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Plant Compartments Shape the Assembly and Network of *Vallisneria natans*-Associated Microorganisms

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Abstract: The submerged plant *Vallisneria natans* can provide an attachment matrix and habitat for diverse microorganisms and plays an important role in maintaining the structure and function of the shallow lake ecosystem. However, little is known about how *V. natans*-related microorganism components, especially bacteria, adapt to specific plant compartments. In this study, we investigated the assembly and network of bacterial communities living in different plant compartments (sediment, rhizosphere, rhizoplane, root endosphere, and leaf endosphere) associated with *V. natans* by 16S rRNA gene sequencing. The results showed that the diversity and network complexity of the bacterial community in the sediment was significantly higher than that in other plant compartments. The bacterial community composition showed that the dominant phyla were Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Desulfobacterota, and Chloroflexi, among which Proteobacteria were extremely abundant in all samples, and there were notable differences in bacterial community composition related to plant compartments. Different networks based on sediment and plant compartments showed distinct co-occurrence patterns and exhibited distinct topological features. Additionally, functional predictions from FAPROTAX indicate that the predominant biogeochemical cycle function of the *V. natans*-related bacterial community is to participate in the carbon and nitrogen cycle. These results strongly suggested how the microbial community adapted to different plant compartments and provided theoretical and technical data for isolating beneficial bacteria from macrophytes in the future.

Keywords: *Vallisneria natans*; plant compartment; diversity; community assembly; co-occurrence networks; function prediction

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1. Introduction

Throughout the plant growth phase, the interrelationships between the plant and the microbial community hold significant importance for plants [1]. These microorganisms, including various mutualistic bacteria, colonize different plant compartments, such as the rhizosphere, rhizoplane, root endosphere, and leaf endosphere [2]. Plant-associated microorganisms play a crucial role in the biogeochemical processes of aquatic ecosystems, providing nutrient sources, enhancing disease resistance, and facilitating pollutant degradation [3,4]. Furthermore, plant-associated microorganisms are enriched by plant secretions and adapt to various ecological niches [5]. Some microorganisms are capable of recognizing signal molecules and settling in specific compartmentalized niches, while other microorganisms are filtered out [6]. This compartment enrichment in particular species is influenced by both biotic and abiotic factors, particularly in plant compartments more closely associated with microbial communities [7]. Previous studies have demonstrated that bacterial communities associated with macrophytes can promote plant growth, nutrient uptake, and tolerance to abiotic and biotic stresses [8]. Additionally, the complex co-associations between bacterial microbiota and macrophytes

play a crucial role in improving water quality [9]. For example, Singh et al. used aquatic plants and root bacteria to remediate the chromium-containing wastewater, and after 72 h, each pollution parameter was significantly reduced [10]. In addition, macrophytes can affect sedimental biogeochemical cycling by increasing the activity of microbes such as methanogens [11].

Previous studies have demonstrated that different plant compartments are colonized by distinct microbial communities [12]. Soil habitats serve as an extremely rich microbial reservoir for host–microbial selection [13]. For the root zone, plant-associated microbial communities originate from the soil, where bacterial populations follow a hierarchical selection mechanism, initially colonizing the rhizosphere, adhering to the rhizoplane, and ultimately entering the root endosphere [14]. Root exudates (such as organic acids) can stimulate bacterial growth and extracellular enzyme activity, thus affecting the biogeochemical cycles of carbon, nitrogen, and other elements in the soil [15]. Therefore, it is important to understand the effect of plant roots on the composition of microbial communities in the rhizosphere and rhizoplane. After entering the plant interior, endophytic bacteria may spread throughout the plant and colonize the leaf tissue. However, due to the physiological requirements necessary to occupy these plant ecological niches, only a few bacteria can colonize the leaf tissue of their host plant [16]. The movement of bacteria inside plants is supported by bacterial flagellum and the plant transpiration stream. Migration along that intercellular space requires the secretion of cell-wall-degrading enzymes such as cellulases and pectinases [17]. Therefore, the bacteria that migrate to the leaf tissue are well adapted to this unique endophytic ecological niche [18]. Microbial endophytes in the leaf are also important to the host plant, promoting plant growth and controlling plant diseases [19]. A better understanding of endophytic bacteria may help elucidate their function and potential role in improving plant performance.

Vallisneria natans, a perennial submerged aquatic herbaceous plant, is one of the most important primary producers in shallow lakes and can provide attachment substrates and habitats for a variety of microorganisms [9]. Its biological characteristics help maintain the structure and function of shallow lake ecosystems [20]. The *V. natans* not only absorbs and accumulates nutrients, organic pollutants, and heavy metal ions in the water through its root system and leaf organs but also secretes oxygen into the sediment to improve the redox environment of the sediment. This species is often selected as an important aquatic plant species when constructing water purification systems and is widely used in the restoration of shallow lake ecosystems and the construction of benign ecosystems [21]. Its sensitivity to high concentrations of environmental pollutants, weak resistance to non-biological stress, and low biological utilization of ions and compounds all restrict its restoration efficiency [22].

In recent years, important progress has been made in the research on the combined remediation technology for submerged plants and microorganisms. However, the application effect of functional bacteria is unstable, and further exploration of functional microbial germplasm resources is needed. In this study, we focused on the following three aspects: (1) revealing the assembly mechanism of microbial communities in different host compartments; (2) mining genetic information of dominant species and key groups in microbial communities; and (3) exploring the potential microbial species in submerged plants that drive the biogeochemical cycle of lakes.

2. Materials and Methods

2.1. Sample Collection and Surface Sterilization

Mature *V. natans* specimens were collected from the experimental field of Anhui University and randomly divided into three parallel groups (30 plants per group). Non-rhizosphere sediments covered with *V. natans* vegetation were collected as sediment samples. The collected samples included sediment, rhizosphere soil, rhizoplane soil, roots, and leaves from this sampling site. All soil samples had the salt crusts and litter layer removed. They were placed in aseptic bags and transported back to the lab. All tissue

samples were washed with 10 mM PBS buffer and surface sterilized with 70% ethanol for 45 s, then washed 5 times with sterile water. Finally, they were soaked in 0.1% mercuric chloride for 5 min and washed with sterile water 5 times. Sterile filter paper was used to absorb water. Next, 100 µL of sterile water from the final wash was applied to an LB agar plate, which was inverted and incubated at 30 °C for 72 h to assess the efficacy of surface sterilization of plant material. If contamination was observed on the surface of the agar medium, the plant material was discarded.

2.2. Microbial Genomic DNA Extraction, PCR, and Sequencing

The sediment and rhizosphere DNA were extracted from 250 mg soil using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). For rhizoplane DNA extraction, microbial cells were dislodged and collected from 5 g of roots and subjected to DNA extraction using the FastDNA® Spin Kit for Soil. All endophytic tissue samples were extracted by the improved CTAB method. The chimeric sequences such as host mitochondria and chloroplasts were removed, and the V5–V7 hypervariable regions of the 16S rDNA of the microorganism were amplified by using nested primers 799 F (5′-AACMGGATTAGATACCCKG-3′) and 1392R (5′-ACGGGCGGTGTGTRC-3′) and 799 F (5′-AACMGGATTAGATACCCKG-3′) and 1193R (5′-ACGTCATCCCCACCT-TCC-3′). The purified PCR products were sequenced on an Illumina Miseq PE300 platform.

