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Article

The Symbiodiniaceae and Bacterial Dynamic Composition of the Coral *Echinopora gemmacea* on Wuzhizhou Island

Zhuoran Li [1], Yushan Li [1], Wentao Zhu [2], Xiangbo Liu [1], Rou-Wen Chen [1,*], Aimin Wang [1,3] and Xiubao Li [1,3,*]

[1] School of Marine Biology and Fisheries, Hainan University, Haikou 570228, China
[2] College of Ecology and Environment, Hainan University, Haikou 570228, China
[3] State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou 570228, China
* Correspondence: chenrouwen@hainanu.edu.cn (R.-W.C.); xiubaoli@hainanu.edu.cn (X.L.)

Abstract: Coral's susceptibility to bleaching is determined by the strength of the intricate mutual relationships among coral symbionts. However, there is limited knowledge about how the symbiotic members of the scleractinian coral *Echinopora gemmacea* respond to changes in their surrounding environmental conditions. In this study, we conducted a survey of seawater characteristics in the south and north zones of Wuzhizhou (WZZ) Island, measured symbiotic microalgal density and chlorophyll-a content in the corals, and performed metabarcoding of the Symbiodiniaceae and bacteria communities within coral tissue. Our findings demonstrated that the seawater in the north zone of WZZ Island had higher levels of turbidity, temperature, salinity, and dissolved oxygen content compared to the south zone. This indicated that the corals in the two zones were subjected to distinctive environmental conditions. Analysis of the Symbiodiniaceae composition revealed that *Cladocopium* sp. C1 and *Cladocopium* sp. C17 were the dominant species in the southern *E. gemmacea*, whereas *Durusdinium* sp. D1a and *Cladocopium* sp. C17 prevailed in the northern *E. gemmacea*. Consequently, symbiotic microalgal density and chlorophyll-a content were diminished in the northern *E. gemmacea*. Furthermore, correlation network analysis revealed the presence of intricate bacterial interactions that potentially mediate coral's adaptation to environmental stress. This study provides insights into the differences in symbiotic members, including Symbiodiniaceae and bacteria, within *E. gemmacea*, and contributes to fundamental knowledge for coral conservation efforts.

Keywords: *Echinopora gemmacea*; seawater characteristics; symbiotic microalgal density; chlorophyll-a; symbiodiniaceae types; bacterial community; Wuzhizhou Island

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1. Introduction

The coral reef system is widely recognized as one of the most productive and biodiverse marine ecosystems on Earth [1]. Unfortunately, the increasing frequency of coral bleaching events has resulted in a significant decline in coral reef coverage, attributed to both natural stressors and anthropogenic impacts [2,3]. In response to these stress events, scleractinian corals exhibit various adaptive responses, including alterations in the growth rate, the loss of Symbiodiniaceae, changes in fecundity, reduced survival of planula larvae, and adjustments in metabolism [4]. It is important to note that the thermal sensitivity of corals may vary in different environments, negatively influencing the natural recovery process of coral reefs. The susceptibility of corals to bleaching is intricately linked to the robustness of the mutualistic relationship within the holobiont [5,6]. Shallow-water corals, for example, host a diverse consortium of microorganisms, including bacteria, archaea, fungi, viruses, and photosynthetic Symbiodiniaceae [7]. Symbiodiniaceae play a vital role in assisting their hosts by providing essential amino acids and aiding in carbon fixation [8]. The ecological and metabolic interactions between coral hosts and their associated bacterial assemblages are believed to be crucial in maintaining the stability and functionality of intact

coral holobionts [9]. In order to survive future bleaching events, corals have the ability to increase their chances of recovery by shifting their symbionts (Symbiodiniaceae and bacteria), a mechanism commonly referred to as the adaptive bleaching hypothesis [10].

