

Original Research

Effects of Sewage Sludge Biochar on Soil Characteristics and Crop Yield in Loamy Sand Soil

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Abstract

Biochar produced from sewage sludge could provide an important alternative to waste management practices while offering an opportunity to improve soil properties and reduce the risk of contamination from direct applications of sewage sludge soil amendments. We assessed the impacts of different rates of biochar application (20, 40, 60 t ha⁻¹) to peanuts grown in a loamy sand soil in the North China Plain on composition of the soil microbial community, soil bulk density (BD), pH, total carbon (TC), total nitrogen (TN), C:N, available phosphorus (P), available potassium (K), dissolved organic carbon (DOC) and crop yield. We found that sewage sludge biochar application increased TC, TN, available K, and C:N, and decreased soil BD and pH and had variable effects on DOC. Amendment with biochar increased microbial biomass and the proportion of Gram-positive bacteria, Gram-negative bacteria, fungi and Actinomycetes, while it decreased the ratios of groups of bacteria. The highest crop yield was achieved under 40 t ha⁻¹ of biochar. Our study suggests that the lower rates of sewage sludge biochar application could improve soil physicochemical properties and increase levels of soil microbes and crop yield; however, the highest rate may induce negative effects on microbe community composition.

Keywords: application rate, field experiment, PLFA, soil properties, peanut productivity

Introduction

Increase of carbon fluxes among soil, plants and the atmosphere is a key driver of global warming [1, 2],

and there has been recent interest in the application of biochar to soil as a potential climate change mitigation strategy, since it may act as a long-term carbon sink [3]. The effects of biochar on soil characteristics such as pH, porosity, bulk density and cation exchange capacity suggest that its application could form part of a long-term climate change adaptation strategy [3].

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Liang et al. [4] found that amending soil with biochar enhanced the specific surface area of a loamy sand soil and consequently had a greater potential cation exchange capacity and retention of dissolved organic matter than untreated, adjacent soil, while Zhao et al. [5] reported that biochar application increased the activity and biomass of soil microorganisms as a result of an associated increase in nutrients. Biochar application may also provide additional benefits to agriculture, since it improves crop yield and plant nutrient availability [6]. Sewage sludge, which is a globally ubiquitous carbon-rich material with reactive surfaces, may be pyrolyzed to biochar that is high in mineral ash content and could serve as a low-cost source of soluble essential plant nutrients [7].

The characteristic of loose texture of sand soil renders it difficult to retain moisture and nutrients, so agricultural productivity of these soils is generally low and insufficient to meet growing food demands. The North China Plain contains more than 25,800 km² of loamy sand soils that is mostly distributed along the old course of the Yellow River in Henan Province. Biochar is generally applied to highly weathered, heavy soils, as an effective alternative for the enhancement of soil fertility and a range of soil physicochemical properties, but the benefits of biochar application to lighter, loamy sand soils have been little studied [8, 9].

Microbial phospholipid fatty acid (PLFA) profiling analysis is commonly used to assess soil microbial community abundance that is widely used as an indicator of soil quality changes due to the sensitivity to environmental change [10]. Microbial community responses to biochar application vary depending on a range of factors, including biochar type [11], where dominant effects of biochar on community composition are changes in substrate availability and pH [12]. For example, Quilliam et al. [13] reported that biochar application could produce and absorb polycyclic aromatic hydrocarbons that are known to interfere with microbes, while Steinbeiss et al. [14], who used PLFA analysis, observed that soil amended with yeast biochar increased fungi and Gram-negative bacterial biomass. Khan et al. [15] reported that sewage sludge biochar increased shoot biomass, grain yield, and the bioaccumulation of phosphorus in rice. To our knowledge, few studies have examined the effects of biochar application on soil characteristics and crop yield in loamy sand soils, particularly in the warm temperate region of the North China Plain, and in those studies that have been reported, the assessed effects of biochar were mostly derived from plants or manure rather than sewage sludge. The objectives of this study were to evaluate the effects of sewage sludge biochar application on soil physicochemical properties, microbial community composition and crop yield in a loamy sand soil, and to illustrate the main factors that drive changes in microbial community composition following biochar addition.

