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The contribution of seasonal variations and *Zostera marina* presence to the bacterial community assembly of seagrass bed sediments

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Abstract

Background Microorganisms play pivotal roles in seagrass ecosystems by facilitating material and elemental cycling as well as energy flux. However, our understanding of how seasonal factors and seagrass presence influence the assembly of bacterial communities in seagrass bed sediments is limited. Employing high-throughput sequencing techniques, this study investigates and characterizes bacterial communities in the rhizosphere of eelgrass (*Zostera marina*) and the bulk sediments across different seasons. The research elucidates information on the significance of seasonal variations and seagrass presence in impacting the microbial communities associated with *Zostera marina*.

Results The results indicate that seasonal variations have a more significant impact on the bacterial community in seagrass bed sediments than the presence of seagrass. We observed that the assembly of bacterial communities in bulk sediments primarily occurs through stochastic processes. However, the presence of seagrass leading to a transition from stochastic to deterministic processes in bacterial community assembly. This shift further impacts the complexity and stability of the bacterial co-occurrence network. Through LEfSe analysis, different candidate biomarkers were identified in the bacterial communities of rhizosphere sediments in different seasons, indicating that seagrass may possess adaptive capabilities to the environment during different stages of growth and development.

Conclusions Seasonal variations play a significant role in shaping these communities, while seagrass presence influences the assembly processes and stability of the bacterial community. These insights will provide valuable information for the ecological conservation of seagrass beds.

Keywords Seagrass (*Zostera marina*) bed, Rhizosphere bacteria, Seasonal variations, Seagrass presence

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Background

Seagrasses are the only flowering angiosperms in the ocean, and seagrass beds are among the ecosystems with the highest level of marine productivity, along with coral reefs and mangroves. Seagrass beds provide critical ecosystem services, including stabilizing sediment, purifying water quality, protecting the coastline, providing a habitat for many marine organisms to survive, capturing and storing organic carbon, and serving as a blue carbon sink [1–3]. Microorganisms, as crucial components of seagrass bed ecosystems, encompass endophytic microorganisms residing within seagrass plants, epiphytic microorganisms attached to seagrass leaves, and rhizosphere microorganisms living around seagrass roots. Together, they actively participate in vital biogeochemical cycling processes within the ecosystem, including the carbon, nitrogen, phosphorus, and sulfur cycles [4–8].

The plant rhizosphere contains many microorganisms and is closely linked to the plant, with far more genes than the plant itself; therefore, the rhizosphere microbiome is also known as the second genome of the plant [9, 10]. Numerous studies on terrestrial plants have shown that different plant root secretions strongly influence the composition of rhizosphere bacterial communities [11–13]. Plants can not only secrete carbon sources into the rhizosphere environment and provide nutrients to microbial communities (thus influencing their composition and activity) but also inhibit the growth of specific rhizosphere microorganisms by secreting secondary metabolites [14–17]. As a result, microbial communities colonizing the rhizosphere environment can interact with plants in a variety of ways, which can improve plant stress resistance and productivity to a certain extent, regulate the biogeochemical cycle of biogenic elements in rhizosphere sediments, and promote plant growth by regulating the rhizosphere ecological environment [18–21].

Seagrasses mostly grow in shallow subtidal to intertidal areas, and their root systems play an important role in the nutrition, presence and spread of seagrasses [22]. To receive oxygen in the anoxic sediments of the ocean, seagrasses diffuse the oxygen produced by photosynthesis from upright stems to roots to release into the sediment, thus creating a gradient of oxygen concentrations between the rhizosphere; moreover, the root tissue also releases photosynthetically-produced reactive organic carbon into the sediment, thus creating the conditions for microbial communities to survive [23–25]. New evidence indicates that seagrass microbial communities have the ability to regulate host growth and modulate their response to environmental stress [4, 5, 26]. The seagrass microbiome not only fixes nitrogen and produces phytohormones [27, 28] but is also thought to mitigate the toxic effects of hydrogen sulfide in sediments, which has been associated with decreased seagrass health and localized mortality events [29–31]. However, compared with the well-studied rhizosphere microbes of terrestrial plants, research on seagrass rhizosphere microbes is still lacking, even though seagrass rhizosphere microbial communities play a multifaceted role in maintaining seagrass health.

In recent decades, seagrass beds have been severely degraded by human activities and natural factors [32, 33]. Bacterial communities in the sediments of seagrass beds that have been degraded may be significantly different from those in the rhizosphere of the original seagrass beds, and differences in microbial community structure may be able to provide indicators of seagrass health status. This study employed high-throughput sequencing to investigate the diversity of bacterial communities in seagrass (*Zostera marina*) rhizosphere and bulk sediment across different seasons. Using the co-occurrence network analysis, we assessed the effects of seasonal variations and seagrass presence on the bacterial community structure in seagrass bed sediments. The findings of this study will deepen our understanding of the interactions between seagrass and its rhizosphere bacterial communities, and will provide new insights into the ecological restoration of seagrass beds.

Materials and methods

Sample collection

In this study, sediment samples were collected from a seagrass (*Zostera marina*) bed in Miaodao village (37.93°N, 120.68°E). A total of 32 samples were collected during different seasons: August (summer), November (autumn) in 2020, and February (winter), May (spring) in 2021 (Fig. 1). Among these samples, 16 were collected from the rhizosphere of seagrass (rhizosphere sediments), while the remaining samples were collected from sediments located 5 m away from the seagrass beds (bulk sediments). For rhizosphere sediments samples collection, we followed the commonly used methods for rhizosphere collection in land plants and tropical seagrass [34–36]. The selected seagrass roots were manually shaken to remove loose sediments, and sediment still attached to the roots (rhizosphere) was collected using a needle rinsed with seawater. Each sample (approximately 200 g) was collected in cryogenic tubes, immediately stored in liquid nitrogen in the field, and subsequently transferred to a -80 °C refrigerator in the laboratory for further analysis.

DNA extraction, 16S rRNA gene PCR and sequencing

For DNA extraction, the FastDNA™ SPIN kit (MP Biomedicals, Solon, USA) was utilized to isolate total genomic DNA from sediment samples. The extracted DNA was then assessed for concentration and purity

