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Effects of Salinity Accumulation on Physical, Chemical, and Microbial Properties of Soil under Rural Domestic Sewage Irrigation

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Abstract: Under irrigation with saline wastewater, SO_4^{2-}, Cl^-, and Ca^{2+} aggregated in the topsoil, and Mg^{2+} was significantly higher in the deeper soil than in the topsoil and 40 cm soil layers. The abundance of *Zoopagomycota*, *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, *Chytridiomycota*, *Rozellomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Mucoromycota* and *Olpidiomycota* in the surface soil was influenced by Mg^{2+}, whereas Ca^{2+} affected the abundance of *Zoopagomycota* and *Chytridiomycota*. In the 40 cm soil layer, Mg^{2+} and Cl^- promoted Actinobacteria, Proteobacteria, *Nitrospirae*, *Firmicutes*, *Entotheonellaeota*, *Myxococcota*, *Gemmatimonadota* and *Methylomirabilota*, whereas they inhibited *Planctomycetota*, *Acidobacteria*, *Chloroflexi*, *Patescibacteria* and *Bacteroidota*. In the 80 cm soil layer, SO_4^{2-} and Cl^- promoted *Rozellomycota*, *Mortierellomycota*, *Chytridiomycota*, *Ascomycota*, and *Mucoromycota*, but had a negative effect on *Glomeromycota*, *Blastocladiomycota*, *Olpidiomycota* and *Monoblepharomycota*. The increase in salinity significantly reduced the abundance of the *Actinomycetes* phylum and the *Amoebozoa* phylum. Both saprophytic and symbiotic fungi decreased with increasing salinity.

Keywords: rural domestic sewage; soil salinity; soil fertility; bacteria; fungi; functional classification

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1. Introduction

Soil salinization has become one of the important factors of soil degradation; 40% of the world's agricultural land has already been contaminated with varying degrees of salinity, and more than 830 million hectares of arable land are affected by soil salt damage [1]. The risk of salinization has become a common problem in more than 100 regions of the world, and about 900 million hectares of soil suffer from salinization or alkalization, more than 60% of which are sodic and salinity-affected soils [2], with 36 million hectares affected by salinity in China. It is noteworthy that soil salinity damage caused by irrigation water is also occurring [3,4].

The rural domestic wastewater discharge in China is increasing, with about 8 billion tons of domestic wastewater discharged annually. To alleviate the water shortage, many regions use rural domestic wastewater as an important resource for irrigation. Since rural domestic wastewater contains a certain amount of salt, agricultural use has direct impacts on soil health due to salinity, representing an important area of study [5]. Previous research found that the soils irrigated with treated wastewater had no salt accumulation for 5 years, while there was a slight salt accumulation in the soil after 15 years [6]. The accumulation of soil surface salts depends on the quality and quantity of irrigation water and the depth of irrigation water [7]. It has been noted that high Na^+ content may result in soil porosity, and

high concentrations of sodium ions may also cause deficiency of other cations, such as K^+, Ca^{2+}, and Mg^{2+} [8]. In areas with high evaporation and low natural precipitation, irrigation with treated wastewater can easily lead to salt accumulation in soils with increasing years of a certain amount of irrigation [9].

Biological indicators are important drivers of matter transformation and formation in soil because soil microorganisms can directly reflect changes in the soil environment [10–12]. Salt can reduce the enzyme activity, microbial quantity, and decomposition rates of organic matter and affect the functional diversity of microorganisms. The soil salinity content has a direct effect on soil nutrients and microorganisms, inhibiting soil microbial activity and affecting microbial community structure. Previous studies have shown that irrigation with saline effluent can increase the abundance of soil bacterial communities, but other studies have also shown a decreasing trend or no significant change [13]. The migration, accumulation, and transport of salts in soil under domestic wastewater irrigation is determined by the complexity of the soil. The topsoil has direct contact with irrigation water, the atmosphere, crop planting, and farming methods; therefore, the state of topsoil is crucial to agricultural farming. The long-term use of saline domestic wastewater for irrigation inevitably impacts the sustainable development of agriculture due to the various adverse effects of salt. Saline wastewater irrigation has specific effects on soil permeability and crop growth; for example, salinity affects the topsoil soil environment, which in turn can also increase chloride and sodium levels in crops. It has been shown that soil salinization can also lead to changes in the physical, chemical, and biological properties of the soil itself [14].

Most of the current studies on the effects of soil salinity have been conducted in agricultural fields or coastal wetland ecosystems and mainly address the relationship between salinity and soil nutrients and microorganisms, estimation of soil water–salt migration, soil salinity distribution, and ionic composition, and effects on crop growth under brackish water irrigation. However, agricultural recycling of saline domestic wastewater has been less studied. Currently, domestic wastewater used for irrigation mainly focuses on nitrogen, phosphorus, COD, and heavy metals [15,16]. Generally, it seems that the research on rural domestic wastewater is scattered. Because rural domestic wastewater recycling is not standardized, many uncertain factors need to be verified, and salt should be one of the important factors to be considered. Irrigation with rural domestic wastewater has impacts on the soil nutrient and microbial environment, especially in different soil environments, under the combined effects of its fertility and pollutants.

Different external factors, such as light, temperature, and moisture, in different soil layers may lead to corresponding changes in the soil environment in these layers, thus affecting soil physicochemical and microbial community structure. The structure and diversity of soil microbial communities in different soil layers after irrigation with saline domestic wastewater change, with increases, decreases, or even irregular changes in parameters. The deep soil microbial community also plays an important role in soil respiration, nutrient cycling, and ecosystem function. Based on this, this study was conducted on topsoil, 40 cm, and 80 cm soil layers, and the limit of soil total salinity in the Standard for Irrigation Water Quality [17] was used as the median. The experiments with different salinities of domestic wastewater were conducted to explore the nutrient, soil bacterial, and fungal community changes in lightly saline soil, as well as the effect of different salinity levels on soil fertility as well as bacterial and fungal communities as a result of different amounts of irrigation. The work aims to provide data support for the safe reuse of rural domestic wastewater and the long-term development of agricultural safety.

2. Materials and Methods

2.1. Sample Test

The soil was taken from Jinghai District, Tianjin, China, and was naturally air-dried and passed through a 2 mm mesh sieve with a soil capacity of 1.2193 $g \cdot cm^{-3}$. The physical properties of the soil are shown in Table 1. The soil samples were then filled with an 80 cm soil column, the physicochemical properties of which are shown in Table 2. The domestic

wastewater was collected from the sewage treatment plant in Jinghai District, Tianjin City. Plastic containers were used to collect the domestic wastewater and transport it back to the experimental site. The physicochemical properties of the domestic wastewater are shown in Table 2.

Table 1. Physical properties of tested soil.

Soil Depth	Soil Particle Composition				Soil Properties	
	Grit/% (0.2~2 mm)	Fine Sand/% (0.02~0.2 mm)	Silt/% (0.002~0.02 mm)	Clay Particle/% (<0.002 mm)	Saturated Moisture Content/%	Soil Bulk Density/g·cm^{-3}
0~20	3.6	35.9	34.4	26.1	30.17~32.24	23.1~32.07
20~40					32.47~33.67	24.9~33.02
40~60					33.09~35.13	25.32~35.91
60~80					33.58~38.61	27.82~37.84

Table 2. The physical and chemical properties of test soil samples.

Test Sample	TN	TP	OM	NH_4^+-N	NO_3^--N	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	SO_4^{2-}
Soil sample/mg·kg^{-1}	0.84	0.62	20.93	0.022	0.68	6.45	2.09	6.65	3.36	2.89
Water sample/mg·L^{-1}	22.62	1.89	-	18.48	2.71	120.88	40.48	121.25	54.6	75

Note: TN, TP, and OM represent total nitrogen, total phosphorus, and organic matter.

