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Combination of Phytoextraction and Biochar Improves Available Potassium and Alters Microbial Community Structure in Soils

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Abstract: This study aimed to assess the effectiveness of combining phytoextraction and biochar for metal-polluted wetland soils by exploring the changes in soil biochemical properties, especially compared to the outcomes of single phytoremediation or biochar application. Soil biochemical properties serve as reliable indicators of soil quality and exhibit a high sensitivity to microbial community dynamics. Phytoextraction is via the native plants *Phragmites australis* (*P. australis*) and *Suaeda salsa* (*S. salsa*). The addition of biochar significantly increased the total organic carbon (TOC) and available potassium (AK) contents in the rhizosphere soil of *P. australis* and more in that of *S. salsa*. The effects of the combined remediation on the composition of the main classes of bacteria are uncertain, and the abundance of the main fungal classes decreased. At the level of OTU, no significant differences were observed in the richness and diversity of microbial communities between the single and combined remediation approaches. On a genus level, the combined remediation of biochar and *S. salsa* had the highest specificity of soil bacteria, while the single biochar remediation gave the highest specificity of soil fungi. At the class level, the four most abundant classes of bacteria were actinobacteria, alphaproteobacteria, gammaproteobacteria, and bacterricilineae. Biochar addition decreased the abundance of actinobacteria in *P. australis* rhizosphere soil but increased the abundance of actinobacteria in *S. salsa* rhizosphere soil. The sordariomycetes and eurotiomycetes were the dominant fungal classes. The combined remediation reduced the abundance of sordariomycetes, and the abundance of eurotiomycetes decreased after single phytoextraction, biochar, and combined remediation.

Keywords: soil heavy metal; phytoextraction; biochar; biochemical properties

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1. Introduction

The Yellow River Delta is a unique, young, and vulnerable wetland ecosystem, which is easily affected by human activities. In recent years, there has been more concerning heavy-metal pollution in the wetland due to upstream industrial pollution, oil exploitation, and excessive use of chemical fertilizers and pesticides. Cadmium (Cd) and lead (Pb) are the typical heavy metals in the study of soil heavy-metal pollution in this area [1–3]. Urgent steps must be undertaken to address the remediation of heavy-metal-contaminated soil.

Phytoremediation is one of the most favorable environmental and economical methods for heavy-metal remediation in soils. It degrades heavy metals in the soil through absorption, immobilization, and redox. Phytoremediation affects the soil's biochemical properties, especially within the rhizosphere, an ecologically crucial zone that extends no more than 2 mm from the root surface. Within the realm of phytoremediation, the rhizosphere emerges as a pivotal ecological field for the study of the intricate relationships between plant growth, soil elements, and microorganisms. It was found that the contents of total carbon (TC), total nitrogen (TN), and available phosphorus (AP) in the rhizosphere soil of *Phragmites australis* and *Suaeda salsa* were significantly higher than those in the non-rhizosphere soil [4], and the plants' root exudates serve as a vital carbon,

nitrogen, and nutrient source for microbes to ensure their normal growth and reproductive functions. This, however, shows the relationship between the chemical properties of soil and the microbial community. Soil physical and chemical properties are the main factors that change the species diversity and community structure of host roots and rhizosphere arbuscular mycorrhizal fungi [5]. Noteworthy studies by Yang et al. [6] underscore how phytoremediation brings about simultaneous shifts in soil characteristics and bacterial and fungal profiles. Other research [7] has also demonstrated that halophytes influence the soil micro-ecosystem by exerting effects on the physicochemical properties of soil. The Yellow River Delta region is facing the challenge of soil salinization, where saline–alkali land occupies more than 70% of the total area [8,9]. This research selected the high-salt-tolerant native plants *P. australis* and *S. salsa* as pioneer plants in remediating the wetland to regulate the content of heavy metals in the soil.

Biochar possesses an abundance of surface functional groups and a high carbon content that promote heavy-metal stabilization of heavy metals in soils. Currently, both local and international scholars have conducted several studies on the effect of biochar application on soil properties and bacterial community structure. Biochar, with high porosity and high carbon content, can effectively absorb nutrients in the soil, enhance water retention capacity, and reduce nutrient leaching [10]. Studies showed that the application of biochar significantly increased available potassium (AK), AP, organic matter content, and soil cation-exchange capacity [11,12]. It was also found that biochar significantly increased soil bacterial richness and changed the bacterial community structure, which stimulated microbial activity and growth [13]. The response of the soil microbial community to biochar was related to a variety of factors, such as soil type, soil depth, microbial species, and biochar addition dosages [14,15]. It was found that the most abundant bacterial phylum in black soil, proteobacteria, increased after biochar addition, while the most abundant phylum in red soil, chloroflexi, decreased [16]. Studies by Zhao et al. [15] showed that there was a decrease in the relative abundance of acidobacteria but an increase in actinobacteria with the biochar application. Meanwhile, the chemical properties of soil could affect the microbial community, and soil microorganisms would also affect its physical and chemical properties. Yan et al. [17,18] confirmed that the increase in soil nutrient content could promote fungal diversity after biochar addition; correspondingly, the arbuscular mycorrhizal fungal (AMF) community composition was significantly impacted by soil chemical properties, such as TC, TN, TP, and AP. And it was found that the root microorganisms of *S. salsa* have a negative correlation with soil salinity and nutrients [19].

