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Responses of soil nutrients and microbial activities to additions of maize straw biochar and chemical fertilization in a calcareous soil

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ABSTRACT

Biochar addition to soil has been proposed as a strategy to enhance soil quality and crop productivity. However, little is known about responses of soil nutrients and microbial activities to additions of chemical fertilizer and biochar with different pyrolysis temperatures. To investigate the effects of control (CK), chemical fertilizer (NPK), and NPK with maize straw biochar (MC) produced at 300, 450, and 600 °C (NPK + MC300, NPK + MC450, and NPK + MC600) on crop yield, soil nutrients, soil enzyme activities, and microbial attributes in a calcareous soil, we conducted a pot experiment. The results showed that the NPK + MC450 treatment obtained the highest wheat yield and N, P, and K uptakes. The NPK + MC300 and NPK + MC450 treatments decreased significantly the soil available K content and increased the C/N ratio, contents of soil organic carbon (SOC) and available P compared to the NPK + MC600 treatment. The NPK + MC450 treatment promoted the increases in soil C- and N-cycling enzyme activities. The total N content, soil MBC and MBN were the main driving factors affecting soil enzyme activities. All the NPK plus MC soils significantly reduced the relative abundance of soil fungi and enhanced soil nutrient contents (excluding soil inorganic nitrogen) and total phospholipid fatty acid concentrations. A redundancy analysis revealed that the changes in soil microbial community depended mainly on the contents of MBC, MBN and available K as well as the C/N ratio. This study provides clear evidence that the co-application of NPK fertilizers and MC produced at 450 °C was more efficient for improving soil quality and potential crop productivity.

1. Introduction

Biochar (BC) is produced by the pyrolysis of organic biomass under relatively low temperature (< 700 °C) and oxygen-limited conditions. Biochar contains large amounts of carbon and macro or micro-nutrients depending on the feedstock and pyrolysis temperature [1]. Some studies have reported that BC as a soil amendment has considerable potential for enhancing soil fertility and crop productivity [2]. The enhancement of soil fertility as a result of BC addition has been attributed to increased soil electrical conductivity (EC), soil organic carbon (SOC), and the soil holding capacity of nitrogen (N), phosphorus (P), and potassium (K), changes to soil pH, or direct nutrient contributions from the BC [1,3,4]. However, other studies have shown either negative effects or no effect of BC on soil fertility parameters and C storage potential, such as short-term reductions in soil mineral N availability [5,6] and decreased performance of crops on calcareous soils [7]. Therefore, the effects of BC on soil quality and nutrient cycling are uncertain.

Soil microbes play very important roles in soil organic matter (SOM) decomposition, nutrient cycling, and other relevant functions [8]. Soil microbial biomass C and N (MBC and MBN) and enzyme activities are related to soil fertility and agricultural productivity [9,10]. Nevertheless, microbial responses to BC addition are uncertain about both the nature of BC and experimental conditions. The meta-analysis of Zhou et al. [11] showed that BC amendments to soil increased MBC by 26% and MBN by 21% for the 413 and 106 pairs of data reported, respectively. Interestingly, the laboratory incubation, pot and field experiments showed that BC addition could increase soil MBC content. Soil MBN increased significantly only in incubation studies (mean: 42%), but did not differ significantly from controls in pot or field studies. Whereas, the divergent change in MBN across the experimental types

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could be attributed to N competition by plants in the pot and field trials [12]. Understanding the effect of BC on the soil enzyme activities is a research priority. Some studies reported that BC 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 [13,14]. Conversely, other studies have found inconsistent results [15,16], which suggest that BC has variable effects on different soils and enzymes.

Soil microbial community abundance and structure are used widely to indicate soil quality changes [17]. BC addition to soil may change the soil microbial community composition and functional groups. Some studies suggest that BC addition to soil may stimulate the activity of soil microorganisms, such as Gram-positive (G^+) bacteria [14], Gram-negative (G^-) bacteria [18,19] and fungi on short timescales [20,21]. However, other studies have found that BC addition to soil has no [22,23], or in some instances even negative [24,25] effects on soil microbial properties. These contradictory results are primarily due to differences in soil type, BC sources, production conditions (pyrolysis temperature and duration), the application rate and time durations used in different studies [16,26].

Generally, understanding BC effects on soil microbial properties is receiving more attention because these soil properties are usually considered to be sensitive indicators of soil quality and function [17,27]. However, the long- and short-term responses of microbial attributes to BC addition are uncertain to some extent and cannot be generalized widely regarding the practical application of BC to different soil types [24,28] This is especially true in calcareous soils of arid regions with low SOM content and water availability [29,30] Therefore, we require a more complete understanding of the effects of different BC production conditions on microbial activity and subsequent nutrient cycling and plant responses in agricultural soils. Our aim was to quantify the responses of soil nutrients, enzyme activities and microbial community composition to combined application of maize straw biochar (MC) and chemical fertilizer in a calcareous soil, and to illustrate the main environmental factors that drive the changes in soil enzyme activities or microbial community composition. Our hypothesis was that MC addition to soil would stimulate soil microbial properties, and the stimulating effects of MC would vary with MC pyrolysis temperatures.

2. Materials and methods

2.1. Soil and biochar

Samples of calcareous soils were obtained from 0 to 20 cm depth in arable fields at the Soil Fertility and Fertilizer Efficiency Monitoring Network Station, Henan Province, China (34°47′02″N, 113°39′25″E), with the soil parent material originating mainly from the alluvial deposits of the Yellow River. The soil samples were naturally air-dried for one week in the room temperature, and filtered through a 2 mm sieve. The basic soil physicochemical characteristics were determined and presented in Table 1.

Maize straw was collected from a maize field at the Soil Fertility and Fertilizer Efficiency Monitoring Network Station, Zhengzhou, Henan Province, China. Maize straw biochars (MCs) were produced at 300, 450 and 600 °C by slow pyrolysis (5 °C min^{-1} heating with a 1 h residence time in a microwave muffle furnace (SX2, Shanghai Rongfeng Scientific Instrument Inc., Shang hai, China)), which were identified as MC300, MC450 and MC600, respectively. All the MC samples were homogenized, ground, and sieved to < 0.154 mm. The physicochemical characteristics of these MCs were measured as described by Wang et al. [31] and shown in Table 1.

