the 0–10 and 10–20 cm soil layers were determined in a two-year experiment in maize field on the Loess Plateau in China. Low-pyrolysis biochar produced from maize straw was applied into soils at rates of 0 (BC0), 10 (BC10) and 30 (BC30) t ha^{-1}. Chemical analysis indicated that the biochar did not change the pH, significantly increased the amounts of organic carbon and nitrogen, and decreased the amount of mineral nitrogen and the microbial quotient. The biochar significantly decreased average well colour development (AWCD) values in Biolog EcoPlates™ for both layers, particularly for the rate of 10 t ha^{-1}. Biochar addition significantly decreased substrate richness (S) except for BC30 in the 0–10 cm layer. Effects of biochar on the Shannon-Wiener index (H) and Simpson's dominance (D) were not significant, except for a significant increase in evenness index (E) in BC10 in the 10–20 cm layer. A principal component analysis clearly differentiated the treatments, and microbial use of six categories of substrates significantly decreased in both layers after biochar addition, although the use of amines and amides did not differ amongst the three treatments in the deeper layer. Maize above ground dry biomass and height did not differ significantly amongst the treatments, and biochar had no significant effect on nitrogen uptake by maize seedlings. H was positively correlated with AWCD, and negatively with pH. AWCD was positively correlated with mineral N and negatively with pH. Our results indicated that shifts in soil microbial functional diversity affected by biochar were not effective indicators of soil quality in earlier maize growth periods in this region.

1. Introduction

Biochar is a carbon-rich by-product from the pyrolysis of biomass under low oxygen concentrations or without oxygen and is widely applied to agricultural soils (Lehmann and Joseph, 2009). Biochar is usually alkaline, rich in recalcitrant C, and has a large surface area, high negative charge density and high porosity (Lehmann et al., 2011). Incorporation of biochar in soil has been promoted as a useful tool to increase soil organic carbon (SOC) and decrease atmospheric CO_2 concentrations. Biochar amendment can alter bulk density, increase net soil-surface area (Chan et al., 2008; Spokas et al., 2012), and increase pH, nutrient content and moisture retention (Laird et al., 2010; Van Zwieten et al., 2010). Biochar resists decomposition in the soil due to its high aromaticity (Lehmann et al., 2011), thereby increasing soil C storage. Biochar would thus affect crop productivity via changes of soil physical and chemical properties (Jones et al., 2012).

Positive and negative effects of biochar on various crops have been reported (Karer et al., 2013; Van Zwieten et al., 2010). Above-ground biomasses of wheat and soybean in a ferrosol soil in Australia (Van Zwieten et al., 2010), and barley in soil classified as Chernozem (Karer et al., 2013) increased after biochar addition while negative responses of wheat and radish were observed in loamy calcarosol soils sourced from a vineyard (Van Zwieten et al., 2010). The different soil conditions combined with biochar application may have contributed to these variable responses of crops to biochar, and the underlying mechanism requires further studies.

Changes of soil physico-chemical properties combined with plant growth would lead to changes of the soil microclimate, thereby

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affecting soil microorganisms and microbial communities. Biochar can alter biological properties such as N mineralisation and nitrification by affecting the bacteria associated with these processes and providing a suitable environment to promote microbial activity (Berglund et al., 2004). Recent studies have addressed effects of biochar on soil microbial communities. Biochar addition caused significant fluctuations in microbial community structure and increased microbial biomass, growth, and activity (Lehmann et al., 2011; Prayogo et al., 2014). Biochar addition can create conditions capable of affecting soil microecosystems and microbial communities, which have been investigated by a variety of profiling methods such as denaturing gradient gel electrophoresis, phospholipids fatty acid analysis (PLFA), terminal restriction fragment length polymorphism analysis, and quantitative polymerase chain reaction analysis. Gomez et al. (2014) observed an increase in microbial abundance proportional to rates of biochar application (0–20% by mass) and a change in microbial community composition towards a community dominated by gram-negative bacteria relative to fungi and gram-positive bacteria at a biochar rate of 20%. PLFA and fungal abundance were strongly reduced at a rate of biochar application of 49 t ha^{-1} in sandy clay loam soils (Ameloot et al., 2014). Microbial functional diversity associated with biochar application, however, is not well understood because it has been rarely investigated (Abujabhah et al., 2016; Imparato et al., 2016; Rutigliano et al., 2014). Microbial functional diversity, as an important indicator to assess soil processes and ecological functions, is an aspect of the overall microbial diversity in soil. Assessing the effects of biochar on soil microbial functional diversity is therefore essential.

Community-level physiological profiles (CLPPs) based on the ability of microorganisms to oxidise various substrates have been used to evaluate the functional diversity of microbial communities (Pignataro et al., 2012). Biolog EcoPlates™ are useful for identifying disturbances in microbial functional diversity from various environmental stresses (Liu et al., 2011), and they can be used to rapid screen the use of specific substrate in soil microbial communities. Previous studies have mostly focused on the changes of soil microbial diversity after biochar addition in calcareous soils (Gomez et al., 2014; Joseph et al., 2013), but CLPPs have not been widely used to assess soil biodiversity after biochar application.

