

Rhizosphere Soil Bacterial Community Structure and Diversity of Four Typical Plants in the Water Level Fluctuation Zone

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Abstract:

The aim of this study was to provide a reference for riparian wetland resource management and ecological restoration of mountain reservoir in Zhejiang province. The soil fungal community structure is critical toward maintaining the function of the wetland ecology. To investigate rhizosphere soil fungal community structure and diversity of four dominate plants (i.e., *Miscanthus floridulus*, *Pterocaryastenoptera*, *Salix babylonica*, *Taxodium distichum*) growing in wetlands of water-level-fluctuation zone of Changtan reservoir, we sequenced and analyzed their structure and composition using Illumina sequencing of the internal transcribed spacer 2 (ITS2) region. The results indicated that 7 phylums, 26 classes, 68 orders, 137 families, 248 genera and 331 species of fungal were obtained, with Ascomycota Basidiomycota and Zygomycota being the dominant phylum, and Dothideomycetes, Agaricomycetes and Sordariomycetes being the dominant class in the rhizosphere soils of different plants growing in wetlands of water-level-fluctuation zone of mountain reservoir in Zhejiang province. The soil fungal community structure of *Salix babylonica* was more similar to *Miscanthus floridulus* at phylum level, while that of *Taxodium distichum* was different from other three plants significantly. The soil bacteria community structure of *Salix babylonica* was more similar to *Miscanthus floridulus* at phylum level, while that of *Taxodium distichum* was different from other three plants significantly. The Shannon index and Chao1 index of the rhizosphere soil fungal of *Pterocaryastenoptera* were 6.67 and 770.44, respectively, which were the highest among the four plants, and that of *Taxodium distichum* were the lowest, with 2.59 and 403.96, respectively. The fungal structures in rhizosphere soil of the four plants were similar, but there was a significant difference in the relative abundance of some fungal at the phylum and class levels.

Keywords: Water level fluctuation zone, Rhizosphere soil, High-throughput sequencing, Fungal community structure

I. INTRODUCTION

The plants in the water level fluctuation zone has the functions of reducing soil erosion, greening and beautifying the reservoir bank landscape, and reducing the pollution of reservoir water bodies. Since the zone is in the alternating water and dry areas of the reservoir ecosystem, it is often under the double stress of flooding and drought adversity, so the ecological environment is very harsh and there are very few suitable plants in the zone^[1]. In the mountainous areas of central and southern Zhejiang Province, the climate is warm,

with abundant rainfall and sunshine, and there are relatively more suitable plants in the fallout zone compared with those in the north and other places. The screening and introduction of suitable plants for each reservoir fallout zone should be the key to the plant construction and ecological restoration of the fallout zone.

Zhejiang Changtan Reservoir is a large reservoir mainly for water supply, flood control and irrigation. It is located at latitude 28°3'~28°40'N, diameter 121°00'~121°04'E, 32 km west of Huangyan District, Taizhou City, Zhejiang Province. Changtan Reservoir mainly provides water for more than 3 million urban and rural residents in "three districts" (Jiaojiang District, Huangyan District, Luqiao District) and Yuhuan and Wenling counties and cities of Taizhou City^[2]. The sedimentation zone of Changtan Reservoir is 86 km long, the depth of water level is from 36 m to 29 m, and the area of sedimentation zone is 0.61 km². three kinds of native plants are widely distributed in the sedimentation zone, such as *Miscanthus floridulus*, which is a perennial herb of the genus *Miscanthus*. *salixbabylonica* Linn is a perennial tree of the genus Willow of the family *Populus*. *Taxodium distichum* is a genus of deciduous fir in the family *Firaceae*, usually with knee-bending respiratory roots at ground level, durable low temperature, salinity, flooding, drought and infertile, pollution and pest resistance. At present, it has been listed as one of the tree species for the construction of protective forest system in many reservoirs in Zhejiang Province. The Changtan Reservoir in Zhejiang Province has planted 120 ha of *Fagus sylvatica* in the zone. It plays a good ecological effect in the whole reservoir ecosystem.

