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The synergistic effect of extracellular polysaccharide-producing salt-tolerant bacteria and biochar promotes grape growth under saline-alkaline stress

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ABSTRACT

Grapes (*Vitis vinifera*) are a vital economic crop worldwide but are severely threatened by soil salinization and alkalization. While extracellular polysaccharides are known to improve soil conditions, it remains unclear how rhizosphere microorganisms that can produce extracellular polysaccharides enhance the tolerance of plants to salt-alkali conditions. This study selected *Bacillus subtilis* B4 and *Pseudomonas resinovorans* B9 based on their high levels of production of extracellular polysaccharides and subjected them to pot and field experiments. Our results demonstrated that both strains significantly promoted the growth of grape shoots, reduced the salinity of soil, and increased the levels of phosphorus and potassium in both the plants and soil. Compared to traditional *B. subtilis*, B9 performed better, and this was further enhanced when the strain was co-applied with biochar. 16S rRNA high-throughput sequencing was used to show that the combination of bacteria and biochar reshaped the native rhizosphere microbial community, altered its functional abundances, and improved the properties of soil, thus, ultimately promoting plant growth and enhancing salt-alkali tolerance. This study expands the microbial species available to improve the tolerance of grape to salt and ameliorate the saline-alkaline soils, thus, providing a theoretical basis for the combined application of microbial inoculants and biochar.

1. Introduction

Grape (*Vitis vinifera*), a highly valuable crop that is widely cultivated worldwide, covers an estimated 8 million hectares and holds a significant position in the global agricultural economy (Liu et al., 2022). However, soil salinization, which has been exacerbated by worsening climate conditions and unsustainable farming practices, severely restricts the growth of grapes and reduces their yield and quality (Lu et al., 2023). Salinization impacts plant physiological growth and worsens these adverse effects by degrading the physical and chemical properties of the soil (Tejada et al., 2006). Physically, salinized soil has a disrupted aggregate structure, dispersed particles, less porosity, and increases in its bulk density, which results in poor aeration (Liu et al., 2020). Chemically, the increase in soil pH (Ren et al., 2023) significantly inhibits microbial activity, which ultimately reduces its fertility (Naz et al., 2022; Sun et al.,

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2021). Therefore, the effective improvement of saline-alkaline soil has become critical for the cultivation of grapes.

In recent years, microbial inoculants have emerged as a key, cost-effective, environmentally friendly, and sustainable approach to improve saline-alkaline soils (Jiang et al., 2020). The addition of these beneficial microorganisms to the soil promotes the restoration of beneficial microbial communities and stimulates root secretions that suppress the proliferation and spread of pathogens in the soil (Salehin et al., 2020). Through their metabolic activities, microorganisms enhance the uptake of nutrients by plants (Lv et al., 2024), increase the quantity and diversity of beneficial soil microbes, and convert insoluble nutrients into forms that are more accessible to plants (Khatoon et al., 2020). This action improves the growth of plants and enhances their resistance to saline stress (Woo et al., 2020). Among these microorganisms, functional strains, such as phosphate-solubilizing bacteria, potassium-solubilizing bacteria, and siderophore-producing bacteria, are widely used to improve the soil (Chen et al., 2020; Zhang et al., 2023), and they offer a continuous supply of nutrients and microbial regulation for the soils (de Oliveira et al., 2024). Additionally, the exopolysaccharides (EPS) secreted during microbial activities significantly enhance the tolerance of plants to stress (Chen et al., 2024) and play a crucial role in enhancing the soil structure (Hale et al., 2021; Paul et al., 2024).

Despite the substantial potential of microorganisms to improve saline-alkaline soils, severe soil conditions often hinder the growth and functionality of microorganisms in the soil. To address this, biochar, an environmentally friendly soil amendment, has garnered considerable attention (Mandal et al., 2024). Biochar provides essential nutrients for plants and soil microbes and supports the absorption of nutrients by plants and microbial activity, while enhancing soil physicochemical properties through various chemical reactions and nutrient cycles (Hou et al., 2022). The critical properties of biochar, such as its ability to adjust the pH, enhance the cation exchange capacity, reduce bulk density, and increase bioactivity (Yu et al., 2019), make it an ideal material to improve the soil. Studies have shown that biochar can effectively mitigate salt stress by reducing the uptake of sodium (Na^+) and promoting the growth and development of plants (Zhang et al., 2019).

Based on the background described above, this study aimed to systematically evaluate the effects of the combined application of two microbial inoculants and biochar on saline-alkali soil, the growth of grapes, fruit quality, and rhizosphere microbial communities. The following hypotheses were proposed and tested: (1) The combined application of microbial inoculants and biochar can significantly enhance the fertility of soil, improve its physical properties, and mitigate the negative impacts of saline-alkali stress on grapes. This, in turn, promotes the growth of grapes and enhances the quality of fruit by enhancing the vitality of roots and increasing the concentrations of nitrogen (N), phosphorus (P), and potassium (K) in the plant tissues. (2) The combined addition of microbial inoculants and biochar can optimize the rhizosphere bacterial community structure of grapes, thereby positively impacting the growth of grape, and (3) Compared to the application of biochar or microbial inoculants alone, their combined use will produce a synergistic effect, which will result in significant improvements.

2. Materials and methods

2.1. Isolation and identification of salt-tolerant bacteria

Salt-tolerant bacteria were isolated from the rhizosphere of grapevines growing in salinized soils in the coastal area of Weifang, Shandong Province, China (119°10'32.977" E, 36°53'29.072" N). During the sampling process, a strict adherence to the sampling standards was maintained, with samples sealed in sterile bags and stored at low temperatures for transport (Li et al., 2022). Different strains were screened using the dilution plating method, followed by secondary selection on highly saline media (with a five-fold higher concentration of salt compared to the standard LB medium) (Zhou et al., 2020). The yield of EPS produced by each selected strain was determined using the phenol-sulfuric acid method (DuBois et al., 1956). Two strains that resulted in significant advantages were identified for subsequent experimental analysis. These dominant strains were sent to Qingke Biotechnology Co., Ltd. (Beijing, China) for 16S rRNA sequencing in Qingdao. The data were compared with the NCBI database, and a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method in MEGA 7 software to determine their taxonomic status and clarify the species of the strains.

2.2. Pot test

The pot experiment was conducted in a sunlight greenhouse at the Horticultural Experiment Station of Shandong Agricultural University (Tai'an, Shandong Province, China) (longitude 117°9′32.760″E, latitude 36°10′15.870″N). Brown plastic pots were used, which were 20 cm high with an outer diameter of 17 cm and an inner one of 15 cm. Each pot was filled with 500 g of garden soil with the following basic physicochemical properties: pH = 7.21, EC = 174.23 μS/cm, and salinity = 2.41 ‰. The plant material used was 'Shine Muscat' grape cuttings. Biochar was added to the soil. It was derived from carbonized rice (*Oryza sativa*) husks with properties, such as an ability to improve the retention of water in the soil and its aeration, and promote the proliferation of microorganisms. Each pot was irrigated every 5 days with 400 mL of a simulated saline-alkali solution that contained 0.42 % salts ($NaCl$: Na_2SO_4: $NaHCO_3$ [4:5:5, v/v/v]). The entire experimental period lasted for 30 days. Six treatment groups were established to evaluate the effects of different treatments on the growth of grapes and the soil properties. The treatments were as follows: (1) CK: 400 mL of water; (2) T: 80 g of biochar + 400 mL of water; (3) B4: 400 mL of *Bacillus subtilis* inoculum; (4) TB4: 80 g of biochar + 400 mL of *B. subtilis* inoculum; (5) B9: 400 mL of *Pseudomonas resinovorans* inoculum; and (6) TB9: 80 g of biochar + 400 mL of *P. resinovorans* inoculum. All the inoculants were diluted 100-fold before application, with the *B. subtilis* and *P. resinovorans* inocula standardized to $OD_{600} = 0.5$. The treatments were applied initially at the start of the experiment and repeated every 10 days for a total of three applications. There were six replicates of each treatment to ensure that the data were reliable and reproducible.

2.3. Field trials

The field experiment was conducted at the grape cultivation base of Shandong Jingqi Agricultural Technology Co., Ltd. in Hanting District, Weifang City, Shandong Province, China (119°10’32.977" E, 36°53’29.072" N). The subjects were 3-year-old ’Shine Muscat’ grapevines, and six treatment groups were established. They were similar to those in the pot experiment and included the following: (1) CK: 10 L of water; (2) T: 1.5 kg of biochar + 10 L of water; (3) B4: 10 L of *B. subtilis* inoculant; (4) TB4: 1.5 kg of biochar + 10 L of *B. subtilis* inoculant; (5) B9: 10 L of *P. resinovorans* inoculant; and (6) TB9: 1.5 kg of biochar + 10 L of *P. resinovorans* inoculant. All the treatments were diluted 100-fold before application, and the inoculants were prepared at a concentration of $OD_{600} = 0.5$. Fertilizer was applied using a trenching and root irrigation method. The trenches were 30–40 cm deep and 30 cm away from the grapevine roots. The biochar was applied by trenching, and the inoculants were applied by the irrigation of roots before they were covered with soil. The area of treatment for each vine was 0.64 m^2. The first application was conducted in April, and the inoculants were re-applied every month for five treatments. Each treatment group had six replicates.

2.4. The sampling and measurement of the soil and plants after treatment with salt-tolerant bacteria

In the pot experiment, we initiated sampling when the grape plants displayed distinct phenotypes. The leaves were collected from the fourth to fifth internode to analyze the contents of chlorophyll and malondialdehyde (MDA). Newly emerged roots were selected to assess their activity, while the remaining roots, stems, and leaves were analyzed for their contents of total nutrients (total N [TN], P, and K). For the field experiment, the plants were sampled during the harvest period of the grapes. For each treatment, 10–20 fruits per plant were randomly selected from different layers to measure the weight per 100 berries and the content of soluble solids. Soil samples were also collected using a three-point sampling method. The sampling points were established within each plot, and the soil was collected from the 20–40 cm layer using a ring knife. These samples were used to measure the physical and chemical properties of the soil. Grapevine roots were sampled from multiple orientations around each plant, and active roots were selected. These were placed in sealed bags, gently shaken to collect the adhering rhizosphere soil, and stored at low temperatures to analyze the microorganisms in the rhizosphere. Contingency was avoided by combining the soil samples from two sampling points within the same treatment to create composite soil samples.

2.5. Determination of the plant indicators and soil physical and chemical indicators

The relative growth rate of the new grapevine shoots was measured with a ruler. Simultaneously, the root vitality was assessed using the triphenyl tetrazolium chloride (TTC) reduction method (Cheng et al., 2022). The content of chlorophyll in the grape leaves was analyzed by ethanol extraction and that of MDA was measured using the thiobarbituric acid (TBA) colorimetric method (He et al., 2018). The contents of TN, P, and K in the roots, stems, and leaves were determined using the Kjeldahl method, vanadium molybdenum yellow colorimetry, and flame photometry, respectively (Zhang et al., 2017; Zhou et al., 2023). The weight of 100 fruits was precisely measured, while the fruit dimensions were measured with a caliper. The contents of soluble solids in the fruits were determined using a WZB45 digital refractometer (Shanghai Yidian Physical Optics Instrument Co., Shanghai, China).

