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Drought Stress Increases the Complexity of the Bacterial Network in the Rhizosphere and Endosphere of Rice (*Oryza sativa* L.)

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Abstract: The root microbiota plays a crucial role in assisting the plant host in combating various biotic and abiotic stresses, notably drought, which poses a significant threat to global food security. Despite extensive efforts to understand the shifts in rhizosphere and endosphere bacteriomes, there remains a gap in comprehending how drought stress influences the co-occurring network patterns within these compartments and their ecological functional potentials. To address this gap, a pot experiment was conducted with two treatments: continuous flooding as a control and drought treatment. Bulk soil, rhizosphere, and endosphere samples were collected and subjected to high-throughput sequencing and bioinformatics analysis. The results revealed that drought stress significantly reduced the rice biomass but increased the Shannon diversity index in both the rhizosphere and endosphere bacterial communities with no observable effect on richness across compartments. Additionally, drought treatment markedly altered the community structure and bacterial assemblages in these compartments, resulting in the specific enrichment of *Actinobacteriota*, *Gemmatimonadetes*, and *Patescibacteria*, while *Bacteroidetes* and *Firmicutes* were depleted in the rhizosphere and endosphere. Furthermore, drought heightened the complexity of the co-occurring networks and the proportions of positive links across all sampling compartments; this effect was accompanied by an increase in the number of connectors in the bulk soil and rhizosphere, as well as module hubs in the rhizosphere. Functional potential prediction indicated that drought stress significantly altered multiple potential ecological functions across all sampling compartments, particularly enriching functions related to the oxidation of sulfur, manganese, and hydrogen in the bulk soil, while functions associated with iron oxidation were significantly depleted in the rhizosphere. Overall, our results demonstrate that under drought stress, rice may specifically enrich certain bacterial taxa and enhance their positive interactions within its root system to improve adaptation.

Keywords: network; drought; stress gradient hypothesis; functional potential

Citation: Wu, C.; Zhang, X.; Liu, Y.; Tang, X.; Li, Y.; Sun, T.; Yan, G.; Yin, C. Drought Stress Increases the Complexity of the Bacterial Network in the Rhizosphere and Endosphere of Rice (*Oryza sativa* L.). *Agronomy* **2024**, *14*, 1662. https://doi.org/10.3390/agronomy14081662

Academic Editors: Carlos Garrido, Victoria E. González-Rodríguez and Hernando José Bolivar Anillo

Received: 23 June 2024
Revised: 13 July 2024
Accepted: 15 July 2024
Published: 29 July 2024

1. Introduction

In the context of climate change, widespread and frequent droughts pose a serious threat to global food security [1]. Uncovering the mechanisms through which crops adapt to drought and then improving their tolerance to drought stress have become a crucial topic in contemporary agricultural science research [2]. Accumulating evidence highlights that rhizosphere and endosphere bacteriomes assume a critical role in mitigating a variety

of stresses in crops, including drought stress, and the diverse beneficial services provided by root microbiota have thus received increasing attention [3]. Accordingly, systematic investigation of the shift patterns of bacterial communities within (i.e., endosphere) and around the crop root (i.e., rhizosphere) in response to drought stress, and deciphering microbes that help crops adapt to drought stress and mechanisms thereof, will help improve crop drought tolerance via engineering the rhizosphere microbiota [4].

It has been extensively demonstrated that there is a conservative successional pattern of root bacterial communities across plants in response to drought stress [4–6]. For example, Naylor et al. [7] showed that the rhizosphere and endosphere of 18 plant species, including several major crops, were generally enriched in *Actinobacteriota* under drought stress. Xu et al. [8] found that the root system of sorghum, a drought-tolerant crop, was specifically enriched in monoderm *Actinobacteriota* and *Firmicutes* under drought treatment, while the relative abundances of diderm Gram-negative taxa such as *Bacteroidetes* and *Proteobacteria* were generally depleted; hence, they proposed the hypothesis of the 'monoderm-diderm' mechanism for plant drought tolerance [8]. Furthermore, there are differences in the response magnitude of rhizosphere and endosphere microorganisms to drought stress [7–11]. For example, Santos-Medellín et al. [9]. found that changes in bacterial assemblage were more pronounced in the endosphere under drought stress compared to the rhizosphere compartment. Fitzpatrick et al. [11] showed that drought stress exerted a stronger influence on the endosphere bacteriome across 30 angiosperm species, and Naylor et al. [7] observed a more conspicuous enrichment of *Actinobacteriota* in the endosphere. Overall, substantial insight has been gained regarding the effects of drought stress on the microbial assemblage of plant roots [2,4,5]; however, the influence of drought stress on the potential ecological functions of rhizosphere and endosphere bacteriomes has rarely been examined. Moreover, as 'hotspots' of microbial activities, microbes residing in the plant rhizosphere and endosphere typically form complex ecological networks through myriads of interactions [12,13]. Recent studies demonstrated that changes in root microbial networks profoundly affected the growth performance and stress resistance of the plant host [13,14]. Therefore, unveiling the impact of drought stress on root bacterial co-occurrence patterns will further deepen our understanding of the mechanisms by which microbes assist crops in enduring drought; however, associated studies are still scarce.