Wetland soil fungal diversity is an important component of biodiversity in wetland ecosystems. Wetland soil fungi are abundantly distributed in the wetland soils of reservoir fallout, participate in the decomposition of soil organic matter and humus formation process, participate in the decomposition of cellulose, hemicellulose, lignin and tannin compounds in plant apoplast, participate in the degradation of some toxic substances in wetland soils, especially some heavy metal pollution, participate in wetland soil nutrient and carbon cycling processes, and play an important role in the ecological restoration of damaged wetlands throughout the reservoir fallout It plays an important role in the ecological restoration and sustainable development of damaged wetlands in the whole reservoir fallout zone^[3]. Vegetation restoration refers to the application of ecological principles to reestablish natural ecosystems by protecting existing vegetation or creating artificial forests, irrigation, and grass vegetation to restore the biodiversity and its ecological service functions in its ecosystem^[4]. Different plant species selection in vegetation restoration produces different inter-rooted soil fungal communities. Therefore, it is of theoretical and practical significance to study the composition and diversity characteristics of the inter-rhizosphere soil fungal communities of reservoir-fading wetland establishment species for the selection of the ecological restoration mode and the evaluation of the ecological effects of fading zone vegetation. At present, most studies on the ecosystem of the degraded wetlands have focused on the changes of soil enzyme activity^[5], characteristics of greenhouse gas emissions^[6], and characteristics of heavy metal pollution^[7]. In contrast, not many studies have been reported on the diversity and community composition of soil fungi under different vegetation in degraded wetlands. In this study, we propose to use high-throughput sequencing technology to analyze the changes in community structure and diversity indices of soil fungi in the inter-rhizosphere of *Miscanthus floridulus*, *Salix babylonica*, *Pterocaryastenoptera* and artificially planted *Taxodium distichum* vegetation in the wetland of Changtan Reservoir in Zhejiang Province, in order to provide a basis for exploring reasonable vegetation restoration techniques in the fallout zone wetlands of mountain reservoirs in Zhejiang Province.

II. MATERIALS AND METHODS

2.1 Sample Collection

Four species of established species of plants, *Miscanthus floridulus*, *Pterocaryastenoptera*, *Salix babylonica* and *Taxodium distichum*, were collected from the inter-root soil of the widely distributed wetlands of the HuangyanChangtan Reservoir in Zhejiang Province. These plants are *Miscanthus floridulus*, *Pterocaryastenoptera*, *Salix babylonica*, and *Taxodium distichum*, among which *Miscanthus floridulus* is a perennial herb with well-developed rhizomes in the family Gramineae and genus *Mangifera*, denoted by Mf in this paper. *Pterocaryastenoptera* is a genus of *Pterocaryastenoptera*, a large tree, and is denoted by Ps. *Salix babylonica* is a deciduous tree of the genus Willow, family *Populus*, and is denoted by Sb. *Taxodium distichum* is a large deciduous tree, a genus of Fir, and is denoted by Sb. *Taxodium distichum* is a deciduous tree of the genus Fir, native to North America and Mexico^[8], and is cultivated in Zhejiang Province, where it is native to low-lying moist areas such as swamps, and has a strong stain tolerance, denoted by Td. Among these four species, except *Taxodium distichum*, which was planted in 2010 on a large scale in the fallout wetlands, the other three species were distributed in their natural wild state on the reservoir fallout wetlands. They are moisture- and drought-tolerant, with high pollution reduction power and good adaptability, and are established species of plants on the floodplain wetlands. Three standard plots of 10 m × 10 m were set up for each plant species, 10 representative plants were selected from each standard plot, and four sampling points were set up around the plants in the southeast and northwest. The samples were mixed well and sieved with a 2 mm diameter mesh sieve. The 10 g soil samples were placed in sterile polyethylene sealing bags and stored in ice boxes at -70°C for high-throughput sequencing.