2.3. Bioinformatics Analysis Process

Firstly, the raw data were subjected to quality control and filtering using Trimmomatic software. Next, the effective data were assembled using FLASH v.1.2.11 software (https://ccb.jhu.edu/software/FLASH/index.shtml, accessed on 14 April 2022). Operational taxonomic units (OTUs) were clustered based on 97% similarity using UPARSE v.11 software (http://www.drive5.com/uparse/, accessed on 14 April 2022). Chimeras were removed using UCHIME v.11 software (https://drive5.com/uchime, accessed on 14 April 2022). Finally, the RDP classifier was employed to annotate species classification for each sequence by comparing it with the Silva database.

2.4. Statistical Analysis

A bipartite network analysis of the bacterial community associated with the *V. natans* was performed using the QIIME script and visualized in Gephi v.0.9.2. Nodes in the network correspond to sample and bacterial OTUs, and the link indicates the presence of OTUs in the sample. For beta diversity, the Bray–Curtis dissimilarity distance matrices were used to perform a principal coordinates analysis (PCoA). Alpha diversity indices were calculated using the mothur v.1.30.2 software (https://www.moth-ur.org/wiki/Download_mothur, accessed on 14 April 2022).

A co-occurrence network analysis was performed for each bacterial community associated with the different plant compartments of *V. natans* to explore the significant relations among the OTUs, using the routine CoNet in Cytoscape v3.9.1. To build the network, we filtered out the OTUs with frequencies less than 0.05 then combined an ensemble of the Pearson and Spearman correlation coefficients and the Bray–Curtis (BC) and Kullback–Leibler (KLD) dissimilarity indices. Based on the topological position of the microbial community within the ecological network, several potential keystones were identified. The topological type of each node was determined using measures of within-model connectivity (*Zi*) and among-module connectivity (*Pi*). Based on the values of *Zi* and *Pi*, four categories were established: peripherals ($Zi < 2.5$, $Pi < 0.62$), connectors ($Zi < 2.5$, $Pi > 0.62$), module hubs ($Zi > 2.5$, $Pi < 0.62$), and network hubs ($Zi > 2.5$, $Pi > 0.62$). The latter three categories played a crucial role in network topology and were deemed key taxa. Network analysis was depicted using Gephi v.0.9.2. The biogeochemical cycle functionality of microorganisms was analyzed using FAPROTAX v2.2 (https://pages.uoregon.edu/slo-uca/LoucaLab/archive/FAPROTAX/lib/php/index.php, accessed on 14 April 2022), a database that was manually constructed to map prokaryotic

taxa, such as genera or species, to ecologically related functions. These functions include nitrification, denitrification, or fermentation, based on literature from culture representatives. Using a Python script, the sample OTU table was converted into the following six tables: sample menu, process report, OTUs for each functional group, table with each functional group overlapping, annotation information used in the analysis process, and sub-table of the input sample OTU table (only OTUs related to a specific function are listed).

3. Results and Discussion

3.1. OTUs Distribution and Diversity

Bacterial diversity was assessed using Illumina MiSeq sequencing of the 16S rRNA gene in five distinct plant compartments, including sediment, rhizosphere, rhizoplane, root endosphere, and leaf endosphere. A total of 833 unique operational taxonomic units (OTUs, cut-off level 97%) were identified at an even sequencing depth of 24,100 sequences per sample. The highest number of different OTUs was found in sediment (774 OTUs), followed by rhizoplane (747 OTUs), rhizosphere (730 OTUs), root endosphere (535 OTUs), and leaf endosphere (325 OTUs). A bipartite network analysis showed that the sediment harbors the highest number of OTUs, which are shared, in part, with the root-associated compartments (Figure 1A). Among the sediment OTUs (774), 56.33% were shared between the rhizosphere and rhizoplane, while only 32.69% were in common with the root and leaf tissues. PCoA based on the Bray–Curtis distance revealed that PCoA1 explained 49.92% and PCoA2 19.98% of the total variance among the different communities (PERMANOVA: $R^2 = 0.878$, $p < 0.001$; Figure 1B). The results suggest that the plant compartments exerted a significant influence on the bacterial community, with distinct samples exhibiting significant clustering. To investigate the effect of plant compartments on bacterial communities, α-diversity indices of bacterial communities in different samples were calculated (Figure 1C–E; Table 1). In all samples, the value of the coverage index was greater than 0.99, indicating that the sequencing results covered almost all bacteria in the samples (Figure 1C). In Figure 1D, bacterial richness (Chao1 index) was significantly greater in sediment (755.950 ± 7.417) than in other plant compartments (rhizosphere, 722.446 ± 28.159; rhizoplane, 709.794 ± 54.305; root endosphere, 515.799 ± 44.392; leaf endosphere, 311.312 ± 51.223). The Shannon index in the sediment samples had the highest value (5.455 ± 0.028), whereas the lowest values (2.546 ± 0.444) were in the leaf endosphere samples (Figure 1E). Contrary to the Chao1 index, the Shannon index increased gradually from rhizosphere (3.882 ± 0.121) to rhizoplane (3.969 ± 0.124) and then to root endosphere (4.050 ± 0.350). The alpha-diversity results showed that the bacterial communities were significantly different in different plant compartments.

To investigate the impact of plant compartments on the bacterial communities, we analyzed the distribution of OTUs and diversity indexes across different plant compartments. We examined the effect of *V. natans* on sediment by comparing the sediment with the rhizosphere. The OTUs number and α-diversity indexes were found to be higher in the sediment, consistent with previous studies that reported significantly lower bacterial community diversity associated with the rhizosphere of submerged macrophytes than that of the sediment [23]. Yan et al. used *V. natans* for rhizodegradation to repair PAH-contaminated sediments, which showed that the Shannon indices gradually decreased from the sediment bacterial community to the endosphere and then to the rhizoplane [24]. These results indicate a significant influence of *V. natans* on sediment. Moreover, it has been demonstrated that sediment is the primary source of the plant-associated microbiome, and the microbiome in all plant compartments is tightly regulated by a rigorous process [25]. This rigorous process is mainly caused by the plant immune defense mechanism; microorganisms must overcome it to survive and colonize in different plant compartments.

