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Effects of Mixed Saline and Fresh Water Sprinkler Irrigation on the Rhizosphere Soil Microbial Community of Summer Maize

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Abstract: Mixing saline and fresh water can help to increase the agricultural water utilization rate and solve the water shortage situation, but its role on rhizosphere microbes is unknown. This study revealed the effects of mixed saline and freshwater sprinkler irrigation at different proportions on the rhizosphere soil microbial community of summer maize. Compared to freshwater sprinkler irrigation, sprinkler irrigation with 2 g·L^{-1} of mixed saline and fresh water significantly increased the bacterial α-diversity and significantly affected the structure and composition of bacterial communities, increasing the number of OTUs, the ACE index, and the Shannon index in the rhizosphere soils, whereas sprinkler irrigation with 3 g·L^{-1} did not lead to significant changes. In addition, there was a significant difference in *β*-diversity between the mixed saline and freshwater sprinkler irrigation and the freshwater sprinkler irrigation. Principal coordinate analysis revealed that the 2 g·L^{-1} and 3 g·L^{-1} sprinkler irrigation treatments were closer to each other, but both were at a greater distance from the freshwater sprinkler irrigation, indicating that mixed saline and freshwater irrigation significantly affected the structure of rhizosphere soil bacterial communities. Ammonium nitrogen, alkaline dissolved nitrogen, and total nitrogen all affected the soil bacterial community structure by more than 10%, with ammonium nitrogen being the most influential environmental factor. The relative abundance of most microbes in the mixed saline and freshwater sprinkler treatments was positively correlated with ammonium nitrogen, especially in the 2 g·L^{-1} treatment, while that of most microbes in the freshwater sprinkler treatment was negatively correlated with ammonium nitrogen. In conclusion, selecting a sprinkler irrigation model with a mineralization level of mixed saline and freshwater not exceeding 2 g·L^{-1} could enhance the rhizosphere soil microbial community while conserving water resources.

Keywords: brackish water irrigation; rhizosphere soil bacteria; biodiversity; community structure; summer maize

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1. Introduction

Maize is vital in the agricultural economy, and imbalances in soil microbial communities can significantly impact crop yields of maize. The rhizosphere serves as a dynamic region where plant roots promote the growth of beneficial microorganisms by regulating soil pH, adding nutrients, and secreting specific compounds. These rhizosphere microorganisms play crucial roles in nutrient effectiveness, stress tolerance, and defense against soil-borne pathogens [1,2]. Consequently, studying the structure and function of rhizosphere microbial communities has become a critical area of research in agro-ecosystems.

Microbial activity is influenced by soil structure and properties [3,4], and soil microorganisms maintain ecosystem functions through the decomposition of organic matter and nutrient cycling [5]. Therefore, soil microbes can be used as indicators of land use change and ecosystem health [6–8].

However, a number of biotic and abiotic factors can significantly affect soil microbial characteristics, community structure, and function [9,10]. Microbial communities are directly involved in important ecological processes, such as organic matter decomposition, soil aggregate formation, and nutrient cycling in the soil, and therefore they are affected by changes in the soil environment [11]. It has been shown [12] that soil microbial biomass, respiration, and enzyme activities are highly sensitive to brackish water irrigation. Brackish water irrigation can affect the metabolic activity of soil microorganisms by altering the soil environment and consequently the metabolic activity of soil microorganisms. Studies have shown that bacteria are particularly sensitive to salt stress associated with brackish water irrigation [13–16]. Salinity significantly inhibited the growth of bacteria. Moreover, salinity under salt tolerant vegetation is the main determinant of community composition [4,17,18]. In addition, studies have also shown that saline irrigation could significantly increase the soil salt content from 1.77% to 8.74% in saline soils [1], and then adverse effects might appear in soil properties. Therefore, suitable salinity levels are beneficial for improving soil microbial diversity and promoting soil health and crop growth.

In this study, large-scale field trials of sprinkler irrigation were conducted, focusing on a brackish water sprinkler irrigation system for summer maize in a locational trial in the central region of the Heilonggang Basin. The experiments used deep-well fresh water (0.5 $g \cdot L^{-1}$), a mixture of salty and fresh water (2 $g \cdot L^{-1}$), and another mixture of salty and fresh water (3 $g \cdot L^{-1}$). The study systematically investigated the effects of different irrigation water sources on the diversity of soil microbial organisms, changes in community structure, and the relationship with soil physicochemical properties within the arable layer (0–20 cm) of summer maize fields during the harvesting period.

2. Materials and Methods

2.1. Test Site Summary

This experiment was conducted from March 2020 to December 2022 at the Long-Term Positioning Experiment Base for Winter Wheat-Summer Maize Continuous Brackish Water Irrigation, located at the Hengshui Irrigation Experiment Station in the central plain area of the Heilonggang Basin (Shenzhou City, Hengshui City, Hebei Province, China) (longitude: 115°38′, latitude: 37°59′, elevation: 26 m above sea level), which has a shallow ground water table of approximately 10 m.

The area is classified as a warm temperate semi-arid continental monsoon climatic zone. The frost-free period lasts between 190 and 200 days, and the region receives an annual sunshine duration of 2500 to 2900 h. Meteorological data were collected from the local meteorological station. Over the years, the average temperature at the site has been 12.8 °C, with an average annual precipitation of 512.5 mm. Notably, approximately 70% of this precipitation occurs during the summer months of June to August. The annual evaporation is 1785.4 mm. The soil at the experimental site was clay loam of tidal soil type, with soil salt content at 0.04% from 0 to 40 cm and 0.05% from 40 to 120 cm, i.e., at the non-salinity level. The basic physicochemical properties of 0~20 cm soil were as follows: bulk weight was 1.32 g cm^{-3}, field holding capacity was 22.1%, saturated water content was 32.7%, organic matter was 11.71 g kg^{-1}, total nitrogen was 0.62 g kg^{-1}, pH value was 8.20, soil electrical conductivity (measured by conductivity meter) was 172.50 μs cm^{-1}, nitrate nitrogen was 9.71 mg kg^{-1}, ammonium nitrogen was 4.96 mg kg^{-1}, available phosphorus was 20.61 mg kg^{-1}, and available potassium was 104.50 g kg^{-1}.