Symbiodiniaceae, also known as zooxanthellae, refers to a family of mutualistic and photosynthetic dinoflagellate algae that form symbiotic relationships with coral hosts. These algae play a vital role in providing nutrients to coral reef ecosystems [11]. They are responsible for supplying photosynthetic products to their coral hosts, ensuring that their energy requirements are met [8]. Current studies on Symbiodiniaceae mainly focus on understanding their response to heat-related stress, with many studies demonstrating the superior performance of *Durusdinium* under such conditions [12,13]. Furthermore, corals display a remarkable ability to adapt to environmental changes due to the flexibility of their symbiotic composition, which can adapt through community recombination [14]. A study revealed that the content of *Durusdinium* significantly increased in bleached corals, and during the subsequent recovery process, the proportion of *Durusdinium* was found to be higher in corals subjected to greater temperature stress [15]. However, although *Durusdinium* exhibits better thermotolerance compared to *Cladocopium*, a higher proportion of heat-resistant symbiotic organisms can also reduce the photochemical efficiency of the community. Research indicates that the total chlorophyll content of *Durusdinium* communities is significantly lower than that of *Cladocopium* [16]. This signifies that alterations in the community structure can have significant implications for the symbiotic performance and stress endurance of the symbionts.

Moreover, it has been observed that bacterial communities undergo changes corresponding to the health status of corals [17]. Research has demonstrated that bacterial communities can enhance coral's resistance and aid it in coping with coral bleaching events [7,18]. Diverse bacteria exist as both stable and transient members in various microenvironments within the coral reef system [19]. These bacterial communities play a critical role in maintaining the ecological balance and facilitating the environmental adaptation of the entire coral holobiont [20]. Additionally, the high diversity of bacterial communities contributes to the healthy growth of corals by providing essential nutrition for the coral hosts [21]. For instance, the bacterial genus *Pseudoalteromonas* is known to produce biofilms and can induce metamorphosis in *Acropora* larvae [22]. Furthermore, certain bacteria isolated from corals, referred to as "beneficial microorganisms for corals" (BMCs), have been found to alleviate coral bleaching by competitively displacing pathogens from the host [23]. Due to their phenotypic plasticity, bacterial communities can adapt to environmental changes more rapidly than their hosts, thereby enhancing the host's immunity responses [24]. Recent studies have also provided evidence that changes in bacterial population structure can enhance the environmental adaptability and resilience of corals [25,26]. BMCs possess the ability to induce the restructuring of the microbial community, resulting in genetic and metabolic changes within the coral hosts, ultimately leading to a reduction in coral bleaching and mortality [27]. However, many questions regarding how coral symbiont members respond to their surroundings and which key factors influence the symbiont community structure still remain open for further investigation.

The scleractinian coral *Echinopora gemmacea* is usually laminar, bifacial, encrusting or forms thick leaves, and it commonly develops contorted branches with irregularly spaced, obvious and prominent corallites [28]. It is found in diverse habitats at uncommon abundance, usually in shallow protected reef environments, but also on reef flats in severe environmental conditions, and on exposed and turbid reef slopes (Corals of the World) (http://www.coralsoftheworld.org (accessed on 8 September 2023)) and World Register of Marine species (https://www.marinespecies.org (accessed on 8 September 2023)). However, studies on *E. gemmacea* are mainly focused on the cells and oocytes in the process of cryopreservation [29,30]. To date, we know that both *Cladocopium* and *Durusdinium* exist within the Symbiodiniaceae community of *E. gemmacea* [31]. Therefore, the objective of this study is to explore the differences in the Symbiodiniaceae and bacterial communities in *E. gemmacea* across various environmental conditions. This study aims to identify the

factors influencing these differences and provide a scientific foundation for local coral conservation efforts.