2.2. Experimental Design

In the pre-test, it was found that Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} were the main salt-based ions in domestic wastewater through preliminary testing, and the solubilizing effect of salt ions in the soil was significant. The increase in soil conductivity was positively correlated with these soluble salt ions. It was subsequently found that Na^+ and Cl^- were the main indicators for the assessment of soil salt damage, SO_4^{2-} and Ca^{2+} had an inhibited effect on each other, and Mg^{2+} was the main indicator for affecting crops. So, this experiment took Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} as the main research ions to explore their effects on the soil, and the salt concentration, with a specified concentration of 1 g·L^{-1} in GB 5084 (China National Water Quality Standards for Agricultural Irrigation) as the median, was determined as 0.03 g·kg^{-1}, 0.04 g·kg^{-1}, 0.06 g·kg^{-1}, 0.08 g·kg^{-1}, and 0.12 g·kg^{-1}. Since no heavy metals were detected in preliminary experiments with domestic wastewater, and the impact of domestic wastewater irrigation on soil heavy metals was negligible [18], the influence of heavy metals was not considered.

Indoor soil column simulation experiments were conducted to study the effects of salinity on soil microbiota. The soil columns were set to 80 cm, with sampling ports set at 0 cm, 40 cm, and 80 cm as shown in Figure 1. Before the experiment began, the soil was thoroughly mixed, and the soil columns were leached with water to remove salts. As the domestic wastewater could not reach the required concentration for the experiment, it was diluted to 0.2 g·L^{-1} (RW). Different salinities were achieved by mixing the diluted domestic wastewater with prepared saline solution ($NaCl:CaCl_2:MgSO_4$ = 2:1:2), including CK (0 g·L^{-1}), W1 (0.5 g·L^{-1}), W2 (0.8 g·L^{-1}), W3 (1.2 g·L^{-1}), W4 (1.6 g·L^{-1}), and W5 (2.0 g·L^{-1}); see Figure 1b. After 72 days of irrigation, the fertility and microbial changes in the irrigated surface soil were measured to provide a basis for the safe reuse of rural domestic wastewater. The irrigation rate was determined by the water requirements of the cabbage in the unit area; the irrigation rate was 15.555 L/m^2, watering once every 2 days.

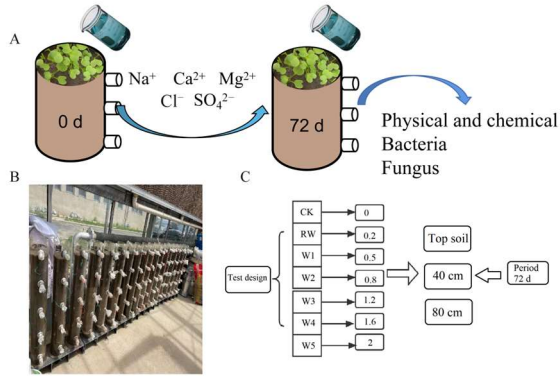

Figure 1. Schematic diagram of test design. (**A**) Schematic diagram of experimental design (**B**), Actual picture of experiment (**C**), Schematic diagram of different treatments.

2.3. Physicochemical Analyses

The physicochemical properties of samples were determined using the following methods: soil TP content was measured by the alkali fusion-molybdenum antimony anti-spectrophotometric method [19]; soil TN content was measured by the Kjeldahl method [20]; soil NH_4^+-N and NO_3^--N contents were determined by the spectrophotometric method [21]; soil OM content was determined according to the Soil Organic Matter Determination Method [22]; soil conductivity was measured by a conductivity meter (SX650, Shanghai San-Xin Instrumentation Factory); Na^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, and Cl^- contents were determined according to [23]; and soil density was determined according to [24].

2.4. Microbial Analyses

On an Illumina HiSeq 2500 platform, high-throughput sequencing for the microbial community study was carried out. The thermocycler PCR system (GeneAmp 9700, ABI, Los Angeles, CA, USA) was used to amplify the V3–V4 hypervariable regions of the bacteria 16S rRNA gene using the primers 338F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR reactions were carried out in a 20 L mixture containing 4 L of 5 FastPf following the manufacturer's instructions; the produced PCR products were extracted from a 2% agarose gel, further purified using the AxyPrep DNA Gel Extraction Kit from Axygen Biosciences in Union City, California, and quantified using QuantiFluorTM-ST from Promega in the United States.

The raw data of bacteria were checked first, and sequences that were less than 200 bp, had a low-quality score (20), contained ambiguous bases, or did not precisely match primer sequences and barcode tags were excluded from consideration. The sample-specific barcode sequences were used to segregate qualified reads, which were then trimmed with Illumina Analysis Pipeline Version 2.6. After that, QIIME was used to evaluate the data collection. To create rarefaction curves, group the sequences into operational taxonomic units (OTUs), and determine the richness and diversity indices, 97% similarity was used to group the sequences into OTUs. All sequences were categorized into several taxonomic groupings using the Ribosomal Database Project (RDP) Classifier program.

2.5. Statistical Analysis

The data were calculated using Excel 2019 (Microsoft, Redmond, WA, USA), and the figures were plotted by OriginPro 2021 (OriginLab, Northampton, MA, USA). SPSS 25 was used for the analysis of variance (ANOVA) of soil and water physicochemical properties and the composition of microbial communities ($p < 0.05$). Major bio was used for processing microbiome data. Redundancy analysis (RDA) (CANOCO 5) and Spearman's correlation analysis were used for assessing correlations among soil properties, salinity, and soil microbial compositions. PICRUST software was used for analyzing the function

of soil bacteria using the KEGG function. FUNGuild prediction was used to predict the function of soil fungi.

3. Results and Analysis

3.1. Soil Salt Accumulation and Soil Fertility Status

To investigate the effect of salinity on soil physicochemicals, this study subjected soil salinity and soil physicochemical indicators to Pearson correlation analysis. Total soil salinity was highly significantly negatively associated with soil TN and OM ($p < 0.01$); total soil salinity was highly significantly correlated with soil TN, TP, and OM in the 40 cm soil layer ($p < 0.01$), with correlation coefficients of −0.80, −0.80, and −0.85, respectively; meanwhile, TN, OM, and TP contents in the 80 cm soil layer showed a significant decrease compared to the topsoil ($p < 0.05$) (Table 3).

Table 3. Correlation analysis of soil physical and chemical properties at different depths.

Depth (cm)		Total Salt	TN	TP	OM
0	Total salt	1	−0.63 **	−0.39	−0.80 **
	TN		1	0.58 **	0.35
	TP			1	0.37
	OM				1
40	Total salt	1	−0.80 **	−0.80 **	−0.85 **
	TN		1	1 **	0.88 **
	TP			1	0.87 **
	OM				1
80	Total salt	1	−0.817 **	−0.041	−0.964 **
	TN		1	−0.305	0.873 **
	TP			1	0.033
	OM				1

Note: ** $p < 0.01$, TN, TP, and OM represent total nitrogen, total phosphorus, and organic matter.

Figure 2 illustrates the differences in soil properties between the different treatments.

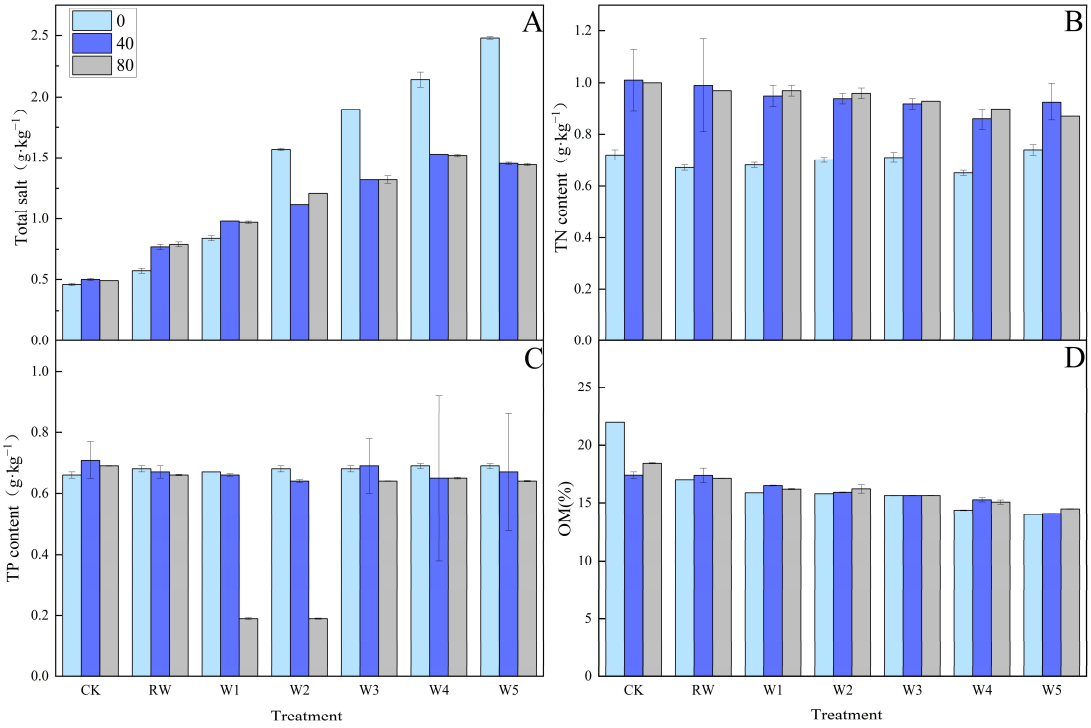

Figure 2. Trend chart of soil physical and chemical indicators in each layer. (**A**) Total salt; (**B**) TN; (**C**) TP; (**D**) OM. Note: TN, TP, and OM represent total nitrogen, total phosphorus, and organic matter.

