Maize biochar addition rate influences soil enzyme activity and microbial community composition in a fluvo-aquic soil

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ABSTRACT

Biochar addition to soil has been proposed as a strategy to enhance soil quality and crop productivity, which may also affect microbial activity. However, the response of soil enzymes and microbial community composition to biochar addition and the main factors that drive their consequent behavior have rarely been studied. Therefore, to investigate the combined effect of different amounts of biochar (0, 0.5, 1.0, 2.5 and 5.0% by mass) and urea application on soil nutrients, enzymatic activities and microbial community in a fluvo-aquic soil, we conducted a 90-day laboratory study. Increased maize biochar addition led to significantly increased soil organic carbon (SOC), total N, and exchangeable K and reduced soil exchangeable Ca. Soil total N and exchangeable Ca were dominant factors affecting soil enzyme activities. Activities of soil extracellular enzymes involved in C and S cycling (except β-xylosidase) suggested lower amounts of biochar addition (0.5% by mass) could increase soil enzyme activities, while higher amounts of biochar addition reduce soil enzyme activities. However, the activities of L-leucine aminopeptidase and urease, both of which are involved in N cycling, increased with the increase of biochar addition rate. Total phospholipid fatty acid content and the relative abundance of bacteria were significantly reduced with increasing biochar addition rate. The relative abundance of fungi in the urea-amended soil was significantly higher than that in the other treated soils, and abundance of actinomycetes did not show a clear response to biochar addition. The changes in the microbial community composition were mainly related to SOC and total N contents, with a significant negative correlation. We concluded that the effect of biochar addition on soil enzymes and microbial community composition was highly variable. There is an urgent need to further estimate both the positive and negative long-term effects of biochar on the soil quality and crop productivity in this region.

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1. Introduction

Biochar is a by-product of biomass pyrolysis under oxygen-limited conditions and at relatively low temperatures ($<700\,^{\circ}$C). Biochar contains large amounts of carbon and macro or micro-nutrients depending on the feedstock and pyrolysis temperature (Enders et al., 2012; Ronsse et al., 2013; Wiedner et al., 2013). Recently, there has been a growing interest in applying biochar to amend acidic or nutrient-poor soil for soil ecological restoration while also sequestering carbon (Lehmann et al., 2003; Xu et al., 2013). Several studies have also reported biochar as a soil conditioner for enhancing soil fertility and crop productivity (Lehmann et al., 2006; Major et al., 2010). The enhancement of soil fertility as a result of biochar addition has been attributed to increased cation exchange capacity (Liang et al., 2006), changes to soil pH, or direct nutrient contributions from the biochar (Enders et al., 2012; Quilliam et al., 2012). However, other studies have shown possible negative effects of biochar on soil quality and fertility parameters, such as short-term reductions in soil mineral N availability (Bruun et al., 2012; Tammeorg et al., 2014) and decreased performance of crops on calcareous soils (Van Zwieten et al., 2010). These results suggest that soil nutrient responses to biochar addition are dependent on soil type, biochar addition rate and other unknown factors (Liang et al., 2014). However, the exact mechanisms for these increases or decreases are still a matter of speculation (Sohi et al., 2010), but are certainly related to changes in soil physicochemical properties and biological functions (Biederman and Harpole, 2013).

Soil extracellular enzymes are the catalysts of organic matter decomposition and are involved in the biogeochemical cycling of

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nutrients (Burns et al., 2013). Understanding the effect of biochar on the activity of these key enzymes has been identified as a research priority. Recently, some studies have reported that biochar addition to soil usually increases the soil enzyme activities related to N and P cycling and reduces the soil enzyme activities involved in C cycling (Bailey et al., 2011). Conversely, other studies have found inconsistent results (Lammirato et al., 2011; Paz-Ferreiro et al., 2014), which suggest that biochar has variable effects on different soils, enzymes, and assay types. In addition, soil enzymes are catalysts that play an important role in modulating ecosystem responses to changes in abiotic (changes in soil nutrient status, or in the quality of soil organic matter) and biotic conditions (Stone et al., 2012; Trasar-Cepeda et al., 2007). However, to date, few studies have explored the response of soil extracellular enzymes to soil environment under combined urea and biochar addition with different levels of biochar application.

Biochar effects on the soil biological processes involved in C and N dynamics are not well understood (Lehmann et al., 2011) and the responses are highly variable (Jones et al., 2011). Biochar amendments to soils have been recently shown to affect the community structure and abundance of soil microorganisms (Meynet et al., 2012). Some studies have reported enhanced (Bamminger et al., 2014; Rutigliano et al., 2014) or inhibited microbial activity (Dempster et al., 2012) in response to biochar additions, whereas other studies have reported no effects on soil microbial biomass as a result of its recalcitrance (Kuzyakov et al., 2009; Zavalloni et al., 2011). In addition, biochar addition to soil can alter soil physicochemical properties, which influence the soil microbial biomass and activity as well as community composition (Lehmann et al., 2011). As a result of their sensitivity to environmental changes, soil microbial community abundance and structure have been widely used as indicators of soil quality changes (Chu et al., 2007; Marschner et al., 2003). Until now, only limited studies on the effects of biochar on individual microbial communities have been conducted, which suggest that community compositional responses to biochar addition vary according to biochar type, among other possible factors (Steinbeiss et al., 2009). However, few studies have explored the individual microbial compositional responses to soil environment changes under combined urea and biochar addition with different levels of biochar application.

