

Microbiota community assembly in wild rice (*Oryza longistaminata*) in response to drought stress shows phylogenetic conservation, stochasticity, and aboveground-belowground patterns

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Abstract

Background

Drought is a global environmental stress that limits crop yields. Microbial communities control many biogeochemical processes, and a predictive understanding of how crop microbial communities assemble in response to drought stress is central to addressing the challenges caused by drought. Little is known about the microbiome assembly processes in rice-ecosystems, particularly with regard to their environmental adaptation. Wild rice may serve as a source of superior drought tolerance candidate for rice breeding. There is an urgent need to explore wild rice resistance mechanisms to drought stress. Here, we evaluated the effect of drought stress on the microbial community recruitment and assembly in the endosphere (leaf, stem, and root) and rhizosphere of *Oryza longistaminata*.

Results

Species replacement was the dominant process shaping microbial community composition under drought stress. *O. longistaminata* recruited the phyla Actinobacteria and Fusobacteria, the genus *Streptomyces*, and phototrophic prokaryotes to improve its fitness. The host exerted strong effects on microbiome assembly, and the responses of the microbial community structure to the drought environment showed above- and belowground patterns. Drought reduced taxonomic α -diversity and destabilized co-occurrence network properties in the leaves and stems, but not in the roots and rhizosphere. Drought promoted the restructuring and strengthening of belowground network links to more strongly interconnect network properties. The drought response of the microbiome was phylogenetically conserved. Stochastic (neutral) processes acted on microbial community reassembly in response to drought stress across all four compartments.

Conclusions

Our results provide new insight into the mechanisms through which drought alters microbial community assembly in drought-tolerant wild rice and reveal a potential strategy for manipulating plant microbiomes to improve crop fitness.

Background

Drastic climate change and increased water scarcity are challenges to global crop production [1]. Drought is one of the most important factors influencing rice production and can lead to a 25% decrease in rice yield [2]. During rice domestication, 50–60% of alleles were lost from the nearest wild relatives of cultivated rice (*Oryza sativa*) [3, 4]. Cultivated rice (*Oryza sativa*) is more sensitive to drought than wild rice [2, 5]. Wild rice may serve as a source of superior drought tolerance alleles for cultivated rice. There is an urgent need to explore wild rice drought stress resistance mechanisms, which can then be used to

improve cultivated rice productivity under unfavorable drought stresses conditions given the limited global land resources.

Drought not only decreases the water available to soil microbial communities and plants but also changes in nutrient availability, specifically N and P uptake [6]. Moreover, microbial communities control many biogeochemical processes [7]. Microbiota may affect soil water availability by altering the hydraulic hydrophobicity and conductivity of water [8]. A large body of evidence demonstrates that major crops can be made dramatically more tolerant of abiotic stresses by transplanting various plant growth-promoting (PGP) microbiota, the best known of which are arbuscular mycorrhizal fungi (AMF) and nitrogen fixation bacteria [9–14]. In nature, plants and their associated microbiota have been interacting with each other for 450 million years, and the assemblage of species is known as the holobiont [15]. The holobiont has a much greater evolutionary potential to tolerate abiotic stress than the plant itself [16]. The plant creates nutrient-rich conditions by exuding complex carbon compounds, mucilage, sloughed root border cells and microbe signaling hormones to recruit the soil microbiota to the rhizosphere. Then, host-plant genetic factors screen a subset of microbiota into the root endosphere [6, 17, 18]. Plants shape their associated microbiota by employing a combinatorially complex system of receptors and signals; conversely, the microbiota can have wide-ranging effects on host plant fitness, including on host drought tolerance and nutrient uptake [17, 19–22].

To date, extensive evidence suggest that microbiota community establishment around plants is not random but is rather controlled by specific assembly rules [23–25]. Nonetheless, it remains unclear whether genomic signatures exist for microbiota community-related fitness phenotypes [15]. Although the mechanisms shaping microbial community structure have been intensively examined, those controlling the ecological processes of crop systems in response to environmental perturbations remain unclear. To understand the mechanisms and factors giving structure to natural and agricultural microbial communities, studies have emphasized two types of processes: stochastic and deterministic [26]. Both stochastic and deterministic components are embedded in four ecological processes, including selection, dispersal, diversification, and drift [27]. The balance between stochastic and deterministic assembly processes is mediated by environmental factors [28]. Several recent studies have provided an excellent overview of the ecological processes controlling microbial community structure and biogeographic patterns in general [29–33].

Red rice, *Oryza longistaminata*, is a perennial wild rice with strong rhizomes[34]. *O. longistaminata* contains various agronomically valuable traits that could be used in rice improvement programs, including drought tolerance, long anthers, large biomass in poor soils, high nitrogen use efficiency, and resistance to insect pests and disease [35]. Along with its useful traits, *O. longistaminata* possesses the AA genome as cultivated rice (*Oryza sativa*), which make it a good candidate for rice breeding [36]. In light of growing concerns over the threat of water stress to agricultural ecosystems, increased emphasis has been placed on a mechanistic understanding of how drought stress conditions influence the composition and functioning of the plant-association microbiome and the ultimate consequences for

plant health [37]. Exploring the interactions between the microbiome and drought-tolerant wild rice under drought stress is critical in efforts to improve rice yields under increasingly frequent droughts.

By exploiting this unique agronomically trait in wild rice, we aimed to disentangle the relationships between microbial communities and host systems and to address the fundamental mechanisms of microbiota maintenance and recruitment strategies in wild rice under drought conditions. Here, we are interested in the following questions: first, how does drought stress influence microbiota community composition and functioning in drought-tolerant wild rice? Second, do the above- and belowground tissues show similar patterns of control over their resident microbiota communities under drought stress? Third, which deterministic and/or stochastic processes structure microbial communities in response to drought perturbations? To answer these questions, we compared the microbial community structures, network structures, and assembly processes within the endospheres of leaves, stems, and roots and the rhizospheres of *O. longistaminata* in response to drought stress. The results from our experiments provide insight into adaptive plant responses to global change by identifying changes in microbial community composition.

Results

The effect of the drought treatments on *Oryza longistaminata* performance

To test the plant-associated microbial community response to drought stress, drought was imposed on *Oryza longistaminata* for two months. Two months after water withdrawal, the soil moisture content in the treated samples decreased from 34.6% to 0.072% (Fig. S1). Notably, the drought-stressed plants remained viable throughout the experiment, which indicated *O. longistaminata*'s strong drought tolerance traits (data not shown).

Drought significantly decreased the leaf photosynthetic rate (P_n) ($P < 0.01$) and the leaf intercellular CO_2 concentration (C_i) ($P < 0.01$), while increasing the stomatal limitation value (L_s) ($P < 0.01$) (Fig. 1A). The results suggest that drought decreased the leaf photosynthesis of *O. longistaminata* through stomatal closure. To test the degree of membrane lipid peroxidation caused by drought stress, the MDA content was measured. Drought increased the MDA content ($P < 0.01$), suggesting that drought decreased photosynthesis through metabolic impairment (Fig. 1B). Drought significantly decreased the antioxidant enzymatic activity levels of SOD and POD and while increased the antioxidant enzymatic activity levels of CAT ($P < 0.05$) (Fig. 1C). Collectively, these data demonstrate that drought treatments lead to a corresponding increase in plant stress.

Figure 1

Drought shapes the microbiome taxonomic community structure in the different compartments

Across plant compartments, the composition of the microbial communities varied significantly. The taxonomic α -diversity increased from the leaf towards the rhizosphere (Kruskal-Wallis test, $P < 0.01$) (Fig.

2A and S2). We quantified microbiome community composition using weighted UniFrac distances with principal coordinate analysis and found clear differences in the compositions of the leaf, stem, root, and rhizosphere compartments (PERMANOVA, $R^2=0.18375$, $P<0.001$) (Fig. 2B). The results indicated that the *O. longistaminata* microbiomes was spatially structured in each distinct compartments.