Total soil salinity, TP, TN, and OM content changed with increasing irrigation salinity. Firstly, there were significant differences between the surface layer and the 40 cm and 80 cm layers in the total salinity of the soil layers. The TN content of the 40 cm and 80 cm soil was higher than that of the surface soil after 72 d of irrigation; the TN content increased by 14.9–28%. Secondly, soil OM under high salinity (>1000 mg·L^{-1}) rural domestic wastewater irrigation was found to be significantly lower ($p < 0.05$) than that under low salinity (<1000 mg·L^{-1}) irrigation, with a range of 19.04% to 36.3%, and the OM content of 40 cm soil layer was significantly different ($p < 0.05$) from that of the surface layer and 80 cm soil layer. Soil TN, OM, and TP are essential sources of information on soil fertility levels and factors determining soil fertility. Prolonged irrigation with domestic wastewater leads to soil salt accumulation, especially in deep soils, thus reducing soil fertility. Soil fertility was also significantly negatively correlated with soil salinity in all relevant studies. Soil OM is one of the essential sources of soil nutrients, playing a vital role in soil formation and fertility cycles. The increase in soil salinity tended to plate the soil, further reducing soil porosity and soil OM content; therefore, irrigation with high saline domestic wastewater did not facilitate soil nutrient enrichment.

3.2. Changes in Major Salt-Based Ions in Surface, 40 cm, and 80 cm Soils

SO_4^{2-}, Cl^-, and Ca^{2+} displayed the phenomenon of surface aggregation, among which Na^+ and Cl^- in the surface soil were considerably different from those in the 40 cm and 80 cm soil layers, and the content of Mg^{2+} in the 80 cm soil was significantly higher than that in the surface and 40 cm soil layers ($p < 0.01$). Rural wastewater irrigation causes accumulation and washing of soil salts, which may both occur in the same soil. When salt accumulation was predominant, irrigation with salt wastewater at this concentration increased the negative impact on the soil environment, while when washing was prevalent, the use of saline wastewater at this concentration did not have a significant negative impact on the soil. The exchange of ions plays a key role; Mg^{2+} and Ca^{2+} are replaced by exchangeable Na^+, resulting in a further increase in the Na^+ content of the surface soil. With the infiltration of NaCl solution, the infiltration properties of the soil are further reduced, so that Na^+ accumulates more on the surface. Irrigation with domestic wastewater containing a salt concentration of (<1 g/L^{-1}) showed insignificant migration changes in sodium ions; irrigation with domestic wastewater having a salt concentration of (>1 g/L^{-1}) caused significant trends in the Na^+ content in topsoil as well as 40 cm and 80 cm soil layers (Figure 3).

The different migration phenomena of sodium ions may be a result of the migration ability of Na+ in soil being primarily governed by environmental factors. When the evaporation effect is strong at high temperatures, the upward migration effect of Na^+ in the soil is noticeable, so that the sodium ion content of shallow soils is higher than that of deep soils. This phenomenon also occurs for Cl^-. Mg^{2+} showed a more obvious desalination phenomenon; Mg^{2+}, which was quickly replaced by Na^+, migrated into the soil solution together with Ca^{2+} and then migrated more profoundly into the soil layer together with water, resulting in a more obvious desalination of surface Mg^{2+}. Mg^{2+} in deep soils has the property of strong adsorption with soil colloids; thus, the Mg^{2+} content in soils increased with the increase of irrigation [25]. The migration pattern in soils of different textures showed that Cl^- had the fastest migration rate in the 0~40 cm soil layer, with the migration rate of Cl^- in deep soils significantly slower. Cl^- has strong mobility and leaching, and its downward migration ability gradually increases with the increase of Cl^- content (Figure 3).

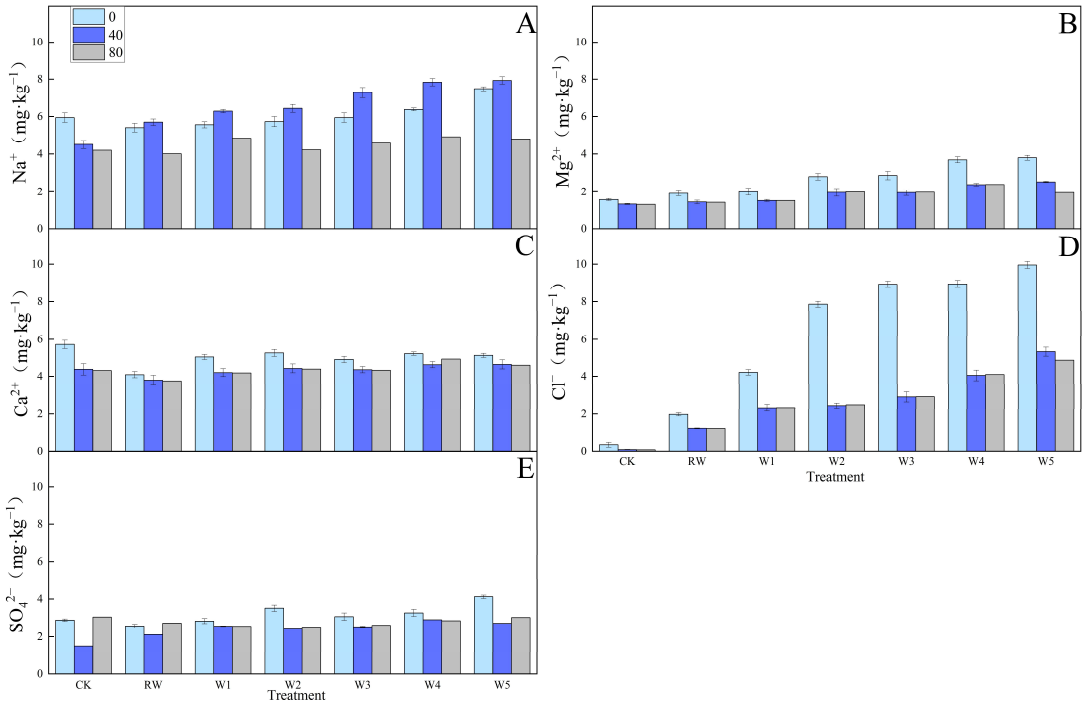

Figure 3. Trend chart of main base ions of soil in each layer. (**A**) Na^+; (**B**) Mg^{2+}; (**C**) Cl^-; (**D**) Ca^{2+}; (**E**) SO_4^{2-}.

3.3. Soil Microbial Environment Analysis

The surface soil fungal diversity indicated that soil salinity in group Z was significantly negatively correlated with the Shannon index ($R^2 = -0.64$, $p < 0.05$) and with the Chao1 index soil bacterial richness ($R^2 = -0.53$, $p < 0.05$). The 40 cm soil layer Na^+ was highly negatively correlated with the Shannon index and Chao1 index ($p < 0.01$); total salt and Ca^{2+} were significantly positively correlated with the fungal Shannon index, Chao1 index, and ACE index ($p < 0.05$). Soil salinity and major salt-based ions were not significantly correlated with bacterial and fungal Alpha diversity in the 80 cm soil layer ($p > 0.05$).