2.2. Experiment Design

The summer maize variety selected for testing in this project is Sanbei No. 63 (Sanbei Seed Co., Hebei, China), with a sowing rate of 17.25 kg/ha, equivalent to approximately

4200 grains per acre. The planting pattern remained consistent across both years: in 2020, summer maize was sown on 8 June and harvested on 30 September; in 2021, it was sown on 10 June and harvested on 2 October. The trial plots were 60 m × 104 m, utilizing a fully mobile pipeline sprinkler system with branch pipes and sprinkler heads spaced 10 m apart. In this experimental station, the salt content of shallow salt water was 3.6 g·L^{-1}, and the salt content of deep fresh water was 0.5 g·L^{-1}. The shallow salt water and deep fresh water were mixed in a certain proportion through a brackish water mixed irrigation automatic regulation system, and the mixed water salinity at the outlet end could be automatically adjusted to maintain the salinity of the mixed water at 2 g·L^{-1} and 3 g·L^{-1} for the irrigation experiment. Three treatments were established in this study: deep-well freshwater irrigation at 0.5 g·L^{-1} (FI), saline and fresh mixed water irrigation at 2 g·L^{-1} (SI), and high level saline and fresh mixed water irrigation at 3 g·L^{-1} (HSI), with three replicates for each treatment. Irrigation schedules were determined based on precipitation forecasts and field soil moisture monitoring, with irrigation initiated when the average soil moisture content from 0 to 40 cm reached 60% to 70% of the field's holding capacity. In 2020, saline and fresh mixed water was applied twice for irrigation; in 2021, due to high precipitation, only one irrigation event occurred in early July. The irrigation quota for each maize sprinkler application was 675 m^3/ha.

2.3. Measuring Indicators

2.3.1. Measurement of Soil Properties

For two consecutive years, during the harvest of summer maize, soil samples were taken from each treatment at a depth of 0–20 cm using a soil auger. A portion of the soil was air-dried and analyzed in the laboratory for the determination of Available Phosphorus (AP) (0.5 mol L^{-1} $NaHCO_3$ leaching—molybdenum antimony resistivity colorimetry), Available Potassium (AK) (ammonium acetate leaching—flame photometer method), Total nitrogen (TN) (AA3 continuous flow analyzer), Soil organic matter (SOC) (Potassium dichromate volumetric method), and Alkali hydrolyzed nitrogen (AIN) (alkali diffusion method); a part of it was frozen in the freezer for the determination of soil nitrate nitrogen (NIN) (dual wavelength UV spectrophotometry) and ammonium nitrogen (AMN) (2 mol L^{-1} KCl leaching-indophenol blue colorimetric method).

2.3.2. Measurements of Soil Microbiology

At the harvest in two consecutive years, three areas with consistent stubble density were randomly selected within each treatment area. Soil samples from the top layer (0–20 cm) were collected by excavation method. The stubble was completely removed from the soil, and a thin layer of soil closely adhering to the residual roots was collected and immediately stored in an ice box and then transported to the laboratory. This were passed through a 2 mm sieve to remove visible root residues and stones. The samples were then stored in a −80 °C refrigerator for high-throughput sequencing analysis.

2.3.3. Bacterial 16SrRNA Sequencing

Soil bacterial communities were analyzed using 16S rRNA gene amplicon sequencing. A total of 0.5 g of fresh homogenized soil was extracted using E.Z.N.A.® Soil DNA Kit (Guangzhou Feiyang Biological Engineering Co., LTD, Guangzhou, China), DNA concentration and purity were detected by 1% agarose gel electrophoresis, and extracted DNA was diluted with TE buffer and stored in a refrigerator at −20 °C. Primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3-V4 region of the bacterial 16S rRNA gene [19,20], and PCR amplification reactions were performed using a 50 μL system (94 °C, pre-denaturation for 5 min and denaturation for 60 s, 27 cycles), annealing (48 °C, 60 s) and stabilization (72 °C, 60 s, 30 cycles), followed by a final extension at 72 °C (10 min). The DNA amplicons were then purified and recovered using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Bedford, MA, USA), quantified using the QuantiFluorTM-ST Blue Fluorescence

Quantification System (Promega Corporation, WI, USA), and sequenced on an Illumina HiSeq 2500 platform.

2.4. Data Processing

Microsoft Office Excel 2019 was used to process the data and create graphs, SAS 8.1 was used for statistical analysis, and Duncan's SSR test was used for multiple comparisons of significance of differences ($p < 0.05$).

