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Bacterial Communities in *Zostera marina* Seagrass Beds of Northern China

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Abstract: Microbial communities associated with seagrass beds play a crucial role in maintaining the balance of seagrass ecosystems. However, the driving mechanisms behind the structure and functional succession of seagrass microbial communities are still unclear despite the close interaction between seagrass and surrounding microorganisms. To enhance our knowledge of the diversity and functional characteristics of microbial communities in seagrass beds, we employed 16S rRNA gene amplicon sequencing to investigate bacterial communities in seagrass leaves, roots, seawater, and sediments in Caofeidian *Zostera marina* seagrass beds of Hebei Province, Northern China. Our results highlighted that specific types of bacteria were enriched in different sample compartments, indicating the importance of habitat in influencing microbial diversity and community structure in seagrass bed ecosystems. Notably, the microbial community structure of seagrass leaves and roots showed more similarity to that found in seawater and sediments. Among all the samples, the phylum Pseudomonadota exhibited the highest relative abundance, particularly in sediment samples where they accounted for over 95% of the total bacterial population. In addition, the enrichment of *Vibrio*, an opportunistic pathogen in several plant samples, alerted us to seagrass and its surrounding marine environments. Finally, functional predictions of microbial communities using PICRUSt2 revealed variations in microbial functions, indicating specific metabolic preferences of microbial communities in different natural environments. The present research sheds light on the mechanisms underlying microbial community succession and their ecological function in seagrass beds.

Keywords: seagrass; *Zostera marina*; bacteria; high throughput sequencing; functional prediction; community structure

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1. Introduction

Seagrass meadows play a vital role in marine ecosystems as they convert carbon dioxide into organic matter through photosynthesis and release oxygen, help mitigate climate change, and maintain oxygen supplies to marine ecosystems [1]. In addition, seagrass meadows serve as important habitats and food sources for a diverse range of marine organisms, contribute to the complexity of the marine food web and ensure the overall health of marine ecosystems [2]. Moreover, seagrass meadows can stabilize sediment at the seabed, effectively reduce erosion caused by water flow, prevent coastal soil erosion, and help maintain the stability of shorelines. Nevertheless, the seagrass ecosystem now encounters increasingly severe challenges stemming from climate change, pollution emissions, overfishing, marine engineering disturbances, and pathogenic invasions [3–6]. Thus, higher requirements have been proposed for environmental monitoring and risk assessment related to marine areas. Seagrass beds must undergo more frequent and comprehensive monitoring than in the past.

Among the various factors influencing seagrass beds, physical and chemical factors are the most studied aspects. Physical factors include coastal construction and typhoons influencing the physical parameters of seagrass beds. Chemical factors include the discharge

of pollutants such as nutrients [7], heavy metals [8,9], sulfides [10,11], polycyclic aromatic hydrocarbons [12], and microplastics [13]. Revealing the adverse effects of these physical and chemical factors can provide supporting data for the protection and restoration of seagrass bed ecosystems. However, biological factors are often neglected in field studies of seagrass beds.

Microorganisms play an important role in maintaining the stability and health of seagrass bed ecosystems. These microorganisms degrade refractory organic matter, dead seagrass, phytoplankton and animals, and release small molecular compounds. They participate in marine carbon, nitrogen, and sulfur cycles by changing the redox state of sediments through metabolic processes, affecting the form and availability of various elements in sediments [14–16]. In addition, microorganisms resist plant pathogen invasions by forming a symbiotic relationship with seagrass. In fact, our knowledge of microbial community structure and function in seagrass beds is still limited. However, a few studies have indicated that seagrass species and the surrounding environments may influence microbial composition and metabolic characteristics [17,18]. More information is needed about the dynamic variation of the seagrass microbial community on a larger scale, as well as diverse environmental conditions to uncover their succession mechanisms in this particular ecosystem.

The diversity and function of microbial communities on seagrass surfaces of various species generally differ from those in surrounding seawater and sediment, exhibiting greater stability and specialization. Due to variations in habitat conditions, resource availability, and interactions with host plants, the diversity and function of microbial communities inhabiting seagrass surfaces exhibit different patterns than those found in the surrounding environment. The microbial community on seagrass surfaces can promote seagrass growth and health through nutrient assimilation assistance and pathogen resistance. By contrast, microbial communities in the surrounding seawater and sediment are more susceptible to environmental factors due to their greater diversity and flexibility in ecological characteristics and biological functions. Microbial communities associated with seagrass leaves participate in nutrient cycling and organic matter degradation and protect seagrass beds. Those surrounding seawater and sediment also have distinct functions [19]. However, a global study of the microbiome of *Z. marina* eelgrass showed a higher degree of similarity between leaf and seawater communities than that of root surfaces [15]. Therefore, elucidating the distribution characteristics of microbial communities among different plant hosts in various habitats is conducive to understanding the specific roles played by microorganisms in the seagrass bed ecosystems they depend on.

In this study, we investigated the diversity and functional characteristics of bacteria on seagrass surfaces (leaves and roots) and in the surrounding environment (seawater and sediment) in the Caofeidian seagrass bed of Hebei Province, China. By analyzing various biological and non-biological factors, we aimed to enhance our understanding of the relationships between microbial communities and their ecological functions in different geographical locations and environmental conditions. The results of this study will help us elucidate the succession mechanisms of bacterial communities. It will also provide significant insights into the conservation and management of seagrass ecosystems.

