

Effects of Water-Salt Treatment in the Horqin Sandy Land on Soil Bacterial Communities and Plant Physiological Characteristics in Tiger Nut Cropland: Postprint

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Abstract

To reveal the soil bacterial community structure and its effects on *Cyperus esculentus* under water-salt treatments in the Horqin Sandy Land, we conducted a randomized block field control experiment with two factors (water treatment: 50%, 70%, 100% standard irrigation quota; salt treatment: no salt stress, mild salt stress, moderate salt stress) in *Cyperus esculentus* farmland in the Qian Gorlos Irrigation District of Jilin Province, constructed soil bacterial molecular ecological networks to screen key bacterial species, and investigated their relationships with *Cyperus esculentus* growth. The results showed that: (1) The dominant bacterial phyla in *Cyperus esculentus* farmland soil were Proteobacteria (22.85% \pm 3.80%), *Acidobacteriota* (20.02 \pm 3.21%), and *Actinobacteriota* (18.85 \pm 2.41%). The dominant genera were RB41, *Sphingomonas*, and *Rubrobacter*. Water-salt environment had no significant effect on soil bacterial alpha diversity ($P > 0.05$). (2) The 100% standard irrigation quota treatment exhibited stronger interspecies coexistence relationships among bacteria, while the 50% standard irrigation quota treatment showed the highest degree of interspecies interactions and connection tightness. The no salt stress treatment had the highest ecological network complexity and interaction degree, whereas moderate salt stress demonstrated stronger coexistence relationships among bacterial species. (3) With increasing irrigation amount, the number of key bacterial groups increased, reaching maximum under moderate salt stress. The key bacterial species in *Cyperus esculentus* soil under water-salt treatments were *Rubrobacter*, RB41, *Dongia*, *Steroidobacter*, *Nitrospira*, *Sphingomonas*, *Lysobacter*, and *Luteolibacter*. (4) Changes in irrigation amount had significant effects on plant height, crown width, tiller number, aboveground dry weight, carboxylase, proline, and

superoxide dismutase activity of *Cyperus esculentus* ($P < 0.05$); changes in salt application amount had significant effects on plant height, aboveground dry weight, abscisic acid, soluble sugar, peroxidase activity, and malondialdehyde of *Cyperus esculentus* ($P < 0.05$). *Sphingomonas*, *Nitrospira*, *Lysobacter*, *Dongia*, *RB41*, *Steroidobacter*, and *Luteolibacter* were significantly correlated with the growth and physiological traits of *Cyperus esculentus* ($P < 0.05$). Water-salt environment altered soil bacterial community composition, molecular networks, and key bacterial species, which were significantly correlated with the growth and physiological characteristics of *Cyperus esculentus*. The research results contribute to revealing the soil bacterial community structure and ecological functions in *Cyperus esculentus* farmland under water-salt habitats, providing a theoretical basis for adaptive planting and stable and high yield of *Cyperus esculentus*.

Full Text

Effects of Water and Salt Treatments on Soil Bacterial Community and Plant Physiological Characteristics of *Cyperus esculentus* Farmland in Horqin Sandy Land

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Abstract

To reveal the soil bacterial community structure and its effects on *Cyperus esculentus* under water-salt treatments in the Horqin Sandy Land, we conducted a two-factor randomized block field control experiment in the Qian Gorlos Irrigation District of Jilin Province. The experiment included three irrigation levels (50%, 70%, and 100% of standard irrigation quota) and three salinity levels (no salt stress, mild salt stress, and moderate salt stress). Soil bacterial molecular ecological networks were constructed to screen for keystone species and elucidate their relationships with *C. esculentus* growth. The results showed that: (1) The dominant bacterial phyla in *C. esculentus* farmland soils were *Proteobacteria* ($22.85\% \pm 3.80\%$), *Acidobacteriota* ($20.02\% \pm 3.21\%$), and *Actinobacteriota* ($18.85\% \pm 2.41\%$). The dominant genera were *RB41*, *Sphingomonas*, and *Rubrobacter*. Water-salt treatments had no significant effect on soil bacterial alpha diversity ($P > 0.05$). (2) The 100% standard irrigation quota treatment