Rice, a crucial staple crop, feeds over 50% of the global population. Due to its semi-aquatic nature, rice production is highly vulnerable to drought stress [15]. In recent years, extreme high temperatures and drought conditions have frequently afflicted multiple rice-growing regions around the world, especially the middle and lower reaches of the Yangtze River, one of the most important crop production regions in China. However, there are currently no reports on the effects of drought stress on the diversity of the rice root bacteriome in this region. Consequently, it is still unclear how the co-occurring patterns and functional potential of the root bacterial community in this region respond to drought stress. Hence, this study focuses on the waterloggogenic paddy soil typical of the lower Yangtze River region and a rice variety widely planted therein. Leveraging the high-throughput sequencing technology combined with bioinformatic analysis, the work aims to reveal shifts in the diversity and potential ecological functions of rice rhizosphere and endosphere bacterial assemblages under drought stress. Additionally, it seeks to explore how drought stress impacts bacterial co-occurring networks, thereby improving our understanding of its effects on crop root microbiomes. Ultimately, this work may serve as a foundation for identifying potential drought-resistant probiotic strains.

2. Materials and Methods

2.1. Soil Sampling and Basic Soil Properties

The tested soil was collected from the Jiaxing Soil Quality National Observation and Research Station (E 120°25′01″, N 30°26′04″) (Figure 1), situated in Jiaxing City, a major rice-producing region in the lower reaches of the Yangtze River. The soil at the experimental station is typical waterloggogenic paddy soil. Soil samples were collected

at a depth of 0–20 cm using a hand shovel subsequently sieved through a 2 mm mesh to remove stones and plant residues. Prior to the potting experiment, the basic physical and chemical properties of the soil were as follows: pH 5.70, soil organic matter 28.7 g kg^{-1}, total nitrogen (TN) 1.45 g kg^{-1}, total phosphorus (TP) 1.48 g kg^{-1}, total potassium (TK) 17.22 g kg^{-1}, alkali-hydrolyzable nitrogen (AN) 138.64 mg kg^{-1}, available phosphorus (Olsen P) 94.56 mg kg^{-1}, available potassium 85.32 mg kg^{-1}.

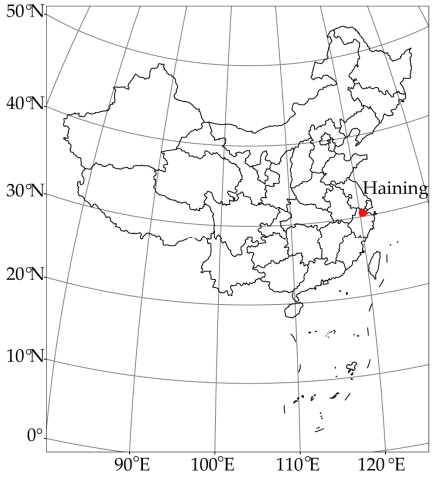

Figure 1. The location of soil sampling site.

2.2. Pot Experiment and Sample Collection

Two treatments comprising continuous flooding control and drought were established for the potting experiment; while corresponding unplanted controls were set up for bulk soil collection, all treatments were composed of four replicates (n = 4). *Oryza sativa* subsp. *japonica* cv. Jia 67, a widely grown rice cultivar in the Jiaxing area, one of the important rice-growing areas in China, was used in this study. Dehulled seeds were surface sterilized by 10% H_2O_2 (*v/v*) for 10 min before being washed five times with sterile water. The seeds were arranged on a Petri dish at 30 °C for germination. Individual germinated seedings were then transformed into Kimura B nutrient solution for another 15 days of growth before planting. For pot establishment, each polyethylene pot (height 17 cm, diameter 15 cm) was filled with fresh soil equivalent to 1.5 kg of dry soil; after one week of flooding, two rice seedlings with uniform growth were transplanted into each pot, followed by the application of one single dose of fertilizer solution. The final fertilizer rates were 100 mg N kg^{-1}, 20 mg P kg^{-1}, and 80 mg K kg^{-1} in the form of ammonium sulphate, calcium superphosphate, and potassium chloride, respectively. The pots were then randomly placed in the growth chamber and rearranged periodically.

After 25 days of continuous flooded incubation, the soil moisture was controlled by weighing to facilitate subsequent implementation of drought treatment. Drought treatments were initiated on day 30 after transplanting; half of the samples were randomly selected to stop irrigation, and the other half remained flooded. During the drought period, the drought treatment was supplemented with 50–200 mL of deionized water every 2 days to avoid possible rice mortality due to excessive dehydration, and the maximum soil moisture content of the drought treatment was 20% after watering in droughted pots, while the control pots were kept flooded. At 28 days after the drought treatment (day 58 after transplanting), plant and soil samples were destructively collected, with two rice plants per pot composited as one sample. Soil samples from the drought treatment were passed through a 2 mm sieve, while soils from the control were collected into sterile plastic bags after thorough mixing with a glass rod in a beaker. Rhizosphere and endosphere samples were collected as proposed by Edward et al. [16], and all samples were quickly stored in a −80 °C refrigerator for subsequent DNA extraction. The remaining samples, including the

shoot compartment, were washed thoroughly using deionized water and dried at 80 °C until constant weight for determination of the plant biomass.

2.3. DNA Extraction and PCR Amplification

DNA was extracted from 500 mg of soil using the Fast DNA SPIN Kit for soil with a FastPrep-24 machine (Qbiogene, Montréal, QC, Canada). The extraction of microbial DNA from the endosphere was performed according to the previous report [17]. All DNA samples were assessed for DNA concentration and purity using 1% agarose gel electrophoresis and a Nanodrop UV spectrophotometer (Thermal fisher, Waltham, MA, USA).