The present study was conducted in a maize field where biochar was added to silt loam soils on the Loess Plateau in China. The effects of biochar on the soil physical properties and crop production in this field have been previously reported (Xiao et al., 2016). Therefore, we used Biolog EcoPlates™ to obtain a snapshot of metabolic activity of microbial assemblages in soils with or without biochar to (1) understand the impact of biochar on soil microbial functional diversity, and (2) determine the correlations between CLPP indices and soil chemical properties.

2. Materials and methods

2.1. Site description and experimental design

The field study was conducted at the Changwu Agricultural and Ecological Experimental Station on the Loess Plateau of China (35.28 °N, 107.88 °E; 1200 m a.s.l.). Maize (*Zea mays* L.) has been cultivated at this site for several decades. Soils at this site are Cumuli-Ustic Isohumosols with a loamy texture. The main properties of the top 20 cm of soil were: bulk density 1.3 g cm^{-3}, pH 7.9, organic-matter content 15.1 g kg^{-1}, total N content 0.99 g kg^{-1}, Olsen-P content 6.6 mg kg^{-1}, NH_4OAc-K content 127.1 mg kg^{-1}, and mineral N (NO_3^--N and NH_4^+-N) content 9.96 mg kg^{-1}. The mean annual air temperature is 9.1 °C. The average precipitation of the last 20 years was 555 mm, with about 79.8% falling during maize growing season.

The experiment was designed to investigate the effects of two rates of biochar addition and a control, each with three replicates, under field conditions. The nine plots (each 7 × 8 m) were arranged in a completely randomised block: three with no biochar addition (BC0, control), three with biochar added at a rate of 10 t ha^{-1} (BC10), and three with biochar added at a rate of 30 t ha^{-1} (BC30). The biochar was applied evenly to the soil surface once, in April 2012, and was then incorporated by shovel to a depth of 20 cm before maize sowing. The treatments were ploughed once each year in spring before sowing. All plots were ploughed on 20 April 2012, 2013, and 2014 and were sowed with spring maize (*Zea mays* L.) at a depth of 5 cm and a density of 65 000 plants ha^{-1}. All plots had alternating wide (60 cm) and narrow (40 cm) row spacing. In each treatment, the same basal fertilizer was evenly applied to the soil surface and then ploughed into the subsurface soil. Basal chemical fertilisers were broadcast over the soil of each plot at rates of 90 kg N ha^{-1} in the form of urea (46% N), 40 kg P ha^{-1} in the form of calcium super phosphate (12% P_2O_5), and 80 kg K ha^{-1} in the form of potassium sulphate (45% K_2O). An additional 67.5 kg N ha^{-1} (urea, 46% N) was applied using a hole-sowing machine at the jointing and tasselling stages. The maize was harvested gradually as it ripened at the end of September each year.

The biochar was produced by a slow pyrolysis of maize straw at 400 °C. The biochar had a total C content of 591.60 g kg^{-1}, total N content of 9.77 g kg^{-1}, pH (1:2.5H_2O) of 9.8, and bulk density of 0.4 g cm^{-3}. The proportions of biochar particles of 0.02–2 mm, 0.002–0.02 mm and < 0.002 mm were 77.76%, 18.78% and 3.46%, respectively.

2.2. Sampling and analysis

Three replicate soil cores (4.0 cm in diameter) per plot were collected with a hand auger at the six-leaf stage of maize growth periods in 2014. The samples were then homogenised to constitute a representative soil sample for each of the 0–10 and 10–20 cm layers. These samples were sieved through a stainless steel 2-mm mesh sieve in the field to remove visible roots and other materials, transported to the laboratory in a cooler and divided into two sets of subsamples. One set was used to determine soil water content (SWC) and microbial properties, and the other set was air-dried for the determination of soil pH and for other chemical analyses. Subsamples of < 2 mm air dried soil were further ground to pass through a 0.25-mm mesh for total C and N analysis.

Soil pH was determined with a pH meter using a soil:water ratio of 1:2.5. SOC content was measured by $K_2Cr_2O_7$-H_2SO_4 oxidation, and total nitrogen (TN) content was measured with the Kjeldahl method. Dissolved organic carbon (DOC) content was determined as described by Guo et al. (2013), and the quantity of DOC in the extract was measured with a total organic carbon analyser (Shimazu TOC-5050, Tokyo, Japan). The microbial quotient q_{mic} represents the ratio of microbial C to SOC, in which microbial C was measured by fumigation-extraction method (Vance et al., 1987). Mineral N was extracted with 1 M KCl solution, and analysed with an automated flow injection analyser. Dissolved inorganic N (DIN) consists of NH_4^+-N and NO_3^--N and was extracted with 0.5 M K_2SO_4 solution (Jones and Willett, 2005). Total dissolved N (TDN) content was determined from the filtrate from a persulphate digestion. Total dissolved organic N was calculated as the difference between TDN and DIN.

