

## Article

# Soil Texture Mediates the Short-Term Influence of Wheat Straw Biochar Amendment on Crop Yield and Soil Bacterial Community

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## Abstract

Optimizing biochar use requires understanding its effects on soil microbes and crop yield, yet the role of soil texture remains unclear. In a two-year field experiment with a winter wheat–summer maize rotation on sandy and fluvo-aquic soils, biochar was applied at rates of 0, 10, and 20 t ha<sup>−1</sup>. In sandy soil, biochar increased maize yield by 9.8% in the first year and by 13.1% in the second year, and wheat yield by 8.0% in the second year. In fluvo-aquic soil, only the 10 t ha<sup>−1</sup> rate increased maize yield by 9.7% in the second year. Biochar altered soil properties and enzyme activities in both soils, but microbial community responses varied with soil type, and soil type exerted a stronger influence than biochar application rate. In fluvo-aquic soil, the highest biochar application rate (20 t ha<sup>−1</sup>) enhanced bacterial richness and diversity with a changed community structure, increasing *Acidobacteria* and *Chloroflexi* while decreasing *Proteobacteria*, whereas no such effects were observed in sandy soil. These community shifts were correlated with available potassium, urease, and sucrase, factors themselves affected by biochar. Fungal diversity and community structure were unaffected in either soil. Biochar increased yields through direct physicochemical improvements in sandy soil, whereas in fluvo-aquic soil it acted via combined physicochemical and microbial effects. Overall, the effects after two years of biochar on soil microorganisms and crop yield strongly depend on soil texture, mediated by complex interactions among soil properties. Given that the study involved a single biochar type and two soil types, further research across broader conditions is needed to verify the generality of these conclusions.

**Keywords:** biochar; soil enzymes; soil physicochemical properties; microbial community structure

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## 1. Introduction

Soil microorganisms serve as engines of plant productivity in terrestrial ecosystems, actively participating in biogeochemical cycling and plant nutrient acquisition, while also

strongly influencing soil fertility and physical structure [1–3]. Soil health and quality are routinely evaluated through the diversity and structural composition of microbial communities [4]. Investigating how soil microbial communities react to soil amendment techniques is crucial for creating suitable agricultural management strategies on a regional level.

Biochar is a carbon-dense, stable, and highly aromatic material with a well-developed pore structure, created by heating agricultural and forestry residues or other organic feedstocks in an atmosphere with limited oxygen [5]. It has been demonstrated to provide numerous agricultural advantages, such as improved soil fertility, carbon sequestration, remediation of acidic soils, and adsorption of heavy metals [6–9]. Moreover, there has been growing interest in how adding biochar affects soil microbial activity and composition [10]. Biochar influences microbial activity through multiple mechanisms. Firstly, Biochar's porous structure provides a refuge for microorganisms, although the bacterial communities colonizing it are strongly shaped by the pyrolysis temperature [11]. Secondly, biochar addition can alter the microbial habitat by augmenting soil physical structure and nutrient retention capacity [12]. Thirdly, certain organic compounds and free radicals retained in biochar may exert toxic effects on microbial cells [13]. The overall impact of biochar on soil microbes thus arises from the combined action of several interacting mechanisms. Indeed, published findings on how biochar influences soil microbial communities remain inconsistent [14,15]. This discrepancy is attributed to variations in feedstock, pyrolysis temperature, application rate across existing studies [16,17].

Soil physicochemical variables are closely linked to microbial growth and metabolism. Consequently, the diversity and composition of soil microbial communities with varying textures and physicochemical properties may respond differently to biochar amendments [18]. Khadem and Raiesi [19] discovered that microbial indicators responded most strongly when biochar was applied to less fertile sandy soil. Liu et al. [20] and Zhang et al. [21] noted highly alkaline biochar effectively neutralized soil pH and significantly altered bacterial community structures in acidic red soil, with a stronger effect than in saline or organic brown soils, due to bacterial sensitivity to pH changes. According to Wang et al. [22], fine-textured soils exhibited stronger responses to biochar in terms of aggregate formation and microbial community shifts than coarse-textured soils. Additionally, a meta-analysis of 265 datasets from 49 studies indicated biochar could increase total PLFAs and enhanced microbial activities, especially in low-nutrient paddy soils with fine texture [17]. The complex interplay among microorganisms, soil, and plants complicates systematic comparisons of results obtained under different environmental conditions and across soil types. Thus, how biochar addition alters soil microbial community composition and function remains poorly understood, especially in soils of comparable pH but contrasting textures. Furthermore, biochar application rate is a significant factor affecting soil microorganisms, as demonstrated by numerous pot and incubation experiments [23,24].

Exploring how soil microbial communities in the sandy and fluvo-aquic soils of the North China Plain respond to biochar amendment is essential for improving agricultural soil management in this major grain-producing region. A two-year field study was conducted to examine how varying biochar application rates affected key soil biochemical and microbial properties in sandy and fluvo-aquic soils. We hypothesized that biochar amendment would improve soil physicochemical properties and alter microbial community structure, but that these effects would differ between the two soil types: (i) in sandy soil, characterized by low organic matter and weak nutrient retention, biochar would directly enhance nutrient availability and crop yield; and (ii) in fluvo-aquic soil, with higher fertility and stronger buffering capacity, biochar effects on yield would be less pronounced, and microbial community responses would be mediated primarily through indirect changes in soil properties.

## 2. Materials and Methods

### 2.1. Experiment Site and Biochar Preparation

Field experiments were conducted in Yanjin County, Xinxiang City, Henan Province, China. This area is part of the North China Plain. Prior to the experiment, both sites had been under a winter wheat–summer maize rotation for at least six years. At both sites, rotary tillage was performed before wheat sowing, and maize was sown simultaneously with fertilizer application. Both sites received conventional mineral fertilization at rates similar to those applied during the experiment. Based on meteorological records from 2006–2015, The average yearly temperature is 17.0 °C, and the average yearly precipitation is 504.4 mm., with 71% concentrated in the maize-growing season from June to September. During the wheat-growing period from October to May, the mean temperature is 8.9 °C, with minimum and maximum temperatures of −11.4 °C and 37.4 °C, respectively, and mean total rainfall of 148.7 mm. During the maize-growing period from June to September, the mean temperature is 25.1 °C, with minimum and maximum temperatures of 8.6 °C and 41.0 °C, respectively, and mean total rainfall of 355.7 mm. Based on the Chinese system for classifying soil, two soil types were identified as sandy soil with a loamy sand texture (86.0% sand, 7.8% silt, and 6.2% clay) and fluvo-aquic soil with a silt loam texture (20.0% sand, 54.6% silt, and 25.4% clay). Prior to the field experiment, the sandy soil (0–20 cm depth) had an organic matter content of 13.06 g kg<sup>−1</sup>, total N of 0.78 g kg<sup>−1</sup>, available N of 63.08 mg kg<sup>−1</sup>, available P of 18.21 mg kg<sup>−1</sup>, available K of 66.27 mg kg<sup>−1</sup>, and a pH of 8.03. The fluvo-aquic soil had an organic matter content of 17.05 g kg<sup>−1</sup>, total N of 0.92 g kg<sup>−1</sup>, available N of 75.55 mg kg<sup>−1</sup>, available P of 14.42 mg kg<sup>−1</sup>, available K of 112.50 mg kg<sup>−1</sup>, and a pH of 7.95.