The bacterial 16S rDNA V3-V4 region was amplified using the barcoded primer pair 322F-A/(5′-ACGGHCCARACTCCTACGGAA-3′)/796R(5′-CTACCMGGGGTATCTAATCC KG-3′) [17], since it can effectively reduce the amplification of mitochondrial and chloroplast 16S rDNA of the plant host while sufficiently covering the rhizosphere and endosphere bacterial diversity [17]. Detailed amplification conditions and reaction mixtures have been described previously [17]. The amplification products were recovered by gel purification and then subjected to high-throughput sequencing using the Illumina-MiseqPE300 platform.

2.4. Bioinformatic Analysis

The raw sequence data were processed with the QIIME2 pipeline [18]. Briefly, raw reads were demultiplexed with the 'demux' plugin, then merged with the 'joined-pairs' function in the 'vsearch' plugin. The merged sequences were denoized with the 'deblur' plugin with a trim-length of 450bp, followed by chimer-filtering with the 'uchime-denovo' script. The final valid sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold and taxonomized with Naïve Bayes classifier based on the SILVA database (release132).

The potential ecological function of each OTU was inferred with FAPROTAX software (version 1.2.10) [19]. The networks were constructed with the 'SpiecEasi' method [20], and the topological properties were calculated with the 'microeco' package [21].

2.5. Statistical Analysis

All statistical analyses were conducted under the R environment (version 4.0.2) [22]. Briefly, one-way ANOVA combined with post hoc multiple comparisons were used to assess differences in moisture content, plant biomass, and bacterial α diversity indexes among groups. Shift in bacterial community structure was assessed with principal coordinate ordination (PCoA) based on the Bray–Curtis dissimilarity matrix, and the significance of the sampling compartment and treatment effects was assessed using permutational multivariate ANOVA (PERMANOVA) (1000 permutations) with the 'vegan' package (version 2.6-4) [23]. The edgeR package (https://bioconductor.org/packages/release/bioc/html/edgeR.html) was used to identify OTUs with significant differences in abundance between treatments [24].

3. Results

3.1. Influences of Drought Stress on Soil Moisture Content and Rice Biomass

Drought treatment resulted in a significant decline in soil moisture content (Figure 2A), and the soil moisture content of planted pots decreased further due to the transpiration of rice, compared with the unplanted control, but did not reach a significant level ($p > 0.05$). Compared with the CK, the drought treatment caused a 40% decrease in the rice biomass (Figure 2B), indicating a symptom of drought stress.

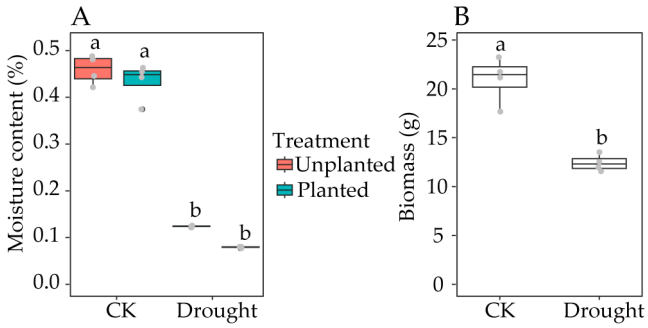

Figure 2. The influence of drought stress on soil moisture (**A**) and rice biomass (**B**). Different letters indicated significant differences among groups ($p < 0.05$).

3.2. Influences of Drought Stress on α- and β-Diversity as Well as Community Assemblage of Bacteria

Final valid sequences were rarefied to 61,610 reads per sample, which were clustered into 1920 OTUs. The richness and Shannon's α-diversity indexes showed a decrease along the order of bulk soil, rhizosphere, and especially endosphere (Figure 3A,B). ANOVA indicated that the richness and Shannon diversity were significantly lower in the endosphere than in the bulk soil and rhizosphere, whereas there was no significant difference in richness between the rhizosphere and bulk soil (Figure 3A). Drought showed no significant influence on the bacterial richness of different compartments (Figure 3A), but significantly increased the Shannon indexes of the rhizosphere and endosphere (Figure 3B)