Figure 2

The microbial taxonomic alpha diversity within each sample was analyzed based on the Shannon diversity index. The mean α -diversity reduced under drought-stress in the leaves and stems, suggesting the decreased growth in a few bacterial groups under drought (Fig. 2C). However, the α -diversity of the root and rhizosphere microbiomes did not differ significantly in response to drought (Kruskal-Wallis test, $P>0.05$) (Fig. 2C). Unconstrained principal coordinate analyses (PCoAs) based on the weighted UniFrac (WUF) distance matrix were performed to investigate patterns of separation among microbial communities between the control and drought treatments within different compartments. The microbiome communities were separated by drought in the leaves, stems, roots, and rhizosphere (PERMANOVA, $P<0.05$) (Fig. 2D).

We analyze the drought-mediated alterations within the individual compartments. There were notable differences in the proportions of various phyla and families influenced by water deprivation. Drought dramatically affected the relative abundances of the microbiome in the endosphere compartments (Fig. S3AB). To more clearly show the patterns of the drought-responsive taxa, we display the first 50 different taxa at the phylum and family levels (Fig. S3CD). At the phylum level, drought-affected not only the major groups but also the minor groups, indicating a broad exclusionary effect of drought on the relative abundances of the microbiome (Fig. S3C). In response to drought, the patterns of control over their resident microbiota communities in the four compartments were divided into two patterns, i.e., aboveground and belowground patterns (Fig. S3CD). In the aboveground tissues, the phyla Euryarchaeota, Actinobacteria, Fusobacteria, and Firmicutes were significantly enriched, and the phyla beta-proteobacteria, Bacteroidetes, Spirochaetes, and Chloroflexi, the genus *Pseudomonadaceae* were significantly depleted under drought stress. In belowground tissues, the phyla Actinobacteria, Fusobacteria, TM7, Tenericutes, and FBP, the genus *Streptomycetaceae* were significantly enriched, and the phyla Spirochaetes, Epsilon-proteobacteria and OD1 were significantly depleted under drought stress (Fig. S3C). The results indicated that aboveground and belowground plant parts host microbiome assemblies with different taxonomical structures in response to drought.

The microbiome co-occurrence networks of ASVs changed with drought stress in compartment-specific patterns

The α -diversity data showed that microbial communities in aboveground tissues are more sensitive to drought than those of belowground compartments (Fig. 2C). We generated a microbial ASV (amplicon sequence variant) co-occurrence network using significant correlations to explore the more detailed changes in potential interactions among microbiota under drought stress.

The diameter, the number of edges, and vertices of the co-occurrence networks increased, while the clustering coefficient decreased, in the order of aboveground tissues, roots and rhizosphere soil (Fig. 3A, Table S1). The data indicated that the co-occurrence networks become larger and more connected and have less modularity from the aboveground plant parts to the rhizosphere soil. The wild rice microbiota formed highly compartmentalized coexistence networks of coexistence within the host.

Surprisingly, the responses of the aboveground and belowground microbial networks to drought was quite opposites. Drought strongly decreased the connectedness of nodes, the number of edges and vertices, and the number of clusters in the aboveground microbial networks, while it increased these properties in the below-ground microbial networks (Fig. 3ABC, Table S1). In particular, the microbial network's negative correlation was strengthened in the rhizosphere under drought stress, which could be interpreted as an increase in competitive relationships within the rhizosphere under drought (Fig. 3A, Table S1). Specific microbes that are highly connected to other microbes within the co-occurrence networks are often defined as "hub" or "keystone" species and likely exert a stronger influence than other species on the structure of microbial communities. We then focused on how the network hubs respond to drought stress in belowground microbial networks. In the roots, Proteobacteria and Firmicutes were the dominant network hubs. Proteobacteria and Firmicutes accounted for 25.0% and 37.5% of the community, respectively, while their relative abundance increased to 54.55% and 9.09% respectively in response to drought (Fig. 3D). In the rhizosphere, drought increased the number of network hubs (Fig. 3D). We also found that the network hub scores were not related to the relative abundance of the ASVs in all four compartments, and ASVs with either low or high relative abundance can be network hubs (Fig. S5). Together, this suggests that the aboveground microbial networks were unstable under drought, but not belowground co-occurrence networks. Drought promoted the restructuring and strengthening of the belowground networks to more strongly interconnect network properties.

Figure 3

Phylogenetic conservation of microbial drought responses

We quantified the strength of the relationship between phylogeny and drought to determine whether phylogenetic information could be predictive of the response of microbial taxa to the global drought stress. The drought responses were strongly phylogenetically conserved in all compartments. The mean genetic depth (τ_D) ranged from 0.07282 to 0.2099. The D-test of Fritz and Purvis also confirmed that drought responses were dispersed in a mode between a Brownian motion and a random model ($0 < D < 1$) in every compartment, suggesting that closely related species exhibited more similar ecological preferences for drought stress (Fig. 4).

Figure 4

Microbial community assembly processes in drought-stressed *Oryza longistaminata*

To understand the assembly processes governing the composition of microbial communities of *Oryza longistaminata* in response to drought stress, we first analyzed changes in the microbial community under drought using Sorensen beta diversity based on the ASV level. The Sorensen beta diversity (β_{SOR}) was the lowest in the rhizosphere, followed by the roots, leaves, and stems (Fig. 5A). To explain those changes in the microbial community, we partitioned the Sorensen beta diversity into turnover (species replacement through dispersal) and nestedness-resultant dissimilarity (species loss through death or emigration) components. We found that species replacement (β_{SIM}) was a much greater contributor to the observed beta diversity than species loss (β_{SNE}) in all four compartments in response to drought (Fig. 5B).

We considered which deterministic and/or stochastic processes give structure to microbial communities. To evaluate the ecological process controlling the composition of each microbial community, the microbial phylogenetic community composition within each community was determined. The phylogenetic alpha diversity (PD) of the rhizosphere microbiome decreased significantly in response to drought (Kruskal-Wallis test, $P < 0.001$) (Fig. 5C). In the leaves and stems, we found that the values of the standardized effect sizes of MNTD (SES_{MNTD} , equivalent to -NTI) calculated using the null model were in the range of -2 to +2 (Fig 5D, Table S2). The values of SES_{MNTD} were < -2 in the roots and rhizospheres, suggesting that microbial communities within these samples were more significantly phylogenetic clustering among co-occurring species than expected by chance ($\text{mntd.obs.z} < 0$, $\text{mntd.obs.p} < 0.05$), and drought significantly increased microbial community clustering in the rhizospheres ($p < 0.001$) (Fig. 5D, Table S2). In addition, based on the AIC values, the fitness of the null, log-normal, pre-emption, Mandelbrot, and Zipf models was compared to investigate which processes were important in shaping the microbial community structure. The results showed that a large amount of data for the leaf and stem communities fit to the null models (Fig S5), while the nonrandom models best fit the data for the roots and rhizospheres (Fig S5), these results are consistent with the MNTD analysis.