Nitrogen fertilizer (N), especially urea, is one of the most important nutritious factors for crop productivity and grain quality. In the North China Plain (NCP), high rates of N fertilizer are often applied and have led to low fertilizer use efficiency and serious environmental problems (Ju et al., 2009). It has been reported that the combination of biochar and N fertilizer is effective for improving crop yield while reducing the N application rate (Steiner et al., 2007). In addition, the fluvo-aquic soil is a typical soil type in NCP, which accounts for 53% of the fluvo-aquic soils in China. However, there are few studies on microbial mechanisms between biochar and chemical N in a fluvo-aquic soil. Therefore, the specific objectives for this work were to: (1) investigate the short-term effects of combined urea and biochar addition with different levels of biochar application (0, 0.5, 1.0, 2.5 and 5.0% by mass) on soil nutrients (pH, electrical conductivity, SOC, TN, NO_3^--N, NH_4^+-N, water soluble and exchangeable K, Na, Ca, Mg) and extracellular enzyme activities and microbial community in a fluvo-aquic soil; (2) illustrate the main factors that drive the changes in soil enzyme activity or microbial community composition after biochar addition. Soil enzyme activities involved in C, N, P, and S cycling and microbial community composition were determined by microbial fluorometric assay and phospholipid fatty acid (PLFA) analysis, respectively.

2. Materials and methods

2.1. Biochar and soil

Biochar was produced at 450 °C by slow pyrolysis (5 °C min^{-1} heating and 1 h residence time in a Microwave Muffle Furnace (SX$_2$, Shanghai Rongfeng Scientific Instrument Inc, China)) of maize straw. Maize straw was taken from the main maize producing area in China; Zhengzhou, Henan Province, in the North China Plain. All biochar samples were mixed evenly, ground and sieved to <0.154 mm. Their physical and chemical properties are shown in Table 1.

Soil was collected in summer 2014, before sowing, from the top layer (0–20 cm) of a fluvo-aquic soil, in the Soil Fertility and Fertilizer Efficiency Monitoring Network Station, Zhengzhou, Henan Province, China (34°47′02″ N, 113°39′25″ E), with the soil parent material mainly originating from the alluvial deposits of the Yellow River. The soil texture is light loamy soil. The basic physical and chemical soil characteristics are shown in Table 1.

2.2. Incubation experiment

An incubation experiment was conducted over 90 days to investigate the effects of biochar on soil nutrients, enzyme activity and microbial community composition. The six treatments were control (CK), urea (U) and urea with maize biochar (MC) added separately at 0.5, 1.0, 2.5 and 5.0% by weight to soil (henceforth termed U + 0.5%MC, U + 1.0%MC, U + 2.5%MC and U + 5.0%MC, respectively). The experiment was arranged in a complete randomized block design with three replicates. Initially, 150 g of air-dried soil (<2 mm) was weighed into 500-ml plastic containers. A urea solution was added to each container (except CK) at the ratio of 200 mg N (kg soil)$^{-1}$. The moisture content of each sample was adjusted to 40–45% of the water-holding capacity, and readjusted by adding deionized water every 3 days. Each individual container was sealed with a polyethylene film containing 3 pin-sized holes to permit aeration. Temperature was kept constant at 25 °C during the entire experiment. The soil was sampled after 90 days and analyzed for SOC, total N, inorganic N, water soluble and exchangeable K, Na, Ca and Mg, extracellular enzyme activities and microbial community composition.

Table 1
The physical and chemical properties of experimental soil and biochar.

	Yield (%)	pH	Ash content (%)	EC (ms cm^{-1})	Surface area (m^{2}g^{-1})	SOC (%)	TN (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (g kg^{-1})	Ws. K (g kg^{-1})	Ws. Na (g kg^{-1})	Ws. Ca (g kg^{-1})	Ws. Mg (g kg^{-1})	Ex. K (g kg^{-1})	Ex. Na (g kg^{-1})	Ex. Ca (g kg^{-1})	Ex. Mg (g kg^{-1})
Biochar	32.60	10.50	22.28	5.37	4.00	53.81	1.22	/	/	1.67	0.08	0.15	0.10	75.45	12.98	13.66	3.52
Soil		8.28	/	0.57	/	0.54	0.07	15.82	0.43	0.07	0.48	0.50	0.05	0.28	12.81	33.21	0.99

Abbreviations: Ex, exchangeable; Ws, water-soluble; TN, total nitrogen; SOC, soil organic carbon; EC, electrical conductivity. "/" not measured. Yield (%) = (weight of biochar)/ (weight of feedstock) × 100.

2.3. Chemical analysis

Soil pH was measured with a compound electrode (PE-10, Sartorius, Germany) using a soil to water ratio of 1:2.5. Electrical conductivity (EC) was determined in 1:5 (w/v; $g\,cm^{-3}$) soil–water mixtures. Inorganic N (NH_4^+-N and NO_3^--N) was extracted with 2 M KCl and determined by flow injection analysis (TRAACA-2000, Germany). The SOC and total N contents were determined using a total organic C/total N analyzer (Multi N/C 3100/HT1300, Analytik Jena AG, Germany). The water soluble K, Na, Ca and Mg were extracted with deionized water at 1:5 (w/v; $g\,cm^{-3}$) soil–water mixtures; the exchangeable K, Na, Ca and Mg were extracted with 1 M ammonium acetate and concentrations were determined by atomic absorption spectrometry (NovAA300, Analytik Jena).