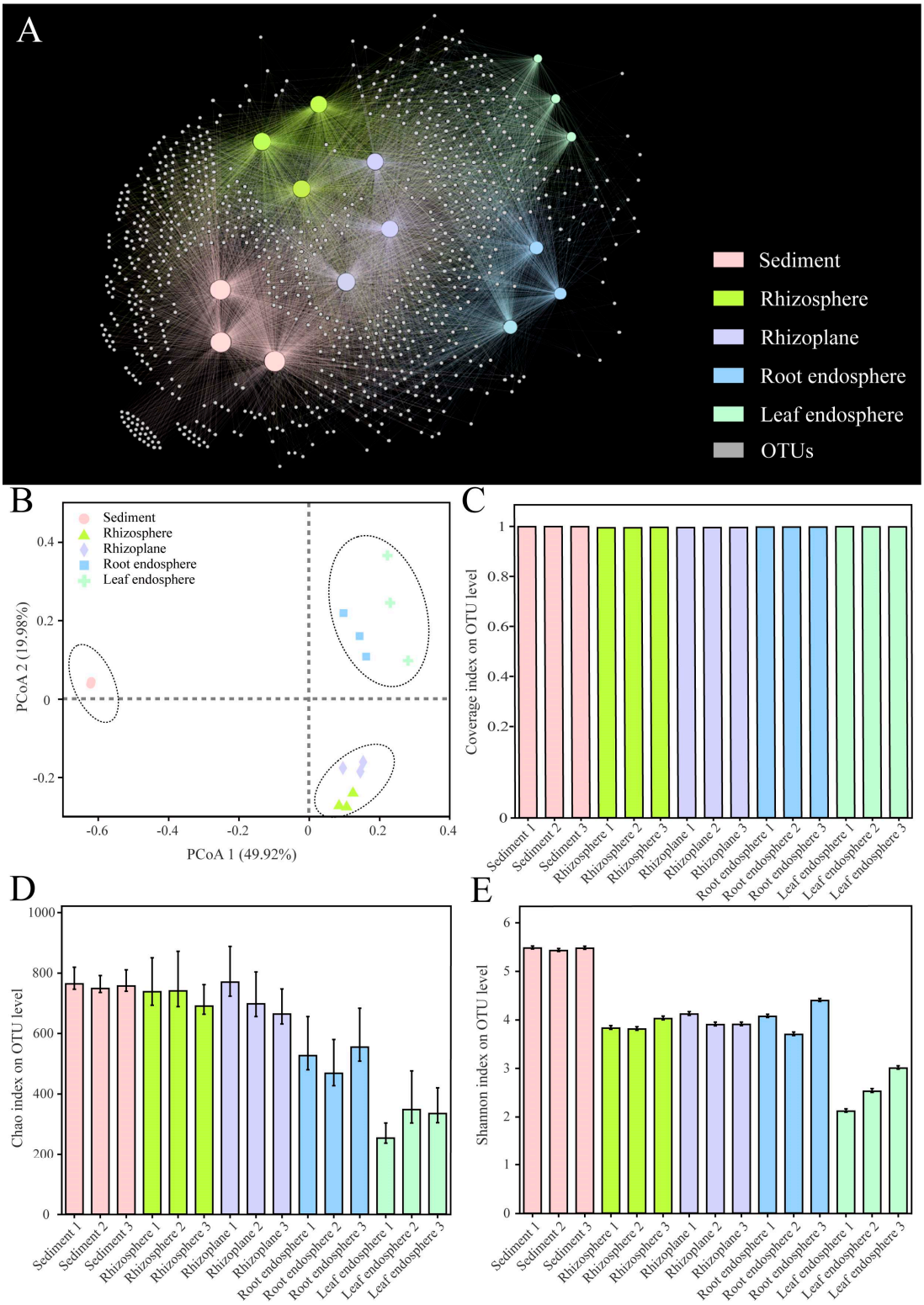

Figure 1. OTU distribution and diversity of bacterial communities in samples from different compartments (sediment, rhizosphere, rhizoplane, root endosphere, and leaf endosphere). (**A**) Bipartite network analysis represented sample/OTU interactions. Different circle colors indicate different sample types of *V. natans*; the OTU node is silver, with the edges connecting sample nodes to OTU. Nodes are colored by sample type. (**B**) Based on the Bray–Curtis distance, principal coordinates analysis (PCoA) was conducted for bacterial communities in different plant compartments. (**C**–**E**) α–diversity index of the bacterial community in different samples.

Table 1. Mean bacterial α-diversity indexes in the different compartments.

Compartment	OTUs Number	Coverage	Chao1	Shannon
Sediment	774	0.998	755.950 ± 7.417	5.455 ± 0.028
Rhizosphere	730	0.994	722.446 ± 28.159	3.882 ± 0.121
Rhizoplane	747	0.994	709.794 ± 54.305	3.969 ± 0.124
Root endosphere	535	0.996	515.799 ± 44.392	4.050 ± 0.350
Leaf endosphere	325	0.997	311.312 ± 51.223	2.546 ± 0.444

An OTU was defined as reads with 97% sequence similarity. Values represent an average number of α-diversity indexes (±standard error).

Most of the observed endosphere bacterial community originates from the rhizosphere, which is consistent with the two-step selection model proposed for the filtration of *Phragmites australis* root endophytic microbiome, where microorganisms colonize the rhizosphere and then select the roots [26]. This filtration effect between different root compartments is due to niche characteristic differences and the selection of the microbial community [14]. Compared to other plant compartments, there are few studies on bacterial communities in submerged plant leaves, and whether the filtration mechanism of bacterial communities in root compartments can also be applied to the filtration mechanism of bacterial communities in leaves has yet to be determined [18]. Since most of the endophytic bacteria originated from the sediment and shared a certain similarity with the sediment microbial community, the diversity of root endophytic bacteria was higher than that of leaf tissue, which might be attributed to the fact that the root system was the main site for the interaction between plants and the sediment [26]. Most bacteria found in leaves were also found in the root endosphere, indicating that the root endosphere may be the major source of bacteria in leaves. This is likely because endophyte bacteria can spread systemically inside the plant and colonize the leaves [27]. However, this may not be the only pathway for the leaf endosphere, as submerged plants are completely submerged in water, which contains a large number of microorganisms that can enter the plant directly via the leaf stomata. The results of this and previous studies support the idea that submerged macrophytes recruit and select bacteria instead of randomly obtaining bacteria from surrounding environments.

3.2. Taxonomic Composition Analysis at Different Levels

A total of 26 phyla and 71 classes were identified, including some unclassified bacteria (Figure 2). In Figure 2A, the predominant phyla included Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Desulfobacterota, and Chloroflexi, which could be detected in all samples with varied values. The most dominant phylum in the six samples was Proteobacteria, which accounted for 33.12~86.80% (mean 61.07%) of the relative abundance value, followed by Bacteroidetes (1.65~45.10%, mean 17.46%), Actinobacteria (1.06~23.99%, mean 6.92%), Firmicutes (1.14~9.59%, mean 5.84%), Desulfobacterota (0.02~9.37%, mean 2.25%), and Chloroflexi (0.02~8.20%, mean 2.13%). We observed significant enrichment ($p < 0.05$) of Proteobacteria in plant-associated communities (mean 68.06%), compared with sediment (33.12%). Bacteroidetes were significantly enriched ($p < 0.05$) in the leaf endosphere (45.10%) compared with other samples (mean 10.56%). In addition, Actinobacteriota, Desulfobacterota, and Chloroflexi were present at higher levels in sediment than in other plant compartments. The relative abundance of Firmicutes in endophytic tissues was lower than that in sediment, rhizosphere, and rhizoplane ($p < 0.05$).

At the class level, there was a significant difference in the relative abundance of dominant classes across plant compartments, as depicted in Figure 2B ($p < 0.05$). Within Proteobacteria, Gammaproteobacteria (22.43~54.15%; mean 36.54%) and Alphaproteobacteria (10.69~45.99%; mean 24.53%) exhibited absolute dominance in all samples, with their combined relative abundance exceeding 60%. However, Bacteroidia (45.10%) were the most dominant class in the leaf endosphere, whereas their abundance was less than 1% in sedi-

ment. Desulfuromonadia (5.96%) and Thermoleophilia (5.04%) were also the predominant dominant classes in sediment, whereas their abundance was less than 1% in other samples.

