RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2022.101

*ScienceAsia* 48 (2022): **673–680**

# Biochar application affects dynamics of soil microbial biomass and maize grain yield

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*Received 7 Jul 2021, Accepted 13 Apr 2022*
*Available online 6 Jul 2022*

**ABSTRACT**: A pot experiment was carried out to determine effects of biochar on soil microbial biomass carbon and nitrogen during maize growing season, and grain yields were also studied after harvest. Biochar (BC) was applied at the rates of 0 (BC0), 10 (BC10), 20 (BC20), and 30 g/kg (BC30). The soil microbial biomass carbon (MBC) and nitrogen (MBN) were assessed; the maize aboveground biomass and grain yields were also evaluated. The MBC was significantly increased by 15.2%–71.8% compared with BC0 in 0–10 cm soil layer, and MBC generally increased with increasing biochar application rates compared with the control in each soil layer. Soil MBN and the ratio of MBC to MBN varied with the maize growing season. Soil MBC and MBN were relatively high at the 6-leaf stage, then decreased slowly, and remained stable across the whole growing season. No significant effect of biochar on microbial quotient was observed across growing stages. BC30 significantly increased aboveground biomass by 11.8% compared with BC0. The grain yields increased with increasing biochar rate, and the increases in BC20 and BC30 reached 11.2% and 14.1%, respectively. The application of biochar could generally increase soil microbial biomass, improving soil fertility and crop yield.

**KEYWORDS**: biochar, maize, seasonal dynamic, soil microbial biomass carbon, soil microbial biomass nitrogen

## INTRODUCTION

Biochar is the solid product of thermal degradation of organic materials without or with limited oxygen [1]. Most of the biochar produced from crop straw has an alkaline pH [2]. Biochar addition to agricultural soils gained much attention in the last decade for the positive effects on soil properties and crop yields [3], which could be attributed to abundant mineral nutrients, improving the textural structure and soil microenvironment [1, 4]. Meanwhile, due to its high surface area and abundant functionality, biochar-based materials also play an important role in environmental protection, having been widely used for $CO_2$ capture [1, 5]. Generally, biochar can increase soil organic carbon (SOC) level, promoting the growth of soil microorganisms in specific groups [6, 7].

Soil microorganisms play a vital role in maintaining crop productivity through their involvement in mineralization and the breakdown of complex organic compounds in soil [8]. Soil microbial activities would be affected by organic material addition for their sensitivity to environmental changes. Biochar has been shown to play a vital role in improving biophysical condition for microbial growth and their performance [9, 10]. Previous studies have shown that biochar as a soil amendment had the potential to improve soil microbial properties [11, 12], for the reason that pores and particles of biochar could provide effective habitat for soil microbes and protect them from predators [1, 6]. Biochar can influence the soil microbial biomass in several ways. It may provide a habitat for microorganisms and serve as a substrate and supplement nutrients from the labile carbon of biochar [6, 7]. Recent evidence has shown that soil microbial biomass was higher in soil applied with biochar than in blank soil [13]. However, some studies revealed no significant effect of biochar on soil microbial biomass [14, 15]. Differently, Dempster et al [16] determined that biochar addition decreased soil microbial biomass due to the toxicity effect. Soil microbial biomass would also vary with crop growing season in a long-term field experiment [17]. Thus, further research should be conducted to evaluate effects of biochar application on soil microbial biomass.

Microbial carbon (C) use efficiency has been generally considered as the fraction of C taken up by microbial cells and retained in biomass as opposed to being respired. Microbial quotient as the earliest and simplest parameter was used to measure microbial C use efficiency. The microbial quotient was the portion of microbial biomass C to total organic C pool, ranging from 1% to 5% [18]. Though the microbial quotient decreased with biochar application rate [10, 17], dynamics of microbial quotient during maize growth stage have not been well understood. Changes of microbial biomass and C use efficiency in soil applied with biochar addition have not been well evaluated during maize growing season in a pot experiment in a short term. Therefore, this study was conducted to (1) assess the biochar effects on soil microbial biomass and microbial C use efficiency in agricultural soil for better crop productivity with suitable biochar application rates and (2) evaluate the maize yield affected by biochar application.