Materials and Methods

Experimental Site and Biochar

Soil samples were collected from the HuaiWei Wind Erosion Observation Point in Yifeng (114°57'12.13"E, 34°48'00.59"N; 66 m elevation), on the Central Great Plain of the Yellow Huai River in northern China that is characterized by a warm temperate monsoon climate with a mean annual temperature of 14.2°C, and mean annual precipitation of c. 678.2 mm, most of which is confined to a 4-month period from June to September. According to the United States Department of Agriculture texture classification system, the soil texture is loamy sand (FAO) with 821 g kg⁻¹ sand, 124 g kg⁻¹ silt, and 55 g kg⁻¹ clay. The observation point was set up in September 2015, and before this the soil was traditionally cultivated under crops for at least 30 years.

The experimental site was prepared by clearing weeds, moldboard ploughing and harrowing to a depth of c. 30 cm. The experiment comprised three replicates of four treatments (Table 1) arranged in a randomized block design in plots (5 x 8 m, with 0.25 m between rows) separated by a 1-m buffer zone. Inorganic fertilizers and biochar amendments were incorporated to a 30-cm depth before peanut (cv. No.16 Huayu) was drilled in 2016 and 2017; there were no amendments in 2017. Mature peanuts were harvested on 20 September 2016 and 26 September 2017.

Biochar was produced from municipal sewage sludge from a treatment plant and prepared using the cracking process in a reactor [16] at the Mississippi International Water Company. The dry sewage sludge was combusted under "no-oxygen" conditions using slow-pyrolysis in a muffle furnace, where the pyrolysis temperature was raised at a rate of 26°C min⁻¹ and then held at 700 to 850°C for 4 h. It is expected that 10% of the mass of sewage sludge is converted to biochar using this process. The biochar was passed through a 2-mm sieve prior to hand-broadcasting it onto the soil surface, and then using a tilling tractor to ensure even mixing within the top 30 cm of soil. The physicochemical properties of biochar are presented in Table 2; metal content did not exceed the threshold of China Environmental Protection Agency standards [17-18].

Table 1. Sewage sludge treatments and associated nitrogen (N), phosphorous (P), and potassium (K) content (kg ha⁻¹)

| Treatment Code | Biochar Application Rate | N | P | K |
|----------------|--------------------------|-----|------|-----|
| CK | 0 t ha ⁻¹ | 0 | 0 | 0 |
| B20 | 20 t ha ⁻¹ | 332 | 342 | 54 |
| B40 | 40 t ha ⁻¹ | 664 | 684 | 108 |
| B60 | 60 t ha ⁻¹ | 996 | 1026 | 162 |

Table 2. Physicochemical properties of pre-treated soils and biochar.

| Elements | Soil | Biochar |
|--|-------|---------|
| pH | 8.8 | 6.4 |
| MC(unit) | 4.5 | 9.7 |
| BD (g cm ⁻³) | 1.39 | / |
| Total carbon (g kg ⁻¹) | 9.3 | 197.7 |
| Total N (g kg ⁻¹) | 0.68 | 16.6 |
| Total P (g kg ⁻¹) | 0.98 | 17.1 |
| Total K (g kg ⁻¹) | 12.79 | 2.7 |
| Volatile matter (wt %) | / | 11.8 |
| Fixed carbon (wt %) | / | 29.4 |
| Ash (wt %) | / | 58.8 |
| BET surface area (m ² g ⁻¹) | / | 14.2 |

Soil Physicochemical Properties and Crop Yield Analysis

Soil samples were collected on 10 May 2016 (prior to drilling) and at the end of growing season in 2017 in order to assess the treatment effects on soil properties after applying biochar for two years. Composite soil samples were taken from a depth of 0-15 cm using a 4-cm diameter soil core sampler sealed in a plastic bag, stored on ice and then shipped to the laboratory within 24 h. A portion of the fresh soil samples was stored at 4°C for dissolved organic carbon (DOC), while the remaining sample was air-dried and passed through a 2-mm sieve. Large plant debris and roots were removed before sieving and there was no visually recognizable loss of biochar from soil during this process.