2. Materials and Methods

2.1. Sampling Sites and Seawater Data

Wuzhizhou (WZZ) Island is a small island renowned for its abundant coral reef resources and is situated to the northeast of Sanya City, Hainan Province, China (109°45′ E, 18°18′ N) (Figure S1). The island spans approximately 1400 m from east to west and 1100 m from north to south. The northern side of the island is closer to the mainland. Previous research has highlighted the distinct natural environment differences between the southern and northern zones of WZZ Island [32–34]. The northern zone possesses a sandy coastline, while the southern zone features a rocky coastline [32]. Additionally, the seawater characteristics, such as temperature, turbidity, and dissolved inorganic nitrogen, exhibit notable disparities between the south and north zones [33,34]. In August 2022, samples of *E. gemmacea* were collected from WZZ Island at a depth of 8 m through scuba diving. Five samples were collected from each of the north and south zones, with each sample being approximately 20 cm^2 in size. One colony was immediately stored in a −20 °C refrigerator for physiological experiments, while the other was preserved in liquid nitrogen at −80 °C for molecular analysis. In situ seawater characteristics, such as seawater temperature, salinity, and dissolved oxygen (DO), were recorded using a multi-parameter water quality probe (Eureka Water Probes, Austin, TX, USA). Turbidity data of the seawater were obtained using an AQUAlogger 210 (Aquatec, Hampshire, UK). All parameters were measured five times for each sample. The seawater samples near the coral were collected by scuba divers using 1 L plastic bottles and the seawater was transported to an indoor laboratory. The specimens were filtered through glass-fiber filters (Whatman GF/F, mm), and 100 mL of filtered seawater was collected and stored in a refrigerator. Finally, inorganic nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, and SiO_4^{3-}) were assessed using a Skalar SANplus automated analyzer (Skalar, Breda, The Netherlands).

2.2. Symbiotic Microalgal Density and Chlorophyll-a Content in E. gemmacea

The coral tissues were rinsed with a dental scaler (Waterpik) containing filtered seawater that was filtered through 0.45 μm membranes (Whatman, Maidstone, UK). The symbiotic microalgal density in *E. gemmacea* was measured using a hemocytometer under a microscope. Each sample was counted eight times, and the average value was calculated. The remaining algal solution was filtered through 0.45 μm membranes (Whatman, Maidstone, UK), and the filter membrane and residues were combined, ground in acetone to form a uniform slurry, and extracted in the dark at 4 °C for 24 h, the purpose being to measure chlorophyll-a. Chlorophyll-a content in the supernatant was measured using a fluorometer (Turner Designs, San Jose, CA, USA).

2.3. DNA Extraction and Sequencing

Due to the unforeseen loss of the sample from station N5 during submission, the study ultimately involved nine coral colonies. Coral tissue samples collected from both the mucus and skeletal parts were carefully transferred into tubes. The designated 16S data analysis program for this study was QIIME (v1.9.1). Total holobiont DNA was extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN, Redwood City, CA, USA) adhering strictly to the manufacturer's instructions. The ITS2 region of the Symbiodiniaceae was amplified using the designated primers, GTL2-F (5′-GAATTGCAGAACTCCGTG-3′) and GTL2-R(5′-GGGATCCATATGCTTAAGTTCAGCGGGT-3′) [35,36]. PCR amplification of the V3-V4 variable region of the bacterial community's 16S rRNA gene utilized the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [37]. The PCR reaction followed the system previously described [38] and was amplified in triplicate before pooling per sample. Subsequently, the corresponding PCR products underwent purification with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Axygen,

San Francisco, CA, USA). Sequencing was executed on the Illumina Miseq PE300 platform based in Shanghai, China. In processing raw sequencing reads for both Symbiodiniaceae and bacteria, the fastp software (https://github.com/OpenGene/fastp (accessed on 8 December 2022), version 0.20.0) [39] was used, and sequences were assembled through the application of FLASH software (http://www.cbcb.umd.edu/software/flash (accessed on 8 December 2022), version 1.2.7) [40]. The reads were handled and analyzed as follows: (1) Filtering: We trimmed bases with a quality value below 20 at the end of reads. We used a 10 bp window, and if the average quality value within the window was lower than 20, we truncated bases starting from the window. We removed reads below 50 bp after quality control and discarded reads containing N bases. (2) Overlap merging: We merged paired-end (PE) reads based on their overlap relationship. The minimum overlap length was 10 bp. (3) Overlap quality control: We allowed a maximum mismatch ratio of 0.2 during sequence overlap merging. We discarded sequences that did not meet this criterion. (4) Sample classification: We differentiated samples based on barcode and primer sequences at the ends of the sequences. We then adjusted the sequence orientation. We allowed 0 mismatches for barcodes and a maximum of 2 mismatches for primers. The operational taxonomic units (OTUs) were clustered at 97% similarity using the UPARSE software (http://drive5.com/uparse/ (accessed on 13 December 2022), version 7.1) [41].