## MATERIALS AND METHODS

### Site description

A pot experiment was conducted in a greenhouse at the Zhoukou Normal University (114°65′ N, 33°62′ E) in China. The soil used for this experiment was collected from a farmland, planted with winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) for more than 20 years. The soil used in this experiment is silt-loam in texture and classified as Aquic Cambosol, containing 13% sand, 72% silt, and 15% clay. Chemical properties of soil were listed below: pH (1:2.5 $H_2O$) 7.9, bulk density 1.33 g/cm$^3$, SOC 10.5 g/kg, total N 1.0 g/kg, available phosphorus (P) 6.6 mg/kg, available potassium (K) 127.1 mg/kg, and mineral nitrogen (N) 10.0 mg/kg.

### Biochar properties

Biochar used for this experiment was derived from maize straw pyrolyzed at 400 °C for 2 h (heating rate 10 °C/min) with limited oxygen in a vertical kiln made of refractory bricks in Sanli New Energy Company, Henan, China. The biochar has an initial density of 0.4 g/cm$^3$, pH of 9.8, C content of 59.16%, N content of 0.98%, mineral N of 2.71 mg/kg, cation exchange capacity of 37.33 cmol/kg, specific surface area of 53.0 m$^2$/g, average pore diameter of 10.38 nm, and total pore volume of 0.09 cm$^3$/g. The available P and available K of biochar were 158.5 and 21518.6 mg/kg, respectively. Furthermore, the biochar particles of 0.02–2, 0.002–0.02, and $< 0.002$ mm with the content of 77.76%, 18.78%, and 3.46%, respectively, were detected.

### Pot experiment

A pot experiment was conducted to assess the effects of biochar on the soil microbial community in the presence of growing maize. Nursery pots were each filled with 15 kg soil, and all pots received calcium superphosphate, potassium sulfate, and urea at rates of 80 mg P/kg soil, 80 mg K/kg soil, and 150 mg N/kg soil. Both P and K fertilizers were applied as the basal fertilizer. The N fertilizer in the form of urea was applied 3 times: 40% applied as basal fertilizer, 30% applied at the jointing stage, and the remaining N was applied at the tasseling stage. Four biochar application rates, that was 0, 10, 20, and 30 g/kg referred as BC0, BC10, BC20, and BC30, respectively, were included in this experiment. Biochar was carefully mixed with soil and placed in plastic pots (0.14 m diameter and 0.9 m high). The experiment consisted of 3 replications for each treatment, and all the 12 pots were set up in a randomized design. The pots were incubated in a greenhouse under the same conditions of 25 °C and 16 h photoperiod.

A commercial variety of maize (Zhengdan 958), widely planted by local farmers, was used as the test crop. All pots were irrigated to field capacity, and 3 corn seeds were sown in each pot and later thinned down to one seedling at the 3-leaf stage. The pots were watered to field capacity every 5 or 7 days according to soil water content during the experiment.

### Soil sample collection

Soil cores were collected from 0–10, 10–20, and 20–30 cm layers at the 6-leaf stage (V6), silking stage (R1), milk stage (R3), and physiological maturity (R6) (Fig. 1). Soil samples were sieved through a 2-mm mesh to remove visible plant roots and transported to laboratory. Samples were divided into 2 sets; one set was used to determine soil microbial biomass and stored at 4 °C in a cooler, and the other set was air-dried and ground to 0.15 mm for the determination of SOC. Additionally, soil samples in 0–20 cm layer collected at the R6 stage were collected to determine soil basic properties after biochar application.