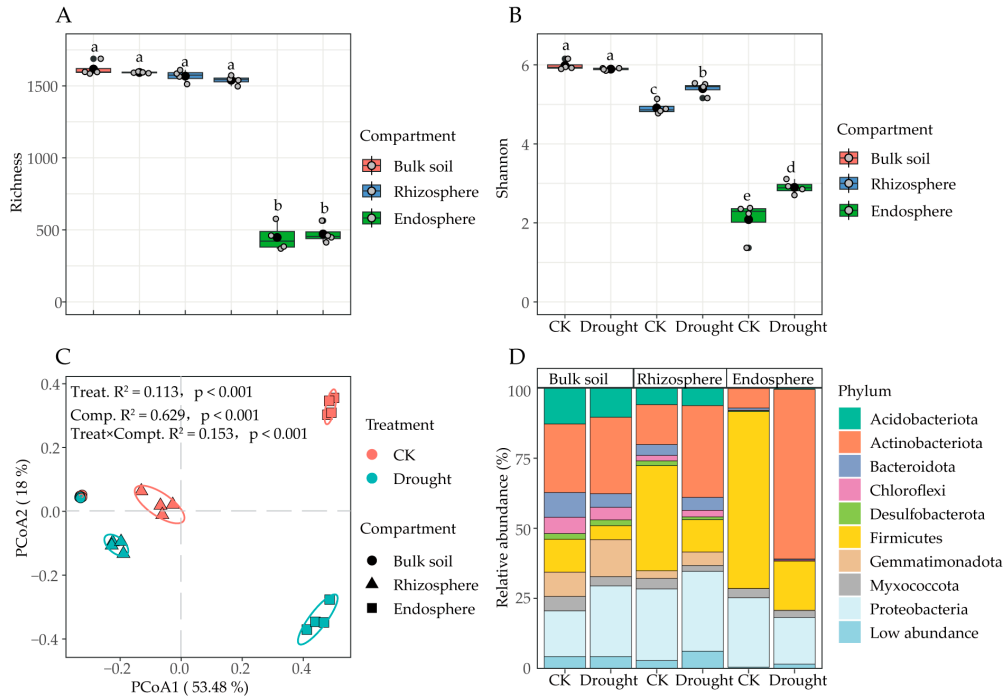

Figure 3. Influences of drought stress on bacterial richness (**A**) and Shannon diversity indexes (**B**), as well as community structure (**C**) and assemblage at phylum level (**D**). The 95% confidence ellipse was draw on PCoA plot (**C**).

PCoA analysis revealed that the first principal axis captured 54% of the total variation in the bacterial community assemblage and primarily reflected the effect of the compartment (Figure 3C); samples of bulk soils, rhizosphere, and endosphere were distributed along this axis, and endosphere samples were far away from other compartments, suggesting a contrasting difference in the community assemblage among compartments. The second axis accounted for 18% of the variance corresponding to the effect of drought, and

endosphere samples from different treatments were further away from each other than the rhizosphere and bulk soil, implying a stronger effect of drought on the endosphere bacterial community than those of the rhizosphere and bulk soil. The effect of drought and compartments were further confirmed by PERMANOVA; meanwhile, the interaction between these factors was also significant ($p < 0.001$, $R^2 = 0.15$).

Clear differences in the community composition were observed among different compartments at the phylum level (Figure 3D). Specifically, the communities of bulk soil were more diverse and homogeneous than other compartments and consisted mainly of several dominant phyla such as *Proteobacteira*, *Actinobacteriota*, *Firmicutes*, *Acidobacteriota*, and *Gemmatimonadota*, while the community of the rhizosphere and endosphere, especially the latter one, were dominated by few phyla including *Proteobacteria*, *Actinobacteriota*, and *Firmicutes*. Drought resulted in the decline of the relative abundance of *Firmicutes* in the rhizosphere and endosphere while it increased the proportion of *Actinobacteriota* in both compartments, particularly the endosphere.

3.3. Community Composition of Bacteria at Genus Level in Different Compartments

At the genus level, ANOVA analysis within each treatment revealed specific enrichments across compartments. In the CK treatment (Figure 4A), 26 genera were notably enriched in the bulk soil, including *Gemmatimonas*, *SJA-15*, *Ellin6067*, *Methylobacter*, and *Methylocaldum*, etc. The rhizosphere showed enriched relative abundances of 12 genera, including *Pseudarthrobacter*, *Reyranella*, *Methylobacter*, and *Methylocaldum*. Meanwhile, the endosphere exhibited specific enrichments of 36 genera, such as *Exiguobacterium*, *Anaeromyxobacter*, *Aeromonas*, and *Streptomyces*. In the drought treatment, 33 genera were significantly enriched in the bulk soil, including *Gemmatimonas*, *Ellin6067*, *Ramlibacter*, and *Thiobacillus*; the droughted rhizosphere specifically enriched *Sphingomonas*, *Paracoccus*, and *Mesorhizobium*, among 9 other genera. Forty genera, including *Streptomyces*, *Exiguobacterium*, *Anaeromyxobacter*, and *Pseudomonas*, etc., were specifically enriched in the endosphere.

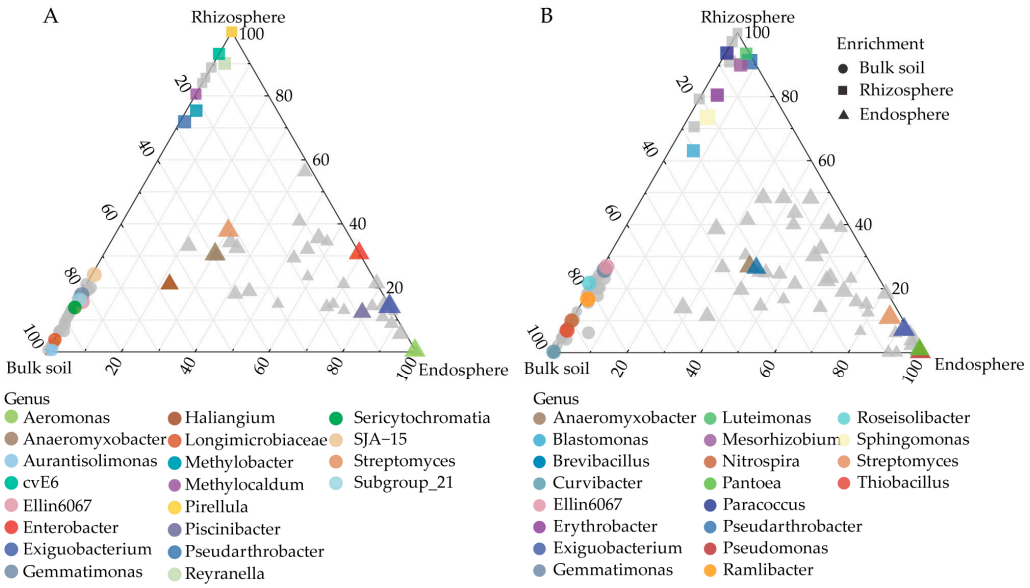

Figure 4. Genera that are specifically enriched in each individual compartment for control (**A**) and drought treatment (**B**). Note: only dominant taxa in each compartment are colored.