2.2 Soil Fungal Genomic DNA Extraction

The total DNA of soil samples was extracted by E.Z.N.A. Soil DNA Kit method, and the quality of DNA extraction was detected by 1% agarose gel electrophoresis, while the DNA was quantified by UV spectrophotometer. The five DNA samples of each sample were randomly mixed in three equal portions to make three parallel samples, which were stored at -20°C and set aside.

2.3 PCR Amplification and High-Throughput Sequencing

The primers used were ITS1FI2 (5'-GTGARTCATCGAATCTTTG-3') and ITS2 (5'-TCCTCCGCTTATTGATATGC-3') for PCR amplification of the variable region of fungal ITS2. PCR amplification products were detected by 2% agarose gel electrophoresis and the target fragments were recovered using the AxyPrep PCR Cleanup Kit recovery kit. The purified PCR products were quantified on the library using Quant-iTPicoGreen dsDNA Assay Kit on a Qbit fluorescence quantification system, and the qualified library concentration should be above 2 nM. The qualified libraries for up-sequencing (Index sequences are not repeatable) were diluted in gradient, mixed in the corresponding ratio according to the required sequencing volume, and denatured to single strand by NaOH for up-sequencing, and double-end sequencing of 2×250bp was performed using NovaSeq sequencer. Technical support was provided by Hangzhou Lianchuan Biotechnology Co.

2.4 Bioinformatics Analysis

Based on the overlapping regions of the double-end sequences, the R1 and R2 sequences were spliced into a long tag sequence using Pear, and the barcode and primer sequences were removed using cutadapter. Then Fqtrim was used to filter the low quality sequences and Vsearch (v2.3.4) was used to filter the chimeras. Alpha diversity and Beta diversity were normalized by sampling the number of sequences from all samples to the number of sequences from the fewest sequence samples, and species annotations were normalized by relative abundance. The images were drawn by the R(v3.5.2) package. Species annotation was performed by QIIME2 plug-in feature-classifier for sequence comparison, and the comparison databases were SILVA and unite databases, and the annotation results of SILVA database were used as the basis.

III. RESULTS AND ANALYSE

3.1 Analysis of Alpha Diversity of rhizosphere soil fungal communities of Four Typical Plants in the Water Level Fluctuation Zone

After Miseq sequencing, the total number of effective sequences obtained for all samples was 254259, and *Miscanthus floridulus* was the most abundant fungus, followed by *Taxodium distichum*, and *Pterocaryastenoptera* was the least abundant fungus. *stenoptera* was the least effective sequence. The proportion of effective sequences was higher than 97% in all samples. Sequence lengths between 200-300 bp and 300-400 bp accounted for 52.41% and 47.47%, respectively, which were greater than 99.88% in total and met the analysis requirements.

After quality control and removal of chimerism, the valid sequences were clustered into OTUs with 97% consistency, and the number of OTUs for the four species of inter-rhizosphere soil fungi was 495-839, with a total of 1532 OTUs. The number of OTUs of *Miscanthus floridulus* was the highest, with a value of 839, followed by *Pterocaryastenoptera*, with a value of 797, and the lowest number of OTUs of *Taxodium distichum* in plantation forest, with only 495. The number of OTUs shared by the four plants was 298, accounting for 19.45% of the total. Although these four plants belonged to different families, they shared a reservoir ecosystem with similar soil parent material and the same climatic conditions, so the number of OTUs shared by the four plants was high, while the ratio of OTUs of various plants to the number of OTUs was low. For example, the number of OTUs specific to inter-root soil fungi of poplar willow was 119, accounting for 7.77% of the total, the number of OTUs specific to *Miscanthus floridulus* was 212, accounting for 13.84% of the total, the number of OTUs specific to *Pterocaryastenoptera* was 135, accounting for 8.81% of the total, and the plantation The number of OTUs endemic to *Taxodium distichum* was 53, accounting for only 3.46% of the total.