Soil microbial levels can reflect soil microbial community characteristics, ecological characteristics, and soil environmental characteristics. The OTU analysis of soil samples was performed by the Uparse software platform, in which the total number of bacterial OTUs was 7974, including 46 phyla, 597 families, 1126 genera, and 2289 species; the total number of fungal OTUs was 3801, including 14 phyla, 265 families, 623 genera, and 1143 species.

To explore the variation of soil microbial species in different soil layers, dominant species were selected according to the gate level and plotted as histograms, as shown in Figure 4A.

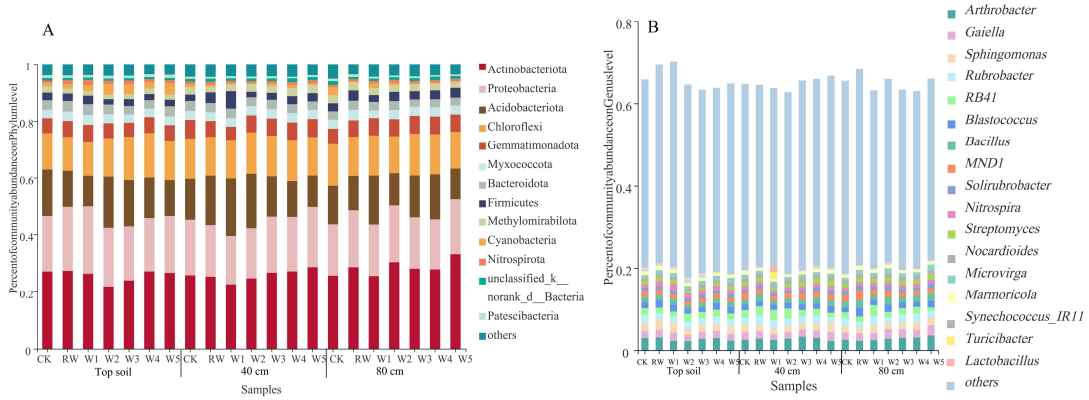

Figure 4. Species composition of soil bacteria in the group (**A**) level of phylum; (**B**) level of genus.

At the bacterial phylum level, the soil bacterial taxa under each treatment were Actinobacteria (21.84~33.15%), proteobacteria (16.89~23.77%), *Chloroflexi* (11.74~15.68%), *Acidobacteriota* (10.60~20.31%), *Gemmatimonadetes* (4.68~6.31%), *Myxococcota* (2.67~3.72%), *Bacteroidetes* (2.53~3.75%), and *Firmicutes* (1.86~6.04%). Figure 4B shows *Arthrobater* (2.21~3.57%), *Gaiella* (1.35~2.48%), *Sphingomonas* (1.36~2.53%), *Rubrobacter* (1.31~2.24%), *RB41* (0.83~2.61%), *Blastococcus* (1.09~2.08%), and *Bacillus* (0.81~1.86%). As shown in Figure 5, at the same irrigation rate, we found proteobacteria, *Gemmatimonadetes*, *Bacteroidota*, *Methylomirabilota*, *Cyanobacteria*, and *Nitrospirota* in the surface, 40 cm, and 80 cm soil layers; *Patescibacteria*, *Entotheonellaeota*, and RCP2-54 relative abundance were significantly different, where the relative abundance of proteobacteria, *Bacteroidota*, *Cyanobacteria*, *Nitrospirota*, and *Patescibacteria* increased with the deepening of the soil layer ($p < 0.05$). The relative abundance of proteobacteria, *Bacteroidota*, *Cyanobacteria*, *Nitrospirota*, and *Patescibacteria* tended to increase significantly ($p < 0.05$), while the relative abundance of *Gemmatimonadetes*, *Methylomirabilota*, *Entotheonellaeota*, and RCP2-54 tended to decrease significantly ($p < 0.05$) as the soil layer deepened. Significant differences were observed for *Gaiella*, *MND1*, *Nitrospira*, and *Streptomyces* in the three soil layers of the genus.

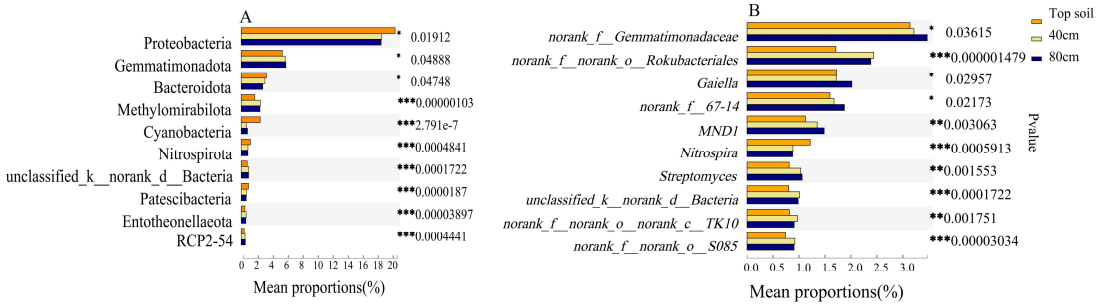

Figure 5. Analysis of differences between dominant bacterial phyla and genus groups in different soil layers ((**A**) level of phylum; (**B**) level of genus). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In the three layers of soil, we took the dominant phylum as an example for illustration, and we found that the trend of the relative abundance of the dominant phylum was consistent with the survival habit and functional characteristics of the phylum, in which proteobacteria had better salt tolerance. As well, it included a variety of pathogenic bacteria and nitrogen-fixing bacteria, which could use nutrients such as ammonia and methane produced by the decomposition of organic matter for growth metabolic activities [26]; proteobacteria were mainly composed of α, β, and γ phyla, with more nitrogen-fixing bacteria, and could play a stabilizing role with regard to residual soil nitrogen in the process of nitrogen cycling. Meanwhile, the relative abundance of proteobacteria in the topsoil was the highest, while *Bacteroidota* had strong resistance to a high salinity environment and was a moderately salinity-loving bacteria, representing the dominant population in saline soils. *Bacteroidota* plays a role in phospholipids in soil and plays a vital role in phosphorus transformation [27]. *Gemmatimonadetes* mainly carries out carbon and nitrogen fixation, and *Cyanobacteria* is also a salt-tolerant phylum that commonly grows in freshwater, seawater, and soil. *Gaiella* belongs to *Actinobacteriota*. *Arthrobacter* is one of the most common bacteria in soil; it has high adaptability to highly saline environments and can be used for bioremediation. The relative abundance of the phylum *Arthrobacter* varied with the deepening of the soil layer.

At the fungal phylum level (Figure 6A), *Ascomycota* (31.90~85.59%), *unclassified_k__Fungi* (2.65~62.63%), *Mortierellomycota* (3.44~12.85%), and *Basidiomycota* (1.30~18.68%) were the dominant phyla in the soil fungal community under rural domestic sewage irrigation at different salinity levels, with *Basidiomycota* (1.30~18.68%) as the dominant phylum. As shown in Figure 6B, *unclassified_k_Fungi*, *Mortierella*, *Neocosmospora*, *Gibberella*, and *Chaetomium* contributed more to the soil fungal community composition at the genus level. We found significant differences in the relative abundance of *Ascomycota*, *Mortierellomycota*, *Rozellomy-*

cota, *Zoopagomycota*, *Monoblepharomycota*, and *Olpidiomycota* in different soil fungal phyla, and the genus groups *Mortierella*, *Neocosmospora*, *Metarhizium*, *Chaetomium*, *Talaromyces*, *Humicola*, *Cephalotrichum*, and *Fusarium* differed significantly; see Figure 7.

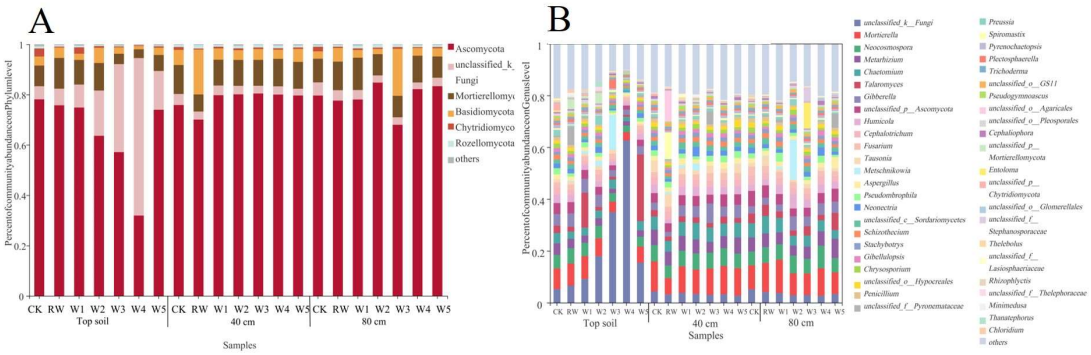

Figure 6. Species composition of soil fungi in the group (**A**) level of phylum; (**B**) level of the genus.