Figure 5

To evaluate the relative influences of stochastic and deterministic processes in controlling community dynamics in response to drought stress, turnover in the phylogenetic composition turnover (phylogenetic β -diversity) between control and drought conditions was calculated using the β nearest taxon index (βNTI) to explore the mechanisms underlying community assembly. The βNTI score for microbial communities was in the range of -2 to +2, indicating that stochastic (neutral) processes dominated microbial community dynamics in response to drought across all four compartments (Fig. 5E). To evaluate the influence of spatial position on phylogenetic turnover, we observed that phylogenetic turnover increased from the leaves to the rhizosphere (Fig. 5F). Density plots showed clear separations among these distributions in different compartments (Fig. 5G). Subsequently, the taxonomic β -diversity metric (Bray-Curtis-based Raup-Crick, RC_{Bray}) was used to partition the pairwise comparisons with an absolute βNTI values of < 2 . The majority of the RC_{Bray} scores were in the range of -0.95 and +0.95 (94.5% in leaves, 92.7% in stems, 66.7% in roots, 86.2% in the rhizosphere) (Fig. 5H), which indicated that weak

selection, weak dispersal, diversification, and/or drift dominated the microbial community dynamics (treated as an “undominated” fraction). The RC_{Bray} scores that were > 0.95 (5.45% in leaves, 7.27% in stems, 33.3% in roots, 13.8% in rhizospheres) indicated that dispersal limitation dominated the microbial community dynamics (Fig. 5H).

Drought affected the functional composition of the microbial community in belowground compartments

Given the evidence for links between microbial communities and biogeochemical cycles, we expected drought-induced changes in microbial communities and networks to influence microbial functions. This expectation was tested using AFPPOTAX. Our data showed that rhizosphere microbiota performed a complete set of biogeochemical processes involving methanogenesis, sulfur-cycling, nitrogen-cycling, carbohydrate metabolism, metal metabolism, and photoautotrophy (Fig. 6A). Drought strongly affected the biogeochemical processes involving a one-carbon cycling, sulfur cycling, iron respiration, and phototrophy. In response to drought stress, the functional composition of the microbial community in the rhizospheres shown more robust than that in the roots (Fig. 6AB). In detail, methylotrophy, sulfur, and iron respiration were significantly decreased under drought in the roots (Wilcoxon test, $P < 0.01$) (Fig 6AB). In the rhizosphere, iron respiration decreased significantly in response to drought (Wilcoxon test, $P < 0.01$), while phototrophy increased significantly ($P < 0.05$) (Fig. 6AB).

Figure 6

Discussion

The plant-associated microbiome extends the functional repertoire of plants in ways that exceed imagination [38-41]. Crops greatly rely on their microbiota for nutrients uptake and stresses protection [1]. Deciphering the microbe-rice interactions under drought, particularly those in drought-tolerant rice, offers great potential for increasing the resilience of rice production to abiotic stress. This study provides a detailed characterization of the effect of drought on microbiome assemblages in the leaves, stems, roots, and rhizosphere of drought-tolerant wild rice (*Oryza longistaminata*).

Many studies have demonstrated that drought can have considerable effects on microbial communities [1]. In terms of recruitment, we found significant changes in the relative abundances of a broad set of bacteria that spanned many prominent phyla and genera in the microbial community. In particular, the phyla Actinobacteria and Fusobacteria were significantly enriched, and Spirochaetes was significantly depleted under drought stress (Fig. 3). Similarly, Santos-Medellín et al. reported root-associated community in rice in which several OTUs belonging to the phyla Actinobacteria and Chloroflexi were significantly enriched under drought, whereas OTUs from the phylum Acidobacteria and classes Deltaproteobacteria were generally depleted [42]. Of note, many works focusing on the root bacterial microbiomes of rice [42], sorghum [43], and diverse lineages of plant species [44, 45] under drought stress have reported enrichment of bacteria from the phylum Actinobacteria [6]. Actinobacteria are gram-positive (G^+), monoderm bacteria. G^+ bacteria are thought to accumulate under drought, and the cell wall

is thought to improve cell drought tolerance by increasing their ability to resist desiccation [6, 46]. Our data also show that G⁺ bacteria were significantly enriched in the rhizosphere under drought conditions (Fig. S7).

Microbiota communities resident in above- and belowground tissues show different response reply patterns under drought stress

Plant adapt to drought by manipulating aboveground-belowground feedbacks between plants and soil microbiota [19]. The aboveground tissue is functionally distinct from the belowground tissue [47]. Plant fitness responses to drought were governed by rapid changes in belowground microbial communities [19]. Our data showed that the patterns of control over their resident microbiota communities in the four compartments could be classified into two patterns, i.e., aboveground and belowground patterns. Drought reduced taxonomic α -diversity and destabilized co-occurrence network properties in the leaves and stems, but not in the roots or rhizosphere. Drought promoted the restructuring and strengthening of belowground network links to more strongly interconnect network properties (Fig. 2, 3). This indicated that the aboveground microbiome was less stable under drought than the belowground microbiome.

1. *O. longistaminata* has shown a stronger survival ability than cultivated rice [34, 35]. *O. longistaminata* showed significantly longer root length and number than the cultivated rice when a water deficit was imposed. *O. longistaminata* had greater stomatal conductance under stress and maintained leaf elongation better under stress than most other rice genotypes (data not shown). Nevertheless, in response to drought, *O. longistaminata* decreased its leaf photosynthetic rate (Pn), leaf intercellular CO₂ concentration (Ci), and leaf stomatal limitation value (Ls) to maintain its basic metabolic functions (Fig. 1). At the same time, *O. longistaminata* developed significantly longer roots and more roots than *O. longistaminata* that was not under drought to take up nutrients from the rhizosphere. This survival strategy led to the further differentiation of the aboveground and belowground functions under drought stress. We showed that a drought-induced reduction in plant photosynthesis has direct effects on microbial co-occurrence networks and communities in aboveground tissues, and has the potential to reinforce changes in microbial community composition in belowground compartments in response to drought (Fig. 3).

Photosynthetic carbon inputs and methane emissions were important factors to driving the microbiome structure

Carbon (C) enters terrestrial ecosystems through photosynthesis. Once it is in the plant, C may be allocated to above- or belowground tissues and to growth, metabolism, protection, or storage. Root exudates (mixtures of carbon compounds) can comprise 10% of plant photosynthate and are a highly dynamic source of carbon inputs into the rhizosphere soil [48]. Plants exchange carbon and nutrients with microbiota and plants have been shown to regulate their carbon allocation to microbiota in response to changes in environmental conditions [48, 49]. To take up nutrients under stress, *O. longistaminata* develops significantly longer roots and more roots and creates nutrient-rich conditions from

photosynthetic carbon inputs to recruit the soil microbiota to the rhizosphere. We observed that the taxonomic α -diversity of the root and rhizosphere microbiomes did not differ significantly in response to drought, and drought promoted the restructuring and strengthening of belowground network links to more strongly interconnect network properties (Fig. 2, 3). Recent reporters have shown that the drought-induced accumulation of root exudates supports the post-drought recovery of microbes [50]. However, soil bacterial networks are unstable under drought without root exudates [1]. These results indicated that photosynthetic carbon inputs strongly shaped the rhizosphere microbial community structure due to the root exudates of *O. longistaminata*.

Methane cycling in rice paddies is an important microbial process that involves methane producers (methanogens) and methane metabolizers (methanotrophs) [51-54]. Flooded rice has well-developed gas-filled aerenchyma tissues in the roots and shoots that facilitate the transfer of gas (O_2 , CO_2) from the leaves to the roots, and CH_4 from the rhizosphere to the roots and leaves. CH_4 is produced from the anoxic zone of rice paddy soils by methanogenic archaea [55, 56]. Up to 80% of the methane produced in rice paddies was found to be transported into the atmosphere through rice aerenchyma [35, 57]. Drought affects the rates of methane emissions. *Oryza longistaminata* decreased the leaf photosynthesis through stomatal closure, which resulted in a reduction in the internal CO_2 concentration (C_i) (Fig. 1A) and the CH_4 emissions concentration under drought. We hypothesize that the change in the internal CO_2 and CH_4 concentrations can result in a progressive shift in the one-carbon microbial community, such as methanogens and methanotrophs. We found that the relative abundance of methylotrophy bacteria was decreased significantly under drought in the roots (Fig. 6, S6). The shift toward a decrease in CH_4 was hypothesized to be one possible explanation for the decrease in the relative abundance of methylotrophy bacteria in the root samples, particularly Methylocystaceae (Fig. 6, S6).