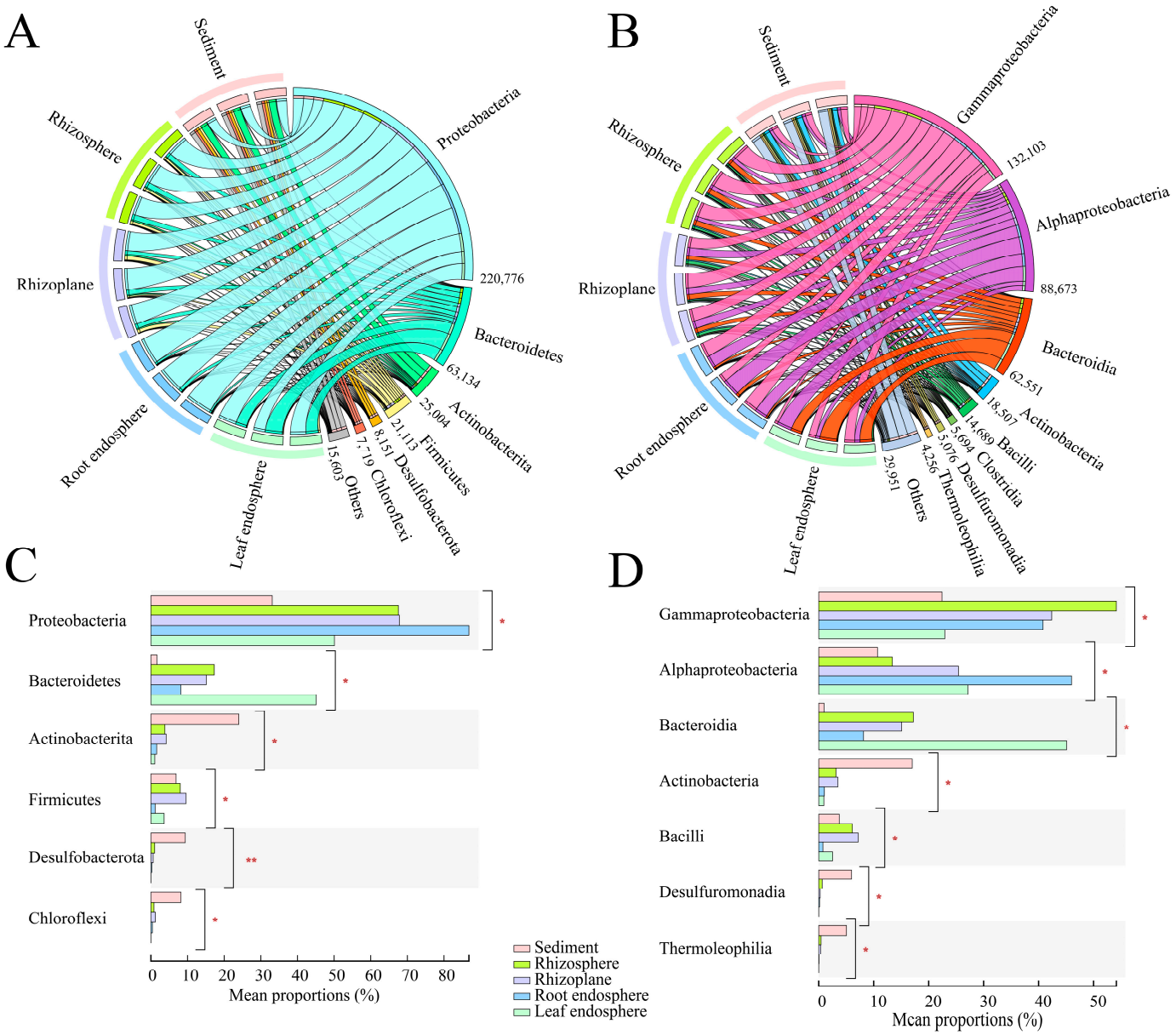

Figure 2. At different taxonomy levels, community composition and relative abundance of dominant bacterial groups in different samples of *V. natans*. (**A**,**B**) represent the community composition of dominant bacterial groups at phylum and class levels, respectively. (**C**,**D**) represent the relative abundance of dominant bacterial groups at phylum and class levels, respectively. (* $p < 0.05$, ** $p < 0.01$).

The hierarchical clustering heat map (Figure 3) drawn according to the top 30 genera of total abundance in five samples exhibited three groups. The sediment samples were separately aggregated, while the rhizosphere and rhizoplane samples, as well as the endophytic bacteria samples from the roots and leaves, were grouped. The results indicate that the endophytic bacterial samples (from roots and leaves) and the rhizosphere and rhizoplane samples have similar bacterial community compositions. Species distributions differed greatly across samples. *Intrasporangium*, *Gaiella*, *Ramlibacter*, *Ellin6067*, *Bacillus*, and *Methylocystis* were mainly distributed in sediment, while the relative abundance of these genera in other samples was low. Species belonging to *Rhizobium*, *Flavobacterium*, *Pseudomonas*, *Massilia*, and *Polaromonas* were particularly enriched in the rhizosphere and

rhizoplane, as well as in root and leaf endospheres, when compared to sediment samples. More globally, the relative abundance of *Limnohabitans* was high in all samples. The relative abundance of unclassified_f_Comamonadaceae was higher in the root endosphere samples than in the other samples.