The simultaneous use of biochar and phytoremediation may seem contradictory, as most research to date suggests that biochar reduces the bioavailability of heavy metals [20], but plants require high concentrations of soluble metals for their extraction and accumulation. We, therefore, have to look at the holistic effect of soil remediation. Studies have shown that biochar and phytoremediation can significantly improve the remediation efficiency of trace metals [21]. This could potentially be attributed to the capacity of biochar-based remediation to bolster plant growth by augmenting soil nutrient levels and inducing alterations in biological attributes [22,23]. Up to now, our study has found that this combined remediation method has a good remediation effect on the soil heavy metals Pb and Cd [24]. However, the effects of the combined remediation on soil biochemical properties are not clear. Thus, the aim of our work is to study the soil biochemical response after the use of biochar and phytoextraction for remediation purposes. We hypothesized that, although the efficiency of phytoextraction in this soil was reduced with the biochar application [25], the biochemical quality of the soil could be improved with a combination of phytoextraction and biochar addition.

2. Materials and Methods

2.1. Site Description

The current study was conducted in the Yellow River Delta restoration zone (37°45′36″–37°46′12″ N and 119°6′36″–119°9′0″ E) in Shandong province, China. This

region bears witness to the significant impact of human activities as it is under the burden of intensive anthropic interventions. In particular, the region faces significant challenges related to heavy-metal pollution and eutrophic pollution of agricultural waters with particular attention to the pervasive presence of Pb and Cd pollutants, which have significant impacts on wetlands. The main properties of the soil are shown in Table 1. The soil of the Yellow River Delta is mainly composed of silt and sand, which account for more than 90% [26]. The dominant vegetation species in this wetland are *Phragmites australis* and *Suaeda salsa* communities, which occupy the most extensive areas. Although their growth cycle is only for approximately six months, they are recognized as highly effective soil-remediation plants.

Table 1. Chemical properties of soil and biochar.

Parameters	Unit	Soil	Biochar
pH	-	8.65	9.15
EC	μS/cm	1725.00	2380.00
NH_{4+}-N	mg/kg	10.89	15.78
NO_{3-}-N	mg/kg	6.13	6.62
TOC	%	0.52	33.56
Available P	mg/kg	1.98	288.79
Available K	mg/kg	132.64	4335.98
Ca	g/kg	46.60	11.42

2.2. Experimental Design

After adding the heavy metals Pb and Cd ($Pb(NO_3)_2$: 125 mg/kg; $Cd(NO_3)_2$: 1.5 mg/kg) at concentrations five times higher than the background value but still within the plant tolerance, half of the contaminated study soils were added to biochar (BC) derived from *P. australis* straw pyrolysis. The pyrolysis temperature was 500 °C, and the pyrolysis time was 120 min, which proved to be a good preparation condition for the soil remediation [27]. The addition of biochar accounted for 1% of the soil. The experimental setup involved plastic pots (13 cm in diameter and 14 cm high) and polymethyl methacrylate columns (15 cm in diameter and 40 cm high). The plastic pots were used for breeding and selecting seedlings with 1.5 kg soil per pot. The height of the experimental columns (10 kg of soil per column) was selected based on the growth of *P. australis* roots, and the columns were shaded with black plastic films to create a dark underground environment.

The experiment was carried out in a greenhouse under natural light and a temperature of 25–30 °C. It was grown in two phases. In the first phase, some *P. australis* rhizomes were collected from the sample pots, and *S. salsa* seedlings were cultivated in the plastic pots (with BC and without BC). The soil moisture content was approximately 60% of the maximum field water capacity of the soil. After one month, eight *P. australis* seedlings with similar growth status were selected and transplanted into columns (four with BC and four without BC) in the second stage. The same treatment was applied to the *S. salsa* seedlings. The six treatments were designed and assayed (4 replicates): (1) No BC + No plant (CK), (2) BC + No plant (BC), (3) No BC + *P. australis* (P), (4) No BC + *S. salsa* (S), (5) BC + *P. australis* (BC-P), and (6) BC + *S. salsa* (BC-S). The soil columns were kept at a field capacity for 80 days and then extracted from surface or rhizosphere (for plants) soil for property tests. Approximately 5 g of fresh soil was collected from each soil column (took rhizosphere soil for plants) and stored in dry ice and an ultra-low temperature freezer (−80 °C) until they were used for high-throughput sequencing tests.

2.3. Determination of Soil Chemical Properties

The pH, EC, TOC, ammonium nitrogen (NH_4^+–N), nitrate–nitrogen (NO_3^-–N), available potassium (AK), available phosphorous (AP), and Ca were measured after 80 days at the end of the experiment. Soil pH was determined using a pH meter (PHS-3C, Shanghai Leici Instrument, Shanghai, China). The conductivity was determined using an EC-meter

(DDS^{-1}1A, Shanghai Leici Instrument, Shanghai, China). The TOC was determined using the high-temperature external hot potassium dichromate oxidation–volumetric method. The NH_4^+–N and NO_3^-–N were determined using a Smartchem200 Automatic Discontinuous Chemical Analyzer. The AK and Ca were determined using a Flame Photometer, and AP was determined using the $NaHCO_3$ extraction method.