2.4. Enzyme activity

The eleven enzymes analyzed included four C-cycling enzymes (β-D-cellobiosidase, β-glucosidase, β-xylosidase and a-glucosidase), 1C and N cycling enzyme (N-acetyl-β-glucosaminidase), 2 N cycling enzymes (leucine aminopeptidase and urease), 1 P cycling enzyme (phosphomonoesterase), 1 S cycling enzyme (sulfatase) and 2 oxidoreductases (peroxidase and phenol oxidase). The potential activities of all enzymes (except phenol oxidase, peroxidase and urease) were quantified according to fluorescence-based protocols as described in Ai et al. (2012, 2015). Phenol oxidase and peroxidase were measured colorimetrically in a clear 96-well microplate according to Ai et al. (2015). Urease activity was determined according to Kandeler and Gerber (1988) and was expressed as $\mu mol\,NH_4^+\,g^{-1}$ dry soil h^{-1}.

2.5. PLFA analysis

Soil microbial community composition and microbial biomass were determined by PLFA analysis according to the procedure described by Wu et al. (2009). Soil samples were freeze-dried and then PLFAs were extracted with a chloroform/methanol/citric acid buffer (1:2:0.8 volume ratios, pH 4.0). Neutral lipids and glycolipids were separated from polar lipids on a silica-bonded phase column (SPE-Si, Supelco, Poole, UK) by elution with chloroform and acetone, respectively. After adding nonadecanoic acid methyl ester (19:0) as the internal standard, the polar lipids were converted to the fatty acid methyl esters (FAMEs) by a mild alkaline methanolysis. Dried FAMEs were redissolved in n-hexane and then quantified and identified by gas chromatography (N6890, Agilent) and MIDI Sherlock microbial identification system version 4.5 (MIDI Inc., Newark, DE, USA), respectively. The internal standard (19:0) peak was used as a reference to calculate the concentration of PLFAs, which was expressed as $nmol\,g^{-1}$ dry soil.

Total microbial biomass was estimated using the total concentration of PLFAs ($nmol\,g^{-1}$). The abundance of individual PLFAs was indicated by their% mole abundance in each sample. PLFAs were divided into various taxonomic groups based on previously published PLFA biomarker data (Ai et al., 2012). Specifically, we used i14:0, i15:0, i16:0, i17:0, a15:0, and a17:0 as Gram positive bacteria biomarkers; cy17:0, cy19:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω5c, and 18:1ω7c as Gram-negative bacteria biomarkers; and the sum of Gram positive and Gram-negative bacteria biomarkers together with 15:0, 17:0, 17:1ω6, and 17:1ω7 as a measure for total bacterial biomass. The unsaturated PLFAs 16:1ω5c, 18:2ω6, 9, 18:1ω9 and 18:3ω3, 6, 9 were used as fungal biomarkers. The fatty acids 10Me-16:0, 10Me-17:0 and 10Me-18:0 were used as markers for actinomycetes.

2.6. Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using software package SAS version 8.0. One-factor ANOVA was deployed to compare treatment effects. The least significant difference (LSD; at 0.05 level of probability) test was applied to assess the differences between the means.

3. Results

3.1. Soil characteristics after maize biochar addition

After 90 days of incubation, the soil pH value in all urea-amended treatments was lower than that in the CK treatment by 0.06–0.21 units (Table 2). The soil pH value increased with the increasing MC addition rate, with the U + 5%MC treatment showing the largest increase at 1.86% larger than the U treatment. Electrical conductivity (EC) in all urea-amended soils was enhanced significantly compared with the CK treatment. The soil EC increased with increasing MC addition rate, and the U + 5%MC treatment showed the largest increase at 16.57% larger than the U treatment (Table 2).

There was a significant effect of different amounts of MC application on soil organic carbon and total N contents (Table 2). The SOC and total N contents both increased significantly with increasing MC addition rate. The SOC content in the U + 5%MC treatment was higher than those in the CK and U treatments by 8.21 and 8.58 times, respectively. The total N content was also higher than those in the CK and U treatments by 78.46 and 52.63%, respectively. However, there was no significant difference between the CK and U treatments ($P > 0.05$).

After 90 days of incubation, soil NH_4^+-N content in all treatments was lower than $1\,mg\,kg^{-1}$ (Table 2). The soil NH_4^+-N content first increased and then decreased with the increase of MC addition rate. The NO_3^--N content in all urea-amended soils was higher than that in the CK soil; however, it showed a marked decrease with increasing MC addition rate, with the U + 5%MC treatment showing the largest decrease at 53.82% lower than the U treatment.

Soil water soluble K and exchangeable K contents showed an increasing trend with increased MC application; the two contents in U+5%MC-treated soil were significantly higher than those in other treatments (Tables 3 and 4). Conversely, both water soluble and

Table 2
Effect of different treatments on chemical properties of the fluvo-aquic soil (mean ± standard error; n = 3).

