

# Microbial Community Structure in the Rhizosphere of *Zostera*

Xiaomin Zhang<sup>1</sup>, Shuai Shang<sup>2</sup>, Jiqing Gao<sup>1</sup>, Jingxi Liu<sup>1</sup>, Lizhu Chen<sup>1</sup>, Chunxiao Sun<sup>1</sup>, Liang Zheng<sup>3</sup>, Bin Li<sup>1,\*</sup>, Huawei Qin<sup>1,\*</sup>

<sup>1</sup>Shandong Provincial Key Laboratory of Marine Ecological Restoration, Shandong Marine Resource and Environment Research Institute, Yantai, China

<sup>2</sup>College of Biological and Environmental Engineering, Binzhou University, Binzhou, China

<sup>3</sup>Marine and Fishery Integrated Service Center of Haiyang City, Yantai, China

## Email address:

albert0722@163.com (Bin Li), 15965178556@126.com (Huawei Qin)

\*Corresponding author

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**Abstract:** Seagrass which evolved from terrestrial plants into marine species plays a vital important role in plant evolution. Although seagrass plays a vital important role in the coastal system, losses in the seagrass population have been observed throughout the world. Many researches have explored the relationship between seagrasses and their rhizosphere microbial community. As more and more plant microbial data become available, a variety of research investigations and applications have been substantially impacted, especially marine ecological protection. Using a meta-analysis, we explored the composition characteristics of rhizosphere microorganisms in *Zostera* on a larger scale and whether there are core microbes in seagrasses. Results showed the phyla Proteobacteria and Bacteroidetes and the genus *Sulfurovum* might be the core microbiome in the *Zostera*. The rhizosphere core microorganisms may be essential for four seagrass species to assist their normal physiological activities. Except the core microbiome, the comparative analysis showed certain differences in the dominant composition of rhizosphere microorganisms in four seagrass species. The rhizosphere microorganisms of the seagrass might be also affected by the plant's species specificity and living environment. In conclusion, the common rhizosphere microorganisms may be essential for four seagrass species to assist their normal physiological activities. Meanwhile, the basis of biological ecosystem and its influencing factors are very complex, and further research is needed.

**Keywords:** Seagrass, *Zostera*, Rhizosphere, Microbial Communities

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## 1. Introduction

Seagrass beds are one of the most productive ecosystems on earth and an important object to be protected. Compared with 250000 species of terrestrial angiosperms, only 72 species of seagrass have fully adapted to the sea lifestyle [11, 18]. Seagrass which evolved from terrestrial plants into marine species plays a vital important role in plant evolution. Seagrass, mangrove and coral reefs constitute three typical marine ecosystems that buffer waves and reduce sediment resuspension [7]. Meanwhile, seagrasses are an important component of coastal ecosystems and provide valuable services to coastal ecosystems such as the storage of blue

carbon, the supply of food, shelter and nursery habitat, and other forms of ecosystem engineering [9]. Seagrasses have important ecological functions and research values. They can filter nutrients and pollutants from estuaries and coastal waters to purify water, stabilize sediments to protect biodiversity, and participate in material recycling and carbon sequestration [5]. Meanwhile, seagrasses also serve as a source of food for many marine species, and protect coastlines from erosion [5]. Although they provide numerous ecosystem services, massive losses in seagrasses caused by natural disturbance and human activities have been observed throughout the world. Marine pollution has led to significant changes in the abundance, composition and structure of species in seagrass bed habitats,

which leads to the loss, degradation and fragmentation of seagrass, and even regional species extinction.