2.2. Pot experiment

The study was conducted in a greenhouse at the Chinese Academy of Agricultural Sciences, in October 2014. The five treatments were control (CK), chemical fertilizer (nitrogen, phosphorous, and potassium or N, P, K), NPK + MC300, NPK + MC450, and NPK + MC600. The pot experiment was arranged in a randomized block design with three replicates. Initially, 5.0 kg of air-dried soil was weighed into a plastic pot (top diameter, 21.5 cm; bottom diameter, 13 cm; depth, 13 cm). Samples of MC300, MC450, and MC600 were all added at 1% by weight to the soil and mixed thoroughly. The N, P and K fertilizers used were $(NH_4)_2SO_4$, $Ca(H_2PO_4)_2$, and KCl, respectively, which were added at the rates of 0.10 g N kg^{-1}, 0.05 g P_2O_5 kg^{-1}, and 0.04 g K_2O kg^{-1} (NPK). All the P fertilizer and one-half of the N and K fertilizers were applied as basal fertilizers, while the rest of the N and K fertilizers were applied evenly as topdressing at the elongating stage. The wheat cultivar "Zhengmai 7698" was used. Twenty wheat seeds were sown in each pot in October 2014, and 15 seedlings were retained in each pot after their emergence. The soil moisture content was adjusted to approximately 60% of the water-holding capacity, and it was readjusted by adding deionized water during winter wheat growth. The wheat was harvested at the maturity stage in June 2015. Soil and plant samples were collected at wheat harvest. Each soil sample was divided into two parts. One part was dried at room temperature, crushed and sieved through a 2.0 mm mesh for chemical analysis; the other part was preserved at 4 °C for enzymatic analysis, and at −80 °C for a phospholipid fatty acid (PLFA) analysis. The aboveground biomass was dried in an oven at 65 °C to constant weight, and the wheat yield and N, P, and K uptakes were measured.

Table 1
The physical and chemical properties of the experimental soil and biochars.

Variables	Unit	Biochar pyrolysis temperature (°C)			Soil
		MC300	MC450	MC600	
pH	/	9.84	10.47	11.37	7.97
EC	mS m^{-1}	340	537	509	46.64
Yield	%	43.63	32.61	29.54	/
Ash content	%	16.34	22.28	27.16	/
Surface area	m^2 g^{-1}	1.00	4.00	70.00	/
Pore volume	mL g^{-1}	0.01	0.01	0.06	/
Organic C	g kg^{-1}	489.03	538.12	629.03	5.3
Total N	g kg^{-1}	12.46	12.20	12.05	0.7
C/N ratio	/	39.25	44.11	52.20	6.46
Available P	g kg^{-1}	0.57	0.62	0.59	0.02
Available K	g kg^{-1}	33.37	66.07	54.93	0.11

Abbreviations: MC300, MC450 and MC600, maize straw biochars that were produced at 300, 450 and 600 °C, respectively. EC, electrical conductivity; "/" not measured.

2.3. Chemical analysis

Soil pH was measured with a compound electrode (PE 10, Sartorius, Goettingen, Germany) using a soil to water ratio of 1:2.5. Soil EC was determined in 1:5 (w/v; g cm^{-3}) soil-water mixtures. SOC was determined by the $K_2Cr_2O_7$ titration method. Soil total N (TN) was determined using the Kjeldahl method [32]. Dissolved organic C (DOC) was extracted with 0.5 M K_2SO_4 and determined by a total organic C/N analyzer (Multi N/C 3100/HT1300, Analytik Jena AG, Germany). Soil inorganic N (SIN) was extracted with 0.01 M $CaCl_2$ and determined by a flow injection analysis (FLA star 5000 Analyzer, Foss, Denmark). MBC and MBN were determined using the chloroform fumigation-extraction protocol. The portion of MBC and MBN were extracted with 0.5 M K_2SO_4 and determined by a total organic C/N analyzer (Multi N/C 3100/HT1300, Analytik Jena AG), the value to calculate biomass from the C and N determinations (K_{EC} and K_{EN}) was 0.45 and 0.38 [33]. Soil available P was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and determined by the Olsen method [34]. Soil available K was extracted with 1 M ammonium acetate, adjusted to pH 7.0, and then measured by atomic absorption spectrometry (NovAA300, Analytik Jena AG). The contents of total N, P and K in the wheat were digested with H_2SO_4-H_2O_2 [35]

Table 2
Effects of different treatments on wheat N, P, K uptakes and yield.

Treatment	N uptake ($g\ pot^{-1}$)	P uptake ($g\ pot^{-1}$)	K uptake ($g\ pot^{-1}$)	Yield ($g\ pot^{-1}$)
CK	0.22 ± 0.01 d	0.015 ± 0.002 c	0.77 ± 0.02 c	8.04 ± 0.39 b
NPK	0.40 ± 0.02 c	0.035 ± 0.004 b	0.85 ± 0.08 c	8.58 ± 0.21 b
NPK + MC300	0.45 ± 0.01 b	0.044 ± 0.002 a	0.95 ± 0.03 bc	10.33 ± 0.42 a
NPK + MC450	0.49 ± 0.01 a	0.045 ± 0.002 a	1.06 ± 0.03 a	11.48 ± 0.57 a
NPK + MC600	0.44 ± 0.01 b	0.041 ± 0.002 a	1.03 ± 0.06 ab	10.94 ± 1.03 a

Data are means ± standard deviation, $n = 3$. Different lowercase letters within a column indicate significant differences among the treatments at $P < 0.05$ (Fisher's LSD test).

and determined using the Kjeldahl method, vanadium molybdenum yellow spectrophotometry, and atomic absorption spectrometry (NovAA300, Analytik Jena, AG), respectively [36].

2.4. Enzyme activity

The analyzed enzymes included three C-cycling enzymes (β-glucosidase, β-D-cellobiosidase and β-xylosidase), one C- and N- cycling enzyme (N-acetyl-b-glucosaminidase), and two N-cycling enzymes (urease and leucine aminopeptidase), and ones P-cycling enzyme (phosphomonoesterase). The potential activities of all the enzymes (except urease) were quantified according to fluorescence-based protocols as described in Wang et al. [31] and Bell et al. [37], which were expressed in units of nmol h^{-1} g^{-1}. Briefly, 1 g of fresh soil was homogenized in 100-mL sterilized water using a polytron homogenizer, and then a magnetic stirrer was used to maintain a uniform suspension. The sterilized water, sample suspension, 10 μM references, and 200 μM 4-methylumbelliferyl-linked substrates were dispensed into the wells of a black 96-well microplate. The microplates were covered and incubated in the dark at 25 °C for 4 h. After incubation, 10 μL of a 1 M NaOH solution was added rapidly to each well of the microplate to stop the enzymatic reaction. Fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo Fisher Scientific, Waltham, MA, USA) with 365 nm excitation and 450 nm emission filters. Urease activity was determined according to Kandeler and Gerber [38] and expressed as mg NH_4^+ g (dry soil)$^{-1}$ h^{-1}.