3. Results

3.1. Analysis of OTUs and Alpha Diversity of Tillage Bacteria

After counting the number of reads for the soil sample of HI, SI, and HSI, USEARCH [21] was employed to cluster the sequences into operational taxonomic units (OTUs) based on sequence similarity, using a threshold of 97%. Specifically, sequences with over 97% similarity were grouped into a single OTU. As illustrated in Figure 1, there was a significant difference in the number of OTUs among the three treatments in the 0–20 cm soil layer in 2020. The SI treatment exhibited the highest number of OTUs, followed by the FI treatment, while the HSI treatment had the lowest count. Notably, the SI treatment significantly increased the number of OTUs in the arable layer compared to the FI treatment. This indicates that the 2 $g \cdot L^{-1}$ saline and freshwater mixed sprinkler irrigation (SI treatment) could substantially enhance the number of soil bacteria in the arable layer. In contrast, the 3 $g \cdot L^{-1}$ saline and freshwater mixed sprinkler irrigation did not significantly affect the number of OTUs in the surface layer of the soil. The Chao1 and ACE indices, which measure species abundance—specifically, the number of species present—were derived from Figure 1. Both indices exhibited a similar trend in relation to the number of operational taxonomic units (OTUs), with the highest values observed in the SI treatment. The ACE index was significantly greater in the SI-treated plough layer compared to the FI treatment, indicating that the 2 $g \cdot L^{-1}$ brackish and freshwater mix sprinkler treatment resulted in the highest species richness. In contrast, the 3 $g \cdot L^{-1}$ brackish and freshwater mix sprinkler irrigation did not significantly affect the number of species in the topsoil layer. Shannon and Simpson indices were used to measure the diversity of microbial species, i.e., the larger the Shannon score, the smaller the Simpson, indicating that the microbial species diversity in the topsoil layer was not as high as in the SI treatment. The Simpson score is smaller, indicating the higher population diversity of microorganisms in that sample [22]. As can be seen in Figure 1, the Shannon index for SI treatment was the largest, indicating that the species diversity for the 2 $g \cdot L^{-1}$ saline and freshwater mix sprinkler irrigation treatment was the highest. This result indicated that 2 $g \cdot L^{-1}$ saline and freshwater mix sprinkler irrigation significantly increased the bacterial diversity of the arable layer of soil, whereas 3 $g \cdot L^{-1}$ saline and freshwater mix sprinkler irrigation did not have a significant effect on the bacterial diversity when compared to deep-well fresh water. The higher the value of Coverage, the higher the probability that the species in its samples were measured and the lower the probability that they were not. The Coverage values of all three treatments were above 99.7%, indicating that the probability of species being measured in the samples was high, and the sequencing results could represent the real situation of the bacterial community structure in the samples. In 2021, compared with freshwater sprinkler irrigation, salt and freshwater mixed-water irrigation did not have a significant effect on the number of OTUs and the α-diversity index.

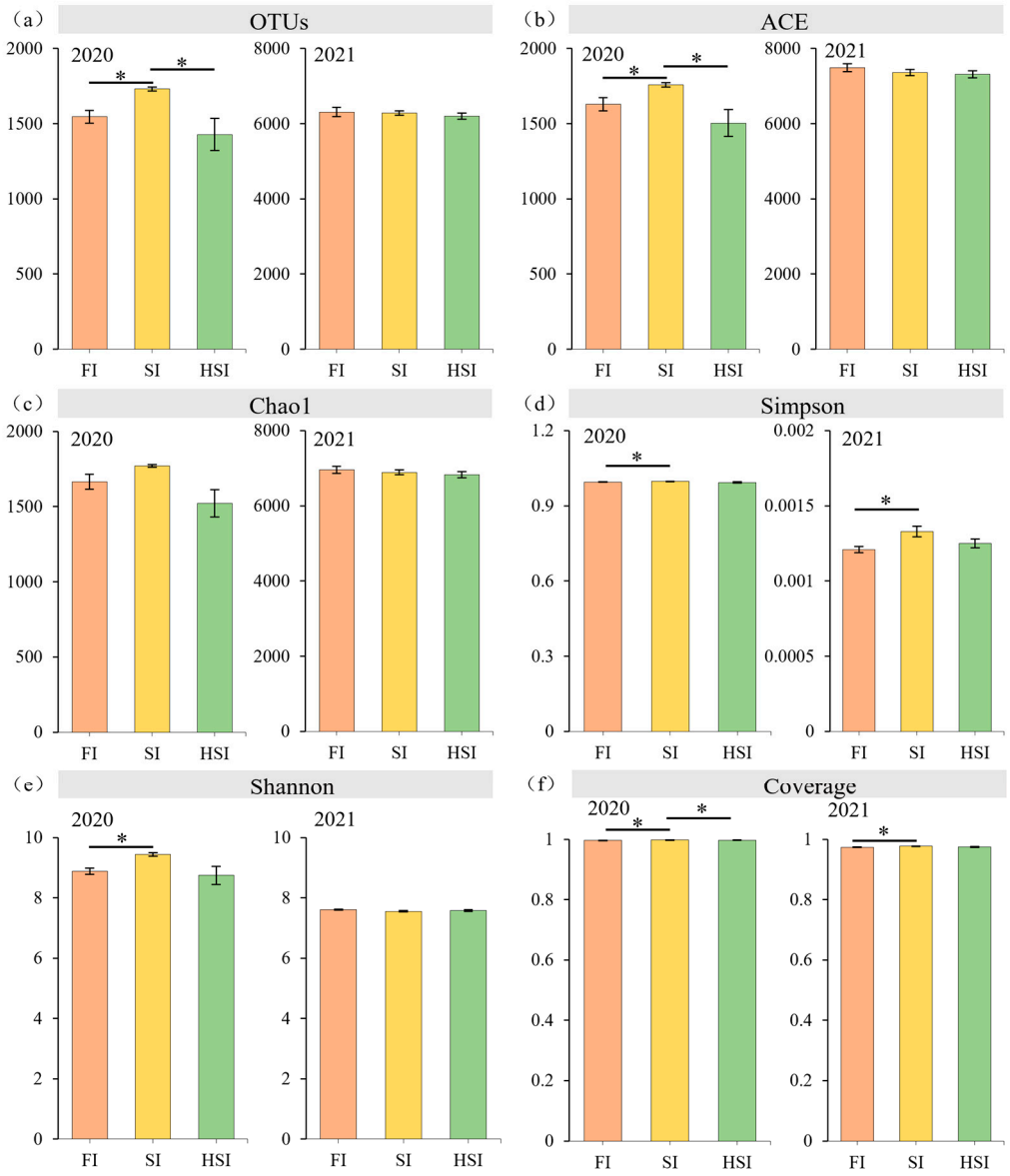

Figure 1. Statistics for bacterial OTUs (**a**) and α-diversity index ((**b**): ACE; (**c**): Chao1; (**d**): Simpson; (**e**): Shannon; (**f**): Coverage) in the plough layer. Note: * represents significant differences between treatments. FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