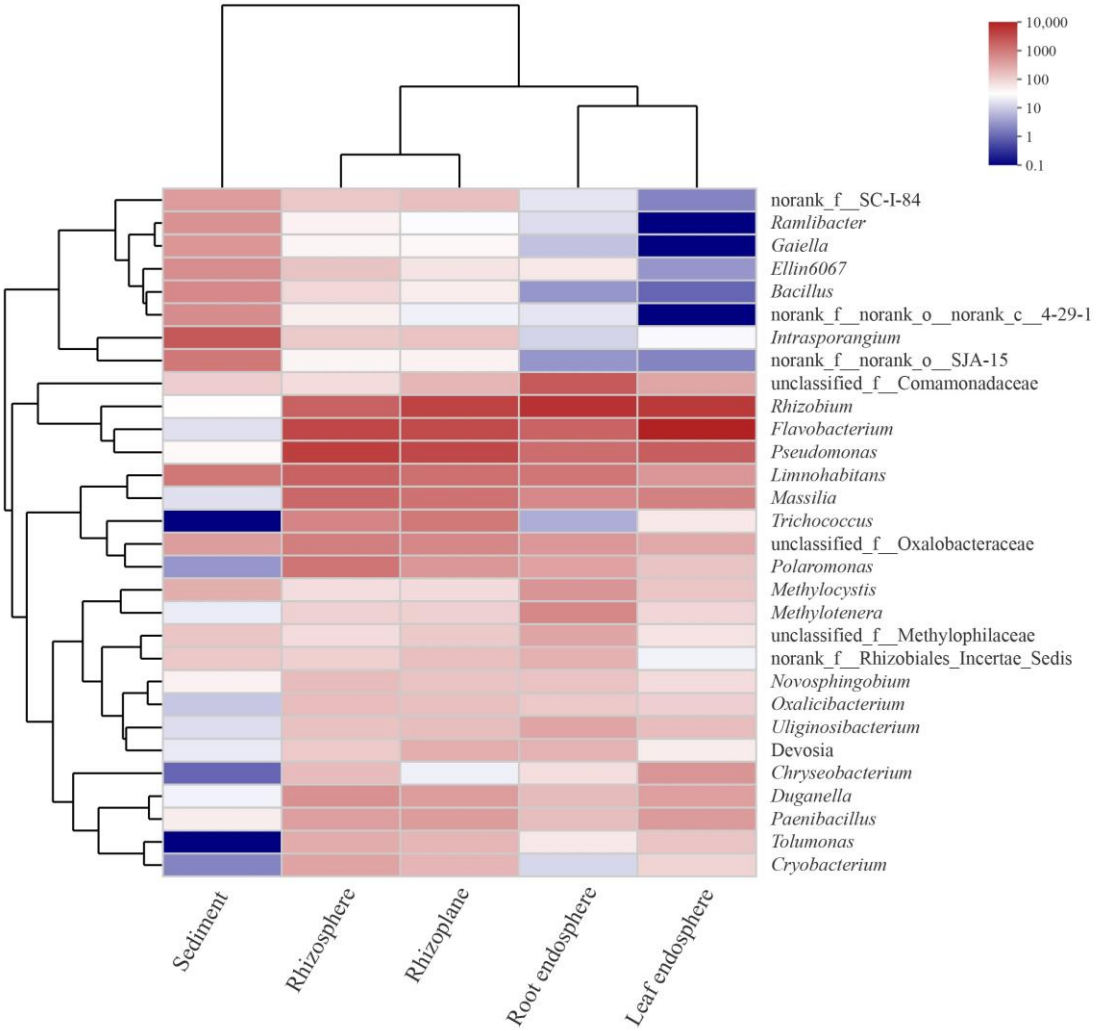

Figure 3. Heat map graph of hierarchy cluster for the top 30 genera. The relative abundance values for the bacteria genera are depicted by the color intensity with the legend indicated at the right of the figure. Brick red and blue, respectively, represent high or poor enrichment of a genus.

Although the results suggest that plant compartments have varying effects on the distribution of operational taxonomic units (OTUs) and α-diversity indices of bacterial communities, a comprehensive understanding of the relationship between plant compartments and bacterial communities requires an examination of the impact of plant compartments on the composition of bacterial communities. Our investigation revealed that each sample contained a unique set of bacteria. Proteobacteria play a crucial role in organic matter decomposition and cycling and are closely related to plant growth. They were dominant in all compartments related to *V. natans* in a similar way to other submerged plants such as *Potamogeton crispus* [28], *Hydrilla verticillatea* [29], and *Potamogeton lucens* [30]. However, we observed that the relative abundance of Alphaproteobacteria and Gammaproteobacteria in root-related compartments was significantly higher than that in sediment and leaf endophytes. Alphaproteobacteria and Gammaproteobacteria, as r-strategy organisms, can utilize a wide range of carbon substrates from roots, which explains their dominance in roots [31]. Several genera belonging to the phylum Proteobacteria were involved in various biogeochemical processes that control nutrient fluxes in aquatic ecosystems. For instance,

Limnohabitans, *Rhizobium*, *Pseudomonas*, and *Massilia*, which are affiliated with Proteobacteria, were found to be positively related to improving metabolic capacity, promoting plant growth, degrading pollutants, and enhancing plant disease resistance [32–34] and were widely distributed in the plant compartments, except in sediment, of *V. natans*. The relative abundance of Bacteroidetes in the leaf endosphere was higher than that in other compartments. These bacteria can decompose soluble organic matter composed of macromolecules, which are widely involved in the biogeochemical cycle and nitrogen removal of freshwater ecosystems [35]. *Flavobacterium*, the most dominant genus of the phylum Bacteroidetes, is a highly abundant aerobic denitrifying microorganism that can obtain energy for its growth by degrading organic matter from algae such as cyanobacteria in the presence of sufficient light [36]. The relative abundance of Actinobacteria and Chloroflexi phyla was significantly higher in the sediment. These bacteria are key to nitrogen removal in the management of wastewater [37] and are involved in the degradation of plant-derived compounds such as cellulose [38]. Strikingly, the dominant genus *Bacillus* in the phylum Firmicutes affects plant growth through biological nitrogen fixation and the production of indole-3-acetic acid (IAA) and gibberellins (GA) [39]. However, *Bacillus* was not detected in root and leaf endosphere samples, suggesting that it fails to enter the internal plant tissues of its host after rhizosphere colonization. Together, these findings confirmed that plant-associated bacterial communities were enriched via tissue selection based on the character of the niche and the adaptation of bacteria to habitat conditions.

3.3. Specific and Shared Bacterial Assemblages

The Venn diagram showed specific OTUs for each plant compartment and a cluster of shared OTUs (Figure 4). The sediment samples contained 28 specific OTUs, accounting for 3.36% of the total OTUs, while other samples contained no specific OTUs. A total of 249 shared OTUs were present in the five samples; 15 of these OTUs were relatively abundant (>1%) (Figure 4A). At the genus level, OTU915 was annotated as *Pseudomonas*, accounting for 11.95% (Figure 4B); OTU548, OTU845, and OTU1436 were annotated as *Flavobacterium*, accounting for 20.50%; OTU975 and OTU543 were annotated as *Rhizobium*, accounting for 16.41%; OTU1144 and OTU1593 were annotated as *Limnohabitans*, accounting for 6.14%; OTU1903 was annotated as *Massilia*, accounting for 4.32%; OTU2741 was annotated as *Intrasporangium*, accounting for 2.72%; OTU1604 was annotated as *Polaromonas*, accounting for 2.24%; and OTU1073 was annotated as *Duganella*, accounting for 1.63%. OTU868 and OTU1001 could not be assigned to a specific genus and were only annotated as Oxalobacteraceae and Comamonadaceae, accounting for 1.26% and 1.01%, respectively. In brief, the shared OTUs (249) were mainly affiliated with Gammaproteobacteria, Alphaproteobacteria, Bacteroidetes, and Actinobacteria, accounting for 66.44% of the relative abundance of all samples sequenced. There were 682 shared OTUs for sediment and rhizosphere samples and 285 shared OTUs for plant compartments. All but 10 of the OTUs in the root and leaf endospheres were also present in the rhizosphere and rhizoplane. By contrast, 227 OTUs were found in the root endosphere but not in leaves; only 17 OTUs were present in leaves but not in the root endosphere. These results indicated that the plant compartments have a significant effect on the distribution of bacterial OTUs.

By defining shared OTUs from different plant compartments in overlapping areas of Venn diagrams, we found that the dominant phyla/classes were mainly Gammaproteobacteria, Alphaproteobacteria, Bacteroidetes, and Actinobacteria. These common groups were defined as the key groups shared among the microbial communities in *V. natans*. The predominance of these phyla or classes was consistent with their ability to use a variety of root carbon matrices from plants, promote plant growth, and degrade pollutants [31,35,37]. These dominant bacteria were also found in other studies, which assessed the composition of rhizosphere bacterial communities of two emergent plants (*Phragmites australis* and *Triarrhena lutarioriparia*) [25]. These dominant groups may have significantly contributed to many major ecosystem processes, including playing a role in organic matter flux and biomass production [40].