2.5. PLFA analysis

The soil microbial community composition and microbial biomass were determined by a PLFA analysis according to the procedure described by Wu et al. [39]. Briefly, the soil samples were freeze-dried, and then PLFAs were extracted with a single-phase mixture of chloroform: methanol: citrate buffer (1:2:0.8 volumetric 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. Nonadecanoic acid methyl ester (19:0) was added as the internal standard, and the polar lipids were converted to fatty acid methyl esters by a mild alkaline methanolysis. Dried fatty acid methyl esters were redissolved in n-hexane and then quantified and identified by gas chromatography (N6890, Agilent Technologies, Santa Clara, CA, USA) 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. The abundance of individual PLFAs was indicated by their % mol abundance in each sample.

PLFAs were divided into various taxonomic groups based on previously published PLFA biomarker data [40]. Specifically, we used i14:0, i15:0, i16:0, i17:0, a15:0, and a17:0 as G^+ bacteria biomarkers, cy17:0, cy19:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω5c, and 18:1ω7c as G^- bacteria biomarkers, and the sum of G^+ and G^- bacteria biomarkers together with 15:0, 17:0, 17:1ω6, and 17:1ω7 as a measure of the total bacterial biomass. The unsaturated PLFAs 16:1ω5c, 18:2ω6, 9, 18:1ω9 and 18:3ω3 were used as fungal biomarkers. The fatty acids 10Me-16:0, 10Me-17:0, and 10Me-18:0 were used as biomarkers of actinomycetes.

2.6. Statistical analyses

The data collected were analyzed by one-way ANOVA with SAS version 8.0 (SAS Institute, Inc., Cary, NC, USA). A least significant difference (LSD; at 0.05 level of probability) test was applied to assess the differences between the means. Standard deviations were computed by root mean square errors. A principal component analysis (PCA) was conducted with Canoco for Windows version 4.5, and figures were drawn by Adobe Illustrator CS4. A redundancy analysis (RDA) with a Monte Carlo permutation test was performed to assess whether the soil enzyme activities or microbial community composition correlated with the soil physicochemical parameters, as implemented by Canoco for Windows version 4.5. Some other figures were generated using MS Excel 2010.

3. Results

3.1. Wheat yield and total N, P, and K uptakes

Wheat yield and total N, P, and K uptakes were lowest in the CK treatment, which in all the MC-amended soils were increased significantly by 10.6–24.2%, 14.7–24.1%, 12.0–24.8% and 20.3–33.7%, respectively, compared with those of the NPK treatment (Table 2) ($P < 0.05$). In all the MC-amended soils, the wheat yield was highest in the NPK + MC450 treatment, but it did not differ significantly among the different MC pyrolysis temperatures ($P > 0.05$). N uptake by the wheat was significantly higher in the NPK + MC450 treatment than the NPK + MC300 and NPK + MC600 treatments, while there was no significant difference between the NPK + MC300 and NPK + MC600 treatments (Table 2). The highest P and K uptakes by the wheat were all observed in the NPK + MC450 treatment, while there was little variation among the different MC pyrolysis temperatures.

3.2. Changes in soil properties

After the wheat harvest, the soil pH values in all the NPK-amended treatments were lower than that in the CK treatment. All MC-amended treatments significantly increased soil pH values by 0.03–0.12 units compared with that of the NPK treatment ($P < 0.05$). The EC, TN, SOC, DOC, SIN and available P and K contents in the CK treatment were lowest, but the EC, TN, SOC, C/N ratio, and available P and K contents were significantly increased in all the MC-treated soils by 3.80–4.61%, 32.09–35.73%, 188.34–260.06%, 118.32–172.09%, 19.57–29.09% and 280.04–343.31%, respectively, compared with those of the NPK-treated soil (Table 3). In contrast, the SIN content was highest in the NPK treatment and decreased by 7.04–55.31% upon MC addition. For all the MC-amended treatments, the contents of soil TN and available P showed increasing and then decreasing trends with increasing pyrolysis temperatures; the pH values and SIN content increased significantly

Table 3
Effects of different treatments on soil physicochemical characteristics after wheat harvest.

Treatment	pH	EC	SOC	TN	C/N	DOC	SIN	AP	AK
		(mS m^{-1})	(g kg^{-1})	(g kg^{-1})		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
CK	8.20 ± 0.02 a	28.63 ± 0.23 c	4.24 ± 0.07 c	0.59 ± 0.00 d	7.17 ± 0.15 c	63.05 ± 3.65 c	6.56 ± 1.26 e	19.94 ± 0.95 d	91.14 ± 1.52 d
NPK	8.06 ± 0.01 d	45.57 ± 1.25 b	4.39 ± 0.07 c	0.62 ± 0.01 c	7.03 ± 0.11 c	66.38 ± 2.57 bc	30.27 ± 0.35 a	32.78 ± 0.46 c	128.73 ± 4.49 c
NPK + MC300	8.09 ± 0.02 c	47.30 ± 0.90 a	15.80 ± 0.15 a	0.83 ± 0.02 b	19.14 ± 0.33 a	74.84 ± 2.95 a	13.53 ± 1.37 d	41.31 ± 1.48 a	497.84 ± 9.80 b
NPK + MC450	8.14 ± 0.02 b	47.67 ± 0.81 a	15.74 ± 0.44 a	0.85 ± 0.00 a	18.59 ± 0.52 a	71.13 ± 3.24 ab	25.23 ± 1.29 c	42.32 ± 0.40 a	489.23 ± 0.89 b
NPK + MC600	8.18 ± 0.00 a	47.50 ± 0.62 a	12.65 ± 0.33 b	0.82 ± 0.01 b	15.36 ± 0.57 b	67.57 ± 3.53 bc	28.14 ± 0.35 b	39.19 ± 0.64 b	570.69 ± 3.51 a

Data are means ± standard deviation, $n = 3$. Different lowercase letters within a column indicate significant differences among the treatments at $P < 0.05$ (Fisher's LSD test).; *Abbreviations*: EC, electrical conductivity; SOC, soil organic carbon; TN, total nitrogen; DOC dissolved organic carbon; SIN, soil inorganic nitrogen.

with increasing MC pyrolysis temperatures ($P < 0.05$), while SOC, DOC and C/N ratio tended to decrease. Soil EC did not differ significantly among the different pyrolysis temperatures.