Plant growth in each plot was monitored after sowing following the system of standardised maize developmental stages when > 50% of the plants reached the six-leaf stage. Three adjacent plants in the same row were randomly selected and cut at ground level in 2014. The plant samples were oven-dried to a constant weight at 75 °C before being ground for chemical analysis. The N concentration of the plant samples was determined (microKjeldahl), and plant N uptake was calculated with the following formula:

$$N_{uptake} = N_c \times DW,$$

where N_c is the N concentration (%) of plant and DW is the plant dry weight (kg ha^{-1}).

2.3. Microbial community-level physiological profilings (CLPPs)

The use of sole C sources was estimated using Biolog EcoPlates™ (Biolog Co., Hayward, USA). The 31 substrates in the plates were classified into six categories (Choi and Dobbs, 1999): carbohydrates (D-cellobiose, i-erythritol, D-galactonic acid γ-lactone, N-acetyl-D-glucosamine, glucose-1-phosphate, β-methyl-D-glucoside, D,L-α-glycerol phosphate, α- D-lactose, D-mannitol, and D-xylose), amino acids (L-arginine, L-asparagine, glycyl- L-glutamic acid, L-phenylalanine, L-serine, and L-threonine), carboxylic acids (γ-hydroxybutyric acid, α-ketobutyric acid, D-galacturonic acid, D-glucosaminic acid, itaconic acid, D-malic acid, and pyruvatic acid methyl ester), polymers (a-cyclodextrin, glycogen, Tween 40, and Tween 80), amines (phenyl ethylamine and putrescine), and phenolic compounds (2-hydroxybenzoic acid and 4-hydroxybenzoic acid). Three replicates of fresh soil samples (equal to 5 g of air-dried soil) were prepared and shaken in 45 ml of sterile 0.85% NaCl for 30 min at 200 rpm and then diluted to 1:1000. Each plate well was inoculated with 150 μL of the dilution, and the plates were incubated at 28 °C. The use of the C sources was recorded with a Biolog MicroStation™ (BIO-TEK Instruments Inc., Winooski, USA) at 590 nm every 24 h for 10 days. Average well-colour development (AWCD) was calculated as a measure of microbial functional diversity.

AWCD was calculated as:

$$AWCD = \sum (C_i - R)/31,$$

where R is the absorbance of the control well (containing water instead of C source) and C_i is the absorbance of plate well inoculated with C source i. To AWCD was assigned a value of 0 when C_i-R < 0. The EcoPlate readings at 96 h were used to calculate the richness (S), Shannon-Wiener diversity index (H), Simpson diversity index (D) and evenness (E). S is the number of C sources used with OD_{590} > 0.5 as the threshold for a positive response. H and D were calculated as:

$$H = -\sum (P_i \times \ln P_i) \text{ and } D = 1 - \sum P_i^2,$$

where $P_i = (C_i - R)/\sum (C_i - R)$.

Evenness (E) was calculated as:

$$E = H/\ln S.$$

2.4. Statistical analysis

Significant differences between treatments were analysed by a one-way ANOVA, followed by Tukey HSD Post Hoc test at $P < 0.05$ using SPSS 19.0 for Windows (SPSS Inc., Chicago, USA). A principal component analysis of the data at 96 h was used to determine the microbial community functions at the various rates of biochar addition. A Pearson correlation analysis was performed to investigate the correlations amongst the measured parameters. Figures were prepared with Sigma Plot 12.0 (Systat Software UK Ltd., London, UK).

3. Results

3.1. Soil chemical and biochemical properties

The soil chemical and biochemical properties in the 0–10 and 10–20 cm layers are shown in Table 1. Soil pH had no significant variations after biochar application. The biochar significantly increased SOC content in both layers relative to BC0. BC30 significantly increased TN content in the 0–10 cm layer, but had no effect on TN content in the 10–20 cm layer. Biochar significantly increased the C:N ratio in the 0–10 cm layer, and BC30 significantly increased the ratio in the 10–20 cm layer. Notably, biochar did not have significant effect on DOC content in the 0–10 cm layer but significantly increased it in the 10–20 cm layer. BC10 significantly increased DON content in both layers, but DON content did not differ significantly between BC0 and BC30. Mineral N concentrations in the 0–10 cm layer did not differ significantly between BC0 and BC30 but was higher than that in BC10. The order of mineral N concentrations was BC0 > BC30 > BC10 in the 10–20 cm layer ($P < 0.05$). Microbial quotient (q_{mic}) decreased significantly with biochar rates in the 0–10 cm layer; q_{mic} in 10–20 cm layer did not differ significantly between BC0 and BC10 but was significantly lower in BC30.