3.2. OTU–Venn Diagram Analysis of Tillage Bacteria

With a similarity level of 97%, the OTU–Venn diagram can identify shared microorganisms across different treatments and explore the similarities and differences in microbial communities among these treatments [23]. As illustrated in Figure 2, the number of overlapping operational taxonomic units (OTUs) among the three treatments in the 2020 tillage soil reached 1715, indicating that the samples shared a substantial number of bacterial species. In the comparison between the freshwater irrigation (FI) treatment and the sprinkler irrigation (SI) treatment, the shared bacterial species totaled 1772, with endemic species numbering 37 and 88, respectively. Notably, the number of endemic species in the SI treatment increased by 1.38 times compared to that in the FI treatment. When comparing the FI treatment to the high-salinity irrigation (HSI) treatment, the shared bacterial species amounted to 1751, with endemic species at 58 and 101, respectively. In this case, the number of endemic species in the SI treatment was 0.74 times higher than that in the FI treatment. These results indicate that brackish water irrigation significantly increased the number of

endemic species in the soil compared to freshwater sprinkler irrigation, particularly in the SI treatment, which had a more pronounced effect on enhancing bacterial species diversity. This finding is consistent with the results of the α-diversity analysis mentioned earlier. Furthermore, the number of overlapping partial OTUs among the three treatments in 2021 in the arable soil was as high as 3682, and brackish water irrigation (SI and HSI treatments) had minimal impact on the changes in the number of endemic species compared to the FI treatment.

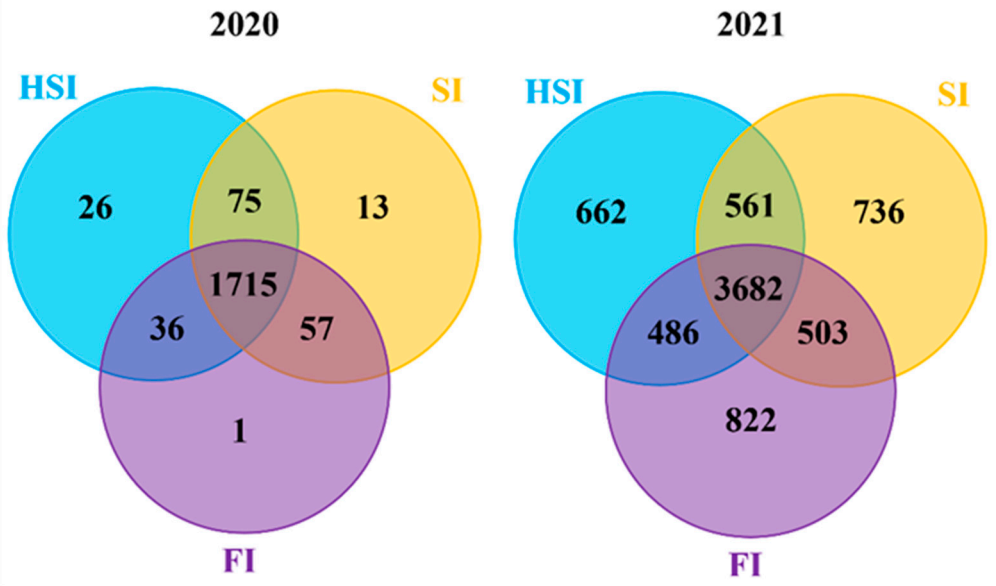

Figure 2. OTU–Venn diagram for each sample of arable soil. Note: FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

3.3. Population Structure Analysis of Bacteria in Each Sample

At the phylum level, 26 and 34 bacterial phyla were detected for each sample in 2020 and 2021, respectively. Figure 3 shows only the top ten phyla at the abundance level for each sample. The overall bacterial composition was similar between treatments. In 2020, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Rokubacteria, Firmicutes, Bacteroidetes, Planctomycetes, and Nitrospirae were the dominant groups, with the combined abundance of these species reaching more than 94%. In 2021, Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Planctomycetes, Nitrospirae, and Firmicutes were the dominant groups.

The relative abundance of these bacterial phyla varied from sample to sample in the soil. In 2020, compared to freshwater sprinkler irrigation, brackish water irrigation reduced the relative abundance of Acidobacteria (mean decrease of 22.5%), Actinobacteria (mean decrease of 18.0%), Gemmatimonadetes (average decrease of 22.3%), and Rokubacteria (average decrease of 31.3%). Conversely, it increased the relative abundance of Chloroflexi (average increase of 84.0%), Firmicutes (average increase of 3.2-fold), Bacteroidetes (mean increase of 1.7-fold), Planctomycetes (mean increase of 1.9-fold), and Nitrospirae (mean increase of 1.6-fold). These findings suggest that brackish water irrigation significantly affects the relative abundance of bacterial taxa in the soil. In 2021, there were no significant differences in bacterial abundance between the treatments.