Soil bulk density (BD) was determined from soil samples that were oven dried at 105°C until a constant weight had been achieved. Soil pH was determined from a soil-to-water ratio of 1:5 using a PB-10 pH meter (Sartorius GmbH, Göttingen, Germany) [11], and content of soil total carbon (TC) and total nitrogen (TN) was determined using an elemental analyzer (Elementar, Vario EL, Germany). Soil-available phosphorus (P) was determined using sodium bicarbonate, and soil-available potassium (K) was determined using ammonium acetate [2]. Soil DOC was measured according to the method reported by Lu and Zhang [19], where fresh soil samples (10 g dry equivalent) and ultrapure water (25%, w/v) were shaken for 30 min on a reciprocal shaker (250 r min⁻¹) at 25°C and then centrifuged at 4000 rpm for 10 min. The supernatant was filtered using a 0.45 µm cellulose ester filter and measured using a TOC analyzer (TOC-VCPH+TNM-1, Shimadzu Inc, Japan).

Crop yield of the plots was determined after harvest at the end of growing seasons in 2016 and 2017, where 10 plants were randomly removed from the center two

rows of each plot, and roots were cleaned and oven-dried at 80°C to a constant dry weight. Yield samples were collected on the same day using an inverting digger, and pods were removed from the plants using a commercial peanut combine harvester.

Phospholipid Fatty Acid (PLFA) Analysis

Soil sampled as above was stored at -70°C prior to extraction and quantification of PLFA based on the method described by Guo et al. [20]. For the microbial analysis, lipids were extracted from 3 g of soil in a solution of methanol (8 ml), chloroform (4 ml) and phosphate buffer (3.2 ml, 0.15 M). Neutral lipids, glycolipids and phospholipids were separated from polar lipids on a silica-bonded phase column (Sep-Pak Silica SPE, Waters Corp, USA) sequentially eluted with chloroform, acetone and methanol, respectively. The purified phospholipids were saponified and methylated to extract the fatty acid methyl esters (FAME) at 37°C in a water bath, and acid levels were reduced using sodium hydroxide before drying under a stream of N₂ at 25°C. FAME were quantified and identified using gas chromatography coupled to mass spectrometry (Hewlett Packard, Agilent 5973 Plus HP6890 Series GC-System) with a cross-linked 5% phenyl methyl rubber phase (film thickness of 0.35 mm). The internal standard (C19:0) peak areas were expressed as nmol g⁻¹ dry soil and used as a reference to calculate the concentration of PLFA.

Total microbial biomass (total PLFA) was estimated using total nmol g⁻¹ dry soil as an index. PLFA was divided into taxonomic groups based on previously published PLFA biomarker data [21] that included Gram-positive (Gram+) bacteria (sum of i14:0, i15:0, i16:0, i17:0, a15:0, a17:0), Gram-negative (Gram-) bacteria (sum of cy17:0, cy19:0, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω5c), total bacterial biomass (sum of Gram+ and Gram- bacteria biomarkers together with 15:0, 17:0, 17:1ω6 and 17:1ω7), fungi (sum of 18:1ω9c and 18:3ω3, 6, 9), and Actinomycetes (sum of 10Me-16:0, 10Me-17:0, 10Me-18:0).

Statistical Analysis

Treatment effects of biochar were analyzed using one-way ANOVA in SPSS 16.0 software package for Windows (SPSS Chicago, IL, USA), with Duncan's multiple range test, based on least significant difference (LSD) values at P<0.05 to test for variation among treatments. Principal component analyses (PCA) and redundancy discriminant analyses (RDA) were used to explore the relationships between the soil microbial community and soil physicochemical properties using Canoco 5. Person correlation tests were applied to explore the association between soil microbial community and crop yield.