### Chemical analysis of soil

The soil bulk density was determined by the method of Xiao et al [4]. The SOC was determined by the wet oxidation method [19], and soil total N was measured using the Kjeldahl method. The available P was extracted with 0.5 mol/l sodium bicarbonate solution at pH 8.5 and measured with a colourimetric method; available K was extracted with 1.0 mol/l ammonium acetate solution (pH 7.0) and determined with flame photometer [4]. Microbial biomass carbon (MBC) and nitrogen (MBN) were determined by the chloroform fumigation extraction method [20]. Briefly, 12.5 g oven dried equivalent field-moist soil subsamples were fumigated with alcohol-free chloroform for 24 h at 25 °C. Then, the soil was extracted with 50 ml 0.5 M $K_2SO_4$ on a shaker at 220 rpm for 30 min and filtered. Total organic C concentrations in the filtrate were measured using an automatic total organic carbon (TOC) analyzer. MBC was calculated by taking the difference between $K_2SO_4$-extractable C of the fumigated and non-fumigated soils with an extraction efficiency factor of 0.45. The concentration of $K_2SO_4$-extractable N was determined by Kjeldahl digestion [21]. MBN was calculated from the increase in the fumigated soil in extractable N with an extraction factor of 0.54. Sample MBC values were divided by MBN to obtain C-to-N ratios of the microbial biomass.

The microbial quotient was calculated from the following equation: Microbial quotient = MBC/SOC, where SOC is the content of total soil organic C in g/kg soil, MBC is the soil microbial biomass C in mg/kg soil as measured using fumigation-extraction method.

### Maize harvest and analysis

Each plant was harvested when it reached physiological maturity. Aboveground biomass was harvested by cutting the stem at the soil surface, dried to a constant weight at 75 °C, and weighed to determine dry biomass production. Maize cobs were plucked, and grain yield was determined after oven drying at 50 °C to a constant weight; the kernel numbers per ear was also determined.

### Statistical analysis

All statistical analyses were carried out using an SPSS 19.0 statistical package program for Windows (SPSS Inc., Chicago, USA). One-way analysis of variance was used to assess the statistical significance in variables between treatments in each sampling time and soil layer at $p < 0.05$ according to least significant difference (LSD) test.

## RESULTS

### Effects of biochar on soil properties

The soil property data in the 0–20 cm soil layer in different treatments were presented in Table 1. SOC, available P, and available K in soil applied with biochar after a growing season, and contents of SOC, available P, and available K increased with increasing biochar rate. Compared with BC0, increases of SOC, available P, and available K were 20.0%, 39.5%, and 53.5% in BC30, respectively. No significance was observed in total N, mineral N, and bulk density among each treatment.

### Dynamics of soil microbial biomass

The MBC did not change very much along with the growing season (Fig. 1). Variability of MBC under different rates of biochar addition was relatively large. Compared with BC0, biochar significantly increased MBC by 15.2%–71.8% across the growing season in the 0–10 cm layer. MBC was also significantly increased by biochar in 10–20 and 20–30 cm layers, and MBC tended to increase with increasing biochar rates, except at the R1 stage.

Different from MBC, MBN showed a large variability throughout the growing season (Fig. 2). Biochar had significant effect on MBN; growth periods and soil depths also contributed to the difference of MBN. BC10 significantly increased MBN at the V6 stage, but BC20 and BC30 had no significant effect on MBN relative to BC0 at the V6 stage in the 0–10 cm layer. Biochar application significantly increased MBN at other growth periods in the same soil layer. In the 10–20 cm layer, BC10 and BC30 significantly decreased MBN by 14.6% and 13.6%, respectively, at the R1 stage compared with BC0. A significant decrease of MBN was also observed in BC20 at the R3 stage, while marked increase was determined in BC10 and BC20 at the V6 stage and in BC30 at the R3 stage in relative to BC0. The MBN was generally decreased by biochar in the 20–30 cm soil layer irrespective of growth periods.

### Changes of microbial indicators

Ranging from 1.7 to 9.4, the ratio of MBC to MBN was significantly affected by biochar rates and growth periods (Fig. 3). Biochar generally increased MBC/MBN at the V6 and R1 stages but decreased it at the R3 and R6 stages in the 0–10 cm soil layer. The MBC/MBN at the R1 stage was increased by biochar compared with BC0 in the 10–20 cm layer, while the ratios at other stages varied with biochar application rates. Differently, biochar significantly increased MBC/MBN across the growing season in the 20–30 cm layer.

Ranging from 2.5% to 4.0%, the microbial quotient maintained at a relatively stable level across the soil layers and the growth periods (Fig. 4). Biochar had no significant effects on microbial quotient though significant decreases were observed in BC10 at the V6 stage and in BC30 at the R1 stage compared with BC0.