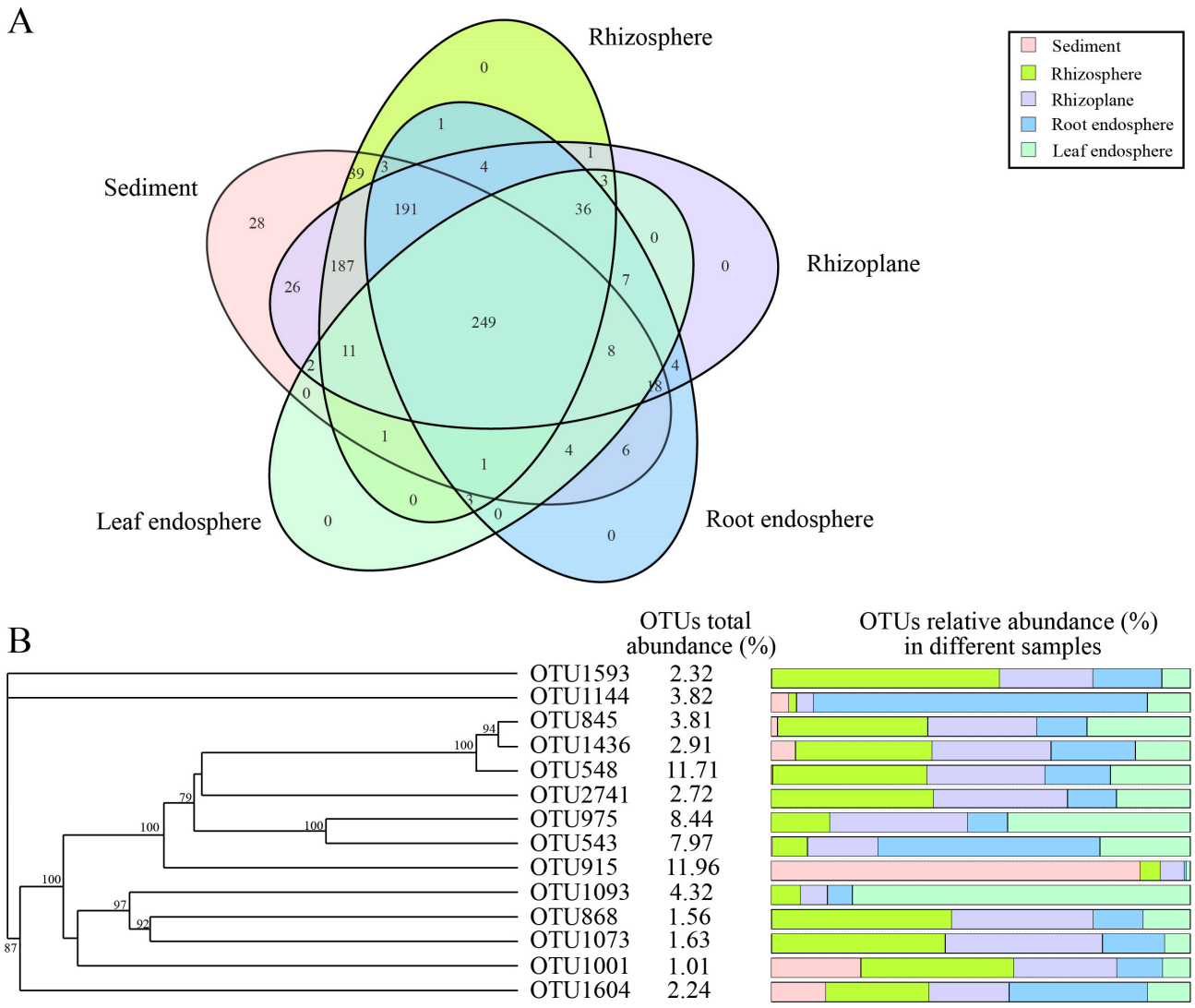

Figure 4. Microbial community shared by different plant compartments of *Vallisneria natans*. (**A**) Venn diagrams showing the shared and specified bacterial OTUs of different plant compartments. (**B**) The phylogenetic trees and distribution of the 14 most abundant (>1%) shared OTUs in all selected different plant compartments of *Vallisneria natans*.

3.4. Co-Occurrence Network of Bacterial Communities in Different Plant Compartments

An analysis of the co-occurrence bacterial networks within different samples of *V. natans* showed different connectivity patterns directly and differently influenced by the plant compartments (Figure 5A). According to the total number of nodes and edges, the network complexity gradually decreased from sediment to rhizosphere, rhizoplane, root endosphere, and finally leaf endosphere (Table 2). We recorded a significantly higher number of co-occurrence interactions than mutual exclusions in all networks. The classification of the most connected nodes in each network is significantly different (Table 3). Actinobacteria mainly shaped the topology of the bacterial network in the sediment (up to 24.93% of the total degree of connection), followed by Gammaproteobacteria (up to 22.74%) and Alphaproteobacteria (up to 10.40%). The connectivity between rhizosphere and rhizoplane bacterial communities was mainly driven by Gammaproteobacteria (56.14% and 43.95%, respectively). In the root endosphere, connectivity among the bacterial community was primarily driven by Alphaproteobacteria (46.98%) and Gammaproteobacteria (42.19%), without Desulfobacterota. By contrast, Bacteroidetes mainly shaped the topology

of the bacterial network in the leaf endosphere (46.66%), followed by Alphaproteobacteria (26.70%) and Gammaproteobacteria (22.88%), but without Desulfobacterot and Chloroflexi.

Table 2. Bacterial co-occurrence network characteristics in each compartment niche.

Niche	Nodes	Edges	Average Degree	Modularity [a]	Average Clustering Coefficient [b]	Average Path Distance [c]
Sediment	516	3437	13.322	0.903	0.773	10.901
Rhizosphere	197	3012	30.579	0.669	0.806	4.673
Rhizoplane	185	2958	33.059	0.643	0.795	3.443
Root endosphere	154	2883	37.442	0.551	0.804	2.628
Leaf endosphere	86	781	18.163	0.570	0.768	2.773

[a]: Degree of nodes tending to differentiate into different network modules. [b]: Degree of nodes tending to cluster together. [c]: Network path distance is the length of the shortest path between two nodes within the network.

Table 3. In that histogram, the relative abundance of the node with connectivity was reported at the phylum/class level for different samples.

Phylum/Class	Sediment	Rhizosphere	Rhizoplane	Root Endosphere	Leaf Endosphere
Alphaproteobacteria	10.40%	13.08%	26.05%	46.98%	26.70%
Gammaproteobacteria	22.74%	56.14%	43.95%	42.19%	22.88%
Bacteroidetes	1.50%	17.91%	15.90%	8.12%	46.66%
Actinobacteria	24.93%	3.26%	3.63%	1.23%	0.79%
Firmicutes	6.99%	8.01%	9.36%	0.60%	2.97%
Desulfobacterota	9.75%	0.61%	0.09%	0	0
Chloroflexi	6.95%	0.40%	0.61%	0.32%	0
Others	16.73%	0.59%	0.41%	0.56%	0

Subsequently, we identified the potential keystone taxa in the networks (peripheral nodes, connectors, module hubs, and network hubs) (Figure 5B). In this study, the majority of nodes in each network are peripheral nodes, without network hubs. Nodes belonging to module hubs and connectors are defined as keystone species. A total of 14 key groups (OTUs) were detected in the networks, including 13 connectors and 1 module hub. Specifically, four connectors were found in the sediment network, whereas only one module hub was detected in the rhizosphere network. The maximum number of keystones was found in the rhizoplane with six connectors. The root and leaf endospheres contain two and one connectors, respectively. Most of these module hubs and connectors were affiliated with four phyla or classes: Gammaproteobacteria, Alphaproteobacteria, Firmicutes, and Actinobacteria. These results suggested that plant compartments significantly affect the network structure and topological properties of individual OTUs and key microbial populations.

Co-occurrence network analysis can provide a better understanding of interactions within functional bacterial communities and enable the assessment of the topological roles of each taxon in the network [41]. Our study's network analysis revealed distinct structural characteristics in various plant compartments, and the differentiation of these compartments' niches may account for the differences in topological traits. The endophytic bacterial community's niche differentiation is low due to the host plant's space and nutrient limitations. Similarly, sediment microbial communities exhibit niche differentiation, which may result from root deposition effects, leading to the selection of specific bacteria combinations [42]. The networks of *V. natans*' various samples were positively correlated, indicating that most bacterial colonies had extensive cooperative and symbiotic potential in their respective compartments. Extensive cooperation among bacterial groups can significantly impact ecological processes and host adaptation [43].

