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Soil nutrient and microbial activity responses to two years after maize straw biochar application in a calcareous soil

Dali Song[a,b], Xiangyin Xi[c], Qin Zheng[a], Guoqing Liang[a], Wei Zhou[a,**], Xiubin Wang[a,*]

[a] Institute of Agricultural Resource and Regional Planning, Chinese Academy of Agricultural Sciences/Key Lab of Plant Nutrition and Nutrient Cycling, Ministry of Agriculture, Beijing, 100081, China
[b] College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, China
[c] Southwest University, Chong Qing, 400715, China

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ABSTRACT

Biochar (BC) addition to soil is a strategy to enhance soil fertility, which may also affect microbial activity. However, little information is available on the responses of soil nutrients and microbial activities to BC in a calcareous soil. This study investigated the changes of soil nutrient contents and microbial activities in a calcareous soil two years after application of biochar at rate of 0, 2.5, 7.5 and 22.5 t/ha. The results showed that the contents of soil organic carbon (SOC), total nitrogen (TN), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and available phosphorus and potassium increased significantly with increasing BC addition rate, but no significant effect on soil pH. Soil microbial biomass carbon and nitrogen (MBC and MBN) had an increased and then decreased trend. BC amendment increased microbial biomass and promoted soil carbon- and nitrogen-cycling enzyme activities, the ratios of β-glucosaminidase/phosphomonoesterase, N-acetyl-β-glucosaminidase plus leucine aminopeptidase/phosphomonoesterase increased significantly with increasing BC addition rate. Redundancy analysis confirmed that DOC and MBN were dominant factors affecting soil microbial biomass, and soil pH, TDN, DOC, MBN and SOC were main factors regulating soil enzyme activities. Besides, principal component analysis revealed that difference in microbial community composition in one year after BC addition was mainly associated with the relative abundance of bacteria and fungi, the relative abundance of bacteria increased, while the ratios of Gram-negative/Gram-positive bacteria and fungi/bacteria, and relative abundance of fungi and arbuscular mycorrhizal fungi decreased in BC-amended soils with control. However, BC had no significant effect on microbial community composition after two years. These results suggest that application of maize BC to calcareous soils may have a great potential for improvements in the soil nutrients and enzyme activity, the changes in soil microbial composition deserve further studies.

1. Introduction

Biochar (BC) is a solid organic substance produced by thermochemical conversion under oxygen-limited conditions. It is estimated that the BC carbon (C) residence time in the soil is hundreds to thousands of years, whereas the residence time of crop residues is several decades (Lehmann et al., 2006). This makes BC attractive as a soil amendment option for C sequestration because it has the potential to improve soil properties and functions relevant to agronomic and environmental performance (Lehmann, 2007; Woolf et al., 2010). Previous studies have reported that BC can increase soil nutrient holding capacity, nutrient supply capacity, nutrient availability, soil fertility, and plant performance, thus exerting a fertilizer effect on crop growth and yield (Butnan et al., 2015; Kameyama et al., 2012; Wan et al., 2014). The effect of BC on soil fertility is predominantly mediated through the increase in pH in acidic soils (Zwieten et al., 2010) or by increasing soil cation adsorption to improve nutrient retention (Liang et al., 2006). However, previous findings are inconsistent, with positive and negative effects of BC reported, and the long-term effects remain uncertain (Bruun et al., 2013; Tammeorg et al., 2014; Viger et al., 2015).

Experimental evidence shows that the addition of BC has an important impact on soil microbial communities and performs a critical role in maintenance of soil health and function (Lehmann et al., 2011; Rondon et al., 2007; Steiner et al., 2010; Warnock et al., 2007). Changes in microbial community composition or activity induced by BC

* Corresponding author.
** Corresponding author.
E-mail addresses: wzhou@caas.ac.cn (W. Zhou), wangxiubin@caas.cn (X. Wang).

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not only affect nutrient cycling and plant growth, but also influence soil organic matter cycling (Kuzyakov et al., 2009; Liang et al., 2010; Wardle et al., 2008). Addition of BC may stimulate microbial growth and improve microbial community composition, such as Gram-positive bacteria (Gomez et al., 2014), Gram-negative bacteria (Gomez et al., 2014; Watzinger et al., 2014), fungi (Steinbeiss et al., 2009), and actinomycetes (Prayogo et al., 2014; Watzinger et al., 2014), and increase biomass. However, other studies have determined that the large amount of easily decomposable organic C in BC promotes biological fixation of nitrogen (N) by soil microorganisms and that BC adsorbs soil available nutrients and hinders the contact of nutrients with microorganisms, which adversely affects soil microbial biomass (Deenik et al., 2010; Gul et al., 2015). It is also reported that addition of BC has no or negative impacts on soil microbial community composition (Elzobair et al., 2015; Mukherjee et al., 2016; Rutigliano et al., 2014). These contradictory results are mainly a result of differences in soil types, BC application rate, and time of use among studies (Farrell et al., 2013; Mitchell et al., 2015; Muhammad et al., 2014).

Before large-scale implementation of BC application to farmland ecosystems, careful consideration must be given to the impact on soil properties, processes and functions, and thorough scientific validation is critical (Mukherjee and Lal, 2014). The long-term and short-term responses of microbial attributes to BC addition remain uncertain and generalizations regarding the practical application of BC to different soil types is presently impossible (Lehmann et al., 2011; Paz-Ferreiro et al., 2012). Although progress has been made in this area (O'Laughlin and Mcelligott, 2009; Slavich et al., 2013; Woolf et al., 2010), empirical results are inconsistent because of differences in experimental conditions and design (Atkinson et al., 2010), and thus much additional research on BC application is required. Therefore, the purpose of this study was to compare the effects of different amounts of BC on the soil microbial community biomass and composition, arbuscular mycorrhizal (AM) fungi and enzyme activities in fluvo-aquic soil in a field trial, as a means of interpreting in the impact on soil fertility. Under a wheat (*Triticum aestivum*)–maize (*Zea mays*) crop-rotation system, the response of soil nutrients and microorganisms to BC addition was quantified from fresh soil samples collected in 2015, which was the first year after BC addition, and from soil samples collected in 2016 two years after BC addition, to assess the effects at different times after BC application.