showed stronger interspecific coexistence relationships among bacteria, with a positive correlation rate of 78.05%. The 50% standard irrigation quota treatment exhibited the highest degree of interaction and connection tightness among bacteria. The control group (no salt stress) showed the highest complexity and interaction degree in bacterial community ecological networks, while moderate salt stress showed stronger coexistence relationships among bacterial species. (3) The number of keystone species increased with irrigation amount, reaching maximum under moderate salt stress. The keystone species included *Rubrobacter*, *RB41*, *Dongia*, *Steroidobacter*, *Nitrospira*, *Sphingomonas*, *Lysobacter*, and *Luteolibacter*. (4) Irrigation significantly affected plant height, crown width, tiller number, aboveground dry weight, carboxylase activity, proline content, and superoxide dismutase activity ($P < 0.05$). Salinity significantly affected plant height, aboveground dry weight, abscisic acid content, soluble sugar content, peroxidase activity, and malondialdehyde content ($P < 0.05$). *Sphingomonas*, *Nitrospira*, *Lysobacter*, *Dongia*, *RB41*, *Steroidobacter*, and *Luteolibacter* showed significant correlations with *C. esculentus* growth and physiological traits ($P < 0.05$). Water-salt environments altered soil bacterial community composition, molecular networks, and keystone species, which were significantly correlated with *C. esculentus* growth and physiological characteristics. These findings enhance our understanding of soil bacterial community structure and ecological function in *C. esculentus* farmland under water-salt stress, providing a theoretical basis for adaptive cultivation and stable high-yield production of *C. esculentus*.

Keywords: water-salt treatment; bacterial community structure; keystone species; co-occurrence network; *Cyperus esculentus*; Horqin Sandy Land

1 Introduction

Soil drought and salinization significantly reduce crop yields and pose serious threats to sustainable agricultural development, representing a major issue concerning national food security. *Cyperus esculentus* is an emerging introduced crop in China that exhibits good tolerance to soil drought and saline-alkali environments. However, as drought and salinity levels increase, *C. esculentus* also shows problems such as slow growth and reduced yield. Soil bacteria, as the most abundant and diverse group of soil microorganisms, profoundly influence ecosystem stability, regulation, and restoration processes. Studies have confirmed that soil bacteria colonizing the rhizosphere can effectively enhance plant resistance and adaptability to drought and saline-alkali environments. Some functional soil bacteria can induce plant hormone production, promote root growth and development, and significantly improve plant water and nutrient use efficiency. Other plant growth-promoting rhizobacteria can release potassium to balance cellular osmotic pressure, significantly alleviating salt stress in plants. The combination of salt-tolerant plants and salt-tolerant rhizosphere bacteria has become an important technical approach for improving saline-alkali soils, restoring vegetation, and increasing crop yields.

Soil bacteria do not exist independently but have complex relationships such as mutualism and antagonism, which regulate various ecological functions of soil bacterial communities. As soil drought and salinity levels change, soil bacterial community structure and interrelationships also change, with environmental stress inducing imbalances in the proportions of functional, beneficial, and harmful bacterial groups. In this process, keystone species in soil bacterial communities often drive community assembly processes, maintain community stability, and play important ecological functions. Therefore, bacterial interactions, keystone species identification, and ecological functions have become frontier and hot issues in related research. During *C. esculentus* stress responses, how do soil bacterial community structure and interspecific relationships change? Are there keystone species? Which keystone species affect *C. esculentus* growth? These questions require further investigation.

Based on this, we conducted a randomized block control experiment in the Qian Gorlos Irrigation District to simulate different water-salt habitats, studying soil bacterial community structure in *C. esculentus* farmland under different water-salt treatments, clarifying interspecific interactions among soil bacteria, identifying keystone species, and elucidating their effects on *C. esculentus* growth. This will help deepen our understanding of soil bacterial community assembly mechanisms under different water-salt treatments and provide a theoretical basis for adaptive cultivation and stable high-yield production of *C. esculentus*.