The biochar for this investigation was acquired from Henan Shangqiu Sanli New Energy Co., Ltd., Shangqiu, China. The biochar was produced from wheat straw in a continuous carbonizer at 450 °C for 3 h. The basic properties of biochar were as follows: ash content of 14.54%, total organic carbon (TOC) content of 65.74%, total N content of 1.28%, C/N ratio of 51.36, total P content of 0.95%, total K content of 2.62%, BET specific surface area of 20.74 m<sup>2</sup> g<sup>−1</sup>, pH of 9.92, electrical conductivity of 4.76 mS cm<sup>−1</sup>, and cation exchange capacity of 35.1 cmol kg<sup>−1</sup>. Before being applied to the soil, the biochar was screened to retain the fraction below 2 mm.

### 2.2. Experiment Design and Treatments

The field trial started in October 2015. Following local farming conventions, the wheat crop occupied the field from October through June of the next year, and the subsequent maize crop extended from June to October. For each soil type, a randomized block design with three replicates was used to establish three biochar treatments: 0 (CK), 10 (BC-1), and 20 t ha<sup>−1</sup> (BC-2). Each treatment plot measured 5 m × 10 m, with adjacent plots separated by 2 m. At wheat sowing, biochar was surface broadcast and incorporated into the 0–20 cm soil layer by rotary tillage. A single application was made for the entire experimental period.

For both wheat and maize, all treatments received identical fertilizer application rates, specifically 180 kg ha<sup>−1</sup> of N (urea, 46% N), 60 kg ha<sup>−1</sup> of P<sub>2</sub>O<sub>5</sub> (calcium superphosphate, 12% P<sub>2</sub>O<sub>5</sub>), and 60 kg ha<sup>−1</sup> of K<sub>2</sub>O (potassium sulfate, 50% K<sub>2</sub>O). For each crop, a basal dressing consisting of the complete doses of P and K fertilizers and 50% of the N fertilizer was broadcast and incorporated. The remaining 50% of the N was sidedressed later at the specific vegetative stages: regreening for wheat and jointing for maize. After harvesting, the straw was fully returned to the soil mechanically. Across the two-year study, which encompassed a pair of wheat and a pair of maize cropping cycles, the total

straw inputs in sandy soil were 28.3, 29.2, and 30.3 t ha<sup>-1</sup> for CK, BC-1, and BC-2, respectively. In fluvo-aquic soil, the corresponding values were 38.2, 39.4, and 39.0 t ha<sup>-1</sup>.

### 2.3. Crop Yield

Crop yields were determined at harvest. In each subplot, a 3 m × 3 m area was randomly selected for maize sampling. The collected maize plants were dried and threshed, and grain weight was recorded to calculate maize yield. In each subplot, a 1 m × 1 m area was randomly selected for wheat sampling, with the samples processed similarly to those of maize.

### 2.4. Soil Samples Collection and Analysis

Soil samples were collected at maize harvest in October 2017, when the field experiment had been ongoing for two years. Soil samples were collected using a soil sampler (3 cm in diameter). In each subplot, five soil cores were randomly taken from a depth of 0–20 cm. The cores were mixed well after debris removal to form a composite sample, which was then transported to the lab on ice. Three subsamples were created from each composite sample. One portion was kept at −80 °C for molecular ecological tests, another was air-dried at room temperature and sieved for physicochemical examination, and the rest was used right away for enzyme activity analysis.

Soil physicochemical properties, including organic matter, pH, and available N, P, and K, were determined as described by Bao [25]. Based on the guidelines in the textbook edited by Li et al. [26], the activities of urease, sucrase, β-glucosidase, and catalase were determined.

### 2.5. DNA Isolation, Amplicon Sequencing, and Sequence Processing

Microbial DNA was extracted from 0.5 g fresh soil with the PowerSoil DNA Isolation Kit (MO BIO, San Diego, CA, USA), verified for quality, eluted in 50 µL buffer, and stored at −20 °C. The V3–V4 region of 16S rRNA and the ITS1 region were amplified with primers 338F/806R [27] and ITS1F/ITS2R [28]. Each 20 µL reaction mix comprised 4 µL 5× TransStart FastPfu buffer, 2 µL dNTPs (2.5 mM), 0.4 µL FastPfu polymerase, 0.2 µL bovine serum albumin, 0.8 µL of each primer (5 µM), 10 ng template DNA, and 11.8 µL ddH<sub>2</sub>O. Cycling began at 95 °C for 3 min, followed by 27 (bacteria) or 35 (fungi) cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s, with a final step at 72 °C for 10 min. Triplicate amplicons were pooled, purified, quantified, and sequenced on an Illumina MiSeq PE250 at Shanghai Majorbio Biopharm Technology Co., Ltd., (Shanghai, China). Barcoded products were combined in equimolar ratios. Raw reads were quality-filtered with Trimmomatic (version 0.39), merged with FLASH (version 1.2.11, minimum overlap 10 bp, sliding window 50 bp, quality cutoff 20), and clustered into OTUs at 97% identity with UPARSE (version 0.1.2) [29]. The dominant sequence per OTU was classified via the RDP classifier (version 2.11, confidence 0.7) against Silva 132 (bacteria) and UNITE 8.0 (fungi). Prior to community analysis, samples were rarefied to an equal depth, resulting in thresholds of 28,053 reads per sample for bacteria and 28,822 reads per sample for fungi. Raw sequence data are available at NCBI under BioProject PRJNA1166880.