Treatment	pH	EC $\mu S\,cm^{-1}$	Total N $g\,kg^{-1}$	SOC $g\,kg^{-1}$	NH_4^+-N $mg\,kg^{-1}$	NO_3^--N $Mg\,kg^{-1}$
CK	8.12 ± 0.02 a	640.67 ± 12.83 e	0.65 ± 0.01 e	3.88 ± 0.89 e	0.31 ± 0.08 b	20.23 ± 1.36 d
U	7.91 ± 0.06 b	863.00 ± 24.04 c	0.76 ± 0.03 d	3.73 ± 0.60 e	0.37 ± 0.01 b	125.29 ± 2.04 a
U + 0.5%MC	7.92 ± 0.04 b	798.00 ± 11.14 d	0.77 ± 0.02 d	7.00 ± 0.32 d	0.65 ± 0.11 a	101.71 ± 13.52 b
U + 1.0%MC	7.92 ± 0.01 b	848.67 ± 21.39 c	0.87 ± 0.02 c	10.30 ± 1.13 c	0.59 ± 0.06 a	93.75 ± 5.65 b
U + 2.5%MC	7.93 ± 0.04 b	914.67 ± 22.03 b	0.96 ± 0.01 b	18.63 ± 1.17 b	0.42 ± 0.09 b	68.78 ± 5.55 c
U + 5.0%MC	8.06 ± 0.05 a	1006.00 ± 36.77 a	1.16 ± 0.01 a	35.75 ± 0.71 a	0.30 ± 0.04 b	57.86 ± 5.67 c

Letters indicate significant difference between the treatments, which was analyzed using a LSD test ($P < 0.05$).

Table 3
The effect of different treatments on water soluble K, Ca, Na and Mg of the fluvo-aquic soil (mean ± standard error; $n = 3$).

Treatment	Water soluble cation (g kg^{-1})			
	K	Na	Ca	Mg
CK	0.025 ± 0.006 d	0.444 ± 0.007 ab	0.498 ± 0.013 c	0.055 ± 0.003 d
U	0.024 ± 0.002 d	0.461 ± 0.021 a	0.707 ± 0.063 a	0.080 ± 0.006 a
U + 0.5%MC	0.054 ± 0.003 d	0.438 ± 0.018 ab	0.608 ± 0.014 b	0.067 ± 0.002 bc
U + 1.0%MC	0.119 ± 0.005 c	0.453 ± 0.013 a	0.605 ± 0.023 b	0.068 ± 0.003 b
U + 2.5%MC	0.358 ± 0.009 b	0.418 ± 0.006 b	0.575 ± 0.045 b	0.061 ± 0.002 cd
U + 5.0%MC	0.737 ± 0.040 a	0.419 ± 0.014 b	0.394 ± 0.048 d	0.042 ± 0.005 e

Letters indicate significant difference between the treatments, which was analyzed using a LSD test ($P < 0.05$).

exchangeable Ca and Mg contents in soil decreased with the increase of MC addition rate. Soil water soluble Ca and Mg for the U + 5%MC treatment reduced significantly by 44.27 and 47.50% compared to U treatment, and soil exchangeable Ca and Mg reduced by 22.53 and 20.07%. For soil water soluble and exchangeable Na, there were little variations between different treatments.

3.2. Changes in soil enzyme activity

The potential activities of 11 soil enzymes involved in C, N, P, and S cycling were determined on the 90th day of incubation (Figs. 1, 2a and b). The activities of β-glucosidase and β-cellobiosidase in the CK and U + 0.5%MC treatments were similar to each other and significantly higher than those in other treatments; whereas, there were no differences between CK and U + 0.5%MC-treated soils ($P > 0.05$). The activities of β-xylosidase and α-glucosidase in CK treated soil were higher than those in all urea-amended soils. In addition, soil enzymes involved in C cycling (except β-xylosidase) showed an initial increase followed by a decrease with increased MC addition and a similar trend was also observed for the activity of sulfatase (Fig. 1). These results indicate that lower addition of MC (0.5% by mass) could increase the activities of soil enzymes involved in C and S cycling, while the higher addition of MC has the opposite effect. The activities of soil enzymes simultaneously involved in C and N cycling (N-acetyl-glucosaminidase), peroxidase and phenol oxidase, were similar to β-xylosidase activity, which showed decreased their activities with increasing MC addition rate. In contrast, the activities of ʟ-leucine aminopeptidase and urease increased with the increase of MC addition rate (Fig. 1). This indicates that MC addition to soil could increase the activities of a series of enzymes related to N utilization (Bailey et al., 2011). In this study, the activity of phosphatase in CK treated soil was higher than that in all urea-amended soils. Phosphatase activity decreased with increasing MC addition rate.

Principal component analysis (PCA) showed that the activities of soil enzymes were significantly different between treatments, and this difference was related to the changes in soil total N and

exchangeable Ca contents (Fig. 2a). Ordination of treatments was primarily related to the first canonical axis (PC1), which separated the samples into two distinct groups, each possessing a specific range of soil total N and exchangeable Ca contents. The first group included the CK, U, and U + 0.5%MC treated soils and had lower total N (0.65–0.77 g kg^{-1}) and higher exchangeable Ca (37.78–39.72 g kg^{-1}) contents. The second group included U + 1.0%MC, U + 2.5%MC and U + 5.0%MC treated soils and had higher total N (0.87–1.16 g kg^{-1}) and lower exchangeable Ca^{2+} (30.47–34.15 g kg^{-1}) contents, indicating that soil total N and exchangeable Ca^{2+} were major factors affecting soil enzyme activity (Fig. 2a). Indeed, redundancy analysis (RDA) confirmed that soil total N ($F = 42.6$, $P = 0.002$), exchangeable Na ($F = 3.6$, $P = 0.006$) and exchangeable Ca ($F = 3.5$, $P = 0.008$) were significantly correlated with soil enzyme activities and explained 72.7, 5.3 and 4.4% of the total enzyme activity variability, respectively (Fig. 2b).