Species annotation was carried out by employing the RDP classifier (http://rdp.cme.msu.edu/ (accessed on 13 December 2022), version 2.2), applying a threshold of 70% to each sequence [42]. Moreover, for Symbiodiniaceae annotation, the OTU sequences derived from previous sequencing were compared with a database (http://sym-its2.marinegenomics.cn/Annotation database (accessed on 13 December 2022)). The most similar type was selected to annotate the OTU [43]. Bacterial annotation was undertaken through comparison with the Silva database (Silva v138) [42].

2.4. Statistical Analyses

Both seawater characteristics and physiological indicators were analyzed using the Wilcoxon rank-sum test to explore differences between the south and north zones. Multivariate analyses, including non-metric multidimensional scaling (NMDS) analysis based on Bray–Curtis dissimilarity matrices, were conducted. The PERMANOVA test was employed via the Adonis package in R. The vegan package used within the R software (version 4.2.3) framework was utilized to assess the explanatory power of the grouping factor (South vs. North) on the sample variations. The Wilcoxon rank-sum test based on the two-sided test was employed when analyzing the main species among the coral symbiotic members (Symbiodiniaceae and bacteria) that differed between the southern and northern zones. The multiple testing correction was applied using the falsely discovery rate (FDR), and the confidence intervals were computed using the bootstrap method with a confidence level of 0.95. The heatmap package in the R environment (version 4.2.3) was used to analyze the association between coral symbiotic members (Symbiodiniaceae and bacteria) and environmental factors, with Spearman's correlation being the correlation coefficient used in the analysis.

The co-occurrence network was constructed using Gephi (version 0.9.2) and visualized in R (version 4.2.3). The correlation coefficient used in the network analysis was Spearman's correlation. In the network, each edge represented the correlation between two nodes, which was undirected. Each node represented a genus or species, where the node sizes corresponded to the proportion of bacteria in the community. The average degree reflected the overall connection density and complexity of the network and was calculated by dividing the sum of edges connected to the node by the number of nodes. The average path length denoted the average distance between any two nodes. This measurement reflected the degree of separation between nodes in the network, with a smaller value indicating a greater connectivity of nodes within the network. The modularity index indicated the modularity of the network graph structure.

3. Results

3.1. Seawater Characteristics and Symbiotic Microalgal Physiology Differ between the Two Zones of E. gemmacea

The overall seawater characteristics between north and south were significantly different, considering seawater temperature, salinity, dissolved oxygen, and turbidity using the Wilcoxon rank-sum test. In general, the south zone presented lower values for these characteristics. In terms of seawater temperature, the average temperature of the south zone (30.30 ± 0.20 °C) was 0.46 °C lower than that of the north zone (30.76 ± 0.01 °C) ($p < 0.05$). The south zone had a salinity of 33.46 ± 0.03 PSU (practical salinity units), which was lower than the salinity of the north zone (33.53 ± 0.01 PSU) ($p < 0.01$), with a difference of 0.07 PSU. There was a significant difference in turbidity ($p < 0.01$), with the turbidity of the south zone being 0.63 ± 0.14 FTU, which was seven times lower than the north zone (4.47 ± 0.23 FTU). The dissolved oxygen content in the south (7.07 ± 0.26 ppm) was also significantly lower than in the north (7.46 ± 0.17 ppm) ($p < 0.05$) (Figure 1). In addition, there were no significant differences in dissolved inorganic nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, and SiO_4^{3-}) between the south zone and the north zone.

Both the symbiotic microalgal density and the chlorophyll-a content of corals in the south zone were significantly higher than those in the north zone. The symbiotic microalgal density of the southern *E. gemmacea* was 2.00 ± 0.54 × 10^6/cm^2, which was also higher than the symbiotic microalgal density of the northern *E. gemmacea* (0.71 ± 0.40 × 10^6/cm^2), with a significant difference ($p < 0.05$) (Figure 1). The chlorophyll-a content of the southern *E. gemmacea* was 3.81 ± 0.92 (μg/cm^2), which was three times higher than that of the northern *E. gemmacea* (1.27 ± 0.44 μg/cm^2), with a significant difference ($p < 0.01$).