### 2.6. Statistical Analysis

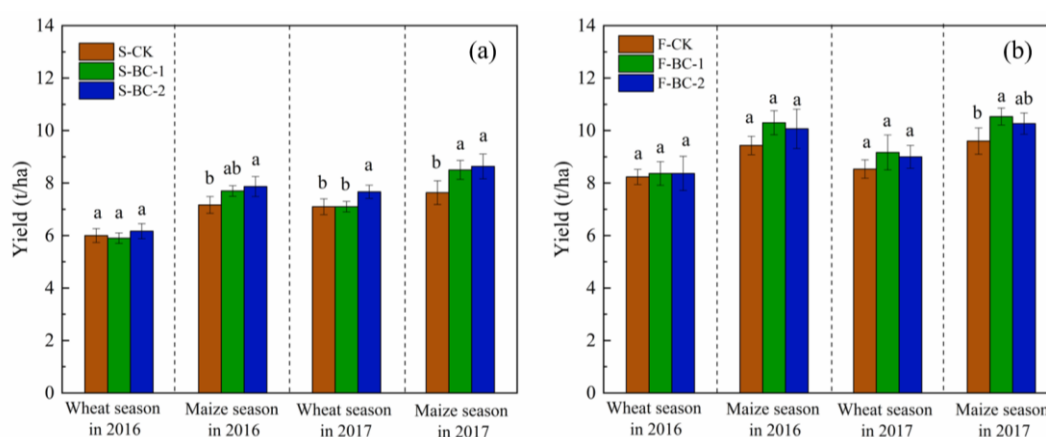
Statistical analysis was performed using two-way ANOVA with Soil type and Biochar rate as fixed factors, including their interaction, to evaluate the overall effects on crop yield and soil properties. Biochar effects within each soil type were compared using one-way ANOVA followed by Duncan's test ( $p < 0.05$ ). Results are expressed as means ± SD. Variable relationships were examined by linear regression. Community composition was visualized via principal coordinate analysis (PCoA) on Bray–Curtis distances, and treat-

ment differences were tested by analysis of similarities (ANOSIM). Distance-based redundancy analysis (db-RDA, vegan package in R, version 3.4.0) with Bray–Curtis distances was employed to link soil variables to bacterial community structure.

### 3. Results

#### 3.1. Crop Yields

In the sandy soil, compared with CK, BC-2 increased maize yields by 9.77% in 2016 and by 13.10% in 2017, and increased wheat yield by 7.98% in 2017; BC-1 increased maize yield by 11.35% in 2017. In the fluvo-aquic soil, yield improvements were observed only in the fourth season, with BC-1 increasing maize yield by 9.72% in 2017 (Figure 1). Two-way ANOVA showed that soil texture significantly affected yield in all four crop seasons ( $p < 0.001$ ), whereas the effect of biochar amendment was significant only in maize seasons ( $p < 0.05$  in 2016;  $p < 0.01$  in 2017). The Soil texture  $\times$  Biochar interaction was not significant in any season (Table 1).



**Figure 1.** Crop yield in 2016–2017 in sandy soil (a) and fluvo-aquic soil (b). Note: Different letters show differ significantly ( $p < 0.05$ ).

**Table 1.** Effects of soil texture and biochar amendment on crop yield in 2016–2017 by two-way ANOVA.

|                                         | Wheat Yield<br>in 2016 | Maize Yield<br>in 2016 | Wheat Yield<br>in 2017 | Maize Yield<br>in 2017 |
|-----------------------------------------|------------------------|------------------------|------------------------|------------------------|
| Soil texture                            | ***                    | ***                    | ***                    | ***                    |
| Biochar amendment                       | NS                     | *                      | NS                     | **                     |
| Soil texture $\times$ Biochar amendment | NS                     | NS                     | NS                     | NS                     |

Note: NS denotes no significant difference, \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

#### 3.2. Soil Physicochemical Properties and Enzyme Activities

In the fluvo-aquic soil, compared with CK, BC-1 and BC-2 significantly increased organic matter (OM) by 22.42% and 54.93%, respectively. In the sandy soil, BC-1 and BC-2 significantly increased OM by 15.16% and 35.78%, respectively (Table 1). These results confirm that biochar amendment improved soil fertility, with higher application rates exerting more pronounced positive effects. Biochar amendment did not affect pH in either soil. Biochar amendment increased available nitrogen only in the fluvo-aquic soil, with the BC-1 and BC-2 treatments raising AN by 16.54% and 21.23%, respectively. Compared with CK, available phosphorus (AP) significantly increased by 40.89% under both BC-1 and BC-2 in the sandy soil, whereas no change was observed in the fluvo-aquic soil. In addition, biochar amendment significantly increased available potassium (AK) in both

soils (Table 2). Two-way ANOVA revealed that soil texture significantly affected all measured physicochemical properties ( $p < 0.01$ ), while biochar amendment significantly affected all properties except pH. The Soil texture  $\times$  Biochar interaction was significant for available N ( $p < 0.01$ ) and available P ( $p < 0.05$ ), indicating that biochar effects on these nutrients differed between the two soils (Table 2).

Biochar amendment also significantly affected soil enzyme activities (Table 3). In the sandy soil, urease activity was significantly decreased by BC-2 but not by BC-1; in the fluvo-aquic soil, it was significantly decreased by both BC-1 and BC-2. Sucrase activity was significantly increased in both soils.  $\beta$ -glucosidase activity was significantly decreased by biochar amendment in the fluvo-aquic soil, while no significant change was observed in the sandy soil. Moreover, BC-2 significantly increased catalase activity in the sandy soil but decreased it in the fluvo-aquic soil. Two-way ANOVA showed that soil texture significantly affected sucrase,  $\beta$ -glucosidase, and catalase activities, whereas biochar amendment significantly affected urease, sucrase, and  $\beta$ -glucosidase activities. The Soil texture  $\times$  Biochar interaction was significant for sucrase,  $\beta$ -glucosidase, and catalase ( $p < 0.01$ ), indicating that biochar effects on these enzymes differed between the two soils (Table 3).

**Table 2.** Effects of soil texture and biochar amendment on soil physicochemical properties.

| Treatment                               | OM<br>g kg <sup>-1</sup> | pH               | AN<br>mg kg <sup>-1</sup> | AP<br>mg kg <sup>-1</sup> | AK<br>mg kg <sup>-1</sup> |
|-----------------------------------------|--------------------------|------------------|---------------------------|---------------------------|---------------------------|
| S-CK                                    | 12.89 $\pm$ 0.57e        | 8.05 $\pm$ 0.06a | 62.30 $\pm$ 3.65bc        | 16.14 $\pm$ 0.62b         | 62.67 $\pm$ 10.07c        |
| S-BA-1                                  | 15.78 $\pm$ 1.11d        | 8.08 $\pm$ 0.05a | 57.98 $\pm$ 1.76c         | 23.00 $\pm$ 2.62a         | 110.33 $\pm$ 19.50b       |
| S-BA-2                                  | 19.97 $\pm$ 0.59b        | 8.05 $\pm$ 0.08a | 60.90 $\pm$ 1.95bc        | 23.00 $\pm$ 4.71a         | 156.00 $\pm$ 10.39a       |
| F-CK                                    | 17.55 $\pm$ 0.46c        | 7.93 $\pm$ 0.02a | 66.27 $\pm$ 2.98b         | 13.32 $\pm$ 1.23bc        | 115.33 $\pm$ 14.84b       |
| F-BA-1                                  | 20.21 $\pm$ 1.28b        | 7.93 $\pm$ 0.08a | 77.23 $\pm$ 4.46a         | 15.74 $\pm$ 1.63bc        | 188.33 $\pm$ 23.44a       |
| F-BA-2                                  | 23.83 $\pm$ 1.18a        | 7.96 $\pm$ 0.13a | 80.27 $\pm$ 1.93a         | 11.03 $\pm$ 2.59c         | 179.00 $\pm$ 28.51a       |
| Significance                            |                          |                  |                           |                           |                           |
| Soil texture                            | ***                      | **               | ***                       | ***                       | ***                       |
| Biochar amendment                       | ***                      | NS               | *                         | *                         | ***                       |
| Soil texture $\times$ Biochar amendment | NS                       | NS               | **                        | *                         | NS                        |

Note: Values in a column with different lowercase letters are significantly different at the  $p < 0.05$  level. The significance of treatments and interactions was assessed using a two-way ANOVA. NS denotes no significant difference. \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . OM organic matter, AN available nitrogen, AP available phosphorus, AK available potassium.