2. Materials and methods

2.1. Study site

A field experiment was conducted in October 2015 in fluvo-aquic soil (Calcaric Cambisol, FAO) at the Fertilizer Efficiency Monitoring Network Station, Henan Province, China (34°47′02″N, 113°39′25″E), where wheat–maize rotation is the predominant cropping system. The site has a temperate and monsoonal climate with annual average temperature and precipitation of 14.4 °C and 640 mm, respectively. The parent material of the experimental soil is derived mainly from alluvial deposits of the Yellow River, which is the typical soil on the North China Plain. In the two years prior to the start of the experiment, the field was not fertilized to ensure uniformity. The basic soil physicochemical characteristics were pH 8.17, organic C 5.38 g kg^{-1}, total N 0.71 g kg^{-1}, NO_3^--N 8.58 mg kg^{-1}, NH_4^+-N 1.97 mg kg^{-1}, available P 24.98 mg kg^{-1} and available K 154.65 mg kg^{-1} (0–20 cm depth).

Biochar was purchased from the Xinfa Agricultural Technology Co. Ltd, Henan Province, and was derived from maize straw, produced at 450 °C by slow pyrolysis under anoxic conditions (5 °C min^{-1} heating with 1 h residence time in a pyrolysis furnace). Additional details regarding the BC determination are provided by Wang et al. (2015c). The physicochemical characteristics of the BC were pH 10.62, surface area 4.00 m^2 g^{-1}, pore volume 0.01 mL g^{-1}, organic C 452.00 g kg^{-1} and total N 15.00 g kg^{-1}.

2.2. Experimental design

The experiment comprised winter wheat and summer maize rotations with a randomized block design initiated in October 2014. The four treatments (three replicates per treatment) included a mineral N, phosphorus (P), and potassium (K) fertilizer combination (Control; BC_0), and mineral fertilizers plus maize BC applied separately at 2.5, 7.5, or 22.5 t hm^{-2} (hereafter designated $BC_{2.5}$, $BC_{7.5}$, and $BC_{22.5}$, respectively). The plot size was 20 m^2 (5 m long and 4 m wide). Each plot was separated by a 0.5-m-wide border. The amounts of fertilizer applied in the wheat and maize rotations were identical. Fertilizer N, P, and K were applied in the form of urea (225 kg N hm^{-2} per season), superphosphate (150 kg P_2O_5 hm^{-2} per season), and potassium chloride (90 kg K_2O hm^{-2} per season), respectively. All fertilizer P and K were applied as a basal dressing, whereas half of the fertilizer N was applied as a basal dressing and the other half as a topdressing for both wheat and maize rotations. Before sowing winter wheat, fertilizer was hand-applied to the soil surface on October 9, 2014 and rototilled to a depth of 20 cm. BC was hand-applied to the soil surface of the appropriate plots and the soil was immediately rototilled again to a depth of 20 cm. BC was applied only once in October 2014 and was not applied subsequently. Winter wheat seeds were sown in rows after fertilization and harvested in early June of the following year. After the winter wheat was harvested, maize seeds were sown in holes between the wheat rows, and manuring and sowing simultaneously applied. After the maize was harvested, the same fertilization and tillage methods, including rotary tillage to 20 cm depth, were applied and winter wheat seeds were sown in early October. The aboveground crop straw was completely removed, therefore the amounts of straw and residue remaining in the field were negligible. Wheat 'Zhengmai 7698' and maize 'Jundan 20' were used in the experiment. Field was managed in accordance with local high-yield practices. Detailed information regarding management practices and irrigation is provided by Ai et al. (2018).

2.3. Soil sampling

Soil was sampled twice in early October 2015 and early October 2016. Four soil cores (0–20 cm depth) were collected on each side of the maize row near the center of each plot (to eliminate potential edge effects) as a composite. The samples were stored on ice and transported to the laboratory for analysis. The samples were sieved through a 2.0 mm mesh to remove wheat roots and non-soil materials. Each soil sample was divided into three portions, which were respectively air-dried at room temperature for chemical analyses, stored at 4 °C for extracellular enzyme analysis (performed within 1 week), and stored at −80 °C for phospholipid fatty acid (PLFA) analysis.

2.4. Soil chemical analyses

Soil pH and electrical conductivity (EC) were determined in mixtures with a soil:water ratio of 1:2.5 and 1:5, respectively. Soil total nitrogen (TN) was determined by means of Kjeldahl digestion and soil organic carbon (SOC) was measured using the $K_2Cr_2O_7$ titration method. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted with 0.5 M K_2SO_4 and determined using a total organic C/N analyzer. Available potassium (AK) was extracted with 1 M ammonium acetate and measured by atomic absorption spectrometry. Available phosphorus (AP) was determined by the Olsen method. Soil microbial biomass carbon and nitrogen (MBC and MBN) were fumigated with chloroform and extracted with 0.5 M K_2SO_4, and determined using a total organic C/N analyzer. Additional details regarding the soil chemical analyses were provided by Song et al. (2018).

2.5. Soil enzyme activities

Potential activities of all soil enzymes except urease were analyzed in accordance with the standard fluorescence assays described by Wang et al. (2015b) and Bell et al. (2013). Fluorimetric assays were used to measure the activity of four C-cycling enzymes (α-glucosidase, β-glucosidase, β-D-cellobiosidase, and β-xylosidase), one C- and N-cycling enzyme (*N*-acetyl-β-glucosaminidase; NAG), one N-cycling enzyme (leucine aminopeptidase; LAM), and one P-cycling enzyme (phosphomonoesterase; Pho). In brief, 1 g fresh soil was homogenized in 100 ml of 50 mM acetate buffer using a polytron homogenizer and a uniform suspension was maintained using a magnetic stirrer. The acetate buffer, sample suspension, 10 μM reference compound, 200 μM 4-methylumbelliferyl-linked substrate, and 200 μM 7-amino-4-methylcoumarin substrate were dispensed into wells of black 96-well microplates. A standard curve for each replicate soil sample was prepared by incubating the soil suspension in the presence of increasing concentrations of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) standards. The microplates were covered and incubated for 4 h at 25 °C in the dark. After incubation, 10 μL of 1 M NaOH solution was quickly added to each well of the microplate to stop the enzymatic reaction. Fluorescence was measured using a microplate fluorimeter with 365 nm excitation and 450 nm emission filters. Activities were expressed as nanomoles per hour per gram (nmol $h^{-1} g^{-1}$). Urease activity was determined in accordance with the method of Kandeler and Gerber (1988) and expressed as milligrams ammonium per gram dry soil per hour (mg NH_4^+ g (dry soil) $^{-1} h^{-1}$).