3.2. Soil microbial communities and diversity

Biochar decreased AWCD in both layers, AWCD for all treatments increased with incubation time during the 10 days and retained stable during the last two days (Fig. 1a and b). AWCD was nearly zero with no difference amongst treatments during the first 24 h of incubation and increased after 48 h in the order of BC0 > BC30 > BC10 for both layers. The shortest incubation time that could identify clear differences amongst the treatments was 96 h for each soil layer. AWCD for the 0–10 cm layer was significantly lower for BC10 than the other treatments at all time points and reached 0.80 at 240 h, 62.0% of that for BC0. Similarly, BC10 had the lowest AWCD for the 10–20 cm layer throughout incubation and only reached 0.57 at 240 h, 54.3% of that for BC0. The AWCDs differed significantly amongst the treatments at the end of incubation.

The diversity indices based on the data at 96 h for the three treatments are shown in Table 2. Biochar had no significant effect on H in either soil layer, but H slightly decreased with soil depths. S was significantly higher in the shallower layer, and lower in BC10 than in BC0 and BC30 in both layers. E did not differ significantly amongst the three treatments in the 0–10 cm layer but was significantly higher in BC10 in the deeper layer. Biochar addition had no significant effect on D in either layer.

The Shannon-Wiener index (H) was positively correlated with AWCD ($R = 0.456$, $P < 0.05$), and negatively with soil pH ($R = -0.685$, $P < 0.01$). AWCD was positively correlated with mineral N ($R = 0.583$, $P < 0.01$), and negatively with pH ($R = -0.580$, $P < 0.05$). Neither AWCD nor H was significantly correlated with q_{mic}, C:N, SOC, TN, DOC, or DON contents.

The first and second principal components (PC1 and PC2) of the data at 96 h for both 0–10 cm and 10–20 cm layers explained 59.56% and 13.34% and 36.52% and 16.64% of the variance in the data, respectively (Fig. 2). The treatments were divided into three groups based on the PCA scores. For both soil layers, BC0 was in the first quadrant, and BC10 and BC30 were in the third quadrant. BC0 was clearly differentiated from BC10 and BC30 for both layers. The scatter graphs for 31 C sources on PC1 and PC2 (graphs not shown) indicated that phenylethylamine, L-phenylalanine, N-acetyl-D-glucosamine, glycyl-L-glutamic acid, glucose-1-phosphate, and D-galacturonic acid made major contributions to PC1 for the 0–10 cm layer, and D, L-a-glycerol and L-asparagine made major contributions to PC2. L-threonine, N-acetyl-D-glucosamine, L-phenylalanine, D-glucosaminic acid, and Tween 40 made major contributions to PC1 for the 10–20 cm layer, and glycogen, L-asparagine, and D-galactonic acid γ-lactone influenced the spread of treatments along the PC2 axis.

The soil microbes used the six substrate categories and the relative substrate use varied from 1.7% to 46.4%. Biochar addition generally significantly decreased utilization of the six categories of substrates (Fig. 3). The use of substrates was significantly higher in the 0–10 cm layer for BC0 but did not differ significantly between BC10 and BC30, except for a relatively high use of amino acids in BC30 (13.6% and 25.2% amino acids were used in BC10 and BC30, respectively) (Fig. 3a). Similarly, the use of all six substrate categories in the 10–20 cm layer was significantly higher in BC0, with no difference between BC10 and BC30 (Fig. 3b). Biochar had no significant effect on the use of amines and amides in the deeper layer.

Table 1
Chemical and biochemical parameters of different treatments at six-leaf stage of maize in year 2014. BC0: no biochar addition, BC10: 10 t ha^{-1} biochar addition, BC30: 30 t ha^{-1} biochar addition. All values represent means ± standard error (n = 3), different letters within a column in the same soil layer indicate statistically significant differences between treatments at $P < 0.05$ according to the Tukey HSD Post Hoc test.