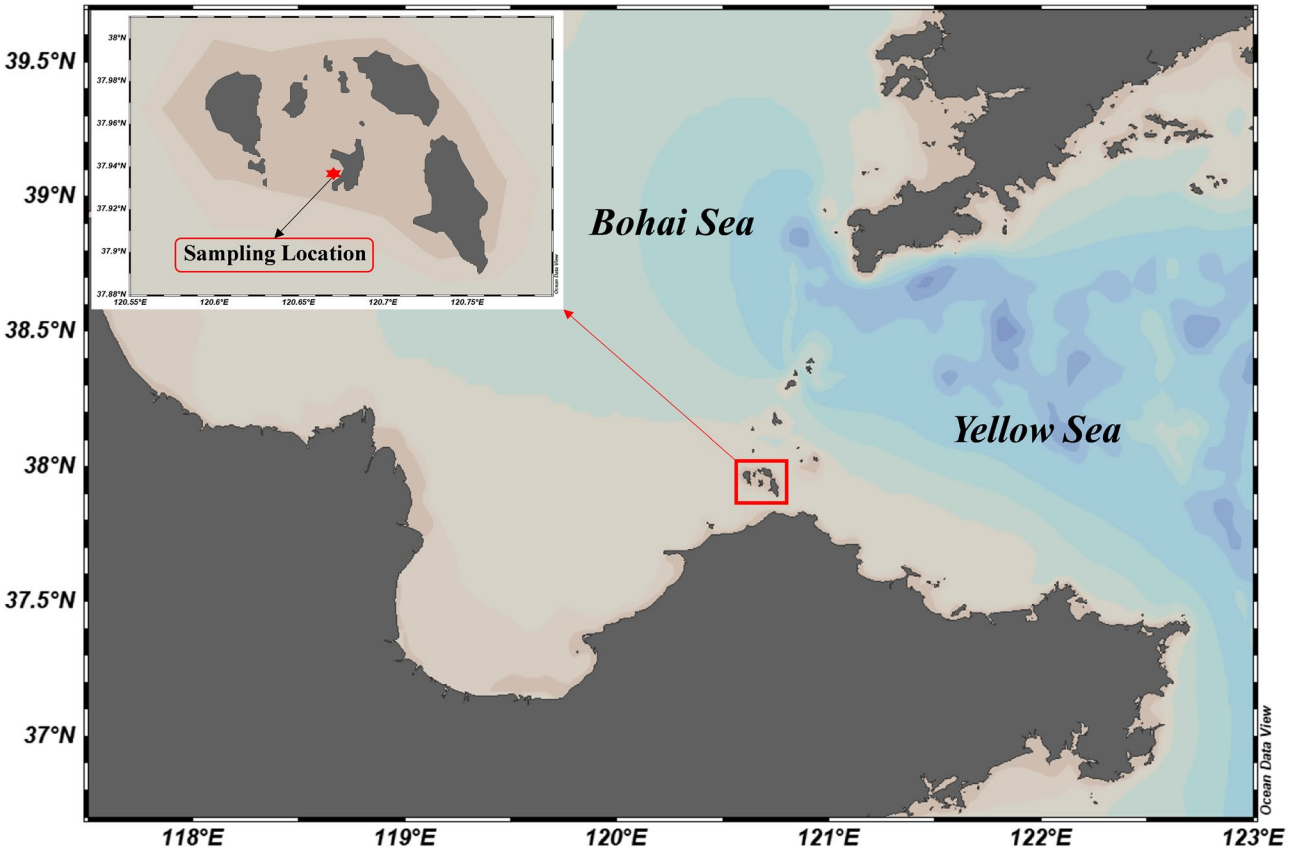

Fig. 1 Sample sampling site map

using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA) after running on a 1% agarose gel. Amplification of the V3-V4 region of the 16S rRNA gene using the primer pair 338 F and 806R, PCR, purification and quality control of the PCR product, and high-throughput sequencing followed the same protocols as in our previous publication [7]. The original reads were deposited in the NCBI Sequence Read Archive (SRA) database (record number: PRJNA929089).

Bioinformatics analysis

The raw sequencing reads of the 16S rRNA gene were processed by demultiplexing, quality filtering, and merging [37, 38]. OTUs with 97% similarity were clustered using UPARSE version 7.1, and chimeric sequences were removed [39]. Representative OTU sequences were annotated using the SILVA bacterial 16S rRNA database (Release138) by referencing the QIIME-based RDP-classifier v.2.2 [40, 41].

Based on the OTU abundance table, α diversity and OTU richness were calculated, and community composition was determined at different taxonomic levels. α diversity indices including OTU richness, Chao 1, Shannon, and Pielou's evenness index were calculated for each sample in vegan 2.4.3 with R v. 3.5.3. One-way analysis of variance (ANOVA) and Student's t test were used to compare alpha-diversity.

Differences in bacterial community composition, based on the OTU abundance table, were reduced using non-metric multidimensional scaling (NMDS) with Bray-Curtis distance matrix and tested through permutational multivariate analysis of variance (PERMANOVA). The PERMANOVA based on the Bray-Curtis distance matrix decomposed the total variance to analyze the significance of bacterial community differences attributed to seasonal variations and the presence of seagrass. Significance of the partitioned statistical outcomes was assessed using permutation tests with 999 permutations. The analysis was carried out using R v. 3.5.3 with the vegan package (version 2.4.3) [7].

The 100 OTUs with higher relative abundance were selected for co-occurrence network analysis, we conducted Spearman correlation analysis to examine the relationships between these OTUs, analysis was performed through R v. 3.5.3 with the psych package. We considered a valid co-occurrence event to be a robust correlation if the Spearman's correlation coefficient (ρ) was both >0.4 and statistically significant ($P<0.05$). Visualization was performed using the Gephi-0.10.1 software [42], nodes are colored according to the phylum level of

the bacteria. Resistance of bacterial co-occurrence networks to interference was tested through removing 50% of the nodes [43]. The contributions of stochastic and deterministic processes to community assembly were assessed using the null model [44]. The nearest-taxon index (NTI) was employed to assess phylogenetic community assembly at the intra-community scale. NTI quantifies the deviation between the observed mean nearest-taxon distance (MNTD) and the null expectation of MNTD. In a single community, NTI>0 indicates phylogenetic clustering, whereas NTI<0 indicates overdispersion. Subsequently, a between-community null modeling approach was adopted to elucidate community assembly processes by computing the β-nearest-taxon index (βNTI). βNTI signifies the discrepancy between observed and expected βMNTD. Values of |βNTI| > 2 (two standard deviations from the null expectation) suggest deterministic selection driving turnover among communities. βNTI>+2 signifies heterogeneous selection, while βNTI < -2 indicates homogeneous selection. Pairwise comparisons falling within the null distributions (|βNTI| < 2) imply stochastic processes predominating, such as dispersal limitation, homogenizing dispersal, and 'undominated' assembly. Additionally, the Bray–Curtis-based Raup–Crick index (RC_{Bray}) was utilized to further categorize pairwise comparisons not attributed to selection (i.e., |βNTI| < 2). Values of |βNTI| < 2 and RC_{Bray} < -0.95 suggest homogenizing dispersal; |βNTI| < 2 and RC_{Bray} > 0.95 indicate dispersal limitation; and |βNTI| < 2 with $|RC_{Bray}|$ < 0.95 denote undominated assembly, primarily characterized by weak selection, weak dispersal, diversification, and drift [45–47]. All computations were performed in R (version 3.5.3). Linear discriminant analysis (LDA>4) combined with effect size measure (LEfSe) analysis was used to search for significantly different biomarkers between groups using Galaxy (http://galaxy.biobakery.org/) [48, 49].