2.6. Phospholipid fatty acid extraction

Phospholipid fatty acids were quantified as a measure of microbial community composition and microbial biomass in accordance with the method described by Wu et al. (2009). Fresh soil samples were freeze-dried and then extracted in a single-phase mixture of chloroform:methanol:citric acid buffer (1:2:0.8, v/v/v; pH 4.0). Neutral lipids and glycolipids were separated from polar lipids by elution with chloroform, acetone, and methanol in a silica-bonded phase column (SPE-Si, Supelco, Poole, UK). The polar lipids were converted to fatty acid methyl esters (FAMEs) by mild alkaline methanolysis after addition of the internal standard methyl dodecanoate (19:0). Nitrogen-dried FAMEs were stored at −80 °C. Dried FAMEs were redissolved in *n*-hexane and then quantified and identified using gas chromatography (N6890, Agilent, Santa Clara, CA, USA) and the MIDI Sherlock microbial identification system version 4.5 (MIDI Inc., Newark, DE, USA), respectively. Concentrations of each PLFA were calculated based on the 19:0 internal standard concentration. The content of individual fatty acids was expressed as nanomoles per gram dry soil (nmol g^{-1} dry soil) and standard nomenclature was used. The abundance of individual PLFAs was represented as the mole percentage (mol %) abundance in each sample.

Phospholipid fatty acids were divided into taxonomic groups in accordance with previously published PLFA biomarkers data (Ai et al., 2015), of which i14:0, i15:0, i16:0, i17:0, a15:0, a17:0, cy17:0, cy19:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω5c, 18:1ω7c, 15:0, and 17:0 PLFAs representing bacteria were present in the samples. Indicators of Gram-positive (G^+) bacteria comprised i14:0, i15:0, a15:0, i16:0, a17:0, and i17:0, Gram-negative (G^-) bacteria biomarkers were cy17:0, cy19:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω5c, and 18:1ω7c, and the PLFAs 15:00 and 17:00 were summed as indicators of other bacteria (Frostegård and Bååth, 1996; Zelles, 1999). The unsaturated PLFAs 18:1ω9c and 18:2ω6,9c were used as fungal biomarkers (Frostegård and Bååth, 1996), and the PLFA 16:1ω5c was regarded as a biomarker of AM fungi (Elzobair et al., 2015; Moeskops et al., 2012). The fatty acids 10Me-16:0, 10Me-17:0, and 10Me-18:0 were used as biomarkers of actinomycetes (Ai et al., 2015). The PLFAs 14:00, 16:00, 18:00, and 16:1 2OH were also included to represent the composition of the microbial community (Cong et al., 2018). In summary, the total PLFA complement of the soil microbial community included all of the above PLFAs. Bacterial stress indices were usually represented by the ratios of cy17:0 to 16:1ω7c, cy19:0 to 18:1ω7c, and total saturated to total monounsaturated fatty acids (sat/mono, 14:00 + 15:00 + 16:00 + 17:00 + 18:00/16:1ω7c + 16:1ω5c + 18:1ω7c + 18:2ω6,9c + 18:1ω9c) (Cong et al., 2018; Fierer et al., 2003; Grogan and Cronan, 1997).

2.7. Statistical analyses

The data were analyzed by one-way ANOVA implemented in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) to test the treatment effects on yield, soil properties, enzyme activities, microbial community composition, and microbial biomass. A least significant difference (LSD; 0.05 level of probability) test was applied to assess the significance of differences between the means. The PLFA profile (percentage of total) was used to represent the soil microbial community composition. To visualize the PLFA profile, principal component analysis (PCA) was performed after standardization of 25 individual PLFAs (mol %) from the PLFA analysis of soil samples. Multi-response blocked permutation (MRBP) tests were used to evaluate the effects of treatments on the PLFA composition. Pearson correlation coefficients were calculated using the PROC CORR procedure in SAS to assess the correlation of PLFAs with community distributions on the first and second principal components (PC1 and PC2) of the PCA, and relationships among microbial biomass parameters, PLFAs (mol %), soil enzyme activities, and soil properties. A redundancy analysis (RDA) with a Monte Carlo permutation test was performed to assess the correlation of microbial biomass with soil physicochemical parameters using CANOCO for Windows version 4.5 (Biometris, Plant Research International, Wageningen, The Netherlands).

3. Results

3.1. Soil physicochemical properties

The effects of BC on soil physicochemical properties after the maize harvests in October 2015 and October 2016 are shown in Table 1. No significant effect of BC addition on soil pH was observed in both years ($p > 0.05$), whereas the contents of SOC, TN, DOC, TDN, MBC, MBN, AP, and AK were significantly influenced by BC addition ($p < 0.05$), which were lowest in the CK treatment for each property. Soil SOC, TN, DOC, TDN, AP, and AK contents increased significantly with increasing rate of BC addition. The soil MBC and MBN contents increased with elevation in the rate of BC addition, peaking in the $BC_{7.5}$ treatment, and thereafter declined in the $BC_{22.5}$ treatment.

3.2. Soil enzyme activities

The potential activities of eight soil enzyme involved in C, N, and P cycling were determined after the maize harvests in October 2015 and October 2016 (Table 2). The activities of all enzymes were significantly affected by soil amendment with BC in 2015 ($p < 0.05$), whereas in 2016 BC addition significantly affected the activities of α-glucosidase, β-glucosidase, β-xylosidase, NAG, LAM, and urease ($p < 0.05$). All BC-amended soils enhanced the activities of soil enzymes compared with those of the NPK treatment in 2015 or 2016. After the maize harvests in October 2015 and October 2016, the activities of soil enzymes involved in C-cycling (α-glucosidase, β-glucosidase, β-xylosidase, and β-cellobiosidase) and NAG all increased with elevation in BC addition rate. In 2015, the activities of Pho, LAM, and urease increased with increasing BC addition rate, with the peak activity observed in the $BC_{7.5}$ treatment, and thereafter declined in the $BC_{22.5}$ treatment; however, in 2016, the activities of the enzymes were elevated with increasing BC addition rate.

After the maize harvest, the ratios of βG:Pho and (NAG + LAM):Pho

Table 1
Physicochemical properties of the studied topsoil (0–20 cm depth) after maize harvest in October 2015 and October 2016, following application of biochar to research plots in October 2014. Values are the mean ± S.D. (n = 3).