The soil was analyzed by mixing 1 g of soil with 5 mL of deionized water to measure the pH and electrical conductivity (EC). The content of soluble salts in the soil was calculated using the mass difference method. The soil pH was measured with an FE28-Standard pH meter (Mettler Toledo, Greifensee, Switzerland), and the EC was determined using an FE30 conductivity meter (Mettler Toledo). The available phosphorus (AP) in the soil was assessed using the sodium bicarbonate extraction-molybdenum antimony colorimetric method (Sahoo et al., 2015), while the available potassium (AK) was determined using ammonium acetate extraction-flame photometry (Muthuraja and Muthukumar, 2022). The bulk density of the soil was calculated as the ratio of the oven-dry soil mass to the volume of fresh soil, and the soil aggregate structure was evaluated using the wet sieving method.

2.6. PCR amplification and high-throughput sequencing

The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with the primer pairs 338 F (5-ACTCCTACGG-GAGGCAGCAG-3) and 806 R (5-GGACTACHVGGGTWTCTAAT-3) by an ABI GeneAmp® 9700 PCR thermocycler (Applied Biosystems, Waltham, MA, USA). The 16S rRNA gene was amplified by PCR as follows: initial denaturation at 95℃ for 3 min, followed by 27 cycles of denaturing at 95℃ for 30 s, annealing at 55℃ for 30 s, extension at 72℃ for 45 s and a single extension at 72℃ for 10 min, and an end at 4℃. The PCR mixtures contained 5 × TransStart FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, TransStart FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng and finally ddH_2O up to 20 μL. The PCR reactions were performed in triplicate. The PCR product was extracted from a 1 % agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer’s instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). A sequencing library was constructed using the NEXTFLEX Rapid DNA seq Kit. The qualified libraries were sequenced using an Illumina MiSeq PE300 (Illumina, San Diego, CA, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.7. Statistical and bioinformatics analysis

High-quality readings were obtained by splicing each sample through overlap by FLASH. Fastp was used to control the quality of original sequencing and obtain optimized data. The nonrepetitive sequences were extracted from the optimized sequences, which could reduce the computational complexity of the intermediate process. The single sequences without duplication were removed, and operational taxonomic units (OTUs) based on the non-repetitive sequences (excluding the single sequence) were clustered according to 97 % similarity using UPARSE. The chimeric sequences were removed during the clustering process, and the representative sequences of the OTUs were obtained. After these steps, the optimized sequence could be obtained for further analysis. All the optimized sequences were mapped to the OTU representative sequence, and the sequences with more than 97 % similarity were selected to generate the OTU table. The sequences obtained from the bacteria that produced EPS were compared with the raw sequences generated by high-throughput sequencing. Corresponding to OTU table, and the relative abundance of bacterial taxa, as determined by high-throughput sequencing, was presented and visualized accordingly.

To accurately assess the diversity of the microbial communities, all the samples were rarefied to the same depth based on the minimum sequence number. The sample data were homogenized using rarefaction by the “vegan” package in R (v. 3.3.1). The subsequent analyses conducted in this study were based on normalized data. To obtain the information on the species that corresponded to each OTU, the RDP classifier Bayesian algorithm was used to analyze the 97 % similarity of the OTU representative sequence against the Silva ribosomal RNA gene database using a confidence threshold of 70 %. The community composition of each sample was analyzed at each level of classification. The Majorbio cloud platform was used to extract the original data. The chloroplasts and mitochondrial sequences were then removed by the platform, and the sequences obtained were annotated for species. To count all the OTUs and the number of OTUs shared and unique in multiple samples, Venn diagrams were created by the “Venn diagram” package in R (v. 3.3.1), and the sparse curves and other richness and diversity indices (ACE, Chao, Shannon, and Simpson) of the bacterial communities were estimated by Mothur. Circos-0.67–7 was used to create the Circos sample and species relationship map. Using the “vegan” package of R language according to the similarity of abundance among the species or samples to generate a community Heatmap diagram and a community Pie diagram (PIE diagram). Difference tests were performed on the multiple groups of samples, and the Kruskal–Wallis H test was used, as well as the “stats” package of R (version 3.3.1) and the “scipy” package of Python, for mapping. QIIME was used to calculate the beta-diversity distance matrix for Hierarchical clustering analysis. A UPGMA algorithm was used to construct the tree structure, using the “pheatmap” package in R (v. 3.3.1) for plotting. A principal coordinate analysis (PCoA) based on the Bray-Curtis distance was applied to reveal the differences in the bacterial communities between the groups. A Gephi network analysis kit was used to obtain the relative information of species and the samples within or between groups and construct a species correlation network. The Pathway (Level 1, Level 2, and Level 3) information in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was obtained by PICRUSt2, and the function information of the Clusters of Orthologous Groups (COG) database was compared to comprehensively predict the function of the assumed microbial community.

The structural equation model (SEM) was developed by AMOS (IBM SPSS Amos 23, Armonk, NY, USA) with maximum-likelihood estimation. The model was used to evaluate the indirect and direct effects of the inoculated strains on the microbial communities, Bacterial function, soil properties, and Plant index. The SEM fitness was examined based on a nonsignificant chi-squared test (P. 0.05), and the root mean square error of approximation (RMSEA). Alpha analysis, Circos analysis, network analysis, and function prediction

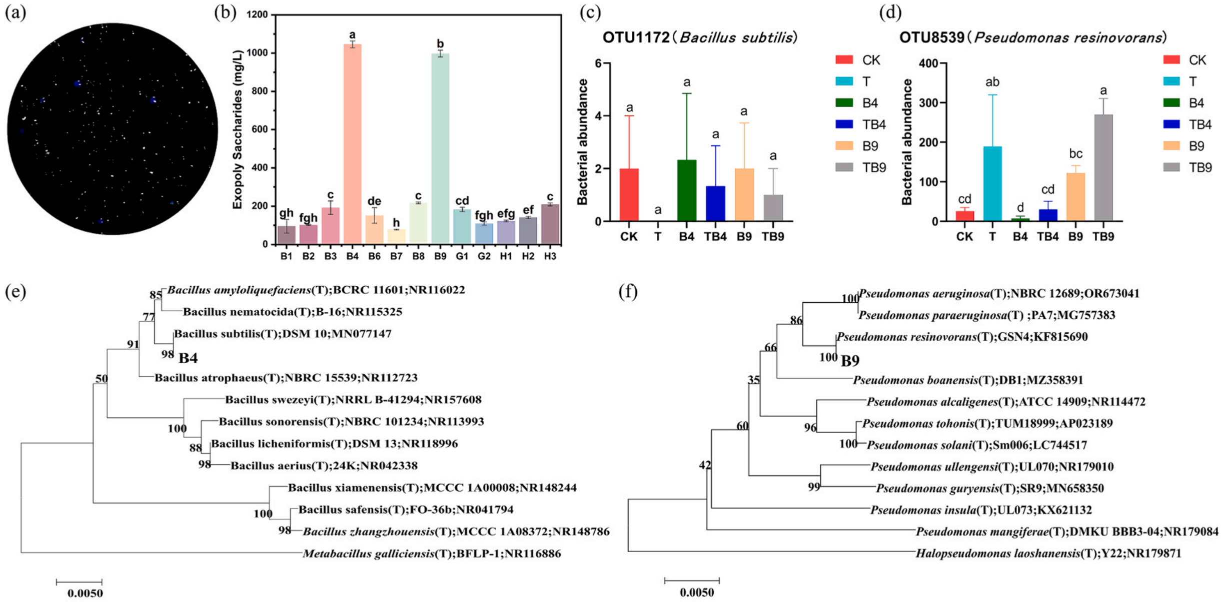

Fig. 1. Microbial screening, functional identification, colonization status and phylogenetic tree. Screening of the aniline blue culture medium (a), The strain grown in high-salt LB liquid medium produces extracellular polysaccharide (b), B4 strain abundance bar chart (c), B9 strain abundance bar chart (d), B4 strain phylogenetic tree (e), B9 strain phylogenetic tree (f). Lowercase letters indicate significant differences between treatments ($P < 0.05$). Values shown are the mean ± SE ($n = 3$).

were all calculated by each sample data, and the mean values of all the samples of each species were then used for mapping and analysis. The raw sequencing data are available on the NCBI Sequence Read Archive under the BioProject accession number PRJNA1174871.

Differences were considered significant when $p < 0.05$ and extremely significant when $p < 0.01$. SPSS 23.0 (IBM, Inc., Armonk, NY, USA) was used for the statistical analysis.

3. Results

3.1. Screening of salt-tolerant functional bacterial strains

Two bacterial strains were obtained based on the extracellular polysaccharide secretion of bacteria as an essential indicator for screening salt-tolerant functional strains. The 16S rDNA sequencing and comparison with the NCBI database, they were identified as *B. subtilis* (B4) and *P. resinovorans* (B9) (Fig. 1c, d). A total of 13 strains that produced extracellular polysaccharides were initially isolated using an aniline blue high-salinity culture medium (Fig. 1a). The content of EPS in these strains was then measured in both the high-salt and non-high-salt LB liquid media. Among them, strains B4 and B9 produced the most EPS (Fig. 1b). Additionally, an analysis of the data revealed that after the treatment with strain B9, the abundance of *P. resinovorans* in the treatment group increased significantly compared to the control group. In contrast, after treatment with B4, the abundance of *B. subtilis* did not increase (Fig. 1e, f). The preliminary conclusion is that B9 can colonize the plant rhizosphere, while B4 cannot. In addition, biochar may also play a role