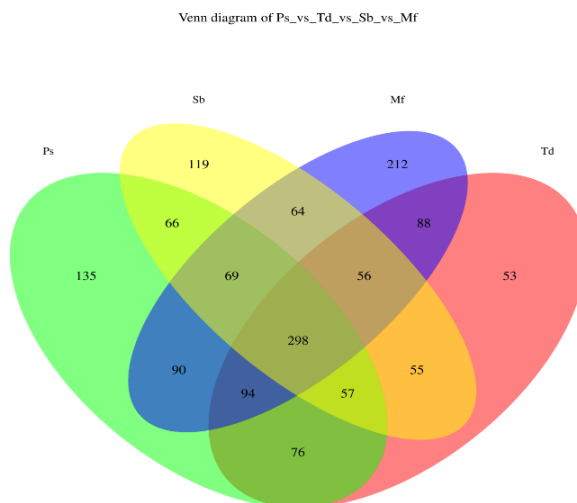


Fig 1: OTUs venn map of fungal community in rhizosphere soil under four plants at phylum level

The dilution curves can reflect the sampling depth of the samples and can evaluate whether the sequencing volume can cover all taxa. As shown in Figure 1, the dilution curves of the inter-root soil samples of the four plant species tended to be flat. The coverage of the library has exceeded 98% for all of them. It indicates that the sampling is basically reasonable and the OTUs coverage of the samples has been saturated. The depth of this sequencing can reflect the actual situation of the inter-rhizosphere soil fungal communities of the four established species of plants in the fading zone wetlands. QILM software was used to calculate the Chao1 index and Shannon index of the samples to characterize their richness and diversity.

TABLE I. Genomic DNA sequencing data and Alpha diversity observations of 4 soil samples

Plant species	Observation	OTUs	Shannon index	Valid-tags	Chao1 index	Good coverage
Pterocaryas tenoptera	617.67	787	6.67	45207	770.44	0.93
Miscanthus floridulus	512.33	839	5.51	84693	758.14	0.95
Taxodium distichum	256.11	495	2.59	68991	403.96	0.97
Salix babylonica	393.67	784	4.39	55364	547.27	0.92

There were significant differences in Chao1 and Shannon indices of soil fungal communities among different plants, as shown in Table I. Both Shannon index and Chao 1 were highest for Pterocaryastenoptera samples, with values of 6.67 and 770.44, respectively, followed by Miscanthus floridulus, with Shannon and

Chao1 were 5.51 and 758.14, respectively, while *Taxodium distichum* was the lowest in the plantation with Shannon and Chao1 of 2.59 and 403.96, respectively.

3.2 Structural characteristics of rhizosphere soil fungal communities of Four Typical Plants in the Water Level Fluctuation Zone

As shown in Figure 2, Ascomycota (50.09-82.59%), Basidiomycota (8.65-45.75%) and Zygomycota (0.70-9.88%) were the dominant fungal groups in the inter-rhizosphere soil of the sedimentation zone wetlands of Changtan Reservoir. The relative abundance of soil unclassified fungi (Fungi-unclassified) ranged from 0.42% to 3.43%. The fungal phylum Glomeromycota, Chytridiomycota and Mortierellomycota were distributed in low abundance in the inter-rhizosphere soils of the established species in the study area.