Year	Treatment	pH	SOC	TN	DOC	TDN	MBC	MBN	AP	AK
			g/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
October 2015	BC0	8.16 ± 0.01	4.25 ± 0.1c	0.52 ± 0.04b	65.44 ± 1.4b	23.21 ± 0.3b	186.52 ± 16.7b	34.98 ± 1.1c	14.25 ± 3.7c	123.88 ± 13.5d
	BC2.5	8.16 ± 0.06	5.36 ± 1.1bc	0.57 ± 0.13b	69.05 ± 7.1b	23.74 ± 0.1b	209.51 ± 14.6a	42.47 ± 4.6b	15.90 ± 1.9bc	143.35 ± 10.4c
	BC7.5	8.19 ± 0.06	6.81 ± 0.5b	0.66 ± 0.04b	72.09 ± 0.6 ab	24.70 ± 1.2a	229.12 ± 4.3a	53.19 ± 2.6a	20.11 ± 2.9 ab	167.38 ± 2.2b
	BC22.5	8.15 ± 0.00	11.26 ± 1.1a	0.90 ± 0.14a	78.00 ± 3.4a	26.71 ± 1.7a	215.68 ± 7.0a	52.94 ± 2.4a	22.53 ± 2.7a	197.30 ± 3.1a
	Pr > F	NS	**	**	*	*	*	**	*	**
October 2016	BC0	8.39 ± 0.02	6.13 ± 0.6c	0.57 ± 0.07b	68.20 ± 2.8b	3.25 ± 0.4c	190.67 ± 11.6b	41.12 ± 4.2b	13.14 ± 0.3b	115.95 ± 4.4c
	BC2.5	8.43 ± 0.08	6.81 ± 0.5bc	0.61 ± 0.09b	74.64 ± 2.8b	7.47 ± 1.4b	206.39 ± 11.1b	48.17 ± 2.0a	20.12 ± 1.0a	136.80 ± 12.4b
	BC7.5	8.44 ± 0.03	7.47 ± 0.7b	0.67 ± 0.08b	85.91 ± 9.9a	10.19 ± 0.2a	234.02 ± 6.9a	48.13 ± 0.1a	22.05 ± 1.8a	140.95 ± 1.1b
	BC22.5	8.37 ± 0.02	9.66 ± 0.9a	0.88 ± 0.04a	85.59 ± 2.0a	11.12 ± 1.6a	200.27 ± 9.0b	42.11 ± 1.0b	21.35 ± 2.4a	180.50 ± 7.6a
	Pr > F	NS	**	**	**	**	**	*	**	**

Within columns by year, means followed by different lower-case letters between the treatments within a column are significantly different at $P < 0.05$. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.
NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

Table 2
Soil enzyme activities in the studied topsoil (0–20 cm depth) after maize harvest in October 2015 and October 2016, following application of biochar to research plots in October 2014. Values are the means ± S.D. (n = 3).

Year	Treatment	α-glucosidase	β-glucosidase	β-xylosidase	β-cellobiosidase	phosphomonoesterase	N-acetyl-β-glucosaminidase	leucine aminopeptidase	urease
		nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	NH_4^+-mg $g^{-1}d^{-1}$
October 2015	BC0	15.34 ± 1.6c	49.67 ± 3.1b	7.15 ± 0.7b	15.02 ± 1.0b	160.49 ± 0.4c	6.26 ± 0.3d	181.62 ± 6.3c	0.22 ± 0.00c
	BC2.5	16.82 ± 1.5c	54.97 ± 2.4b	8.64 ± 0.1b	14.92 ± 0.4b	174.83 ± 9.4bc	8.85 ± 1.1c	221.21 ± 0.8b	0.28 ± 0.05b
	BC7.5	19.59 ± 1.3b	79.59 ± 5.1a	11.71 ± 0.7a	18.71 ± 1.2a	200.69 ± 15.1a	11.09 ± 0.7b	251.99 ± 10.8a	0.36 ± 0.03a
	BC22.5	24.15 ± 0.3a	82.46 ± 1.7a	12.09 ± 1.6a	19.40 ± 0.2a	184.67 ± 8.6 ab	14.23 ± 1.5a	239.65 ± 4.3a	0.29 ± 0.02b
	Pr > F	**	**	**	**	**	**	**	**
October 2016	BC0	13.93 ± 2.1b	57.89 ± 3.2b	9.70 ± 1.4c	15.74 ± 0.1	163.46 ± 9.6	5.99 ± 0.7b	251.51 ± 19.1c	0.11 ± 0.05c
	BC2.5	16.05 ± 1.3b	61.32 ± 7.5 ab	11.33 ± 1.7bc	16.30 ± 2.3	166.80 ± 11.6	8.35 ± 2.4b	270.23 ± 6.3c	0.16 ± 0.02bc
	BC7.5	16.61 ± 0.3b	69.85 ± 2.9a	12.42 ± 0.9b	17.13 ± 0.6	155.35 ± 0.8	8.23 ± 0.5b	372.86 ± 16.0b	0.20 ± 0.04b
	BC22.5	21.87 ± 1.9a	70.53 ± 6.3a	16.35 ± 0.9a	19.72 ± 2.2	176.54 ± 0.9	11.37 ± 0.6a	414.49 ± 14.5a	0.32 ± 0.06a
	Pr > F	**	*	**	NS	NS	**	**	**

Within columns by year, means followed by different lower-case letters between the treatments within a column are significantly different at $P < 0.05$.
NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

in the BC-amended soils were significantly higher than those in the NPK treatment ($p < 0.05$), and the ratio increased with elevation in BC addition rate (Fig. 1). The changes in soil enzyme activities among the treatments after the maize harvest were analyzed by RDA (Fig. 1). In 2015, the soil MBN ($F = 34.1$, $P = 0.002$) was the most highly significant variable selected by forward selection explaining 77.3% of the variance in enzyme activity, followed by pH (7.8%, $F = 4.8$, $P = 0.014$), TDN (4.6%, $F = 3.6$, $P = 0.022$), and DOC (2.8%, $F = 3.4$, $P = 0.026$) (Fig. 1C). After the maize harvest in October 2016, RDA confirmed that soil TDN ($F = 27.5$, $P = 0.002$) and pH ($F = 7.0$, $P = 0.028$) were significantly correlated with soil enzyme activities and explained 73.3% and 11.7% of the total variability in enzyme activity, respectively (Fig. 1D).