2. Materials and Methods

2.1. Study Area

The sampling area is located in the Caofeidian seagrass beds in Hebei Province, Bohai Bay, Northern China (Figure 1). The Caofeidian seagrass bed is the largest seagrass bed found in the Yellow Sea and Bohai Sea of China, mainly consisting of *Z. marina*. The seagrass population is patchily distributed throughout the entire seagrass bed, covering an area of 43.16 km^2. In the last few decades, there has been a significant degradation zone at the edge of the Caofeidian seagrass bed [20]. The overall distribution trend from densely populated continuous seagrass beds to peripheral bare sand areas can be described as "continuous bed-hollow zone-patchy distribution zone-bare sand area", with smaller

patch areas observed toward the outer periphery of the seagrass bed (https://baijiahao.baidu.com/s?id=1768653032372973780&wfr=spider&for=pc (14 June 2023)). Since 2022, the natural resources protection agency of Hebei Province initiated the Caofeidian seagrass bed protection and restoration project, following the principle of "natural restoration as the main approach, supplemented by human intervention" (https://zrzy.hebei.gov.cn/heb/xinwen/stdt/10866296544852197376.html (accessed on 14 June 2023)). Damaged seagrass beds are being restored using nature-oriented measures such as seed sowing, substrate restoration, and seedling transplantation, aiming to effectively alleviate seagrass bed degradation and promote the healthy and sustainable development of relevant marine ecosystems (https://zrzy.hebei.gov.cn/heb/xinwen/stdt/10866296544852197376.html (accessed on 14 June 2023)).

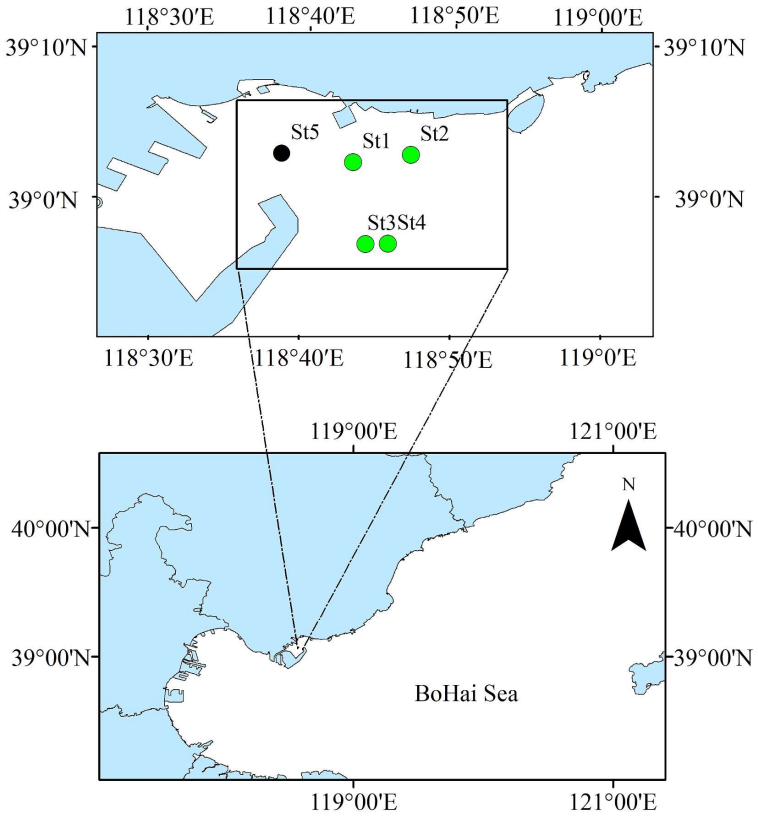

Figure 1. Sampling stations in the present study.

2.2. Sample Collection and Pretreatment

Five stations (St1~St5) were set up in the seagrass bed area of *Z. marina* seagrass bed on 28 September 2022, and 16 samples were collected (Supplementary Table S1). Among them, St1~St4 were seagrass stations and St5 was a control station without seagrass. Sample types included seagrass leaves (L1, L3, and L4), roots (R1, R3, and R4), seawater (W1, W2, W3, W4, and W5), sediment samples (S1, S2, S3, and S4), as well as leaf surface attachments from station St3 (L3F) (Figure 2). A 0.09 m^2 sample of the seagrass bed was carved and collected using a spade and placed in a bucket. Sediment samples with a depth of about 5 cm were collected using a vibrating drill and transferred to a 50 mL conical tube. The rest of the seagrass was placed in a sterile bag. Seawater was collected from above the seagrass meadow at the sampling site using a 2 L sterile glass bottle. All samples were placed on ice and quickly transferred to the lab. They were stored at 4 °C until the next day [21].

Figure 2. Seagrass sample. The above are seagrass leaves. The lower left image features roots, and the lower right image features leaf surface attachments.

2.3. Determination of Environmental Parameters

Physical parameters, such as depth, temperature, transparency, suspended matter, salinity, and dissolved oxygen, as well as chemical parameters, such as active phosphate, nitrite, nitrate, ammonium salt, and petroleum hydrocarbon were measured in seawater (W1, W2, W3, and W4) at experimental stations. Except for transparency and suspended matter, the other physical parameters were determined using a portable YSI Pro Plus Multiparameter instrument (YSI Inc., Yellow Springs, OH, USA). Transparency, suspended matter, and chemical parameters were determined according to the Specification for the Oceanographic Survey (GB/T 12763.4-2007).

2.4. DNA Extration

2.4.1. Pretreatment of Seagrass Leaf and Root Samples

Seagrass was gently rinsed with autoclaved seawater to remove any loose sediment. The leaves and roots were subsequently separated using a sterile blade. For the blade, 4 cm long segments were cut from the center of the blade. To analyze the rhizosphere, the roots were separated from the rhizomes and shoots, then cut to a length of 4 cm. Approximately 10 cut leaves and 30 cut roots were placed into individual 50 mL conical test tubes containing 30 mL of sterile seawater. Subsequently, these tubes were placed in an ultrasound bath for 12 s [21]. The sample was transferred to a new conical tube containing 30 mL of sterile seawater using sterile tweezers. It was then subjected to a second ultrasound treatment for 12 s at a frequency of 42 kHz. The third ultrasonic treatment followed the same procedure, lasting for 1 min. Finally, the eluent obtained after three ultrasonic treatments was combined. The eluent was pre-filtered using a 5 μm cellulose acetate membrane, which was subsequently discarded. The filtrate was then coated with 0.22 μm polycarbonate film. The filter membrane was collected, and DNA extraction was performed using the Omega water DNA kit. The DNA extract was stored at −20 °C until it was used for high-throughput sequencing analysis.