Stochastic processes control community dynamics under drought stress

Across microbial ecology, there is a limited understanding of the mechanisms that govern the relative influences of stochastic and deterministic processes in crop systems [29, 33]. This lack of understanding prevents the manipulation of plant microbiota to improve crop fitness. We estimated the relative contributions of each assembly process over the four compartments in response to drought. The weighted β NTI was used in combination with RC_{Bray} and null analysis to quantify the ecological processes that influence microbial community diversity. The results showed that the contribution of dispersal limitation was 5.45%, 7.27%, 33.3%, and 13.8% in the leaves, stems, roots, and rhizosphere, respectively. The contribution of undominated processes (weak selection, weak dispersal, diversification, and drift) was 94.5% in leaves, 92.7% in stems, 66.7% in roots, and 86.2% in the rhizosphere (Fig. 5).

In general, environmental perturbations have been classified into two categories: increased nutrient inputs (especially from complex carbon substrates) and disturbances. Nutrient input is believed to increase compositional stochasticity. In contrast, it is generally believed that extreme disturbances such as drought often decrease compositional stochasticity [33]. Our results indicated that stochastic (neutral) processes act on microbial community assembly in response to drought stress across all four

compartments (Fig. 5). Other studies show similar results, e.g., that fungi community assembly in drought-stressed sorghum is governed by stochastic processes [26]. It has been suggested that the complex carbon substrates released by roots provide a resource-rich environment that reduces competitive pressures, which increases compositional stochasticity [29, 58]. Consequently, we hypothesize that drought-tolerant crops (e.g., wild rice and sorghum) employ stochasticity strategies that are probably mediated by the root exudation to adapt to global change.

Manipulating microbiomes to improve crop fitness

Given the beneficial services provided to crops by their microbial symbionts, understanding how these plant-associated microbial communities respond to drought conditions could be an important step in the development of microbial strategies to help increase crop drought tolerance. Plant microbiota control many biogeochemical processes [7]. Microbial responses to changing environmental conditions appeared to be phylogenetically conserved [59]. For example, microbial responses to soil nitrogen addition were phylogenetically conserved at a genetic depth (τ_D) of 0.018 [60]. Microbial responses such as the ability to produce particular extracellular enzymes ($\tau_D < 0.010$) and the use of simple carbon compounds for growth ($\tau_D < 0.010$) are less phylogenetically conserved [61]. While complex metabolic pathways such as oxygenic photosynthesis ($\tau_D = 0.101$) and aerobic methane oxidation ($\tau_D = 0.046$) are more phylogenetically conserved [7]. Our data showed that the mean genetic depth (τ_D) ranged from 0.07282 to 0.2099 in the four compartments of *O. longistaminata*, suggesting a microbial response to drought stress exhibited strong phylogenetic conservation (Fig. 4). Therefore, a phylogenetic approach may be useful in predicting how microbial communities respond to environmental changes, and ultimately for the alteration the biodiversity-driven ecosystem functioning.

The study could improve our understanding of the maintenance of microbial diversity, and facilitate the prediction of microbial responses to global change in agricultural ecosystems and provide some strategies for improving crop production. Such strategies may include: 1) the discovery and inoculation of PGP microbes into agricultural fields, such as the phyla Actinobacteria and Fusobacteria, the genus *Streptomyces*, and phototrophic prokaryotes; 2) the manipulation of crop genetic pathways that regulate microbiota homeostasis, such as the genes that regulates roots exuding complex carbon compounds, could lead to a more beneficial and drought-resilient microbiota, which could, in turn, improve the performance of natural ecosystems and crops; and 3) the management of soil microbiota through agricultural practices that promote plant drought tolerance. We hope that our results will assist in integrating microbiota into the practices and tools used in modern agriculture.

Conclusions

The perennial wild rice *Oryza longistaminata*, which is characterized by various agronomically valuable traits, could be used in rice improvement programs. *O. longistaminata* provided a stress-tolerant crop system for evaluating endo-rhizosphere recruitment processes and elaborating new general concepts in crop-microbe interactions. The present study provides a comprehensive perspective on how drought-

adapted *O. longistaminata* reconstruct the microbial communities under drought stress. Although it is generally believed that extreme disturbances such as drought drive a strong deterministic process of selection, our data show that *O. longistaminata* facilitated a stochastic (random) recruitment process in all four compartments in response to drought stress. The microbiota community assembly of wild rice (*O. longistaminata*) in response to drought stress shows phylogenetic conservation, and aboveground-belowground patterns. These results lead to a better mechanisms understanding and future modeling of crop-microbe interactions, which could be fundamental in predicting crop adaptations to global climate change.

Methods

Sample collection and processing

The site of the rice experiment is in Nanchang city in China (28°40'04"N, 115°49'31"E). Seeds of *Oryza longistaminata* were grown in well-mixed soil batches in May 2017. The wild rice was watered with tap water. Drought was imposed on 3-month-old plants by ceasing irrigation and letting the soils progressively dry down. The drought treatment lasted for two months. The water content of the soil samples was measured gravimetrically by drying 5 g of fresh soil until the soil reached a constant weight [62].

At the end of October, samples were collected from four compartments: the rhizosphere, leaf, stem, and root. Loosely bound soil was shaken off from the plant roots, and tightly bound soil samples (rhizosphere soil) were collected by brushing off the soil that tightly adhered to the roots. All samples were packed into polyethylene bags and shipped on ice packs (4°C) to the laboratory. Ethanol-sodium hypochlorite was used for surface sterilization [63-65]. Fragments of the roots, stems, and leaves were washed with sterile water and separated. Then the rice samples were ultrasound-treated. All the samples were washed successively in 70% ethanol for 1 min and 0.3% sodium hypochlorite with 0.01% Tween 20 for 15 min to further clean the surfaces of living microorganisms, and the samples were subsequently washed three times in sterile water. Finally, the sterile filter paper was used to absorb any extra moisture. The water used for the final wash was spread on the LB and PDA plates to verify examine the surface sterilization effect. Each sample was stored at -80°C for DNA extraction.

Photosynthetic parameter analysis

To evaluate the effect of the drought treatments on *Oryza longistaminata* performance, we measured photosynthetic parameters. The gas exchange parameter was measured through a portable photosynthesis gas exchange system (IRGA, Model LI-6400XT, Li-Cor, Lincoln, Nebraska, USA) [66]. All measurements were performed in the morning between 8 AM and 11 AM, on a fully expanded leaf area and with due phytosanitary measures.

Analysis of the MDA level

Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) levels [56]. Leaf tissues (0.5 g) in 1.2 ml of 0.1 (w/v) trichloroacetic acid (TCA) were centrifuged at 12000 rpm for 20 min. An aliquot of the supernatant (0.3 ml) was mixed with 0.3 ml of 0.5% (w/v) thiobarbituric acid (TBA), incubated at 100°C for 20 min, quickly cooled, and centrifuged at 10,000×g for 10 min. The A532, A600, and A450 values of the supernatant were then recorded.

Determination of antioxidative enzyme activities

The activities of the antioxidant enzyme were determined by homogenizing 0.5 g of leaf tissue in 4 ml of extraction buffer containing 1% polyvinylpolypyrrolidone, 50 mM cold phosphate buffer (pH 7.8), 1 mM ascorbic acid, and 10% glycerol. The homogenate was centrifuged at 12,000 rpm for 15 min, and the supernatant was assayed. The SOD activity was measured as described by Kumar et al [67]. The POD and CAT activities were determined as described by Chen et al [56].