3.3. Microbial community biomass

The microbial biomass of the topsoil (0–20 cm depth) after the maize harvest was higher in all BC-amended soils compared with those of the NPK treatment in 2015 or 2016 (Table 3). In 2015, microbial biomass was significantly affected by BC application ($p < 0.05$). With increasing BC addition rate, the content of total PLFAs, and contents of the PLFAs of bacteria, fungi, actinomycetes, and AM fungi were elevated, with the peak contents observed in the $BC_{7.5}$ treatment, and thereafter declined in the $BC_{22.5}$ treatment. The contents of the PLFAs were significantly increased in the BC-amended soils by 2.9%–135.1% compared with those of the NPK treatment. However, no significant treatment effects were detected in 2016 (Table 3).

Redundancy analysis was performed using the soil physicochemical properties as explanatory variables and the PLFA profiles as response variables. In the RDA of changes in soil microbial PLFA patterns among the treatments after the maize harvest in October 2015 (Fig. 2A), the first and second axes accounted for 98.14% and 0.09%, respectively, of the total variation in microbial community biomass. The results confirmed that soil MBN ($F = 40.1$, $P = 0.002$) and DOC ($F = 16.2$, $P = 0.004$) were significantly correlated with soil microbial community biomass and explained 80.0% and 12.8%, respectively, of the total variability in microbial community biomass. In the RDA of changes in soil microbial PLFA patterns after the maize harvest in October 2016 (Fig. 2B), the first and the second axes accounted for 89.76% and 1.23%, respectively, of the total variation in microbial community biomass. Soil MBN ($F = 6.5$, $P = 0.018$) and DOC ($F = 6.3$, $P = 0.034$) were the most highly significant variables selected by forward selection and explained 89.76% and 1.23%, respectively, of the variance in the PLFA data.

3.4. Microbial community composition

Microbial community composition after maize harvest in October 2015 and October 2016 was differentially affected by the BC treatments (Table 4). In 2015, bacterial stress indices were significantly higher in

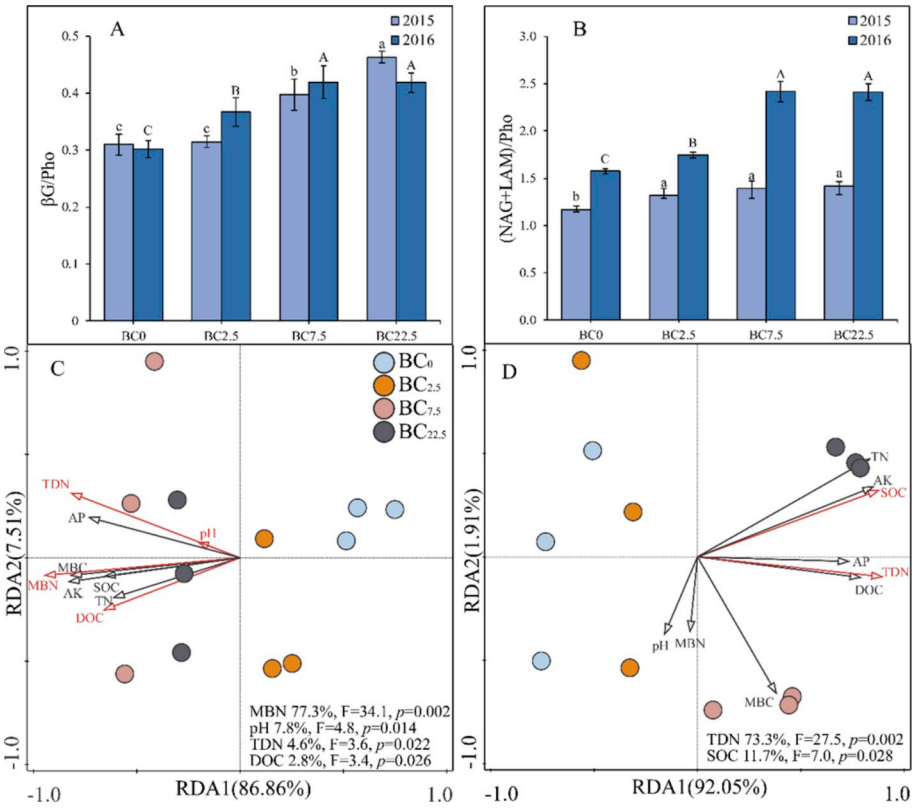

Fig. 1. Stoichiometry of soil microbial enzyme activities, (A) Ratio of β-glucosidase (βG) activity to phosphomonoesterase (Pho) activity; (B) ratio of the sum of *N*-acetyl-β-glucosaminidase (NAG) and leucine aminopeptidase (LAM) activities to Pho activity. Bars labeled with the same letter, within an individual year, are not significantly different ($P > 0.05$). Error bars represent the standard deviation of the mean ($n = 3$). Redundancy analysis of the correlations between soil parameters and enzyme activity after maize harvest in October 2015 (C) and October 2016 (D). The red arrows indicate the soil parameters that were significantly correlated with enzyme activities ($P < 0.05$). The corresponding proportion of the variability explained is shown in the lower right corner. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the BC treatments. The 17:0 cyclo/16:1 ω7c and 19:0 cyclo/18:1 ω7c ratios were significantly higher in the $BC_{2.5}$ and $BC_{22.5}$ treatments than those of the NPK treatment, and were increased by 6.99%–14.9% compared with those of the NPK treatment ($P < 0.05$). The sat/mono ratios increased significantly with increasing BC addition rate ($P < 0.05$). The ratios of G^-/G^+ and fungi/bacteria, and the relative abundance of fungi and AM fungi PLFAs in the BC-amended soils were significantly lower than those in the NPK treatment ($P < 0.05$). The relative abundance of bacterial PLFAs was significantly higher in the BC-amended soils than that in the NPK treatment ($P < 0.05$), and the relative abundance of other bacterial PLFAs was significantly higher in the $BC_{22.5}$ treatment than that of the other treatments. However, after the maize harvest in October 2016, differences in the ratios (except 19:0 cyclo/18:1 ω7c ratio) or relative percentages of soil biomarker PLFAs in the BC treatments were less evident than those observed in 2015.