Layer (cm)	Treatments	pH	SOC (g kg^{-1})	TN (g kg^{-1})	C:N ratio	DOC (mg kg^{-1})	DON (mg kg^{-1})	Mineral N (mg kg^{-1})	q_{mic} (%)
0–10	BC0	7.87 ± 0.11^{a}	9.55 ± 0.06^{c}	1.10 ± 0.05^{b}	8.67 ± 0.21^{c}	42.8 ± 1.7^{a}	19.7 ± 0.9^{b}	5.31 ± 0.17^{a}	2.21 ± 0.91^{a}
	BC10	7.91 ± 0.07^{a}	11.40 ± 0.13^{b}	1.14 ± 0.02^{ab}	9.97 ± 0.07^{b}	44.9 ± 1.5^{a}	27.5 ± 0.6^{a}	4.78 ± 0.19^{b}	2.08 ± 0.08^{b}
	BC30	7.79 ± 0.18^{a}	15.33 ± 0.10^{a}	1.22 ± 0.03^{a}	12.61 ± 0.20^{a}	41.1 ± 1.9^{a}	22.3 ± 1.0^{b}	5.07 ± 0.28^{a}	1.71 ± 0.02^{c}
10–20	BC0	8.01 ± 0.08^{a}	9.01 ± 0.12^{c}	1.07 ± 0.03^{a}	8.46 ± 0.13^{b}	32.2 ± 1.1^{b}	13.1 ± 0.5^{b}	4.85 ± 0.30^{a}	2.06 ± 0.12^{a}
	BC10	8.15 ± 0.18^{a}	9.90 ± 0.11^{b}	1.09 ± 0.02^{a}	9.07 ± 0.24^{b}	40.8 ± 2.8^{a}	19.0 ± 0.4^{a}	4.34 ± 0.11^{c}	1.93 ± 0.04^{a}
	BC30	7.99 ± 0.08^{a}	11.81 ± 0.52^{a}	1.15 ± 0.03^{a}	10.36 ± 0.63^{a}	38.9 ± 0.9^{a}	15.5 ± 1.9^{b}	4.60 ± 0.09^{b}	1.59 ± 0.03^{b}

Note: SOC: soil organic carbon, TN: total nitrogen, C:N ratio: the ratio of soil organic carbon to total nitrogen, DOC: dissolved organic carbon, DON: dissolved organic nitrogen, q_{mic}: microbial quotient.

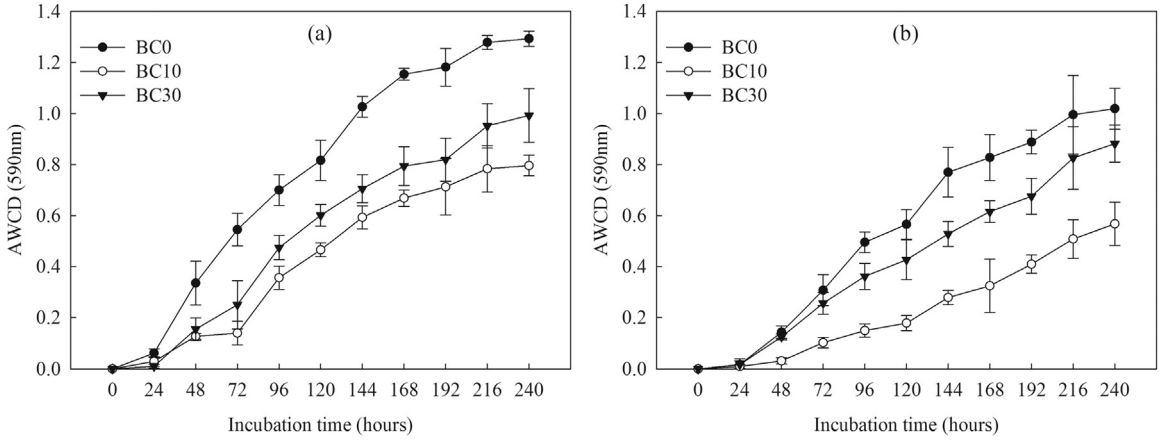

Fig. 1. Variation in average well colour development (AWCD) over time in Biolog EcoPlates™ in year 2014 based on 240 h incubation of soil extracts deriving from 0 to 10 cm (a) and 10–20 cm (b). BC0: no biochar addition, BC10: 10 t ha^{-1} biochar addition, BC30: 30 t ha^{-1} biochar addition. Values represent means ± standard error (n = 3).

3.3. Plant responses to biochar addition

The responses of the maize are shown in Fig. 4. Growth performance of the maize crop did not differ significantly amongst the three treatments ($P > 0.05$). Biochar amendment had no significant effect on maize above ground biomass or height (Fig. 4a). Similarly, N uptake by the maize seedlings did not differ significantly amongst the three treatments (Fig. 4b).

4. Discussion

4.1. Effects of biochar on soil chemical properties and plant growth

Soil physico-chemical properties play an important role in crop productivity. This study aimed to assess the changes in soil properties since the application of biochar. Our results showed that the biochar had no significant effect on soil pH in this study, but increased pH in the first year in our previous study (Xiao et al., 2016). The lack of a difference in pH after two years might have been due to the oxidation of the biochar surface over time (Cheng et al., 2006). A long-term study also suggested that responses of soil pH to biochar may be transient (Jones et al., 2012). Prayogo et al. (2014) found that a lower rate biochar application also had no effect on pH. Soil pH differed by only 0.15 units at biochar application rates of 20 t ha^{-1} and 40 t ha^{-1} in a calcareous soil (Zhang et al., 2012). The low variation of soil pH indicated that the calcareous soils had a large buffering capacity.