Table 1 Statistical analysis of the bacterial α diversity in seagrass meadow sediments by using ANOVA and t tests

Groups	α diversity	Method	*P*-value
Season	Chao1	Anova	0.001213 **
	Shannon	Anova	5.546e-05 ***
	Pielou_e	Anova	0.06063
Seagrass presence	Chao1	T-test	0.0004696***
	Shannon	T-test	0.001911**
	Pielou_e	T-test	0.003041**
Season (rhizosphere sediments)	Chao1	Anova	0.1023
	Shannon	Anova	0.002426 **
	Pielou_e	Anova	0.06063
Season (bulk sediments)	Chao1	Anova	1.68e-07 ***
	Shannon	Anova	2.194e-06 ***
	Pielou_e	Anova	6.83e-05 ***

Note * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Results

Characterization of illumina sequencing data

Within this study, a total of 32 samples were analyzed, resulting in 1,525,150 raw reads. After quality filtering and removal of low-quality sequences and mismatches, 1,365,811 clean sequence reads were obtained. Following the removal of chimeras, 879,567 effective sequences remained, with an average of 27,486 per sample (range: 22,812−31,161). The normalized dataset, with 22,812 reads at 97% similarity, yielded a total of 6,148 OTUs. Rhizosphere sediments samples contained 5,141 OTUs, while bulk sediments samples contained 5,138 OTUs (Supplementary Table 1). The rarefaction curve analysis demonstrated sufficient sequencing depth to capture bacterial richness and diversity in all samples (Figure S1).

Richness and diversity analyses of microbial communities

Both seasonal variations and seagrass presence significantly affected the α diversity indices (Chao1, Shannon, and Pielou evenness indices) in the bacterial communities of seagrass meadows, except for the effect of seasonal variations on the Pielou evenness index (Table 1, $P<0.05$). Moreover, when seagrass meadow samples were distinguished into rhizosphere and bulk sediments, seasonal variations had significant effects on all of the α diversity indices for bulk sediments (but not on the Chao1 and Pielou evenness indices for rhizosphere sediments), thus indicating that seasonal variations had a greater effect on bulk sediments α diversity than on rhizosphere sediments α diversity. For seasonal variations, the Chao1, Shannon and Pielou evenness indices of bacterial communities in both rhizosphere and bulk sediments showed a trend of winter>spring>autumn>summer (the pattern is regarding the mean value of those indices) (Fig. 2AB), thus indicating that seagrass meadow samples had greater bacterial species richness and evenness in winter and lower richness and evenness in summer. For seagrass presence, the bacterial communities in rhizosphere sediments had greater Chao1, Shannon and Pielou evenness indices than those in bulk sediments, thus indicating that the species richness and evenness in rhizosphere sediment were greater than those in bulk sediments (Fig. 2C).

Bacterial community composition

In different seasons, the dominant taxa at the phylum and genus levels in the rhizosphere sediments and bulk sediments were essentially the same (Fig. 3). At the phylum level, Proteobacteria were the most dominant taxa in each group (22.43–33.13%), followed by Bacteroidota (8.50–31.16%), Actinobacteria (11.28–21.09%), Desulfobacterota (11.28–21.09%) and Acidobacteriota (4.67–8.19%) (Fig. 3A). ANOVA results showed significant differences in the relative abundance of the top 10 phylum in bacterial composition in bulk sediments in

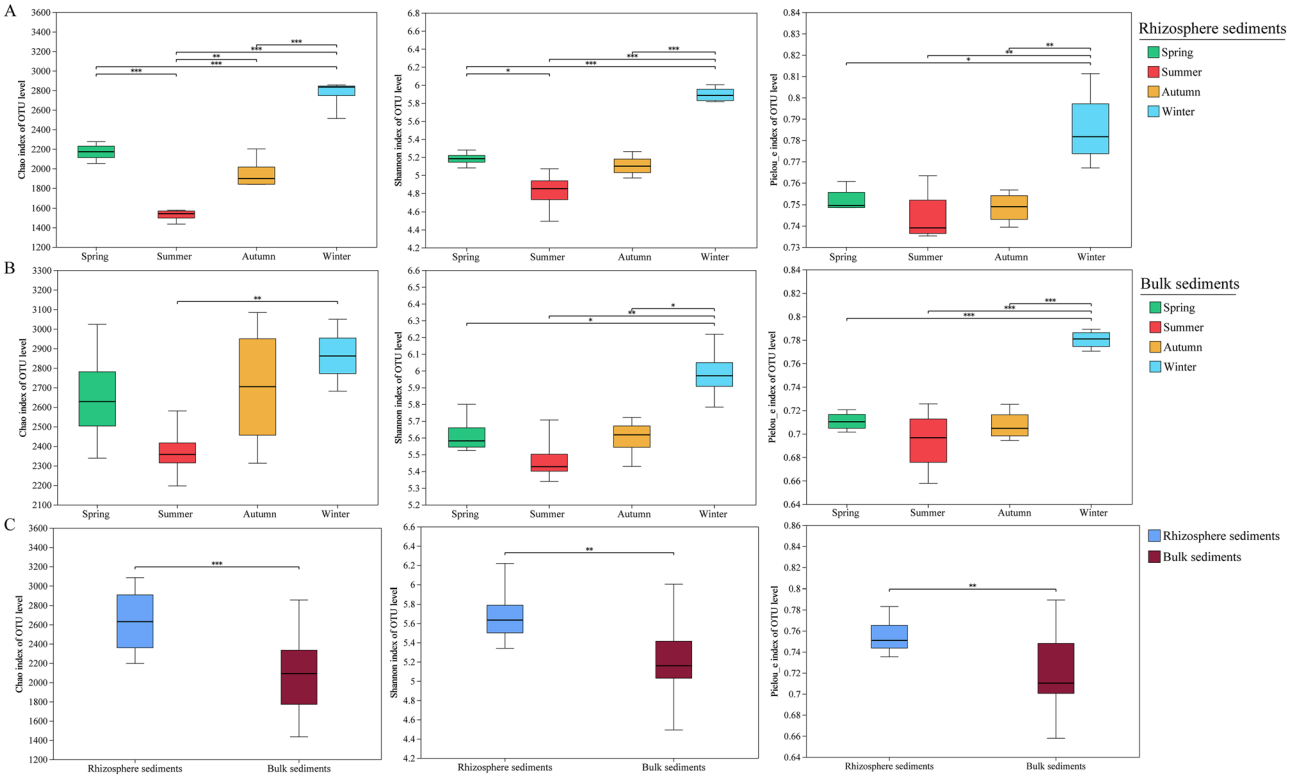

Fig. 2 The results of the α diversity indices. (**A**) Comparison of α diversity among the four seasons in the rhizosphere sediments. (**B**) Comparison of α diversity among the four seasons in bulk sediments. (**C**) Comparison of α diversity in rhizosphere and bulk sediments. Statistical analyses were performed with Student's t test between the two groups. * ($P<0.05$), ** ($P<0.01$) and *** ($P<0.001$)

different seasons ($P<0.05$) (Figure S2A), whereas the relative abundance of the phylum Bacteroidetes, Chloroflexi and Campilobacterota significantly differed in the rhizosphere sediments (Figure S2B). These results indicated that the relative abundance of the dominant phyla in the bulk sediments was more responsive to seasonal variations than that in the rhizosphere sediment, which was consistent with the β diversity results. In addition, t test analysis of the relative abundances of the dominant phylum in the rhizosphere and bulk sediments demonstrated that the relative abundances of six of the top 10 dominant phylum significantly differed between the two groups, with seagrass presence significantly increasing the relative abundances of the phylum Desulfobacterota, Campilobacterota and Firmicutes and decreasing the relative abundances of the phylum Proteobacteria, Actinobacteria and Myxococcota (Figure S3A).