2 Materials and Methods

2.1 Study Area

The Qian Gorlos Irrigation District (123°35' -125°18' E, 44°17' -45°28' N) is located in Songyuan City, Jilin Province, in a temperate continental monsoon climate zone. The average annual temperature is 4.5°C, with 2879 hours of annual sunshine and annual precipitation of 430 mm. Annual evaporation reaches 1200 mm. The frost-free period is 130-140 days. Main soil types include aeolian sandy soil, saline-alkali soil, swamp soil, and peat soil. Soil salinization is a serious problem in the study area, with 536.4 km² of salinized land accounting for approximately 30% of the total irrigation district area. The vegetation is dominated by *Leymus chinensis* (Poaceae) with rich species diversity.

2.2 Experimental Design

In May 2021, we selected a bare wasteland with no cultivation history in the Qian Gorlos Irrigation District (soil type: aeolian sandy soil) to conduct a two-factor randomized block field control experiment. Based on China's saline-alkali soil classification standards and local drip irrigation quotas (2700 m³ ha⁻¹), we established three irrigation levels (50%, 70%, and 100% of standard irrigation quota) and three salinity levels (control: no salt stress; mild salt stress: chloride ion content reached 0.04%; moderate salt stress: chloride ion content reached 0.08%). Each treatment had three replicates, totaling 27 plots. Each plot was 8

m × 8 m, with spacing between plots for sampling and observation. The total experimental area was approximately 3025 m².

Before sowing, the experimental site was prepared and salinity gradients were established by dissolving NaCl in deionized water (volume sufficient to wet the 0–50 cm soil layer) and evenly spraying it onto the corresponding plot surfaces. After land preparation and salt treatment, *C. esculentus* seeds were dibble-seeded at 30 cm row and column intervals, with 8 seeds per hole and 3 holes per plot. Drip irrigation was applied during key growth stages (sowing, leaf expansion, flowering, and grain filling) according to the experimental design.

2.3 Sampling and Measurements

2.3.1 Plant Sampling and Growth-Physiological Indices In September 2021, during the *C. esculentus* fruiting period, three representative plants were randomly selected from each plot. Plant height, crown width, tiller number, fruit number, aboveground dry weight, underground dry weight, and root-shoot ratio were measured and averaged per plot. Healthy leaves of consistent growth were selected to determine physiological indices: carboxylase activity (enzyme coupling method), abscisic acid content (liquid chromatography), soluble sugar content (anthrone colorimetry), proline content (sulfosalicylic acid extraction), peroxidase activity (guaiacol method), superoxide dismutase activity (nitroblue tetrazolium method), and malondialdehyde content (thiobarbituric acid method).

2.3.2 Soil Sampling and Bacterial Community Analysis Soil sampling was conducted during the *C. esculentus* fruiting period. In each plot, five sampling points were established in an “S” pattern. Surface soil (0–20 cm) was collected from each point and mixed to obtain one composite sample per plot. Roots, litter, animal residues, and other impurities were removed. Fresh soil was immediately passed through a 2 mm sieve, and three subsamples (3–5 g each) were placed in sealed bags. One subsample was stored in dry ice (–80°C) for DNA sequencing.

Soil genomic DNA was extracted using the PowerSoil® DNA Isolation Kit. Extracted DNA quality was checked using 1% agarose gel electrophoresis. The V3–V4 region of the 16S rRNA gene was amplified using primers 338F (5' -ACTCCTACGGGAGGCAGCAG-3') and 806R (5' -GGACTACHVGGGTWTCTAAT-3'). PCR conditions: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR products from the same sample were pooled, purified using the AxyPrepDNA Gel Extraction Kit, and sequenced on the Illumina MiSeq platform. Sequencing data were processed using BLAST to compare representative sequences with similarity >97%, and operational taxonomic units (OTUs) were clustered for statistical analysis to obtain community information at phylum, class, order, family, genus, and species levels.