2.4.2. Pretreatment of Seawater Samples

Seawater samples were also filtered using a 5 µm cellulose acetate membrane and a 0.22 µm polycarbonate membrane, respectively. A 0.22 µm filter membrane was retained for DNA extraction using Omega water DNA kit (Omega Bio-tek, Inc., Norcross, GA, USA). The DNA extract was stored at −20 °C until used for high-throughput sequencing analysis.

2.4.3. Pretreatment of Sediment Samples

Approximately 0.3 g of sediment was obtained from the surface of a conical tube containing sediment samples. DNA was extracted using the Omega Soil DNA kit (Omega Bio-tek, Inc., Norcross, GA, USA) [21]. The DNA extract was kept at −20 °C until used for high-throughput sequencing analysis.

2.5. High-Throughput Sequencing and Statistical Analysis

High-throughput sequencing with an Illumina NovaSeq platform and analysis work was completed at Hangzhou Lianchuan Biotechnology Co., LTD (Hangzhou, China). The bacterial 16S rRNA gene was amplified using universal primers 341F (5′-CCTACGGGNGG CWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) with total DNA as the template. The PCR protocol was as follows: initially 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 54 °C for 30 s and 72 °C for 45 s, and finally 72 °C for 10 min. The PCR products were confirmed by 2% agarose gel electrophoresis and AMPure XT beads recovery kit (Beckman Coulter, Inc., Pasadena, CA, USA) was used for recovery. The NovaSeq 6000 sequencer (Illumina, Inc., San Diego, CA, USA) was used for 2 × 250 bp paired-end sequencing. For paired-end data obtained by sequencing, the sample should first be separated according to barcode information and the joint and barcode sequence should be removed. The data was then spliced (http://ccb.jhu.edu/software/FLASH/), filtered (http://ccb.jhu.edu/software/fqtrim/), and denoised using Qiime2 (https://qiime2.org/). Thus, the amplicon sequence variant (ASV) feature sequences were obtained.

The alpha and beta diversity analyses were conducted based on the obtained ASV feature sequences. Alpha diversity was used to analyze species diversity complexity within one sample. We employed four indices, including observed species, Shannon, Simpson, and Pielou's evenness index. All the indices for our samples were computed using QIIME2. Additionally, one-way ANOVA analysis and Duncan's multiple range test were adopted to determine pairwise comparisons of bacterial communities in *Z. marina* seagrass beds with a significance of $p < 0.05$. Beta diversity was also calculated using QIIME2, and the resulting graphs were generated using the R package (v3.5.2). According to the ASV (feature) sequence file, species annotation was conducted using the SILVA and NT-16S databases. The abundance of species in each sample was counted according to ASV abundance. The confidence threshold for species annotation was 0.7. Functional predictions of microbial communities were performed using PICRUSt2 software. Additionally, OmicStudio tools (https://www.omicstudio.cn) were used to perform the unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering analysis based on unweighted UniFrac, non-metric multidimensional scaling analysis (NMDS), and figure creation. Major bacterial taxa in different seagrass leaf samples were examined by one-way analysis of variance using SPSS Statistics 19 software (International Business Machines Corporation, Armonk, NY, USA). Pearson's correlation analysis was conducted using OriginPro 2016 software to determine correlations between the top 30 bacterial species in the genus and class levels in abundance and environmental factors in the present study.

3. Results

3.1. Alpha Diversity

A total of 8116 ASV feature sequences were obtained based on samples in the present study. We calculated the alpha diversity of all the samples, including the Shannon index, Simpson index, and Pielou's evenness index, as shown in Table S2. The rarefaction curves indicate that the sample size and sequencing depth are adequate (Figure S1). Among all the

seagrass stations, the Simpson index and number of bacterial species in the seagrass leaves, roots, and seawater were significantly higher than in sediment ($p < 0.05$) (Figure 3A,C). In addition, the Shannon index and Pielou's evenness index of bacteria on leaf surfaces and roots were significantly higher than that in seawater and sediments ($p < 0.05$) (Figure 3D).

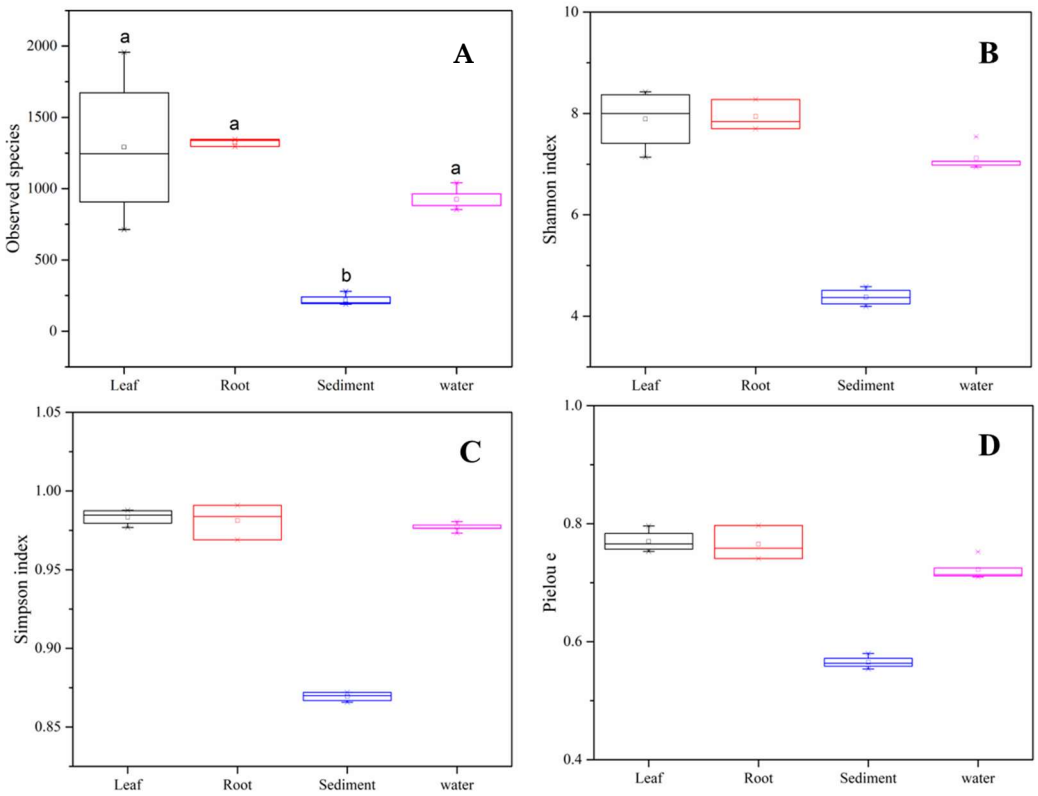

Figure 3. Alpha diversity index of bacteria communities in *Z. marina* seagrass beds, including (**A**) observed species, (**B**) Shannon index, (**C**) Simpson index, and (**D**) Pielou's evenness index. The same letter on the box indicates that the difference is not significant and vice versa.