Results and Discussion

Soil Physicochemical Properties

Physicochemical properties of the sewage sludge biochar are presented in Table 2. After two years, biochar had reduced soil BD (Fig. 1a), although there was no difference between the two high rates (B40 and B60). Biochar is an effective soil conditioner and its effect on BD may be explained by a simple dilution effect on the soil of the low bulk density biochar [22]. In our study, all biochar treatments decreased soil BD, which was consistent with many other field studies [23-24]. However, we found that there was no difference in treatment effect between the two high rates of biochar (40 and 60 t ha⁻¹). Similar results have been reported by Zhang et al. [25], who showed that wheat straw biochar applied to soil at 20 and 40 Mg ha⁻¹ had no effect on soil BD. This indicated that excessive rates of biochar application would not help continue to decrease soil BD.

We found that sewage sludge biochar reduced soil pH by 0.2-0.55 units, where pH decreased with increasing rate of biochar addition (Fig. 1b). While this result is consistent with some studies [26-27], others have shown that soil pH was increased [28] or was not

influenced [11] by biochar application. This difference in response of soil pH to biochar addition may be due to differences in biochar production material and soil types [29]. Biochar amendment has been reported to have a neutralizing effect on soil pH in acidic soil [30], which may be attributed to the content of carbonates of alkali and alkaline earth metals, variable amounts of silica, phosphates and small amounts of organic and inorganic N. As found in this study, decreased soil pH is favorable to microbial activities that are known to contribute to N and P availability in soils [31] and this was found in our study.

Although soil TC and TN concentrations were increased by the addition of sewage sludge biochar, there was no difference in TN content among the treatments (Fig. 1c-d). The higher levels of TC and TN content under biochar application are consistent with previous reports [32-33] and may be a result of the response of non-decomposed C and N in the organic sewage sludge biochar after two years.

Available P concentrations in the B40 and B60 treatments were increased by 17.74 and 24.37%, respectively (Fig. 1e), and while available K and soil C:N ratio were increased by the addition of sewage sludge biochar, there were no differences among the treatments (Fig. 1f-g). The increased in soil C:N ratio in the biochar treatments may be due to the inherent high C:N ration of biochar, as reported by Laghari et al. [34]. Nelissen et al. [35] similarly reported a positive effect of biochar addition on soil K availability in a sandy loam soil during a two-year field experiment, possibly due to its enhancement of cation exchange [2]. The increase in the release of K cations from the soil surface colloid to the soil solution subsequently increased available K concentration in the biochar treatments. The lack of difference in available K among the biochar treatments indicates that biochar application rate should be controlled. Since the B20 treatment had no effect on soil available P, as also reported by Lentz and Ippolito [36], it is possible that only higher rates of biochar increase available P in acidic soils.

The concentration of soil DOC was decreased by the lowest rate of biochar addition and increased by the highest rate, but there was no difference at the mid-rate of application (Fig. 1h). The addition of biochar has been shown to stimulate microbial use soil DOC [37], but the physical adsorption of DOC by biochar may explain the decrease in DOC concentration at the low rate of biochar addition (B20) [37]. High rates of biochar application contain toxic elements that may suppress microbial community composition and associated use of DOC; this may explain our results that showed higher DOC concentrations under the highest rate of biochar application.

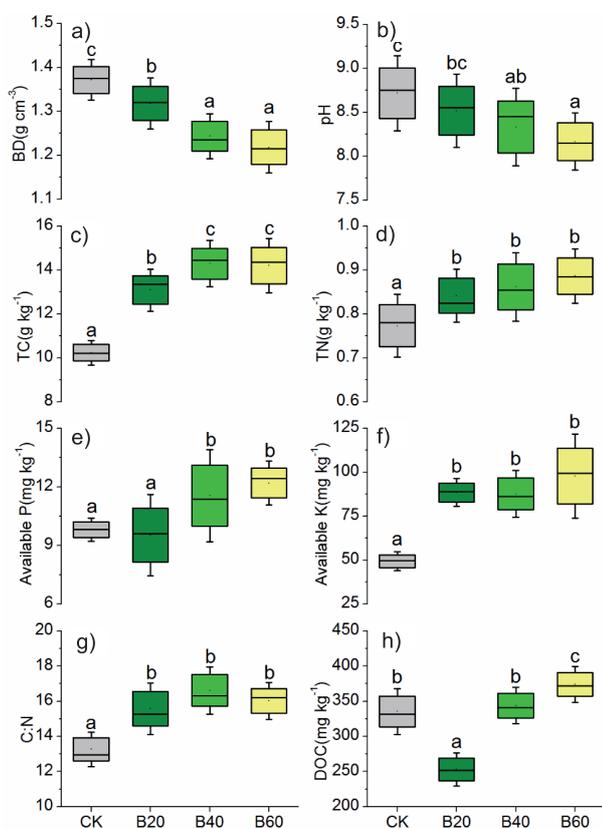


Fig. 1. Soil physicochemical properties under different treatments; boxes represent half of the data, the thick line within each box represents median value, and whiskers represent interquartile ranges