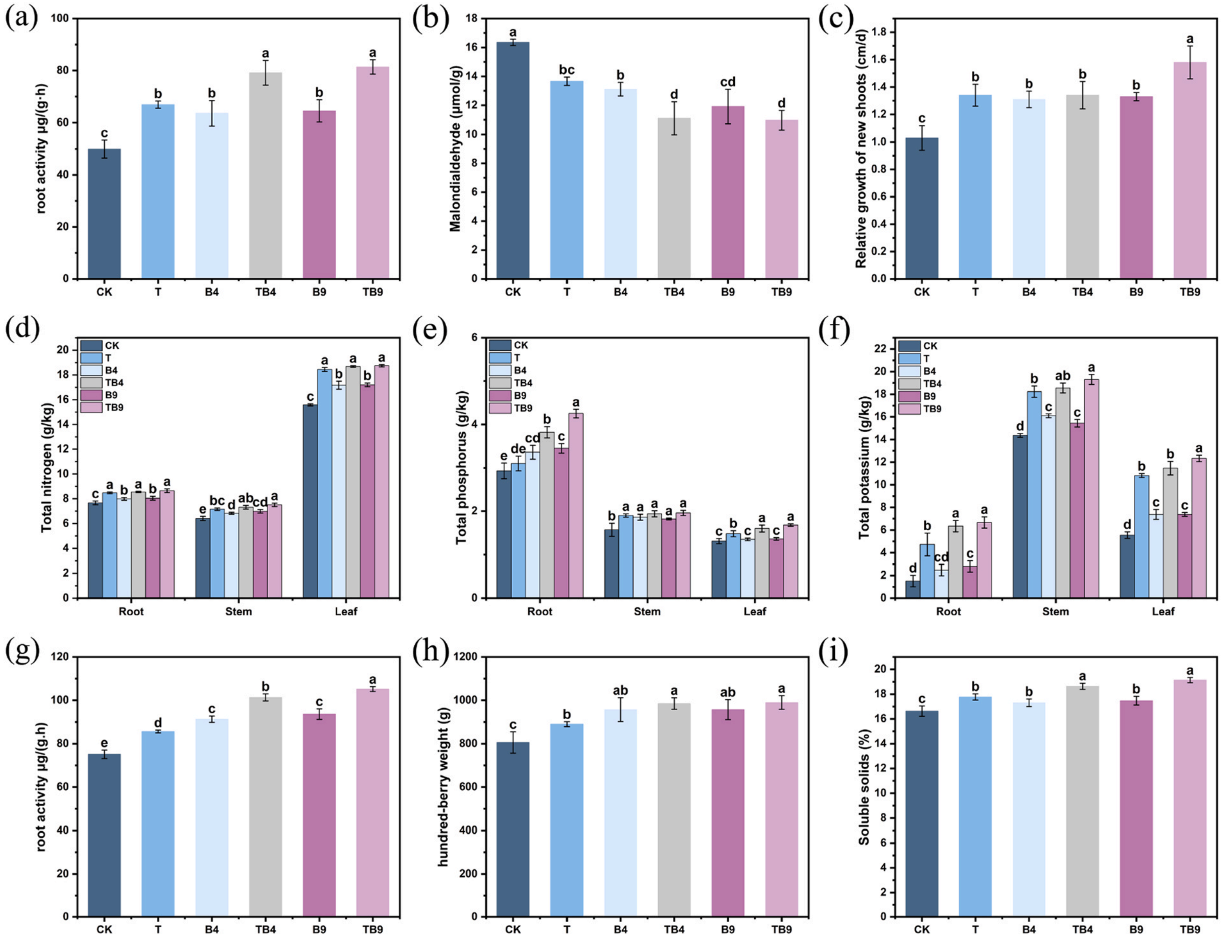

Fig. 2. Changes of grape growth and physiological indexes after different treatments. Physiological and biochemical indicators of the potted plants: Root activity (a), Leaf malondialdehyde (b), Relative growth of new shoots (c), Total nitrogen (d), Total phosphorus (e), Total potassium (f). CK: No fertilizer. T: 80 g of biochar + 400 mL of water. B4: 400 mL of *B. subtilis* inoculum. TB4: 80 g of biochar + 400 mL of *B. subtilis* inoculum. B9: 400 mL of *P. resinovorans* inoculum. TB9: 80 g of biochar + 400 mL of *P. resinovorans* inoculum. Physiological and biochemical indicators of field-grown plants: Root activity (g), 100-berry weight (h), Soluble solids (i). CK: No fertilizer. T: 1.5 kg of biochar + 10 L of water. B4: 10 L of *B. subtilis* inoculant. TB4: 1.5 kg of biochar + 10 L of *B. subtilis* inoculant. B9: 10 L of *P. resinovorans* inoculant. TB9: 1.5 kg of biochar + 10 L of *P. resinovorans* inoculant. Lowercase letters indicate significant differences between the treatments (P < 0.05). Values shown are the mean ± SE (n = 3).

in the ability of B9 to colonize the rhizosphere.

3.2. *Effect of the salt-tolerant bacteria with biochar on plant metrics*

B4 and B9 can produce relatively high concentrations of EPS in highly saline environments. However, their effectiveness at enhancing the tolerance of plants to salt merits further examination. Both the pot and field experiments were conducted to assess the salt tolerance effects of B4 and B9. Under the conditions of saline-alkaline stress, the application of B4 and B9 microbial inoculants significantly increased the root vitality, new shoot growth, 100-fruit weight, and contents of soluble solids in ’Sunshine Rose’ grapes (Fig. 2a, c, h, i). Furthermore, the use of B4 and B9 notably improved the content of total nutrients in the roots, stems, and leaves (Fig. 2d–f).

Additionally, the application of B4 and B9 significantly reduced the adverse effects of salt-alkaline stress as indicated by a marked decrease in the content of MDA compared to the control group (Fig. 2b). In practical production settings, microbial inoculants are often combined with organic materials. Thus, we chose biochar, a common organic amendment, to be used with the inoculants. It was observed that the sole application of biochar could significantly improve most of the plant-related parameters measured and substantially reduce the levels of stress. The combination of microbial inoculants with biochar was more effective, and the co-application of B4 and B9 with biochar led to significant enhancements to the parameters of the plant described above and reduced the damage from salt stress. Among the treatments, TB9 performed the best, followed by TB4, and some indicators surpassed those achieved with the sole application of microbial inoculants. Overall, both B4 and B9 can enhance the tolerance of plants to salt. B9 was slightly more

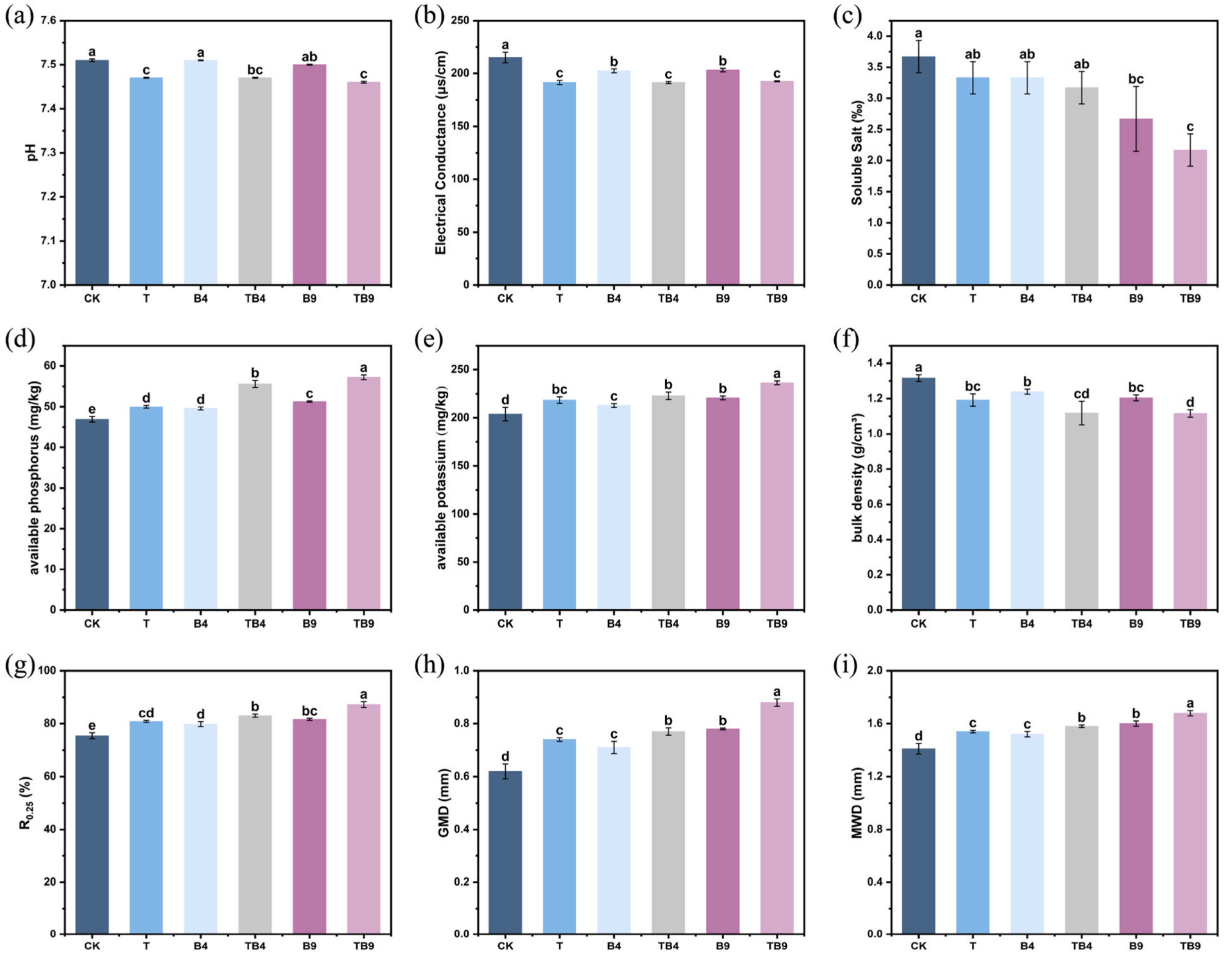

Fig. 3. Changes of grape soil indexes after different treatments. Physiological indicators of potted soil: pH (a), EC (b), Soluble salt (c). CK: No fertilizer. T: 80 g of biochar + 400 mL of water. B4: 400 mL of *B. subtilis* inoculum. TB4: 80 g of biochar + 400 mL of *B. subtilis* inoculum. B9: 400 mL of *P. resinovorans* inoculum. TB9: 80 g of biochar + 400 mL of *P. resinovorans* inoculum. Physiological indicators of field soil: Available Phosphorus (d), Available k (e), Soil bulk density (f), $R_{0.25}$ (g), GMD (h), MWD (i). CK: No fertilizer. T: 1.5 kg of biochar + 10 L of water. B4: 10 L of *B. subtilis* inoculant. TB4: 1.5 kg of biochar + 10 L of *B. subtilis* inoculant. B9: 10 L of *P. resinovorans* inoculant. TB9: 1.5 kg of biochar + 10 L of *P. resinovorans* inoculant. $R_{0.25}$: > 0.25 mm agglomerate ratio; EC: electrical conductivity; MWD: Average weight diameter; GMD: Geometric mean diameter. Lowercase letters indicate significant differences between treatments ($P < 0.05$). Values shown are the mean ± SE ($n = 3$).

effective than B4, and the combination with biochar resulted in significantly greater efficacy than the microbial inoculants alone.

3.3. Effect of the application of salt-tolerant bacteria and biochar on the soil physicochemical properties

The combination of the bacterial strains B4 and B9 with the application of biochar significantly impacted the physical and chemical properties of the soil. The initial treatments directly affected the physical characteristics of the soil, particularly with the addition of biochar. The bacterial treatments notably reduced the soil bulk density (Fig. 3f), and TB9 caused the most significant decrease of 15.20 %. These treatments significantly enhanced the soil $R_{0.25}$, Average weight diameter (MWD) and Geometric mean diameter (GMD) (Fig. 3g–i), with further improvements noted following the application of biochar. Other conventional physical and chemical indicators, such as the AP and AK in the soil, also increased significantly compared to the control (Fig. 3d, e). TB9 showed the most significant effects of 21.93 % and 15.91 %, respectively. The application B4 and B9, along with biochar, lowered the pH, EC, and salinity levels of the soil (Fig. 3a–c), particularly with the treatments T, TB4, and TB9, which significantly reduced the indicators that are related to salt. Notably, the soil salinity after the TB9 treatment decreased by up to 40.87 % compared to the control, thus, it was the most effective at reducing the soil salinity. Overall, the use of bacterial agents notably impacted the soil structure, physicochemical properties, and the indicators related to salt, with biochar enhancing their effectiveness, which is consistent with their effects on the plant indicators.