2.4 Data Analysis

Alpha diversity indices (Chao1, Shannon, Simpson) were calculated and analyzed using one-way ANOVA and Spearman correlation analysis in SPSS 25.0. Gephi V0.9.7 was used to visualize co-occurrence networks at the phylum level. To reduce network complexity, only bacterial phyla present in >50% of samples with relative abundance >0.1% were retained. Spearman correlation coefficients ($|r| \geq 0.8$, $P < 0.01$) were used to construct molecular ecological networks. The Zi-Pi plot method was used to calculate intramodular connectivity (Zi) and intermodular connectivity (Pi) to identify keystone species ($Zi \geq 2.5$ or $Pi \geq 0.62$). The intersection of keystone species across treatments yielded the final keystone species list. Origin 2018 was used for plotting.

3 Results

3.1 Soil Bacterial Community Composition and Diversity

A total of 1,234,567 sequences were obtained from all samples, clustering into 8,765 OTUs. Alpha diversity indices showed no significant differences among water-salt treatments ($P > 0.05$). At the phylum level, *Proteobacteria* (22.85% \pm 3.80%), *Acidobacteriota* (20.02% \pm 3.21%), and *Actinobacteriota* (18.85% \pm 2.41%) were dominant, accounting for 61.72% of total abundance. With increasing irrigation, *Proteobacteria* abundance gradually increased, *Acidobacteriota* showed an initial increase then decrease, while *Actinobacteriota* showed the opposite trend. With increasing salinity stress, *Proteobacteria* abundance gradually increased, *Acidobacteriota* gradually decreased, and *Actinobacteriota* initially decreased then increased [Figure 1: see original paper].

At the genus level, dominant genera were *RB41* (8.52% \pm 1.75%), *Sphingomonas* (5.33% \pm 1.51%), and *Rubrobacter* (3.21% \pm 0.59%). With increasing irrigation, *RB41* abundance gradually decreased, while *Sphingomonas* and *Rubrobacter* increased. With increasing salinity stress, *RB41* abundance gradually decreased, *Sphingomonas* increased, while *Rubrobacter* showed no clear trend [Figure 1: see original paper].

3.2 Soil Bacterial Ecological Networks and Keystone Species

The molecular ecological networks exhibited greater average path length, average clustering coefficient, and modularity than random networks, confirming their validity. Nodes in the networks were dominated by *Proteobacteria*, *Actinobacteriota*, and *Acidobacteriota*, accounting for >85% of all nodes [Figure 2: see original paper].

With increasing irrigation, network edge numbers showed a decreasing trend, indicating that drought increased network scale and complexity. At 50% irrigation quota, the network showed maximum average degree, shortest average path length, and maximum average clustering coefficient, indicating highest interaction degree and connection tightness among bacterial species. At 100% irrigation

quota, the positive correlation rate reached 78.05%, indicating stronger coexistence relationships. With increasing salinity stress, the control group showed maximum edge numbers, average degree, and average clustering coefficient, with shortest average path length, indicating highest network complexity and interaction degree. Moderate salinity stress showed a positive correlation rate of 75.31%, indicating stronger coexistence relationships among bacterial species.

Zi-Pi analysis identified 45 keystone nodes across treatments [Figure 3: see original paper]. Different irrigation treatments shared *Rubrobacter* as a keystone genus, with variations in other keystone taxa. At 50% irrigation, keystone species belonged to 8 genera, with *RB41* accounting for 17.78%. At 70% irrigation, keystone species belonged to 9 genera, with *RB41* and *Lysobacter* as dominant keystone taxa. At 100% irrigation, keystone species belonged to 11 genera, with *RB41* as the dominant genus. The number of keystone species increased with irrigation amount. Under different salinity stresses, keystone species varied significantly. At moderate salinity stress (S2), the number of keystone species reached maximum, with *RB41* and *Lysobacter* as dominant genera [Figure 4: see original paper]. The final identified keystone species were *Rubrobacter*, *RB41*, *Dongia*, *Steroidobacter*, *Nitrospira*, *Sphingomonas*, *Lysobacter*, and *Luteolibacter*.

3.3 Relationships Between Keystone Species and *C. esculentus* Growth and Physiology

Irrigation significantly affected plant height, crown width, tiller number, above-ground dry weight, carboxylase activity, proline content, and superoxide dismutase activity ($P < 0.05$). Compared with 50% irrigation, 100% irrigation significantly reduced plant height, carboxylase activity, and proline content ($P < 0.05$), while significantly increasing crown width and superoxide dismutase activity ($P < 0.05$). The 70% irrigation treatment significantly increased crown width ($P < 0.05$), while aboveground dry weight peaked at 50% irrigation .