Biochar can alter soil C and N pool because it contains relatively high C and low N concentrations (Laird et al., 2010). Our results indicated that biochar increased soil organic C and N, demonstrating that applying organic matter to soil can offset the loss of organic matter due to long term cultivation and can thus help to improve soil properties (Abujabhah et al., 2016). Tammeorg et al. (2014) also showed that biochar significantly increased SOC, and the increase lasted for years in a sandy clay loam soil, likely because most of the biochar-C is aromatic and recalcitrant for soil microorganisms (Lehmann et al., 2011). Biochar significantly decreased q_{mic} might be due to changes in SOC and

Table 2
Diversity and evenness indices of soil microbial communities based on the Biolog data of different treatments (data at 96 h). BC0: no biochar addition, BC10: 10 t ha^{-1} biochar addition, BC30: 30 t ha^{-1} biochar addition. All values represent means ± standard error (n = 3), different letters within a column in the same soil layer indicate statistically significant differences between treatments at $P < 0.05$ according to the Tukey HSD Post Hoc test.

Layer (cm)	Treatments	H	S	E	D
0–10	BC0	3.07 ± 0.03^{a}	16.3 ± 1.2^{a}	1.10 ± 0.02^{a}	0.92 ± 0.01^{a}
	BC10	2.75 ± 0.05^{a}	13.3 ± 0.3^{b}	1.06 ± 0.02^{a}	0.90 ± 0.01^{a}
	BC30	2.82 ± 0.12^{a}	16.0 ± 0.6^{a}	1.02 ± 0.05^{a}	0.94 ± 0.01^{a}
10–20	BC0	2.64 ± 0.22^{a}	8.3 ± 0.3^{a}	1.25 ± 0.14^{b}	0.91 ± 0.02^{a}
	BC10	2.41 ± 0.15^{a}	5.0 ± 0.6^{b}	1.50 ± 0.07^{a}	0.86 ± 0.03^{a}
	BC30	2.62 ± 0.16^{a}	6.3 ± 0.3^{b}	1.42 ± 0.08^{ab}	0.90 ± 0.02^{a}

Note: H: Shannon-Wiener index, S: richness, E: evenness, D: Simpson's Dominance.

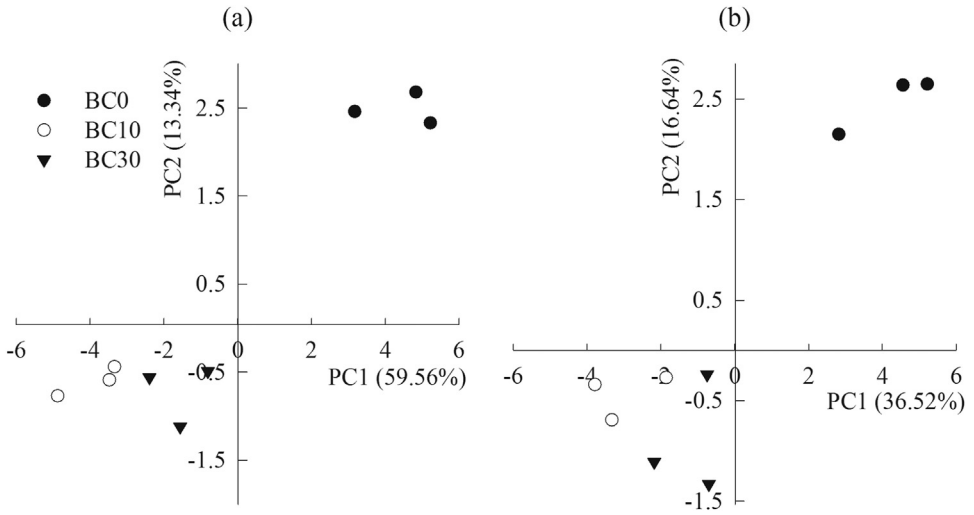

Fig. 2. PCA of Biolog EcoPlate™ data for three biochar addition rates soils at two soil layers. (a) 0–10 cm layer, PC1 and PC2 accounted for 59.56% and 13.34% of variance, respectively. (b) 10–20 cm layer, PC1 and PC2 accounted for 36.52% and 16.64% of variance, respectively. BC0: no biochar addition, BC10: 10 t ha^{-1} biochar addition, BC30: 30 t ha^{-1} biochar addition.

the composition of microbial community (Stark et al., 2008). We attributed it to a dilution effect; the increase in SOC content was due to the dilution of microbial biomass C in the SOC from the addition of biochar.

Biochar addition in our study reduced soil mineral N, consistent with Prayogo et al. (2014), who reported that biochar significantly reduced the rate of N mineralisation and could decrease NO_3^- contents throughout the soil profile (Singh et al., 2010). The suppression of biochar on mineral N might be attributed to the sorption of base functional groups. Moreover, biochar surfaces may develop negative charges at high pH values, and the competition of OH^- and NO_3^- could limit the sorption of mineral N (Chintala et al., 2013). The addition of biochar to soil can potentially change the growth of microorganisms involved in N cycling, thereby altering soil N dynamics.