DNA extract

Plant tissues were fully ground into powder in a mortar with liquid nitrogen. Then DNA was extracted from rice samples and rhizosphere soil using PowerSoil DNA isolation kit (Mo Bio Laboratories) according to the manufacturer's instructions. DNA was quantified with a Qubit Fluorometer by using Qubit dsDNA BR assay kit (Invitrogen, USA), and the quality was checked by running an aliquot on a 1% agarose gel.

16S rRNA library construction

We performed 16S rRNA gene amplification for archaea and bacteria. Barcoded primers targeting the variable V4 regions of the 16S rRNA genes were used for amplification by the universal primer pairs, 515F (GGACTACNVGGGTWTCTAAT) and 806R (GGACTACHVGGGTWTCTAAT). Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR enrichment was performed in a 50 µL reaction containing 30 ng template, fusion PCR primer and PCR master mix. The PCR cycling conditions were as follows: 95°C for 3 minutes, 30 cycles of 95°C for 45 seconds, 56°C for 45 seconds, 72°C for 45 seconds and final extension for 10 minutes at 72°C for 10 minutes. The PCR products were purified using Agencourt AMPure XP beads and eluted in the elution buffer. The libraries were qualified by the Agilent Technologies 2100 bioanalyzer. The validated libraries were used for sequencing on the Illumina HiSeq 2500 platform (BGI, Shenzhen, China) following the standard Illumina pipelines, and 2 × 250 bp paired-end reads were generated.

Bioinformatics processing and statistical analysis

Amplicon sequences were analyzed using the QIIME 2 (version 2019.7, <https://qiime2.org>). We employed the DADA2 pipeline. All actual sequence variants (ASVs) identified as belonging to chloroplasts and mitochondria were removed from the data set. Sample metadata were predicted with random forest classification and regression models in QIIME 2 [68]. The potential microbial phenotypes were predicted with FAPROTAX and Bugbase [69, 70]. All data were checked for normality and log-transformed if

necessary. Statistical analyses of the 16S rRNA microbiome sequencing data were conducted in the Qiime2 environment (version 2019.7) and R version 3.5.1[71-73]. The co-occurrence networks were inferred based on the Spearman correlation matrix constructed with R using the igraph package. To meet assumptions of normality and homogeneity of variance, data were log10-transformed when required.

To evaluate the phylogenetic community composition, the phylogenetic diversity (PD) and the standardized effect size of MPD vs. null communities (mntd.obs.z, equivalent to -NTI) were calculated for each sampling plot, and the β nearest taxon index (β NTI) was calculated for paired joined plots. All MNTD analyses were calculated in the R 'picante' package. For both metrics, values between -2 and +2 values indicate the expectation under neutral community assembly while the individual values below -2 or above +2 are statistically significant [62]. To confirm whether the niche or neutral processes determined the microbial structure within a sample, pre-emption, broken stick, log-normal, and Zipf-Mandelbrot models [74-76] were selected to identify the rank species abundance distributions and were calculated in the vegan package in R [77, 78]. All models were compared based on the Akaike information criterion (AIC), which measures the relative quality of a statistical model [79].

The null-model-based β -diversity metric (β_{RC}) [80] was used to evaluate the differences in species richness by modifying the Raup-Crick measure [27]. β_{RC} can be estimated for each pair of communities based on taxonomic cooccurrence data. If the RC_{Brey} value is >0.95 (alpha = 0.05 by a two-tailed test), the given pair of communities shares significantly fewer species. If the RC_{Brey} value is less than 0.95, the given pair of communities shares significantly more species than expected by random chance.

The phylogenetic signals of binary traits that reflect the environmental preferences of various taxa were measured by the D-test of Fritz and Purvis. The phylogenetic dispersion (D) of the drought response (positive or negative) was determined using the 'phylo.D' function in the "caper" R package [81]. This phylogenetic dispersion (D) value, developed by Fritz and Purvis, compares the observed sister-clade differences in a trait against those of a random phylogenetic pattern [82]. Simulated values of D to set points of 0 (as phylogenetically conserved as expected under a Brownian threshold model) and 1 (random). Given a rooted phylogenetic tree and the presences/absences of a binary trait for each tip, the mean phylogenetic depth (τ_D) at which the trait is conserved across clades is calculated, in terms of the consenTRAIT metric [7].

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All sequence data and sample metadata are publicly available under the NCBI SRA database with the accession number as No. PRJNA631648.

Competing interests

The authors declare that they have no competing interests

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Author contributions

Xiaojue Peng and Zhichao Chen have contributed equally to this work. XD conceived and designed the study. XP, ZC, YJ and LM contributed to the data acquisition and carried out the data analysis. HX and YC drafted the manuscript. All authors were involved in revision of the final version. All authors read and approved the final manuscript.

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Not applicable

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Figures

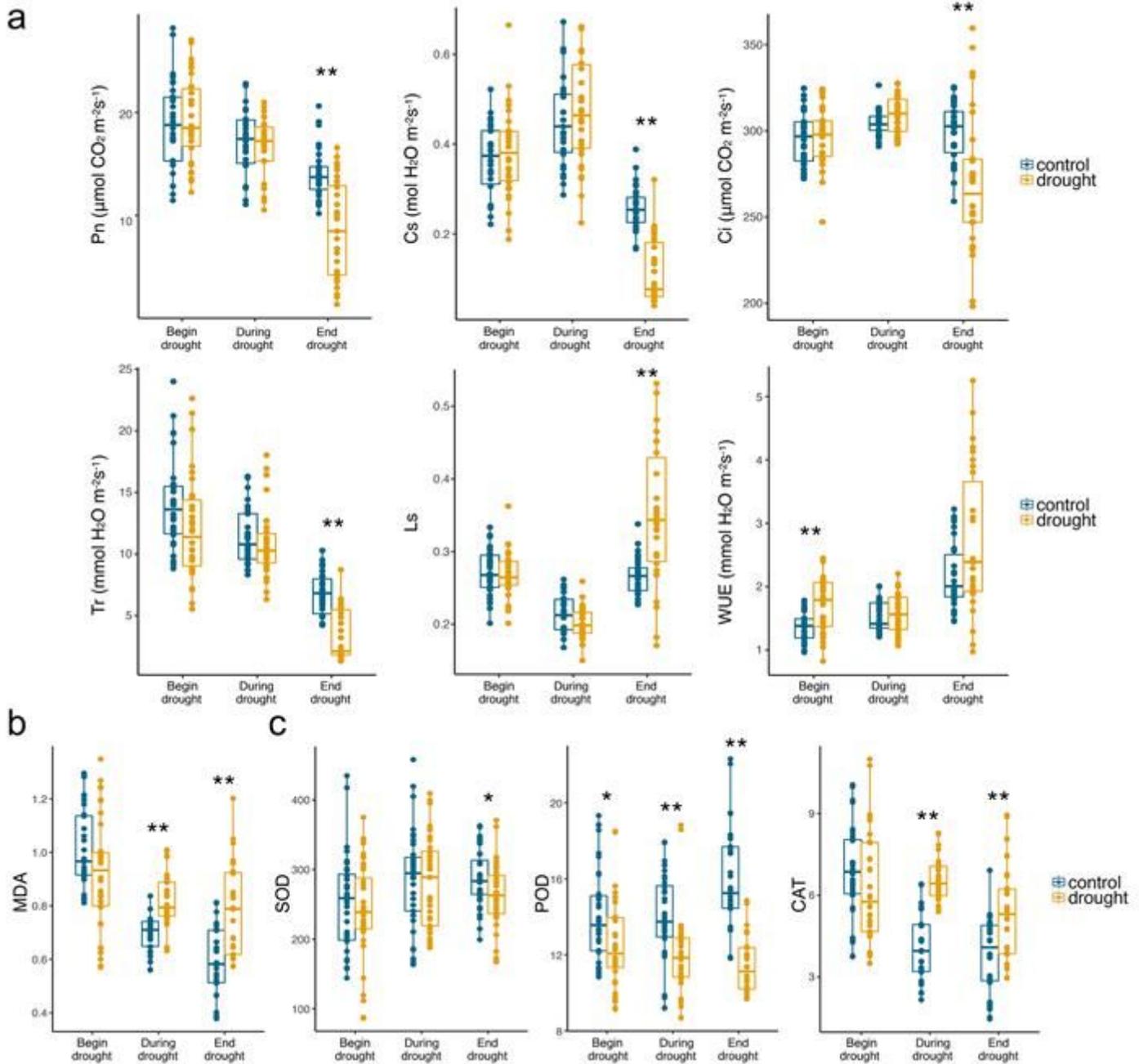


Figure 1

Drought-induced physiological response phenotypes of *Oryza longistaminata* over time. (A) Photosynthetic parameters. Pn: net photosynthetic rate, Cs: stomatal conductance, Ci: intercellular CO_2 concentration, Tr: transpiration rate, Ls: stomatal limitation value, WUE: water use efficiency. (B) MDA content. (C) Antioxidant enzyme activity. * $P < 0.05$, ** $P < 0.01$.