3.3. Changes in the abundance and composition of microbial communities

On the 90th day of incubation, total PLFA content in all biochar-amended treatments were lower than in the CK treatment, which was significantly reduced with the increase of MC addition rate. Conversely, there were no differences between the CK and U treatments (Fig. 3a). A similar trend was also observed for the relative abundances of bacteria. The ratio of Gram-positive to Gram-negative bacteria was significantly decreased in the
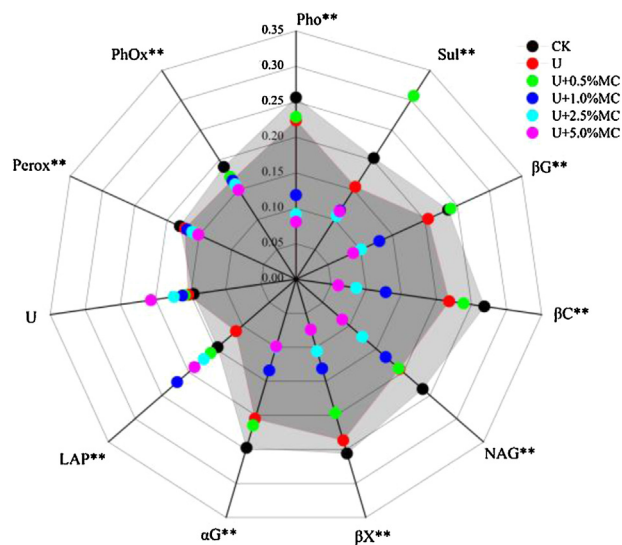
Table 4
The effect of different treatments on exchangeable K, Ca, Na and Mg of the fluvo-aquic soil (mean ± standard error; $n = 3$).

Treatment	Exchangeable cation (g kg^{-1})			
	K	Na	Ca	Mg
CK	0.62 ± 0.15 c	12.31 ± 0.46 ab	39.72 ± 0.92 a	0.93 ± 0.06 a
U	0.72 ± 0.30 c	11.76 ± 0.77 b	39.33 ± 0.70 ab	0.89 ± 0.04 a
U + 0.5% MC	1.90 ± 0.29 b	13.27 ± 0.72 a	37.78 ± 1.20 b	0.84 ± 0.04 ab
U + 1.0% MC	1.94 ± 0.36 b	12.27 ± 0.57 ab	34.15 ± 0.99 c	0.78 ± 0.03 bc
U + 2.5% MC	2.14 ± 0.12 b	11.62 ± 0.59 b	31.33 ± 1.12 d	0.75 ± 0.04 c
U + 5.0% MC	4.77 ± 0.72 a	13.06 ± 0.55 a	30.47 ± 1.24 d	0.71 ± 0.05 c

Letters indicate significant difference between the treatments, which was analyzed using a LSD test ($P < 0.05$).