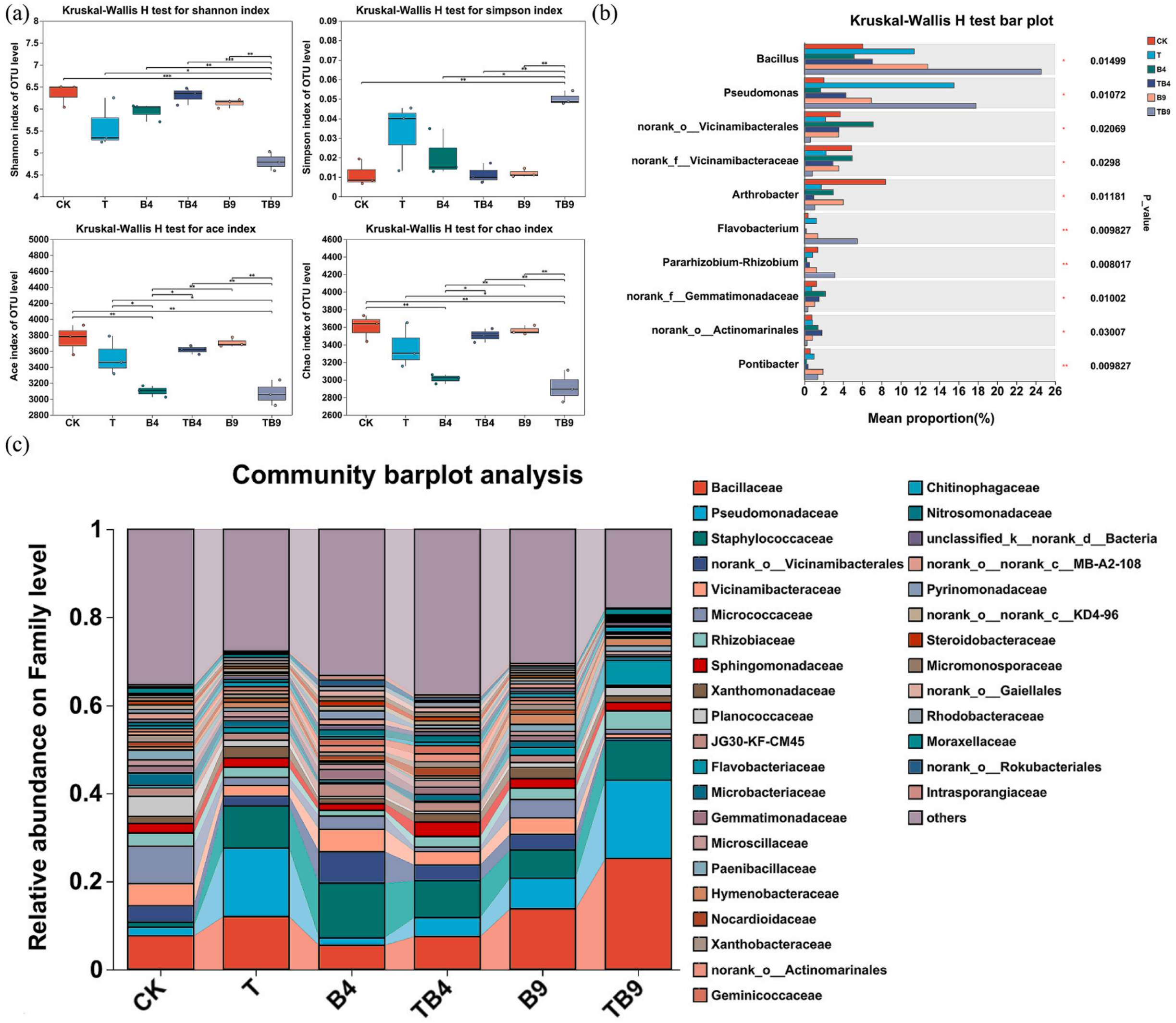

Fig. 4. Changes of microbial communities after different treatments. Alpha-diversity (a), Column chart for a significance test analysis of the differences among multiple groups of all the samples (b), Species composition at the soil bacterial family level (c).

3.4. Effects of the application of the salt-tolerant bacteria on the pre-existing microbial communities

The 18 samples were sequenced using the Illumina MiSeq PE300 platform. After trimming, quality control, redundancy removal, clustering analysis (based on 97 % sequence similarity), and the removal of chimera, 1,378,253 paired reads were obtained (Table S1). To accurately assess the diversity of the microbial community, all the samples were rarefied to the same depth, which normalized the sequence counts to 46,268. The rarefaction curves plateaued, which indicated that the sequencing depth was sufficient for the subsequent data analysis (Fig. S1).

The application of salt-tolerant bacteria and biochar affected the microbial community by altering the bacterial diversity, composition, and relationships. Alpha-and beta-diversity analyses revealed that all the treatments altered the bacterial diversity. In particular, there was a decrease in the average Shannon, Chao, and Ace indices for the samples treated with salt-tolerant bacteria and biochar. This indicated that there was a reduction in bacterial diversity and abundance. However, except for TB9, the diversity indices for the other treatments showed only slight decreases compared to the control group (CK), particularly for TB4 and B9. This suggested that were was minimal impact on the bacterial diversity and abundance. The average Simpson index for all the treatments was higher than that of the CK, and TB9 exhibited a significantly higher Simpson index. This indicated that certain microbial groups became enriched, and the dominant populations increased (Fig. 4a). A PCoA based on the Bray-Curtis distance showed that the samples from different treatments clustered separately and were distantly located from the CK. This confirmed that the treatments induced differences in the bacterial community composition (Fig. S2a). The application of salt-tolerant bacteria and biochar also altered the microbial community composition. At the phylum level, there were notable changes in the proportions of dominant phyla compared to the CK, particularly for TB9, where the relative abundance of the Proteobacteria and Firmicutes phyla exceeded 73.51 %, which were both significantly higher than the CK (Fig. S3a). At the family level, the Bacillaceae, Pseudomonadaceae, and Staphylococcaceae were the three most abundant families, and their proportions increased significantly after the treatment, particularly for TB9 (Fig. 4c). Similar trends were observed at the genus level, and the Top 3 dominant genera were *Bacillus*, *Pseudomonas*, and *Staphylococcus* (Fig. S3b). These genera exhibited substantial increases in abundance following the application of salt-tolerant bacteria and biochar, which indicated their potential impact on changes in the soil and plant properties. Differential analyses revealed that genera, such as

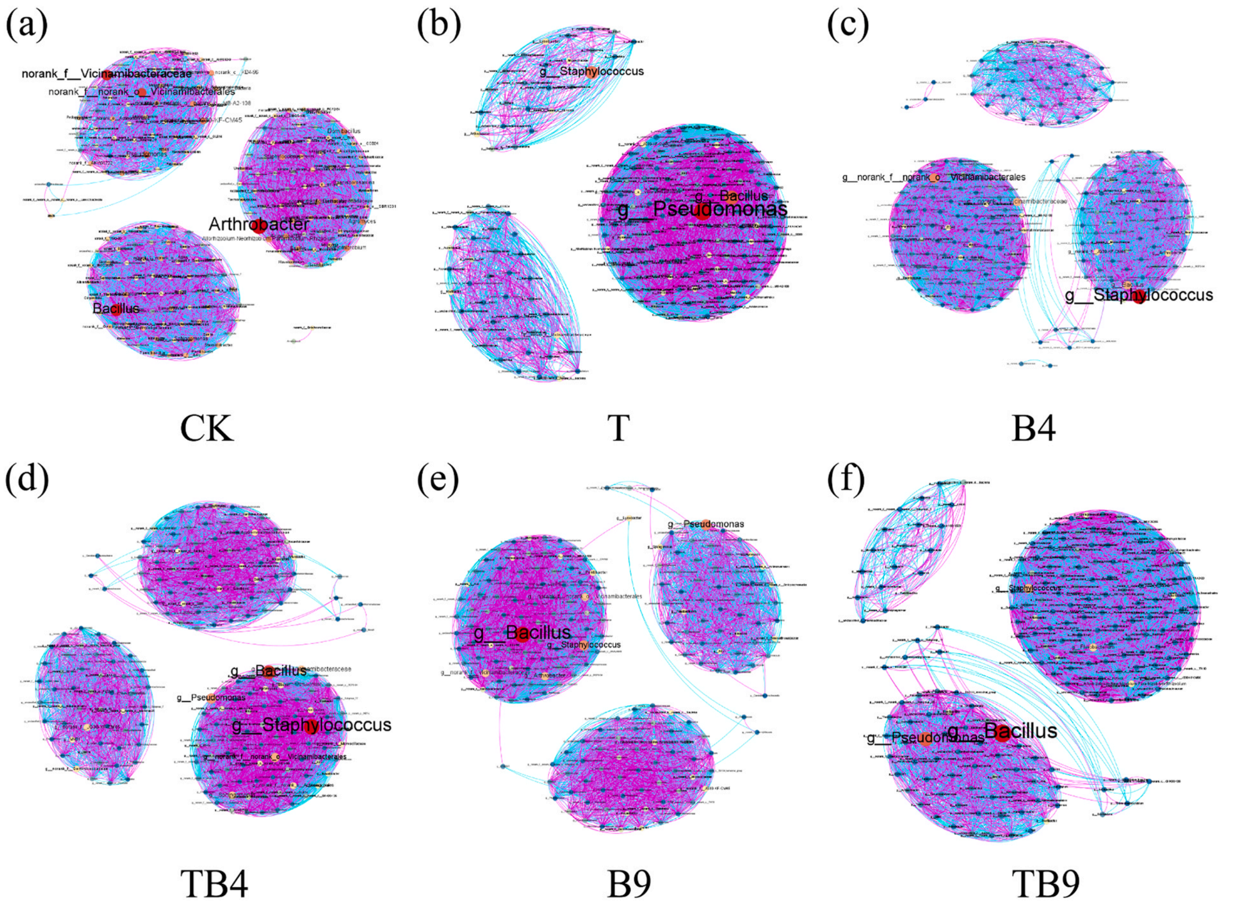

Fig. 5. Single-factor network relationship of bacteria in different treatments. Bacterial single-factor networks treated by CK (a), Bacterial single-factor networks treated by T (b), Bacterial single-factor networks treated by B4 (c), Bacterial single-factor networks treated by TB4 (d), Bacterial single-factor networks treated by B9 (e), Bacterial single-factor networks treated by TB9 (f).

Bacillus, *Pseudomonas*, *Arthrobacter*, and *Flavobacterium,* changed significantly after treatment (Fig. 4b and S4). At the OTU level, the Venn diagram analysis indicated that there were many unique OTUs between the CK and each treatment, particularly for the CK, which suggested that the treatments led to changes in the composition of OTUs (Fig. S2b).

The application of salt-tolerant bacteria and biochar affected the relationships between the bacterial communities and transformed previously closed microbial communities into three distinct groups (Fig. S2c). Biochar was notable in this transformation, and B9 changed in a similar manner. Furthermore, the treatments significantly impacted the positive and negative correlations among the Top 200 microbial communities, which altered the original relationships. In particular, the proportion of positive correlations among the bacteria in samples T, B4, and TB9 increased significantly, and TB9 had the most pronounced positive correlation rate of 67.69 % (Fig. 5f). The application of salt-tolerant bacteria and biochar significantly altered the diversity of the microbial community, composition, and relationships.

