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Keystone Microbial Species Drive the Responses of Saline–Alkali Soil to Three-Year Amendment Measures

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Abstract: Saline–alkali soils exhibit ionic toxicities associated with neutral salinity, as well as a high pH that hinders the exclusion of sodium ions and absorption of vital nutrients; thus, obstructing the development of coastal shelterbelts. A three-year field experiment using a high-soil-pH site was conducted for this study to investigate the influences of five prospective amendments on the soil microenvironments of different soil layers compared to a control. Firstly, the bacterial phyla Proteobacteria, Firmicutes, and Actinobacteria were found to be the most predominant in the samples. As for the fungi phylum, Ascomycota was identified as the most abundant. Similar to Module 1's findings, the relative abundances of Ascomycota varied across treatments. Additionally, differences in the ACE index were primarily observed in the deeper soil layers, where all five soil amendments increased the bacterial ACE index compared to the CK (no additive). Only the BA (biochar mixed with arbuscular mycorrhizal fungi) and AM (arbuscular mycorrhizal fungi on its own) treatments significantly increased the fungal ACE index. In the 20–40 cm soil layer, the pH value of the control group was significantly higher than that of all other treatments, except for the AM treatment. However, the AM treatment induced significantly higher soil enzyme activities and available nutrients compared to the CK. Moreover, the Mantel test showed significant correlations between the Module 1 community, the generalist (microbial species that serve as module hubs and connectors, primarily for Acidobacteria) community and soil pH, electrical conductivity, enzyme activities, as well as bacterial and fungal ACE indices. Pearson's correlation revealed a significantly positive association between enzyme activities and available nutrients. Our findings suggested that keystone microbial species have the potential to improve the availability of soil nutrients through the regulation of microbial diversity and stimulation of soil enzyme activities, to ultimately ameliorate saline–alkali soil. Furthermore, the application of AM in combination with an appropriate amount of biochar is a preferred strategy for the improvement of saline–alkali soils.

Keywords: amendment measure; saline–alkali soil; soil environment; microbial community structure; keystone microbial species

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1. Introduction

With intensifying global warming, rising sea levels have accelerated the terrestrial invasion of seawater, which exacerbates the salinity of coastal lands [1]. Currently, there are more than 1 billion ha of coastal soils damaged by salt, ~60% of which are considered sodic, high in pH, and rich in sodium ions [2]. Even more troubling is that high soil pH decreases the pace at which vital nutrients are absorbed and the rate at which sodium ions are excluded, in addition to the fact that saline–alkali soils possess the ionic toxicity of neutral salinity [3]. These soil conditions have more serious detrimental effects on plant

growth in contrast to salinity alone [4]. However, the current lack of understanding of alkali stress has severely restricted afforestation in saline–alkali soils.

To improve saline–alkali soils, agrohydrotechnical land and water management techniques, bioengineering methods, and chemical amelioration have long been employed and extensively used worldwide [5]. However, these expensive procedures can inflict long-lasting damage on ecosystems [6]. Biochar and straw have garnered increasing attention due to their accessibility and affordability as potential materials for the remediation of saline–alkali soils [7–10]. They are eco-compatible organic materials that have been shown to be effective for increasing the organic matter content of soil [11,12]; enhancing soil porosity and aeration [13]; optimizing the structures and stability of soil aggregates [12]; minimizing nutrient leaching [14,15]; and stimulating enzyme activities [16]. Moreover, the release of nutrients and organic matter during their decomposition has various positive effects on the soil microenvironment [17,18]. Further, straw serves as a capillary barrier to prevent the evaporation or transpiration of dissolved salts from water in the subsoil to the topsoil [19]. Unfortunately, many studies have focused on the control of salt buildup; thus, the effects of high pH and alkalinity on soil physicochemical properties and microbial communities have been somewhat neglected [10,20].

Arbuscular mycorrhizal fungi (AMF) are widely distributed symbiotic partners of plant roots in various terrestrial ecosystems, which facilitate the exchange of nutrients and water between plants and soil [21]. Mycorrhizal symbionts created by AMF play a critical role in the regulation of rhizospheric nutrition [22] by altering the microecological environment, and modifying soil microbial communities through various enzymes and growth regulators secreted by mycelia [23]. Simultaneously, mycorrhizal symbiosis secretes glomalin, which stabilizes the overall structure of the microenvironment, regulates the activities of soil enzymes, and promotes nutrient absorption [24]. As soil remediation techniques become more available, researchers have focused on the combined application of AMF and organic materials [25,26]. However, most of these studies involved only pot trials; thus, validations through field trials have been absent. Further, the high sensitivity of AMF to fertilizers [27] makes the restorative benefits of mixing AMF with organic materials in saline–alkali soils more unpredictable. In our previous studies, we demonstrated that AMF mixed with organic materials promoted plant growth by enhancing the structures of saline–alkali soil and regulating enzyme activities [28]. Unfortunately, there are no data on how the application of mixed AMF and organic materials in saline–alkaline soils affect microbial communities.