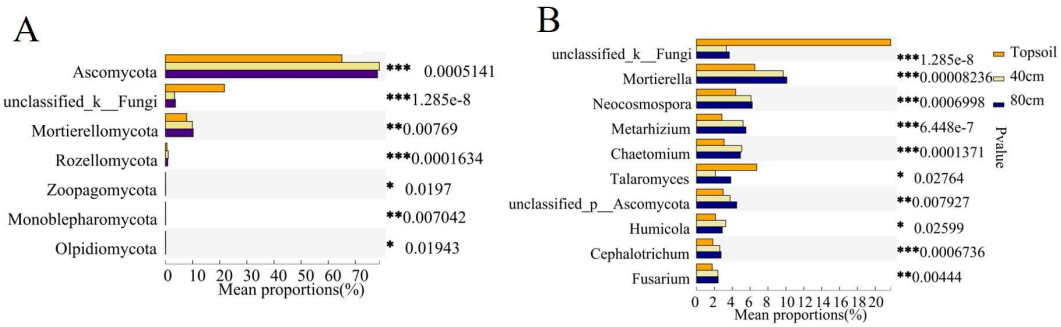

Figure 7. Analysis of differences between dominant fungus phylum and genus groups in different soil layers ((**A**) level of phylum; (**B**) level of the genus). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 6 *Ascomycota* and *Mortierellomycota* showed significant ($p < 0.05$) decreasing trends in their relative abundance with soil deepening and salinity reduction, indicating that saline domestic sewage irrigation played a limiting role in *Ascomycota* and *Basidiomycota* activity. The soils under irrigation with different salinity gradients all had *Ascomycota* as the core node. In contrast, irrigation with highly saline domestic sewage reduced the proportion of *Ascomycota*, damaging the decomposition of some soil residues. Also, *Basidiomycota* is a harmful fungus, and its relative abundance in raw water is significant; the direct use of raw water without treatment for watering might increase the risk of pathogenic soil bacteria. Further analysis of microbial community changes at the fungal genus level showed significant differences in the relative abundance of each group at the fungal genus level, and irrigation with saline water caused changes in soil pH and soil water content, which further affected soil fungal community changes. It could be further inferred that irrigation with domestic saline sewage could change the composition and relative abundance of microbial communities and that irrigation with raw water might create conditions for harmful bacteria to survive.

In the surface soil, TN and OM were significantly positively correlated with *Methylomirabilota*, *Cyanobacteria*, *Gemmatimonadota*, and *Myxococcota* and negatively correlated with *Acidobacteriota*, *Firmicutes*, and *Chloroflexi*. TP was significantly negatively correlated with *Methylomirabilota*, *Cyanobacteria*, *Gemmatimonadota*, *Myxococcota*, and *Acidobacteriota* and positively correlated with *Acidobacteriota*, Firmicutes, and Proteobacteria (Figure 8A). The correlation between dominant fungal phyla in the surface soil showed that TP was significantly positively correlated with *Chytridiomycota*, *Rozellomycota*, *Ascomycota*, *Zoopagomycota*, and *Mortierellomycota*; TN was significantly negatively correlated with *Ascomycota* and *Zoopagomycota* (Figure 8B).

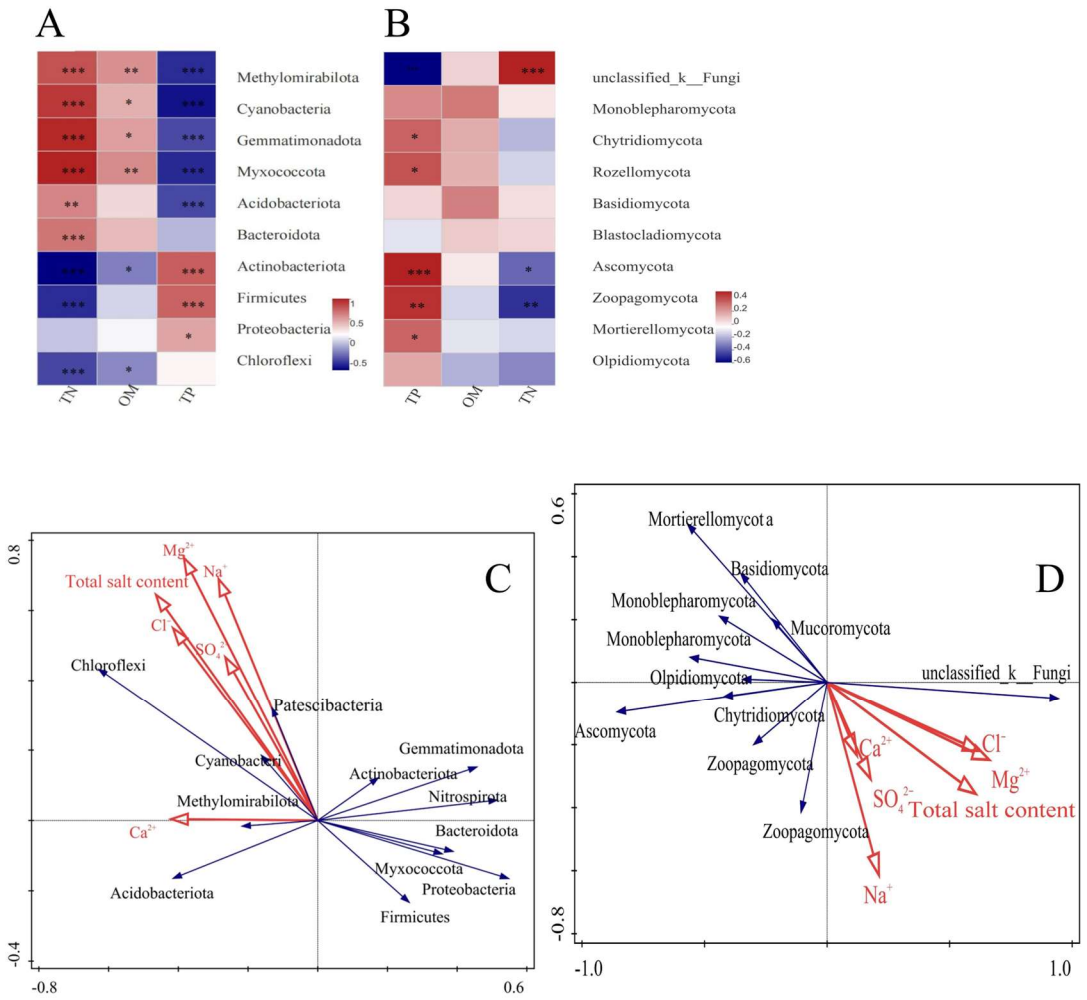

Figure 8. (**A**) Surface soil chemical factor and soil bacterial dominant gate heat map, (**B**) surface soil chemical factor and soil fungal dominant gate heat map, (**C**) surface soil salt ion and soil bacterial dominant gate redundancy analysis, and (**D**) surface soil salt ion and soil fungal dominant gate redundancy analysis. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In Figure 8C, the explanatory rate of the first axis was 16.34%, and soil salinity and major salt-based ions were not significantly correlated with bacterial phyla. By analyzing the correlation between salt-based ions and dominant fungal phyla, it was found, as shown in Figure 8D, that the explanatory rate of the first axis was 80.49%. At this time, Mg^{2+} and Ca^{2+} were the main salt-based ions affecting the dominant fungal phyla. Mg^{2+} was significantly positively correlated with *Zoopagomycota* and significantly negatively correlated with *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, *Chytridiomycota*, *Rozellomycota*, *Blastocladiomycota*, *Monoblepharomycota*, *Mucoromycota*, and *Olpidiomycota* ($p < 0.05$). Ca^{2+} was significantly positively correlated with *Zoopagomycota* and *Chytridiomycota* ($p < 0.05$) and significantly negatively correlated with other dominant fungal phyla ($p < 0.05$).