**Table 3.** Effects of soil texture and biochar amendment on soil enzyme activities.

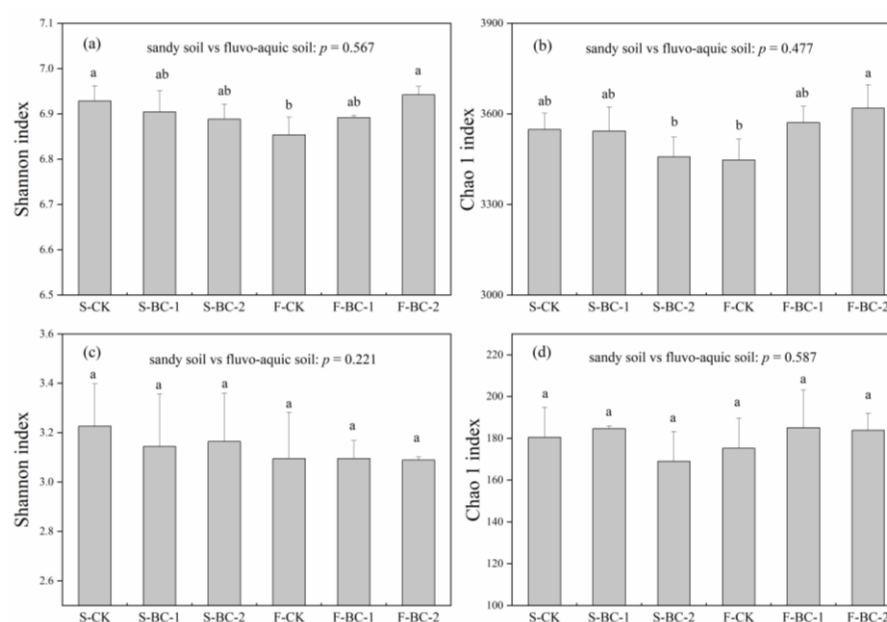
| Treatment                               | UA<br>$\mu\text{g NH}_4^+\text{-N g}^{-1} \text{h}^{-1}$ | SU<br>mg Glucose g <sup>-1</sup> h <sup>-1</sup> | GLU<br>$\mu\text{g } n\text{-Nitrophenol g}^{-1} \text{h}^{-1}$ | CA<br>ml g <sup>-1</sup> |
|-----------------------------------------|----------------------------------------------------------|--------------------------------------------------|-----------------------------------------------------------------|--------------------------|
| S-CK                                    | 1.87 $\pm$ 0.06b                                         | 12.12 $\pm$ 1.45e                                | 40.07 $\pm$ 1.19ab                                              | 6.43 $\pm$ 0.89d         |
| S-BA-1                                  | 1.82 $\pm$ 0.08bc                                        | 15.84 $\pm$ 0.88d                                | 36.33 $\pm$ 2.76bc                                              | 6.96 $\pm$ 1.20d         |
| S-BA-2                                  | 1.69 $\pm$ 0.02d                                         | 18.18 $\pm$ 0.62c                                | 42.91 $\pm$ 1.43a                                               | 10.44 $\pm$ 0.74c        |
| F-CK                                    | 1.97 $\pm$ 0.08a                                         | 19.97 $\pm$ 0.67b                                | 43.69 $\pm$ 5.65a                                               | 20.75 $\pm$ 0.28a        |
| F-BA-1                                  | 1.78 $\pm$ 0.02bcd                                       | 23.15 $\pm$ 0.37a                                | 33.32 $\pm$ 2.54c                                               | 19.89 $\pm$ 0.30a        |
| F-BA-2                                  | 1.73 $\pm$ 0.05cd                                        | 22.13 $\pm$ 0.98a                                | 24.39 $\pm$ 1.79d                                               | 17.14 $\pm$ 0.63b        |
| Significance                            |                                                          |                                                  |                                                                 |                          |
| Soil texture                            | NS                                                       | ***                                              | **                                                              | ***                      |
| Biochar amendment                       | ***                                                      | ***                                              | **                                                              | NS                       |
| Soil texture $\times$ Biochar amendment | NS                                                       | **                                               | ***                                                             | ***                      |

Note: Values in a column with different lowercase letters are significantly different at the  $p < 0.05$  level. The significance of treatments and interactions was assessed using a two-way ANOVA. NS

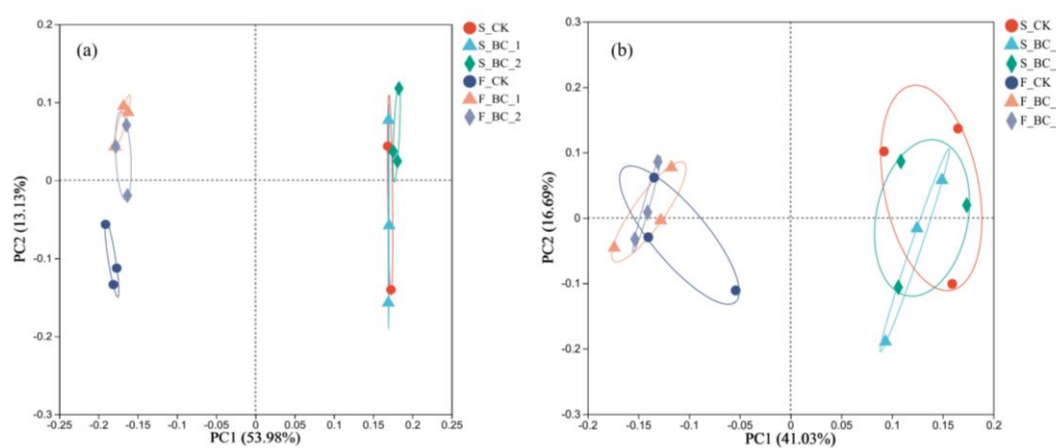
denotes no significant difference, \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . UA: urease; SU: sucrose; GLU:  $\beta$ -glucosidase; CA: catalase.

### 3.3. The Alpha and Beta Diversities of Bacterial and Fungal Community

The alpha diversity of both bacterial and fungal communities was not affected by biochar amendment in the sandy soil (Figure 2). In the fluvo-aquic soil, a higher biochar application rate significantly increased bacterial community alpha diversity. PCoA revealed significant differences in the beta diversity of the bacterial community between the sandy and fluvo-aquic soils (Figure 3a). Biochar amendment did not alter the beta diversity of the bacterial community in the sandy soil, whereas it was significantly altered in the fluvo-aquic soil (Figure 3a). In addition, the beta diversity of the fungal community significantly differed between the sandy and fluvo-aquic soils, whereas biochar amendment did not alter it in either soil (Figure 3b).