Alpha diversity analysis suggests that bacterial communities exhibited higher abundance and evenness in leaves and roots than in sediment samples. Additionally, higher bacterial diversity occurred in the leaves and roots, while sediment exhibited lower diversity. However, the similarity of Pielou's evenness index between leaf and root samples indicates that the species composition in the bacterial community is similar. Additionally, compared to the seawater samples in the control station without seagrass (W5), the Shannon index and number of observed species in the experimental seawater samples with seagrass (W1~W4) were lower (Figure 3A,B). This finding suggests that seagrass may contribute to bacterial enrichment in this particular ecosystem.

3.2. Beta Diversity

Figure 4 illustrates a UPGMA hierarchical clustering analysis using unweighted UniFrac and NMDS analysis based on Bray–Curtis. The UPGMA analysis shows that all samples are clustered into two branches, with sediment samples (S1–S3) forming a separate branch (Branch I), and leaf, root, and seawater samples clustering together in another branch (Branch II) (Figure 4A). Within Branch II, the leaf and root samples are closer to each other, but farther from the seawater samples. The NMDS results are similar to the UPGMA analysis (Figure 4A,B). All samples are scattered and clustered into three regions, with sediment samples in the upper right corner, seawater samples below, and leaf and root samples on the left side.

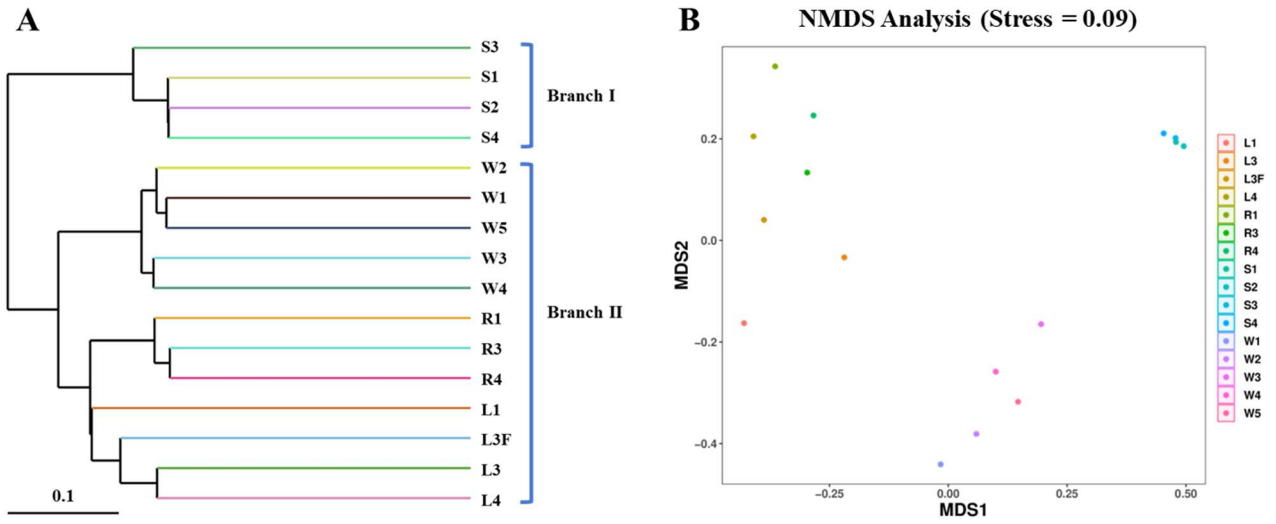

Figure 4. Unweighted pair group method with arithmetic mean (UPGMA) using unweighted UniFrac and Non−metric Multidimensional Scaling Analysis (NMDS) based on Bray−Curtis. (**A**) Hierarchical clustering analysis; (**B**) NMDS analysis.

The bacterial community structure of leaf, root, and seawater samples is more similar than sediment samples. The leaf and root samples were clustered away from seawater samples, indicating greater similarity in bacterial community composition between the two. However, leaf and root samples are also clustered separately, suggesting a higher similarity in bacterial community structure among samples of the same type. NMDS analysis indicated a higher similarity in bacterial community structure between leaf and root samples. However, the leaf and root samples were close to each other, suggesting that sample type is a determining factor in bacterial community structure. These results indicate that sample type is an important factor in determining bacterial diversity and community composition in seagrass beds. Different bacterial communities are distributed in varied niches of seagrass habitats, collectively maintaining seagrass ecosystem stability.

3.3. Bacterial Community

There are significant differences in bacterial community compositions at the phylum level in different types of samples (Figure 5A). The relative abundance of Pseudomonadota, especially in sediment samples, was the highest, accounting for over 95% of total bacterial abundance (Figure 5A). Except for Pseudomonadota, sediment samples showed relatively high abundance in the phyla of Actinobacteriota and Firmicutes (Figure 5A). In seawater, the other abundant phyla, apart from Pseudomonadota, were Actinobacteriota, Bacteroidota, Cyanobacteria, and Firmicutes (Figure 5A). In root samples, Campylobacterota, Bacteroidota, Firmicutes, and Desulfobacterota also accounted for a relatively high proportion. In addition, there were significant differences in bacterial community composition among different leaf samples (Figure 5A). Apart from Pseudomonadota, Bacteroidota had a relatively high abundance in all leaf samples. Compared to other leaf samples, Firmicutes, Patescibacteria, and Desulfobacterota had significantly higher relative abundance in leaf sample L1 ($p < 0.05$) (Figure 5A). Cyanobacteria showed significantly high relative abundance in leaf sample L3. It is worth noting that the bacterial community composition of the L3F sample collected from the leaf attachment of St3 was significantly different from L3 but more similar to L4 (Figure 5A).