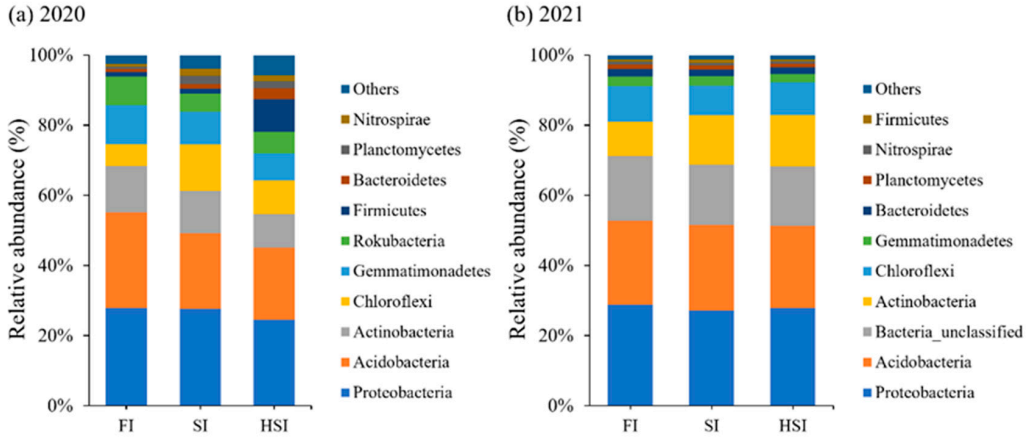

Figure 3. Species distribution of the top 10 microorganisms at the phylum level for bacteria in each sample from the plough layer. Note: FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

At the class level, 86 and 81 bacterial classes were detected in each sample in 2020 and 2021, respectively. Figure 4 displays only the top ten classes by abundance for each sample. The top ten classes in terms of abundance across all samples in both years included Acidobacteria, Subgroup 6, α-Alphaproteobacteria, γ-Alphaproteobacteria, β-Alphaproteobacteria, δ-Alphaproteobacteria, Gemmatimonadetes, NC10, Acidimicrobiia, Blastocatellia Subgroup 4, Thermoleophilus, Thermoleophilia, Actinobacteria, and KD4-96.

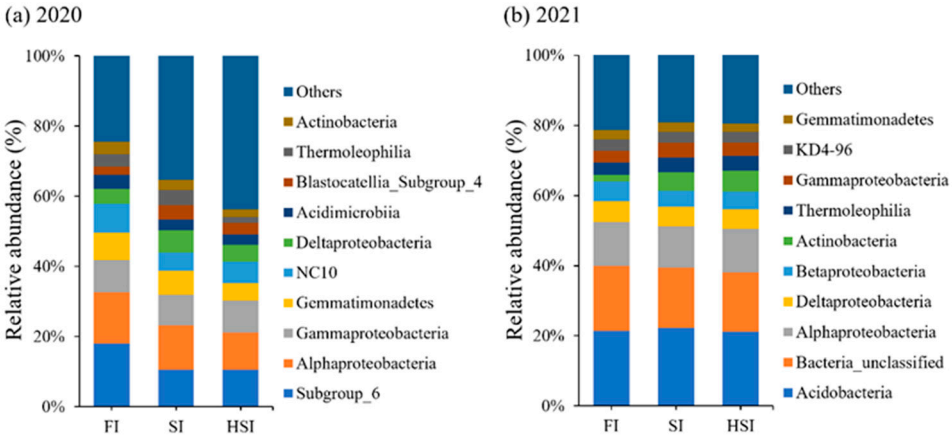

Figure 4. Species distribution of the top 10 microorganisms at the class level for each sample of bacteria from the plough layer. Note: FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

The relative abundance of these bacterial classes varied among the soils of different samples. In 2020, compared to freshwater sprinkler irrigation (FI treatment), brackish water irrigation (SI treatment and HSI treatment) reduced the abundance of Subgroup_6 (41.6% lower on average), Alphaproteobacteria (19.8% lower on average), Gemmatimonadetes (decreased by 24.6% on average), NC10 (decreased by 31.3% on average), Acidobacteria (decreased by 24.9% on average), and Actinobacteria (decreased by 31.1% on average); and an increase in the relative abundance of Deltaproteobacteria (increased by 27.8% on average), Blastocatellia_Subgroup_4 (increased by 62.7% on average); and for Thermoleophilia, compared to the FI treatment, the SI treatment showed a 19.6% increase and the HSI treatment a 52.6% decrease. In 2021, there were no significant differences in bacterial abundance between treatments.

At the genus level, 446 and 459 bacterial genera were detected in each sample in 2020 and 2021, respectively. Figure 5 displays only the top ten genera based on relative abundance for each sample. The top ten bacterial genera with high relative abundance in 2020 and 2021 accounted for 39.5% (ranging from 32.8% to 47.3%) and 50.2% (ranging from 49.6% to 50.1%) of the total sequences in the samples, respectively. The dominant bacterial genera included *MND1*, *RB41*, *Sphingomonas*, and *Gaiella*. The relative abundance of these bacterial genera varied among the soil samples. Compared to freshwater sprinkler irrigation (FI treatment), brackish water irrigation (SI treatment and HSI treatment) decreased the relative abundance of *MND1* and *Sphingomonas*, while increasing the relative abundance of *RB41* and *Gaiella*.