Rhizosphere is the narrow zone of soil around the roots of the plant, which is directly affected by root secretions [13]. Microbial groups which are able to competitively colonize the rhizosphere soil are rhizosphere microorganisms. Rhizosphere microorganisms play an important role in the fitness, growth and survival of plants [23]. In recent years, the rhizosphere bacterial community structures have been studied in a variety of marine and freshwater ecosystems for a range of plant species. A previous study on the composition, activity and association of terrestrial rhizosphere bacterial communities indicates that different exudates of the plant roots have influenced the rhizosphere [4]. Rhizosphere microorganisms can interact with plants in a variety of ways. Microbes can be pathogens or they are beneficial for plant growth, nutrient absorption and resistance to pathogens [2, 13]. In turn, the plants secrete carbon sources to the rhizosphere environment and provide nutrients for the microbial community. Besides, plants can provide root exudates for rhizosphere microorganisms to be used as signaling molecules [13]. The rhizosphere microbiome of seagrass has been well studied. Several studies have shown a symbiotic relationship between the seagrass and their microbiome, such as a highly specialized coupling between *Zostera muelleri* and bacterial metabolism and ecology. The rhizosphere bacteria of two tropical seagrass species (*Thalassia hemprichii* and *Enhalus acoroides*) were affected by habitats but not plant species [25]. A study of the microbial communities of *Tshalassia testudinum* (turtle grass) and manatee grass (*Syringodium filliforme*) showed similar microbial composition between species of two seagrasses, but distinct from seawater and sediment communities [21]. Plant survival and performance depend on the stability of the microbial community state. Therefore, understanding the interaction between rhizosphere microorganisms and seagrasses can provide theoretical guidance for the restoration of seagrass beds.

High-throughput sequencing is dramatically extending our knowledge of plant microbiome diversity and ecological functions. As more and more plant microbial data become available, a variety of research investigations and applications have been substantially impacted, especially marine ecological protection. In order to explore the composition characteristics of rhizosphere microorganisms in *Zostera* on a larger scale, and to better understand whether there are core microbes in seagrass, we obtained the microbial community composition of different plant sample-types including *Zostera japonica*, *Zostera muelleri*, *Zostera marina* and *Zostera mucronata*.

## 2. Materials and Methods

### 2.1. Seagrass Samples

The seagrass microbiomes of four seagrass species (*Zostera japonica*, *Zostera muelleri*, *Zostera marina* and *Zostera mucronata*) were obtained from NCBI (National Center for

Biotechnology Information) database which was available online (<https://www.ncbi.nlm.nih.gov/>). After preliminary data screening, totally 60 samples were obtained for next analysis. The information and seagrass species are shown in Table 1. The accession number and data parameters of these samples were supplied as supplementary material S1.

Table 1. Information of the seagrass samples.

Species	Group	Sample Number	Geographic location
<i>Zostera japonica</i>	A	15	Bohai sea in China
<i>Zostera muelleri</i>	B	15	Brisbane Water Estuary in Australia
<i>Zostera marina</i>	C	15	Yaquina Bay in USA
<i>Zostera mucronata</i>	D	15	Perth in Australia

### 2.2. Diversity Analysis

The data of seagrass were collected according to the following steps. Firstly, the readings were assembled based on the overlap, and the Fastq files were processed to generate quality scores, analyzing by standard methods. Then, the MOTHUR software was used to reduce the noise base [16], and delete the sequences which were shorter than 496bp. MOTHUR was also used for richness and diversity analysis in species composition and structure, including Chao1, observed species, Simpson and Shannon indices. Sequences with greater than 97% similarity were clustered for OTU (operational taxonomic unit). The abundance of OTU in different samples were obtained through the OTU cluster analysis to evaluate the microbial diversity in each sample.

### 2.3. Species Annotation and Statistical Analysis

According to the comparison results of each OTU representative sequence with the database, species classification statistics were performed on the OTU to obtain species abundance at different classification levels. Analyze specific species composition of samples at different taxonomic levels, and test the species with significant differences among groups, so as to find important microbes that influence different samples.

## 3. Results

### 3.1. Alpha Rarefaction Curves and Alpha Diversity

Comparison of high-throughput sequencing results of 16S rRNA gene was carried out to obtain diversity indices (Observed OTUs, Shannon, Simpson and Chao1). Chao1 which refers to the number was the richness of OTUs estimated, the observed OTUs was the actual observed OTU number, and the Shannon index and Simpson index were used to reflect the diversity of OTUs. The Chao1, Observed OTUs, Shannon, and Simpson were higher in *Zostera muelleri* samples, and the differences within the group were small (Figure 1). The number and diversity of OTUs in *Zostera mucronata* samples were relatively low, and the differences among groups were large.