According to hierarchical clustering analysis of bacterial communities at the genus level, samples of the same type are grouped together. These patterns indicate that the habitat environment is an important factor affecting seagrass beds' microbial communities. Different sample types enrich specific bacteria types (Figure 5B). The sediment sample forms a separate branch and is separated from other samples, with a high abundance of

Herminiimonas (Gammaproteobacteria) from the phylum Pseudomonadota, accounting for more than 30% and occupying an absolute advantage (Figure 5B). Additionally, the sediment samples contain relatively higher proportions of certain bacteria, such as *Pseudomonas* (Gammaproteobacteria), *Hafnia-Obesumbacterium* (Gammaproteobacteria), *Herbaspirillum* (Gammaproteobacteria), *Stenotrophomonas* (Gammaproteobacteria), *Bosea* (Alphaproteobacteria), and *Ralstonia* (Gammaproteobacteria) (Figure 5B).

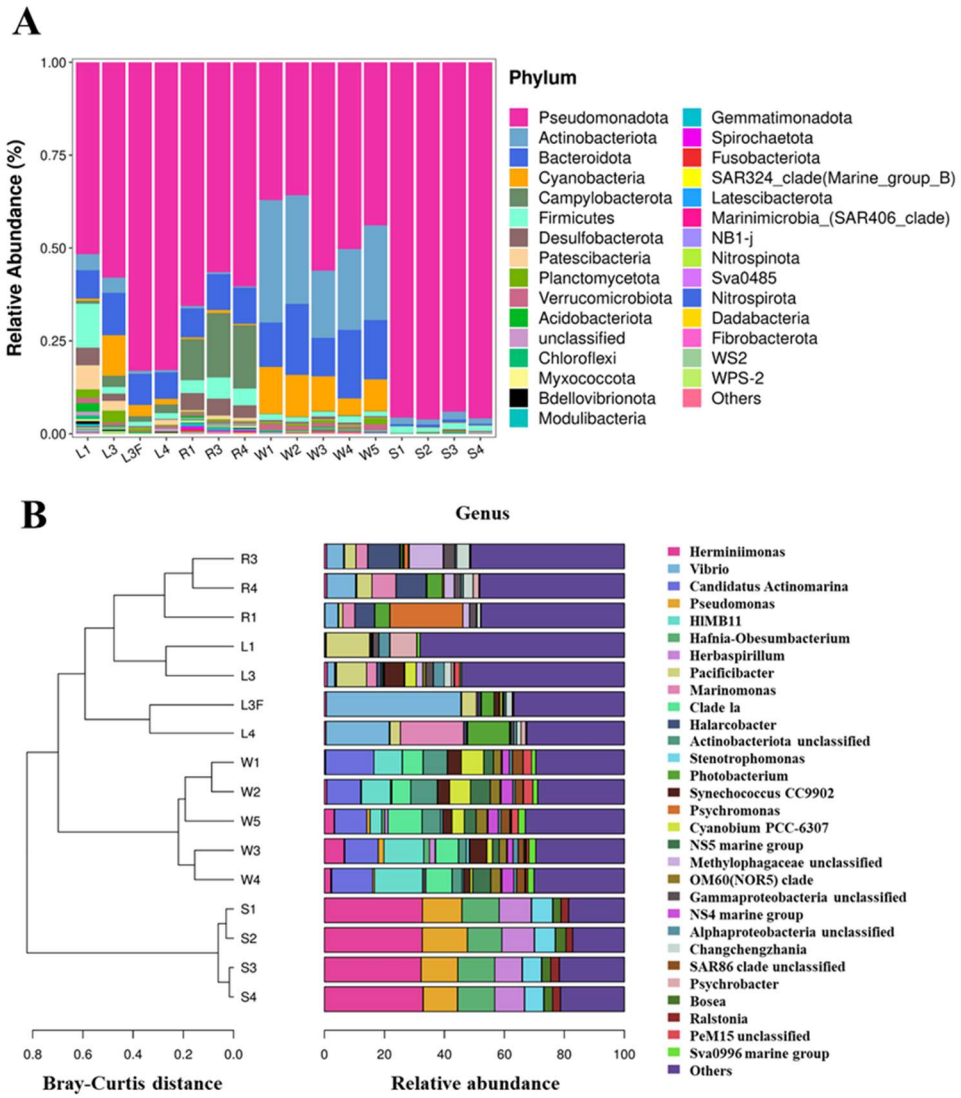

Figure 5. Bacterial community composition of seagrass beds at the phylum and genus levels. (**A**) Bacteria communities at the phylum level; (**B**) bacterial communities at the genus level.

The leaf and root samples were grouped together, with L3F and L4 forming a separate branch, and were rich in *Vibrio*, particularly with a relative abundance of over 40% in L3F (Figure 5B). In the L4 sample, *Marinomonas* and *Photobacterium* were significantly enriched. *Halarcobacter* was significantly enriched in the root sample, and *Psychromonas*, a psychrophilic bacterium of the genus Flavobacteriaceae, dominated in the R1 sample (Figure 5B). The seawater samples were grouped together, with significant enrichment of *Candidatus Actinomarina* (Actinobacteriota), HIMB11 (Alphaproteobacteria), Clade Ia (Alphaproteobacteria), unclassified PeM15 (Actinobacteriota), NS5 marine group (Bacteroidota), NS4 marine group (Bacteroidota), unclassified Actinobacteriota (Actinobacteriota), unclassified SAR86 clade (Gammaproteobacteria), and *Cyanobium* PCC-6307 (Cyanobacteria) (Figure 5B).