The different abundance of the fungal groups in the different samples indicated that there were differences in the community structure of the fungal groups among the samples. The highest relative abundance of the inter-rhizosphere soil fungal ascomycota was found in willow with a value of 82.59%, followed by *Pterocaryastenoptera* with a value of 80.71%, while the lowest relative abundance value of 50.09% was found in the inter-rhizosphere soil fungal ascomycota of *Taxodium distichum* in plantations. The highest relative abundance of the phylum *Taxodium distichum* was found in the plantation with a value of 45.75%, followed by *Miscanthus floridulus* with a value of 19.56%, while the lowest relative abundance of the phylum *Pterocaryastenoptera* was found in the plantation with a value of 8.65%. The highest relative abundance of the inter-rhizosphere soil fungal jointing phylum of each plant was found in maple poplar with a value of 9.88%, while the lowest was in willow, while the relative abundances of *Taxodium distichum* and *Miscanthus floridulus* were only 0.62 and 0.70%, respectively.

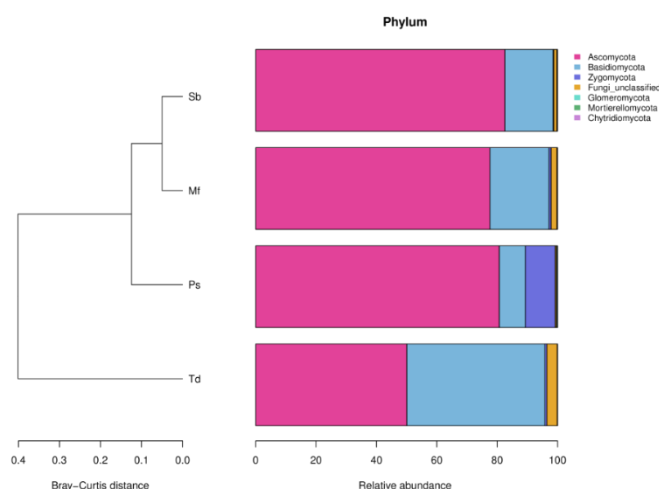


Fig 2: The fungal community in rhizosphere soil under four plants at the phylum level

The microbial structure heat map reflects the species composition and differences among the different samples. As can be seen in Figure 3, the community structure composition of the inter-root soil fungi of four plant species in the reservoir abatement zone wetlands at the gate level showed similarities between

Miscanthus floridulus and willow, while artificial Taxodium distichum differed from the other three plants.

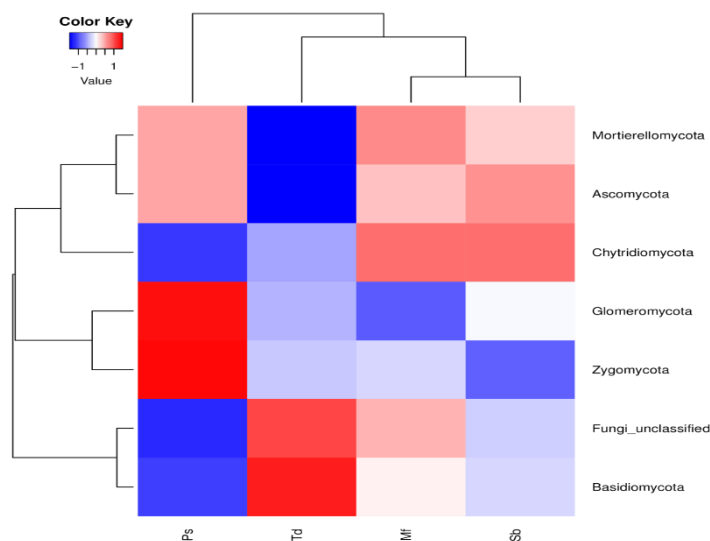


Fig 3: Heat map of plant rhizosphere soil fungal community composition at the phylum level