2.4. Determination of Soil Microbial Community Structure

The diversity of the soil bacteria and fungi was tested using 16S rRNA and ITS, respectively. The specific methods are as follows.

2.4.1. Soil Total DNA Extraction and PCR Amplification

First, the DNA was extracted from the soil using the FastDNA@SPIN Kit. After genomic DNA extraction, 1% agarose gel electrophoresis was used to detect the content and purity of the extracted genomic DNA. After qualification, the primers 338F(5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R(5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the bacterial DNA. The fungi DNA was amplified using ITS1F(5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R(5′-GCTGCGTTCTTCATCGATGC-3′).

2.4.2. Database Construction, Sequencing, and Processing

After the purification of the PCR products, they were quantified and homogenized with a Picogreen dye fluorometer and were sequenced using the Illumina Miseq sequencing platform of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The sequence quality was controlled and filtered. After the samples were distinguished, the RDP Classifier Bayesian algorithm was used to conduct OUT (Operational Taxonomic Units) clustering and taxonomic analysis on OTU representative sequences at 97% similar level (the confidence threshold was 0.7). The Silva database was used for bacterial 16S rRNA, and the Unite database was used for fungal ITS.

2.5. Statistical Analyses

Statistical analyses were performed using SPSS 17.0 and Excel 2019. Analysis of variance was used for testing the differences in the soil pH, EC, TOC, NH_4^+–N, NO_3^-–N, AK, AP, and Ca among treatments. Differences were considered significant at $p < 0.05$. The alpha diversity was analyzed using Mothur software (Version 1.30.2). Qiime software (Version 1.9.1) was used to calculate the abundance and Beta diversity distances. The relationships between the soil microbial communities and soil properties were conducted using library corrplot in R. Origin 2018 software was used for mapping.

3. Results

3.1. Soil Chemical Characteristics

In each treatment, the pH value and concentrations of AP and Ca did not change significantly but increased slightly compared with the control group (Figure 1). After adding biochar, the soil salinity in the rhizosphere soil of *P. australis* and *S. salsa* decreased by 9.84% and 8.72%, respectively, compared to that in the P and S treatments. However, the adding of biochar significantly increased the total organic carbon content of the plant rhizosphere soil ($p < 0.05$). The total soil organic carbon content in the *P. australis*- and *S. salsa*-planted soils increased by 48.51% and 51.75%, respectively. Compared to CK, the TOC in the BC-treated soil increased by 31.13%. There were no significant differences observed in the contents of NH_4^+–N and NO_3^-–N among all the treatments. Furthermore, the concentration of AK had a similar variation trend as TOC in the treatments. The biochar significantly increased the AK content in the plant rhizosphere soil ($p < 0.05$), especially in the *S. salsa* rhizosphere soil, by 39.72%.