The changes in PLFA composition of the microbial community in response to BC soil amendment were analyzed by PCA (Fig. 2). In 2015, the PC1 accounted for 37.88% of the total variation (Fig. 2C) and was correlated with indicators of bacteria and fungi (Table 5). The PC2 accounted for 30.59% of the total variation and was correlated with indicators of AM fungi and other bacteria (Table 5). Significant differences in the microbial community composition of the treatments was detected, with good aggregation among repeats along the PC1 axis. The MRBP test also detected the significant influence of BC addition ($F = 13.66$, $p = 0.0031$) on soil microbial community composition. The *t*-tests revealed significant differences between treatments for BC_0 versus the other treatments ($p < 0.05$). In 2016, the difference in soil microbial community composition caused by BC treatments was less evident than that observed in 2015 (Fig. 2D). The MRBP analysis showed that BC addition had no significant effect on soil microbial community composition ($F = 3.34$, $P = 0.0869$), but the latter was significantly correlated with some PLFAs in the PC1 (Table 5). The PC1 accounted for 44.77% of the total variation and was correlated with indicators of bacteria, fungi, and actinomycetes.

4. Discussion

4.1. Biochar effects on soil nutrients

Biochar has a positive effect on soil quality improvement, which is attributed to changes induced in soil physicochemical properties and

Table 3
Responses of the mean concentration of soil biomarker phospholipid fatty acids (PLFAs) to biochar addition in the studied topsoil (0–20 cm depth) after maize harvest in October 2015 and October 2016, following application of biochar to research plots in October 2014.

PLFAs		October 2015				October 2016			
		$BC_{2.5}$	$BC_{7.5}$	$BC_{22.5}$	Pr > F	$BC_{2.5}$	$BC_{7.5}$	$BC_{22.5}$	Pr > F
Total PLFAs	% Change	+29.9	+127.4	+88.4	**	+8.2	+6.3	+5.9	NS
Bacteria		+30.8	+135.1	+94.3	**	+8.3	+7.0	+6.5	NS
Fungi		+4.5	+77.0	+51.1	**	+7.5	+13.4	+2.9	NS
Actinomycetes		+34.5	+114.5	+95.8	**	+8.3	+5.3	+12.0	NS
AM fungi		+2.9	+74.6	+38.9	**	+13.9	+17.9	+9.0	NS

NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

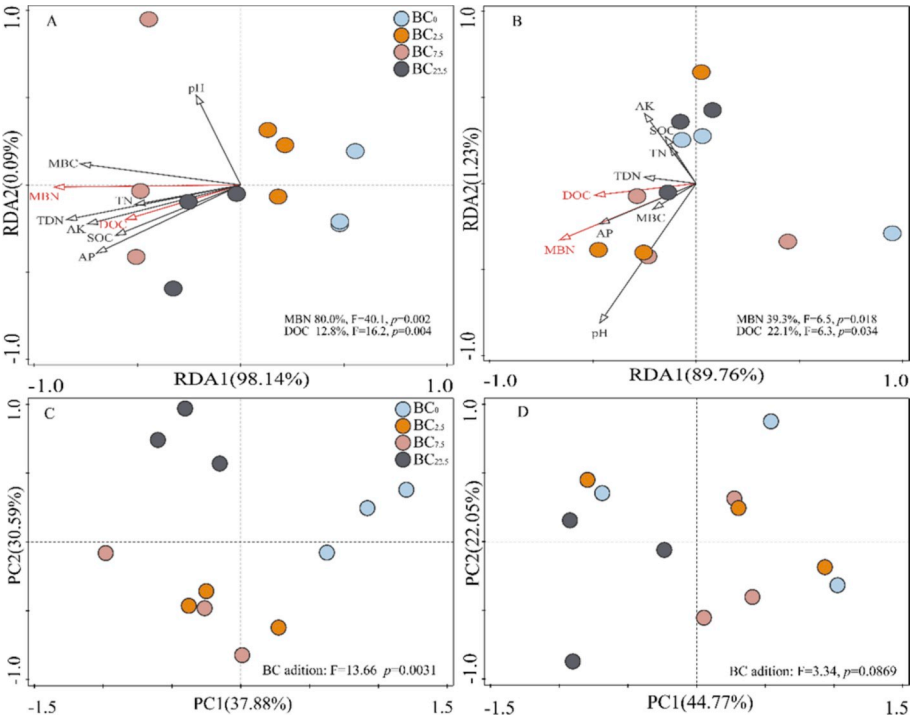

Fig. 2. Redundancy analysis of the correlations between soil parameters and the community biomass of microbial phospholipid fatty acids (PLFAs) after maize harvest in October 2015 (A) and October 2016 (B). The red arrows indicate the soil parameters that were significantly correlated with microbial community biomass ($P < 0.05$). The corresponding proportion of the variability explained is shown in the lower right corner. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. Principle components analysis of the topsoil (0–20 cm depth) microbial community fatty acid methyl esters (PLFAs) after maize harvest in October 2015 (C) and October 2016 (D), following application of biochar to research plots in October 2014 ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

biological functions (Biederman and Harpole, 2013). The present results suggest that BC addition enhances soil quality, as evidenced by increases in the contents of SOC, TN, DOC, TDN, MBC, MBN, and available P and K (Table 1), which is consistent with previous results (Chen et al., 2013; Zhou et al., 2017). However, no significant effect on soil pH with BC addition was observed, in agreement with Elzobair et al. (2015) and Lu et al. (2015), who reported that BC does not affect the pH of an already neutral or alkaline soil. The low variation in soil pH indicates that calcareous soils show a large buffering capacity (Zhu et al., 2017). BC is most effective at changing soil pH in acidic soils, which would be particularly beneficial at low latitudes where soils are acidic (Biederman and Harpole, 2013). Therefore, pH-induced changes to nutrient availability (Rondon et al., 2007), which is often observed after BC is added to acidic soils, are unlikely to be a factor in the current study (Steiner et al., 2007). We consider that the contribution of BC to the improvement of soil nutrient contents is a direct impact, and not a result of indirect effects (Lei et al., 2014; Zheng et al., 2013). Griffin et al. (2017) observed that, although BC caused a slight increase in soil pH, higher concentrations of exchangeable K, calcium, and PO_4-P were most likely due to direct effects rather than to indirect mechanisms. Other researchers have reported that the increases in soil nutrients may be attributed to BC containing labile C, N, P, and K, and the subsequent release of these nutrients into the soil (Lei et al., 2014; Zheng et al., 2013). In addition, BC can adsorb soil organic molecules and promote the polymerization of small organic molecules through surface catalytic activity to improve the soil SOC content (Liang et al., 2010).