Soil microbes are crucial for the health of the soil [29], as they provide the ecosystem with services required for plant growth. Thus, there is an urgent need to elucidate the responses of key microorganisms to different soil amendments in saline–alkali soils. For this study, field trials were established to investigate the responses of keystone microbial species to amendment measures in coastal saline–alkali soil with high pH. The aim was to demonstrate the influences of five amendment measures (biochar on its own (B), biochar mixed with arbuscular mycorrhizal fungi (AMF) (BA), straw mixed with AMF (SA), straw on its own (S), and AMF on its own (AM)) on the soil pH, electrical conductivity, available nutrients, enzyme activities, microbial diversity, microbial community structures, and keystone microbial species in different soil layers compared with a control. Our hypotheses, which were in line with past investigations, were as follows: (1) soil amendment measures improve saline–alkali soils to varying degrees; (2) soil amendment measures affect microbial community structures and increase microbial diversity; (3) the mixed application of organic materials with AMF has the greatest impact on the soil microenvironment.

2. Materials and Methods

2.1. Study Area and Experimental Design

This study was conducted in Yancheng City, of Jiangsu Province, China (32°56′~33°36′ N, 120°13′~120°56′ E) from March 2018 to October 2021. This region is home to a typical sub-tropical monsoon climate, with an original soil electrical conductivity that ranges from

1.6 to 5 mS/cm. The average annual temperature is 14.1 °C, with 1042.2 mm of rainfall, and 230 frost-free days [28]. Six treatments were established for the experiment, including biochar on its own (B), biochar mixed with arbuscular mycorrhizal fungi (AMF) (BA), straw mixed with AMF (SA), straw on its own (S), and AMF on its own (AM), and a no additive control (CK).

After weeding and land preparation, the experimental field was divided into six treatment plots demarcated by soil ridges, which corresponded to the six treatments above, after which eighteen planting holes (40 × 40 × 40 cm^3) were dug at a planting density (3 m × 3 m) for each treatment. The research samples consisted of two-year-old *Taxodium* '*zhongshanshan*' saplings with average heights of 2.06 m and basal diameters of 2.90 cm, which were rooted and combined with 0.5 kg of organic materials and AMF, respectively, in March 2018. Straw (Dafeng Forestry Farm) was uniformly cut to cover the bottoms of the planting holes, while the biochar from rice husks (pyrolyzed at 800 °C) was fully mixed with the soil and loaded into the planting holes. The AMF inoculant was *Funneliformis mosseae* (Beijing Academy of Agricultural and Forestry Sciences). Subsequent to three months of propagation, the inoculant (colonized root segments, hypha, and spores (>7/g) mixed with yellow sand) was harvested and applied to the *Taxodium* '*zhongshanshan*' root systems.

2.2. Sample Collection and Preservation

Samples were extracted from the field in October 2021, where in each treatment plot, three *Taxodium* '*zhongshanshan*' plants with comparatively uniform growth were randomly selected. A total of 36 soil samples were extracted from the 0–20 cm and 20–40 cm soil layers of the root zone soil, preserved with ice packs, and transferred to the laboratory. Once all impurities were removed, the samples were divided into two portions. One portion was dried naturally to quantify the soil pH, electrical conductivity, nutrients, and enzyme activities, while the other was screened with a 2 mm mesh and frozen at −80 °C for DNA extraction.

2.3. Soil Physical and Chemical Analysis

The soil pH and electrical conductivity were tested using pH and conductivity meters, respectively, at a 1:5 (*w*:*v*) soil to water ratio, while the soil enzyme activities were determined following the description of Xue et al. [30]. Briefly, the soil urease activity was determined by quantifying the amount of ammonium released from the soil. The soil alkaline phosphatase activity was assessed using the phenyl phosphate disodium colorimetric method. The soil sucrase activities were measured via 3,5-diyl salicylic acid colorimetry, whereas the soil catalase activities were determined using the $KMnO_4$ titrimetric method. Further, the available soil nutrients were quantified according to the technique of Lu [31]. Briefly, the alkali-hydrolyzed nitrogen was measured using the alkali-hydrolyzed diffusion technique. The concentrations of available phosphorus and potassium were determined using $NaHCO_3$ extraction-colorimetry and the NH_4OAc extraction-flame photometry method, respectively.

2.4. DNA Extraction, Amplification, and MiSeq Sequencing

The total DNA from each of the 36 soil samples was extracted using a FastDNA® Spin Kit for Soil (MP Biomedicals, Irvine, CA, USA). The DNA extracts were examined using 1% agarose gel, and their concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The amplification of the bacterial 16S rRNA and fungal ITS rRNA genes was performed using primers as described by Liu et al. [32]. The purified PCR products were sequenced utilizing the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA). Finally, the sequence data were processed and analyzed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China, including gene splicing, clustering, classification annotation, and database comparisons.

2.5. Bioinformatics Analysis

The operational taxonomic unit (OTU) abundance data of all samples were drawn flat for bacteria and fungi, respectively, using the samples with the smallest quantity of sequencing data for each. Only OTUs that met certain abundance criteria were used for the co-occurrence network analyses to ensure the relative abundance of keystone species. RMT (random matrix theory) was successful in identifying 0.77 as the microbial network threshold, and iNAP (http://mem.rcees.ac.cn:8081/ (accessed on 18 June 2023)) was employed to determine all pair-wise Spearman correlations between OTUs. Gephi version 0.9.7 was used for the visualization of co-occurrence networks. The threshold values of Zi and Pi were set at 2.5 and 0.62, respectively, to determine the topological roles of individual nodes in the network.