3.5. Relationship between the applied and affected microflora on the plant indicators and soil indicators

The application of salt-tolerant bacteria and biochar significantly impacts the soil bacterial community and its relationship with plant and soil indicators. A Canonical Correspondence Analysis (CCA) revealed a complex interaction between the bacterial communities and environmental factors, thus, highlighting that the AP, AK, soil bulk density, soil aggregate structure, electrical

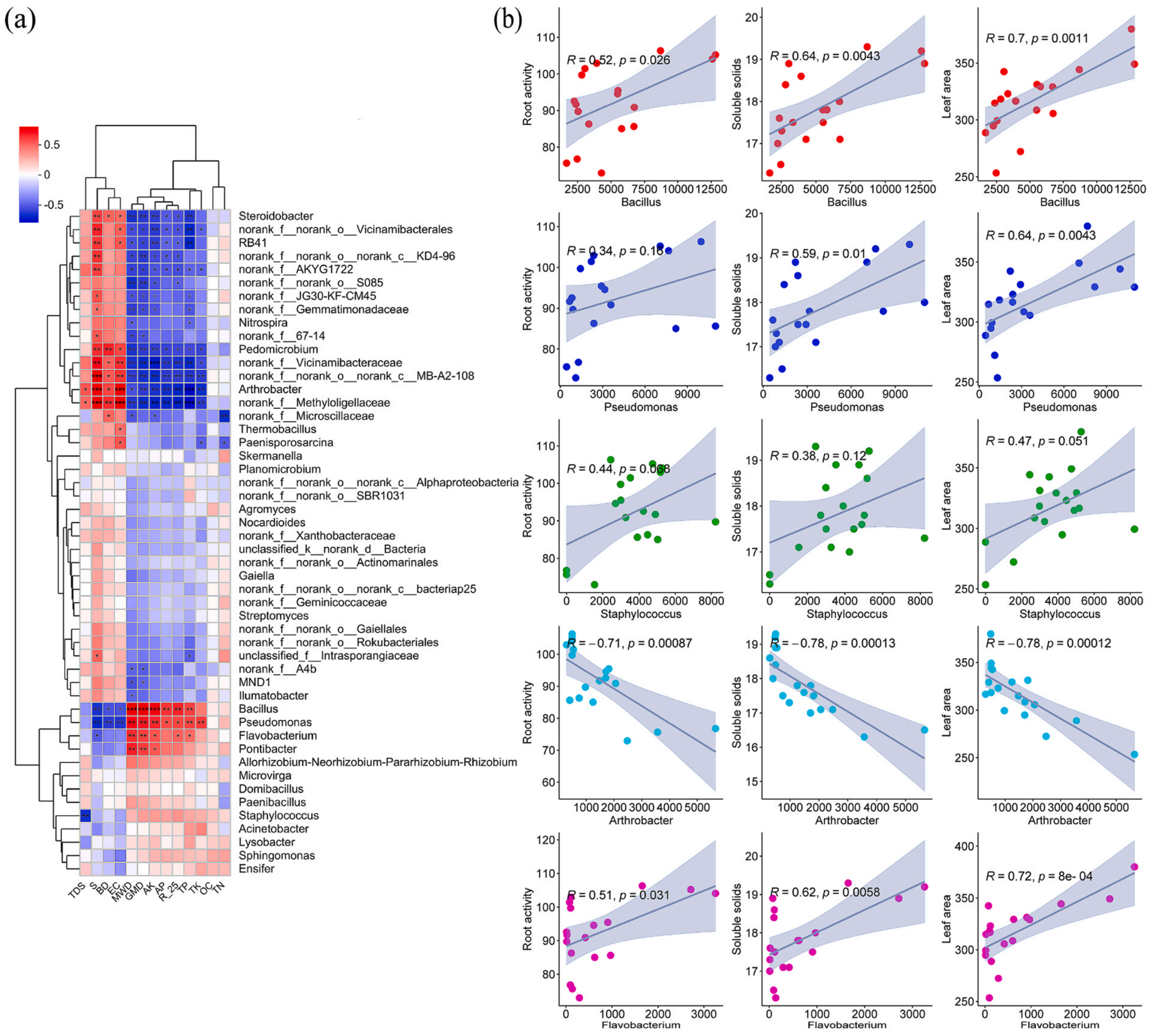

Fig. 6. Relationship of bacteria to soil and plant indicators. Relationship between bacteria and soil physicochemical indexes (a), Relationship between bacteria and plant physiological indexes (b). S: Sodium; AP: Available phosphorous; AK: Available k; BD: bulk density; $R_{0.25}$: > 0.25 mm agglomerate ratio; MWD: Average weight diameter; GMD: Geometric mean diameter; EC: Electrical conductivity; OC: Soil organic matter; TDS: Soluble salts; TN: Total nitrogen; TK: Total potassium.

conductivity, salt content, TP, and TK were the significant influencing factors on the bacterial communities (Fig. S5). A Spearman's rank correlation coefficient analysis of the Top 50 bacterial genera with environmental factors showed notable positive and negative correlations among the various genera and factors. The treatments with salt-tolerant bacteria and biochar notably increased the abundances of *Bacillus*, *Pseudomonas*, *Staphylococcus*, and *Flavobacterium*, while that of *Arthrobacter* decreased significantly. These shifts suggest distinct relationships between the microbial taxa and soil properties. *Bacillus*, *Pseudomonas*, *Staphylococcus*, and *Flavobacterium* positively correlated with the beneficial soil properties, such as the improved soil structure and availability of nutrients, and negatively correlated with the salinity-related indicators. In contrast, *Arthrobacter* displayed the opposite trend (Fig. 6a). A Pearson correlation analysis between these bacterial genera and the key plant indicators—leaf area, root activity, and soluble sugars—further highlighted these relationships. *Bacillus*, *Pseudomonas*, *Staphylococcus*, and *Flavobacterium* positively correlated with the leaf area, root activity, and soluble sugars, while *Arthrobacter* negatively correlated with these indicators (Fig. 6b).

3.6. Impact of the changes in the bacterial communities on function

To understand the functional changes in the native bacterial community after treatment with the salt-tolerant bacteria and biochar, a functional assessment was performed using PICRUSt2 to analyze the KEGG pathways. The results revealed that all the treatments influenced the functional abundance of the relevant bacterial pathways. Treatments T, B9, and TB9 significantly increased the

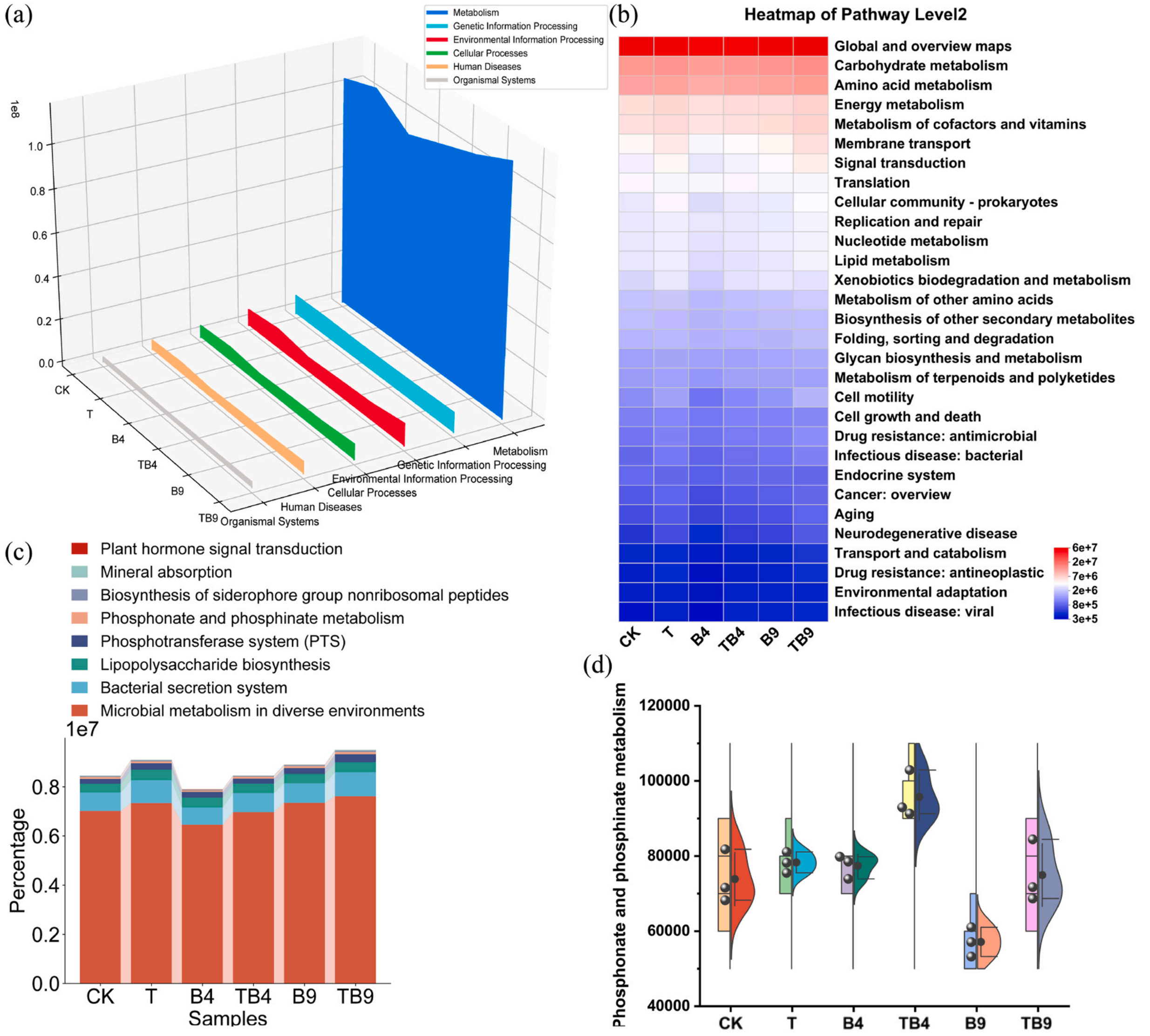

Fig. 7. Changes of bacterial community function after different treatments. KEGG stacking area of primary channels (a), KEGG secondary channel heat map (b), KEGG three-level path dominance stack bar chart (c), Violin diagram of phosphate and phosphate metabolism (d). KEGG, Kyoto Encyclopedia of Genes and Genomes.

functional abundance of Metabolism, Environmental information processing, and Cellular processes in the primary functional pathways of rhizosphere soil bacteria. In contrast, treatments B4 and TB4 showed no change or had a decrease in their functional abundance (Fig. 7a). Moreover, the salt-tolerant bacteria and biochar treatments also increased the functional abundance of most of the