### Changes of plant biomass and crop yield

Biochar significantly increased the aboveground biomass and maize yield, and both values tended to increase with increasing biochar rates (Table 2). A significant increase of 11.8% in aboveground biomass was observed in BC30, but BC10 and BC20 had no effect on aboveground biomass compared with BC0. The increases of maize grain yields were 11.2% and 14.1% in BC20 and BC30, respectively, relative to BC0. Similarly, the kernel numbers per ear were also significantly increased with biochar increasing rates.

## DISCUSSION

Due to the high C content, biochar application significantly increased SOC in the present study. This was in line with the previous studies [4, 17]. Biochar could increase SOC generally via introducing organic C into soil or promoting mineralization of biochar itself [1]. In general, biochar produced at low pyrolysis temperatures was found to enhance SOC content to a significantly greater degree than that produced at high pyrolysis temperatures [1]. This may be because of the contribution of the partially pyrolyzed portion of the biomass to SOC in biochar made at low temperature [2]. However, total N and mineral N were not significantly affected by biochar though it tended to increase with increasing application rate of biochar. This was in line with results of Jones et al [22]. Similarly, our previous study also showed that there were no significant differences in content of $NO_3^-$ and $NH_4^+$ between plots with or without biochar application in the first year [23]. Thus, biochar addition could reduce the risk of mineral N leaching and promote N accumulation in crops, which would contribute to increases of crop yields. In addition, the enhanced

**Table 1** Influence of biochar addition on soil properties after maize harvest.

| Treatment | SOC (g/kg) | Total N (g/kg) | Available P (mg/kg) | Available K (mg/kg) | Mineral N (mg/kg) | Bulk density (g/cm$^3$) |
|---|---|---|---|---|---|---|
| BC0 | $9.63^c$ | $1.09^a$ | $6.55^b$ | $150.64^d$ | $23.15^a$ | $1.38^a$ |
| BC10 | $9.88^c$ | $1.15^a$ | $7.35^b$ | $182.38^c$ | $24.13^a$ | $1.37^a$ |
| BC20 | $10.24^b$ | $1.18^a$ | $8.52^a$ | $205.42^b$ | $24.35^a$ | $1.37^a$ |
| BC30 | $11.56^a$ | $1.22^a$ | $9.14^a$ | $231.25^a$ | $24.61^a$ | $1.36^a$ |

Values (means of 3 replications) followed by the different letters within the column are significantly different at $p < 0.05$. BC0, BC10, BC20, and BC30, biochar applied at rate of 0, 10, 20, and 30 g/kg, respectively. SOC, soil organic carbon; and Total N, total nitrogen.

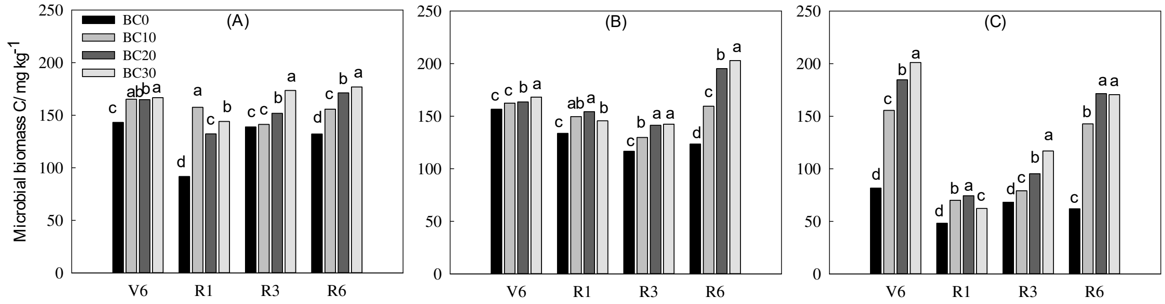

**Fig. 1** Effects of biochar on soil microbial biomass C during the growth periods in 0–10 (A), 10–20 (B), and 20–30 (C) cm soil layer. Different letters above the bar at the same growth period are significantly different at $p < 0.05$. Values are means of 3 replications. BC0, BC10, BC20, and BC30, biochar applied at rate of 0, 10, 20, and 30 g/kg, respectively; V6, the 6-leaf stage; R1, the silking stage; R3, the milk stage; and R6, the physiological maturity.