There are obvious differences in microbial communities between seawater and sediment samples. The seawater samples contained a relatively high abundance of *Candidatus Actinomarina* (Actinobacteriota), HIMB11 (Alphaproteobacteria), Clade Ia (Alphaproteobacteria), unclassified PeM15 (Actinobacteriota), NS5 marine group (Bacteroidota), NS4 marine group (Bacteroidota), unclassified Actinobacteriota (Actinobacteriota), unclassified SAR86 clade (Gammaproteobacteria), and *Cyanobium* PCC-6307 (Cyanobacteria) (Figure 5B). In addition, the sediment was enriched with *Pseudomonas* (Gammaproteobacteria), *Hafnia-Obesumbacterium* (Gammaproteobacteria), *Herbaspirillum* (Gammaproteobacteria), *Stenotrophomonas* (Gammaproteobacteria), *Ralstonia* (Gammaproteobacteria), and *Bosea* (Alphaproteobacteria) (Figure 5B).

From the perspective of spatial distribution, except for station St1, the leaf and root samples at other stations contained abundant *Changchengzhania* (Bacteroidota), *Marinomonas* (Gammaproteobacteria), *Photobacterium* (Gammaproteobacteria), Methylophagaceae unclassified (Gammaproteobacteria), *Vibrio* (Gammaproteobacteria), and *Halarcobacter* (Campylobacterota) (Figure 5B). The root samples of station St1 also contained a relative abundance of *Psychrobacter* (Gammaproteobacteria), *Pacificibacter* (Alphaproteobacteria), and *Psychromonas* (Gammaproteobacteria) (Figure 5B).

3.4. Indicator Species Analysis

Bubble charts use changes in bubble size and color to visually represent species annotation and abundance data in a two-dimensional matrix. In this study, the top 30 abundant genera were analyzed at different taxonomic levels to identify species that could serve as biomarkers (Figure 6). When comparing the genera composition of different sample types, significant enrichment in sediment samples was observed for the genera *Hafnia-Obesumbacterium*, *Herbaspirillum*, *Herminiimonas*, and *Pseudomonas* belonging to the class Gammaproteobacteria. A marked enrichment in seawater samples was noted for Clade Ia, HIMB11, *Candidatus Actinomarina* of the phylum Actinobacteria, as well as for the NS5 and NS4 marine groups of the phylum Bacteroidota. In addition, *Halarcobacter* was enriched in root samples and can be considered a candidate indicator.

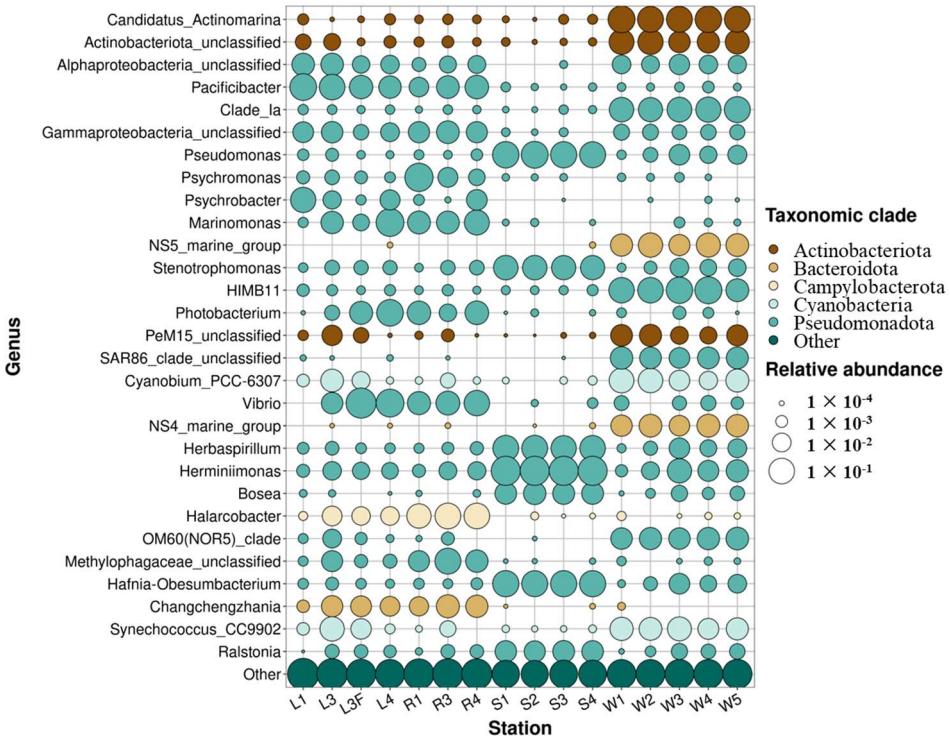

Figure 6. Bubble map using the top 30 bacterial genera in abundance of seagrass bed bacteria.

3.5. Correlation Analysis between Bacterial Communities and Environmental Parameters

This study examined the physical factors (depth, temperature, transparency, suspended matter, salinity, dissolved oxygen) and chemical factors (active phosphate, nitrite, nitrate, ammonium, petroleum hydrocarbons) of seawater samples from four experimental sites in Caofeidian seagrass beds (Supplementary Table S3). Pearson's correlation analysis revealed that, among the top 30 bacterial genera in abundance, significant correlations ($p > 0.05$) were observed between various environmental factors, such as transparency, nitrite, nitrate, ammonium, and active phosphate (Table 1). *Vibrio* showed a significant positive correlation with transparency ($p < 0.05$), while unclassified Alphaproteobacteria and *Bosea* showed a significant positive correlation with nitrite ($p < 0.05$). Unclassified Alphaproteobacteria showed significant negative correlations with nitrate and ammonium ($p < 0.05$), and *Photobacterium* showed a significant negative correlation with ammonium. Unclassified Methylophagaceae showed a significant negative correlation with active phosphate ($p < 0.05$). Furthermore, at the class level, Parcubacteria showed a significant positive correlation with DO ($p < 0.05$), and Moduliflexia showed a significant negative correlation with salinity ($p < 0.05$) (Table S4). Both Anaerolineae and Desulfovibrionia exhibited significant positive correlations with nitrite ($p < 0.05$) but significant negative correlations with nitrate ($p < 0.05$).