3.4. OTUs Significantly Changed under Drought Treatment

Differential analysis indicated that 691 OTUs were significantly affected by drought treatments across compartments (Figure 5). Among these, the proportions of 140, 102, and 180 OTUs decreased in the bulk soil, rhizosphere, and endosphere, respectively (Figure 5A), whereas 168, 123, and 240 OTUs were enriched in their droughted counterparts, respectively

(Figure 5B). Only a limited number of OTUs decreased or increased across all compartments simultaneously, while around 70% of altered OTUs in the endosphere were specific to this compartment.

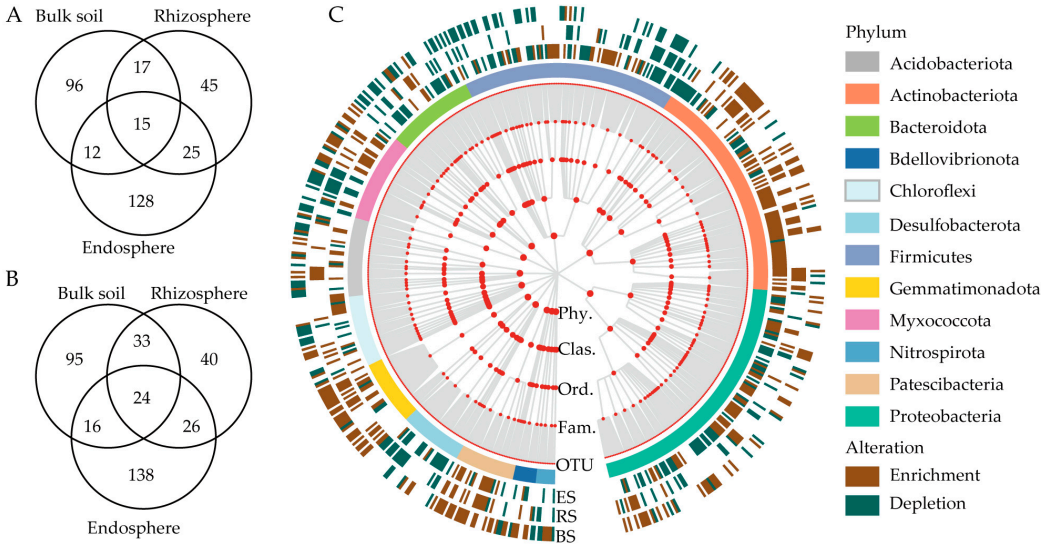

Figure 5. The numbers of OTUs significantly depleted (**A**) or enriched (**B**) by drought treatment, along with the taxonomical composition of OTUs showing significant changes in relative abundances due to drought treatment (**C**). Only the top 12 dominant phylum are shown in Figure (**C**); the innermost ring is colored by corresponding phylum of each taxon; the three outermost rings indicate the shift in relative abundance of each taxon in endosphere (ES), rhizosphere (RS), and bulk soil (BS) in response to drought treatment.

In detail, OTUs that were significantly altered by drought were distributed in 25 phyla including *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Myxomycota*, and *Bacteroidota* (Figure 5C). Among these, OTUs enriched by drought treatment primarily came from *Actinobacteriota*, *Gemmatimonadota*, and *Patescibacteria* (Figure 5C). The enrichment of *Actinobacteriota* was particularly conspicuous in the endosphere, with many unique to this compartment. At the genus level, these primarily included *Nocardioides*, *Streptomyces*, and *Conexibacter*. In contrast, *Patescibacteria* enriched in either the rhizosphere or endosphere were largely consistent and belonged to *Saccharimonadales.* OTUs significantly depleted under drought treatment primarily belonged to *Firmicutes*, *Bacteroidetes*, and *Desulfobacterota*; however, approximately 44% of *Firmicutes* OTUs were also enriched in the droughted endosphere. Unlike others, altered OTUs from *Myxomycota*, *Chloroflexi*, and *Proteobacteria* did not exhibit consistent response patterns to drought treatment.

3.5. Network Analysis

Co-occurring network analysis revealed a decrease in topological complexity across treatments in the order of bulk soil, rhizosphere, and endosphere (Figure 6). Topological attributes such as node number, edges, and average degree were markedly smaller in the endosphere compared to the rhizosphere and bulk soil (Table 1). Specifically, drought conditions increased the number of nodes and edges in all compartments, indicating heightened complexity in bacterial co-occurring networks. However, drought decreased the proportion of negative edges (Table 1) and increased the average degree and average path length in the bulk soil and rhizosphere networks, as well as the clustering and modularity coefficients in the bulk soil and endosphere networks. Node attribute analysis revealed no network hubs across compartments and treatments. Drought increased the number of connectors in the bulk soil and rhizosphere, as well as module hubs in the

rhizosphere, while decreasing module hubs in the bulk soil. No module hubs or connectors were identified in the endosphere networks.