At the genus level, the top 10 dominant genus were *norank_f__norank_o__Actinomarinales*, *Eudoraea*, *norank_f__Sandaracinaceae*, *Woeseia*, *Sva0081_sediment_group*, *norank_f__Desulfocapsaceae*, *Ilumatobacter*, *norank_f__unclassified*, *norank_f__norank_o__B2M28*, and *norank_f__norank_o__norank_c__KD4-96* (Fig. 3B). Furthermore, the relative abundance analysis at the genus level, conducted through analysis of variance and t-tests, aligns with the findings at the phylum level mentioned above. Specifically, these results indicate that the relative abundance of dominant bacterial genera in bulk sediments from areas lacking seagrass is more responsive to seasonal variations compared to those in rhizosphere sediments where seagrass is present (Figure S3B; Figure S4).

β diversity and interactions of bacterial communities

An NMDS ordination plot was generated using the Bray-Curtis distance, illustrating clear dissimilarities in the structure of bacterial communities across the different groups (Fig. 4). The application of NMDS, along with PERMANOVA based on Bray-Curtis distance for community structure, elucidates pronounced disparities in bacterial community structures among various groups (Supplementary Table 2). Both seagrass presence and seasonal variations exerted a notable influence on the β diversity of the bacterial community structure ($P<0.01$); however, seasonal variations had a greater impact (Season: $R^2=0.373$; Seagrass presence: $R^2=0.202$).

In addition, a bacterial co-occurrence network was constructed based on the 100 OTUs with higher relative abundance (Fig. 5). The nodes of rhizosphere and bulk sediments were similar in the co-occurrence network at 88 ~ 97 and 88 ~ 93, respectively (Supplementary Table 3). The bacterial network was partitioned into distinct

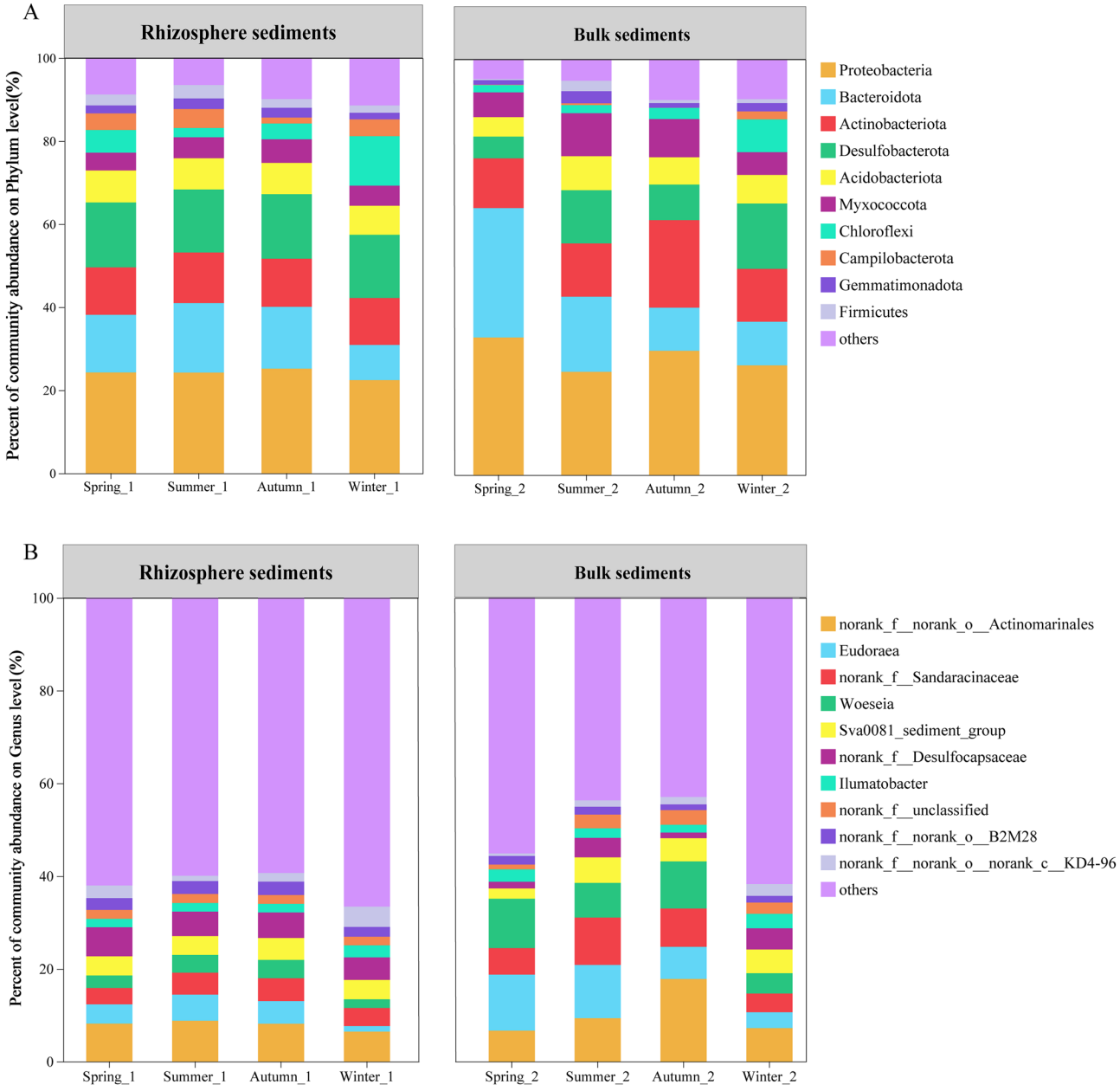

Fig. 3 Relative abundances of bacterial communities in rhizosphere and bulk sediments in four seasons. (**A**) Bacterial community composition at the phylum level. (**B**) Bacterial community composition at the genus level. The top 10 abundant phylum/genus are shown

phylum, with Proteobacteria and Desulfurobacteria representing the most abundant nodes, comprising over 48% of the total nodes. In the rhizosphere and bulk sediments, positive proportions were greater than negative proportions in four seasons (except for spring rhizosphere sediments). In spring and summer, the edges in the bacterial community in the rhizosphere sediments was greater than that in the bulk sediments (1.3- and 1.1- fold, respectively), with equal positive and negative proportions in the rhizosphere sediments (Fig. 5A, B, C **and D**). In autumn and winter, the edges in the bulk sediments slightly exceeded that in the rhizosphere sediments. Notably, the bacterial communities in the bulk sediments showed a predominant positive correlation. (Fig. 5E, F, G **and H**). By removing the nodes of the bacterial co-occurrence network, observing the changes of the network edges, and knowing the resistance of the bacterial network to interference, we found that the interference resistance of bulk sediments bacterial communities was lower than that of rhizosphere sediments (except in winter) (Supplementary Table 4). A combination of seagrass β diversity analysis and co-occurrence network analysis