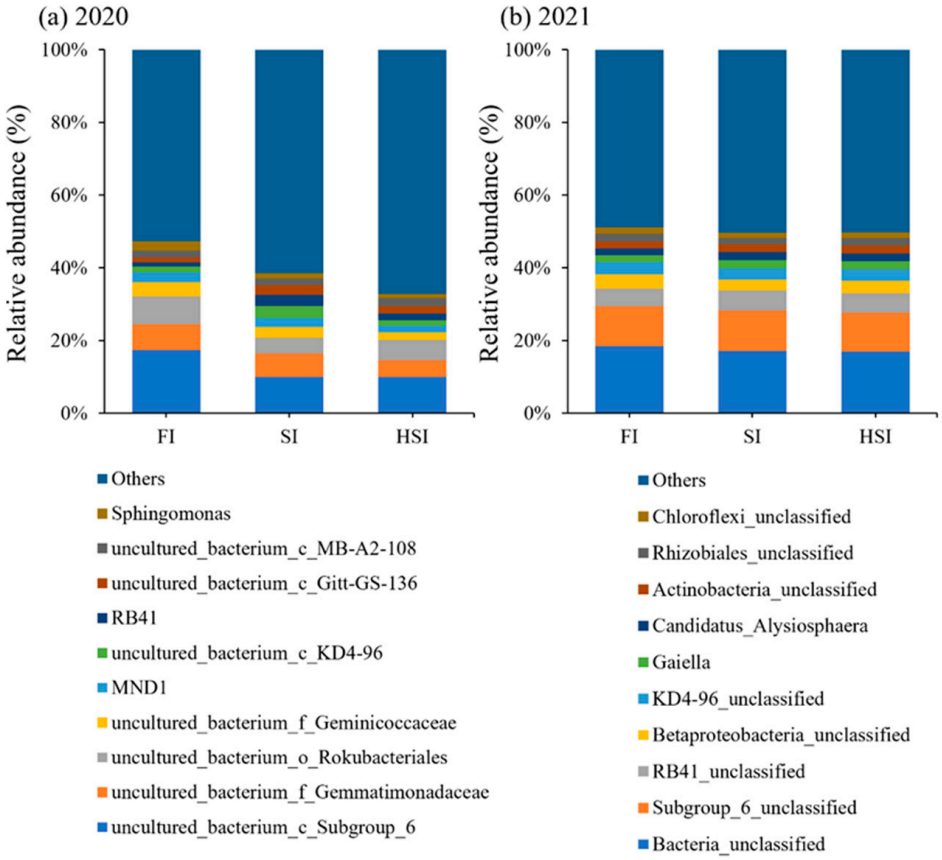

Figure 5. Species distribution of the top 10 microorganisms at the level of genus in each sample from the plough layer. Note: FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

3.4. Beta Diversity Analysis of Bacteria

The β value was calculated by the distance between the samples using weighted unifrac and unweighted unifrac analysis [24]. The unweighted unifrac analysis between different treatments in this study showed no significant difference, so only the β diversity analysis of the weighted unifrac diagram is displayed in Figure 6.

Species variation across multiple samples was visualized graphically using principal coordinate analysis (PCoA) [25]. As illustrated in Figure 6, in 2020, principal coordinates 1 and 2 accounted for 66% and 20.1% of the total variation in community structure, respectively, with the saline irrigation (SI) and high saline irrigation (HSI) treatments distinctly separated from the freshwater irrigation (FI) treatment along axis 2. In 2021, principal coordinates 1 and 2 explained 30.9% and 24.6% of the total variation in community structure, respectively, with the SI and HSI treatments separated from the FI treatment along axis 1. Furthermore, the SI and HSI treatments were closer together in both 2020 and 2021, while both were positioned further away from the FI treatment. These results indicate

that brackish water irrigation can significantly influence the composition of soil bacterial communities in the arable layer.

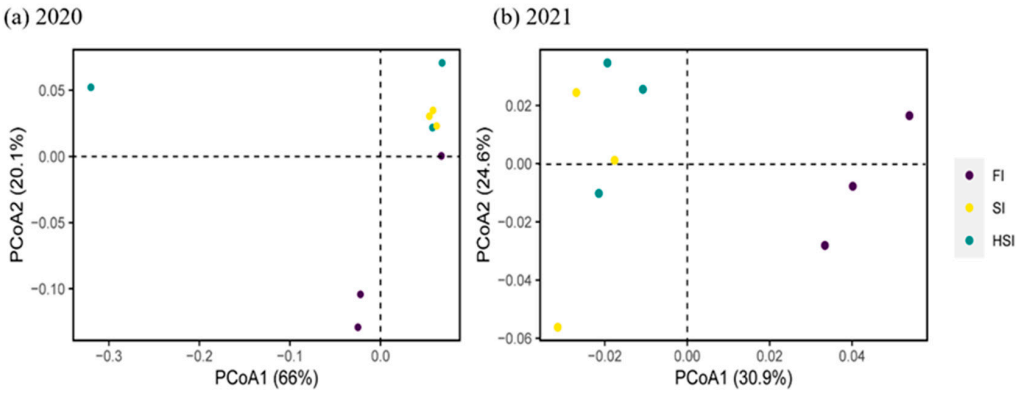

Figure 6. PCoA analysis of soil bacteria in the arable layer. Note: FI: deep-well freshwater irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.

3.5. Correlation Analysis Between Bacterial Microbial Communities and Soil Physico-Chemical Properties

The ecological multivariate data ordering and analysis software CANOCO 5.0 was utilized to investigate the relationship between bacterial microbial communities and soil physicochemical properties. Species-sample data, comprising a table of operational taxonomic units (OTUs) with 97% similarity among samples, were initially employed to conduct Detrended Correspondence Analysis (DCA) for community sorting. The gradient length in the analysis results was found to be less than 3.0 standard deviations, indicating a correlation between soil microbial taxa and the corresponding soil properties, including pH, soil organic carbon (SOC), electrical conductivity (EC), total nitrogen (TN), alkaline nitrogen (AIN), nitrate nitrogen (NIN), ammonium nitrogen (AMN), available phosphorus (AP), and available potassium (AK). The results of the Redundancy Analysis (RDA) are presented in the form of ordination plots. The environmental variables analysis option in the software was employed to identify the environmental variables with the most significant effects.

In 2020, results showed that environmental factors had a greater influence on soil bacterial community structure. The order of influence is as follows: ammonium nitrogen > available phosphorus > alkaline dissolved nitrogen > total nitrogen > pH > EC > nitrate nitrogen, with contribution rates of 27.4%, 23.3%, 14.6%, 14.4%, 11.7%, 4.4% and 2.2%, respectively, and a cumulative contribution rate of 98%. The available potassium and soil organic matter were excluded from the selection of predictor variables using the partial Monte Carlo substitution test.