Soil Microbial Community

PLFA concentrations were increased by biochar two years after application (Fig. 2). Although we

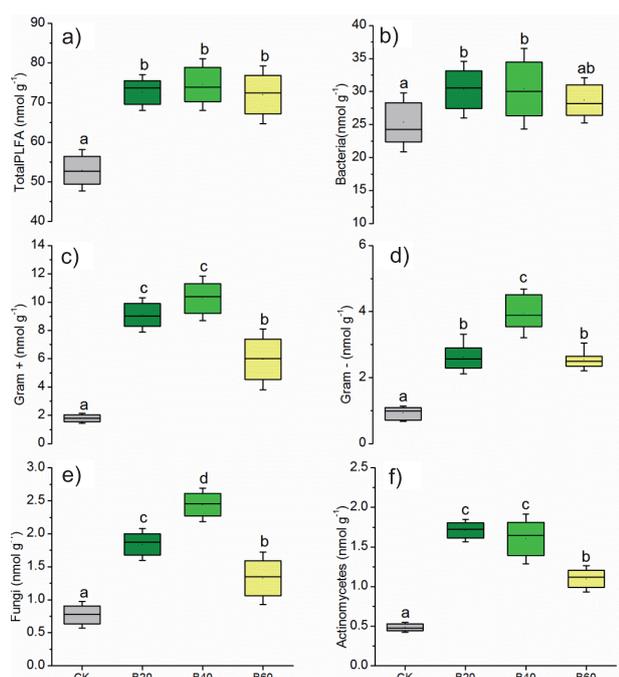


Fig. 2. Relative abundance of different microbial groups under different treatments; boxes represent half of the data, the thick line within each box represent median value, and whiskers represent interquartile ranges.

found there were no differences in total PLFA and bacteria among the biochar treatments (Fig. 2a-b), there were lower densities of Gram⁺, Gram⁻, fungi and Actinomycetes under the highest rate of biochar (Fig. 2c-f). Biochar increased the proportion of Gram⁺, Gram⁻, fungi and Actinomycetes, but decreased the proportion of bacteria (Table 3). Among the biochar treatments, B40 had the highest proportion of Gram⁺, Gram⁻, fungi and Actinomycetes; the increase of biochar rate from 40 to 60 t ha⁻¹ led to a decrease in the proportions of Gram⁺, Gram⁻, fungi and Actinomycetes. These results may be due to the effects of toxic elements of biochar, which are known to suppress microbial community composition [38-40], and indicate that the application of high amounts of sewage sludge biochar (60 t ha⁻¹) could inhibit soil microbial biomass for at least two years in a sandy loam soil.

PCA is a statistic technique to determine treatment differentiation of combinations of variables or parameters [41]. We found that principal components (PCs) 1 and 2 accounted for 84.56% and 6.59% of the total variation in soil microbiological and physicochemical properties, respectively (Fig. 3a). PC1 tended to separate the biochar treatments from the control, where the biochar treatments were associated with high levels of microbial biomass (Gram⁺ and Actinomycetes), and high levels of BD and pH were associated with the untreated control soils. In general, PCA showed distinct microbial and physicochemical properties of the untreated soils, and each biochar treatment after two years, where those of the biochar treatments were more similar to each other.