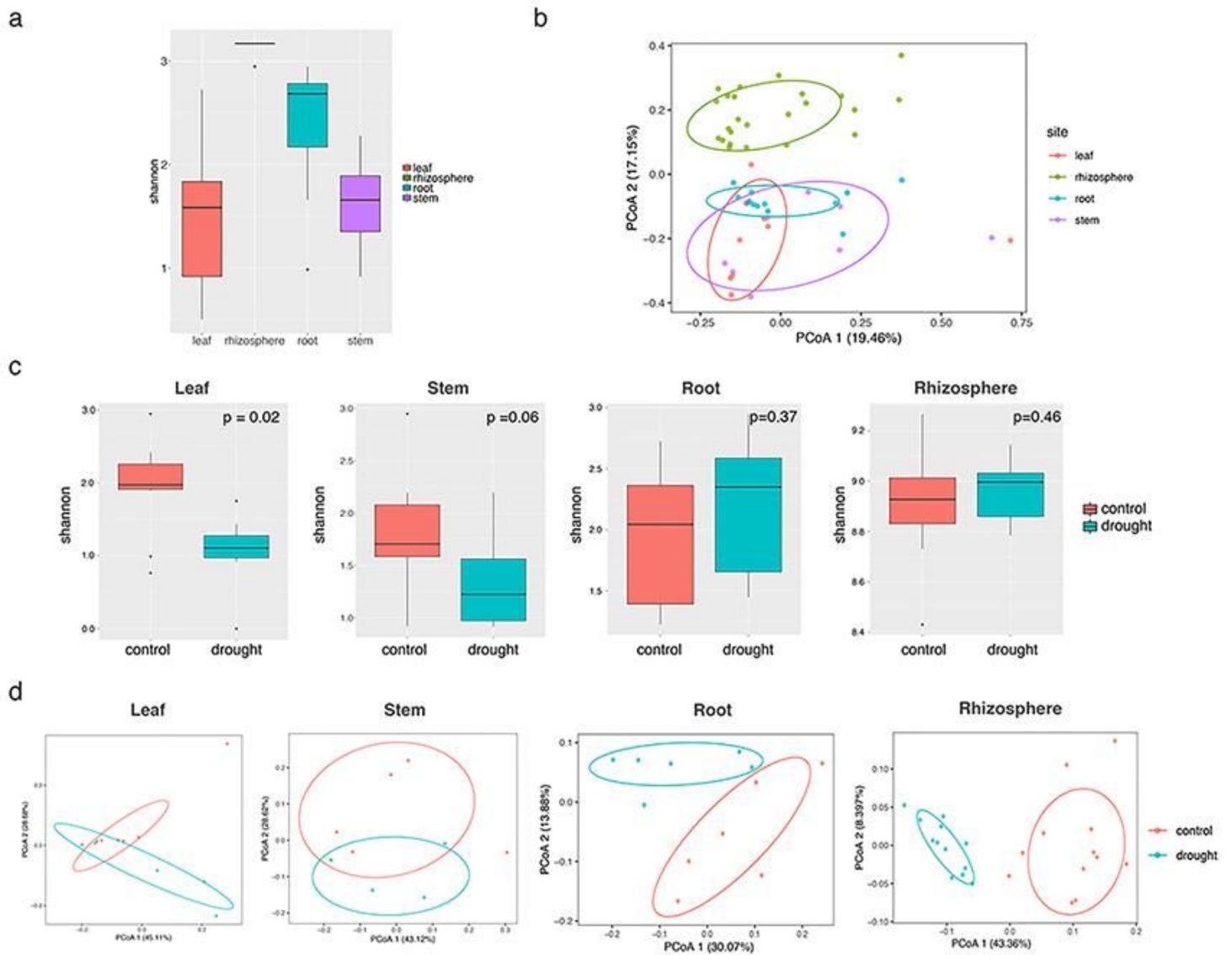


Figure 2

Taxonomic alpha and beta diversity estimates. (A) Taxonomic alpha diversity indices (Shannon) for the 60 samples from the 4 compartments. (B) Unconstrained principal coordinate analyses (PCoAs) of microbial community composition among the four different compartments of *Oryza longistaminata* at the ASV level based on the weighted UniFrac (WUF). (C) Alpha diversity indices (Shannon) of the drought and control treatments in the four compartments. (D) PCoAs of microbial community composition of drought and control treatments among the four different compartments based on the weighted UniFrac (WUF).