In the dominant bacterial phyla, TP was significantly positively correlated with *Actinobacteria* and *Chloroflexi* ($p < 0.01$) and significantly negatively correlated with *Gemmatimonadota*, *Myxococcota*, *Bacteroidota*, and *Methylomirabilota* ($p < 0.01$); TN was significantly negatively correlated with *Actinobacteria* and *Chloroflexi* ($p < 0.01$) and significantly positively correlated with *Acidobacteriota*, *Gemmatimonadota*, *Myxococcota*, *Bacteroidota*, and *Methylomirabilota* ($p < 0.05$) (Figure 9A). As shown in Figure 9B, among the dominant fungal phyla, TN was significantly negatively correlated with *Chytridipmucota* and *Olpidiomycota* ($p < 0.01$), while TP was significantly positively correlated with them ($p < 0.01$).

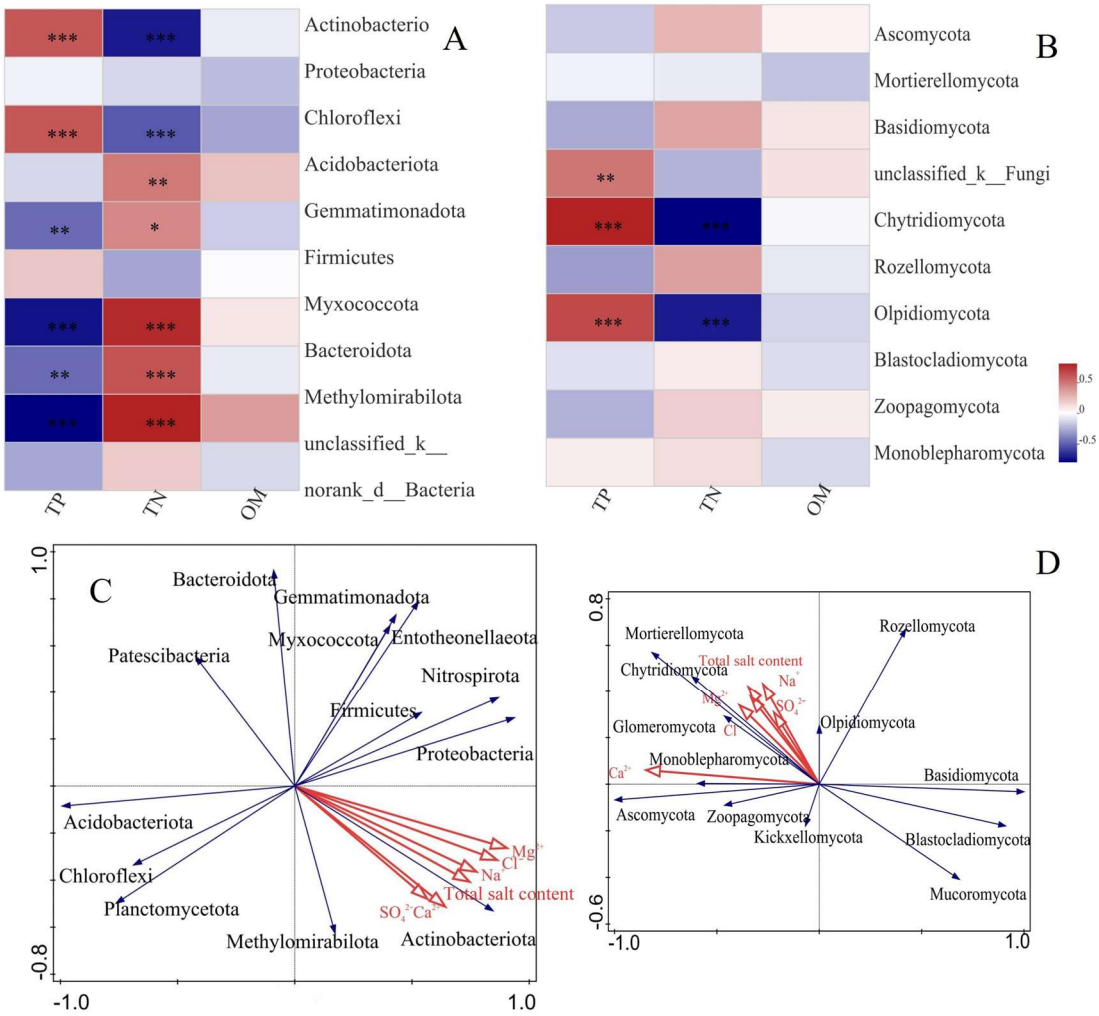

Figure 9. (**A**) The 40 cm soil chemical factor and soil bacterial dominant gate heat map, (**B**) the 40 cm soil chemical factor and soil fungal dominant gate heat map, (**C**) the 40 cm soil salt ion and soil bacterial dominant gate redundancy analysis, and (**D**) the 40 cm soil salt ion and soil fungal dominant gate redundancy analysis. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In Figure 9C, the explanatory rate of the first axis was 86.04%. It was found that Mg^{2+} and Cl^{-} were the main salt-based ions affecting the dominant bacterial phyla in the 40 cm soil. Mg^{2+} and Cl^{-} were significantly positively correlated with Actinobacteria, Proteobacteria, *Nitrospirta*, *Firmicutes*, *Entotheonellaeota*, *Myxococcota*, *Gemmatimonadota*, and *Methylomirabilota* ($p < 0.05$) and significantly negatively correlated with *Planctomycetota*, *Acidobacteria*, *Chloroflexi*, *Patescibacteria*, and *Bacteroidota* ($p < 0.05$). Mg^{2+} and Cl^{-} were found to be the main saline ions in the 40 cm soil layer. In addition, studies have shown that calcium and magnesium ions are the main salt-based ions influencing soil microbial structure. Excessive levels of Mg^{2+} can cause soil colloids to expand, disrupt aggregates, and accelerate the loss of soil organic matter. In this study, total soil salinity was significantly negatively correlated with organic matter ($p < 0.05$). Organic matter is the main source of soil fertility, and the loss of soil fertility further limits the growth of soil microbes.

Soil TN and TP were the main physicochemical indicators influencing the dominant bacterial and fungal phyla in the 80 cm soil layer (Figure 10A,B). Soil TN and TP were significantly negatively correlated with *Proteobacteria* and *Actinobacteria* ($p < 0.01$) and significantly positively correlated with *Myxococcota* and *Methylomirabilota* ($p < 0.01$). OM was significantly negatively correlated with Proteobacteria; TP was significantly negatively correlated with *Chloroflexi*. Soil TN and TP were the main nutrient indicators affecting the dominant bacterial phyla.