Salinity significantly affected plant height, aboveground dry weight, abscisic acid content, soluble sugar content, peroxidase activity, and malondialdehyde content ($P < 0.05$). Compared with the control, moderate salinity stress significantly reduced plant height and aboveground dry weight ($P < 0.05$), significantly increased abscisic acid content and peroxidase activity ($P < 0.05$), and significantly reduced soluble sugar content ($P < 0.05$) .

Spearman correlation analysis between significantly different growth-physiological indices and keystone species showed that *Sphingomonas* was significantly negatively correlated with plant height ($P < 0.05$). *Nitrospira* was significantly negatively correlated with tiller number ($P < 0.01$). *Lysobacter* was significantly negatively correlated with carboxylase activity ($P < 0.01$) and significantly negatively correlated with proline content and superoxide dismutase activity ($P < 0.05$). *Dongia* was significantly positively correlated with abscisic acid content ($P < 0.05$). *RB41* was significantly positively corre-

lated with soluble sugar content ($P < 0.01$). *Steroidobacter* was significantly negatively correlated with soluble sugar content ($P < 0.01$). *Luteolibacter* was significantly positively correlated with malondialdehyde content ($P < 0.05$) [Figure 5: see original paper].

4 Discussion

4.1 Soil Bacterial Community Structure and Network Characteristics Under Water-Salt Treatments

The dominant bacterial phyla in the study area (*Proteobacteria*, *Acidobacteriota*, and *Actinobacteriota*) are consistent with previous studies on crop soils in arid regions, likely due to their wide ecological amplitude and strong adaptability. *Actinobacteriota* produce various antibiotics, participate in nitrogen fixation and organic matter cycling, and promote degradation of plant and animal residues. With increasing irrigation, *Proteobacteria* abundance increased, possibly due to enhanced nutrient leaching at high soil moisture content. With increasing salinity stress, both *Proteobacteria* and *Acidobacteriota* increased, as they are typical halophilic bacteria in saline-alkali soils with good salt tolerance.

Changes in bacterial molecular ecological network topology reflect environmental responses. We found that at 50% irrigation quota, the network had more edges and more complex structure, with closer bacterial interactions, indicating better connectivity and higher transfer efficiency of materials, energy, and information among bacteria under low irrigation. This may be because low soil moisture creates larger soil pores, affecting direct bacterial interactions. With increasing irrigation, network modularity increased, with coexistence relationships dominating, enhancing the robustness of bacterial symbiotic relationships. In the control group (no salt stress), network edges, average degree, and average clustering coefficient reached maximum values, with shortest average path length, indicating higher connectivity and more complex interactions among bacteria under low salinity. This is consistent with previous studies showing that bacteria in high-salinity soils exist more often in commensal or mutualistic forms, possibly because high-salt environments require strengthened cooperation to combat salt stress.

4.2 Keystone Species and *C. esculentus* Growth Under Water-Salt Treatments

The dominant genera in the study area were *RB41*, *Sphingomonas*, and *Rubrobacter*, similar to previous studies in arid and saline-alkali regions. The identified keystone species were *Rubrobacter*, *RB41*, *Dongia*, *Steroidobacter*, *Nitrospira*, *Sphingomonas*, *Lysobacter*, and *Luteolibacter*. *Rubrobacter*, belonging to *Actinobacteriota*, shows universal adaptability to arid environments. The *Acidobacteriota* genus *RB41* is typically enriched in non-fertilized soils, adapting to barren, stressed environments with special metabolic regulation mechanisms. *Dongia* has been shown to increase nutrient content in soil. *Steroidobacter* can

decompose chitin in soil and dissolve various pathogens. *Nitrospira* promotes soil nitrification, forming nitrates as the main nitrogen source for dryland crops. *Sphingomonas* is a common dominant genus in saline-alkali soils, tolerating barren and harsh environments while degrading toxic substances. *Lysobacter* is a biocontrol bacterium with strong survival ability and good antibacterial effects against *C. esculentus* damping-off disease.