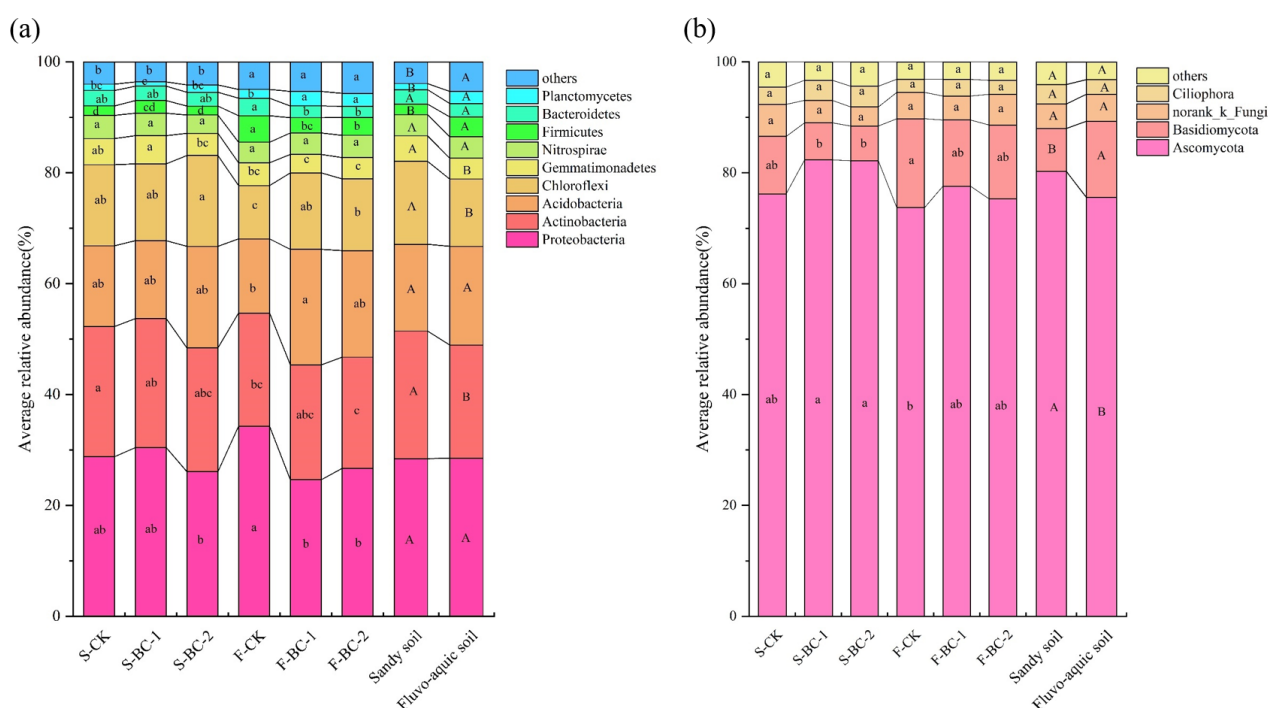
**Figure 2.** Bacterial (a,b) and fungal (c,d) alpha diversity under different biochar treatments across two contrasting soils. Note: Different lowercase letters indicate significant differences among the biochar treatments ( $p < 0.05$ ).



**Figure 3.** Principal coordinate analysis (PCoA) of bacterial (a) and fungal (b) community composition. Ordinations were generated from Spearman distance matrices calculated at the OTU level (97% identity).

### 3.4. Bacterial and Fungal Community Compositions

A total of nine bacterial phyla displayed average relative abundances above 1% in the examined soils, collectively representing over 95% of all recovered sequences (Figure 4a). *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* were predominant members of the bacterial community. Among these predominant members, the relative abundances of *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* responded significantly to biochar amendment in the fluvo-aquic soil but not in the sandy soil. In the fluvo-aquic soil, the average relative abundance of *Proteobacteria* was significantly decreased by both BC-1 and BC-2, whereas that of *Acidobacteria* was significantly increased by BC-1 but not by BC-2. The average relative abundance of *Chloroflexi* was significantly increased by both BC-1 and BC-2. *Ascomycota* dominated the fungal community, accounting for nearly 80% of total recovered sequences across treatments (Figure 4a). However, in both soils, no significant change was observed in the average relative abundance of *Ascomycota*.



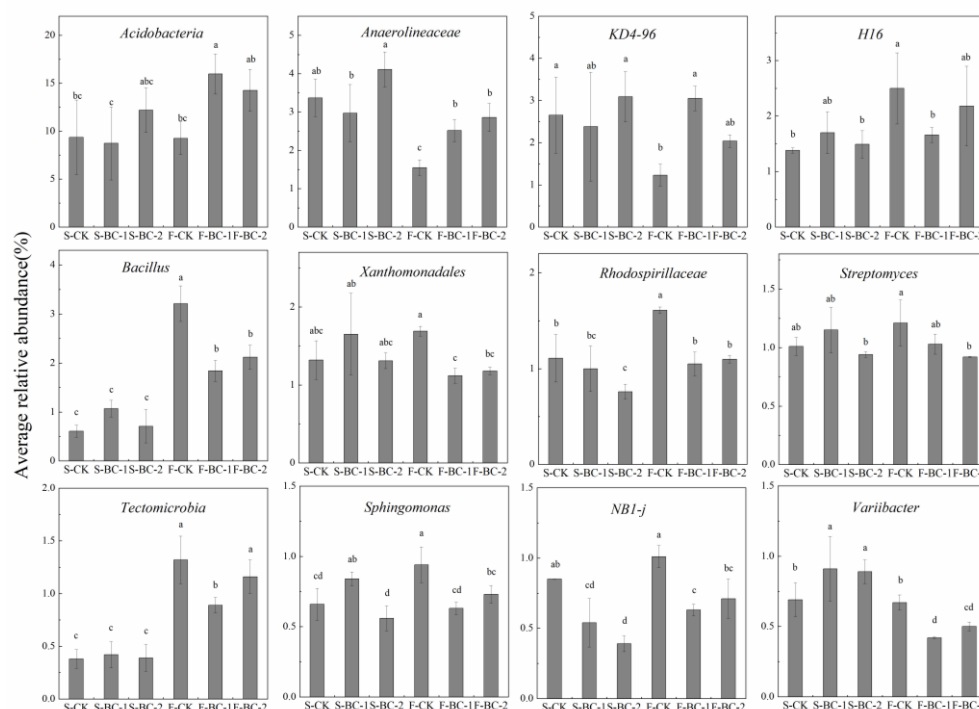
**Figure 4.** Dominant bacterial (a) and fungal (b) phyla composition across all treatments. Note: Different lowercase letters indicate significant differences among the biochar treatments for a given microbial taxon, and uppercase letters denote significant differences between two soils for a given microbial taxon ( $p < 0.05$ ).