After the wheat harvest, the MC addition to soil had significant effects on soil MBC and MBN. All the MC-amended treatments increased significantly both the soil MBC by 20.68–46.00% and MBN by 189.95–233.11% compared with those of the NPK treatment (Fig. 1a and b). The soil MBC and MBN were significantly higher in the NPK + MC300 treatment than those of the NPK + MC450 and NPK + MC600 treatments, while it did not differ significantly between the NPK + MC450 and NPK + MC600 treatments. The MBC/MBN ratio in the soil was highest in the CK treatment, while there was no significant difference between the CK and NPK treatments. The MBC/MBN ratio in all the MC-amended treatments were significantly decreased by 56.17–58.50% compared with that in the NPK treatment (Fig. 1c). The MBC/MBN ratio did not differ significantly (mean: 5.05) among the different MC pyrolysis temperatures.

3.3. Changes in soil enzyme activities

The potential activities of seven soil enzymes involved in C, N, and P cycling were determined after the wheat harvest (Fig. 2), the activities of soil enzymes were lowest in the CK. All the MC-amended soils enhanced the activities of soil enzymes involved in C cycling compared with those of the CK and NPK treatments. The activities of β-glucosidase, β-cellobiosidase, β-xylosidase and N-acetyl-glucosaminidase in the NPK + MC300 and NPK + MC450 treatments increased significantly by 23.26–27.54%, 31.48–43.49%, 11.01%–15.89% and 15.23–21.90%, respectively, compared with those of the NPK treatment ($P < 0.05$), while there were no significant difference between the NPK and NPK + MC600 treatments (Fig. 2a–d). The highest β-glucosidase and β-cellobiosidase activities were observed in the NPK + MC450 treatment, while the activities of β-xylosidase and N-acetyl-glucosaminidase showed a decreasing trend with increasing MC pyrolysis temperatures. All the MC-amended treatments increased significantly the activities of soil enzymes involved in N cycling by 9.14–13.51% (urease) and 11.82–47.74% (leucine-aminopeptidase) compared with those of the NPK treatment (Fig. 2e and g). The activities of urease and leucine-aminopeptidase all showed an initial increase, followed by a decrease with increasing MC pyrolysis temperatures, with the highest values observed in the soils amended with the MC produced at 450 °C. Soil phosphatase activity was significantly higher in the NPK + MC300 treatment than in the other treatments ($P < 0.05$), and it showed a decreasing trend with increasing MC pyrolysis temperatures, while there were no significant differences among the different MC pyrolysis temperatures (Fig. 2f).

A principal component analysis (PCA) showed that the soil enzyme activities differed significantly between the different treatments (Fig. 3a). Ordination of the treatments was primarily related to the first canonical axis (PC1 = 96.30%). Each group on the first canonical axis was distinguished, which possessed a specific range of soil TN values. The first group included the CK treatment that had lowest TN values of 0.59 g kg^{-1}. The next group included the NPK, NPK+300 and NPK +600 treatments that had TN values of 0.62, 0.83 and 0.82 g kg^{-1}, respectively. The final group included the NPK + MC450 treatment that had highest TN values of 0.85 g kg^{-1}. A clear separation was also found when comparing the soil enzyme activities from the NPK + MC300 to the other treatments along the PC2 axis, with the NPK + MC300 treatment showing the highest MBC content (258.32 mg kg^{-1}). RDA was performed using soil physical and chemical properties as explanatory variables and all seven enzyme activities as response variables. The RDA confirmed that soil TN ($F = 31.5$, $P = 0.002$) and MBC ($F = 36.2$, $P = 0.002$) and MBN ($F = 6.2$, $P = 0.034$) correlated significantly with the soil enzyme activities and explained 70.8, 21.9 and 0.6% of the total enzymatic activity variability, respectively (Fig. 3b).