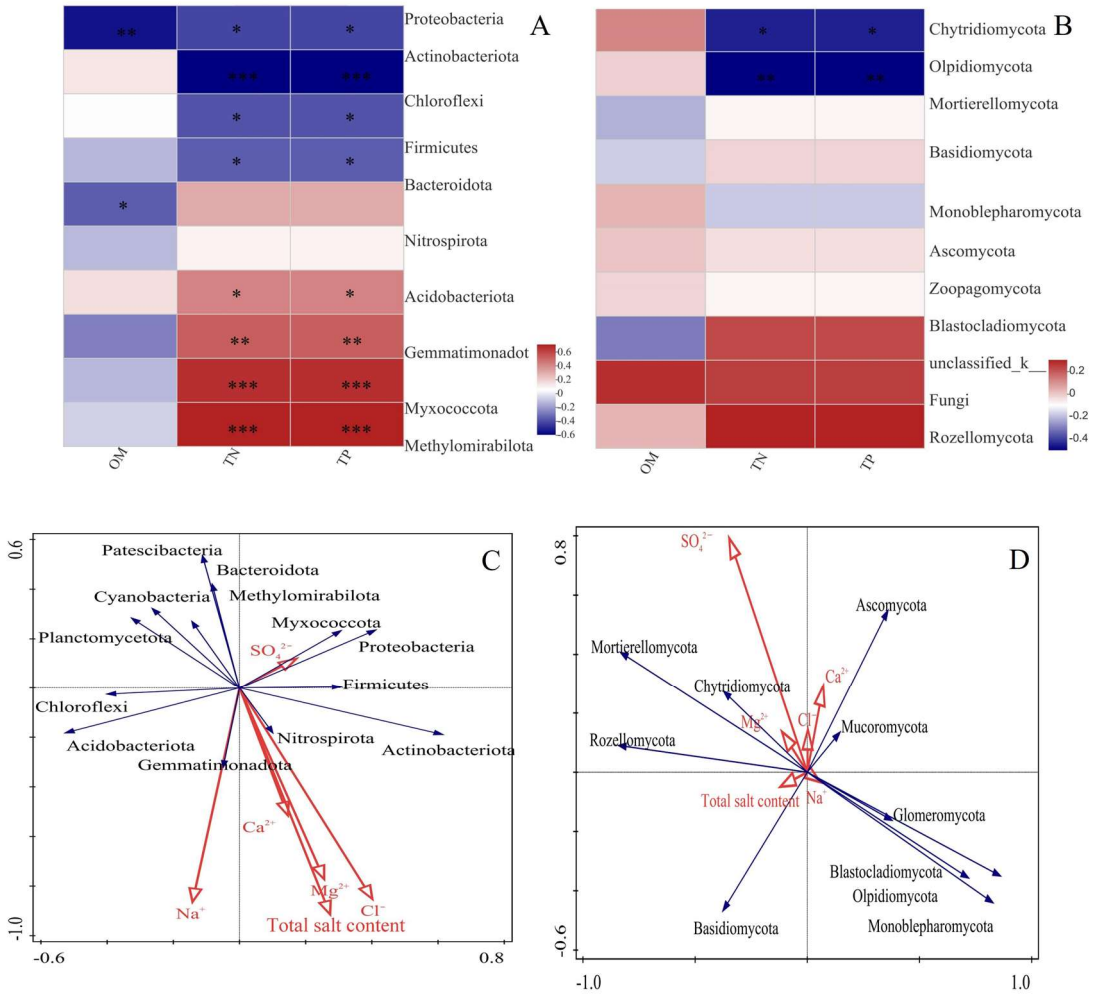

Figure 10. (**A**) The 80 cm soil chemical factor and soil bacterial dominant gate heat map, (**B**) the 80 cm soil chemical factor and soil fungal dominant gate heat map, (**C**) the 80 cm soil salt ion and soil bacterial dominant gate redundancy analysis, and (**D**) the 80 cm soil salt ion and soil fungal dominant gate redundancy analysis. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Cl^- and SO_4^{2-} were significantly correlated with the dominant fungal phyla, with correlation coefficients of ($p < 0.05$, $R^2 = 7.8$; $p < 0.05$, $R^2 = 4.3$) respectively. In Figure 10D, SO_4^{2-} and Cl^- were significantly positively correlated with *Rozellomycota*, *Mortierellomycota*, *Chytridiomycota*, *Ascomycota*, and *Mucoromycota* and significantly negatively correlated with *Glomeromycota*, *Blastocladiomycota*, *Olpidiomycota*, and *Monoblepharomycota*. Excessive SO_4^{2-} in the soil significantly limits the activity of calcium ions.

Mg^{2+} and Ca^{2+} were the main controlling factors for the dominant fungal phyla in the surface soil layer. Mg^{2+} and Cl^- were the main controlling factors for the dominant bacterial phyla in the 40 cm soil layer, while there were no associated factors for the bacterial phyla in the 80 cm soil layer. The main controlling factors for the fungal phyla were SO_4^{2-} and Cl^- (Table 4).

Table 4. Table of the main controlling factors affecting the dominant bacteria gate in each soil layer.

	Bacteriodominant Bacteria			**Fungal Dominance Bacteria**		
Soil layer	Surface layer	40 cm	80 cm	Surface layer	40 cm	80 cm
Influence ion		Mg^{2+}, Cl^-		Mg^{2+}, Ca^{2+}		Cl^-, SO_4^{2-}

There were seven types of metabolic pathways in soil bacteria, which were Cellular process, Environmental information processing, Genetic information processing, Human diseases, Metabolism, Organism systems, and Unclassified. There was no significant difference between treatments and metabolic pathways in the topsoil with increasing salinity of domestic sewage; in the 40 cm soil layer, there were significant differences in Genetic information processing and Metabolism between CK and W5 treatments ($p < 0.05$); in the 80 cm soil layer, there were significant differences in Human diseases and Organismal systems between CK and W5 treatments ($p < 0.05$). We found that the relative abundance of different functions under each treatment tended to increase as the salinity of irrigated domestic wastewater increased, and the relative abundance of metabolic functions under irrigation with highly saline wastewater was significantly higher than that under irrigation with low-salinity water ($p < 0.05$) (Table 5, Figure 11).

In establishing prediction results for secondary functions, with each treatment soil containing 41 types of secondary metabolic pathways, the top ten major functional pathways were selected for comparative analysis in relative abundance, namely Membrane transport, Amino acid metabolism, Carbohydrate metabolism, Replication and repair, Energy metabolism, Poorly characterized, Metabolism of cofactors and vitamins, Translation, Lipid metabolism, and Xenobiotics biodegradation repair. The relative abundance of each secondary function in the 40 cm soil layer also tended to increase as the salinity of irrigated domestic wastewater increased, with significant changes ($p < 0.05$) in the relative abundance of Membrane transport, Amino acid metabolism, Carbohydrate metabolism, Replication and repair, and Energy metabolism, while the relative abundance of the remaining secondary functions did not increase significantly ($p > 0.05$). Metabolism and Carbohydrate metabolism were significantly different between CK and W5 treatments ($p < 0.05$), and the relative abundance of their functions decreased with increasing soil salinity (Table 5, Figure 11).

Table 5. First-level functional classification of soil bacteria.

	Cellular Processes	Environmental Information Processing	Genetic Information Processing	Human Diseases	Metabolism	Organismal Systems	Unclassified
CK0	1,391,270	5,006,581	5,754,990	290,127	19,079,370	292,706	4,606,705
CK40	1,340,220	4,827,094	5,554,030	272,867	18,328,160	278,957	4,431,096
CK80	1,369,520	4,623,373	5,662,434	280,745	18,673,016	285,723	4,509,798
RW0	1,385,445	4,986,383	5,686,718	296,638	18,982,803	292,936	4,573,362
RW40	1,386,267	5,098,672	5,780,204	283,987	19,301,018	294,968	4,611,424
RW80	1,367,160	4,833,606	5,612,101	277,072	18,508,842	282,947	4,460,730
W10	1,396,217	5,034,973	5,724,850	303,135	19,059,202	292,647	4,624,886
W140	1,360,274	5,016,912	5,768,770	286,594	18,685,132	279,505	4,649,175
W180	1,372,578	4,904,324	5,738,065	280,231	18,824,906	285,000	4,548,505
W20	1,399,375	4,877,123	5,751,892	297,077	18,763,323	287,237	4,610,208
W240	1,394,558	4,879,426	5,736,009	282,224	18,726,545	284,815	4,541,129
W280	1,371,227	5,090,576	5,719,080	281,393	19,203,817	296,846	4,557,991
W30	1,388,706	4,970,516	5,773,085	289,402	18,906,468	286,027	4,592,289
W340	1,367,168	4,947,549	5,685,237	279,732	18,742,700	284,963	4,521,507
W380	1,388,957	5,036,150	5,777,576	279,834	19,097,158	289,926	4,573,274
W40	1,378,053	5,125,373	5,885,250	289,365	19,465,571	295,305	4,674,622
W440	1,391,058	5,017,000	5,780,449	279,857	19,132,885	290,944	4,575,153
W480	1,384,923	5,021,269	5,743,019	281,792	18,983,140	287,673	4,584,119
W50	1,363,880	5,000,114	5,754,629	291,827	19,108,757	294,398	4,598,483
W540	1,358,700	5,156,995	5,740,063	281,163	19,441,270	301,990	4,556,737
W580	1,403,623	5,323,602	5,786,163	290,088	19,321,747	294,177	4,628,459