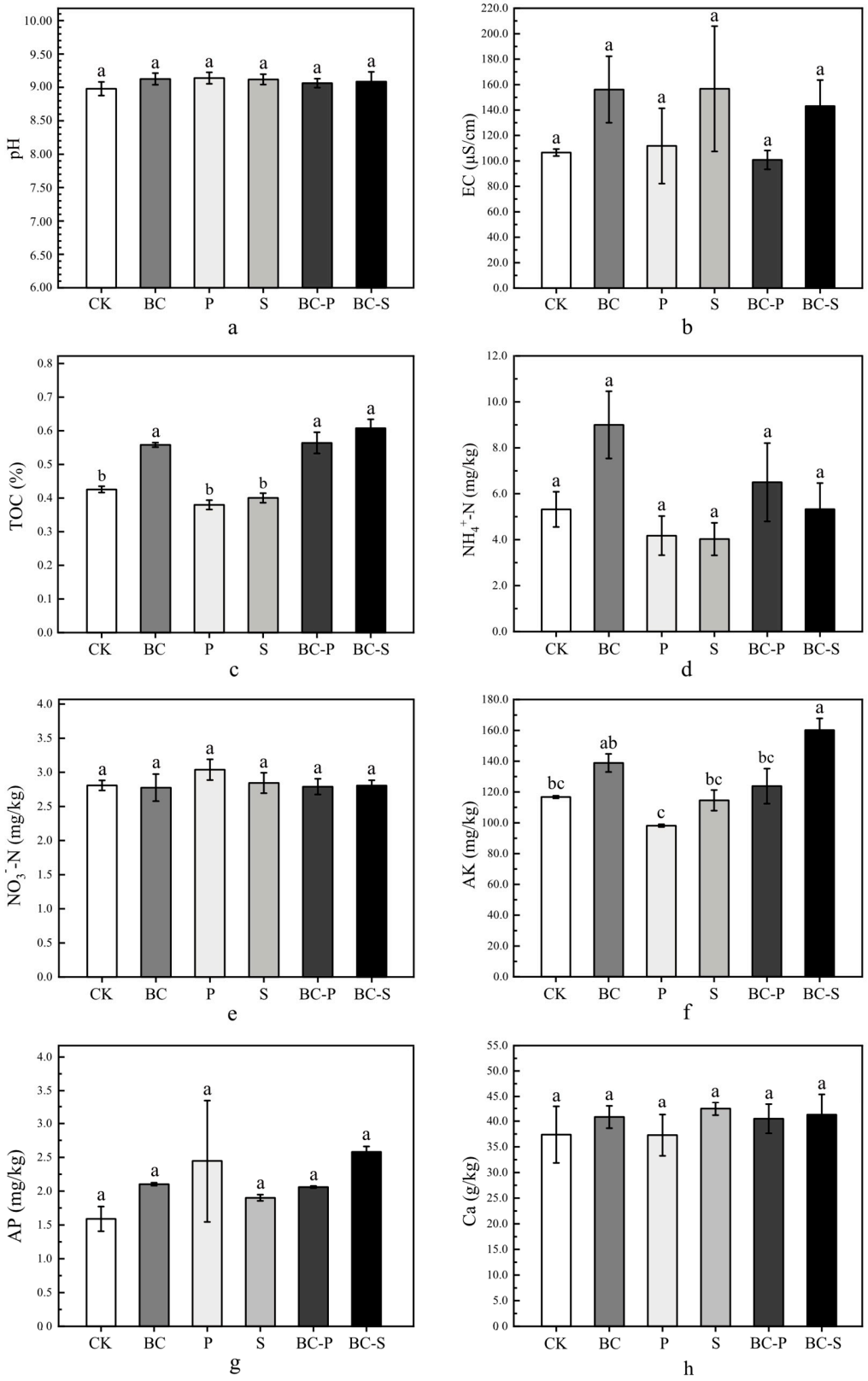

Figure 1. Figure (**a**–**h**) shows the differences in soil chemical properties across treatments. The six treatments represent CK (No BC + No plant), BC (BC + No plant), P (No BC + *P. australis*), S (No BC + *S. salsa*), BC-P (BC + *P. australis*), and BC-S (BC + *S. salsa*). The letters abcd were used to indicate the significant differences in the data. Non-shared letters indicate a significant difference ($p < 0.05$).

3.2. Bacterial and Fungal Community Richness and Diversity

OTU clustering on non-repetitive sequences, selected sequences that are more than 97% like the representative sequence, was performed, and the RDP classifier Bayes algorithm was used to analyze the OTU with 97% similarity. A total of 5804 bacterial OTUs were obtained from whole soil samples. We used the Chao richness index and Shannon diversity index to characterize and compare the richness and diversity of the bacterial communities in the different treatments. The Chao richness index ranged from 4078.57 to 4892.90, while the Shannon diversity index ranged from 5.86 to 6.53. The Wilcoxon rank-sum test showed no significant differences between the index groups for Chao and Shannon ($p > 0.05$) (Figure 2a,b). Compared to the blank control, the richness and diversity of the soil bacterial communities showed no variation across the experimental treatments.