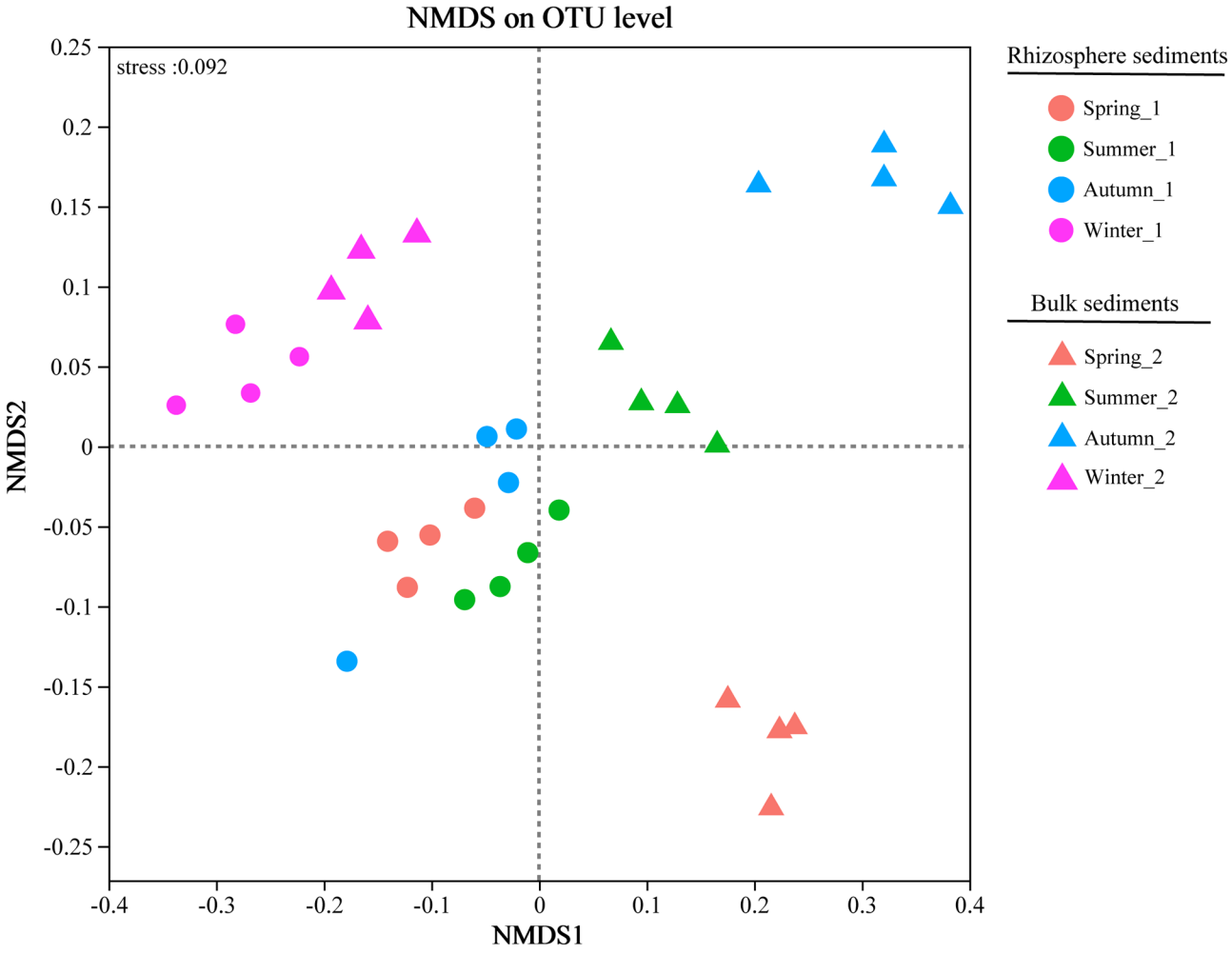

Fig. 4 Non-metric multidimensional scaling (NMDS) is based on the Bray-Curtis distance (at the OTU level) between samples harvested at seasonal variation and from the two habitats (rhizosphere and bulk sediments)

demonstrated that seasonal variations had greater effects on the diversity and stability of microbial communities in bulk sediments than in rhizosphere sediments.

The main process of community assembly

In this study, the null model was used to investigate the assembly processes of bacterial communities in seagrass meadow sediments. Results demonstrated that deterministic processes ($|\beta NTI| > 2$) were the principal drivers shaping the bacterial community assembly in rhizosphere sediments (Fig. 6A), while stochastic processes ($|\beta NTI| < 2$) played a predominant role in the assembly of bacterial community in bulk sediments (Fig. 6B). Notably, during the winter period in the bulk sediments, the process of bacterial community assembly shifted from stochastic processes to deterministic processes. Subsequent identification revealed that deterministic processes in both rhizosphere and bulk sediments were predominantly driven by heterogeneous selection, whereas stochastic processes were primarily influenced by non-dominant processes, including drift and others.

Biomarker discovery

For the different seasons, the LEfSe analysis of bulk sediments revealed 21 biomarkers for spring, 12 for summer, 12 for autumn, and 15 for winter (Fig. 7A). At the family level, the abundant microbes in spring were Flavobacteriaceae, Woeseiaceae, Rhodobacteraceae, Saprospiraceae and Pseudomonadaceae; the abundant microbes in summer were Sandaracinaceae, Desulfocapsaceae, and Thermoanaerobaculaceae; the abundant microbes in autumn were f__norank_o__Actinomarinales, f__unclassified_c__Gammaproteobacteria and f__unclassified_o__Gammaproteobacteria_Incertae_Sedis; and the abundant microbes in winter were Desulfosarcinaceae and Chromatiaceae.

Moreover, the LEfSe analysis of the rhizosphere sediments revealed 3 biomarkers for spring, 12 for summer, 1 for autumn, and 8 for winter (Fig. 7B). At the