At the phylum level (Fig 4), among the major fungal phyla of the inter-rhizosphere soil of *Miscanthus floridulus*, Dothideomycetes had the highest relative abundance of the population, with a value of 43.1%, followed by Agaricomycetes, with a relative abundance value of 18.29%, and The others were Sordariomycetes (14.66%), Lettiomycetes (7.13%), and Eurotiomycetes (3.63%) in descending order of relative abundance. Sordariomycetes had the highest relative abundance with a value of 40.62%, followed by Dothideomycetes with a relative abundance value of 16.76%, and the others in descending order according to their relative abundance values were Mucoromycotina-Incertae-sedis (9.77%), Pezizomycotina-Incertae-sedis (7.44%), and Agaricomycetes (5.59%). The relative abundance of Sordariomycetes was also the highest, with 46.06%, followed by Agaricomycete, with 14.69%, among the major fungal phyla in the inter-rhizosphere soil of poplar in the degraded wetlands. The others were Dothideomycete, with a relative abundance value of 6.32%, and aleotiomyota, with a relative abundance value of only 1.46%. The relative abundance of the main fungal phyla in the inter-rhizosphere soil of *Taxodium distichum* was the highest in Agaricomycetes (45.23%), while the relative abundance of other phyla was lower.

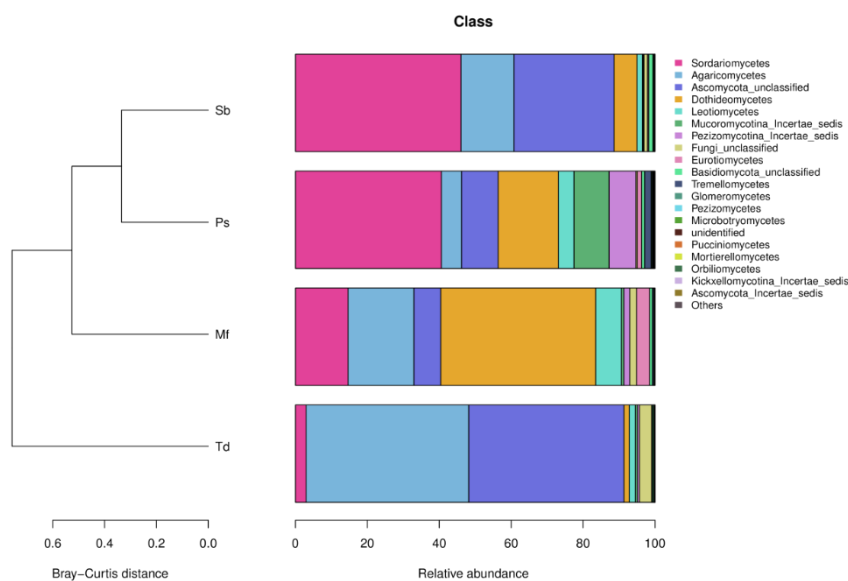


Fig4: The fungal community in rhizosphere soil under four plants at the class level

IV.DISCUSSION

Cysticercus phylum fungi have the ability to degrade a wide range of plant cellulose and hemicellulose^[9]. It can adapt to survive and be highly active in wetland environments. Studies by many scholars have concluded that Cysticercus phylum fungi are the dominant phylum on wetland soils. For example, Chen^[10] found Ascomycota to be the dominant phylum in the soil fungal community of the Napa Sea Plateau wetlands. Zhao^[11] found that Ascomycota, Eygomycota, Basidiomycota and Glomeromycota were the dominant soil fungal groups in alpine meadows of the Tibetan Plateau. Sui^[12] found that the relative abundance of Cysticercales phylum in soil fungi of Little Leaf Chapter wetland in the Sanjiang Plain was 56.56%, and the results of this study showed that at the phylum level, the Cysticercales phylum was the most dominant phylum in the inter-rhizosphere soil fungal community of all four established species of plants in the Changtan Reservoir fallout zone, with relative abundance varying between 50.09-82.59% for various plants. The results of the present study are consistent with those of the aforementioned scholars on wetlands.