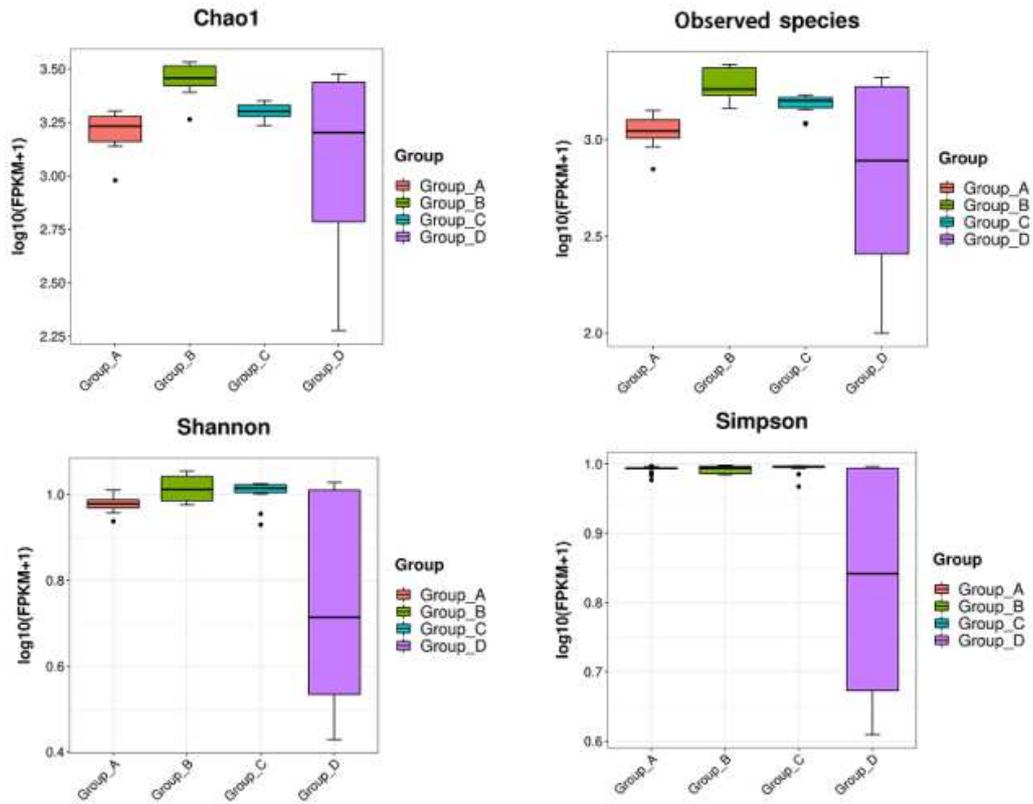


Figure 1. Diversity index of microbial communities of four species.

### 3.2. Bacterial Community Diversity

In the present study, a total of 565112 OTUs were obtained after clustering taxa. The Venn diagram (Figure 2) showed the number of common and unique species (OTU) among groups, visually representing similarity and overlap of composition.

1903 unique OTUs were identified in sediments occupied by *Zostera japonicathe*, 806 unique OTUs were identified in sediments occupied by *Zostera muelleri*, 80 unique OTUs were identified in sediments occupied by *Zostera marina*, and 2232 unique OTUs were identified in sediments occupied by *Zostera mucronata*.