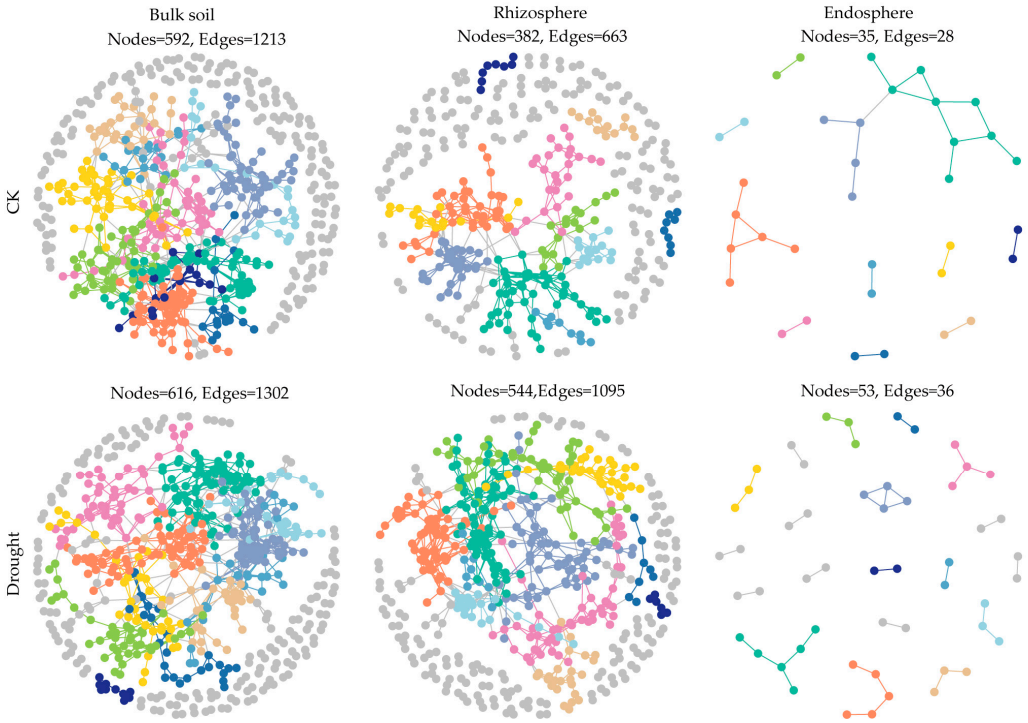

Figure 6. The co-occurring networks of different compartments under different treatments. The modules of networks were presented in different colors.

Table 1. The topological properties of networks of different compartments under different treatments.

Topological Attributes	Bulk Soil		Rhizosphere		Endosphere	
	CK	Drought	CK	Drought	CK	Drought
No. of nodes	592.000	616.000	382.000	544.000	35.000	53.000
No. of edges	1213.000	1302.000	663.000	1095.000	28.000	36.000
Average degree	4.098	4.227	3.471	4.026	1.600	1.359
Average path length	6.897	6.906	6.007	7.200	2.578	1.529
Diameter	18.000	18.000	16.000	19.000	7.000	4.000
Clustering coefficient	0.456	0.459	0.503	0.438	0.182	0.261
Density	0.007	0.007	0.009	0.007	0.047	0.026
Centralization coefficient	0.022	0.021	0.030	0.020	0.071	0.032
Modularity	0.790	0.791	0.803	0.794	0.752	0.921
Proportion of negative edges	0.525	0.489	0.425	0.258	0.464	0.361
No. of module hubs	4.000	1.000	-	4.000	-	-
No. of connectors	6.000	13.000	4.000	7.000	-	-
No. of network hubs	-	-	-	-	-	-

3.6. Effect of Drought on Ecological Functional Potentials

FARPROTAX-based analysis revealed that drought induced significant alterations in 17, 6, and 6 functional pathways within the bulk soil, rhizosphere, and endosphere, respectively, with predation and ectoparasitism being the only pathways that increased significantly across all compartments (Figure 7). Specifically, drought increased the prevalence of the chitinolytic pathway in both the rhizosphere and endosphere, as well as aerobic chemoheterotrophy and chemoheterotrophy in the bulk soil and endosphere. Conversely, drought decreased fermentation in both the bulk soil and rhizosphere, and aromatic compound degradation in the bulk soil and endosphere. Notably, the drought treatment significantly

enriched the functional potential associated with sulfur oxidation while decreasing iron respiration, specifically in the rhizosphere.