Our results showed that the biochar had no significant effect on maize growth compared with the control. Hagner et al. (2016), however, found that biochar produced at 300 °C negatively affected the germination and biomass of lettuce, but none of the biochar types (produced at 300, 375 and 475 °C, respectively) affected the production of barley. These differences may largely be attributed to soil conditions, crop types and rates of biochar application (Calderón et al., 2015). Plant productivity is limited by P, Fe, and Mg when pH > 8, but biochar has provided little benefit in calcareous soils despite the potential to supply necessary nutrients (Farrell et al., 2014b).

4.2. Effects of biochar on the functional diversity of soil microbial communities

The AWCD value in the well of an EcoPlate™ is an important index of microbial functional diversity, because it represents the ability of soil microorganisms utilizing different carbon sources. Previous studies have demonstrated that the application of organic matter to soil can stimulate microbial populations and activities (Gomez et al., 2006). Our results, however, found that biochar generally significantly decreased AWCD and had no effect on the CLPP indices except for S. The decrease in AWCD indicated a lower rate of carbon source use and lower functional diversity compared to soil without biochar. A decrease in soil microbial activity (Chan et al., 2008) and richness of soil microorganisms after biochar addition might be due to a lack of available substrates in the soil. Liao et al. (2016), though, reported an increase in metabolic capacity after biochar application and AWCD increased with biochar rates. Zhang et al. (2013) reported an increase in the activities of soil microorganisms with biochar addition. A significant change in functional diversity was observed three months after biochar addition, but this effect disappeared 14 months later (Rutigliano et al., 2014). Amendment with gasified biochar had no influence on functional diversity (Imparato et al., 2016), and the lack of a significant effect of biochar on soil microbial biomass has also been reported (Castaldi et al., 2011). Liao et al. (2014) found a higher bacterial diversity and lower fungal diversity after biochar application in an incubation of 96 days, suggesting that the biochar may have released toxic compounds

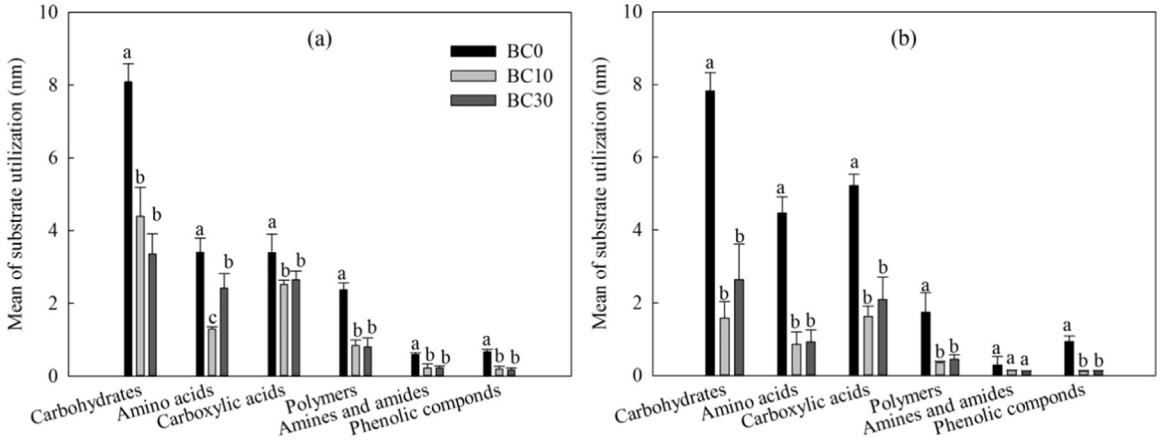

Fig. 3. Means (± standard error; n = 3) of substrate utilization from different biochar addition rates based on 96 h incubation of soil extracts deriving from 0 to 10 cm (a) and 10–20 cm (b). BC0: no biochar addition, BC10: 10 t ha^{-1} biochar addition, BC30: 30 t ha^{-1} biochar addition. Different letters indicate statistically significant differences between treatments comparing the same substrate at $P < 0.05$ according to the Tukey HSD Post Hoc test.