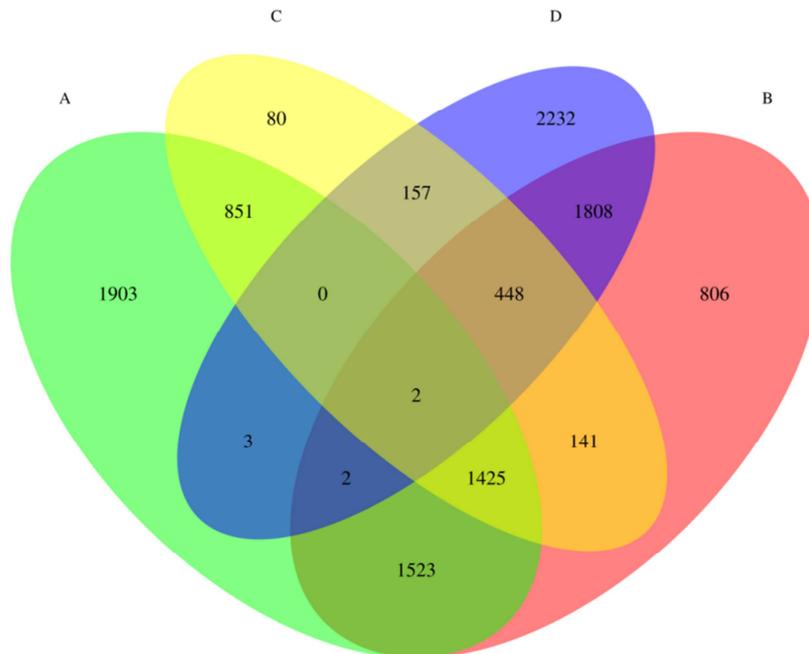


Figure 2. Venn Diagram of operational taxonomic units (OTUs) among different groups.

The analysis of diversity metrics showed that there were significant differences in the microbial communities from seagrass species (Figure 3). At the phylum level, the Proteobacteria (37.65%), Cyanobacteria (20.7%) and Bacteroidetes (13.88%) were dominant in *Zostera japonicathe* samples, the Proteobacteria (60.3%), Bacteroidetes (8.12%)

and Firmicutes (8.05%) were dominant in *Zostera muelleri* samples, the Proteobacteria (40.33%), Bacteroidetes (24.21%) and Epsilonbacteraeota (12.27%) were dominant in *Zostera marina* samples, the Proteobacteria (40.47%) and Cyanobacteria (36.8%) were dominant in *Zostera mucronata* samples.

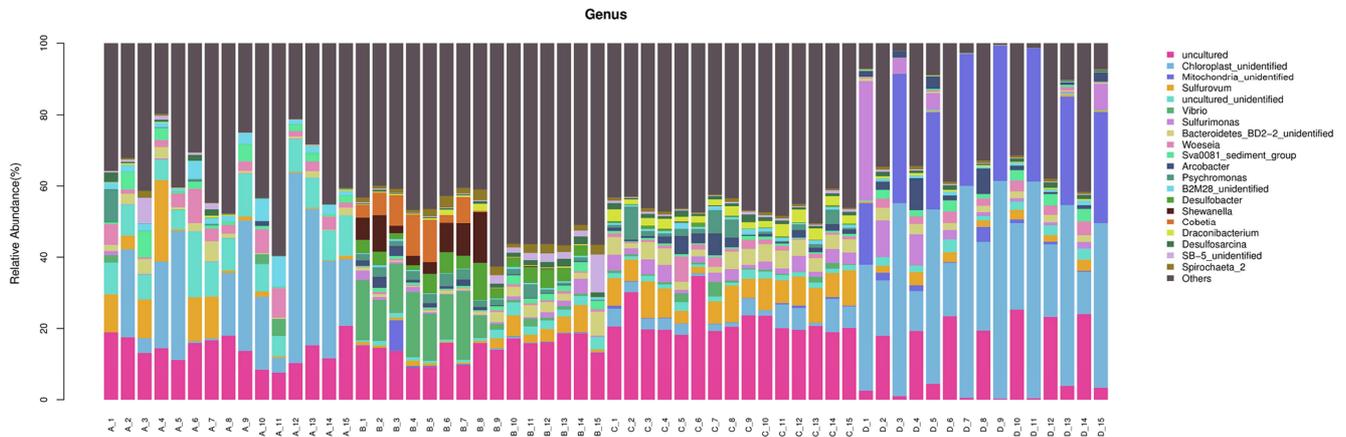


Figure 3. Bacterial community composition of four species at phylum level.