Soil microbial community composition has been widely used as an indicator of soil characteristics because of its sensitivity to environmental change [10]. Potential mechanisms of the effects of biochar on soil microbial community composition include the provision of carbon material, production and/or adsorbance of substances that stimulate or inhibit microbes, and provision a suitable habitat for microbial growth and protection from predators [37]. In our study, additional organic matter in biochar stimulated different groups of soil microorganism, and as a result, soil TC was positively correlated with all biomarker PLFAs. All biomarker PLFAs were influenced by biochar, regardless of application rate, possibly due to effects on pH that stimulated soil microbial biomass and changed the microbial community composition [42, 43].

RDA combines regression and PCA to allow direct analysis of the structuring of a set of response variables by explanatory variables [44]. We found that axes 1 and 2 accounted for 82.89% and of the variation in microbial community composition of PLFA profiles related to soil physicochemical properties, respectively, where Monte Carlo permutation tests of the first canonical axis and the sum of all canonical axes were $F = 72.7$, $P = 0.002$ and $F = 12.0$, $P = 0.002$, respectively (Fig. 3b); microbial community composition was positively correlated with soil TC, C:N and available K, and negatively correlated pH and BD. It is widely accepted that BD and pH affect soil microbial community composition [42, 45], and in our study RDA showed that biochar altered soil characteristics by decreasing soil BD and pH and affecting the soil nutrient pool. Biochar

Table 3. Effect of sewage sludge biochar application rate on proportion (%) of microbial groups characterized by phospholipid fatty acids.

| Treatment | Bac/PLFA | Gram ⁺ /PLFA | Gram ⁻ /PLFA | Fungi/PLFA | Act/PLFA |
|-----------|---------------|-------------------------|-------------------------|-------------|-------------|
| CK | 47.86±4.45 a | 3.39±0.32 c | 1.76±0.35 c | 0.92±0.12 c | 1.46±0.26 d |
| B20 | 41.90 ±5.50 b | 12.54±0.76 a | 3.64±0.76 b | 2.35±0.11 a | 2.53±0.15 b |
| B40 | 40.63±3.20 b | 13.79±1.43 a | 5.34±1.02 a | 2.15±0.29 a | 3.28±0.29 a |
| B60 | 40.24±6.05 b | 8.40±2.39 b | 3.54±0.45 b | 1.53±0.21 b | 1.86±0.45 c |

Different letters within a column indicate treatment differences at $P < 0.05$.

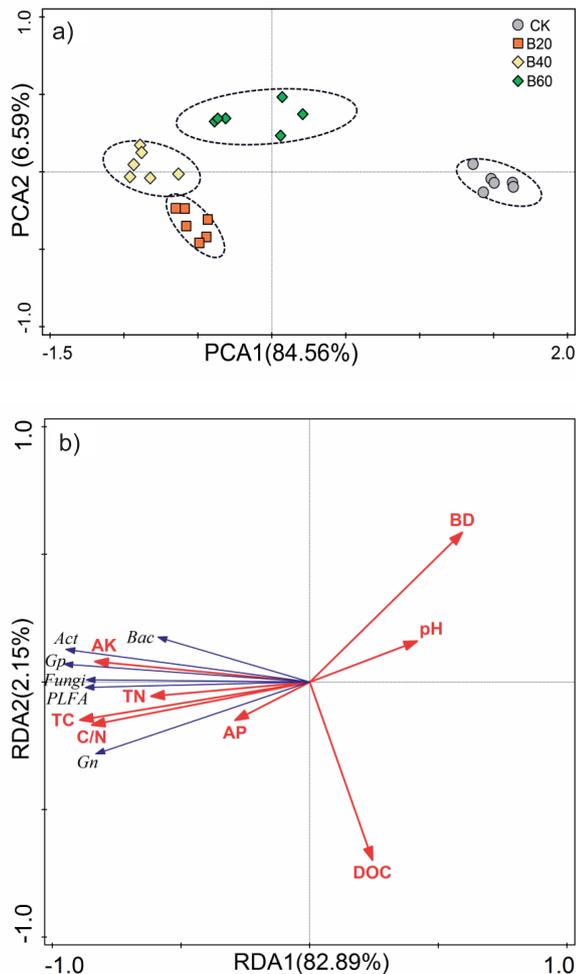


Fig. 3. Principal component analysis of the soil microbiological and physicochemical prosperities of the treatments a) and redundancy analysis of the correlations among soil parameters and microbial community composition b); blue arrows are microbial groups and red arrows indicate the parameters that were related to microbial community composition ($P < 0.05$); the angle and length of the arrows indicate the direction and strength of the relationship; PLFA – total PLFA, Bac – bacteria, Gp – Gram positive bacteria, Gn – Gram negative bacteria, Act – actinobacteria, BD – bulk density, TC – total carbon, TN – total nitrogen, AP – available phosphorus, AK – available potassium, DOC – dissolved organic carbon.