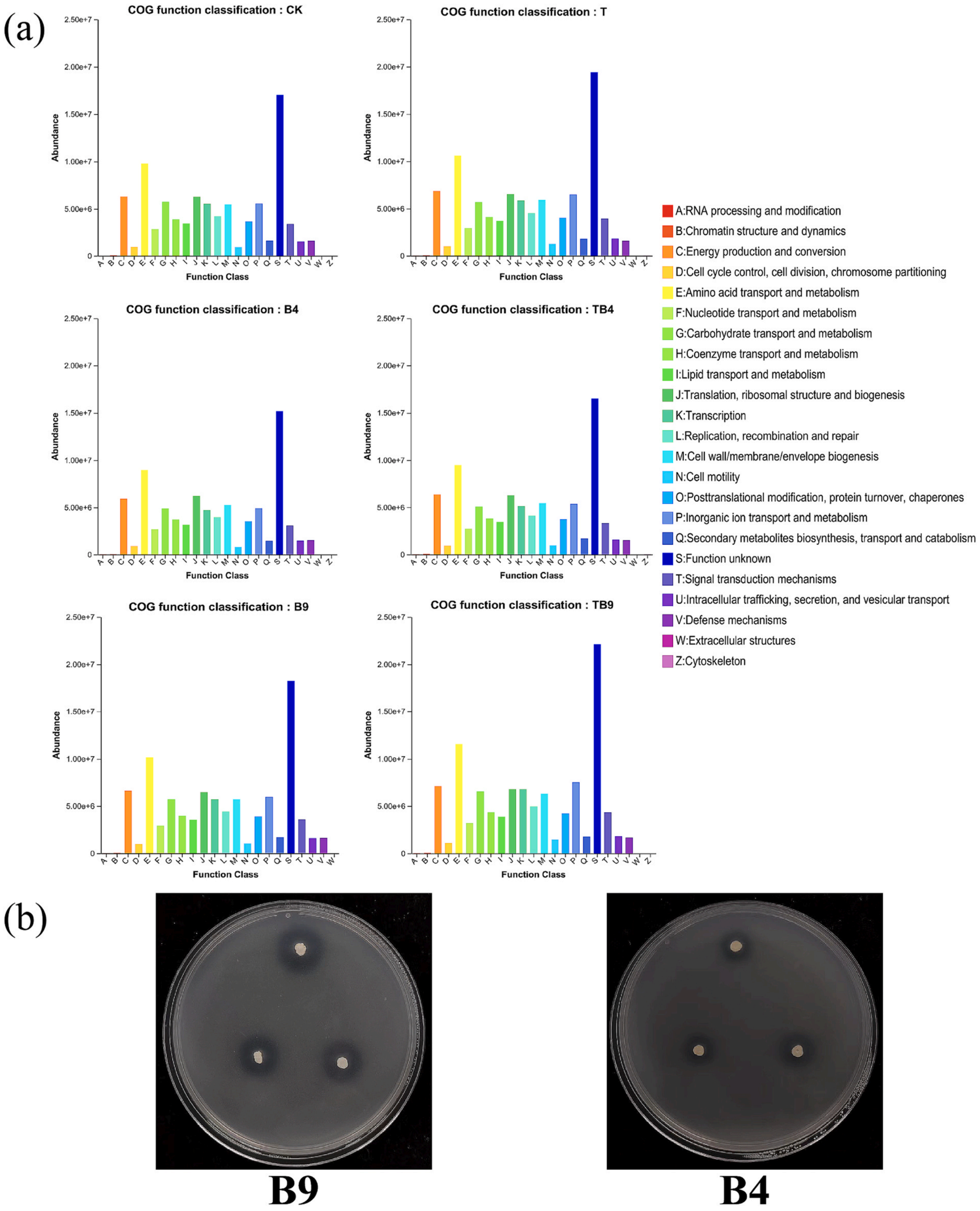

Fig. 8. Changes of bacterial community function after different treatments. Functional abundance column of COG bacteria (a), the Phosphorolytic circles of strains B4 and B9 (b). COG, Clusters of Orthologous Groups.

level 2 and 3 bacterial pathways. In particular, they enhanced the functional abundance in the level 2 pathways, such as Glycan biosynthesis and metabolism, and in the level 3 pathways, such as Bacterial secretion system, Biosynthesis of siderophore group nonribosomal peptides, Lipopolysaccharide biosynthesis, Microbial metabolism in diverse environments, Mineral absorption, Phosphonate, and phosphinate metabolism, Phosphotransferase system (PTS), and Plant hormone signal transduction. Among the treatments, TB9 showed the most significant increase in functional abundance (Fig. 7b–d).

A comparative analysis of the data from the COG database indicated that treatments B4 and B9 enhanced the abundance of specific functional pathways. The addition of biochar further increased the functional abundance of a substantial proportion of bacterial functions (Fig. 8a). A simple validation of the effects of B4 and B9 revealed that both treatments were able to solubilize phosphate, and B9 was more effective at solubilizing phosphate than B4 (Fig. 8b).

3.7. Bacterial community, function, vegetation, soil co-simulation

Structural equation modeling (SEM) was used to analyze the direct and indirect effects of inoculation with microorganisms on the microbial community composition, bacterial functional abundance, soil physicochemical properties, and plant indicators. The three models fitted the data well and explained 76.3 %, 91.8 %, and 91.7 % of the variation in leaf area, root activity, and fruit sweetness, respectively (Fig. 9a-c). These results demonstrate the impact of salt-tolerant bacteria and biochar on the microbial diversity and how they influence the three key plant indicators. For the root activity and content of soluble solids, the treatment with salt-tolerant bacteria and biochar affected the bacterial diversity, which, in turn, directly influenced the soil physicochemical properties, such as TP ($\lambda = -0.469$, $P < 0.01$) and bacterial functions ($\lambda = 0.036$, $P > 0.05$). Owing to the significant variation in functional abundance, only changes in a specific bacterial function were used as a proxy, which, although having a lower explanatory power, still substantially contributed to the overall model. This treatment directly or indirectly affected the TP ($\lambda = 0.402$, $P < 0.01$), which significantly influenced the AP ($\lambda = 0.572/0.682$, $P < 0.01$). The AP, in turn, had a direct effect on the root activity ($\lambda = 1.116$, $P < 0.001$) and content of soluble solids ($\lambda = 0.739$, $P < 0.001$). Phosphorus only had a minor impact on the leaf area, while the indicators related to K played a more prominent role. Changes in the bacterial diversity directly affected the TK ($\lambda = -0.573$, $P < 0.01$) and AK ($\lambda = -0.400$, $P < 0.01$), and the TK directly influenced the AK ($\lambda = 0.420$, $P < 0.01$). Together, the amounts of TK and AK significantly affected the leaf area.

4. Discussion

4.1. The screening of microorganisms that produce extracellular polysaccharide and their combined application with biochar can improve the quality of soil and alleviate the stress levels of plants

Extracellular polysaccharides (EPS) are critical for microbial survival and ecological functions, and they play essential roles in material cycling, nutrient uptake, and stress responses (Thakur and Yadav, 2024). EPS improves the soil structure by binding soil particles and retaining moisture, thus, creating a more favorable environment for the plants to grow. They also form biofilms on the root surfaces, which isolates stress factors and enhances the tolerance of plants to stress (Liu et al., 2022). This study isolated two bacterial strains that produced large amounts of EPS under salt stress, *Bacillus subtilis* and *Pseudomonas resinovorans*, from the rhizosphere soils of coastal grape. The effects of the microbial inoculants and biochar on the plants and saline-alkaline soils were analyzed. Plant roots are susceptible to salt-alkali stress, which manifests as inhibition to the new root growth, the browning of older roots, and a decrease in the absorption of nutrients (Cheng et al., 2020). Studies have shown that certain microorganisms improve the water potential of the roots and their metabolic activity under stress (Romero-Munar et al., 2024). Our findings demonstrate that the application of microbial inoculants significantly enhanced the root vigor, increased the content of dry matter, alleviated the salt-alkali

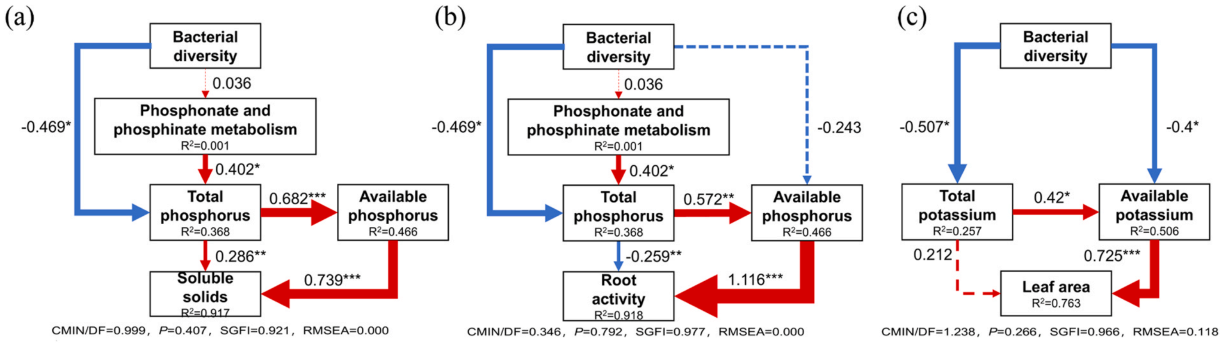

Fig. 9. The linkages between the microbial communities, bacterial functional abundance, soil physicochemical properties, and plant indicators are displayed by structural equation modeling (SEM). Relationships between bacterial diversity, function, soil physicochemical indices and soluble solids (a), Relationships between bacterial diversity, function, soil physicochemical indices, and root activity (b), Relationship between bacterial diversity, soil physicochemical indices and leaf area (c). Dotted arrows, non-significant paths ($P > 0.05$); Red and blue arrows, positive and negative relationships, respectively. The path widths are scaled proportionally to the path coefficient. $0.01 < P \leq 0.05$, $P < 0.01$.

stress, improved the antioxidant capacity, and reduced the levels of MDA, thereby maintaining the integrity of the cellular membrane and providing optimal growth conditions for the plants (Benito et al., 2024). The electrical conductivity (EC) of the soil and its content of salts are the key indicators of the severity of salinization (Liu et al., 2024). Salinization negatively impacts the soil aggregates and structural stability (Wang et al., 2019). Existing research indicates that EPS promotes the formation of soil aggregates (Wang et al., 2024a). This study shows that the application of microbial inoculants under salt stress reduced the soil EC and salinity, increased the proportion and stability of $>$ 0.25 mm soil aggregates, and released soluble K and P, which enhanced their uptake by the plant (Zhang et al., 2022). As a nutrient source, biochar improves the soil properties and provides essential resources for the survival of microorganisms (Palansooriya et al., 2019; Ding et al., 2016). The combined application of microbial inoculants and biochar outperformed their use individually and significantly improved the soil properties, microbial community structure, plant growth, and quality (Qi et al., 2022). Research has shown that species of *Bacillus* regulate the microbial balance in the soil and enhance the health of soil, while species of *Pseudomonas* promote plant growth, stress tolerance, and the efficient uptake of nutrients (Santoyo et al., 2012). This probably explains why B9 exhibited a more pronounced effect on plant growth than B4 in this study. Additionally, the successful colonization of B9 in the rhizosphere contributed to its sustained effects, whereas B4 was competitively displaced by the native microbial communities after a short-term impact, which provides additional evidence for the superior performance of B9 over B4.

4.2. The combined application of microbial fertilizers and biochar optimized the microbial community diversity, composition, and network relationships

The application of microbial inoculants offers numerous advantages in agricultural and environmental contexts. However, challenges remain, such as difficulties competing with the indigenous rhizosphere microbiota and achieving effective long-term colonization (Haskett et al., 2021). Multiple inoculations are often necessary to ensure sustained functionality. The colonization of plant roots by microorganisms depends on their ability to form biofilms, which contain EPS as a primary component (Beauregard et al., 2013; Xu et al., 2019). The composition of rhizosphere microbial communities is shaped by competitive and mutualistic interactions among microbes, with competition typically dominating (Klein et al., 2022; Shi et al., 2016). With its porous structure, biochar provides an ideal environment for the microorganisms to attach to it and subsequently proliferate, thus, enhancing their colonization efficiency (Zheng et al., 2022; Lü et al., 2016). Although *Bacillus subtilis* strain B4 exhibited only short-term effects in this study, it significantly improved the soil conditions and plant growth. Conversely, *Pseudomonas resinovorans* strain B9 adapted more readily, successfully colonized the rhizosphere, and exerted more substantial long-term effects on improvements to the soil. Microbial communities in the rhizosphere promote the growth of plants and improve the health of soil (Zhao et al., 2021). These findings are consistent with those from previous studies (Li et al., 2024), which suggest that highly adaptable bacterial strains dominate under competitive conditions, which enhances the stability of ecosystems (Yang et al., 2023). The combined application of microbial inoculants and biochar increased the abundance of metabolically active and stress-tolerant bacterial groups, such as *Pseudomonas*, *Flavobacterium*, and *Bacillus* (Feng et al., 2023; Moreira et al., 2020). The native adaptability of *Pseudomonas* may explain its more minor impact on microbial diversity compared to *Bacillus subtilis*. Biochar provides sources of carbon that enhance the metabolism of microorganisms while stabilizing the soil structure and microbial communities (Zhao et al., 2020; Qi et al., 2021). Interestingly, the interaction of biochar with different strains produced variable outcomes. For *Pseudomonas resinovorans*, biochar provided a “sheltering” environment that facilitated its growth and reduced the community diversity (Ng et al., 2023; Zhao et al., 2020). In contrast, the application of biochar with *Bacillus subtilis* enriched the sources of carbon (C), which promotes microbial diversity (Qi et al., 2021). A network analysis indicated that biochar increased the positive correlations among the microbial taxa, which enhanced the cooperative interactions and optimized the community functionality (Ren et al., 2015). This microbial interaction optimized the growth environment and promoted the performance of plants (Cheng et al., 2024).