At the genus level, except for unclassified species, the *Sulfurovum* and *Woeseia* were dominated in *Zostera japonicathe* samples, the *Vibrio*, *Desulfobacter*, *Shewanella*, *Cobetia*, *Sulfurovum*, *Psychromonas*, *Spirochaeta\_2* and *Sulfurimonas* were dominated in *Zostera muelleri* samples,

the *Sulfurovum*, *Sulfurimonas*, *Draconibacterium*, *Psychromonas*, *Arcobacter*, *Desulfosarcina* and *Woeseia* were dominated in *Zostera marina* samples, the *Sulfurimonas*, *Arcobacter*, *Woeseia* and *Sulfurovum* were dominated in *Zostera mucronata* samples.

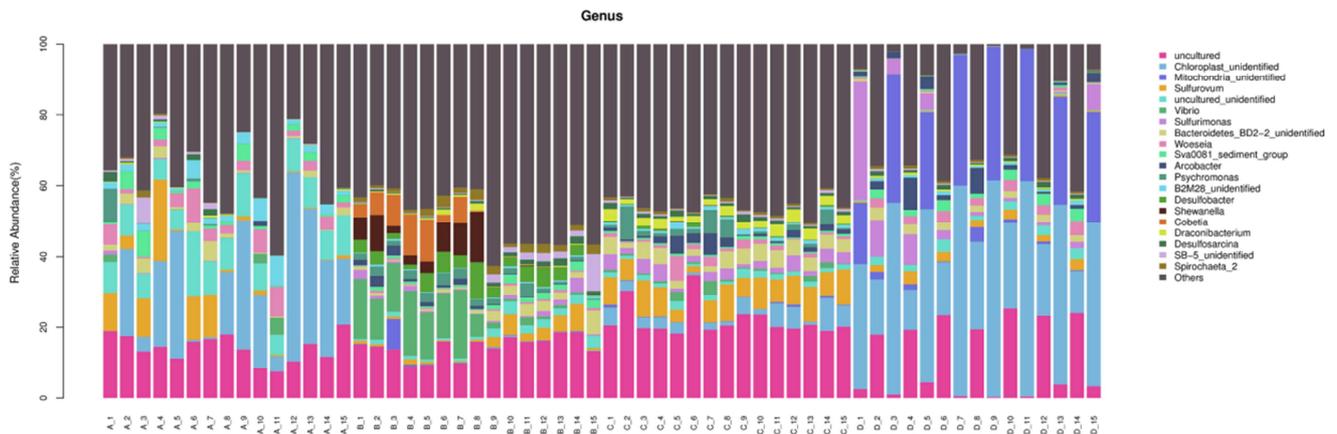


Figure 4. Bacterial community composition of four species at genus level.

### 3.3. Beta Diversity

Beta diversity refers to the turnover of species among different environments. In the present study, the principal coordinates analysis (PCoA) and cluster analysis were used to analyze the beta diversity among different seagrass species and samples with high community structure similarity were

clustered together, while those with large differences in community structure appear more distant. The PCoA results showed that the same seagrass species clustered together (Figure 4). The species abundance of different samples was intuitively seen in the histogram of species composition, but it was impossible to directly observe the similarity and distance between specific samples.