2.6. Statistical Analyses

The soil-available phosphorus, alkali-hydrolyzed nitrogen, available potassium, as well as catalase, alkaline phosphatase, urease, and sucrase activities were Z-score-transformed, respectively. Subsequently, the standardized Z-score rates of the soil nutrients and enzyme activities were individually averaged to create indices for available nutrients and enzyme activities. The specific calculation formula was referenced to Li et al. [33].

The differences in soil pH, electrical conductivity, available nutrients (Z-score), enzyme activities (Z-score), and microbial community alpha diversity between the various amendments were analyzed using one-way ANOVA followed by a Duncan test, whereas the different soil layers were evaluated using *t*-test (SPSS 26.0 Inc., Chicago, IL, USA) and visualized using GraphPad Prism 9 (GraphPad Software, Inc., La Jolla, CA, USA). Co-occurrence network analysis was performed by RMT-based MENA (molecular ecological network analysis). To examine the relationships between the soil environment, microbial community alpha diversity, as well as the Module 1 and generalist communities, the Mantel test and correlation analysis were conducted using the statistical program R 4.2.2.

3. Results

3.1. Soil pH and Electrical Conductivity

The results indicated that, excluding the S and AM treatments, the pH of the soil in the 20–40 cm layer was significantly higher compared with the 0–20 cm layer ($p < 0.05$) (Figure 1A). Conversely, the electrical conductivity of the soil in the 20–40 cm layer was significantly lower under the B, SA, and AM treatments in contrast to the 0–20 cm layer ($p < 0.05$) (Figure 1B).

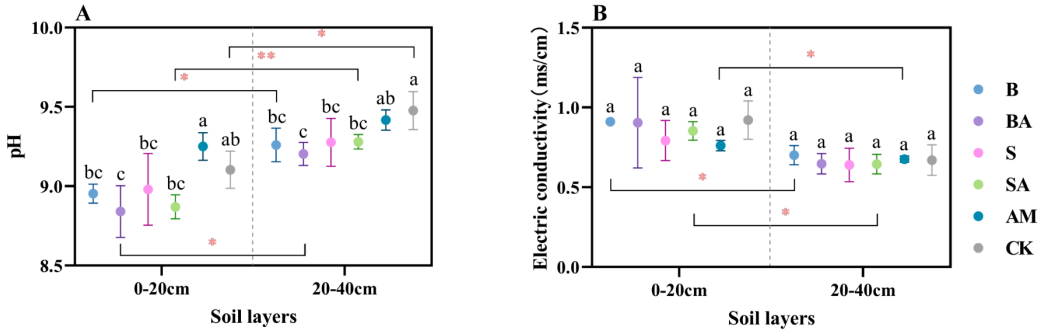

Figure 1. Variations in pH and electrical conductivity between soil layers and soil amendment treatments. (**A**) Changes in pH between soil layers and soil amendments; (**B**) changes in electrical conductivity between soil layers and soil amendments. Different lowercase letters indicate significant differences between various soil amendments in the same soil layer ($p < 0.05$). Error bars represent the standard deviation. * indicates $p < 0.05$; ** indicates $p < 0.01$. Abbreviations: B, biochar on its own; BA, biochar mixed with AMF; SA, straw mixed with AMF; S, straw on its own; AM, AMF on its own; CK, no additive.

For the 0–20 cm soil layer, the soil pH was found to be lower under the BA treatment compared with the CK treatment ($p < 0.05$), while for the 20–40 cm soil layer, the soil pH under the CK treatment was higher in contrast to the soil amendments, except for the AM treatment ($p < 0.05$) (Figure 1A). Further, there were no significant differences observed in the soil electrical conductivity under the various treatments between both soil layers (Figure 1B).

3.2. Soil Enzyme Activities and Available Nutrients

Enzyme activities in the 0–20 cm soil layer were generally higher than those in the 20–40 cm soil layer, except under the B and S treatments ($p < 0.05$) (Figure 2A). In terms of available nutrients, there was only a significant difference between the two soil layers under the BA amendment ($p < 0.05$) (Figure 2B).

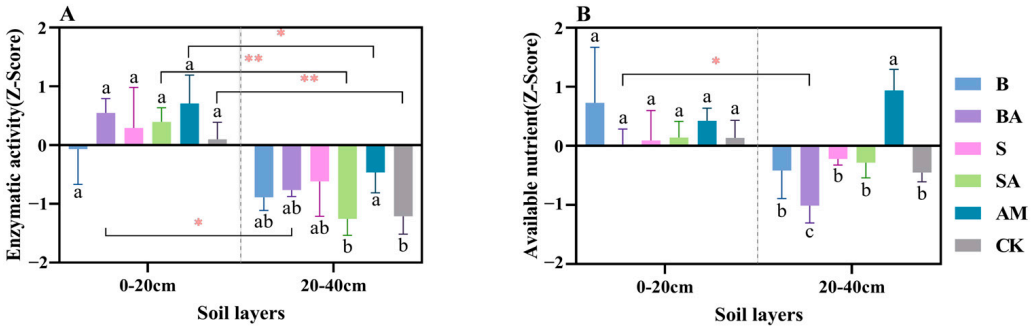

Figure 2. Variations in enzyme activities (Z-score) and available nutrients (Z-score) between soil layers and soil amendments. (**A**) Changes in enzyme activities (Z-score) between soil layers and soil amendments; (**B**) changes in available nutrients (Z-score) between soil layers and soil amendments. The interpretations of the letters, error bars, and asterisks are consistent with Figure 1. Abbreviations: B, biochar on its own; BA, biochar mixed with AMF; SA, straw mixed with AMF; S, straw on its own; AM, AMF on its own; CK, no additive.