When the two soils were compared ( $n = 9$  per group), *Actinobacteria*, *Chloroflexi*, and *Gemmatimonadetes* were more abundant in the sandy soil, whereas *Firmicutes* and *Planctomycetes* were enriched in the fluvo-aquic soil. Among fungi, *Ascomycota* dominated the sandy soil, while *Basidiomycota* showed greater representation in the fluvo-aquic soil.

The 35 most abundant bacterial genera together constituted approximately 60% of all sequences recovered at the genus level. In the sandy soil, only four genera exhibited significant changes in average relative abundance following biochar amendment. Specifically, biochar amendment enriched *Sphingomonas* and *Variibacter* but reduced *Rhodospirillaceae* and *NB1-j* (Figure 5). Conversely, the relative abundance profiles of 12 genera in the fluvo-aquic soil were significantly reshaped by biochar application. Specifically, biochar amendment enriched *Acidobacteria*, *Anaerolineaceae*, and *KD4-96*, but reduced *H16*, *Bacillus*, *Xanthomonadales*, *Rhodospirillaceae*, *Streptomyces*, *Tectomicrobia*, *Sphingomonas*, *NB1-j*, and

*Variibacter* (Figure 5). Among these significantly changed genera, four showed obvious changes in both soils: *Sphingomonas*, *Variibacter*, *Rhodospirillaceae*, and *NB1-j*. *Rhodospirillaceae* and *NB1-j* were suppressed by biochar amendment, whereas the remaining two genera were enriched.

Approximately 90% of all recovered sequences were attributed to the 20 most abundant fungal genera. However, none of them showed a significant response in average relative abundance to biochar amendment.



**Figure 5.** Significantly changed bacterial genera after biochar application. Note: Different lower-case letters indicate significant differences among the biochar treatments ( $p < 0.05$ ).

### 3.5. Correlation Analysis

According to the RDA, no measured soil property accounted for significant variation in bacterial community structure in sandy soil (Figure 6; Table 4). Bacterial community structure in fluvo-aquic soil was most strongly correlated with AK, SU, and UA (Figure 6; Table 4), whereas in sandy soil, SU alone accounted for a significant portion of fungal community variation; in the fluvo-aquic soil, no soil variable correlated with fungal community structure (Table 5). In the sandy soil, crop yield was not significantly correlated with dominant bacterial or fungal phyla but was positively correlated with OM, AK, and SU (Figure 7a). Conversely, in the fluvo-aquic soil, yield was positively correlated with *Chloroflexi* and *Acidobacteria*, as well as with AK and SU (Figure 7b).

**Table 4.** Correlations between bacterial community structure and soil properties.  $R^2$  and  $p$ -values were retrieved from the RDA on the community structures.

| Soil Variables | Sandy Soil |           | Fluvo-Aquic Soil |              |
|----------------|------------|-----------|------------------|--------------|
|                | $R^2$      | $p$ Value | $R^2$            | $p$ Value    |
| pH             | 0.233      | 0.488     | 0.095            | 0.766        |
| OM             | 0.251      | 0.431     | 0.348            | 0.261        |
| AN             | 0.202      | 0.471     | 0.594            | 0.079        |
| AP             | 0.464      | 0.162     | 0.098            | 0.716        |
| AK             | 0.101      | 0.774     | <b>0.787</b>     | <b>0.010</b> |

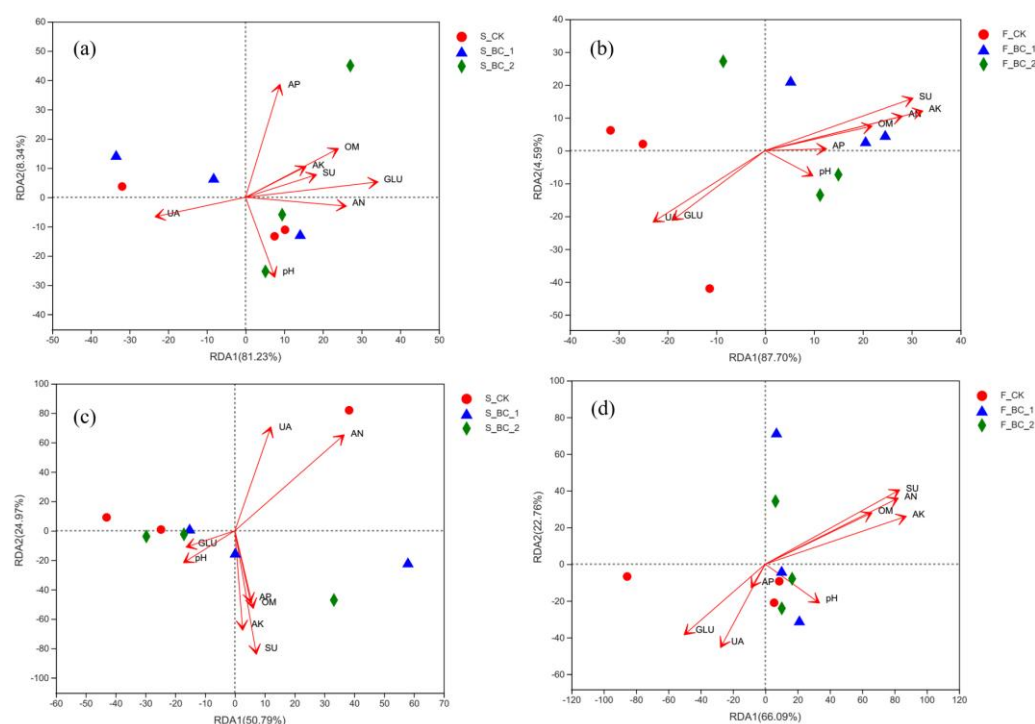
|     |       |       |              |              |
|-----|-------|-------|--------------|--------------|
| UA  | 0.172 | 0.577 | <b>0.657</b> | <b>0.040</b> |
| SU  | 0.114 | 0.708 | <b>0.775</b> | <b>0.016</b> |
| GLU | 0.357 | 0.290 | 0.530        | 0.111        |

Significant correlations at the  $p < 0.05$  level are indicated in bold.