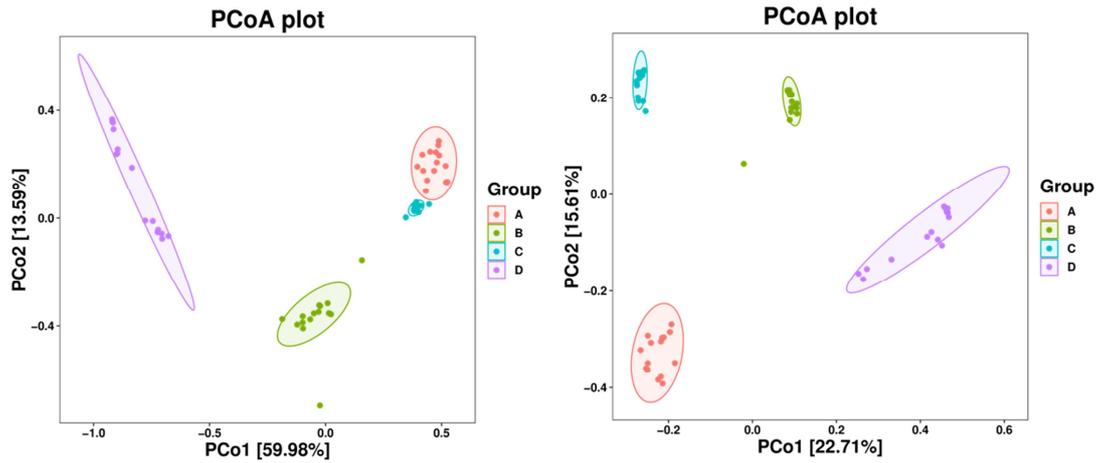


Figure 5. Principal Coordinates Analysis (PCoA) of bacterial community structures of different species using unweighted (left) and weight (right) Unifrac distances.

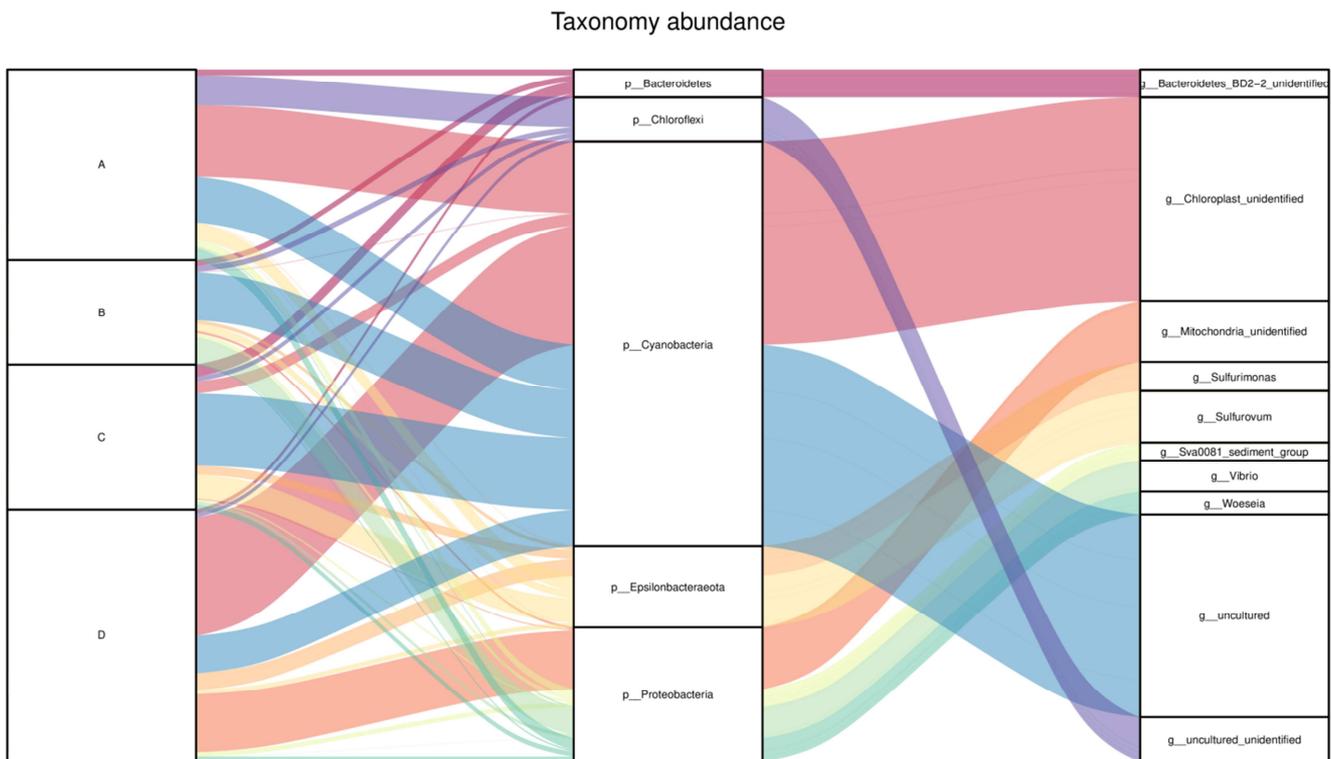


Figure 6. The taxonomy abundance.

