



## 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<sup>-1</sup> 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

**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 <sup>-1</sup>	340	537	509	46.64
Yield	%	43.63	32.61	29.54	/
Ash content	%	16.34	22.28	27.16	/
Surface area	m <sup>2</sup> g <sup>-1</sup>	1.00	4.00	70.00	/
Pore volume	mL g <sup>-1</sup>	0.01	0.01	0.06	/
Organic C	g kg <sup>-1</sup>	489.03	538.12	629.03	5.3
Total N	g kg <sup>-1</sup>	12.46	12.20	12.05	0.7
C/N ratio	/	39.25	44.11	52.20	6.46
Available P	g kg <sup>-1</sup>	0.57	0.62	0.59	0.02
Available K	g kg <sup>-1</sup>	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.

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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, and KCl, respectively, which were added at the rates of 0.10 g N kg<sup>-1</sup>, 0.05 g P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>, and 0.04 g K<sub>2</sub>O kg<sup>-1</sup> (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.

### 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<sup>-3</sup>) soil-water mixtures. SOC was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> titration method. Soil total N (TN) was determined using the Kjeldahl method [32]. Dissolved organic C (DOC) was extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub> 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<sub>2</sub>SO<sub>4</sub> 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<sub>EC</sub> and K<sub>EN</sub>) was 0.45 and 0.38 [33]. Soil available P was extracted with 0.5 M NaHCO<sub>3</sub> (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<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> [35]

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