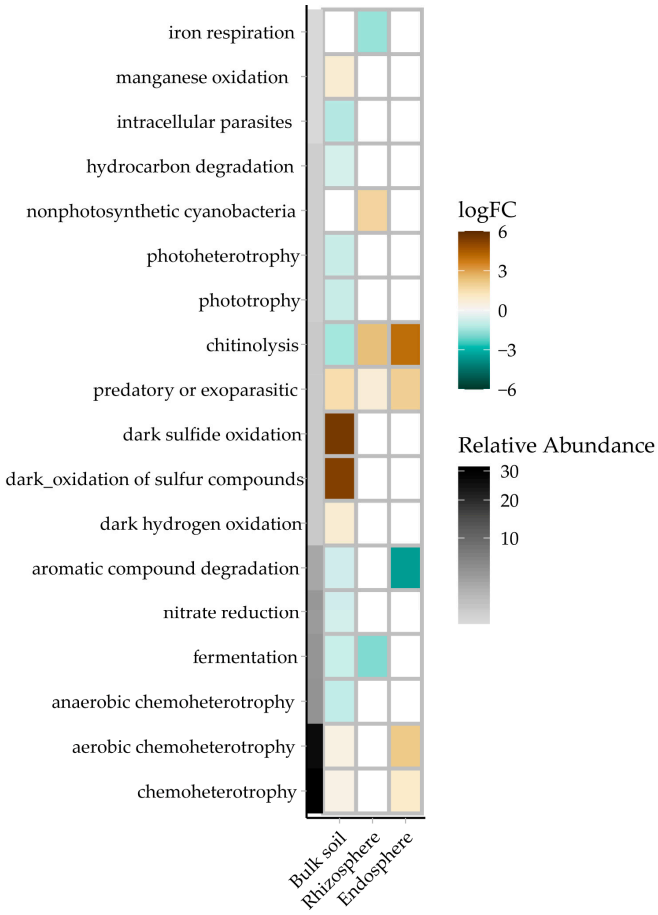

Figure 7. The influence of drought treatment on the functional potentials of different compartments.

4. Discussion

The rhizosphere and endosphere bacteriomes are integral components of the 'holobiont', playing crucial roles in soil nutrient transformation and aiding the plant host in resisting myriads of biotic and abiotic stresses [25]. Consequently, microbial diversity, including community structures and co-occurrence patterns, in these environments is strongly influenced by the plant host and increasingly differs from that of the surrounding soil (i.e., bulk soil) due to the ever-strengthening selection pressure exerted by the plant host [3,13,26]. Our work reaffirms this phenomenon, as evidenced by several key findings: (i) α-diversity, particularly the Shannon index, sequentially decreased in the rhizosphere and endosphere bacterial communities compared to the bulk soil; (ii) network complexities similarly decreased; and (iii) the community structure and dominant bacterial taxa in the rhizosphere and endosphere differed markedly from those in the bulk soil. Specifically, the rhizosphere and endosphere bacteriomes were dominated by *Proteobacteria* (e.g., genera such as *Pseudomonas*, *Mesorhizobium*, and *Sphingomonas*, etc.), *Firmicutes* (e.g., *Exiguobacterium*), and *Actinobacteriota* (e.g., *Streptomyces*). While similar findings have been extensively reported [8,9,13,16,27], our results revealed a notable difference: the typically dominant phylum *Bacteroidetes* was underrepresented in the roots of the tested rice cultivar. This discrepancy may be attributed to its low abundance in the soil from which the plant was cultivated; after all, the soil serves as a microbial "seed bank" [16,23]. Alternatively, it might be due to the primer pairs used in this study. Nonetheless, Chen et al. demonstrated that their amplification bias was slightly different from those of other conventional primers, particularly affecting *Chloroflexi* and *Verrucomicrobia* [17]. Our findings thus lend

further support for the perspective that the soil environment plays a pivotal role in shaping microbial communities in the rhizosphere and endosphere of plant hosts.

Drought treatment significantly altered the Shannon diversity index of the rhizosphere and endosphere bacterial communities but had no significant effect on the richness index. This suggests that the plant host reshaped the community structure by changing the population evenness in these compartments under drought stress. While shifts in the α-diversity of the root bacteriome in response to drought have been widely reported across various crops and rice varieties [7–11], our results differ from previous studies in that drought increased rather than decreased the α-diversity of the root bacteriome [5,7,11]. Undoubtedly, the soil type and rice cultivar are crucial factors governing the response patterns of root bacterial α-diversity to drought treatment [28]. Furthermore, in paddy soils, drought can reduce nutrient diffusion and availability, increase oxygen supply and soil microsite heterogeneity, alter the quality and quantity of root exudates [28,29], and elevate root slough-offs [30]. These changes contrast sharply with the relatively homogenized conditions found under flooded environments [6], thus potentially providing more diversified niches for microbes [30]. As such, drought treatment in the tested soil may prevent a few phyla from dominating the community assemblage.

In consistence with earlier findings [4,5,7,9,10], our results confirm that drought stress significantly alters the community composition of rhizosphere and endosphere bacteria, consistently enriching taxa such as *Gemmatimonadetes*, *Patescibacteria*, and *Actinobacteriota*, among others. Accumulating evidence suggests that *Gemmatimonadetes* thrives in environments with low moisture content [31], while *Actinobacteriota* (e.g., *Streptomyces* and *Nocardioides*), adhering to the 'monoderm–diderm' hypothesis, possess thicker cell walls and can produce spores and osmoregulatory compounds to adapt to and tolerate drought conditions [4,5,8]. Meanwhile, *Streptomyces* strains, known for synthesizing antibiotics and plant hormones [32], may aid in modulating plant adaptation to drought by altering the rhizosphere microbial community and influencing plant growth performance [8,10]. Similarly, *Patescibacteria* isolates have been demonstrated to own capabilities in enhancing plant growth under drought stress [33]. However, although *Firmicutes*, a representative of monoderm bacteria, has been identified as an indicator taxon for drought stress in various plants, we did not observe their enrichment in the roots of the tested rice cultivar. Likewise, Santos-Medellín et al. [9] and Li et al. [28] reported a decrease in the relative abundance of most *Firmicutes* genera in other rice cultivars under drought stress. Hence, it appears that the response of root *Firmicutes* to drought stress is more influenced by the plant host than their intrinsic abilities to drought tolerance. Therefore, the enrichment with specific bacterial taxa under drought stress largely reflects the mechanism of 'crying for help' of the plant host [34].