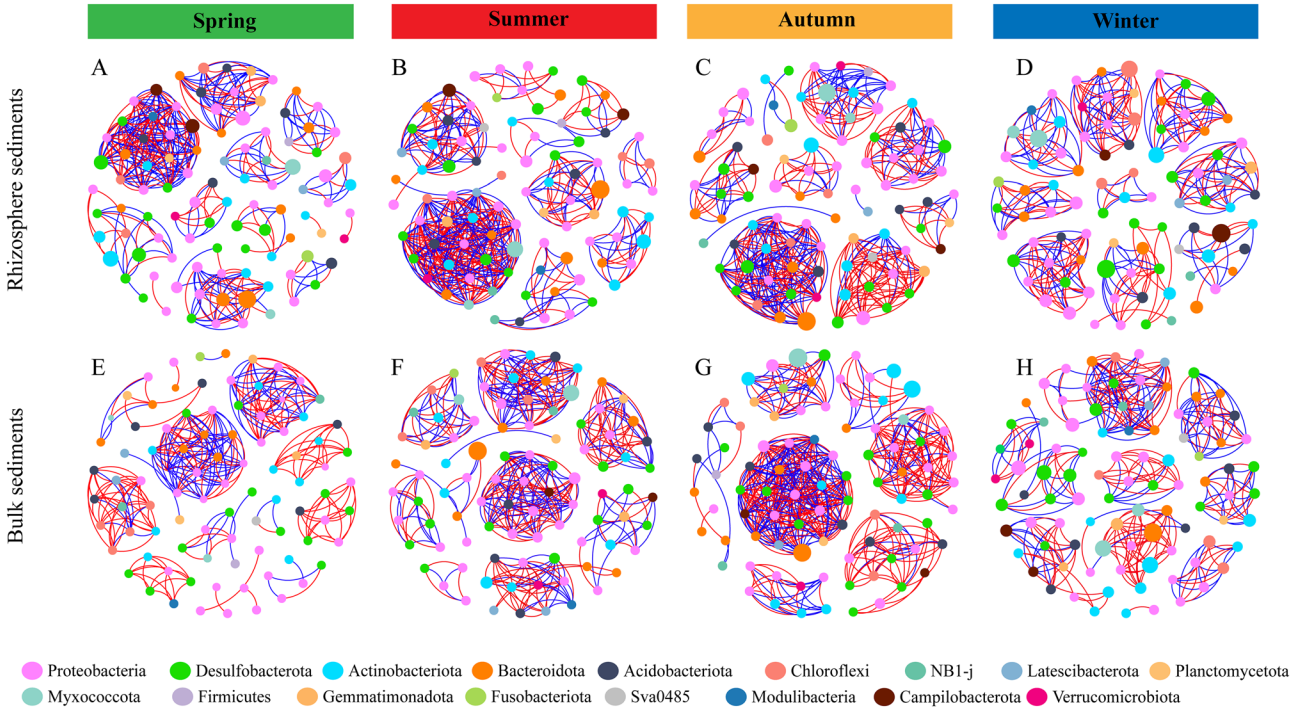

Fig. 5 Co-occurrence network graphs of bacterial communities at the OTU level in rhizosphere sediments (**A**, **B**, **C**, and **D**) and bulk sediments (**E**, **F**, **G**, and **H**) across four seasons. The 100 OTUs with higher relative abundance were selected for each sample. The colors of the nodes represent different bacterial phylum, and the lines between nodes represent the correlations between OTUs. Red lines indicate positive correlations, whereas blue lines indicate negative correlations

family level, the abundant microbes in spring were Pseudomonadaceae; the abundant microbes in summer were Flavobacteriaceae and Sulfurovaceae; the abundant microbes in autumn were *Woeseia* (genus); and the abundant microbes in winter were f__norank_o__norank_c__KD4-96.

For the rhizosphere and bulk sediments communities, the LEfSe analysis identified 12 and 15 biomarkers, respectively (Fig. 7C). At the family level, the abundant microbes of the rhizosphere sediments were Desulfocapsaceae and Sulfurovaceae, and the abundant microbes of the bulk sediments were Flavobacteriaceae, Woeseiaceae and Sandaracinaceae.

Discussion

Seagrasses can establish mutualistic relation with its microbiome that facilitate the exchange of energy sources, nutrients, and hormones, thus ultimately impacting plant stress resistance [50]. However, compared with well-studied terrestrial bacteria, studies related to seagrass bed sediment bacteria are relatively rare. Here we explore the influence of seasons and the presence of seagrass on the bacterial community structure in seagrass bed sediments, providing a theoretical foundation from a microbial perspective for the ecological restoration of seagrass beds.

Effects of seasonal variations and seagrass presence on the diversity and structure of bacterial communities in seagrass bed sediments

In this study, we observed significant effects of seasonal variations and seagrass presence on bacterial α diversity indices and co-occurrence network stability. We tested bacterial network stability by removing nodes and showed consistently higher complexity and stability of bacterial species in rhizosphere sediments except in winter. These results indicate that bacterial α diversity and co-occurrence network complexity and stability may vary in response to seagrass life history, with maximum bacterial abundance and diversity occurring during seagrass withering. In autumn, fragmented seagrass accumulates in the sediment and decomposes during winter and spring, thus providing particulate organic carbon for microorganisms (such as cellulose and hemicellulose) [51]. We speculate that in summer, seagrasses may supply more organic matter to the growth of the underground parts, For example, *Posidonia australis* growing in summer at Oyster Harbour, Australia had more lateral roots than in winter [52]. Furthermore, when seagrasses are in decline period, the accumulation of detritus in the sediment leads to the peak decomposition rate of seagrass debris and bacterial OTU diversity over time [53]. Due to the consumption of organic matter in winter and spring [53], the amount of available organic matter in summer

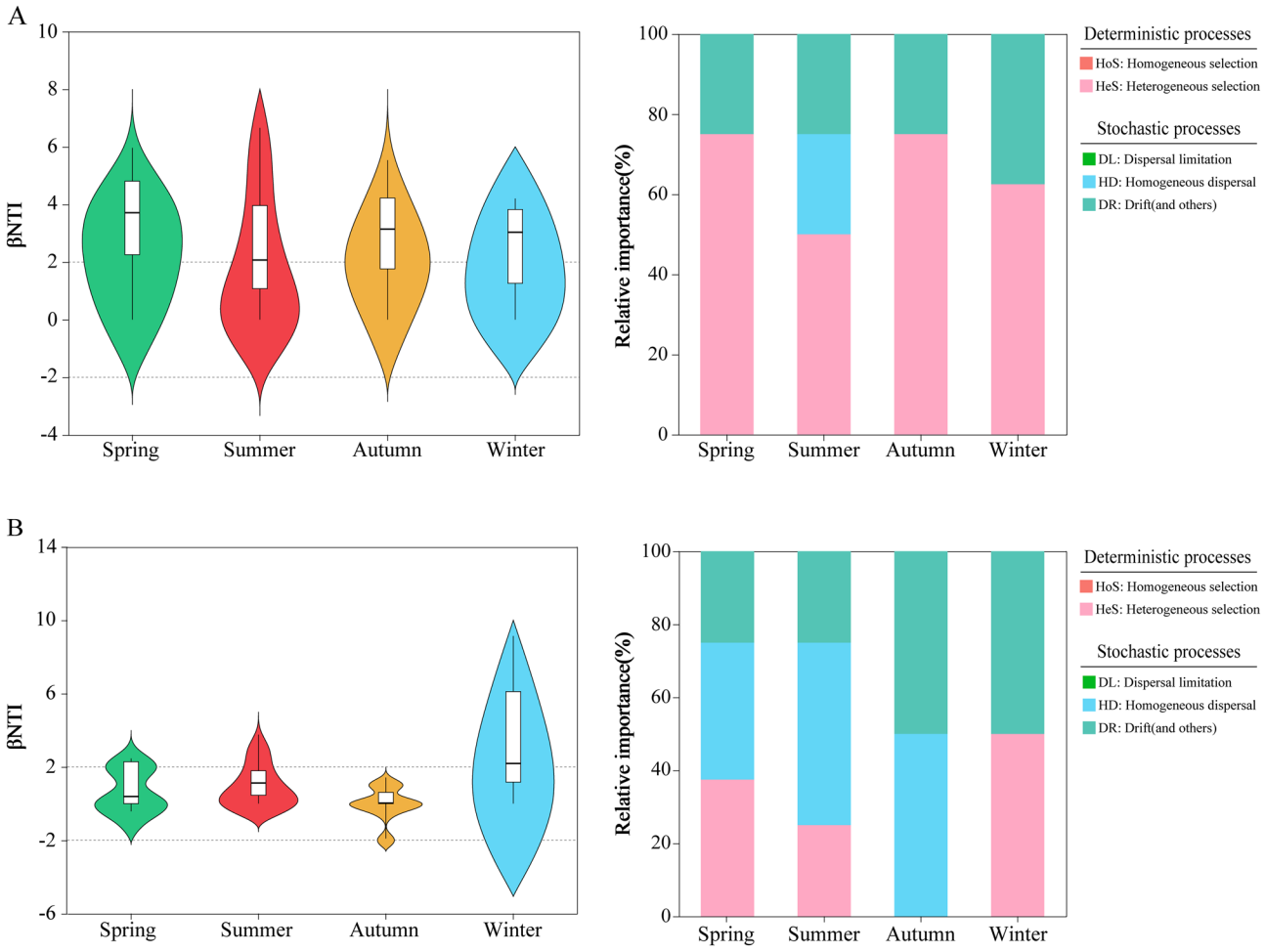

Fig. 6 Evaluation of the assembly mechanisms of bacterial communities in in rhizosphere sediments, bulk sediments by using null model analysis. The contributions of deterministic processes (|βNTI| ≥ 2) and random processes (|βNTI| < 2) to the assembly of bacterial communities in seagrass meadow sediments across four seasons (**A**). The relative contributions of deterministic processes, homogeneous dispersal, dispersal limitation, and drift(and others) processes driving the assembly of bacterial communities in seagrass meadow sediments across four seasons (**B**)