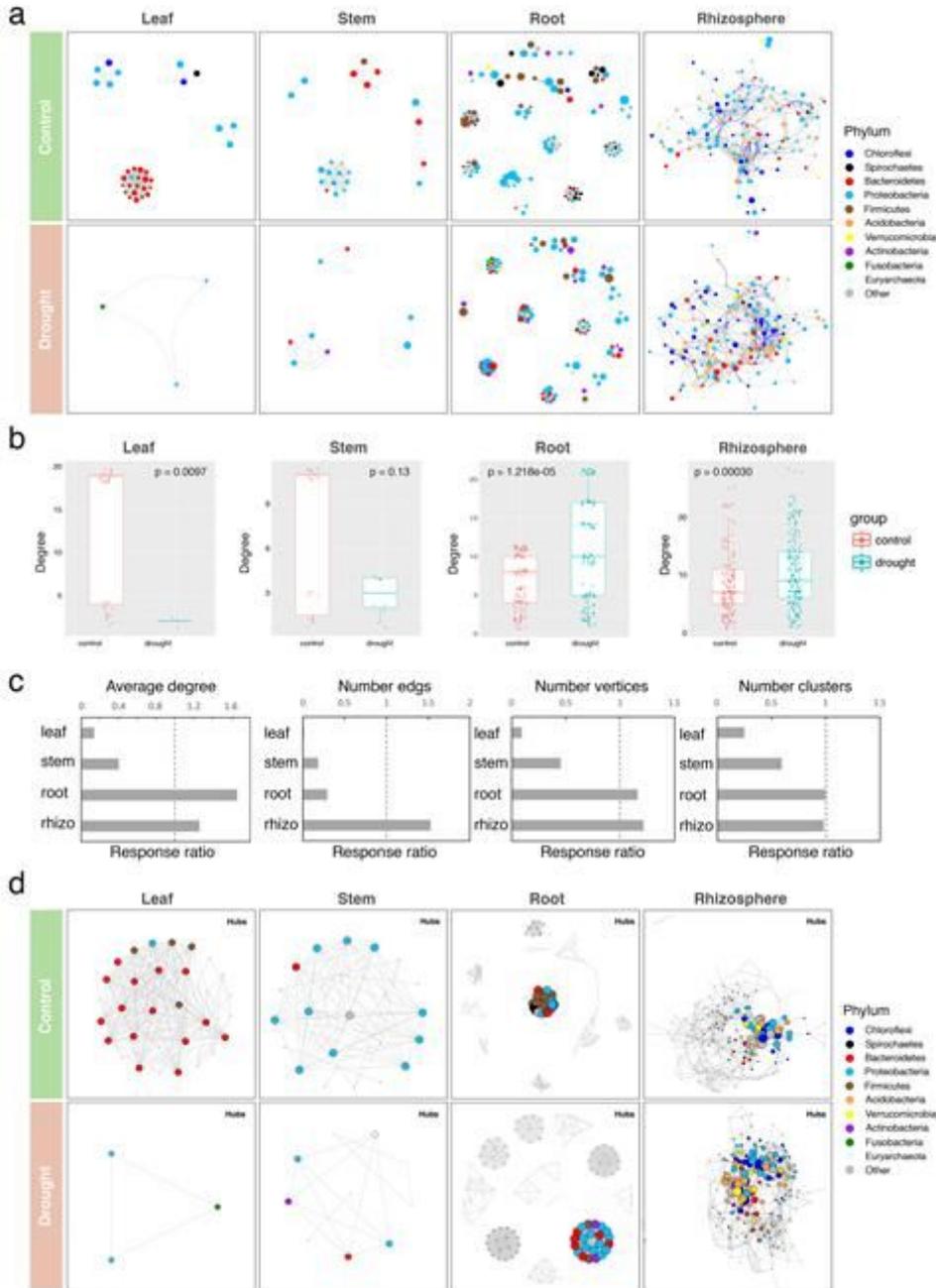


Figure 3

Drought affects the ASV co-occurrence network interactions of the leaves, stems, roots, and rhizosphere of *Oryza longistaminata*. Co-occurrence relationships with strong Spearman's correlation values (P -value < 0.5 and $\text{abs}(r) > 0.6$) are depicted for each compartment. The nodes represented a unique ASV in the data sets. (A) ASV co-occurrence network affected by drought. Networks are colored by phylum and sized based on the relative abundance of the ASV (\log_{10} -fold change in relative abundance). The negative correlations of edges are colored with blue. (B) Node degree of the co-occurrence network. (C) Response ratios (treatment/control) of the average degree, number of edges, vertices, and clusters within the co-occurrence network. (D) Hub (keystone) microbiome species within the co-occurrence network. Networks are colored by phylum and sized based on the hub score of the ASV.

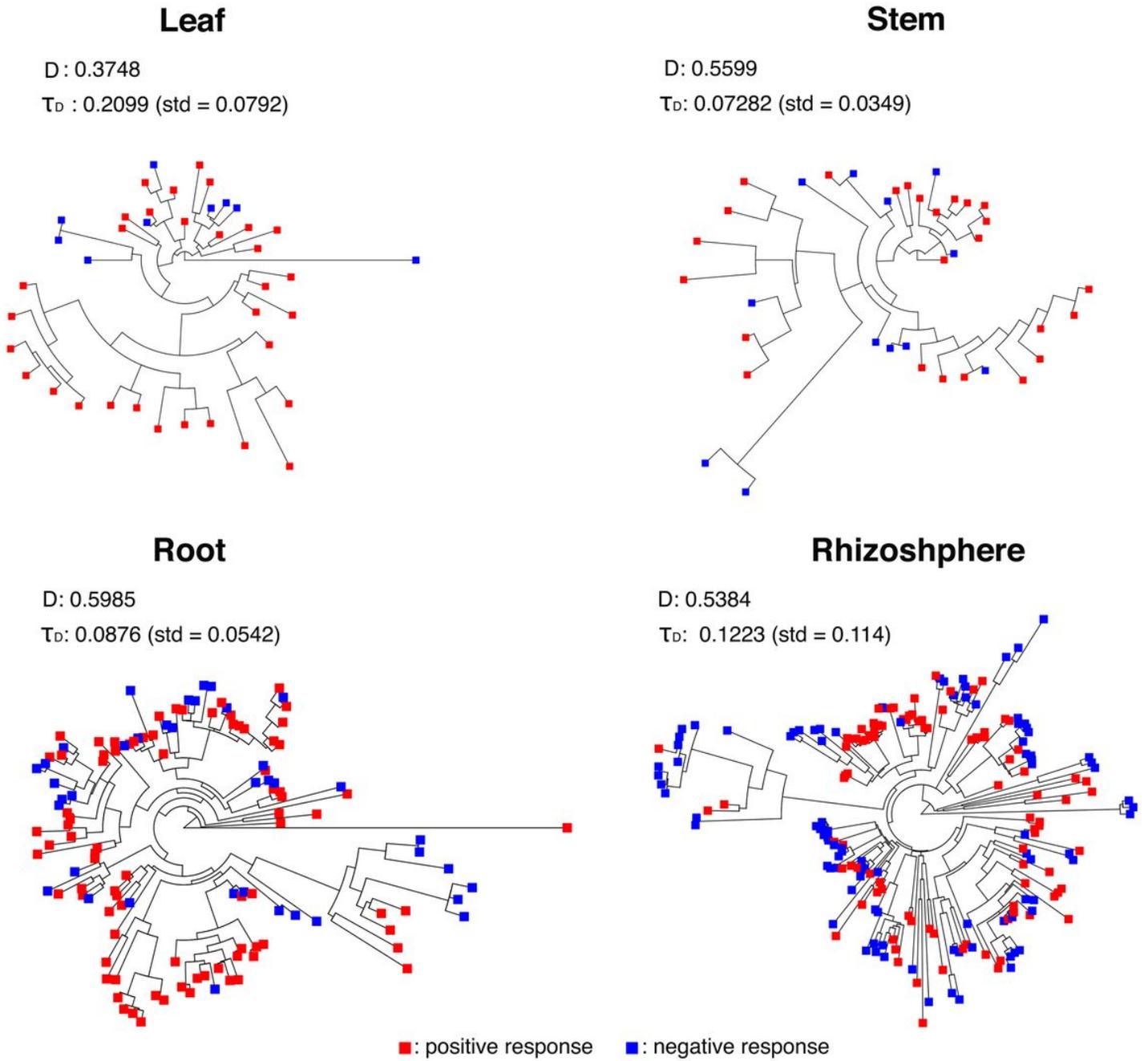


Figure 4

Phylogenetic distribution showing the level of trait conservatism under drought. D: Fritz and Purvis index. τ_D : genetic depth.