Fig. 1. Radar graph illustrating the relative response of enzyme activity to biochar and urea application (normalized value). Asterisks indicate significant differences among different treatments based on Fisher's LSD test (*$P < 0.05$, **$P < 0.01$). Enzyme abbreviations: Pho, phosphomonoesterase; Sul, sulfatase; βG, β-glucosidase; βC, β-cellobiosidase; NAG, N-acetylglucosaminidase; βX, β-xylosidase; αG, α-glucosidase; LAP, ʟ-leucine aminopeptidase; U, urease; Perox, peroxidase, and PhOx, phenol oxidase.
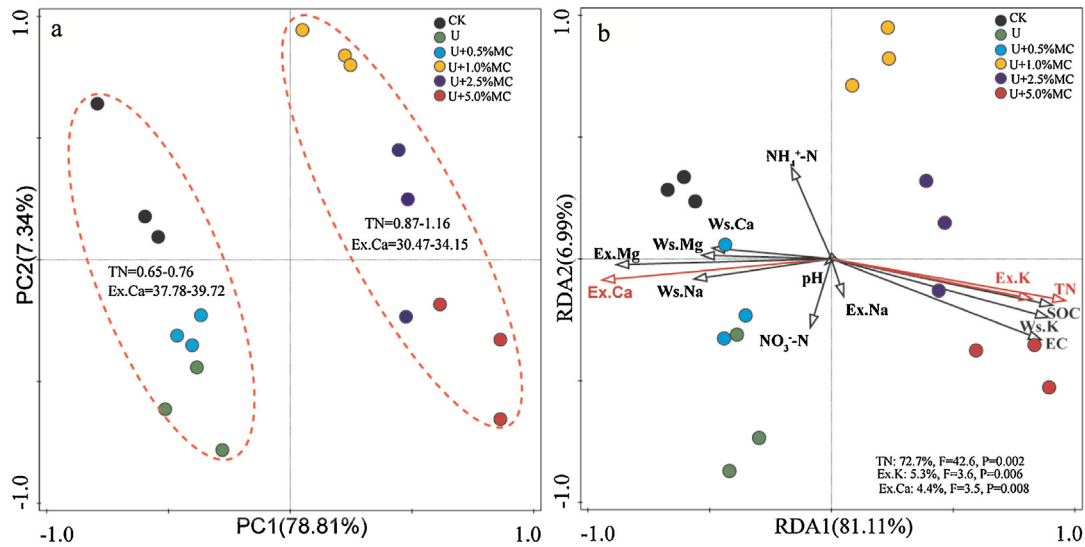
Fig. 2. Principal component analyses (PCA) of enzyme activities in soils from different treatments (a), and redundancy analyses (RDA) of the correlations between soil parameters and enzyme activity (b). The red arrows indicate the soil parameters that had a significant impact on enzyme activities ($P < 0.05$), and the corresponding explained proportion of variability is shown in the lower right corner. Abbreviations: Ex, exchangeable; Ws, water-soluble; TN, total nitrogen; SOC, soil organic carbon and EC, electrical conductivity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).
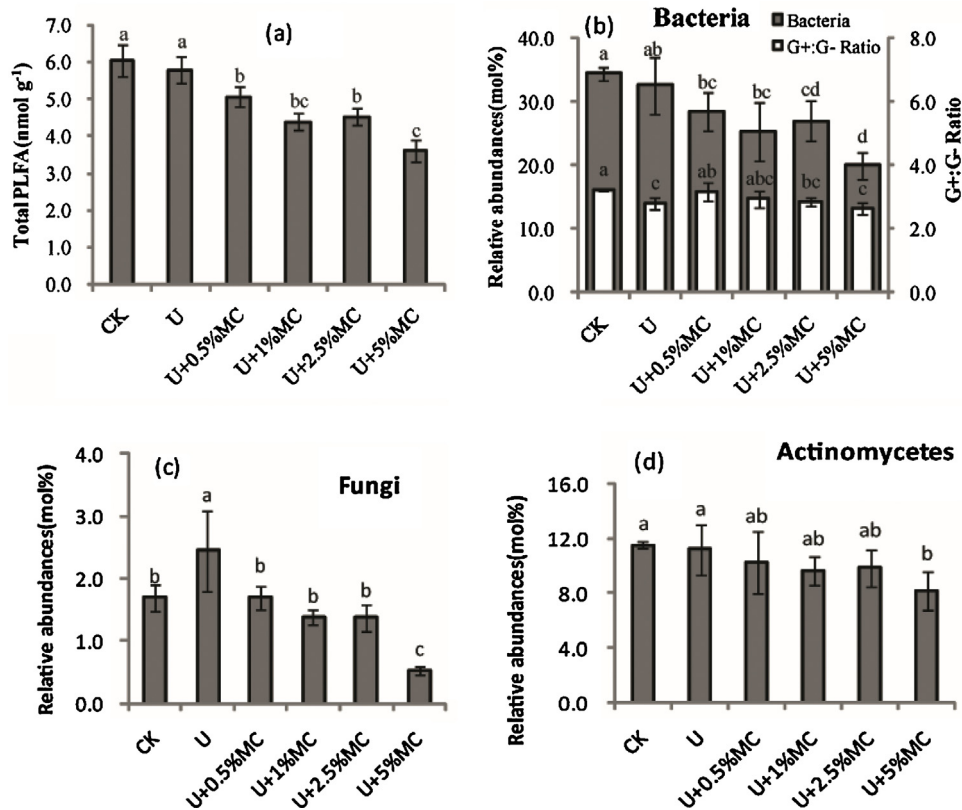

Fig. 3. Comparisons of total PLFA (a), the relative abundance of bacteria, and the ratio of Gram positive to Gram-negative bacteria (b), and the relative abundances of fungi (c) and actinomycetes (d). Vertical bars represent the standard error ($n = 3$) and lower case letters indicate significant differences between treatments at the $P < 0.05$ level.

U, U + 2.5%MC and U + 5.0%MC treatments compared with the CK treatment, and there was an initial increase followed by a decrease with increased biochar addition. There were no differences between the U and U+5.0%MC treatments (Fig. 3b). The relative abundance of fungi in the U treatment was significantly higher than that in the other treated soils, and these showed a declining trend with increasing MC addition (Fig. 3c). The relative abundance

of Actinomycetes did not show a clear trend in response to MC addition, except for a small but significant difference between soils with a U + 5.0%MC and U treatment (Fig. 3d).

The PCA showed that the composition of the microbial community was significantly different between different treatments, and this difference was related to the changes in soil total N and SOC contents (Fig. 4a). The PLFA profiles of the CK, U, and
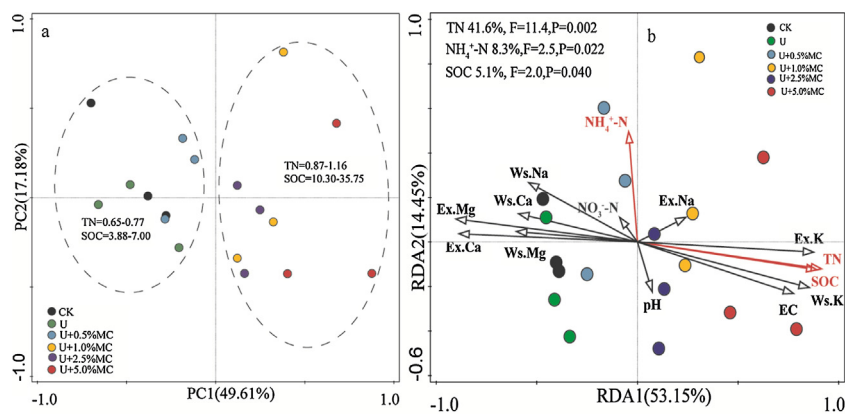
Fig. 4. Principal component analyses (PCA) of microbial community composition (relative content of individual PLFA molecules) in soils from different treatments (a), and redundancy analyses (RDA) of the correlations between soil parameters and microbial community composition (b) .The red arrows indicate the soil parameters that had a significant impact on enzyme activities ($P < 0.05$), and the corresponding explained proportion of variability is shown in the upper left corner. Abbreviations: Ex, exchangeable; Ws, water-soluble; TN, total nitrogen; SOC, soil organic carbon; EC, electrical conductivity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