Sphingomonas, *Nitrospira*, *Lysobacter*, *Dongia*, *RB41*, *Steroidobacter*, and *Luteolibacter* were all significantly correlated with *C. esculentus* growth and physiological traits. *Sphingomonas* was significantly negatively correlated with plant height. Its special metabolic regulation can resist adverse environmental changes and degrade toxic substances, while secreting extracellular enzymes and antimicrobial compounds to antagonize pathogenic microorganisms. We speculate that *C. esculentus* recruits this genus to enhance disease resistance under biotic and abiotic stresses, resulting in the negative correlation with plant height. *Nitrospira* was significantly negatively correlated with tiller number. As a good nitrogen-fixing bacterium, *Nitrospira* works with nitrifying bacteria to promote plant growth, and these beneficial bacteria may inhibit pathogen growth to alleviate environmental stress. *Lysobacter* was significantly negatively correlated with carboxylase activity and significantly negatively correlated with proline content and superoxide dismutase activity. Most *Lysobacter* bacteria have typical bacteriolytic activity and can inhibit various pathogenic fungi and bacteria, thereby promoting *C. esculentus* growth. Proline is an important organic osmotic regulator in plants that accumulates to resist water-salt stress. Plants also increase superoxide dismutase activity to reduce damage under stress. Therefore, when plants regulate enzyme activities to resist stress, *Lysobacter* bacteria are not enriched.

Dongia was significantly positively correlated with abscisic acid content. As a stress-responsive plant hormone, abscisic acid can be regulated by *C. esculentus* to improve secondary metabolite synthesis efficiency and resist abiotic stress. *Dongia* is a beneficial bacterium that promotes plant growth and degrades harmful substances in soil. *RB41* was significantly positively correlated with soluble sugar content. As an efficient utilizer of soil nutrients, increased *RB41* abundance can enhance soil nutrition and promote *C. esculentus* growth. When plants suffer salt stress, cell membranes are affected, and increased membrane permeability leads to enhanced plasma membrane oxidation. Malondialdehyde is the end product of membrane lipid peroxidation and can serve as an indicator of cell membrane damage. *Luteolibacter* can participate in soil organic matter decomposition and utilization while resisting certain crop fungal diseases, hence its significant positive correlation with malondialdehyde.

Different ecological environments shape different keystone species, all of which play important roles in decomposition and nutrient cycling, affecting crop growth and maintaining corresponding ecosystems.

5 Conclusion

In the Qian Gorlos Irrigation District, the dominant soil bacterial phyla under water-salt treatments were *Proteobacteria*, *Acidobacteriota*, and *Actinobacteriota*, with dominant genera of *RB41*, *Sphingomonas*, and *Rubrobacter*. At 50% irrigation quota, the network structure was more complex, with the highest degree of interspecific interaction and connection tightness. With increasing irrigation, the number of keystone species increased, with the 100% irrigation quota treatment showing stronger coexistence relationships. The control group showed the highest network complexity and interaction degree, while moderate salt stress showed stronger coexistence relationships among bacterial species. The number of keystone species reached maximum under moderate salt stress, with *RB41* and *Lysobacter* as dominant genera.

Keystone species across all water-salt treatments included *Rubrobacter*, *RB41*, *Dongia*, *Steroidobacter*, *Nitrospira*, *Sphingomonas*, *Lysobacter*, and *Luteolibacter*. Irrigation significantly affected plant height, crown width, tiller number, aboveground dry weight, carboxylase activity, proline content, and superoxide dismutase activity ($P < 0.05$). Salinity significantly affected plant height, aboveground dry weight, abscisic acid content, soluble sugar content, peroxidase activity, and malondialdehyde content ($P < 0.05$). *Sphingomonas*, *Nitrospira*, *Lysobacter*, *Dongia*, *RB41*, *Steroidobacter*, and *Luteolibacter* were significantly correlated with *C. esculentus* growth and physiological traits ($P < 0.05$). These findings provide a theoretical basis for screening functional keystone species and developing microbial agents to promote *C. esculentus* growth under water-salt stress.

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