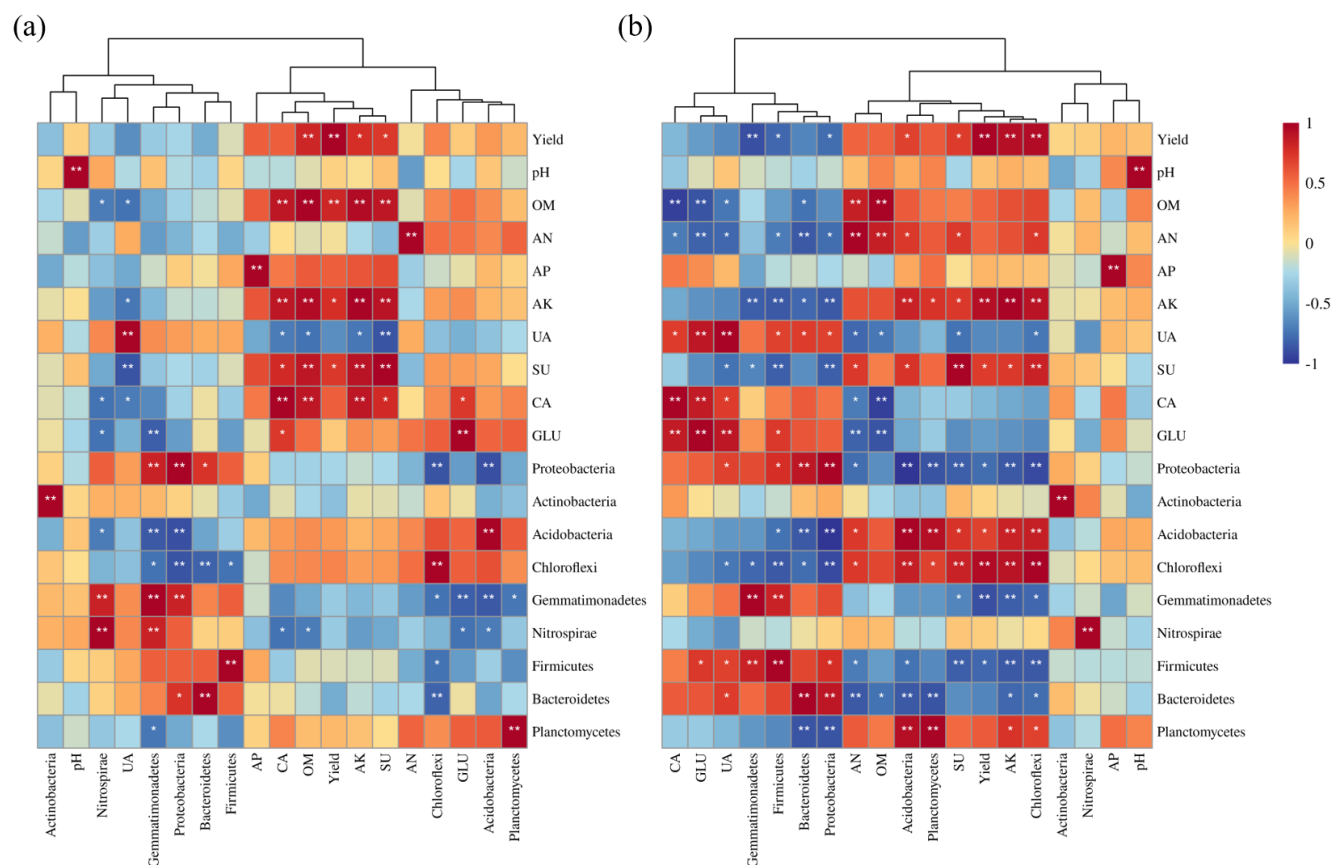
**Table 5.** Correlations between fungal community structure and soil properties.  $R^2$  and  $p$ -values were retrieved from the RDA on the community structures.

| Soil Variables | Sandy Soil   |              | Fluvo-Aquic Soil |           |
|----------------|--------------|--------------|------------------|-----------|
|                | $R^2$        | $p$ Value    | $R^2$            | $p$ Value |
| pH             | 0.070        | 0.829        | 0.079            | 0.723     |
| OM             | 0.266        | 0.406        | 0.270            | 0.387     |
| AN             | 0.545        | 0.131        | 0.429            | 0.138     |
| AP             | 0.228        | 0.474        | 0.011            | 0.976     |
| AK             | 0.437        | 0.160        | 0.441            | 0.142     |
| UA             | 0.493        | 0.096        | 0.147            | 0.625     |
| SU             | <b>0.688</b> | <b>0.022</b> | 0.455            | 0.113     |
| GLU            | 0.035        | 0.891        | 0.209            | 0.496     |

Significant correlations at the  $p < 0.05$  level are indicated in bold.



**Figure 6.** RDA analyses between community structures and soil physicochemical variables at the genus level: (a) bacteria in sandy soil; (b) bacteria in fluvo-aquic soil; (c) fungi in sandy soil; (d) fungi in fluvo-aquic soil.



**Figure 7.** Correlation heatmap of crop yield with soil properties in sandy soil (a) and fluvo-aquic soil (b). Note: \*  $p < 0.05$  and \*\*  $p < 0.01$ .

#### 4. Discussion

Biochar has a unique porous structure, is abundant in mineral nutrients, and possesses a relatively high C/N ratio, can enhance soil permeability, water retention, and nutrient retention, thereby promoting microbial activity [30–32]. In this study, biochar increased organic matter content in both fluvo-aquic and sandy soils in a dose-dependent manner (Table 2), consistent with previous findings [33]. This phenomenon can be attributed to two mechanisms. First, biochar directly contributes stable organic carbon to the soil [34]. Second, its large surface area and porous structure enhance the adsorption and retention of dissolved organic carbon, reducing carbon loss. Notably, the increase in organic matter was greater in sandy soil than in fluvo-aquic soil at the same application rate, likely due to the lower buffering capacity of sandy soil.

Biochar can alter soil pH through direct neutralization by its alkaline components [35] and by forming a buffer system via oxygen-containing functional groups [36]. Although biochar is commonly used to amend acidic soils [8,21], it had no significant effect on pH in the two alkaline soils tested (Table 2), indicating its limited pH-adjusting capacity under alkaline conditions. Biochar is rich in phosphorus and potassium, which can directly increase availability of P and K in soil, but its pyrolysis process leads to nitrogen volatilization, limiting direct N contribution. Nevertheless, biochar improved available N in fluvo-aquic soil, likely due to its ability to adsorb and retain fertilizer N, thereby inhibiting leaching [37,38]. Unexpectedly, biochar significantly increased available P in sandy soil but had minimal effect in fluvo-aquic soil (Table 2). Sandy soil has limited P sorption sites due to low clay and Fe/Al oxide content, allowing biochar-derived P to remain available. In fluvo-aquic soil, higher clay and oxide contents promote P adsorption and precipitation, buffering the increase in available P [39]. Overall, biochar affects soil physicochem-

ical properties through both direct and indirect pathways, with effects highly dependent on soil type.