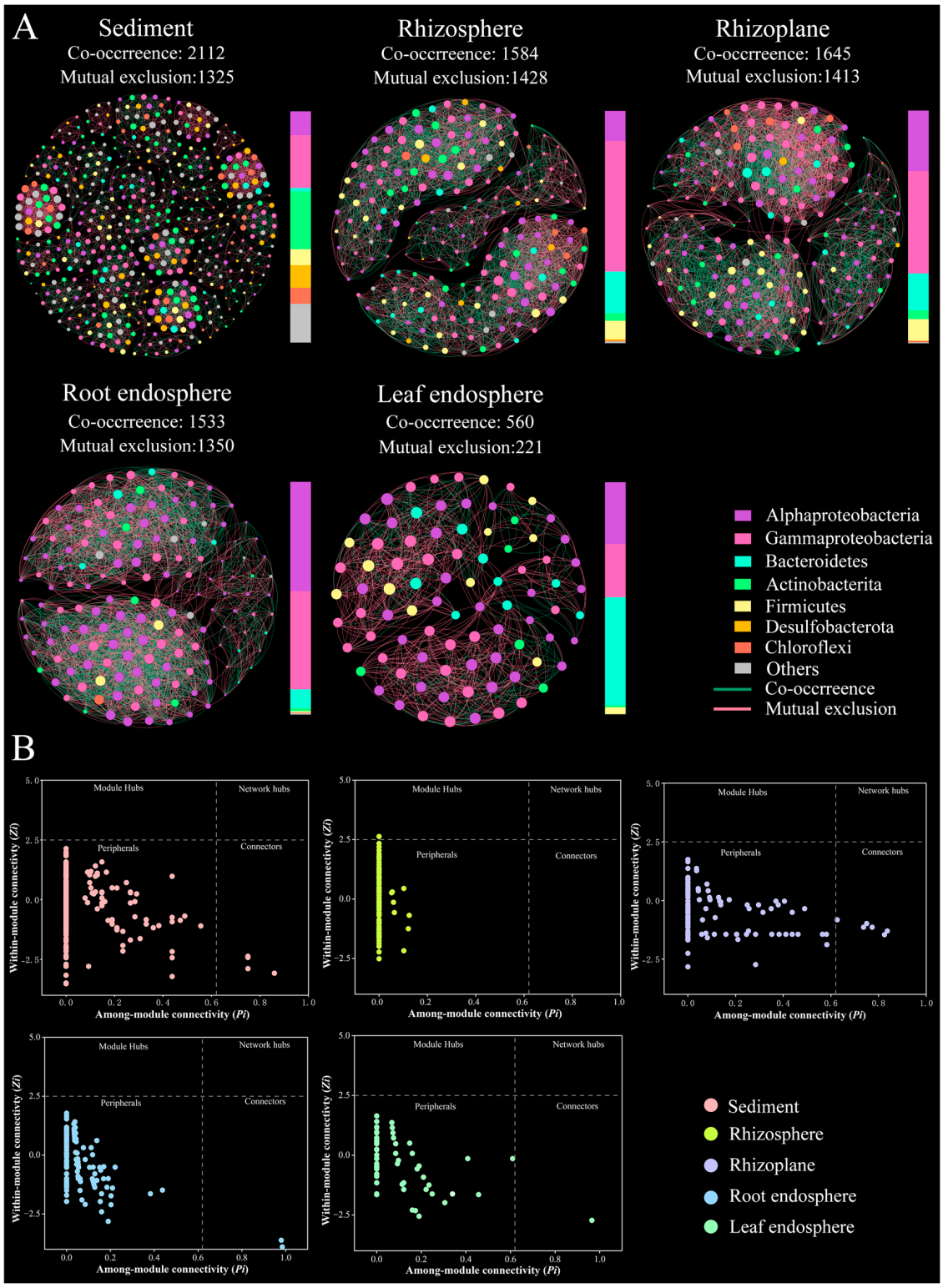

Figure 5. The correlation networks of bacteria in different plant compartments. (**A**) co-occurrence network of bacterial communities associated with different plant compartments. The colors and sizes of network nodes correspond to bacterial phylum/class and the degree of connectivity of each node. The colors of the network links represent the correlations between nodes (green: positive correlation; red: negative correlation). (**B**) Within-module connectivity (*Zi*) and among-module connectivity (*Pi*) plots show the distribution of OTUs based on their topological roles. Each node represents an OTU in the bacterial community. The topological role of each OTU depends on the scatter plot of *Zi* and *Pi*.

Research has demonstrated that hubs or connectors can act as keystone groups in stabilizing ecosystems [44]. The five networks of *V. natans* were found to have varying numbers of module hubs and connectors. The rhizosphere network had only one module hub, indicating a higher level of orderliness compared to other networks. It has been established that more module hubs can maintain and stabilize microbial community structure [45]. The rhizoplane network had the highest number of connectors, allowing it to organize a series of modules into a complete community. This enhances the efficiency of energy metabolism, nutrient cycling, and material transformation in the environment [46]. Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, and Firmicutes were identified as key OTUs in the five networks. As mentioned earlier, these key phyla or classes play crucial ecological roles within bacterial communities. These findings highlight the significant influence of keystone species on bacterial community structure and functioning [8].

3.5. Functional Prediction of Bacterial Communities in Different Plant Compartments

Many microorganisms are involved in crucial biogeochemical processes and interspecies interactions. The putative functions of FAPROTAX are mainly used to analyze the functions of biogeochemical cycles of microorganisms, such as the circulatory functions of sulfur, carbon, hydrogen, and nitrogen. According to the classification annotation of 16S rRNA sequences, 833 OTUs were classified into 28 functional groups (Figure 6). Although the most common functions of bacterial communities in different plant compartments were aerobic chemoheterotrophy, chemoheterotrophy, and nitrogen fixation, there was no significant difference in relative abundance between the three functional types ($p > 0.05$; Figure 7). Chemoheterotrophy and aerobic chemoheterotrophy were mainly contributed by the abundant Proteobacteria. A comparison of the different compartments shows that the functional populations related to human and animal diseases (such as animal parasites or symbionts, human pathogens (all), and human pathogens (pneumonia)), ureolysis, and nitrate reduction were more abundant in the rhizosphere, while methylotrophy, methanol oxidation, methanotrophy, and hydrocarbon degradation were higher in the endosphere. These results indicate that the bacterial community in the *V. natans* samples mainly participates in biogeochemical functions, where the carbon cycle, nitrogen cycle, and fermentation are rich in autotrophic functions (phototrophy, photoautotrophy, oxygenic photoautotrophy), as well as in animal parasites or symbionts, human pathogens (all), methylotrophy, methanol oxidation, methanotrophy, and hydrocarbon degradation.