In 2021, results showed that environmental factors affected the soil bacterial community structure in the following order: ammonium nitrogen (AN) > EC > alkaline dissolved nitrogen (ADN) > total nitrogen (TN) > available potassium (AP) > soil organic matter (SOM) > pH, with contribution rates of 31.5%, 31.4%, 12.5%, 12.2%, 4.9%, 4.1% and 2.2%, respectively, and a cumulative contribution rate of 98.8%. The available phosphorus and nitrate nitrogen were excluded when using the partial Monte Carlo substitution test during the selection of predictor variables.

The environmental factors that affected the soil bacterial community structure by more than 10% in both years were ammonium nitrogen, alkaline dissolved nitrogen and total nitrogen, of which ammonium nitrogen was the most influential environmental factor in both years.

In 2020, RDA analyses (Figure 7) indicated that soil available phosphorus, total soil nitrogen, and pH levels were higher, with a greater clustering of bacterial communities observed in the freshwater sprinkler irrigation treatment (FI treatment). In contrast, soil

microbial communities in the brackish sprinkler irrigation treatments (SI and HSI treatments) were primarily clustered in areas characterized by high electrical conductivity (EC) and ammonium nitrogen. In 2021, total soil nitrogen, organic matter, alkaline dissolved nitrogen (ADN), and available potassium (AK) levels were higher, leading to increased clustering of bacterial communities. Conversely, soil microbial communities in the brackish water sprinkler irrigation treatments (SI and HSI treatments) were predominantly clustered in areas with elevated EC, pH, and ammonium nitrogen. These findings suggest that the bacterial community structure was more similar among the brackish water sprinkler treatments, while it significantly differed from that of the freshwater sprinkler treatments.

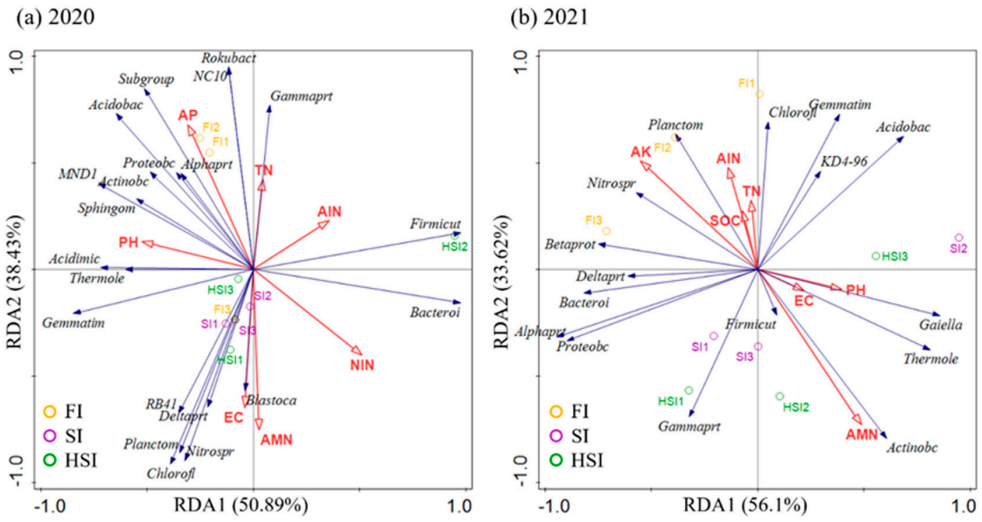

Figure 7. RDA analysis of soil properties and microbial communities of different treatments. Note: FI: deep-well fresh water irrigation at 0.5 g·L^{-1}, SI: saline and fresh mixed water irrigation at 2 g·L^{-1}, HSI: high level saline and fresh mixed water irrigation at 3 g·L^{-1}.Note: The bacterial flora are abbreviated as Proteobacteria = Proteobc, Acidobacteria = Acidobac, Actinobacteria = Actinobc, Chloroflexi = Chlorofl, Gemmatimonadetes = Gemmatim, Rokubacteria = Rokubact, Firmicutes = Firmicut, Bacteroidetes = Bacteroi, Planctomycetes = Planctom, Nitrospirae = Nitrospi, Subgroup_6(Class) = Subgroup, Alphaproteobacteria(Class) = Alphaprt, Gammaproteobacteria(Class) = Gammaprt, NC10(Class) = NC10, Deltaproteobacteria(Class) = Deltaprt, Acidimicrobiia(Class) = Acidimic, Blastocatellia_Subgroup_4(Class) = Blastoca, Thermoleophilia(Class) = Thermole, Betaproteobacteria(Class) = Betaprot, KD4-96(Class) = KD4-96, MND1 (Genus) = MND1, RB41 (Genus) = RB41, Sphingomonas (Genus) = Sphingom, Gaiella = Gaiella.

In 2020, the relative abundance of Rokubacteria, NC10, Subgroup 6, Acidobacteria, Alphaproteobacteria, Proteobacteria, Actinobacteria, Sphingomonas, MND1, and Gammaproteobacteria in freshwater sprinkler irrigation was correlated with a high level of soil available phosphorus, total soil nitrogen, and pH. In the brackish sprinkler irrigation treatment, the relative abundance of RB41, Deltaproteobacteria, Planctomycetes, Chloroflexi, and Nitrospirae, Blastocatellia Subgroup 4 were associated with high level of electrical conductivity (EC) and ammonium nitrogen. The relative abundance of Firmicutes was linked to a high level of total nitrogen, alkali-dissolved nitrogen, and nitrate nitrogen. In 2021, in the freshwater sprinkler irrigation treatment, the relative abundance of Planctomycetes, Nitrospirae, and Betaproteobacteria was associated with a high level of total soil nitrogen, organic matter, alkaline-dissolved nitrogen, and available potassium. In the brackish water sprinkler treatment, Gammaproteobacteria were correlated with a high level of ammonium nitrogen and EC, while Firmicutes, Actinobacteria, Thermoleophilia, and Gaiella were associated with increased ammonium nitrogen, EC, and pH.