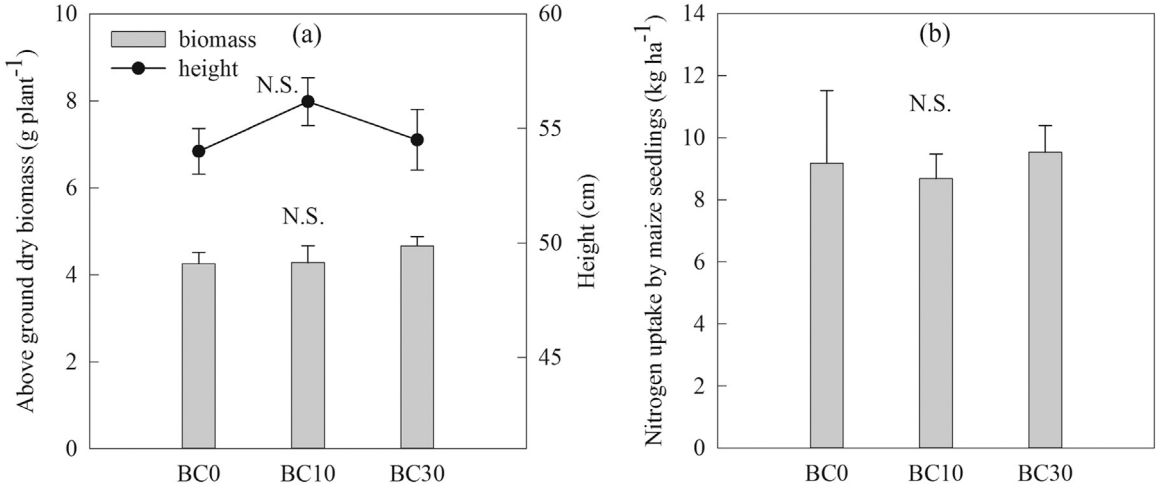

Fig. 4. Effects of biochar dose rate (0–30 t ha^{-1}) on above ground dry biomass (bars), height (line and scatters) (a) and nitrogen uptake by maize seedlings (b) at six-leaf stage in year 2014. Values represent means ± standard error (n = 3). N.S.: no statistically significant differences between treatments at $P < 0.05$ according to the Tukey HSD Post Hoc test.

(Spokas et al., 2012; Rutigliano et al., 2014), which can restrain the activities of some microbes. Biochar application may thus have variable effects on soil microbial communities; physiological differences of the microbes and variable soil textures may have contributed to some of the differences amongst the studies (Castaldi et al., 2011).

Soil hydrothermal conditions can affect microbial activities, which would in turn affect availability of soil nutrient. Consistent with Shen et al. (2010), our results also showed that AWCD was positively correlated with mineral N, suggesting that available N in soil is a key factor influencing soil microbial functional diversity. The absence of correlation between AWCD and organic C might be attributed to a microbial selection favouring certain microbes more than others and to differences in the chemical characteristic of the C fractions (Lagomarsino et al., 2012). Moreover, Yan et al. (2000) reported a threshold effect of soil organic matter on microbial diversity. SOC was not significantly correlated with microbial diversity in our study, suggesting that the content of soil organic matter in our study area was higher than the threshold. The variable correlations between soil physico-chemical parameters and microbial functional diversity indicated that soil environment is biodiverse and complex.

The PCA was able to distinguish between the control and the samples treated with biochar. The PCA plots suggested that the pattern of C-substrate use was significantly affected by biochar. The use of the substrates differed markedly between the two soil layers. Biochar can increase the quantity and diversity of microorganisms (Chan et al., 2008), but Calbrix et al. (2007) reported that organic amendments had no significant effect on functional diversity due to the stability of the microbial communities. Soil texture, crops and organic materials may contribute to variations of microbial communities between assays.

Biochar has the potential to change use of microbial substrate, but the appropriate rate of biochar addition needs to be determined before it can be widely applied in a given region. The application of biochar to silt-loam soils decreased the metabolic capacity of the soil microbial communities and use of substrates, in accordance with the results reported by Gómez-Luna et al. (2012), who reported that amino acid C and carboxylic acid C were not well metabolised in soil amended with charcoal, which decreased C-use efficiency and functional diversity. The addition of biochar in situ may cause fluctuations in the amount of organic material and environmental stress. Liao et al. (2016), however, reported different trends amongst microbes after biochar application. Farrell et al. (2014a) found that biochar addition could increase the C-use efficiency of five low molecular weight organic substrates, especially in soil with low fertility, indicating that the biochar contained a small but important amount of labile organic matter, which may account for the higher use of amino acids in BC30 than BC10 in the 0–10 cm layer. Microbial diversity is variable and dependent on soil properties, but the actual changes in nutrient sources remain unknown, and a general understanding of soil microbial functional diversity after biochar application remains lacking. Multivariate analyses may therefore be required, and only broad groups may be realistically differentiated.

5. Conclusions

The addition of biochar to agricultural land had variable effects on soil chemical properties; soil pH did not significantly change, organic C and N contents increased, and mineral N content and q_{mic} decreased significantly in both soil layers after biochar addition. Biochar decreased AWCD and C substrate utilization, which were more evident at the lower dose of biochar addition. The biochar notably did not significantly affect maize growth, such as above-ground dry biomass, height, and plant N uptake. H was positively correlated with AWCD and negatively with pH, and AWCD was correlated positively with mineral N and negatively with soil pH. Our results indicated that soil microbial functional diversity alone might not be an effective indicator of soil fertility for evaluating the effects of adding stable C in the form of biochar, especially for the early growth periods of maize. Further research is thus required to evaluate the structural and genetic diversity of microbial communities in long-term semiarid agricultural ecosystems, and appropriate rates of biochar amendment needs further exploration.

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