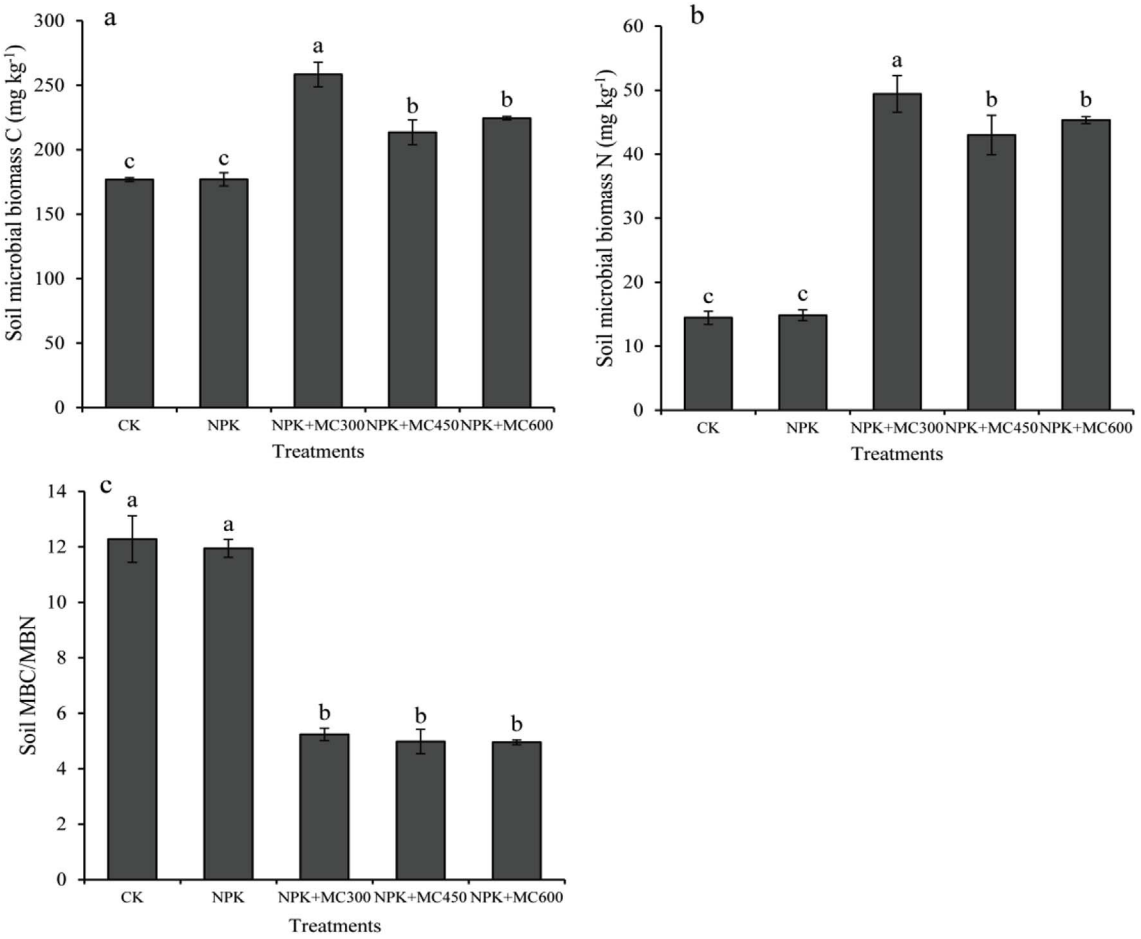

Fig. 1. Changes in microbial biomass carbon (MBC) (a), microbial biomass nitrogen (MBN) (b), and the ratio of MBC to MBN in soil after wheat harvest. Vertical bars represent the standard deviation ($n = 3$), and lowercase letters indicate significant differences among the different treatments at the $P < 0.05$ level.

3.4. The abundance and composition of microbial communities

After the wheat harvest, the total PLFA in the CK treatment was significantly lower than that in other treatments, and all the MC-amended soils exhibited significantly higher (6.73%–12.07%) total PLFA contents compared with that of the NPK treatment (Fig. 4a). The total PLFA content was significantly higher in the NPK + MC300 and NPK + MC600 treatments than in NPK + MC450 treatment, while there were no significant differences between the NPK + MC350 and NPK + MC600 treatments ($P > 0.05$). The ratio of G^-/G^+ bacteria was significantly lower in the CK and NPK treatments than in the other treatments, while it did not differ significantly between the CK and NPK treatments. The G^-/G^+ ratio showed a decreasing trend with increasing MC pyrolysis temperatures (Fig. 4b). The relative abundances of bacteria in the NPK + MC300 and NPK + MC450 treatments were significantly higher than those of the other treatments ($P < 0.05$), while there were no significant differences among the three other treatments (Fig. 4c). The relative abundance of fungi in the NPK treatment was significantly higher than that of the other treatments ($P < 0.05$), while there were no significant differences among the different pyrolysis temperatures (Fig. 4d). The relative abundances of actinomycete was significantly higher in the NPK + MC450 and NPK + MC600 treatments than the other treatments, and the largest value was observed in the soils amended with MC produced at 450 °C (Fig. 4e).

A PCA showed that the PLFA profiles differed significantly among the different treatments (Fig. 5a). The CK and NPK treatments were well separated from the other treatments along PC1, which had the lowest soil C/N ratio and available K contents, whereas all the MC-amended soils clustered together. An RDA was performed using the soil physical and chemical properties as explanatory variables and the PLFA profiles as response variables (Fig. 5b). The first and second axes accounted for 76.2 and 16.0% of the total variation in the microbial community structure, respectively. The C/N ratio ($F = 28.2$, $P = 0.002$) was the most significant variable selected by forward selection explaining 68.4% of the variance of the PLFA data, followed by available K (10.8%, $F = 6.2$, $P = 0.006$), MBC (7.3%, $F = 5.9$, $P = 0.002$), and MBN (3.0%, $F = 4.2$, $P = 0.042$).

4. Discussion

4.1. Biochar effects on soil nutrients and yield

BC is widely proposed as a means to enhance soil quality and sequester C, which is attributed to changes in soil physicochemical properties and biological functions [41,42]. Our results suggest that MC addition could enhance soil quality, as evidenced by increases in SOC, TN, MBC, MBN, and available P and K, which is similar to previous results [11,43]. These increases could be attributed to BC containing labile C, N, P and K, and the subsequent release of these nutrients into soil [44]. However, the SIN content in present study was significantly lower in the MC-amended soils than in the NPK-treated soil, in agreement with Lehmann et al. [12] and DeLuca et al. [45], who confirmed that reduction of SIN was due to a high C:N ratio of BC (N immobilization) and a high surface area (adsorption). Recently, Yao et al. [46] has reported that BC adsorbs inorganic N (NH_4^+ and NO_3^-) in leachates, depending on the production temperature (adsorption of NO_3^- at > 600 °C) and feedstock. The lowest SIN content in all the MC-