In the 20–40 cm soil layer, the AM amendment resulted in higher soil enzyme activities than under the SA and CK amendments ($p < 0.05$) (Figure 2A). Moreover, the AM amendment led to higher available soil nutrients compared with the other amendments ($p < 0.05$). On the other hand, the BA amendment decreased the available soil nutrients in contrast to the CK amendment ($p < 0.05$) (Figure 2B).

3.3. Alpha Diversity of Soil Microbial Communities

Under the BA, S, and SA amendments the fungal ACE index for the 0–20 cm soil layer exceeded that of the deeper soil layer ($p < 0.05$) (Figure 3C). Conversely, under the AM amendment ($p < 0.05$) the bacterial ACE index for the 0–20 cm soil layer was lower than that of the deeper soil layer (Figure 3D).

Compared with the CK in the 20–40 cm soil layer, the B amendment decreased both fungal and bacterial Shannon indices, whereas the SA amendment increased the bacterial Shannon index ($p < 0.05$) (Figure 3A,B). Meanwhile, in the 20–40 cm soil layer, the fungal ACE index was higher under the BA and AM amendments than for the CK ($p < 0.05$) (Figure 3C), and all five soil amendments increased the bacterial ACE indices, in contrast to the CK ($p < 0.05$) (Figure 3D).

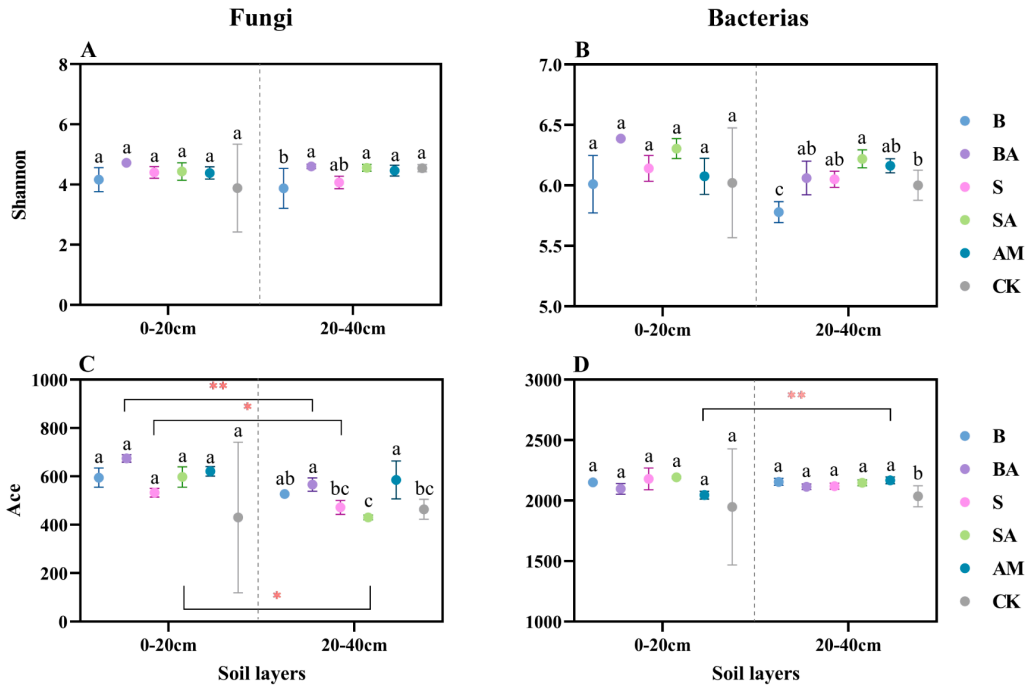

Figure 3. Variations in microbial community diversity indices between soil layers and soil amendments. (**A**) Changes in fungal Shannon index between soil layers and soil amendments; (**B**) changes in bacterial Shannon index between soil layers and soil amendments; (**C**) changes in fungal ACE index between soil layers and soil amendments; (**D**) changes in bacterial ACE index between soil layers and soil amendments. The interpretations of the letters, error bars and asterisks are consistent with Figure 1. Abbreviations: B, biochar on its own; BA, biochar mixed with AMF; SA, straw mixed with AMF; S, straw on its own; AM, AMF on its own; CK, no additive.

3.4. Soil Microbial Community Composition and Beta Diversity

The composition of fungal communities varied significantly between amendments as revealed by Ascomycota, which was the most prevalent fungal phyla. Relative to the CK (47.59% in the 0–20 soil layer, and 65.31% in the 20–40 soil layer), each soil amendment increased the relative abundance of Ascomycota in the 0–20 soil layer, while in the 20–40 soil layer their relative abundances were 38.31% (B), 64.87% (BA), 43.32% (S), 57.94% (SA), and 58.20% (AM), respectively (Figure 4A).