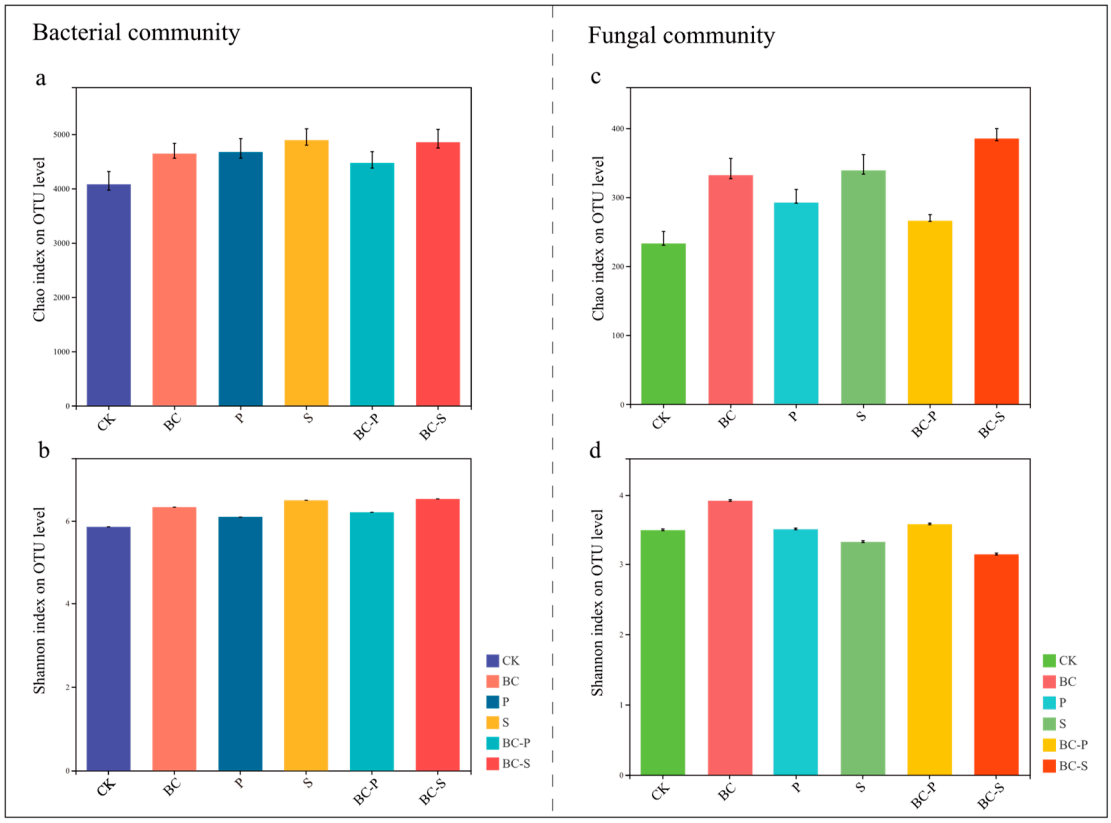

Figure 2. Comparative analysis of the alpha diversity of the bacterial and fungal communities in different treatments. (**a**) Chao index for bacterial community: the index of chao1 algorithm to estimate the number of bacterial community OTU contained in the sample. (**b**) Shannon index for the bacterial community. (**c**) Chao index for fungal community: the index of the chao1 algorithm to estimate the number of fungal community OTU contained in the sample. (**d**) Shannon index for the fungal community.

The OTU acquisition method of soil fungi was consistent with that of bacteria. The richness, represented by the Chao index and ranging from 233.00 to 385.20, was similar among the different treatments. As shown in Figure 2c, all treatments increased the soil fungal species richness compared to that of the control, and the fungal species richness was higher in the soil treated with *S. salsa* and biochar. The Shannon index indicated that the soil surface fungal community diversity was higher only with biochar addition (Figure 2d).

3.3. Soil Bacterial and Fungal Community Composition

A total of 5805 OTU sequences were detected with 16S rRNA sequencing in 51 phyla, 168 classes, 391 orders, 637 families, 1173 genera, and 2224 species of bacteria. There were

799, 899, 906, 949, 879, and 983 bacterial genera in the soils of the CK, BC, P, S, BC-P, and BC-S treatments, respectively. The BC-S treatment had the highest number of bacterial genera in the soil. The species Venn diagram represents the similarity and difference of species classification in the different bacterial communities of the treatments at different levels. The color overlapping part is the number of common genera in the different treatments. As shown in Figure 3a, at the genus level, there were 631 common genera of soil bacteria in all treatments, accounting for 53.79% of the total bacterial genera. Among them, there were 38 specific bacterial genera in the soil treated with BC-S, and in addition, 9, 17, 12, 17, and 13 genera were specific to the soil of the CK, BC, P, S, and BC-P treatments. The combined remediation of biochar addition and *S. salsa* planting had the highest specificity of soil bacteria.

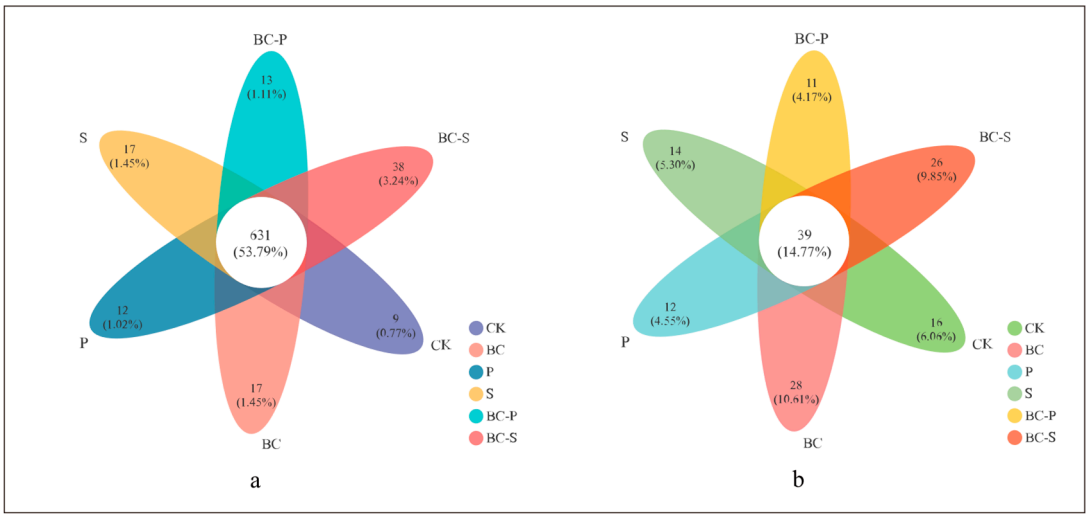

Figure 3. (**a**) Venn of soil bacterial genus level composition in the phytoremediation and biochar remediation of soil treatment; (**b**) Venn of soil fungal genus level composition in the phytoremediation and biochar remediation of soil treatment.