4.2. Biochar effects on soil enzyme activities

Soil enzyme activity has been identified as a key indicator of microbial function in nutrient retention and conversion associated with soil fertility and quality (Tripathi et al., 2007), mainly as urease and hydrolase were known (GarcíA-Gil et al., 2000). Previous studies have revealed considerable uncertainty in the effect of BC addition on the activity of soil hydrolases involved in the C cycle (Bailey et al., 2011; Elzobair et al., 2015). It is generally reported that the addition of BC reduces soil enzyme activities associated with ecological processes, such as synthetic C mineralization (Lehmann et al., 2011). BC has the capacity to adsorb a variety of organic and inorganic molecules and inhibits some soil enzymes or their substrates by adsorbing or blocking the reaction sites (Bailey et al., 2011; Lehmann et al., 2011). This response did not occur in the present study, which revealed that BC addition to the soil resulted in increased activities of a suite of enzymes associated with C and N utilization (Table 2), in agreement with the findings of Bailey et al. (2011) and Ameloot et al. (2013). Bailey et al. (2011) suggested that volatile compounds in BC stimulate enzyme activity in sandy loam, including dehydrogenase and β-glucosidase activities, but this mechanism was not investigated in the present study. BC may also adsorb toxic substances in the soil, thereby increasing soil enzyme activity (Lammirato et al., 2011). However, no indication of toxicity was detected in the soil of the present study. The shift in soil enzyme activity in response to BC also may be a result of soil property changes (Lehmann et al., 2011; Liang et al., 2005); the significant correlation of soil enzyme activities with soil nutrient contents in the present experiment may indirectly support this suggestion (Fig. 1). Therefore, the higher activities of soil enzymes caused by the addition of BC may reflect the contribution of BC to the improvement of soil nutrient availability and physicochemical interaction with extracellular soil enzymes (Elzobair et al., 2015; Lu et al., 2015).

The relative abundance of enzymes involved in the C, N, and P cycles reflects the biogeochemical balance between microbial biomass stoichiometry and organic matter elemental composition (Sinsabaugh and Shah, 2012; Waring et al., 2014). The average (NAG + LAM):Pho ratio (1.2–2.4) of the BC treatments in the present study (Fig. 1) was significantly higher than the global average (0.44) reported by Sinsabaugh et al. (2010a). In contrast, the β-glucosidase:Pho ratios observed in the current experiments were 0.03–0.46, which were considerably lower than the 'global' soil β-glucosidase:Pho ratio of 0.62 ± 0.04 (Sinsabaugh et al., 2010b). We speculate that variation in β-glucosidase:Pho and (NAG + LAM):Pho ratios among ecosystems may be influenced by soil nutrient availability and climatic conditions (Waring et al., 2014).

4.3. Biochar effects on soil microbial biomass and microbial community composition

Changes in soil microbial biomass and microbial community composition are responses to environmental changes and soil nutrient

Table 4
Ratio or relative percentage of soil biomarker phospholipid fatty acids (PLFAs) after maize harvest in October 2015 and October 2016, following application of biochar to research plots in October 2014. Values are the mean ± S.D. (n = 3).

Year	Treatment	Ratio of soil biomarker PLFAs				The relative abundance PLFAs (mol %)				
		17:0 cyclo/16:1 w7c	19:0 cyclo/18:1 w7c	Sat/mono	Fungi/bacteria	G-/G+	Bacteria	Fungi	Actinomycetes	AM fungi	Other Bacteria
October 2015	BC0	0.46 ± 0.01 b	0.69 ± 0.02 c	0.44 ± 0.02 b	0.24 ± 0.02 a	1.69 ± 0.04 a	54.50 ± 0.3 b	12.95 ± 0.9 a	13.29 ± 0.9	4.98 ± 0.2 a	1.44 ± 0.05 b
	BC2.5	0.49 ± 0.01 a	0.80 ± 0.01 a	0.49 ± 0.04 b	0.18 ± 0.01 b	1.50 ± 0.04 c	54.85 ± 0.9 b	9.72 ± 0.7 b	13.30 ± 0.2	4.73 ± 0.1 ab	0.64 ± 0.03 c
	BC7.5	0.46 ± 0.01 b	0.70 ± 0.01 c	0.53 ± 0.01 a	0.18 ± 0.01 b	1.60 ± 0.02 b	56.34 ± 0.7 a	10.04 ± 0.3 b	13.06 ± 0.6	4.96 ± 0.4 a	0.69 ± 0.05 c
	BC22.5	0.50 ± 0.02 a	0.75 ± 0.02 b	0.54 ± 0.01 a	0.18 ± 0.01 b	1.59 ± 0.07 b	56.20 ± 0.2 a	10.37 ± 0.3 b	13.24 ± 0.4	4.35 ± 0.2 b	1.63 ± 0.07 a
	Pr > F	**	**	**	**	**	*	**	NS	*	**
October 2016	BC0	0.49 ± 0.01	0.64 ± 0.03 b	0.52 ± 0.05	0.23 ± 0.03	1.68 ± 0.08	51.69 ± 0.7	12.10 ± 1.6	12.53 ± 0.5	4.85 ± 0.1	0.69 ± 0.04
	BC2.5	0.48 ± 0.01	0.64 ± 0.03 b	0.50 ± 0.01	0.23 ± 0.03	1.63 ± 0.04	51.79 ± 2.1	11.61 ± 0.9	12.58 ± 0.4	5.08 ± 0.5	0.68 ± 0.01
	BC7.5	0.48 ± 0.01	0.65 ± 0.03 b	0.49 ± 0.01	0.23 ± 0.01	1.72 ± 0.04	51.94 ± 0.03	11.99 ± 0.2	12.61 ± 0.3	5.34 ± 0.1	0.66 ± 0.04
	BC22.5	0.47 ± 0.02	0.73 ± 0.01 a	0.54 ± 0.02	0.20 ± 0.02	1.53 ± 0.11	52.17 ± 0.03	10.64 ± 0.7	13.37 ± 0.3	5.08 ± 0.2	0.66 ± 0.04
	Pr > F	NS	**	NS	NS	NS	NS	NS	NS	NS	NS

Within columns by year, means followed by different lower-case letters between the treatments within a column are significantly different at $P < 0.05$. Sat/mono, ratio of total saturated to total monounsaturated fatty acids; G-/G+, the ratio of Gram-negative to Gram-positive bacteria.
NS, * and ** indicate ANOVA results of $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

Table 5
Pearson's correlation coefficients between soil phospholipid fatty acid (PLFA) variables with community positions associated with principal component (PC) 1 and 2 after maize harvest in October 2015 and October 2016, following application of biochar to research plots in October 2014 (n = 12).