Proteobacteria, Firmicutes, and Actinobacteriota comprised the majority of bacteria at the phylum level, which accounted for more than half of the relative abundance of all phyla. Compared with the CK (13.23% in the 0–20 soil layer and 24.76% in the 20–40 soil layer), all soil amendments increased the relative abundance of Firmicutes in the 0–20 soil layer, while in the 20–40 soil layer their relative abundances were 31.85% (B), 25.80% (BA), 26.87% (S), 21.94% (SA), and 21.64% (AM), respectively (Figure 4B).

The microbial communities were found to be clustered by soil amendment treatments and soil layers, according to principal coordinate analysis, which explained 30.21% and 46.97%, respectively, of the total variations for fungi and bacteria (Figure 4C,D). Regarding fungal beta diversity, the first PCoA axis clearly distinguished the soil amendment treatments and soil layers (Figure 4C). Conversely, as regards bacterial beta diversity, the differentiation of soil amendment treatments and soil layers in the first PCoA axis was not obvious (Figure 4D).

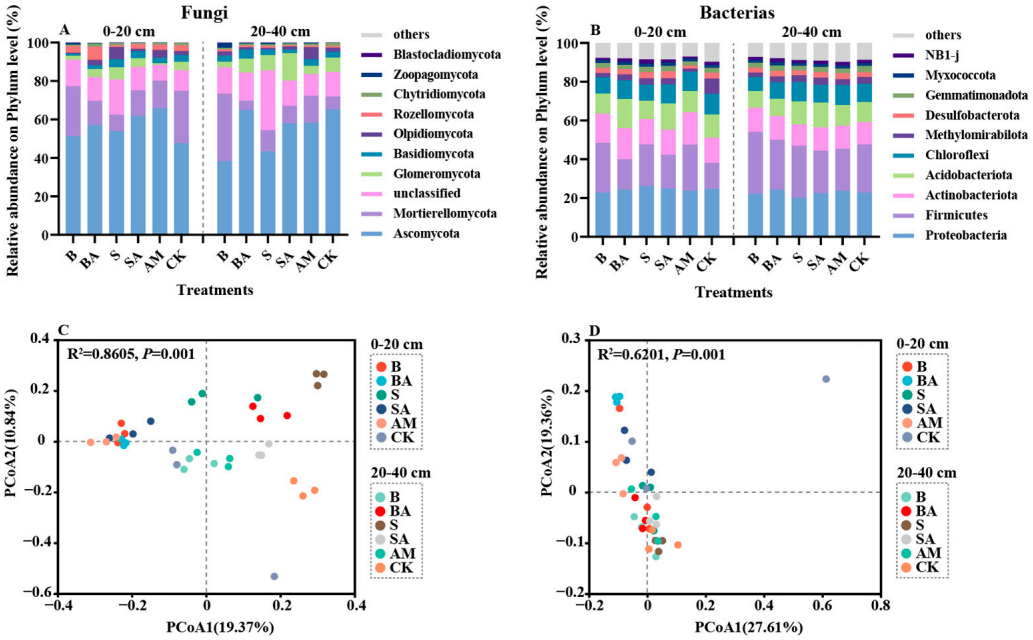

Figure 4. Variations in relative microbial abundance between soil layers and amendments. (**A**) Changes in relative fungal abundance at phylum level between soil layers and soil amendments; (**B**) changes in relative bacterial abundance at phylum level between soil layers and soil amendments; (**C**) principal coordinate analysis plot displaying the Bray–Curtis distance of fungi; (**D**) principal coordinate analysis plot displaying the Bray–Curtis distance of bacteria. Abbreviations: B, biochar on its own; BA, biochar mixed with AMF; SA, straw mixed with AMF; S, straw on its own; AM, AMF on its own; CK, no additive.

3.5. Co-Occurrence Network Analysis

The microbial network contained a total of 238 nodes and 441 linkages, with BOTU9061 having the maximum degree. Module 1 accounted for 23.11% of the co-occurrence microbial network, and its dominant phylum was Ascomycota (Figure 5A). Among them, the relative abundance of Ascomycota under the AM amendment was highest in the 0–20 cm soil layer, while that under the SA amendment was highest in the 20–40 cm soil layer. Further, in Module 1, the relative abundances of Actinobacteriota under the soil amendments were higher than those of the CK for both soil layers (Figure 5C).

Three nodes (BOTU7135, BOTU9061, and BOTU10239) were identified as module hubs in the microbial network and were highly interconnected to multiple nodes in their respective modules. Five nodes (BOTU3177, BOTU1489, BOTU1787, BOTU10624, and FOTU3213) were categorized as connectors and had strong connections to several modules. These nodes were the generalists, which might resemble the keystone species of the microbial communities predicted in network theory (Figure 5B). The generalists were primarily concentrated in Acidobacteria, and for each soil layer the relative abundances of BOTU3177 under the B, BA, SA, and AM amendments were higher than that of the CK (Figure 5D).