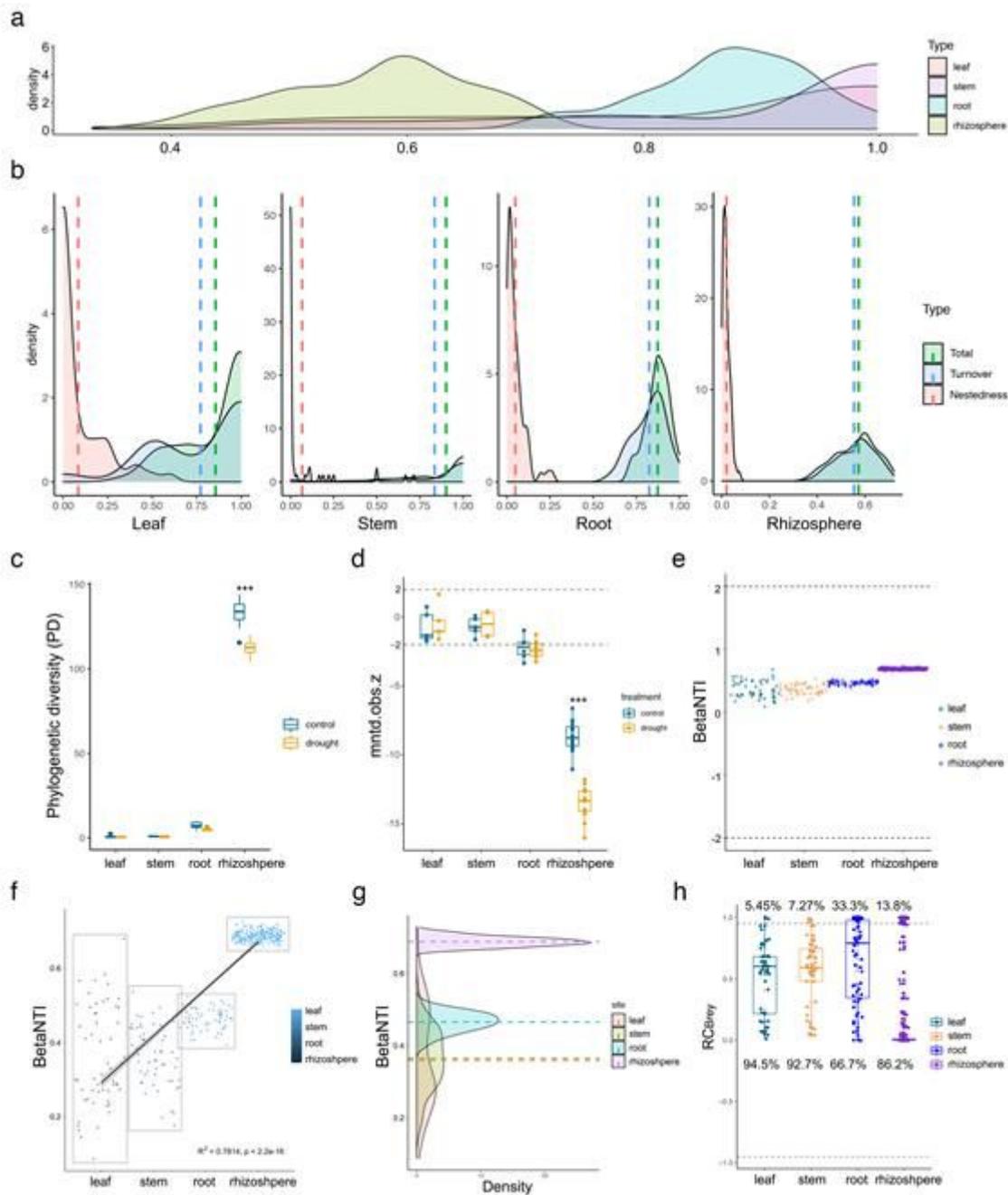


Figure 5

Ecological process analysis. (A) Distributions of Sorensen beta diversities among microbial communities, based on the taxonomic profiles at the ASV level. (B) Sorensen beta diversity (β SOR) was decomposed into the turnover component (β SIM) and the nestedness component (β SNE). (C) Phylogenetic alpha diversity (PD) between drought and control conditions. (D) Variation in the standardized effect sizes of the MNTD (SESMNTD) of microbial communities between drought and control conditions. Horizontal dashed lines indicate the upper (+2) and lower (-2) significance thresholds. (E) Distribution of β nearest taxon index (β NTI) according to the compartments that indicate community assembly processes under drought. Horizontal dashed lines indicate the upper (+2) and lower (-2) significance thresholds. (F) β NTI patterns and (G) distributions of β NTI for all pairwise community comparisons between control and

drought across the different compartments. The linear regression model is shown as a black line, and the statistics are provided in the panel. (H) Distribution of RCBrey (modified Raup-Crick index) based on a null model test of the Bray-Curtis taxonomic β -diversity index. Horizontal dashed lines indicate the upper (+0.95) and lower (-0.95) significance thresholds. P values are coded as *** 0.001, ** 0.01, * 0.05.

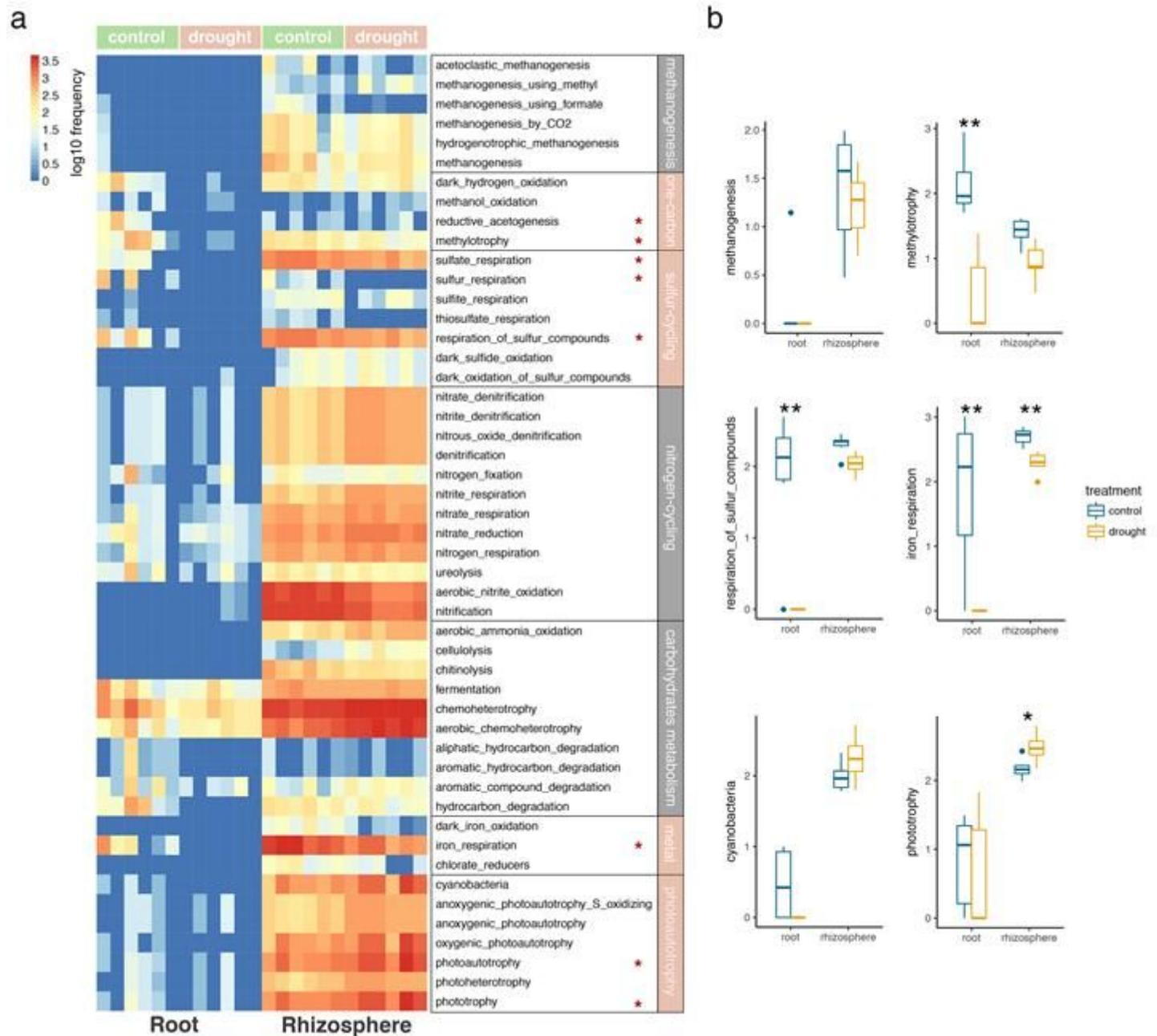


Figure 6

Drought affects the functional composition of the microbial community in the roots and rhizosphere of *O. longistaminata*. P values are coded as ** 0.01, * 0.05.

Supplementary Files

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