Our results indicate that drought treatment led to changes in the bacterial co-occurring networks across compartments, resulting in the increased complexity of networks and a greater proportion of positive connections. This suggests that microorganisms may strengthen their collaboration in response to environmental pressures during drought conditions. A similar pattern was observed in a study on the rhizosphere and endosphere bacteria of wild rice (*Oryza longistaminata*) [35]. Yuan et al. [36] also noted that prolonged warming coupled with decreasing soil moisture content amplified the complexity of soil bacterial co-occurrence networks, particularly the proportion of positive links. Hernandez et al. [37] reported a progressive rise in the proportion of mutualistic relationships in soil microbial networks under environmental stresses across a gradient of soil water availability and nutrient levels in a shrub stand. Furthermore, these results align well with the predictions of the 'stress gradient hypothesis', which suggests that positive microbial interactions would be enhanced under stressful conditions [38]. However, it is worth noting that inconsistent findings have been documented as well. For instance, Gao et al. [39] found that drought reduced the complexity of rhizosphere and endosphere bacterial networks of sorghum after 6 weeks of drought treatment. In the work of Hernandez et al. [37], they also found chronic stress environments reduced the complexity and stability of soil

microbial networks. This discrepancy may be associated with the variations in soil type and duration of drought among studies, as ecosystems that were moderately disturbed could accommodate a greater number of species coexisting and exhibited the highest biodiversity, according to the 'intermediate disturbance hypothesis' [40]. As such, the shorter duration of drought treatment in this study might have created more favorable conditions for the coexistence of the root bacteriome, thereby increasing the network complexity and Shannon diversity indexes.

Drought stress significantly altered several ecological functions in different compartments, particularly in the bulk soil. Notably, the biological oxidation processes of elements such as manganese, hydrogen, and sulfur were promoted to varying degrees exclusively in the droughted bulk soil. Previous studies have shown that the oxidized forms of these elements under flooded conditions can serve as important electron donors for anaerobic microbes in paddy soils, leading to the accumulation of their reduced forms [41,42]. In contrast, improved soil aeration induced by drought can enhance both the biotic and abiotic transformation of these reduced compounds [41]. Additionally, the strong stimulatory effect of drought on sulfur/sulfide oxidation may be closely related to the substantial input of ammonium sulfate as a nitrogen fertilizer in this study. Intriguingly, drought significantly reduced the iron (Fe) respiration in the rhizosphere. Earlier research has indicated that increased soil oxygen partial pressure under drought conditions drives the oxidation of rhizosphere Fe(II) to Fe(III) minerals with low bioavailability, thereby reducing the substrate for Fe(II)-metabolizing microbes [43]. This not only diminishes the availability of Fe in the rhizosphere but also affects the assembly of the rhizosphere bacterial community [44,45]. For instance, taking advantage of multi-omics approaches, Xu et al. [45] demonstrated that under drought conditions, the decrease in iron availability within the sorghum root environment was tightly associated with the specific enrichment of *Streptomyces* spp. On the other hand, it is well established that *Actinobacteria*, particularly *Streptomyces*, produce a variety of siderophores to increase the availability of Fe, which may assist the plant host in mitigating drought stress [8,10,46]. Overall, our results illustrate that drought stress profoundly impacts the biogeochemical processes in paddy soils.

5. Conclusions

Our results revealed that drought stress increased the Shannon diversity of rice rhizosphere and endosphere bacteriomes and altered their community assemblages. This led to the specific enrichment of *Gemmatimonadetes*, *Patescibacteria*, and notably *Actinobacteriota*, while depleting the populations of *Firmicutes*, *Bacteroidetes*, and *Desulfobacterota*, among others, in the rhizosphere and endosphere. Additionally, drought stress increased the complexity of microbial networks and the proportion of positive interactions across compartments, thereby supporting the "stress gradient hypothesis." Furthermore, drought altered multiple ecological functions associated with the transformations of elements such as Fe, S, and Mn, suggesting that drought stress may profoundly affect the biogeochemistry of paddy soils.

Author Contributions: Conceptualization, C.W., X.Z. and C.Y; methodology, investigation and formal analysis, C.W., X.Z., Y.L. (Yinxiu Liu) and X.T.; software, Y.L. (Yan Li) and T.S.; writing—original draft preparation, C.W., X.Z., G.Y. and C.Y.; writing—review and editing, G.Y. and C.Y; supervision, C.Y.; funding acquisition, Y.L. (Yan Li) and C.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was jointly supported by Zhejiang Province's 'Three Rural and Nine Aspects' Technology Collaboration Plan (grant numbers 2022SNJF004 and 2024SNJF079), funded by Department of Agriculture and Rural Affairs of Zhejiang Province, and by the Zhejiang Provincial Natural Science Foundation of China (grant number Y23D010011), funded by Department of Science and Technology of Zhejiang Province.

Data Availability Statement: All raw sequences have been deposited into the GSA database under the accession number CRA012577.

Conflicts of Interest: The authors declare no conflicts of interest.

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