3.6. Correlations between Microbial Community Diversity, Soil Conditions, and Keystone Species

The Mantel test revealed that there was a significant correlation between the Module 1 community and various soil properties such as pH, electrical conductivity, enzyme activities, and bacterial and fungal Ace indices. Meanwhile, the generalist community was strongly related to the soil pH, electrical conductivity, enzyme activities, and microbial community alpha diversity (Figure 6).

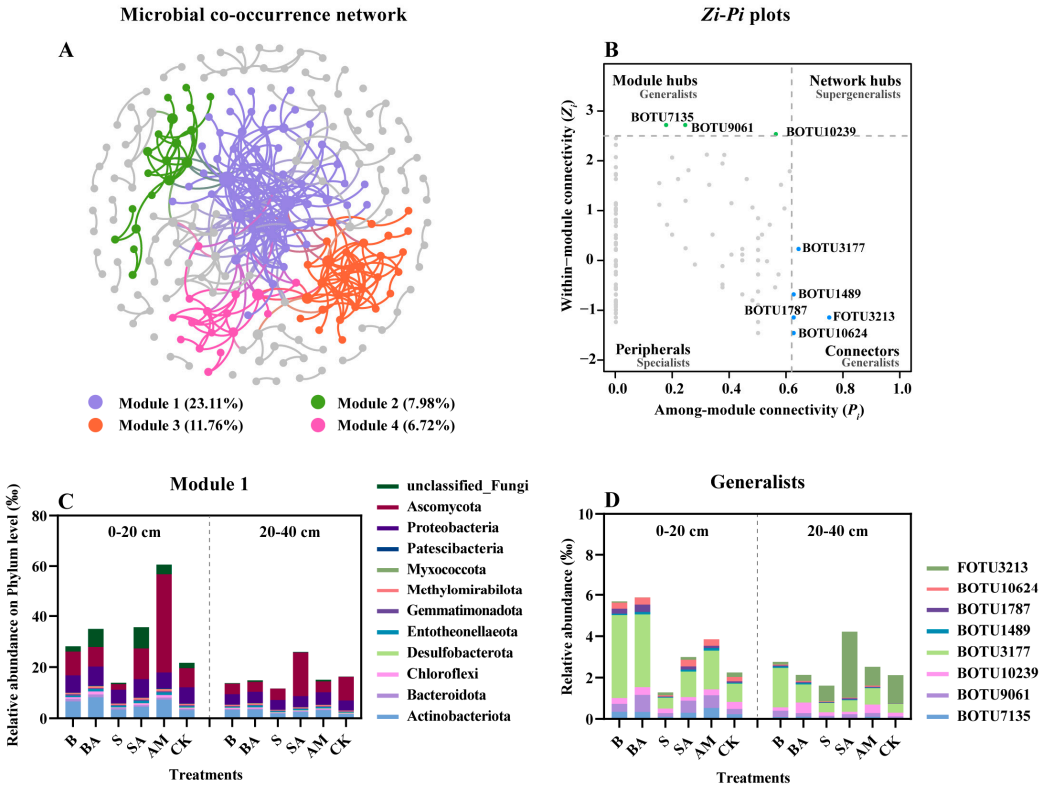

Figure 5. Microbial co-occurrence network analysis. (**A**) Co-occurrence networks of microorganisms from the soil based on RMT analysis from OTU profiles; (**B**) *Zi-Pi* plots of microorganisms based on OTUs topological roles in microbial networks; (**C**) hanges in the relative abundance of microorganisms in Module 1 between soil layers and soil amendments; (**D**) changes in the relative abundance of keystone species between soil layers and amendments. Abbreviations: B, biochar on its own; BA, biochar mixed with arbuscular mycorrhizal fungi (AMF); SA, straw mixed with AMF; S, straw on its own; AM, AMF on its own; CK, no additive.

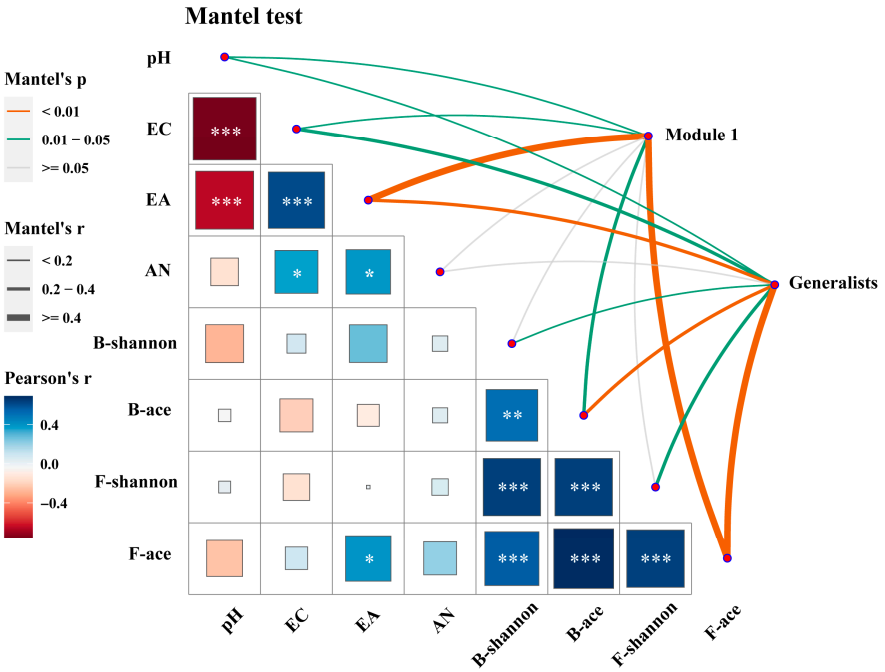

Figure 6. Correlations between the main microbial community structures (Bray–Curtis distance), soil pH, electrical conductivity, enzyme activities, available nutrients, and microbial community diversity.