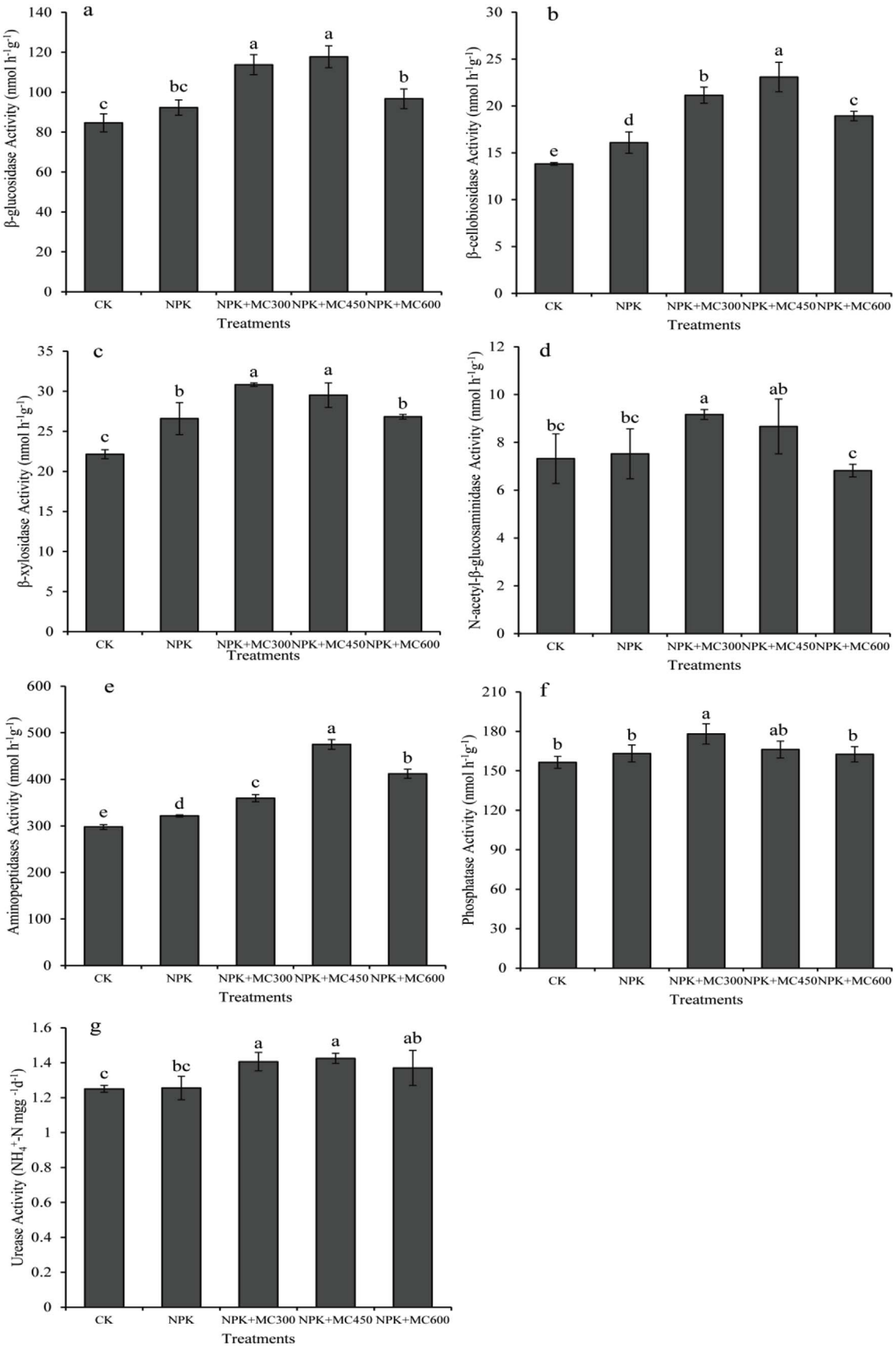

Fig. 2. Effects of different treatments on the soil enzyme activities. Vertical bars represent the standard deviation ($n = 3$) and lowercase letters indicate significant differences among the different treatments at the $P < 0.05$ level.

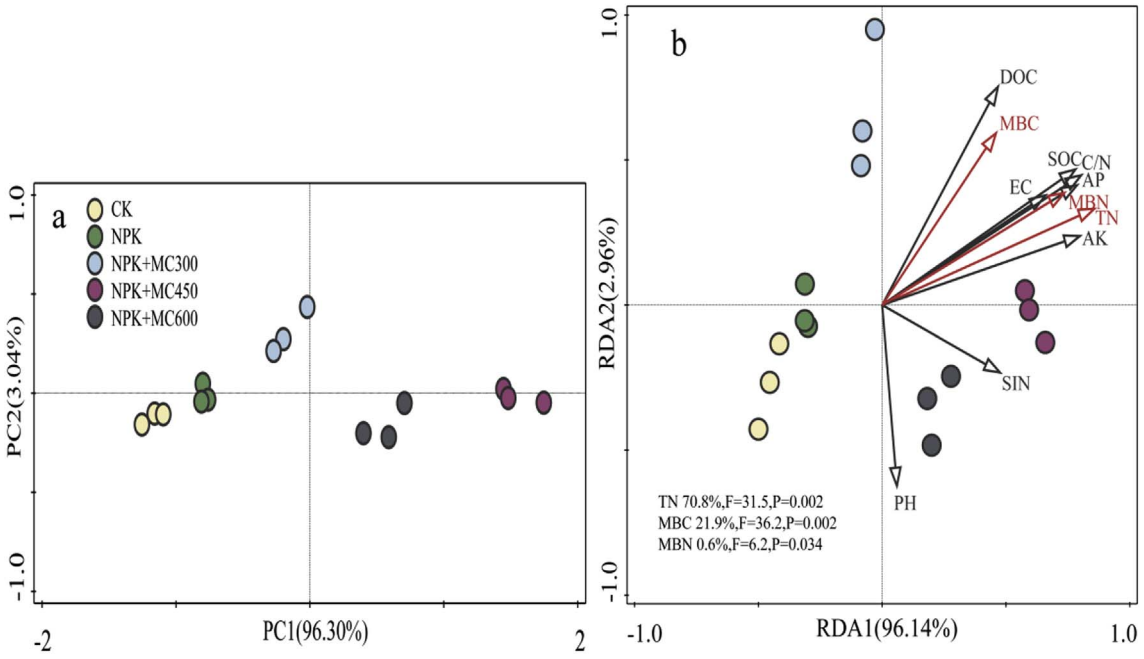

Fig. 3. Principal component analyses (PCA) of soil enzyme activities from different treatments (a), and redundancy analyses (RDA) of the correlations between soil parameters and enzyme activities (b). Orange arrows indicate that the soil parameters have a significant impact on enzyme activities ($P < 0.05$), and the corresponding explained proportion of variability is shown in the lower left corner. *Abbreviations*: EC, electrical conductivity; SOC, soil organic carbon; TN, total nitrogen; DOC dissolved organic carbon; SIN, soil inorganic nitrogen; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amended soils was observed in the MC300 + NPK treatment, in agreement with the previous findings of Zhang et al. [47], who found that soil amended with BC produced at 200 °C had significantly lower inorganic N contents due to inorganic N immobilization, which can be attributed to increased microbial activity and decomposition of labile C substrates.

BC addition to soil has a significant effect on crop yield and nutrient availability. Our results indicate that the co-application of MC and NPK promotes higher wheat yields in the calcareous soil, which is similar to the findings of Zhang et al. [48], who found that BC compound fertilizer increased the maize yield 10.5% compared to inorganic compound fertilizer in a calcareous soil. Our results also indicate that the co-application of MC and NPK promotes the uptakes of N, P, and K by wheat, and the largest values were all observed in the NPK + MC450 treatment, which is similar to the findings of previous studies [49,50]. BC has been reported to contain a significant amount of P, and it has tendency to modulate soil properties, which increases P availability resulting in improved plant growth [45,51]. The increases in P desorption is true for alkaline soil possibly due to Ca-ion induced chemical reactions [45]. Tan et al. [50] reported that the plant growth vigor followed the trend: maize BC produced at 400 °C > maize BC produced at 800 °C > uncharred maize residue. This suggests that plants have a good ability to absorb K from BC.