U + 0.5%MC treated soils with lower total N (0.65–0.77 g kg^{-1}) and SOC (3.88–7.00 g kg^{-1}) contents were well separated from those soils receiving the U + 1.0%MC, U + 2.5%MC and U + 5.0%MC treatments with higher total N (0.87–1.16 g kg^{-1}) and SOC (10.30–35.75 g kg^{-1}) contents along PC1. This indicates that soil total N and SOC contents were major factors affecting the microbial community composition (Fig. 4a). The RDA showed that total N ($F = 11.4$, $P < 0.01$), NH$_4^+$-N ($F = 2.5$, $P = 0.02$) and SOC ($F = 2.0$, $P = 0.04$) were significantly correlated with soil microbial community composition and explained 41.6, 8.3 and 5.1% of the total community variability, respectively (Fig. 4b).

3.4. Correlations of environment factor and soil microbial properties

Correlation analysis revealed that eleven enzyme activities (excluding L-leucine aminopeptidase and Urease) were negatively correlated with soil total N, SOC and exchangeable K and positively correlated with soil exchangeable Ca (Table 5). The activities of L-leucine aminopeptidase and Urease were the exact opposite of other enzyme activities. A similar trend was also observed for the Gram-positive bacteria biomarkers (except i 14:0), Gram-positive bacteria biomarkers (cy17:0 and cy19:0 w8c), fungi biomarkers (18:3 w6c (6, 9, 12) and 18:1 w9c) and the actinomycetes biomarker (10Me16:0) (Table 5). In addition, soil total N, SOC and exchangeable K were significantly increased, whereas soil exchangeable Ca reduced with increased MC addition (Tables 2 and 4). These results indicate that MC addition to soil might generally increase the activities of a series of enzymes related to N utilization (Bailey et al., 2011) while impeding soil microbial activity involved in C cycling.

Table 5
Correlations of microbial properties (enzyme activities or PLFA biomarkers) and soil environment factors.

Enzymes/PLFA biomarkers		TN	SOC	Ex.K	Ex.Ca
G+	i 14:0	−0.18	−0.09	−0.27	0.26
	i 15:0	−0.76**	−0.68**	−0.76**	0.62**
	a 15:0	−0.68**	−0.65**	−0.71**	0.62**
	i 16:0	−0.62**	−0.64**	−0.61**	0.56*
	i 17:0	−0.70**	−0.69**	−0.65**	0.68*
	a 17:0	−0.55*	−0.58*	−0.59**	0.54*
G−	cy17:0	−0.59*	−0.55*	−0.55*	0.56*
	cy19:0 w8c	−0.53*	−0.52*	−0.55*	0.57*
Fungi	18:3 w6c (6,9,12)	−0.59**	−0.67**	−0.76**	0.50*
	18:1 w9c	−0.74**	−0.78**	−0.73**	0.68**
Actinomycetes	10Me16:0	−0.67**	−0.68**	−0.64**	0.60**
	10Me17:0	−0.36	−0.45	−0.45	0.38
	10Me18:0	−0.31	−0.35	−0.29	0.22
Enzyme	Phosphomonoesterase	−0.90**	−0.82**	−0.75**	0.95**
	Sulfatase	−0.56*	−0.48*	−0.28	0.60**
	β-glucosidase	−0.89**	−0.82**	−0.70**	0.92**
	β-cellobiosidase	−0.92**	−0.84**	−0.76**	0.93**
	N-acetylglucosaminidase	−0.94**	−0.88**	−0.81**	0.86**
	β-xylosidase	−0.93**	−0.87**	−0.83**	0.97**
	α-glucosidase	−0.90**	−0.80**	−0.73**	0.95**
	L-leucine aminopeptidase	0.52*	0.48*	0.54*	−0.64**
	Urease	0.87**	0.86**	0.86**	−0.79**
	Phenol oxidase	−0.79**	−0.71**	−0.63**	0.68**
	Catalase	−0.83**	−0.79**	−0.74**	0.75**

Significant correlations are highlighted with asterisks *$P < 0.05$; **$P < 0.01$.

4. Discussion

The effects of biochar on soil properties vary widely, depending on the characteristics of both the underlying soil and the biochar. In this study, soil pH in the urea only treatment showed a decrease, while soil pH showed an increase with the increase of biochar addition rate over a short term period (90 d), which is consistent with previous results (Jones et al., 2012). This result confirmed that biochar could serve as a liming agent resulting in increased pH for a number of different soil types (Jones et al., 2012). In our study, the organic C and total N contents in soils were increased with increasing MC addition rate, which is consistent with previous results (Liang et al., 2014). This increase may be because biochar contains labile C and N and could release organic C and N into the soil (Ouyang et al., 2014). Nelson et al. (2011) found that biochar application increased soil NH_4^+ concentration and decreased NO_3^- recovery, whereas other studies have reported that the contents of NH_4^+ and NO_3^- markedly decreased with increasing amounts of biochar (Shenbagavalli and Mahimairaja, 2012). In this study, soil NH_4^+-N content at the 90th day of incubation first increased and then decreased along with the increase of MC addition rate, while, NO_3^--N content showed a marked decrease, which was consistent with the results of Shenbagavalli and Mahimairaja (2012). Novak et al. (2009) found that soil available Ca, K, Mn, and organic carbon increased whereas soil available S and Zn decreased after biochar addition in a soil column experiment. We found that soil water soluble K and exchangeable K contents increased as the amounts MC applied increased; however, both water soluble and exchangeable Ca and Mg contents in soil showed a decrease with the increase of MC addition rate. This may be because MC has both a high surface area per unit mass and a high charge density, contributing to a higher capacity to adsorb bivalent cations than monovalent cations.