The widths of the arcs in the plot correspond to the Mantel r value, while the colors of the arcs indicate the statistical significance. Pairwise correlations between these variables are depicted using a color gradient, representing the Pearson's correlation coefficients. Abbreviations: EC, electrical conductivity; EA, enzyme activity (Z-score); AN, available nutrient (Z-score); B-Shannon, bacterial Shannon index; B-ace, bacterial ACE index; F-Shannon, fungal Shannon index; F-ace, fungal ACE index. * indicates significant correlation at $p < 0.05$; ** indicates significant correlation at $p < 0.01$; *** indicates significant correlation at $p < 0.001$.

According to the Pearson correlation analysis, the electrical conductivity and soil enzyme activities were significantly negatively correlated with the soil pH. Additionally, all microbial alpha diversity indices had significantly positive correlations with each other. However, only the fungal ACE index among them exhibited a significantly positive correlation with soil enzyme activities (Figure 6).

4. Discussion

Alkalinization, which frequently occurs in conjunction with soil salinization, negatively affects the physical, chemical, and biological characteristics of soil by decreasing the diversity and abundance of microbial communities [34]. However, investigations into the influences of organic materials and the application of AMF on microbial communities and soil microenvironments in saline–alkali soils (based on microbiological approaches) remain in their nascent stages. In this study, we investigated the changes in soil microbial communities and core microorganisms after three years of soil amendments at a high pH site, to enhance our understanding of the effects of alkaline stress on soil microbial communities.

4.1. Responses of pH and Electrical Conductivity to Soil Amendments

Saline–alkali soil often exhibits high salinity and pH, which can induce ionic toxicity, as well as osmotic, oxidative, and high pH stress [35]. In view of the loose porous properties of biochar [7] and the capillary barrier attributes of straw [36], they have been extensively utilized for the remediation of saline soil, as their capacities for reducing salt and alkalinity have been confirmed by numerous studies [10,37]. Nevertheless, in this study the amendments did not significantly reduce the electrical conductivity of the soil, which may have been due to the long-term interment and further decomposition of biochar and straw, which resulted in decreased adsorption and salt resistance efficiencies [38]. In alignment with prior research, the single application of biochar, straw, and their mixed application with AMF in this study significantly reduced the soil pH in the 20–40 cm soil layer while the application (AMF on its own) did not produce a significant effect. Thus, the pH reduction observed in response to the mixed AMF application was primarily due to the organic acids generated through the decomposition of organic materials, which effectively neutralized CO_3^{2-} and HCO_3^-; thus, reducing the soil pH [10].

4.2. Responses of Enzyme Activities and Available Nutrients to Soil Amendments

Since nutrients are mineralized by soil enzymes and made available to plants and microorganisms, their activities are a critical indicator of soil fertility [39]. This was reflected through the significantly positive correlations observed between enzyme activities and available nutrients in our study. Earlier research revealed that the application of organic materials (e.g., biochar and straw) effectively stimulated the activities of soil enzymes and improved the soil nutrient content [40,41]. Surprisingly, after more than three years of field decomposition, the application of biochar or straw on their own, and their combination with AMF did not drastically increase the activities soil enzymes and available nutrients, while the application of AMF on its own continued to play a role. On one hand, AMF affects root growth and rhizospheric metabolism [42], while altering microecological systems through the mycelium mediated secretion of various enzymes and growth regulating substances, which assists with disrupting microbial communities and the available phosphorus content [43]. This was reflected in the current study as evidenced by the significant

enhancement of phosphatase activities and available phosphorus content in the 20–40 cm soil layer, through the application of AMF on its own (Figures S1 and S2). Conversely, the application of large quantities of organic material inhibited AMF colonization, biomass, and diversity [44] due to nutrient overload, which translated to weakening the influence of AMF on soil activation.

4.3. Responses of Soil Microbial Community Diversity and Structures to Amendments

Plant–microbe interactions, microbial community structures and functions, as well as their reactions to biotic and abiotic stimuli are impacted by the soil environment [45]. Studies have demonstrated that the application of organic materials and AMF can alter the activities of certain soil microbes and entire microbial communities [17,46–48]. However, the modification of soil resident microbial community structures may impact their functions, namely the conversion of soil nutrients and acquisition of plant nutrients; thus, directly impacting plant production [49,50]. In this study, the effects of soil amendments on the diversity of microbial communities were observed via the ACE index in the 20–40 cm soil layer. In keeping with the findings of previous studies [51], both the application of AMF on its own, and its combination with biochar significantly enhanced the species richness of soil fungi and bacteria in the 20–40 cm soil layer. This was mainly attributed to the large surface area of the proliferating mycelium in the soil, which provided a nutrient-rich ecological niche for the colonization and growth of other soil microorganisms, particularly bacteria [52], while biochar served as a micro-shelter for mycelial consumers [53].