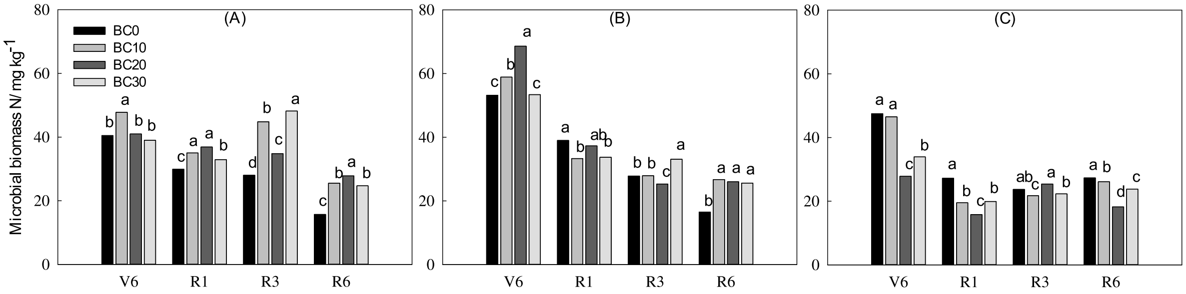

**Fig. 2** Effects of biochar on soil microbial biomass N during the growth periods in 0–10 (A), 10–20 (B), and 20–30 (C) cm soil layer. Different letters above the bar at the same growth period are significantly different at $p < 0.05$. Values are means of 3 replications. BC0, BC10, BC20, BC30, V6, R1, R3, and R6 are defined as in Fig. 1.

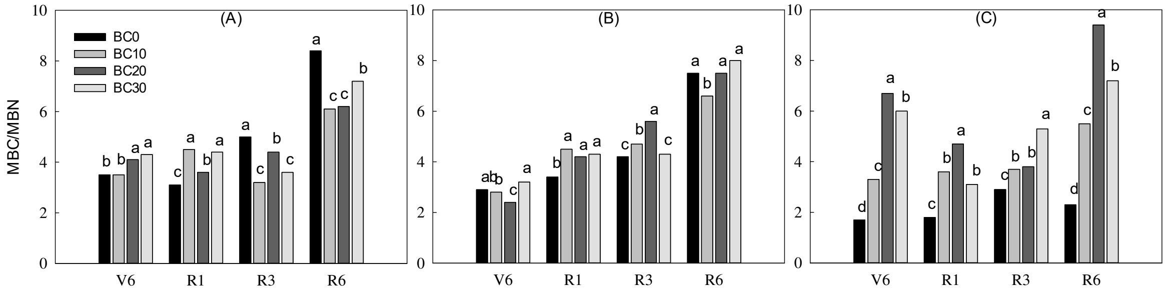

**Fig. 3** Effects of biochar on MBC/MBN during the growth periods in 0–10 (A), 10–20 (B), and 20–30 (C) cm soil layer. Different letters above the bar at the same growth period are significantly different at $p < 0.05$. Values are means of 3 replications. BC0, BC10, BC20, BC30, V6, R1, R3, and R6 are defined as in Fig. 1. MBC/MBN, the ratio of soil microbial biomass C to microbial biomass N.