4. Discussion

Soil salinity is a key factor affecting soil microbial diversity and community composition. Especially under high salinity conditions, the increase in salinity leads to changes in soil osmotic pressure, which results in the loss of water from microbial cells, thus inhibiting bacterial growth and even leading to bacterial death. Experimental results showed that bacteria in the tillage soil under 2 $g \cdot L^{-1}$ mixed saline and freshwater treatment exhibited higher species richness and diversity, and the number of bacterial OTUs, Chao1 index and Shannon index were significantly higher than that of freshwater treatment. However, when salinity was further increased to 3 $g \cdot L^{-1}$, these indices decreased significantly, i.e., excessive salinity showed a negative effect on bacteria.

Changes in the chemical composition of the soil and plant root secretions can also affect microbial diversity, as plant roots alter the rhizosphere chemical environment through the secretion of specific compounds, which not only help themselves to adapt to salt stress [12], but also have an impact on the composition and diversity of rhizosphere microorganisms [11]. Meanwhile, plant-associated microorganisms are able to promote plant growth by competing for nutrients [3,26,27], thus affecting the composition of the entire microbial community. Therefore, the relationship between salinity and microbial diversity is complex, involving the interaction of multiple factors [28,29].

The effect of soil salinity on microbial community structure has been demonstrated in several studies. Under high salinity conditions with 1.5–2% NaCl, the mixed treatment of *P. pseudoalcaligenes* and *Bacillus pumilus* showed a highly positive response to the adverse effects on salinity [30]. For example, the rhizosphere bacterial communities of saline plants showed a significant correlation with soil salinity in arid and semi-arid regions, and these salt-tolerant bacteria mainly belong to Gammaproteobacteria, Alphaproteobacteria, Bacteroidetes, and Verrucomicrobia. These microorganisms in high salinity soil not only help plants to resist salt stress, but also reflect the ability of microbial communities to adapt to saline environments [31]. This study showed that 2 $g \cdot L^{-1}$ mixed saline and freshwater irrigation decreased the relative abundance of Acidobacteria, Actinobacteria, Gemmatimonadetes, and Rokubacteria, while increasing the relative abundance of Chloroflexi, Firmicutes, Bacteroidetes, Planctomycetes, and Nitrospirae, suggesting that it had a significant impact on the relative abundance of soil bacteria. In addition, the increase in salinity promoted the growth of certain microbial taxa, such as Bacteroidetes and Gemmatimonadetes, which are highly salinity-adapted [32]. In saline soils, structural changes in microbial communities not only affect their functions, but also have a profound impact on soil health and ecological balance [33]. Previous study showed that the abundance of Acidobacteria and actinomycetes decreased with the increase of salt in hypersaline soils and sediments [17]. Hence, long-term saline irrigation could inhibit soil microbial activity, and then affect the decomposition and mineralization of soil organic matter.

Soil salinity is also closely related to soil physico-chemical properties. Factors such as soil pH, salinity and organic matter content together determine the soil microbial ecological environment. Results showed that environmental factors with more than 10% influence on soil bacterial community structure in both years were mainly ammonium nitrogen, alkaline dissolved nitrogen and total nitrogen, of which ammonium nitrogen was the most influential environmental factor in both years.

Soil pH had a significant effect on the microbial community, especially in alkaline soils, where bacterial diversity was positively correlated with pH [34], suggesting that soil alkalinity may promote the growth of microbial taxa [35]. In addition, changes in soil salinity occurred under irrigation with a mixture of brackish and fresh water of different mineralization levels, which also affects microbial activity and metabolic efficiency. High salinity environments usually inhibit microbial respiration [36] and affect soil biological activity. Moreover, salinity may also lead to a decrease in the functional diversity of microbial communities [37], which in turn affects the nutrient cycling and ecological balance of the soil. Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_{3^-}) are the two main forms of inorganic nitrogen required for plant growth, and they are also inextricably

linked to plant growth and soil microbial communities. In the root zone of maize, the spatial distribution of bacterial communities is affected by a combination of nitrogen forms and rhizospheres, which is closely related to changes in soil pH during NH_4^+ and NO_{3^-} uptake by the maize root system [38].

In summary, the effects of soil salinity on microorganisms are multifaceted, involving both changes in microbial diversity, and reflecting the interrelationships between microbial community structure and physicochemical properties.

5. Conclusions

In this study, summer maize was taken as the test crop. The bacterial diversity and community structure in the rhizosphere soil of brackish water irrigation at different concentrations were analyzed using high-throughput sequencing technology. The conclusions were as follows: compared to freshwater sprinkler irrigation, sprinkler irrigation at 2 $g \cdot L^{-1}$ significantly increased the number of bacteria, the number of endemic species, and the species diversity in the ploughed soil, whereas sprinkler irrigation at 3 $g \cdot L^{-1}$ did not have a significant effect on these indices in the surface soil. In addition, mixed saline and freshwater irrigation decreased the relative abundance of Acidobacteria, Actinobacteria, Gemmatimonadetes, and Rokubacteria, while increasing the relative abundance of Chloroflexi, Firmicutes, Firmicutes, Bacteroidetes, Planctomycetes and Nitrospirae. This indicated that mixed saline and freshwater irrigation significantly affected the relative abundance of soil bacterial taxa. Results also showed that soil physicochemical properties, such as ammonium nitrogen, alkaline dissolved nitrogen and total nitrogen, all had a more than 10% effect on the bacterial community structure. It can be seen that sprinkler irrigation with a mixture of salty and fresh water will have a certain effect on the growth environment of soil bacterial microorganisms in summer maize, and 2 $g \cdot L^{-1}$ sprinkler irrigation was chosen as a more suitable method.

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