4.2. Biochar effects on soil pH and EC

The soil pH value directly influences the distribution of large amount and trace elements in soil, and also affects the nutrient status of soil [52]. The effect of BC addition on soil pH is often reported [53,54]. In a meta-analysis of BC studies, BC was shown to increase the soil pH on average [41]. Cai et al. [55] also found that mineral fertilizer additions decreased significantly soil pH compared with the CK. Our results showed that the soil pH value in the CK treatment was higher than that in all NPK-amended treatments, and it was significantly higher in all the MC-amended soils than the only NPK-treated soil. The soil pH value increased significantly with increasing MC pyrolysis temperatures, in agreement with the findings of Purakayastha et al. [56] and Khadem et al. [57], who suggest that the greatest increase in pH is due to a high BC pyrolysis temperature. The other explanation for the pH increase in the BC-amended soils is that the high surface area and porous nature of BC increased the cation-exchange capacity of the soil [58]. In addition, Soil EC is the basic index of soil electrochemical properties and fertility characteristics, and the soil EC value and yield are significantly correlated [59]. Soil EC was significantly higher in all the MC-amended treatments than in the CK and NPK-only treatments, which was mainly due to the higher contents of water soluble cations (K^+, Ca^{2+}, Na^+, and Mg^{2+}) released from the BC [31,60], while there was no significant difference in soil EC among the different pyrolysis temperatures, in agreement with the findings of Purakayastha et al. [56], who indicates that the increased EC is not related to the pyrolysis temperature or ash content of BC.

4.3. Biochar effects on soil enzyme activities

Soil enzyme activities control the rate of SOM decomposition and nutrient cycling processes [61]. Previous studies have stated that there is great uncertainty about the impacts of BC addition on the activities of hydrolytic enzymes involved in C cycling [16,28]. Our results showed that the activities of β-glucosidase, β-cellobiosidase, β-xylosidase and N-acetyl-glucosaminidase in the MC300 + NPK and MC450 + NPK treatments were significantly higher than those of the CK and NPK-only treatments, in agreement with the findings of Bailey et al. [13] and Ameloot et al. [14], who suggest that volatile compounds in biochar produced at low temperatures (350–500 °C) stimulate enzymatic activity in a sandy loam soil, including dehydrogenase activity and β-glucosidase activity. However, Wu et al. [62] found that BC addition had no effect on β-glucosidase activity in a chernozemic soil. The highest β-glucosidase and β-cellobiosidase activities in the present study were observed in the MC450 treatment, but the activities of β-xylosidase, and N-acetyl-glucosaminidase, and phosphatase all showed a decreased trend with increasing MC pyrolysis temperatures. The higher activities of soil enzymes reported in the present study may be due to physicochemical interactions of the BC with extracellular soil enzymes, which thereby enhances their activity [28,29].

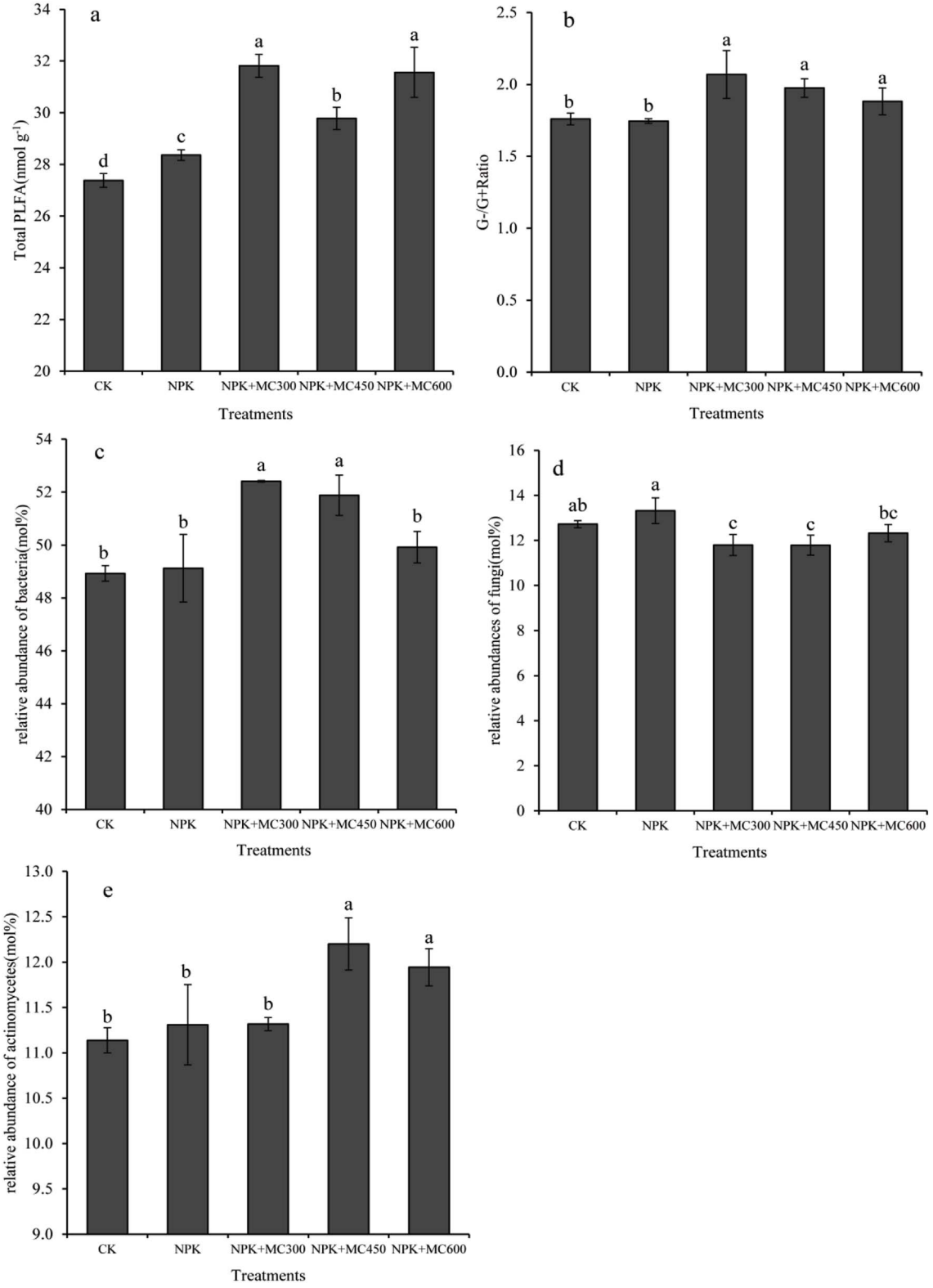

Fig. 4. Comparisons of the total PLFA concentration (a), the ratio of Gram-negative to Gram-positive bacteria (b), the relative abundances of bacteria (c), fungi (d), and actinomycetes (e). Vertical bars represent the standard deviation ($n = 3$) and lowercase letters indicate significant difference among the different treatments at the $P < 0.05$ level.