The soil samples also contained abundant fungal community information (Phylum: 13, class: 36, order: 78, family: 159, genus: 264, species: 408, OTU: 820). There were 101, 135, 104, 123, 100, and 145 fungal genera in the soils of the CK, BC, P, S, BC-P, and BC-S treatments, respectively. The soils treated with BC and BC-S had the most genera. As shown in Figure 3b, analyzed at the genus level, there were 39 fungal genera in the soils of each treatment, accounting for 14.77% of the total number of fungal genera, reflecting a great difference in the level of fungal genera under the different treatments. Among them, the BC-treated soil had the most specific fungal genera at 28, while the BC-P-treated soil had the least number of unique fungal genera at 11. The number of unique fungal genera in the CK-, P-, S-, and BC-S-treated soils was 16, 12, 14, and 26 genera. From the genus level, only the biochar treatment had the highest specificity of soil fungi.

In decreasing order of abundance, the four most abundant bacterial classes were actinobacteria, alphaproteobacteria, gammaproteobacteria, and bacterricilineae (Figure 4a). The abundance of actinobacteria decreased after treatment, especially in the S- and BC-S-treated soils, while the abundance of alphaproteobacteria was higher than that in the other treatments. The similarity of the soil bacterial community composition was divided into two parts (Figure 4a): BC-, P-, and BC-P-treated soils had similar bacterial community composition, while S and BC-S had similar bacterial community composition. It showed that *S. salsa*-planted soil had a great difference compared to the other treatments.

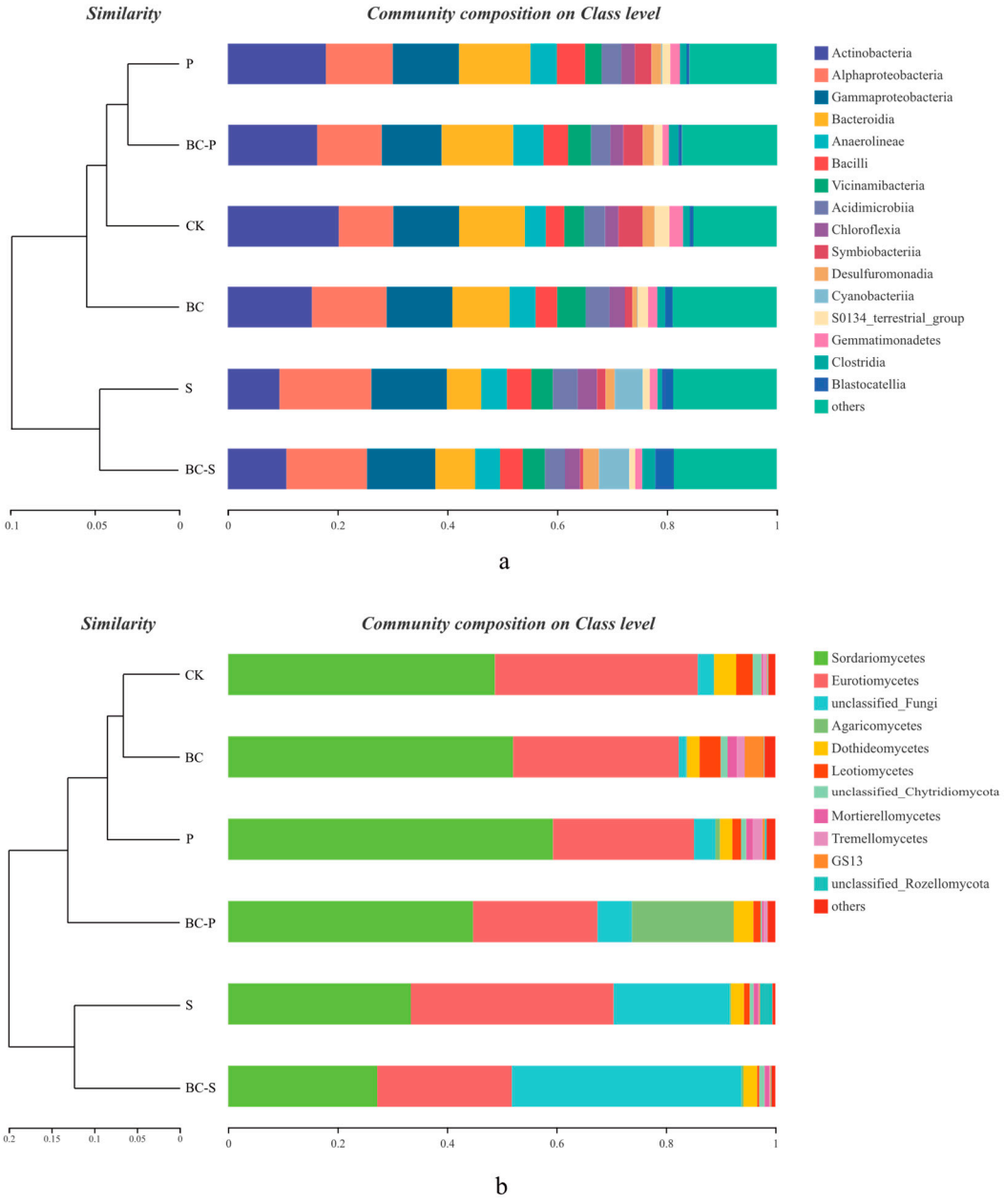

Figure 4. (**a**) Soil bacterial community composition on the class level in the different treatments; (**b**) Soil fungal community composition on the class level.