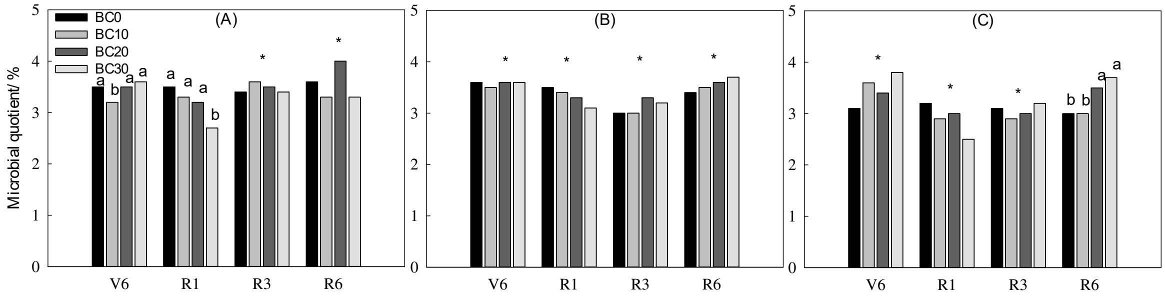

**Fig. 4** Effects of biochar on microbial quotient during the growth periods in 0–10 (A), 10–20 (B), and 20–30 (C) cm soil layer. Different letters above the bars at the same growth period are significantly different at $p < 0.05$. Values are means of 3 replications. BC0, BC10, BC20, BC30, V6, R1, R3, and R6 are defined as in Fig. 1.

**Table 2** Effects of biochar application rates on maize aboveground biomass and crop yield.

| Treatment | Aboveground biomass (g/plant) | Maize yield (g/plant) | Kernel no. per ear |
|---|---|---|---|
| BC0 | $110^{b}$ | $150.8^{c}$ | $442^{c}$ |
| BC10 | $112^{b}$ | $160.0^{bc}$ | $451^{bc}$ |
| BC20 | $118^{ab}$ | $166.2^{ab}$ | $470^{ab}$ |
| BC30 | $123^{a}$ | $170.8^{a}$ | $481^{a}$ |

Values (means of 3 replications) followed by the different letters within the column are significantly different at $p < 0.05$. BC0, BC10, BC20, and BC30 are defined as in Table 1.

microbial activity might also be an explanation of the unchanged soil N concentrations [13, 24].

It is well known that biochar could affect soil microbial activity via improving soil physical and chemical properties. The organic C content predominantly influenced the soil biological parameters such as MBC and MBN [1]. Changes in soil microbial biomass reflect the process of microbial growth, death, and organic matter degradation. Our results showed that effects of biochar on MBN differed according to growth periods and soil layers. MBC increased with increasing biochar application rates compared with BC0 in our study, suggesting that microbial growth could be accelerated by biochar addition. This was consistent with results of Ifran et al [13] and Khadem and Raiesi [25]. A positive linear relationship between microbial biomass and biochar addition rate was also observed in a highly weathered soil [26]. Additionally, the Terra Preta soils have greater biomass and diversity than adjacent soils, which may be due to the positive priming effects of biochar [6, 24]. Biochar produced at low temperature (250–400 °C) would result in mineralization of soil organic matter and the labile components of the biochar [1, 24], which provided large amount of nutrients for microorganisms. The increased soil microbial biomass after biochar application may also partly be due to biochar surfaces supplying nutrients and inhabits for the soil microbes [27] since the biochar has good pore structure and large surface area of 53.0 $m^2/g$. The alkalinity of biochar could promote the establishment of soil microbes and facilitate the growth of both bacteria and fungi within the pores of biochar [28]. The increases in the microbial biomass with biochar application indicated improvements in the functioning and quality of the soil due to a sustainable microbial community. In addition, the increased physical contact between biochar particles and microbial community enhanced by soil C might also contribute to increases of soil microbial biomass [29]. However, different biochar effects on MBC were also observed in other studies. Dempster et al [16] showed decreased soil microbial activities and soil microbial biomass in a coarse textured soil. No significant changes of soil microbial biomass were also reported in other studies[15, 30]. These discrepant results might be explained partly by the variation of biochar and soil texture in different experiments.

The ratios of MBC to MBN reflect the changes in relative availability of C and N to soil microbes and show changes of soil microbial community structure. Biochar addition generally increased MBC/MBN in our study, except at the R3 and R6 stages in the 0–10 cm soil layer. This result might suggest increased microbial N limitation. Our previous study showed MBC/MBN increased in the first year after biochar application but decreased in the second year [17]. While a pot experiment conducted by Dempster et al [16] showed no significant change of MBC/MBN after biochar addition. The MBC/MBN was significantly positively related to the C/N value of the added organic matter [31]. Biochar might change soil nutrient availability and soil physiochemical properties, which would cause shifts in ratios of soil MBC to MBN. Biochar had no effect on the values of microbial quotient across the growing season and soil layers in our study. Dilution effect might be an explanation, namely that the increase of SOC was consistent with the increase of MBC after biochar application. The changes of soil microbial community composition might also contribute to changes of MBC/MBN and no changes of microbial quotient. We only evaluated changes of soil microbial biomass in the present study; further studies are required to assess effects of biochar on soil microbial properties.