The activities of soil enzymes are widely recognized as sensitive proxies for both soil quality and microbial function [40]. In this study, biochar significantly increased sucrase activity in both soils but decreased urease activity (Table 3). The increase in sucrase activity aligns with previous reports [41,42] and may reflect enhanced mineralization of active organic carbon, as the labile fraction of biochar and its porous structure can stimulate enzyme-producing microorganisms. The inhibitory effect on urease may reflect differences in biochar type and soil properties. Notably, biochar had contrasting effects on catalase activity: BC-2 increased catalase activity in sandy soil but decreased it in fluvo-aquic soil (Table 3). Catalase is an oxidation-reduction enzyme sensitive to soil aeration, and the effects of biochar on soil permeability vary with both biochar properties and soil type [43]. However, the precise mechanisms underlying these enzyme responses remain to be fully elucidated, as the observed changes may reflect a combination of direct effects of biochar on soil physical conditions and indirect effects mediated by nutrient availability and microbial community shifts.

Biochar can influence microbial diversity by providing favorable habitats [44,45] and indirectly by improving soil properties such as nutrient content and water holding capacity [46]. In this study, BC-2 increased bacterial alpha diversity in fluvo-aquic soil but had no effect in sandy soil (Figure 2). Similarly, biochar altered bacterial beta diversity in fluvo-aquic soil but not in sandy soil (Figure 3). Although fluvo-aquic soil is generally more fertile, sandy soil exhibited higher bacterial diversity in the absence of biochar (Figure 2), possibly due to years of fertilization and better permeability. Notably, despite having comparable organic matter and available N, sandy soil had higher available P (Table 2). The effects of biochar on bacterial diversity are context-dependent, with studies reporting both positive [47,48] and negligible [49,50] effects, highlighting the importance of biochar type, soil properties, and experimental conditions.

Compared with biochar application, soil type was the primary factor shaping bacterial and fungal community composition (Figure 3), underscoring the fundamental role of soil physicochemical properties. Biochar significantly altered the bacterial community composition in fluvo-aquic soil but had minimal effect in sandy soil (Figure 3). This differential response may be attributed to the higher organic matter content of the fluvo-aquic soil and its greater straw inputs resulting from higher crop yields, both of which would have shaped distinct initial microbial communities over time. At the phylum level, biochar decreased the relative abundance of *Proteobacteria* in fluvo-aquic soil, a trend reflected at the genus level by reductions in *H16*, *Xanthomonadales*, *Rhodospirillaceae*, *Sphingomonas*, *NB1-j*, and *Variibacter* (Figures 4a and 5). *Proteobacteria* typically thrive in organic-matter-rich environments [51]. Although biochar increased soil organic matter, the added carbon is largely recalcitrant and may not be readily utilized by many *Proteobacteria*. Indeed, soluble carbon in biochar is primarily consumed within one year [52]; our study examined microbial responses after two years, when labile carbon had likely been depleted. Additionally, biochar's porous structure may adsorb soluble organic carbon [53], further limiting its availability.

A marked enrichment of *Acidobacteria* was observed in the biochar-treated fluvo-aquic soil, which was contrary to expectations (Figure 4a), contrasting with many previous studies [47,48]. Although most *Acidobacteria* are considered acidophiles, different subphyla exhibit contrasting pH preferences [54], and some may respond positively to biochar [55]. The relative abundance of *Chloroflexi* in fluvo-aquic soil also rose significantly following biochar amendment (Figure 4a), consistent with previous reports [48,56].

In contrast to bacteria, fungal diversity and community composition were not significantly affected by biochar in either soil (Figures 2 and 3), diverging from several previous

studies [47,49,57]. Fungi are highly dependent on active organic carbon for metabolism and may be less responsive to mineral nutrients. A previous study suggested that easily mineralizable carbon in biochar, rather than insoluble aromatic carbon, drives fungal community composition [58]. However, >97% of biochar carbon consists of insoluble aromatic carbon, and labile carbon is typically depleted within one year [52]. Our study examined microbial responses two years after biochar addition, when the remaining carbon is largely recalcitrant. This observation should therefore be considered specific to the conditions of this study rather than a general rule, and transient effects occurring shortly after biochar application may have been missed by sampling at the two-year mark. Evidence from a three-year field trial indicated that biochar influences fungal diversity indirectly over the long term, primarily through alterations in soil properties [46]. Although biochar improved soil properties such as organic matter and available K in this study, these factors may not be the primary regulators of fungal communities in these soils.

Soil edaphic heterogeneity is recognized as a principal architect of microbial community assembly. In the present work, neither fungal community composition in either soil, nor bacterial community composition in the sandy soil, exhibited statistically discernible linkages to the measured edaphic variables or enzymatic activities (Figure 6; Tables 4 and 5). However, in fluvo-aquic soil, available K, urease, and sucrase were the variables most strongly correlated with bacterial community structure, and all of these were significantly affected by biochar addition (Tables 2 and 3). Consistently, correlation analysis showed that crop yield was correlated only with soil properties in sandy soil, whereas in fluvo-aquic soil, yield was correlated with both available K and the relative abundance of specific bacterial phyla (e.g., Chloroflexi and Acidobacteria) (Figure 7). These findings support recent evidence that the efficacy of biochar is strongly modulated by intrinsic soil properties [59]. It should be noted that the cost of biochar remains relatively high, and application rates of 10 and 20 t ha<sup>-1</sup> may hinder adoption at large scales. In the present study, however, biochar was applied only once, meaning that both the material cost and the application cost can be amortized over multiple growing seasons. Furthermore, yield increases in sandy soil were observed in three of the four crop seasons, indicating a reasonably consistent effect that strengthens the economic rationale for single-application strategies.

Nonetheless, the experimental design included one site per soil type and one biochar produced from wheat straw at a single pyrolysis temperature, and the findings may not extend to other soil types, climatic conditions, or biochar types. Multi-site studies using diverse biochars across broader geographic regions are needed to validate the generalizability of these results. Nonetheless, the clear contrasts observed between the two soil types provide a useful basis for hypothesis generation and future experimental design.

## 5. Conclusions

This two-year field study demonstrated that biochar significantly influences the physicochemical properties and enzyme activities of sandy and fluvo-aquic soils. Soil type exerted a stronger influence than biochar application rate, underscoring the critical role of soil properties in mediating the effects of biochar. Biochar did not significantly affect fungal diversity or community structure in either soil type. In sandy soil, biochar had negligible effects on bacterial diversity and community composition, whereas in fluvo-aquic soil it increased bacterial diversity and significantly altered community composition. At the phylum level, biochar markedly increased the relative abundance of Acidobacteria and Chloroflexi but decreased that of Proteobacteria in fluvo-aquic soil. Redundancy analysis (RDA) revealed that variations in bacterial community composition in fluvo-aquic soil were most strongly correlated with available potassium, urease activity, and sucrase activity. Furthermore, yield increases were accompanied by soil-type-specific changes: di-

rect physicochemical improvements in sandy soil, and combined physicochemical and bacterial community changes in fluvo-aquic soil, suggesting that the underlying mechanisms may differ between the two soil types. Collectively, these findings indicate that the effects of biochar on crop yield and soil microorganisms observed over a two-year period are strongly dependent on soil type, mediated by complex interactions among soil properties.

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