Our results also showed that all the MC-amended soils increased significantly the activities of urease and leucine aminopeptidase compared with those of the CK and NPK-only treatments. Increases in the activities of N-cycling enzymes are reportedly due to microorganisms that accelerate N mineralization from the soil to compensate for the high C/N ratios after BC addition [13,63]. However, there were no significant differences in the urease activity among the different MC pyrolysis temperatures, which accords with the results obtained by Wu et al. [62]. This demonstrates that the ability of BC to stabilize enzymes is dependent on the pyrolysis temperature and the specific enzyme. The MC prepared at different pyrolysis temperatures had different effects on leucine aminopeptidase activity, which showed an initial increase,

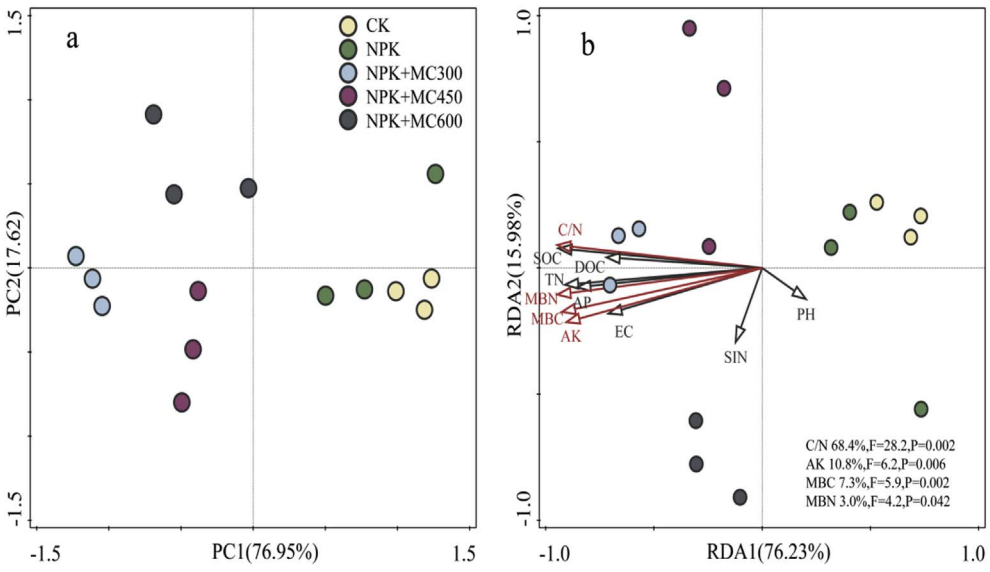

Fig. 5. Principal component analyses (PCA) of the microbial community composition (relative content of individual PLFA molecules) in soils from different treatments (a), and redundancy analyses (RDA) of the correlations between soil parameters and microbial community composition (b). Orange arrows indicate that the soil parameters have a significant impact on enzyme activities ($P < 0.05$), and the corresponding explained proportion of variability is shown in the lower right corner. Abbreviations: EC, electrical conductivity; SOC, soil organic carbon; TN, total nitrogen; DOC dissolved organic carbon; SIN, soil inorganic nitrogen; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

followed by a decrease with increasing pyrolysis temperatures of the applied MC, which is similar to the results obtained by Awad et al. [64]. In addition, the RDA analysis confirmed that the TN, MBC, and MBN values in the soil correlated significantly with the soil enzyme activities (Fig. 3b). Generally, these different results suggest that the effects of BC on soil enzyme activities are dependent mainly on soil type, pyrolysis temperature, the types of BC, and the interactions of substrates and enzymes with BC [15].

4.4. Biochar effects on the soil microbial community composition

PLFA analyses are used to determine the soil microbial community structure and responses to environmental change and soil nutritional quality. Recently, BC addition to soil was found to affect the community structure and abundance of soil microorganisms [57]. Our results found that MC addition to soil stimulated the activity of soil microorganisms, as evidenced by increases in the total PLFA concentration, and the relative abundances of bacteria and actinomycetes in the calcareous soil, although there was a decrease in the fungal population, in agreement with the findings of Khadem and Raiesi [57] and Ippolito et al. [30], who concluded that BC might supply labile C substrates that favored fast-growing bacteria over fungi. Conversely, Dempster et al. [10] found that BC could inhibit soil microbial activity and reduced microbial abundance. These different results suggest that the availability of nutrients and C may increase or decrease microbial abundance, depending on the physicochemical differences of BC and microbial community in the soil [28,65] The RDA analysis in the present study confirmed that the C/N ratio, MBC, MBN and available K were dominant factors affecting soil abundance and the composition of microbial communities (Fig. 5b), which is similar to a previously studied PLFA pattern within different sites and chemical properties of soils [66]. In addition, the G^-/G^+ ratio decreased with increasing MC pyrolysis temperatures in the calcareous soil, which is consistent with previous results [67], who demonstrated that a lower G^-/G^+ ratio was observed in neural and alkaline soils. This was due to the stronger adsorption capacity for DOC and enzymes in high-temperature-pyrolyzed BC.

5. Conclusions

Our short-term pot experiment clearly demonstrated the responses of wheat yield, soil nutrients, soil enzyme activities and microbial community structure to co-application of NPK fertilizers and MC in the calcareous soil. Our results suggest that the additions of NPK fertilizers and MC produced at 450 °C are more efficient for improving soil quality and potential crop productivity. The co-application of NPK fertilizers and MC450 promotes the increases in soil C- and N-cycling enzyme activities, and that the contents of TN, MBC and MBN in the soil are the main driving factors affecting soil enzyme activities. The additions of NPK and MC to soil favored fast growing bacteria over fungi, changes in the soil microbial community depended mainly on the contents of MBC, MBN, available K, and the C/N ratio. However, the long-term influence of MC on soil physicochemical and biological properties is unlikely to be similar to the short-term effects described herein. Therefore, future studies are needed to investigate the long-term influence of MC and fertilization on soil microbial function, soil nutrients, and potential crop productivity in the BC-soil system.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2017.11.003.

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