Compared with bacteria, fungi are more sensitive to the soil environment [54]. This was evident in the PCoA axis I, where differences in fungal communities were more pronounced between treatments than bacteria. The dominant groups at the soil bacterial phylum level under each amendment were primarily Proteobacteria, Firmicutes, and Actinobacteriota, where Firmicutes are ubiquitous in saline-alkali soils and thought to be resistant to extreme conditions [34]. In this study, each amendment treatment increased the relative abundance of Firmicutes in the 0–20 cm soil layer, which may have been correlated with the lower soil pH. Meanwhile, the most abundant fungal species in this study was Ascomycota, which is generally the most abundant fungal group in soils that is rich in organic matter [55]. In the 0–20 cm soil layer, the increased relative abundance of Ascomycota under each amendment represented augmentations in soil regulation and nutrient cycling functions to some extent [43]. Interestingly, in this study the application of biochar on its own in the 20–40 cm soil layer significantly decreased the Shannon indices of fungi and bacteria, as well as the relative abundance of Ascomycota. It is plausible that harmful substances were generated during the decomposition of biochar [56], which led to the destruction of microbial communities, where the additional application of AMF could weaken this effect.

4.4. Keystone Microbial Species and their Relationships with the Soil Environment

The application of organic materials (e.g., biochar, straw) and AMF altered the abundance of key species in the microbial symbiotic network, which provided critical ecological functions in microbial communities. Module 1 was the most dominant module in the symbiotic network with Ascomycota as the dominant phyla, where the highest relative abundance of AMF on its own was applied in the 0–20 cm soil layer. However, Ascomycota can adapt to adverse conditions (e.g., low nutrient availability) and utilize resources more effectively in challenging environments [57]. When combining significant correlations between Module 1 microorganism communities with the soil pH, conductivity, enzyme activities, and bacterial-fungal ACE index, we believed that the Module 1 microorganisms were of particular importance toward enriching microbial communities and enhancing saline–alkali soil.

The results of this study also indicated that all soil amendments, except for the application of straw on its own, increased the relative abundance of key species in the microbial co-occurrence network. The higher relative abundances of BOTU7135, BOTU9061, and

BOTU3177 were classified as Actinobacteriota, which concurrently improved soil structures and increased plant tolerance under stress [48]. These three microorganisms, particularly BOTU3177, were found to be increased to a certain degree in both soil layers under the B, BA, SA, and AM amendments. Actinobacteria have physiological attributes that generate secondary metabolites such as extracellular enzymes and potent antibiotics [58], which are crucial for the breakdown and transformation of refractory organic matter. Therefore, the activities of Actinobacteria are critical for saline ecosystems in conjunction with the addition of organic material. Future studies should examine the specific contributions of each Actinobacteria genus and species for the improvement of saline–alkali soils. There were significant correlations between the generalist communities with the soil pH, electrical conductivity, enzyme activities, microbial alpha diversity, and the substantial positive correlations of soil enzyme activities with the fungal ACE index and soil available nutrients in this study. This suggested that changes in the abundance of key microorganisms could improve saline–alkali soils through the modification of microbial diversity, stimulation of enzyme activities, and enhanced availability of nutrients.

Since pH values can indirectly impact microbial communities through the availability of nutrients and organic matter [59], attention should be paid (when considering the ecological restoration of saline–alkali land) to the enhancement of soil enzyme activities and available nutrients, rather than only the impacts of salt and alkali reduction. Considering the degradation cycle and unsustainability of straw, the mixed application of AMF with biochar was the preferred solution for the improvement of saline–alkali soil, which aligned with the growth promotion results of our field experiment with *Taxodium* '*Zhongshanshan*' [28]. It is worth mentioning that more attention should be devoted to the investigation of soil microfauna (i.e., soil mites, springtails, and nematodes), which plays important roles in the decomposition of organic matter [60] and can influence microbial communities. Further, this study revealed the negative impacts of biochar on soil microbial communities. To avoid the deterioration of AMF functions induced by hypertrophication and the negative effects of excessive biochar, as well as to ensure the efficacy of salt and alkali reduction, it was of particular importance to determine the optimal amount of applied biochar. In the future, we will conduct further research on the effects of various biochar dosages on the colonization and functionality of AMF, while combining metabonomics and metagenomics to reveal the kinetics of mixed applications of biochar and AMF on saline–alkali soils.

5. Conclusions

Subsequent to three years of soil amendments, the microorganisms contained in Module 1 played an important role in enriching soil microbial communities and enhancing saline–alkali soil environments. Meanwhile, keystone microbial species were observed to improve the availability of soil nutrients by regulating microbial diversity and stimulating soil enzyme activities to ultimately ameliorate saline–alkali soil. Further, the application of AMF combined with a suitable quantity of biochar was the preferred solution for the improvement of saline–alkali soils.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14122295/s1, Figure S1: Variations in enzyme activities between soil layers and soil amendments; Figure S2: Variations in available nutrients between soil layers and soil amendments.

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