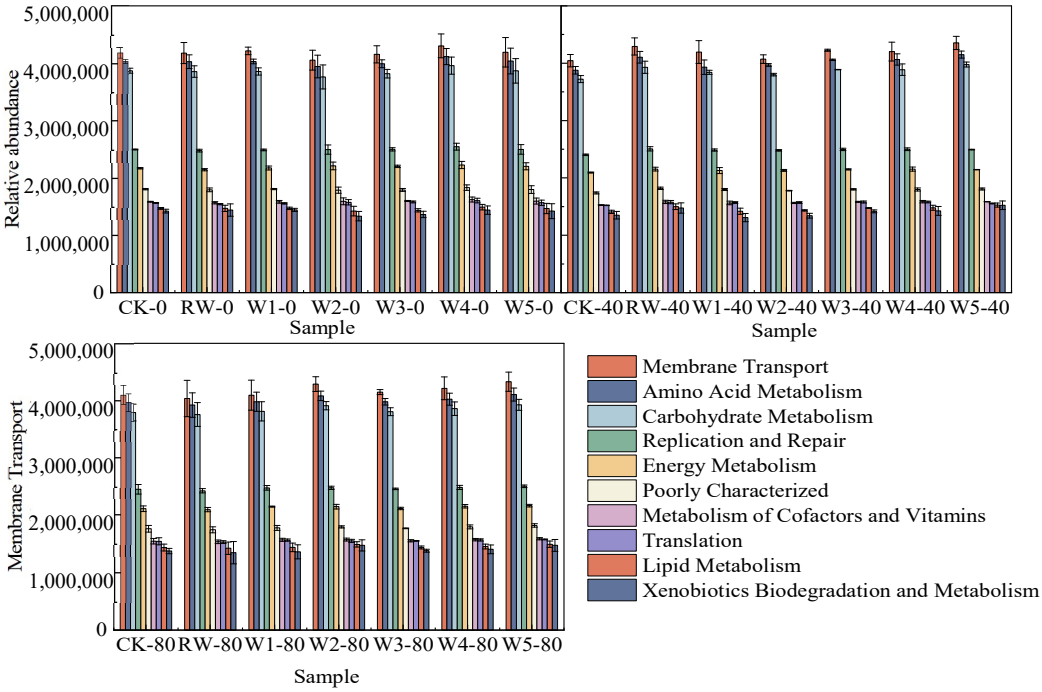

Figure 11. Map of secondary functional classification of soil bacteria.

The relative abundance of Membrane Transport in soil tended to decrease after an increase in soil salinity, and the significant decrease in the functional abundance of Membrane transport may have been due to the increase in soil salinity caused by irrigation with saline domestic sewage. Carbohydrate metabolism was related to nitrogen fixation and phosphorus solubilization in soil and was associated with *Actinomycetes* and *Aspergillus*, which were involved in nitrogen cycling and nitrogen fixation in soil and had a great correlation with them. The phyla *Actinomycetes* and *Amoebacteria* were the dominant phyla in this salinity range under irrigation with saline sewage; the phylum Actinomycetes had more aerobic saprophytes that promoted the decay of plant and animal remains, and the phylum *Amoebacteria* had many metabolic species, with the relative abundance of these two phyla decreasing significantly with the increase of irrigation ($p < 0.05$). The results demonstrated that the prediction analysis of the bacterial function in this study was similar to the dominant phylum of the bacterial community, which could verify the reliability of its prediction results.

FUNGuild was used to predict soil fungal community functions under irrigation with different salinity levels of rural domestic wastewater (Figure 12), and fungi were classified into three major groups, namely saprophytic, pathotrophic, and symbiotic, belonging to 12 functions according to their nutrient mode. Soil fungal communities under different treatments were dominated by undefined saprophytic fungi, followed by plant pathogens (8.66~9.20%), Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph (6.52~10.09%), Animal pathogens (3.30~6.11%), Animal pathogen-Dung Saprotroph-Endophyte-Epiphyte-plant Saprotroph-Wood Saprotroph (3.25~5.34%), Animal pathogen-Endophyte-Wood Saprotroph (3.25~5.34%), and Animal pathogen-Endophyte-Lichen parasite-plant pathogen-Soil Saprotroph-Wood Saprotroph (1.76~2.42%), and Dung Saprotroph-plant Saprotroph (1.20~1.57%). Among them, Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph, Animal pathogen, and Animal pathogen-Dung Saprotroph-Endophyte-Epiphyte-plant Saprotroph-Wood Saprotroph functional abundance significantly decreased with increasing irrigation ($p < 0.05$). Compared to bacteria, fungi absorbed nutrients in multiple ways in the soil and lived in more complex environments, and saprophytic functional fungi had a significant advantage in this study. In terms of nutrient type, the treatments were dominated by Saprotrophs, which may have been a

result of the fact that the *Cysticercus* phylum was mostly saprophytic, the most dominant phylum, and the most important decomposer in the soil. The other part was composed of phototrophs, which obtained nutrients mainly by damaging host cells; this part was prone to causing plant diseases. *Symbiotrophs* obtained nutrients by exchanging resources with host cells, which had beneficial effects on plant growth and quality. Still, the abundance value of the *Symbiotroph* phylum was not found in the test results, so the salt-containing sewage had an inhibitory effect on *Symbiotrophs*, which might have been harmful to crop growth if used for a long time.

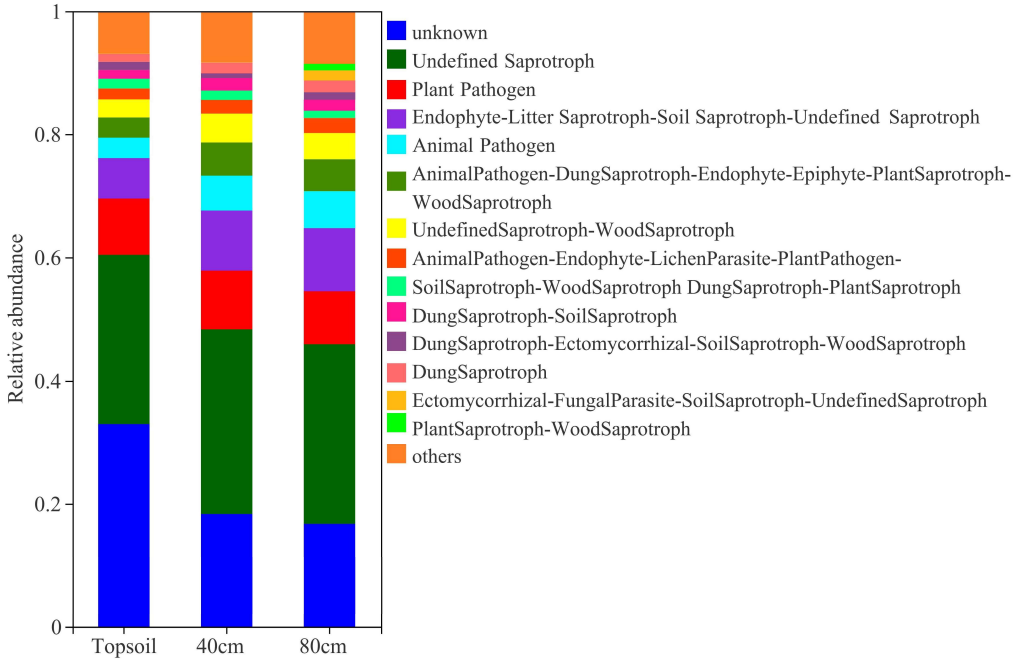

Figure 12. Changes in the functional community composition of soil fungi.

4. Conclusions

In this study, domestic wastewater with a salt concentration in the range of 0 to 2 $g \cdot L^{-1}$ was used for irrigation. SO_4^{2-}, Cl^-, and Ca^{2+} aggregated in the topsoil, and Mg^{2+} was significantly higher in the deeper soil than in the topsoil and 40 cm soil layers. Ca^{2+} and Mg^{2+} affected the fungi in the topsoil. Mg^{2+} and Cl^- affected the bacteria in the 40 cm layer. SO_4^{2-} and Cl^- affected the fungi in the 80 cm layer. As irrigation salinity increased, the relative abundance of pathogenic genera in the soil increased, but the abundance of beneficial genera decreased (more pronounced above 0.8 $g \cdot L^{-1}$). The long-term irrigation of domestic sewage may increase the risk of soil pathogenicity.

Based on the results of the experiment, prolonged irrigation of soil with high concentrations of saline water (above 0.8 $g \cdot L^{-1}$) is not recommended.

Due to the limited duration of this experiment, long-term trials were not conducted, thus limiting the scope of studying the effects of long-term saline wastewater irrigation on soil.

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