bacterial communities decreased compared with the two seasons. Consequently, the abundance and diversity of bacterial communities decrease, impacting the stability of the bacterial communities. This study found that the proportion of most bacterial composition in the bulk sediments decreased in summer, and only the proportion of Acidobacteriota, Myxococcota, Gemmatimonadota and Campilobacterota increased. Moreover, according to the null model analysis, a certain proportion of homogeneous dispersal was observed in the bacterial community in seagrass rhizosphere sediments during the summer. It is speculated that in summer seagrass meadows, nutrient limitations and other factors may lead to the occurrence of homogenization within the bacterial community, resulting in a reduction in bacterial diversity, consistent with the α diversity results. Similar effects of seasonal variations and seagrass presence on α diversity have been reported in previous studies on seagrass beds and mangroves [54–56], although the specific results may differ due to variations in sampling location and sampling months.

Furthermore, we found that both seasonal variations and seagrass presence significantly influenced β diversity, with seasonality having a greater impact, thus suggesting the importance of seasonal factors in regulating bacterial community structure in seagrass bed sediments. Seagrass meadows create favorable conditions for bacterial community growth by reducing water turbulence, inhibiting substrate resuspension, promoting fine sedimentation, and releasing dissolved organic carbon [57, 58]. In addition, seagrass roots can release dissolved organic carbon and capture carbon particles from the overlying water to increase the organic carbon load through shed leaves, decaying roots and stems; therefore, there is abundant carbon material in seagrass beds [59, 60]. The growth and developmental state of seagrass determine the source and composition of organic matter in sediments, which

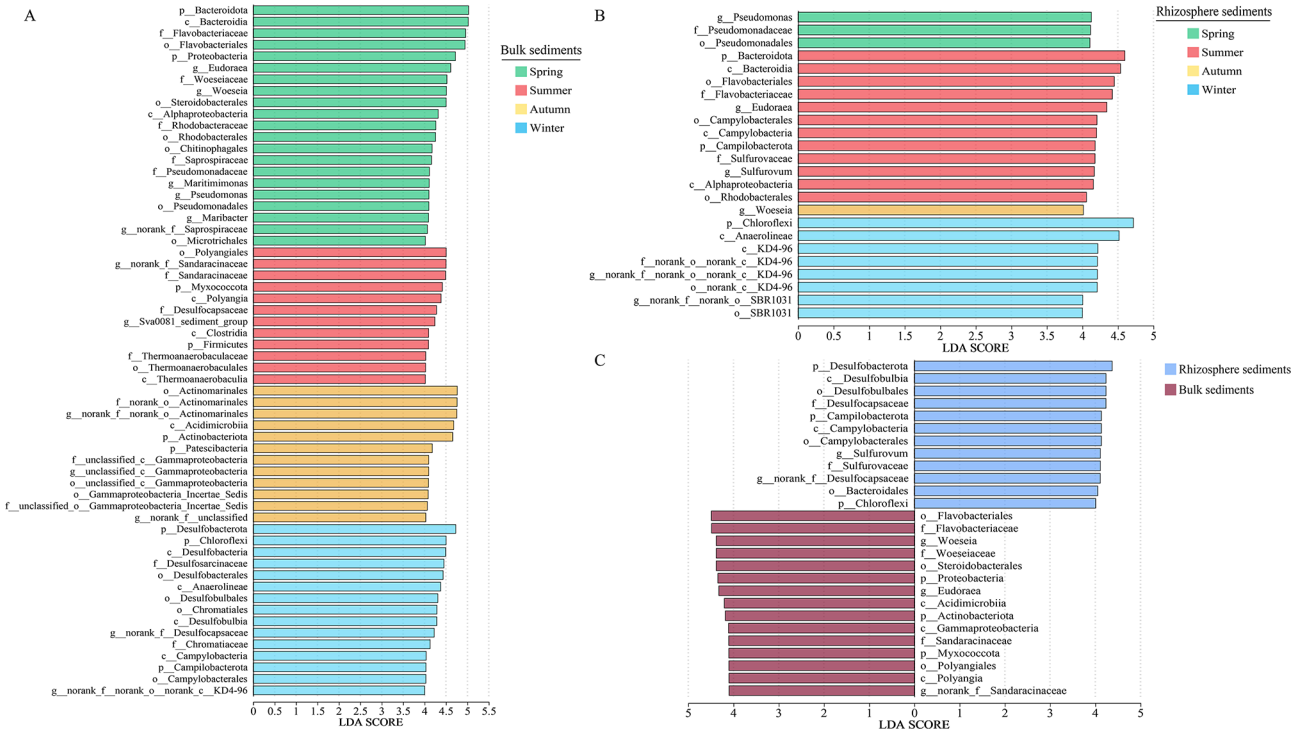

Fig. 7 Linear discriminant analysis (LDA) scores of the biomarkers in the bacterial communities of rhizosphere and bulk sediments. The colors of the bars represent the groups, and the lengths of the bars represent the contributions of the biomarkers ($P < 0.05$). (**A**) LDA scores of bulk sediments in the four seasons. (**B**) LDA scores of the rhizosphere sediments in the four seasons. (**C**) LDA scores in rhizosphere and bulk sediments

correspondingly shapes bacterial community dynamics [61, 62].

Considering the potential limitations associated with studying seasonal variations within a one-year timeframe, extending the research duration to three years in subsequent investigations could help alleviate these temporal replication constraints. By lengthening the study period, we can comprehensively capture the breadth of seasonal fluctuations, allowing for a more in-depth exploration of how bacterial communities adapt to prolonged seasonal dynamics and seagrass phenology. Such investigations hold promise for providing valuable insights into the sustainability and resilience of seagrass-associated ecosystems within the realm of ecological research.