The response of soil microbial biomass to seasonal changes is vital in regulating the turnover of soil microbes, which would affect nutrient availability and soil productivity [32]. The microbial indicators generally varied with growth periods, except for the microbial quotient. Soil labile C increased when maize root generally perished, which resulted in an increase of soil organic materials. The decomposition of these organic materials would provide extra nutrients and energy for soil microbes, which increased soil MBC in turn. However, the MBN showed a decrease trend along with the growth periods; this might be partly due to the competition between plant and soil microbes. Maize would need large amount of N for the formation of yield, which decreased available N in the soil.

Studies have shown that biochar could promote soil micro-environment which was beneficial for crop growth and nutrient accumulation in crop [33, 34]. Maize yield is positively correlated with kernel number, which was established during early stage of grain filling and reflected the maize growth conditions during flowering [35]. In this study, the fact that kernel number was significantly increased by biochar application, which contributed to increases of maize grain yield, was in line with the results of Xiao et al [4]. In the present study, both the aboveground biomass and maize grain yield were increased by biochar application in silt loamy soil, and the increases tended to increase with increasing biochar application rates. In a previous study, both the aboveground biomass and maize grain yield had an additional increase of 16.5% and 11.4%, respectively, in soil applied with chemical fertilizer and biochar [36]. The improvement of soil water content and nutrient availability generally contributed to the increased maize aboveground biomass and grain yield [1, 4].

Biochar is widely used to improve the retention of nutrients in soil, particularly in nutrient poor soils [34]. However, the effects of biochar application on crop biomass and grain yield are complex and depend on properties of biochar, soil texture, crop types, and management practice. The effect of biochar on crop growth may thus be different. No significant effect of biochar was observed on spring wheat yield [37]. In a pot experiment, Butnan et al [38] reported that the accumulation of maize biomass decreased in the first cycle in soil with a single amendment of 2% biochar but increased in the second cycle. Similarly, a single application of biochar at a rate of 90 t/ha caused a decrease of 80% in maize yield [39]. Hence, the application of biochar combined with chemical fertilizer may be a better measurement in agricultural production, which was helpful for the improvement of biomass and grain yields. Considering the average cost of chemical fertilizer used in this experiment was 2.6-yuan/kg, and the amount was 51.57 g per pot; biochar used in this experiment was 1.0-yuan/kg, and the extra costs were 0.15, 0.30, and 0.45 yuan in BC10, BC20, and BC30, respectively. Given that the average maize price was 2-yuan/kg, and the increases in maize grain yield in BC10, BC20, and BC30 were 9.2, 15.4, and 20.0 g; the net benefits were 0.02, 0.03, and 0.04 yuan, respectively. It was obvious that the net benefits were less than the extra cost. Biochar application in this specific soil was not an effective practice for increasing farmer income. However, incubation experiment in greenhouse might be different from field experiment. Thus, it was required to evaluate effects of biochar application on soil conditions, crop yield, and economic feasibility in field in the future.

## CONCLUSION

The utilization of biochar derived from crop straw is a potential sustainable waste management option for the organic waste of dry land areas. Soil applied with biochar significantly increased the aboveground biomass and maize yield. The reason for the increase in biomass and grain yield was mainly a result of promoted microbial activity. Soil microbial biomass was generally increased by biochar application. The MBC tended to have a little increase trend along with the growth periods, while MBN tended to decrease irrespective of biochar addition rates. Application of 30 g/kg yielded the best crop yield and higher microbial biomass under the conditions of this experiment. For economic feasibility, biochar application was not an effective practice. However, whether biochar applied in the farmland would show the similar effect was not clear. Further research about biochar application in natural agricultural system is required, and additional field studies on the long-term effect of biochar on soil microbial community and diversity are also needed.

*Acknowledgements*: This research was funded by the Foundation of He'nan Science and Technology Committee (222102320276; 202102310154) and the School-based project of Zhoukou Normal University (ZKNUC2018012).

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