application that reduced soil BD and improved root growth and increased root biomass probably explain the higher microbial biomass in the biochar treatments [24]. We also found changes to microbial community composition among the treatments, and these changes that were correlated with soil C content were consistent with previous reports [38, 42]. It is possible to reason

that the mineralization of organic matter increased the biomass of soil microorganisms.

Crop Yield

We found that the addition of biochar at 20, 40 and 60 t ha⁻¹ increased crop yield by 34.72, 60.43 and 52.29%, respectively, where crop yield of the B20 treatment was lower than the B40 and B60 treatments, and there was no difference in crop yield between these higher rates (Table 4).

Biochar decreased soil BD and may have acted as a soil conditioner that retained more available water and nutrients, and improved root growth which subsequently enhanced crop yield [24]. Biochar application may have increased soil temperatures during seed germination, mitigate drought, improved soil structure and aggregate formation and reduced weed competition [18] – which are all beneficial to crop yield. The higher rates of available P and K indicate that the biochar amendment may provide a slow-release of P and K, in addition to that provided by the application of conventional fertilizer, through mineralization reactions [46]. Although studies have shown a clear enhanced effect of biochar on crop yield in sandy soil with low fertility [46], we found that the increase in application rate of biochar from 40 to 60 t ha⁻¹ did not change crop yield, and we suggest the rate should be optimized to 40 t ha⁻¹ of sewage sludge biochar. The highest positive correlation with crop yield was Gram- bacteria ($r = 0.71^{**}$) (Table 5), probably because various Gram- bacteria families contain a plant growth-promoting member [47]. In our study, the increase in crop yield may have been due to improved nutrient availability mediated by biochar amendment, as a result of effects on soil physicochemical properties.

Conclusions

Our two-year consecutive field experiment in the North China Plain revealed that the application of sewage sludge biochar positively affected soil characteristics and crop yield. Strong correlations among microbial community composition, soil physicochemical properties and crop yield indicated that microbial community composition and crop yield were strongly affected by physicochemical properties. The stimulation of the microbial community composition by biochar was primarily due to changes in soil physicochemical properties – especially decreases in BD and pH. The strong positive correlation between crop yield and microbial community composition indicated that the

Table 4. Effect of sewage sludge biochar application rate on crop yield.

| Treatment | CK | B20 | B40 | B60 |
|-----------------------------------|------------|------------|------------|------------|
| Crop yield (kg ha ⁻¹) | 2863±214 a | 3857±305 b | 4593±152 c | 4360±217 c |

Different letters indicate treatment differences at $P < 0.05$.

Table 5. Pearson's correlation between microbial community composition and crop yield.

| Microbial composition | Total PLFA | Bacteria | Gram+ | Gram– | Fungi | Actinomycetes |
|-----------------------|------------|----------|--------|--------|--------|---------------|
| Yield | 0.50** | 0.23 | 0.65** | 0.71** | 0.64** | 0.60** |

change of soil microbial community composition may have led to the increase in crop yield. Our results demonstrate that the biochar application is beneficial to the improvement of soil properties and has potential as an effective measure for improving crop yield. It is likely that high rates of sewage sludge biochar amendment (60 t ha⁻¹) would not continue to elicit benefits in soil properties or crop yield, but application them at 40 t ha⁻¹ would likely be an optimal rate of amendment to loamy sand soils for at least two years. In the future, long-term comprehensive evaluations are needed to improve knowledge about the effect of sewage sludge biochar on the composition of the soil microbial community in the midland area of China.

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Conflict of Interest

The authors declare no conflict of interest.

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