Seasonal effects on the composition of bacterial communities in seagrass bed sediments

The study findings indicate significant seasonal variations in the relative abundance of the top 10 phylum in rhizosphere and bulk sediments. Bacteroidetes, Chloroflexi, and Campilobacterota exhibit marked seasonal changes in the rhizosphere sediments. Bacteroidetes, in particular, are renowned for their role in the degradation of complex soluble polysaccharides and proteins, thereby enhancing the host's access to nutrients and facilitating storage [63–65]. Moreover, notably, the varying relative abundance of the top 10 genus shows substantial seasonal variations in rhizosphere sediments, particularly with *Eudoraea* and *Sulfurovum* demonstrating distinctive trends. *Eudoraea* significantly contributes to the proliferation of Bacteroidetes and plays a crucial role in polysaccharide metabolism [66]. Additionally, given the accumulation of reduced sulfur compounds in eutrophic sediments known to be plant toxins [67], sulfur-metabolizing microbes (*Sulfurovum*) may play a crucial role in promoting seagrass adaptation. The variations in organic matter secretion by seagrass in different growth states can explain the seasonal fluctuations in the abundance of Bacteroidetes in sedimentary bacteria, as well as the differences in bacterial network structure between rhizosphere and bulk sediments within the same season. Based on co-occurrence network analysis conducted on 100 OTUs with relatively high abundances, the results indicate that in winter, the bacterial community of bulk sediments exhibits greater complexity and stability compared to rhizosphere sediments. Specifically, the phylum Firmicutes showed negative correlations within the bacterial co-occurrence network of bulk sediments, thus indicating competition among bacteria. It is speculated that the increase in lignin content resulting from seagrass decay in winter may contribute to the involvement of Firmicutes in lignin metabolism in the marine environment [68, 69]. In rhizosphere sediments with relatively high lignin content, Firmicutes were abundant and primarily

involved in lignin metabolism in seagrass. Conversely, the lower lignin content in bulk sediments led to competition between bacteria, which influenced the complexity and stability of the bacterial community.

Moreover, LEfSe analysis revealed distinct candidate biomarkers within the bacterial communities of bulk sediments across different seasons, diverging from those in the rhizosphere sediments, exhibiting higher abundance levels. Notably, Desulfocapsaceae and Sulfurovaceae were enriched in rhizosphere sediments, potentially implicated in sulfur metabolism processes facilitated by the seagrass presence, as corroborated by prior studies [70, 71]. Conversely, Flavobacteriaceae, Woeseiaceae, and Sandaracinaceae likely play a predominant role in nitrogen cycling within bulk sediments. Based on the distinct biomarkers observed in rhizosphere sediments and bulk sediments, we speculate that the physicochemical and biological attributes of seagrass rhizosphere and bulk sediments could influence the microbial community composition, potentially interacting with nutrient cycling dynamics and ecological functions within these habitats [72, 73].

Effects of seagrass presence on bacterial community composition in seagrass bed sediments

Previous studies have demonstrated notable differences between seagrass rhizosphere microbial communities and the bulk sediments, including variations in network stability and microbial dispersal capacity [4, 5, 74]. In this study, seagrass presence enhanced the stability and richness of the bacterial communities. The results of the null model analysis suggested that the presence of seagrass increases the proportion of deterministic processes in the bacterial community. It is hypothesized that the secretion of root exudates and the decomposition of tissues from seagrass roots provide abundant nutrients for microorganisms [75], recruiting relevant bacterial groups and enhancing the diversity of bacterial communities in rhizosphere sediments. This finding is consistent with results from agricultural ecosystems, where plant rhizosphere exhibit stronger selective effects on bacterial communities [76]. Additionally, differences in organic matter content and sources may partially influence the dispersal of bacterial communities, thus leading to selective enrichment of bacterial populations in both rhizosphere and bulk sediments [56]. Specifically, we found that Desulfocapsaceae and Sulfurovaceae were significantly enriched in rhizosphere sediments. Both Desulfobulbaceae and Sulfurovaceae are functional microorganisms involved in the sulfur cycle; however, the former type are sulfate-reducing bacteria (SRB), and the latter type are sulfur-oxidizing bacteria (SOB) [77]. Studies have shown that seagrass roots can release organic matter to stimulate SRB proliferation [78]; consequently, seagrass plants use nitrogen fixed by SRB, which can meet approximately 50% of seagrass nitrogen requirements [79]. The incidence of nitrogen fixation in seagrass beds is greater than that in nearby non-grass-vegetated sediments [80].

In addition, Flavobacteriaceae, Woeseiaceae and Sandaracinaceae were significantly enriched in the bulk sediments. Flavobacteriaceae and Sandaracinaceae are widely recognized as being important degraders of complex organic matter, such as rhizome sediments and algal cell wall polysaccharides [81–83]. The bulk sediments putatively possess larger amounts of particulate organic matter, which explains why Flavobacteriaceae and Sandaracinaceae were more abundant in the bulk sediments. Additionally, numerous studies have shown that Woeseiaceae is the dominant bacterial taxon in both offshore and deep-sea sediments and is considered one of the core taxa in the global marine sediment microbiota [84, 85]. This aligns with our study's finding of significant enrichment of Woeseiaceae in bulk sediments. In future research, it is essential to consider seagrass root metabolites. Integrating seagrass root exudates, sediment characteristics, and seagrass metabolomic profiles with our research data can provide insights into understanding specific compounds released by seagrass roots, how these compounds impact microbial communities, and the reciprocal relationships between seagrass hosts and associated bacteria.

Conclusion

This study analyzed the bacterial communities in rhizosphere and bulk sediments during different seasons. We found that seasonal variations significantly influence the bacterial communities in seagrass bed sediments. However, the presence of seagrass enhances the stability of the bacterial community structure, indicating that seagrass reinforces the deterministic processes that control bacterial community assembly, thereby increasing the abundance and diversity of the bacterial community. Complex bacterial co-occurrence networks were observed in spring and summer, suggesting close interactions among bacteria during the growth of seagrass. The results of this study will provide a theoretical data from microbial perspective to support the ecological restoration of eelgrass beds.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03558-0.

Supplementary Material 1: **Supplementary Figure S1**. Rarefaction curve analysis of all of the samples

Supplementary Material 2: **Supplementary Figure S2**. ANOVA of the relative abundances of the dominant phylum in seagrass meadows in different seasons. (A) Rhizosphere sediments. (B) Bulk sediments. Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Supplementary Material 3: **Supplementary Figure S3**. The t test analysis of the relative abundances of the dominant phylum and genus in the rhizosphere and bulk sediments. Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Supplementary Material 4: **Supplementary Figure S4**. ANOVA of the relative abundances of the dominant genus in seagrass meadows in different seasons. (A) Rhizosphere sediments. (B) Bulk sediments. Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Supplementary Material 5: **Supplementary Table 1.** Bacterial community sequencing data of all of the samples

Supplementary Material 6: **Supplementary Table 2.** Analysis of seasonal variation and seagrass presence explaining bacterial community structure (PERMANOVA based on Bray-Curtis distance)

Supplementary Material 7: **Supplementary Table 3.** The properties of co-occurrence networks of bacterial communities in seagrass meadow sediment samples across four seasons

Supplementary Material 8: **Supplementary Table 4.** The properties of the bacterial community co-occurrence network in seasonal seagrass meadow sediment samples after removing 50% of the nodes

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Author contributions
Jun Chen: Investigation, Formal analysis, Methodology, Writing – original draft, Data curation, Writing – review & editing, Funding acquisition. Xinqi Li: Investigation, Formal analysis, Writing – original draft. Hongzhen Wang: Investigation, Resources, Formal analysis. Liuqing Tang: Investigation, Resources. Song Xue: Investigation, Resources. Jiayi Xin: Investigation, Resources.: Investigation, Resources. Yu Zang: Investigation, Resources, Funding acquisition. Xuexi Tang: Conceptualization, Writing – review & editing, Funding acquisition. All authors read and approved the final manuscript.

Data availability
The raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) database with the record number PRJNA929089.

Declarations

Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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