4.3. The integrated analysis of soil, plants, and microbial communities

The diversity and abundance of soil microorganisms are closely associated with pH, salinity, nutrient availability, and soil aggregate structure (Wang et al., 2024c). This study identified five key bacterial genera, including *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Flavobacterium*, and *Acinetobacter*. Among these, the first four were beneficial rhizosphere bacteria that positively correlated with environmental factors, which was consistent with the findings of previous studies (Wang et al., 2019). *Flavobacterium* enhanced the levels of nutrients in the soil by increasing the activity of phosphatase and mitigating salt stress (Radziemska et al., 2023). Plant growth-promoting rhizobacteria (PGPR), such as *Pseudomonas* and *Bacillus*, improved the availability of P and the soil structure, which sustained the growth of plants (Li et al., 2022; Xie et al., 2024). Although the abundance of *Arthrobacter* decreased after treatment, the reduction might reflect changes in the rhizosphere community composition since some potentially harmful *Arthrobacter* populations increased owing to alterations in conditions. The functional analysis based on the KEGG and COG databases revealed significant enhancements in microbial community functions, including Bacterial secretion systems and Biosynthesis of siderophore group nonribosomal peptides, which indicated the enrichment of beneficial microbes. The bacterial secretion system can be an essential weapon for bacteria against competition, while helping them to colonize the natural environment (Purtschert-Montenegro et al., 2022; Stringlis et al., 2019). The siderophores improved iron solubility and uptake by microbes and plants (Roskova et al., 2022; Qin et al., 2016). The enhanced biosynthesis of lipopolysaccharides contributed to the stress-induced microbial resilience, which improved the tolerance of plants to salt (Cao et al., 2024; Sperandeo et al., 2017). Additionally, microbial inoculants and biochar enhanced the P cycling pathways, including Phosphonate and phosphinate metabolism and the Phosphotransferase system (Feng et al., 2023), and increased Plant hormone signal transduction, which enabled the plants to grow well in the presence of saline stress (Yu et al., 2020). Treatments, such as T (biochar alone), B9 (B9 alone), and TB9 (B9 + biochar), significantly enriched the microbial functions related to plant growth and soil improvement. Conversely, the B4 and TB4 treatments showed limited or no functional enhancement, probably owing to the weaker environmental adaptability of B4. The SEM demonstrated that microbial inoculants and biochar indirectly improved the soil properties and plant traits by modulating the microbial diversity and functional activity. Enhanced microbial cooperation and optimized community structure supported robust plant root systems and improved the quality of fruit. Over time, despite a reduction in microbial diversity, the enrichment of beneficial taxa optimized the microbial community structure, which significantly increased the growth of plants and the functions related to soil health. This synergy between the functional microbial inoculants and biochar highlights its potential for sustainable agriculture in saline-alkaline environments.

5. Conclusion

This study isolated two strains with high salt tolerance and elevated extracellular polysaccharide production—*Bacillus subtilis* and *Pseudomonas resinovorans*—from saline-alkaline soils. This study further explored the effects of biochar as a soil amendment when co-applied with these microbial inoculants on the soil environment and the growth of grapevines. The results indicated that combining the microbial inoculants and biochar significantly improved the physicochemical properties of the soil. Key indicators, such as the soil aggregate structure, readily available nutrients, salinity, grapevine root activity, and physiological and biochemical indicators, such as malondialdehyde, showed notable positive responses. At the microbial level, the co-application of the microbial inoculants and biochar promoted the enrichment of dominant strains and increased the positive correlations within the microbial community, which enhanced the functions of certain beneficial microbes and optimized the microbial community structure in the rhizosphere. Overall, the application of microbial inoculants and biochar improved the soil microbial community and effectively alleviated the negative impacts of saline-alkali stress on grapes. This demonstrates that their use in saline-alkali soils has significant benefits and holds potential for sustainable development.

CRediT authorship contribution statement

Yeqi Li: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Jiqiang Zhang:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Xindong Wang:** Writing – original draft, Visualization, Project administration, Investigation. **Zhangzhang Feng:** Investigation. **Enshuai Yang:** Investigation. **Mengzhen Wu:** Investigation. **Yuqing Jiang:** Investigation. **Jianquan Huang:** Resources, Project administration. **Zhen Gao:** Supervision, Resources, Funding acquisition. **Yuanpeng Du:** Validation, Supervision, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104070.

Data availability

Data will be made available on request.

References

Beauregard, P.B., Chai, Y.R., Vlamakis, H., Losick, R., Kolter, R., 2013. Bacillus subtilisbiofilm induction by plant polysaccharides. Proc. Natl. Acad. Sci. 110.
Benito, P., Celdrán, M., Bellón, J., Arbona, V., González-Guzmán, M., Porcel, R., et al., 2024. The combination of a microbial and a non-microbial biostimulant increases yield in lettuce (Lactuca sativa) under salt stress conditions by up-regulating cytokinin biosynthesis. J. Integr. Plant Biol.
Cao, T.T., Luo, Y.C., Shi, M., Tian, X.J., Kuzyakov, Y., 2024. Microbial interactions for nutrient acquisition in soil: miners, scavengers, and carriers. Soil Biol. Biochem. 188, 109215.
Chen, Y.H., Yang, X.Z., Li, Z., An, X.H., Ma, R.P., Li, Y.Q., et al., 2020. Efficiency of potassium-solubilizing for the growth of apple seedling. J. Integr. Agric. 19, 2458–2469.

Chen, E.N., Yang, C.S., Tao, W.Y., Li, S., 2024. Polysaccharides produced by plant growth-promoting rhizobacteria strain burkholderia sp. BK01 enhance salt stress tolerance to arabidopsis thaliana. Polymers 16, 145.

Cheng, Y.W., Kong, X.W., Wang, N., Wang, T.T., Chen, J., Shi, Z.Q., 2020. Thymol confers tolerance to salt stress by activating anti-oxidative defense and modulating Na homeostasis in rice root. Ecotox Environ. Safe 188, 109894.

Cheng, X.Y., Li, X.Y., Cai, Z.H., Wang, Z.K., Zhou, J., 2024. The structural and functional responses of rhizosphere bacteria to biodegradable microplastics in the presence of biofertilizers. Plants 13, 2627.

Cheng, Y., Li, X., Fang, M.Y., Ye, Q.J., Li, Z.M., Ahammed, G.J., 2022. Systemic H_2O_2 signaling mediates epigallocatechin-3-gallate-induced cadmium tolerance in tomato. J. Hazard. Mater. 438, 129511.

Ding, Y., Liu, Y.G., Liu, S.B., Li, Z.W., Tan, X.F., Huang, X.X., et al., 2016. Biochar to improve soil fertility. A review. Agron. Sustain. Dev. 36.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Feng, Q.J., Cao, S.L., Liao, S.J., Wassie, M., Sun, X.Y., Chen, L., et al., 2023. Fusarium equiseti-inoculation altered rhizosphere soil microbial community, potentially driving perennial ryegrass growth and salt tolerance. Sci. Total Environ. 871, 162153.

Hale, L., Curtis, D., Leon, N., McGiffen, M., Wang, D., 2021. Organic amendments, deficit irrigation, and microbial communities impact extracellular polysaccharide content in agricultural soils. Soil Biol. Biochem. 162, 108428.

Haskett, T.L., Tkacz, A., Poole, P.S., 2021. Engineering rhizobacteria for sustainable agriculture. ISME J. 15, 949–964.

He, F., Wang, H.L., Li, H.G., Su, Y.Y., Li, S., Yang, Y.L., et al., 2018. PeCHYR1, a ubiquitin E3 ligase from, enhances drought tolerance via ABA-induced stomatal closure by ROS production in. Plant Biotechnol. J. 16, 1514–1528.

Hou, J.B., Pugazhendhi, A., Sindhu, R., Vinayak, V., Thanh, N.C., Brindhadevi, K., et al., 2022. An assessment of biochar as a potential amendment to enhance plant nutrient uptake. Environ. Res. 214, 113909.

Jiang, N.J., Tang, C.S., Hata, T., Courcelles, B., Dawoud, O., Singh, D.N., 2020. Bio-mediated soil improvement: the way forward. Soil Use Manag. 36, 185–188.

Khatoon, Z., Huang, S.L., Rafique, M., Fakhar, A., Kamran, M.A., Santoyo, G., 2020. Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. J. Environ. Manag. 273, 111118.

Klein, M., Stewart, J.D., Porter, S.S., Weedon, J.T., Kiers, E.T., 2022. Evolution of manipulative microbial behaviors in the rhizosphere. Evol. Appl. 15, 1521–1536.

Li, Q., Chang, J.J., Li, L.F., Lin, X.Y., Li, Y.C., 2024. Soil amendments alter cadmium distribution and bacterial community structure in paddy soils. Sci. Total Environ. 924, 171399.

Li, Q., Li, H.C., Yang, Z., Cheng, X., Zhao, Y.C., Qin, L., et al., 2022. Plant growth-promoting rhizobacterium sp. CM11 specifically induces lateral roots. N. Phytol. 235, 1575–1588.

Li, Q., Wan, F., Zhao, M., 2022. Distinct soil microbial communities under ageratina adenophora invasions. Plant Biol. 24, 430–439.

Liu, X.T., Chai, J.L., Zhang, Y.C., Zhang, C., Lei, Y., Li, Q.P., et al., 2022. Halotolerant rhizobacteria mitigate the effects of salinity stress on maize growth by secreting exopolysaccharides. Environ. Exp. Bot. 204, 105098.

Liu, B.S., Huang, L.H., Jiang, X.T., Liu, Y., Huang, G.Z., Yang, C., et al., 2024. Quantitative evaluation and mechanism analysis of soil chemical factors affecting rice yield in saline-sodic paddy fields. Sci. Total Environ. 929, 172584.

Liu, S., Shan, B., Zhou, X., Gao, W., Liu, Y., Zhu, B., et al., 2022. Transcriptome and metabolomics integrated analysis reveals terpene synthesis genes controlling linalool synthesis in grape berries. J. Agric. Food Chem. 70, 9084–9094.

Liu, M.L., Wang, C., Liu, X.L., Lu, Y.C., Wang, Y.F., 2020. Saline-alkali soil applied with vermicompost and humic acid fertilizer improved macroaggregate microstructure to enhance salt leaching and inhibit nitrogen losses. Appl. Soil Ecol. 156, 103705.

Lu, X., Chen, G.P., Ma, L., Zhang, C.C., Yan, H.K., Bao, J.Y., et al., 2023. Integrated transcriptome and metabolome analysis reveals antioxidant machinery in grapevine exposed to salt and alkali stress. Physiol. Plant. 175.