### 4. Discussion

Seagrass roots release soluble organic carbon and leaves capture organic carbon particles from the overlying water [22]. The organic carbon load to sediments is increased through leaves, roots and stems in vitro, thus organic carbon concentration tends to be higher in seagrass beds. The rhizosphere mainly contains microorganisms involved in the biochemical cycles of nitrogen, sulfur, and carbon that are supported in part by the root exudates [14]. Rhizosphere microbes play an important role for the growth of plants and regulate the decomposition rate of soil organic matter [24]. There are interactions between microorganisms and plants,

and the growth secretions or chemical signal molecules of plants impact the rhizosphere soil microbial community [13]. Previous studies found that the root microbiota was mainly influenced by the local environmental parameters, notably soil properties [17, 19]. However, the presence of a core microbiome was indicated to exist in the seagrass [1, 6]. In order to explore the plant dependence on the microbiotic components, we determined the core microbiota among different seagrass species. Our results showed that the Proteobacteria and Bacteroidetes dominated in four seagrass species, which was consistent with previous studies on marine plants [10, 21]. The *Sulfurovum* was identified in four seagrass species at genus level. The Proteobacteria and Bacteroidetes (at phylum level), and the *Sulfurovum* (at genus level) might

be the core microbiome in the *Zostera* (Figure 6). A previous study confirmed that the Proteobacteria was more adapted to the rhizosphere environment than the Firmicutes in two tropical seagrass species (*Thalassia hemprichii* and *Enhalus acoroides*) [25]. The *Sulfurovum* which was involved in the sulfur metabolism was the main type of anaerobic degradation of organic matter in offshore marine environment group and offered rich organic carbon source for its reproduction in the seagrass [3]. A previous study found that the sulfate-reducing bacteria occupied the key position in the seagrass ecosystem. The sulfate reducing bacteria of the seagrass sediment also performed nitrogen fixation. As other coastal marine ecosystems, rapid consumption of oxygen by microorganisms in seagrass bed root sediments created an anoxic environment below the surface [20]. Thus, sulphate-reducing bacteria dominated organic mineralization due to the high concentration of sulfate in sediment porewater. Meanwhile, the seagrass offered rhizosphere exudates for sulfate metabolism, and in return, the bacteria could produce about 50 percent of fixed nitrogen for seagrass [8].

Except the core microbiome, the comparative analysis showed certain differences in the dominant composition of rhizosphere microorganisms in four seagrasses. The Actinobacteria was only dominant in the *Zostera japonica*. The Cyanobacteria was only dominant in *Zostera japonica* and *Zostera mucronata*. We speculated that the difference in the composition of rhizosphere microorganisms in four seagrass species might be caused by the sampling-site. The surrounding environment of seagrasses might also play an important role in the rhizosphere microorganisms. Different seagrass species can adapt to complex and diverse environments, and the difference among rhizosphere microorganisms may be due to their different habitats and distribution. Meanwhile, the rhizosphere microorganisms of the seagrass might be also affected by differences among seagrass species. The seagrass will attract beneficial bacteria and repel harmful ones [15].

## 5. Conclusions

In the present study, the core microbiome was identified in four seagrass. The common rhizosphere microorganisms may be essential for four seagrass species to assist their normal physiological activities. Meanwhile, the basis of biological ecosystem and its influencing factors are very complex, and further research is needed.

## Ethics Approval and Consent to Participate

Not applicable.

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## Competing Interests

The authors declare that they have no competing interests.

## Availability of Data and Material

The data of four seagrass species were obtained from NCBI database which was available online (<https://www.ncbi.nlm.nih.gov/>).

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