Table 1. Significant correlations ($p < 0.05$) between the top 30 bacterial genera in abundance and environmental factors.

Bacteria	Transparency	Nitrite	Nitrate	Ammonium	Active Phosphate
Vibrio	0.964 *	0.039	−0.302	−0.497	−0.859
Alphaproteobacteria unclassified	0.539	0.962 *	−0.996 *	−0.973 *	0.165
Bosea	0.356	0.951 *	−0.926	−0.918	0.375
Photobacterium	0.724	0.844	−0.939	−0.998 *	−0.061
Methylophagaceae unclassified	0.741	−0.331	0.072	−0.050	−0.985 *

Note: * indicates a significant correlation ($p < 0.05$).

3.6. Functional Analysis

Functional predictions of microbial communities in leaf, root, seawater, and sediment were conducted using PICRUSt2 (Figure 7). The top 10 predicted functions and their relative abundance ranges were as follows: overall overview (66.80~68.84%), membrane transport (6.43–14.13%), amino acid metabolism (9.04–11.55%), carbohydrate metabolism (8.17–9.89%), replication and repair (7.02–8.38%), energy metabolism (5.10–6.54%), translation (4.03–5.71%), cellular processes and signaling (2.90–4.59%), coenzyme factors and vitamin metabolism (3.73–4.65%), and cell motility (2.21–4.81%) (Figure 7). Among the top 22 predicted functions at level 2, microbial communities in all sample types (leaf, root, seawater, and sediment) were enriched in metabolic pathways (amino acid metabolism, carbohydrate metabolism, energy metabolism, coenzyme factors, and vitamin metabolism, nucleotide metabolism, lipid metabolism, biodegradation, and metabolism of polysaccharides, enzyme families, terpenoids, and polyketides metabolism), gene information processing (replication and repair, translation, folding, sorting and degradation, genetic information processing, transcription), environmental information processing (membrane transport and signal transduction), and cellular processes (cellular processes, signal transduction as well as cell motility).

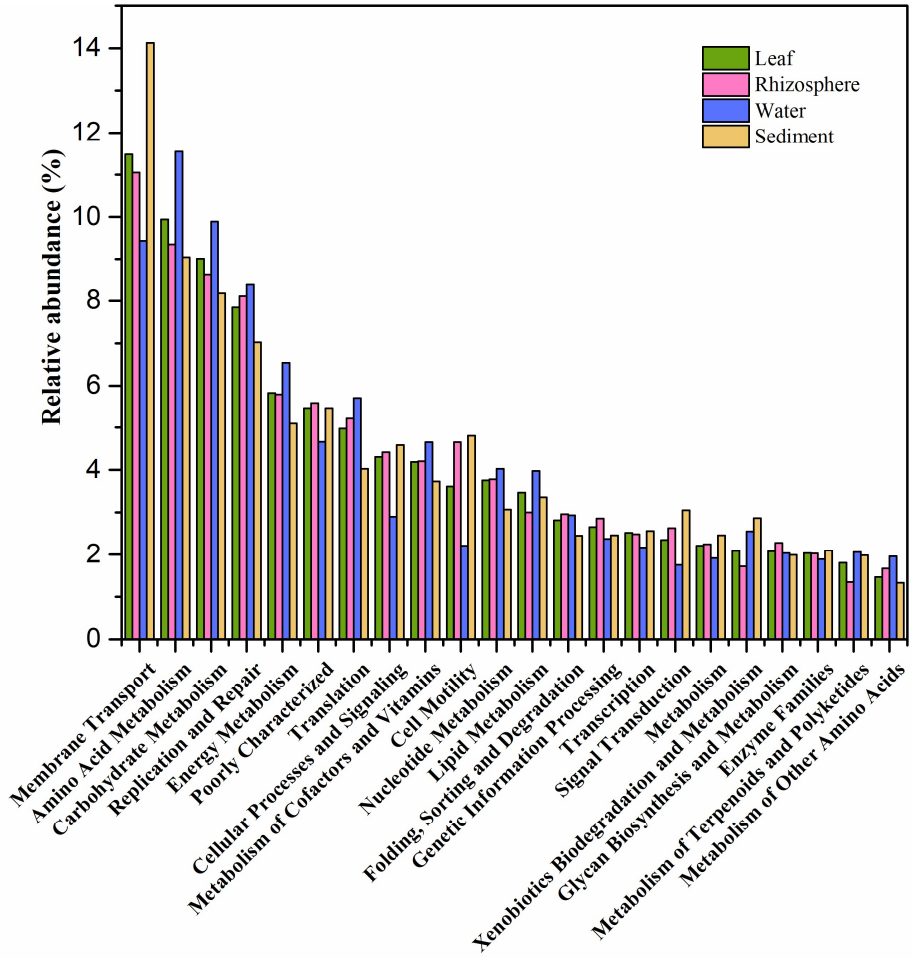

Figure 7. Results of functional prediction of bacterial communities in leaves, roost, seawater, and sediment by PICRUSt2.

4. Discussion

Studies have shown that the composition of leaf-associated microorganisms in terrestrial plants differs from the microbial communities in the surrounding air samples, whereas the composition of leaf-associated microorganisms in macroalgae is similar to the microbial communities in the surrounding seawater [22–24]. However, we found that bacterial community compositions between the leaves of seagrass *Z. marina* and the seawater samples. In addition, the bacterial diversity and abundance in the sediment samples were lower than in other sample types (leaves, rhizosphere, and seawater), which is contrary to other studies on seagrass microbials [21]. These results can be attributed to differences in seagrass beds' environmental conditions in different marine areas. Therefore, future studies on the distribution patterns of microbes across different ecological niches and habitats associated with various seagrass species will help further uncover the ecological significance of microbial communities. Furthermore, the composition of seagrass microbial communities may change with varying environmental conditions. In the seagrass meadow of Aqaba Bay in the Northern Red Sea, the composition of microbial communities associated with the leaf surface of the seagrass *Halophila stipulacea* varied among different sampling sites and different plant assemblages [25]. Gammaproteobacteria and Bacteroidetes were more abundant under light-limiting conditions, whereas Cyanobacteria and Rhodobacteraceae were more abundant under strong light and hydrodynamic conditions [25]. Seagrass plants and microbial communities interact with each other. The composition of seagrass-associated microbial communities may serve as early indicators

for assessing the ecological health of seagrass ecosystems, as well as comprehensively and timely identifying environmental pressures.