Variable	2015		2016	
	PC1	PC2	PC1	PC2
% Bacteria	−0.828**	0.259	0.689*	0.309
% Fungi	0.800**	0.354	−0.962**	−0.043
% Actinomycete	0.424	0.073	0.882**	−0.225
% AM fungi	0.541	−0.706**	−0.212	−0.299
% Other Bacteria	0.309	0.913**	−0.129	0.930**

* and ** indicate of $P < 0.05$ and $P < 0.01$, respectively.

quality (Cong et al., 2018). The effects of BC addition to the soil on microbial biomass and soil microbial abundance are often reported (Elzobair et al., 2015; Gomez et al., 2014). Gomez et al. (2014) and Dempster et al. (2012) observed that the microbial biomass of BC-amended soil was significantly reduced, which they consider was mainly caused by the sorption of BC. However, in the present study BC addition to the soil stimulated the activity of soil microorganisms, as evidenced by increases in microbial biomass (Table 3), in agreement with the findings of Lehmann et al. (2011) and Khadem and Raiesi (2017). In the majority of studies, microbial biomass increases in response to BC addition and microbial community composition is changed significantly (Jones et al., 2012; Lehmann et al., 2011). These responses may be because BC benefits soil microbial biomass and microbial communities by providing suitable habitat for microbial growth and protection from predators (Pietikäinen et al., 2000; Quilliam et al., 2013), production of irritating molecules to stimulate microbial growth (Bamminger et al., 2014), absorbance of toxic substances to prevent microbial poisoning (Gomez et al., 2014; Kasozi et al., 2010; Kolb et al., 2009), enhancement of soil physicochemical properties (Jindo et al., 2012), enhanced availability of soil nutrients (Atkinson et al., 2010), or provision of a C substrate for degradation (Khadem and Raiesi, 2017; Smith et al., 2010). The current results showed that in the first year after BC addition, a significant impact on microbial biomass and community composition was observed, whereas the impact in the second year was not significant, which was similar to the results of Elzobair et al. (2015) and Jones et al. (2012). The RDA analysis in the present study confirmed that DOC and MBN were dominant factors affecting soil microbial biomass (Fig. 2). Wang et al. (2015a) and Biederman and Harpole (2013) consider that the labile portion of BC, which is typically 3% of the total mass, is available for mineralization by soil microbes and, on average, a small increase in soil microbial biomass is observed following BC application. Therefore, in the present study, we consider that DOC played a leading role of microbial biomass changes caused by BC addition (Khadem and Raiesi, 2017; Smith et al., 2010). The present results showed that BC did not have a significant effect on soil microbial community composition with greater duration after BC addition (2 years) (Fig. 2). Other authors have observed no effect of BC on microbial communities when the BC addition does not affect the pH of an already neutral or alkaline soil (Elzobair et al., 2015; Paola et al., 2012). Elzobair et al. (2015) and Domene et al. (2014) noted that BC had no effect on soil microbial biomass and microbial community composition compared with that of control soils, measured 4 and 3 years after addition, respectively. These authors believed that the high variability among repetitions may prevent detection of significant BC effects; however, the high variability among repetitions in the current study was not as great as that of the above-mentioned studies. Thus, the inconsistent effects of BC on microbial biomass and microbial communities suggests that the effects of BC may reflect change in the physicochemical properties of the soil and BC, associated with the timing of application and the rate applied to the soil (Elzobair et al.,

2015; Jindo et al., 2012; Jones et al., 2012).

The most suitable habitats differ among microbial groups, and BC addition to a specific soil environment tends to preferentially change the biomass and abundance of certain or several types of microorganisms (Khadem and Raiesi, 2017; Steinbeiss et al., 2009). In the present study BC addition changed the soil microbial community composition, and especially the relative abundance of bacteria increased and the relative abundance of fungi and AM fungi decreased one year after BC addition (Table 4). These results are in agreement with the findings of Khadem and Raiesi (2017) and Ippolito et al. (2014), who concluded that BC may provide an unstable C substrate that favors fast-growing bacteria rather than fungi. Turlapati et al. (2013) consider that the increased content of soil available N may reduce the allocation of plant C to fine roots, resulting in reduced fungal colonization of roots and abundance of fungi. Soil amendment with BC also increased the available P content in the soil, which explains the negative impacts of BC on the relative abundance of AM fungi one year after BC addition. When P and other nutrients are abundant in the soil, plants rely less on AM fungi to obtain nutrients and the abundance of AM fungi in soil is reduced (Covacevich et al., 2006; Elzobair et al., 2015). In addition, we observed higher stress indices in the BC-amended plots one year after BC addition (Table 4), which indicated that microbes in BC-amended plots suffered from intensified environmental stresses. The significant reduction in the G^-/G^+ ratio under BC addition may support the hypothesis of stress-based community shifts, because G^+ bacteria are more stress-tolerant than G^- bacteria and enhanced environmental stress inhibits G^- bacteria, but may have little effect on G^+ bacteria (Cong et al., 2018; Silhavy et al., 2010). In addition, we noted that BC significantly increased the relative abundance of other bacteria in the $BC_{22.5}$ treatment, which may indicate that certain bacteria are highly adapted to BC-induced environmental stress in calcareous soils. The PCA was able to distinguish between control and BC-treated samples (Fig. 2C). The PCA plot suggested that the microbial community composition was significantly affected by BC in the short term (Zhu et al., 2017).

5. Conclusions

In a 2-year field experiment, our results indicate that the changes in SOC, TN, DOC, TDN, available P and K depended mainly on the BC addition rate and increased with the increase of BC addition rate, but the soil pH did not significantly change. The higher amount of BC promoted the activities of enzymes involved in C and N cycling. BC amendment increased microbial biomass, redundancy analysis confirmed that DOC and MBN were dominant factors affecting soil microbial biomass. Our results also suggest that changes in soil community composition significantly affected by time since BC application. Principal component analysis revealed that differences in microbial community composition in one years after BC addition, but which had no significant influence after two years. Further study is needed to illustrate the potential mechanism of change in soil microbial community composition to several years (> 2 years) since BC application in the calcareous soil, and determine the optimal rate of BC application.

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