In decreasing order of abundance, the two most abundant fungal classes were sordariomycetes and eurotiomycetes. Meanwhile, there was a high abundance of unclassified classes in the S- and BC-S-treated soils (Figure 4b). The agaricomycetes were the special class in the BC-P-treated soils, while sordariomycetes were most abundant in the P-treated soils. Fungi and bacteria showed agreement on the similarity of community composition across the treatments. The fungal community composition of the S- and BC-S-treated soils was different from that of the others, which showed greater similarity.

4. Discussion

4.1. Combining Phytoextraction and Biochar Significantly Improved Soil Nutrients

As a polyhaline plant, *S. salsa* root can accumulate a high amount of salt in the soil and change soil salinity levels [28]. However, biochar addition had a negative effect on the soil salinity of the plant roots in this study. This could be attributed to the fact that

biochar helps plants grow and imbibe some of the salt. Furthermore, biochar contains a large amount of alkali metal ions and abundant cation-exchange sites, which can alter the content of salt-based ions when applied [29]. The results showed that the soil TOC content decreased slightly after planting *P. australis* and *S. salsa*, while it increased significantly after adding biochar. Since plant growth absorbed many nutrients in the soil, it would result in a decrease in soil fertility. Biochar, as an essential biomass, contains numerous nutrients after the pyrolysis of protoplasts and cell walls. When these nutrients are released into the soil, they can replenish soil fertility. The properties of *P. australis* biochar in this study were shown in Table 1, indicating abundant nutrients, such as TOC, AP, AK, etc. Most of the nitrogen in the biochar was organic nitrogen and could not be absorbed and utilized directly. The biochar had specific functional groups on its surface, which adsorbed NH_4^+–N to the soil and, thus, significantly improved its nitrogen-retention capacity [30,31]. And there was no significant effect with NO_3^-–N, which was probably because the biochar could decrease NO_3^-–N leaching and reduction in the soil and promote its absorption and reduction by the root [32]. In this study, the biochar addition significantly increased the content of AK, especially in the *S. salsa*-planted soil. On the one hand, the biochar produced a large amount of soluble potassium after high-temperature pyrolysis, which directly supplied the soil's available potassium and promoted plant growth, including the absorption and utilization of potassium [33]. Also, it could effectively reduce the leaching of available nutrients in the soil. The abundant acidic functional groups on its surface could adsorb free potassium in the soil and form multi-molecular layers [34]. Novak et al. [35] showed that the addition of 2% biochar can increase AK in low-fertility soil by 106%, which was consistent with the results of this experiment.

4.2. Combining Phytoextraction and Biochar Changed Bacterial Community Composition

In the level of OTU, the richness and diversity of the bacterial community showed no significant differences between the groups. There is uncertainty in the effect of biochar on soil microbial abundance. Moderate amounts of biochar increased the abundance and diversity of the soil bacterial communities, but beyond a certain dose, the number of bacterial OTUs decreased, and the abundance of pathogenic bacteria increased [36]. At the genus level, the soil planted with *S. salsa* and biochar had the most specific bacterial genera. Studies showed that biochar addition could promote the growth of some types of bacteria while inhibiting the growth of some bacteria, resulting in changes in the structure of the soil bacterial community [11,37]. On the class level, the abundance of the dominant bacterial community changed to some extent. The biochar addition reduced the abundance of actinobacteria, promoted the decrease in the actinobacteria abundance in the *P. australis* rhizosphere soil, and inhibited the decrease in the actinobacteria abundance in the *S. salsa* rhizosphere soil. In this study, after biochar addition, the abundance of actinobacteria was significantly negatively correlated with TOC ($p < 0.05$) and AK ($p < 0.001$) (Figure 5), which could be due to the increase in TOC and AK after biochar addition that led to its abundance decline. The alphaproteobacteria and gammaproteobacteria both belong to proteobacteria. In this study, proteobacteria were the dominant phylum. It had been revealed that proteobacteria had strong tolerance and stability in soils contaminated by heavy metals, especially in soils contaminated with Pb and Cd in this study [38]. The research showed that biochar addition had little effect on the relative abundances of proteobacteria, while phytoremediation could increase the relative abundances of alphaproteobacteria and gammaproteobacterial [39], which was consistent with the results of this study. The abundance of bacteroidia was not affected by biochar addition. However, it decreased in the *S. salsa* rhizosphere soil and increased in the *P. australis* rhizosphere soil. This was probably related to the characteristics of the vegetation. It was that the abundance of bacteroidia was significantly correlated with soil salt content, and it was lower in the soil of the heavy salt-tolerant vegetation than in the light salt-tolerant vegetation [39].