To further explore the relationship between plant compartments and microbial communities, we compared the functional potential of the bacterial communities in different plant compartments. FAPROTAX can effectively predict the variations of bacterial ecological functional profiles [47]. The dominant functions were chemoheterotrophy and aerobic chemoheterotrophy involved in C cycling, which is consistent with the research on the main potential functions of other aquatic plants [48]. Heterotrophic bacteria are often used as decomposers and are responsible for in situ pollution repair and degradation of organic matter in ecosystems [49]. This observation agrees with the idea that most bacteria have evolved flexible and diverse strategies to use available carbon sources [50]. Nitrogen is one of the key indicators of water quality in lake ecosystems, and its excessive emissions will lead to global problems, such as eutrophication, water deterioration, and even harm to human health [51]. Nitrogen fixation is the primary reaction in the biogeochemical cycling of nitrogen [52], and it is also one of the most important functions of the bacterial community related to *V. natans*. Similar studies reported that the sediment and epiphytic bacterial community of *V. natans* had important functions in nitrogen fixation [53]. Therefore, the functions of *V. natans*-related bacterial communities were mainly concentrated within the lake biochemical cycle, especially the cycle of carbon and nitrogen.

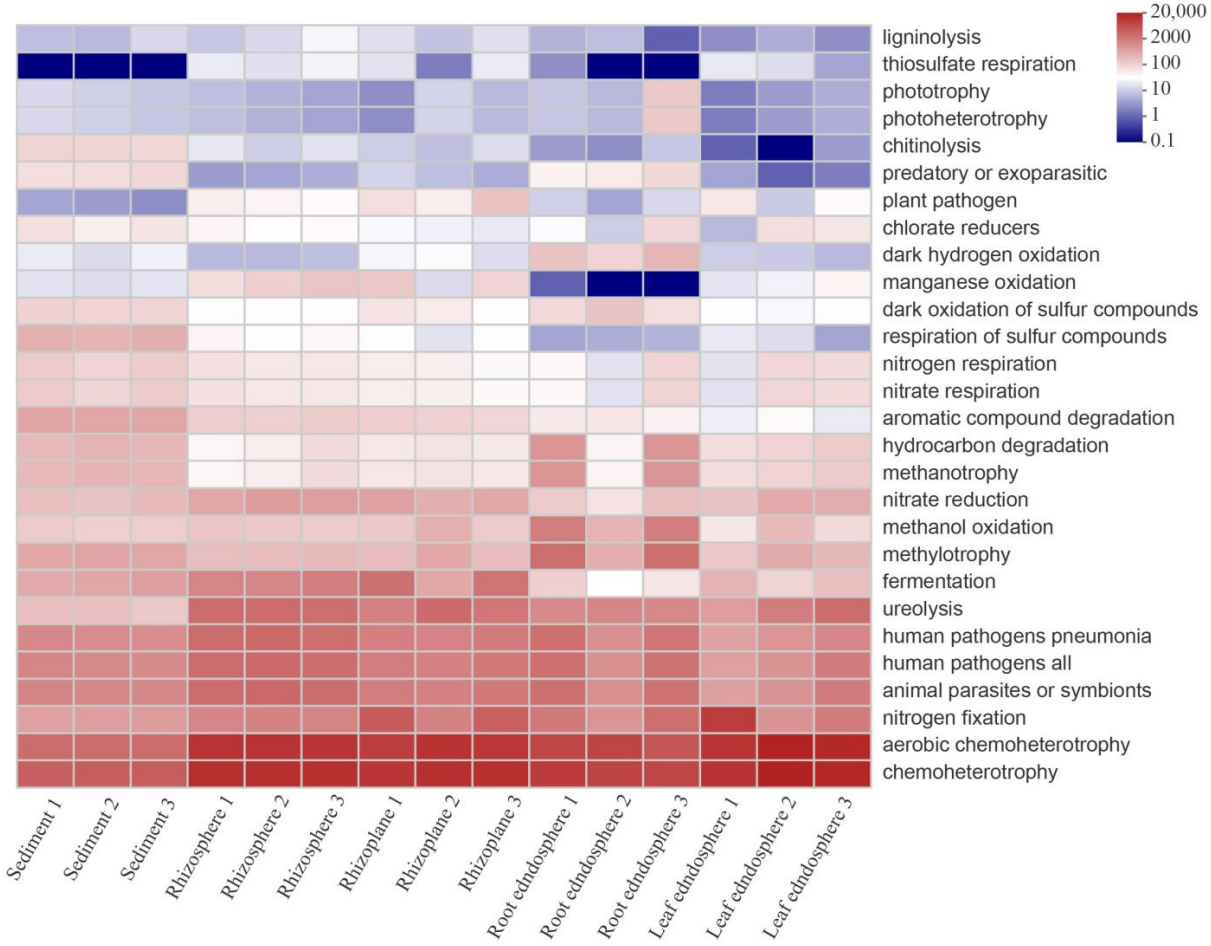

Figure 6. Based on the FAPROTAX database, the potential functions of bacterial communities in different plant compartments were displayed by heatmap.

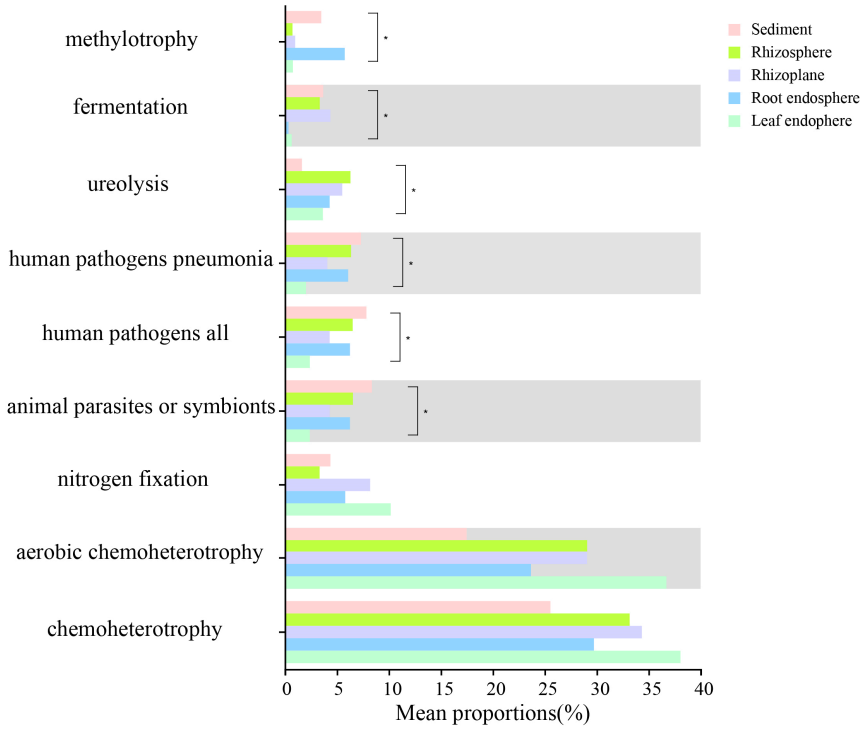

Figure 7. The top ten functional groups in relative abundance in different samples. (* $p < 0.05$).

4. Conclusions

In summary, we observed significant effects of plant compartments on plant-related microorganisms. These effects extend to all aspects of the bacterial community, including the OTU distribution, diversity, community composition, co-occurrence networks, and the mutualism between the potential function of the microbiome and different plant compartments of the *V. natans*. There were significant differences between sediment and other plant compartments, although two endosphere samples (root and leaf endospheres) had some similarities. It was proven that different plant compartments of *V. natans* greatly influenced its microbial community in sediment. Our network and functional prediction analyses revealed different co-occurrence patterns of bacterial communities in different plant compartments and the relative abundance of functional groups. Although the application value of *V. natans* in water environment improvement and ecological restoration is gradually being explored, the technology based on *V. natans* for aquatic plant restoration inevitably has certain limitations. This study provides theoretical and technical data for the future work of isolating beneficial bacteria from plants.

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