Soil enzyme activities control the rate of soil organic matter decomposition and nutrient cycling processes (Nannipieri et al., 2012). Biochar addition has generally been found to reduce the soil enzymatic activities associated with ecological processes such as soil C mineralization (Lehmann et al., 2011). In our study, soil enzymes involved in C cycling (except β-xylosidase) showed an initial increase and then decreased with increasing MC addition rate (Fig. 1), while the potential activities of β-xylosidase decreased with increasing MC addition rate (Fig. 1), which is consistent with the results of Elzobair et al. (2015). These results indicated that a lower MC addition (0.5% by mass) could increase the activities of soil enzymes involved in C and S cycling, while a higher amount of MC addition would reduce their activities. The decreased activities were 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). The activities of N-acetyl-glucosaminidase showed a decrease with the increase of MC addition rate, which supports the results of Awad et al. (2012), while biochar did not affect N-acetyl-glucosaminidase activity, regardless of the application rate (Elzobair et al., 2015). In addition, the activities of L-leucine aminopeptidase and urease increased with the increase of MC addition rate after a 90-day incubation (Fig. 1), which confirmed the previous findings of Bailey et al. (2011) that adding biochar to soil could increase the activities of a series of enzymes related to N utilization. However, Elzobair et al. (2015) found that L-leucine aminopeptidase activity was reduced by lower rates of biochar (1–5% by mass) but not at the highest rate (10% by mass).

Once added to the soil, abiotic and biotic surface oxidation of biochar results in increased surface carboxyl groups, a greater negative charge, and subsequently an increased ability to sorb cations (Cheng et al., 2008). Several studies have found that biochar has the potential to sorb a wide range of organic and inorganic molecules and may affect enzymes (and consequently their activity) by sorbing them and/or their substrates (Jin, 2010).

In our study, both PCA and RDA showed that these alterations depended on soil total N and exchangeable Ca contents (Fig. 2). Correlation analysis revealed that eleven enzyme activities (excluding L-leucine aminopeptidase and urease) were negatively correlated with soil total N, SOC and exchangeable K while positively correlated with soil exchangeable Ca (Table 5). These results indicate that the ability of biochar to sorb enzymes is dependent on the amount of biochar addition, soil nutrient content and the specific enzyme, but further research is necessary to understand the mechanisms of enzyme sorption by biochar.

Potential mechanisms of the effects of biochar on soil microbes include (i) providing a C substrate (Smith et al., 2010), (ii) producing/adsorbing substances that stimulate (Bamminger et al., 2014) or inhibit microbes (Dempster et al., 2012), and/or (iii) providing a suitable habitat for microbial growth and protection from predators (Quilliam et al., 2013). In this study, total PLFA content and the relative abundances of bacteria and fungi in all biochar-amended soils showed a decrease with increased MC addition (Fig. 3a), which is consistent with the results of Dempster et al. (2012). These results suggest that an alkaline soil amended with high amounts of biochar could inhibit soil microbial activity during the short-term (Marks, 2013).

Because of their sensitivity to environmental change, soil microbial community abundance and structure have been widely used as indicators of soil quality change (Chu et al., 2007; Marschner et al., 2003). Recently, biochar addition to soils was found to affect the community structure and abundance of soil microorganisms (Meynet et al., 2012). Many studies have shown that soil microbial biomass and community composition were changed by organic amendments and that these changes were related to the soil C content (Ai et al., 2012; Bowles et al., 2014), because the mineralization of organic matter increased the activity of soil microorganisms. In this study, both PCA and RDA showed that these alterations were dependent on soil total N and SOC contents (Fig. 4). We found that the individual PLFA biomarkers of Gram-positive bacteria (except i 14:0), Gram-negative bacteria, fungi, and actinomycetes (10Me 16:0) were significantly negatively correlated with soil total N and SOC contents.

5. Conclusions

This study clearly demonstrated the soil nutrients, enzyme activity and microbial community composition change in responses to varying amounts of MC addition in a fluvo-aquic soil, after 90 days of incubation. The SOC and total N contents increased with the increase of MC addition rate and were dominant factors affecting soil microbial community composition. Soil extracellular enzymes involved in C and S cycling (except β-xylosidase) indicated that amendment with the lower amount of maize biochar (0.5% by mass) could increase soil enzyme activities, while amendment with the higher amount of maize biochar reduces their activities. The activities of L-leucine aminopeptidase and urease further confirmed that adding biochar to soil could increase the activities of a series of enzymes related to N utilization. Furthermore, soil total N and exchangeable Ca were dominant factors affecting soil enzyme activities. As biochar cannot practically be removed from soil after application, future studies are needed to further estimate soil ecosystem functioning response and to focus on both the positive and negative long-term effects of biochar on the soil quality and crop productivity in this region.

Boon for his help in English editing and proof reading of our manuscript.

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