Lü, F., Luo, C.H., Shao, L.M., He, P.J., 2016. Biochar alleviates combined stress of ammonium and acids by firstly enriching methanosaeta and then methanosarcina. Water Res. 90, 34–43.

Lv, J.Y., Yang, S.Y., Zhou, W., Liu, Z.W., Tan, J.F., Wei, M., 2024. Microbial regulation of plant secondary metabolites: Impact, mechanisms, and prospects. Microbiol. Res. 283, 127688.

Mandal, J., Sharma, P.K., Mondal, D., Wood, M.D., Hutchinson, S.M., Kirby, J., et al., 2024. Meta-analysis of biochar as an amendment for arsenic mitigation in paddy soils. Curr. Pollut. Rep. 10, 105–118.

Moreira, H., Pereira, S.I.A., Vega, A., Castro, P.M.L., Marques, A.P.G.C., 2020. Synergistic effects of arbuscular mycorrhizal fungi and plant growth-promoting bacteria benefit maize growth under increasing soil salinity. J. Environ. Manag. 257, 109982.

Muthuraja, R., Muthukumar, T., 2022. Co-inoculation of halotolerant potassium solubilizing bacillus licheniformis and aspergillus violaceofuscus improves tomato growth and potassium uptake in different soil types under salinity. Chemosphere 294, 133718.

Naz, M., Dai, Z.C., Hussain, S., Tariq, M., Danish, S., Khan, I.U., et al., 2022. The soil pH and heavy metals revealed their impact on soil microbial community. J. Environ. Manag. 321, 115770.

Ng, C.W.W., Liao, J.X., Lau, S.Y., San So, P., Hau, B.C.H., Peprah-manu, D., 2023. Coupled effects of elevated CO2 and biochar on microbial communities of vegetated soil. J. Environ. Manag. 342, 118136.

de Oliveira, L.M., Kavamura, V.N., Clark, I.M., Mauchline, T.H., De Souza, J.T., 2024. Diversity and multifunctional potential for plant growth promotion in bacteria from soil and the rhizosphere. Soil Use Manag. 40.

Palansooriya, K.N., Ok, Y.S., Awad, Y.M., Lee, S.S., Sung, J.K., Koutsospyros, A., et al., 2019. Impacts of biochar application on upland agriculture: a review. J. Environ. Manag. 234, 52–64.

Paul, S., Parvez, S.S., Goswami, A., Banik, A., 2024. Exopolysaccharides from agriculturally important microorganisms: conferring soil nutrient status and plant health. Int. J. Biol. Macromol. 262, 129954.

Purtschert-Montenegro, G., Cárcamo-Oyarce, G., Pinto-Carbó, M., Agnoli, K., Bailly, A., Eberl, L., 2022. Pseudomonas putida mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. Nat. Microbiol. 7, 1547–1557.

Qi, X., Gou, J.L., Chen, X.M., Xiao, S.Q., Ali, I., Shang, R., et al., 2021. Application of mixed bacteria-loaded biochar to enhance uranium and cadmium immobilization in a co-contaminated soil. J. Hazard. Mater. 401, 123823.

Qi, X., Xiao, S.Q., Chen, X.M., Ali, I., Gou, J.L., Wang, D., et al., 2022. Biochar-based microbial agent reduces U and Cd accumulation in vegetables and improves rhizosphere microecology. J. Hazard. Mater. 436, 129147.

Qin, Y., Druzhinina, I.S., Pan, X.Y., Yuan, Z.L., 2016. Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. Biotechnol. Adv. 34, 1245–1259.

Radziemska, M., Gusiatin, M.Z., Cydzik-Kwiatkowska, A., Blazejczyk, A., Holatko, J., Brtnicky, M., 2023. Does biochar in combination with compost effectively promote phytostabilization of heavy metals in soil under different temperature regimes? Sci. Total Environ. 882, 163634.

Ren, D.W., Madsen, J.S., Sorensen, S.J., Burmolle, M., 2015. High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. ISME J. 9, 81–89.

Ren, H.L., Zhang, F.Y., Zhu, X., Lamlom, S.F., Zhao, K.Z., Zhang, B.X., et al., 2023. Manipulating rhizosphere microorganisms to improve crop yield in saline-alkali soil: a study on soybean growth and development. Front. Microbiol. 14.

Romero-Munar, A., Muñoz-Carrasco, M., Balestrini, R., De Rose, S., Giovannini, L., Aroca, R., et al., 2024. Differential root and cell regulation of maize aquaporins by the arbuscular mycorrhizal symbiosis highlights its role in plant water relations. Plant Cell Environ.

Roskova, Z., Skarohlid, R., McGachy, L., 2022. Siderophores: an alternative bioremediation strategy? Sci. Total Environ. 819, 153144.

Sahoo, R.K., Ansari, M.W., Tuteja, R., Tuteja, N., 2015. Salt tolerant SUV3 overexpressing transgenic rice plants conserve physicochemical properties and microbial communities of rhizosphere. Chemosphere 119, 1040–1047.

Salehin, A., Hafiz, M.H.R., Hayashi, S., Adachi, F., Itoh, K., 2020. Effects of the biofertilizer OYK (Bacillus sp.) inoculation on endophytic microbial community in sweet potato. Horticulturae 6, 81.

Santoyo, G., Orozco-Mosqueda, M.C., Govindappa, M., 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species ofBacillusandPseudomonas: a review. Biocontrol Sci. Technol. 22, 855–872.

Shi, S.J., Nuccio, E.E., Shi, Z.J., He, Z.L., Zhou, J.Z., Firestone, M.K., 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. 19, 926–936.

Sperandeo, P., Martorana, A.M., Polissi, A., 2017. Lipopolysaccharide biogenesis and transport at the outer membrane of Gram-negative bacteria. BBA Mol. Cell Biol. Lipids 1862, 1451–1460.

Stringlis, I.A., Zamioudis, C., Berendsen, R.L., Bakker, P.A.H.M., Pieterse, C.M.J., 2019. Type III secretion system of beneficial rhizobacteria WCS417 and WCS374. Front. Microbiol. 10.

Sun, X.Q., She, D.L., Fei, Y.H., Wang, H.D., Gao, L., 2021. Three-dimensional fractal characteristics of soil pore structure and their relationships with hydraulic parameters in biochar-amended saline soil. Soil Tillage Res. 205, 104809.

Tejada, M., Garcia, C., Gonzalez, J.L., Hernandez, M.T., 2006. Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. Soil Biol. Biochem. 38, 1413–1421.

Thakur, R., Yadav, S., 2024. Biofilm forming, exopolysaccharide producing and halotolerant, bacterial consortium mitigates salinity stress in Triticum aestivum. Int. J. Biol. Macromol. 262, 130049.

Wang, M., Chen, S.B., Chen, L., Wang, D., Zhao, C.M., 2019. The responses of a soil bacterial community under saline stress are associated with Cd availability in long-term wastewater-irrigated field soil. Chemosphere 236, 124372.

Wang, C.Q., Pei, J., Li, H., Zhu, X.L., Zhang, Y.A., Wang, Y.J., et al., 2024a. Mechanisms on salt tolerant of Paenibacillus polymyxa SC2 and its growth-promoting effects on maize seedlings under saline conditions. Microbiol. Res. 282, 127639.

Wang, X.L., Riaz, M., Babar, S., Eldesouki, Z., Liu, B., Xia, H., et al., 2024c. Alterations in the composition and metabolite profiles of the saline-alkali soil microbial community through biochar application. J. Environ. Manag. 352, 120033.

Woo, O.G., Kim, H., Kim, J.S., Keum, H.L., Lee, K.C., Sul, W.J., et al., 2020. Bacillus subtilis strain GOT9 confers enhanced tolerance to drought and salt stresses in Arabidopsis thaliana and Brassica campestris. Plant Physiol. Biochem. 148, 359–367.

Xie, X.F., Liu, Y., Chen, G.F., Turatsinze, A.N., Yue, L., Ye, A.L., et al., 2024. Granular bacterial inoculant alters the rhizosphere microbiome and soil aggregate fractionation to affect phosphorus fractions and maize growth. Sci. Total Environ. 912, 169371.

Xu, Z.H., Zhang, H.H., Sun, X.L., Liu, Y., Yan, W.X., Xun, W.B., et al., 2019. Wall teichoic acids are required for biofilm formation and root colonization. Appl. Environ. Microbiol. 85.

Yang, X.L., Cheng, J., Franks, A.E., Huang, X.W., Yang, Q., Cheng, Z.Y., et al., 2023. Loss of microbial diversity weakens specific soil functions, but increases soil ecosystem stability. Soil Biol. Biochem. 177, 108916.

Yu, Z.P., Duan, X.B., Luo, L., Dai, S.J., Ding, Z.J., Xia, G.M., 2020. How plant hormones mediate salt stress responses. Trends Plant Sci. 25, 1117–1130.

Yu, H.W., Zou, W.X., Chen, J.J., Chen, H., Yu, Z.B., Huang, J., et al., 2019. Biochar amendment improves crop production in problem soils: a review. J. Environ. Manag. 232, 8–21.

Zhang, J.H., Bai, Z.G., Huang, J., Hussain, S., Zhao, F.T., Zhu, C.Q., et al., 2019. Biochar alleviated the salt stress of induced saline paddy soil and improved the biochemical characteristics of rice seedlings differing in salt tolerance. Soil Tillage Res. 195, 104372.

Zhang, P., Bing, X., Jiao, L., Xiao, H., Li, B.X., Sun, H.W., 2022. Amelioration effects of coastal saline-alkali soil by ball-milled red phosphorus-loaded biochar. Chem. Eng. J. 431, 133904.

Zhang, C.A., Chen, H.M., Dai, Y., Chen, Y., Tian, Y.X., Huo, Z.L., 2023. Isolation and screening of phosphorus solubilizing bacteria from saline alkali soil and their potential for Pb pollution remediation. Front. Bioeng. Biotechnol. 11.

Zhang, Z.H., Wang, Q., Wang, H., Nie, S.M., Liang, Z.W., 2017. Effects of soil salinity on the content, composition, and ion binding capacity of glomalin-related soil protein (GRSP). Sci. Total Environ. 581, 657–665.

Zhao, L., Xiao, D.L., Liu, Y., Xu, H.C., Nan, H.Y., Li, D.P., et al., 2020. Biochar as simultaneous shelter, adsorbent, pH buffer, and substrate of to promote biodegradation of high concentrations of phenol in wastewater. Water Res. 172, 115494.

Zhao, M.L., Zhao, J., Yuan, J., Hale, L., Wen, T., Huang, Q.W., et al., 2021. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. Plant Cell Environ. 44, 613–628.

Zheng, X.M., Xu, W.H., Dong, J., Yang, T., Shangguan, Z.C., Qu, J., et al., 2022. The effects of biochar and its applications in the microbial remediation of contaminated soil: a review. J. Hazard. Mater. 438, 129557.

Zhou, L.X., Liu, W., Duan, H.J., Dong, H.W., Li, J.C., Zhang, S.X., et al., 2023. Improved effects of combined application of nitrogen-fixing bacteria azotobacter beijerinckii and microalgae Chlorella pyrenoidosa on wheat growth and saline-alkali soil quality. Chemosphere 313, 137409.

Zhou, G., Wang, X., Zhao, H., Zhang, W., Liu, G., Zhang, X., 2020. Isolation of two salt-tolerant strains from activated sludge and its COD degradation characteristics from saline organic wastewater. Sci. Rep. 10, 18421.