The activity of the soil fungus Potylobacter phylum was closely related to the water content in the soil. In this study, we detected the presence of a small amount of Potylobacter phylum in the inter-rhizosphere soil of all the four sedimentation zone building species plants. The relative abundance of the four species varied from 0.00235 to 0.059749%, with the highest relative abundance of Miscanthus floridulus samples at 0.059749%, followed by poplar willow at 0.051987%, and Pterocaryastenopectera had the lowest relative abundance value of 0.00235%, indicating that under the same environmental conditions, poplar willow and Miscanthus floridulus rhizosphere soils could absorb relatively more water, while Taxodium distichum and poplar willow rhizosphere soils absorbed relatively less water.

The diversity of soil fungi in the inter-rhizosphere of the established species in the degraded wetlands was significantly different, with the highest diversity index of soil fungi in the inter-rhizosphere of the native plant

Pterocaryastenoptera, followed by *Miscanthus floridulus* and willow, and the lowest diversity index of soil fungi in the inter-rhizosphere of the artificially grown *Taxodium distichum*. The lowest diversity index was found for *Taxodium distichum*. This is because the distribution density, root fresh weight and surface area of the root systems of different plants are different^[13, 14]. And the root secretion is also different for different plants^[15]. Plant roots in soil will form a symbiosis with soil fungi, and the morphological distribution of the root system will directly affect the diversity of fungi.

The four fading zone building species of plants belong to different families, and the structural and physiological characteristics of the root system vary greatly, as do the composition of the secretions released by the root system into the soil, and thus the species of fungi gathered from the inter-root soil. Therefore, the proportion of photosynthetic products secreted in the roots and the metabolites produced are different in the wetlands of the fading zone due to the different plant species, and the soil environment between the roots of the plants also changes, thus causing differences in the composition and diversity index of the soil fungal community between the roots of different plants.

The soil microorganism *Gibberella* spp. is a phytopathogenic fungus that has a damaging effect on plants. Infection of plants with this disease will cause the plant to grow slowly or even wither, while causing other plants to grow faster and replace the dominant species in the ecosystem, resulting in changes in the plant community and affecting the ecological balance of the original ecosystem. The results of this study showed that the relative abundance of Pterocaryastenoptera was the highest, with a value of 1.96%, followed by *Miscanthus floridulus*, with a value of 0.73%, and the relative abundance of *Pterocarya distichum* in the inter-rhizosphere soil fungi of four planted species in the wetlands of Changtan Reservoir. *distichum* inter-rhizosphere soil had the lowest relative abundance values of *Erysipelothrix* spp. Therefore, the planting of *Taxodium distichum* has a good ecological effect in terms of maintaining the ecological balance of the wetland ecosystem in the fading zone and resisting plant diseases.

The high-throughput sequencing results showed that the inter-rhizosphere soil of four established species of plants in the wetland of Changtan Reservoir in Zhejiang Province obtained a total of 7 phyla, 26 classes, 68 orders, 137 families, 248 genera and 331 species of fungi. There were significant differences in ChaoI and Shannon index of fungal communities in inter-root soil of different plants. Both indices were highest for Pterocaryastenoptera samples, followed by *Miscanthus floridulus*, and lowest for artificial *Taxodium distichum* samples. At the phylum level, Cysticercia, Stenotrophomonas and Joints were the common dominant fungal groups in the inter-rhizosphere soils of the plants of each of the established species in the Changtan Reservoir Floodplain Wetland. The community structure composition of the inter-rhizosphere soil fungi of the four plant species in the reservoir abatement zone wetland showed that *Miscanthus floridulus* was similar to that of poplar willow, while the artificial *Taxodium distichum* differed more from the other three plants. At the phylum level, the ascomycetes, corymbis, and dungal crustaceans were the common dominant fungal groups in the inter-rhizosphere soil of wetland plants. The relative abundance of *Erysiphae* spp. was highest in maple samples, followed by *Miscanthus floridulus*, willow, and lowest in the inter-rhizosphere soil of artificially grown *Taxodium distichum*, indicating that *Taxodium distichum* has a certain disease avoidance effect in the fading zone wetlands.

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