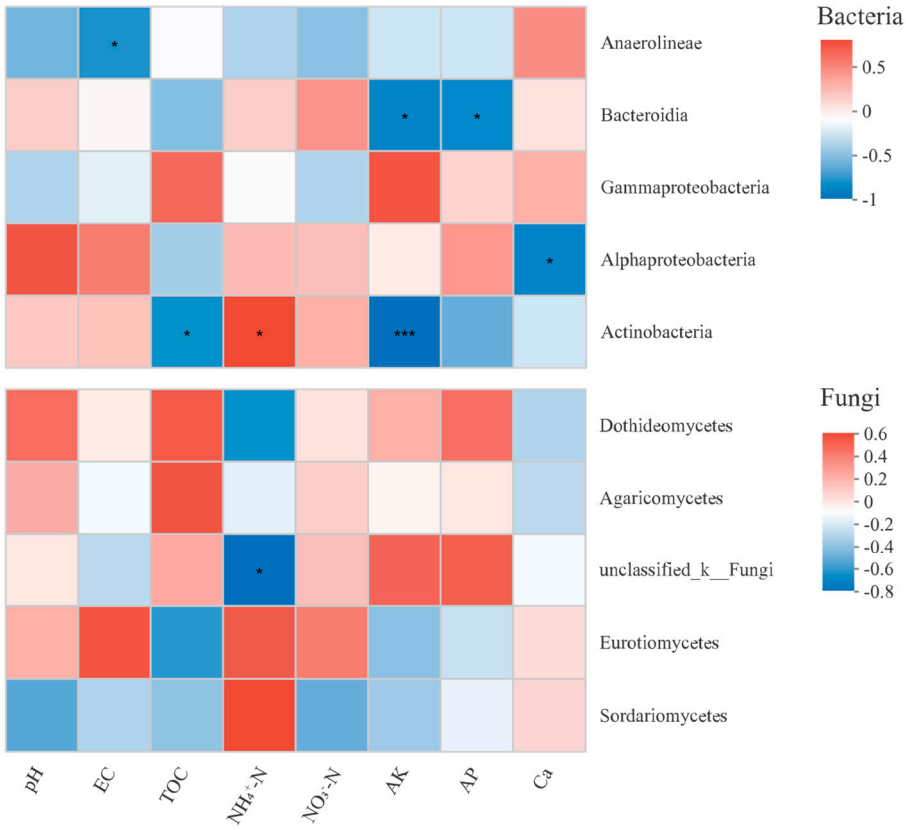

Figure 5. Spearman correlation heatmap of soil properties and soil bacterial (**up**) and fungal (**down**) community structures in the BC, BC-P, and BC-S treatments. * $p \leq 0.05$, *** $p \leq 0.001$.

4.3. Combining Phytoextraction and Biochar Changed Fungal Community Composition

At the OTU level, no differences in the fungal community richness and diversity were detected between the groups. At the genus level, the soil with the addition of only biochar had the most specific fungal genera. As decomposers in the soil, the fungi showed a higher utilization capacity of biochar and a more significant change in community structure compared to bacteria [40]. On the class level, sordariomycetes, eurotiomycetes, and dothideomycetes were the dominant fungal classes. They both belong to the ascomycota, which can decompose refractory organic matter [41]. The only-biochar addition and only-*P. australis* planting increased the abundance of sordariomycetes. It was obvious that the combined remediation reduced the abundance of sordariomycetes. In Zhao's [19] study, the fungi with the highest relative abundance in the *S. salsa* wetland and *P. australis* wetland were sordariomycetes, which had a significant negative correlation with soil organic matter. For this study, the abundance of sordariomycetes had a non-significant negative correlation with TOC and AK in the soil-added biochar (Figure 5). Perhaps that was why the combined remediation reduced its abundance. For eurotiomycetes, all treatments reduced their abundance, and the combined remediation had a more significant decreasing effect. This is consistent with previous studies, which reported that biochar addition not only reduces the abundance of eurotiales in plant roots and promotes the growth of biomass but also suppresses plant pathogenic fungi in soils and improves plant resistance [42,43].

5. Conclusions

Adding biochar significantly increased the TOC and AK contents in the *P. australis* rhizosphere soil and more in the *S. salsa* rhizosphere soil. On a genus level, the combined remediation of biochar and *S. salsa* had the highest specificity of soil bacteria, while the single biochar addition had the highest specificity of soil fungi. At the class level, the four most abundant bacterial classes were actinobacteria, alphaproteobacteria, gammaproteobac-

teria, and bacterricilineae. The biochar addition promoted the decrease in actinobacteria abundance in the *P. australis* rhizosphere soil and inhibited the decrease in actinobacteria abundance in the *S. salsa* rhizosphere soil. At the class level, sordariomycetes and eurotiomycetes were the dominant fungal classes. The single biochar and single *P. australis* remediation increased the abundance of sordariomycetes, while the combined remediation reduced its abundance. The abundance of eurotiomycetes decreased after single phytoextraction, biochar, and combined remediation. In conclusion, the combined remediation increased soil nutrients. The effects of the combined remediation on the composition of the main classes of bacteria were uncertain, while the abundance of the main classes of fungi was decreased.

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