The high abundance of Cyanobacteria in seawater samples can be ascribed to the favorable light and hydrodynamic conditions prevalent in marine environments. In this study, the relative abundance of Cyanobacteria was also high in several leaf samples. This observation can be attributed to environmental factors, particularly oligotrophic conditions resulting from deeper water levels. Such conditions enhance hydrodynamic properties and reduce nitrate and ammonium salt concentrations. Similar to Mejia et al. (2016), our results prove that Cyanobacteria's relative abundance is higher in oligotrophic sites with higher effective light and lower chlorophyll content [25].

Vibrio is widely distributed in both coastal and deep-sea environments with high metabolic flexibility [26]. In the present study, *Vibrio*, which is reportedly rare in healthy seagrass, was enriched in several samples [27]. This finding suggests that the studied area of these samples may be under anthropogenic pressure from coastal development and pollution. Although some *Vibrio* species benefit seagrass ecosystems, certain strains can also be pathogenic. They may cause seagrass diseases and negatively impact the health and stability of seagrass beds, which are related to the specific genomic and environmental conditions of *Vibrio* strains [28]. Furthermore, leaves and roots have similar microbial compositions, and both are different from seawater and sediment. These results differ from those in published papers, which asserted that root communities were more similar to sediments than water samples [21]. However, Pseudomonadota (Gammaproteobacteria and Alphaproteobacteria) is usually the dominant group in sediment, as was observed in the present study, since Gamma-proteobacteria were also enriched in the sediment samples.

There was an obvious difference in microbial communities between seawater and sediment samples. The genus *Pseudomonas* is significantly enriched in sediment samples from the Caofeidian seagrass bed. This genus is also an important taxonomic group for the nitrogenase gene (*nifH*) in coastal marsh sediments where the northern eelgrass *Z. marina* is planted in China [14]. Previously detected species closely related to *P. stutzeri* are known for their involvement in various biogeochemical processes, including nitrate-dependent Fe(II) oxidation [29] and thiosulfate oxidation to tetrathionate and elemental sulfur [30,31]. Most bacterial species contribute to decomposing organic matter; the sediment bacterium *Herbaspirillum* also promotes plant growth and development by producing plant hormones [32]. Furthermore, bacterial communities with specific functions could rapidly and sensitively indicate the environmental state of the natural habitat. In the present study, indicator species analysis enriched some bacteria groups that might serve as representatives for investigating the microbial function of seagrass bed ecosystems.

Functional predictions using PICRUSt2 suggested relatively similar predicted functions among different sample types. It is worth noting that the most abundant predicted function in terms of relative abundance in the leaf (11.49%), root (11.05%), and sediment (14.13%) samples was membrane transport, with sediment having the highest relative abundance. In seawater samples, the most abundant predicted function was amino acid metabolism (11.55%), indicating that microbial communities in different natural environments have specific metabolic preferences, and the functional composition of microbial communities is closely related to their habitat. However, there is still a lack of a significant correlation between the actual metabolic functions and the predicted metabolic functions of environmental microbial communities [33]. However, our knowledge of seagrass bed microbial communities' actual metabolic functions is still limited. Therefore, further analyzing the actual metabolic functions of seagrass bed microbial communities more comprehensively and systematically by expanding the sampling area and using metagenomics and metabolomics techniques is crucial. These efforts will help elucidate the ecological status and environmental pressures faced by seagrass ecosystems in the future.

This study highlights the intricate interplay between seagrass-associated bacterial communities and their surrounding environments, underscoring the ecological significance of their microbial inhabitants. The distinct microbial compositions observed on

seagrass (leaves and roots) and in the surrounding seawater emphasize the unique ecological niches these plants offer, which diverge significantly from ambient marine conditions. The variability in microbial communities, influenced by factors such as light availability and hydrodynamic conditions, suggests that these microorganisms are closely attuned to their habitats' environmental nuances. The presence of specific bacterial groups, such as Cyanobacteria and *Vibrio*, as well as their association with the environmental conditions and health status of seagrass beds, further underscores these bacterial communities' potential as indicators of ecological health. This insight is particularly valuable for monitoring and preserving the ecological integrity of seagrass ecosystems, which is vital for marine biodiversity and environmental stability. Our study also provides key information for further research to explore the functional roles of microbial communities in seagrass ecosystems worldwide. These findings deepen our understanding of microbial communities and marine habitats' ecological resilience and health.

In conclusion, seagrass bed ecosystems play important roles in carbon sequestration, oxygen production, marine biodiversity maintenance, and coastline stabilization. Distinct sample types selectively enrich particular bacterial species, underscoring the critical role of habitat in shaping the microbial diversity and community structure of seagrasses. However, compared to seawater and sediments, the bacterial community structure of seagrass leaves and roots is more similar. Among all samples examined, Pseudomonadota, especially within sediment samples, exhibited the highest relative abundance at over 95% of the total bacterial population. The genus *Herminiimonas* of Pseudomonadota is significantly enriched in sediment samples. Additionally, there are differences in microbial functional profiles among different sample types, indicating microbes' specific metabolic preferences in different natural environments. Therefore, further studies are needed to gain a deeper understanding of microbial communities' roles in seagrass bed ecosystems.

Supplementary Materials: The Supplementary Material for this article can be found online at: https://www.mdpi.com/article/10.3390/w16070935/s1, Table S1: Sample information, Table S2: Alpha diversity, Table S3: Physical and chemical parameters of seawater samples, Table S4: Significant correlations ($p < 0.05$) between the top 30 bacterial class in abundance and environ-mental factors, Figure S1: Rarefaction curves. The variation of observed species (A), Shannon index (B), Pielou's evenness index (C), and Simpson index (D) with increased number of sequences.

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Data Availability Statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found as follows: https://www.ncbi.nlm.nih.gov/, SAMN38338616~SAMN38338631.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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