3.2. Symbiodiniaceae Types and Dynamic Composition in E. gemmacea

A total of 109,125 valid sequences were preserved after quality control across nine samples. Furthermore, a total of 17 operational taxonomic units (OTUs) were grouped at a similarity threshold of 97%. Annotation information for all OTUs can be found in Table S1. OTU rarefaction curves indicated that most of the bacterial communities were nearly fully sampled (Figure S1). The southern *E. gemmacea* possessed over 80% affiliation with the Symbiodiniaceae of *Cladocopium*. In contrast, the northern *E. gemmacea* demonstrated a mix of *Cladocopium* and *Durusdinium* in the Symbiodiniaceae, with *Cladocopium* and *Durusdinium* accounting for 51.71% and 48.24%, respectively (Figure 2a). The primary Symbiodiniaceae in the southern *E. gemmacea* include *Cladocopium* sp. C1, *Cladocopium* sp. C17, *Cladocopium* sp. C116, and *Cladocopium* sp. C115. However, dominant species in those from the north included *Durusdinium* sp. D1a, *Cladocopium* sp. C17, and *Cladocopium* sp. C1. It is noteworthy that *Cladocopium* sp. C17 and *Cladocopium* sp. C1 were prevalent in both the southern and northern *E. gemmacea*. The relative abundance of *Cladocopium* sp. C17 exceeded 35% in both regions of *E. gemmacea*. Meanwhile, the relative abundance of *Cladocopium* sp. C1 in the southern and northern *E. gemmacea* displayed percentages of 46.45% and 13.40%, respectively. Interestingly, *Durusdinium* sp. D1a was barely observed in the southern *E. gemmacea* but was predominant in the northern *E. gemmacea*, reflected by a relative abundance of 48.24% (Figure 2b).

The NMDS analysis highlighted disparities in beta diversity between the southern and northern sections within the Symbiodiniaceae communities (stress: 0, $r^2 = 0.306$, $p < 0.05$) (Figure 2c). Through Wilcoxon rank-sum test analysis, OTU10 (*Durusdinium* sp. D1a) was found in the most significant differences in Symbiodiniaceae between the southern and northern *E. gemmacea* (Figure 2d, $p = 0.019$). The correlation between the seawater characteristics, symbiotic microalgal density, and Symbiodiniaceae OTUs was analyzed (Figure 2e). The result show that OTU10 (*Durusdinium* sp. D1a) was positively correlated with salinity (Spearman, r = 0.864, $p < 0.01$), turbidity (Spearman, r = −0.845, $p < 0.01$), seawater temperature (Spearman, r = 0.752, $p < 0.05$), and DO (Spearman, r = 0.695, $p < 0.05$), and was negatively correlated with chlorophyll-a (Spearman, r = −0.812, $p < 0.01$). OTU11 (*Cladocopium* sp. C1232) was positively correlated with DO (Spearman, r = 0.748, $p < 0.05$).

Likewise, OTU12 and OTU14, both affiliated with *Durusdinium* sp. D1a, generally showed a positive correlation with seawater characteristics but a negative correlation with the symbiotic microalgal density and chlorophyll-a. Most OTUs associated with *Cladocopium* revealed an inverse correlation with seawater characteristics and were positively correlated with the symbiotic microalgal density and chlorophyll-a. OTU7 (*Cladocopium* sp. C1) was negatively correlated with seawater temperature (Spearman, r = −0.740, $p < 0.01$) and was positively correlated with symbiotic microalgal density (Spearman, r = 0.817, $p < 0.05$). OTU69 (*Cladocopium* sp. C1) was also negatively correlated with seawater temperature (Spearman, r = −0.667, $p < 0.05$). OTU75 (*Cladocopium* sp. C115) was positively correlated with chlorophyll-a (Spearman, r = 0.667, $p < 0.05$).

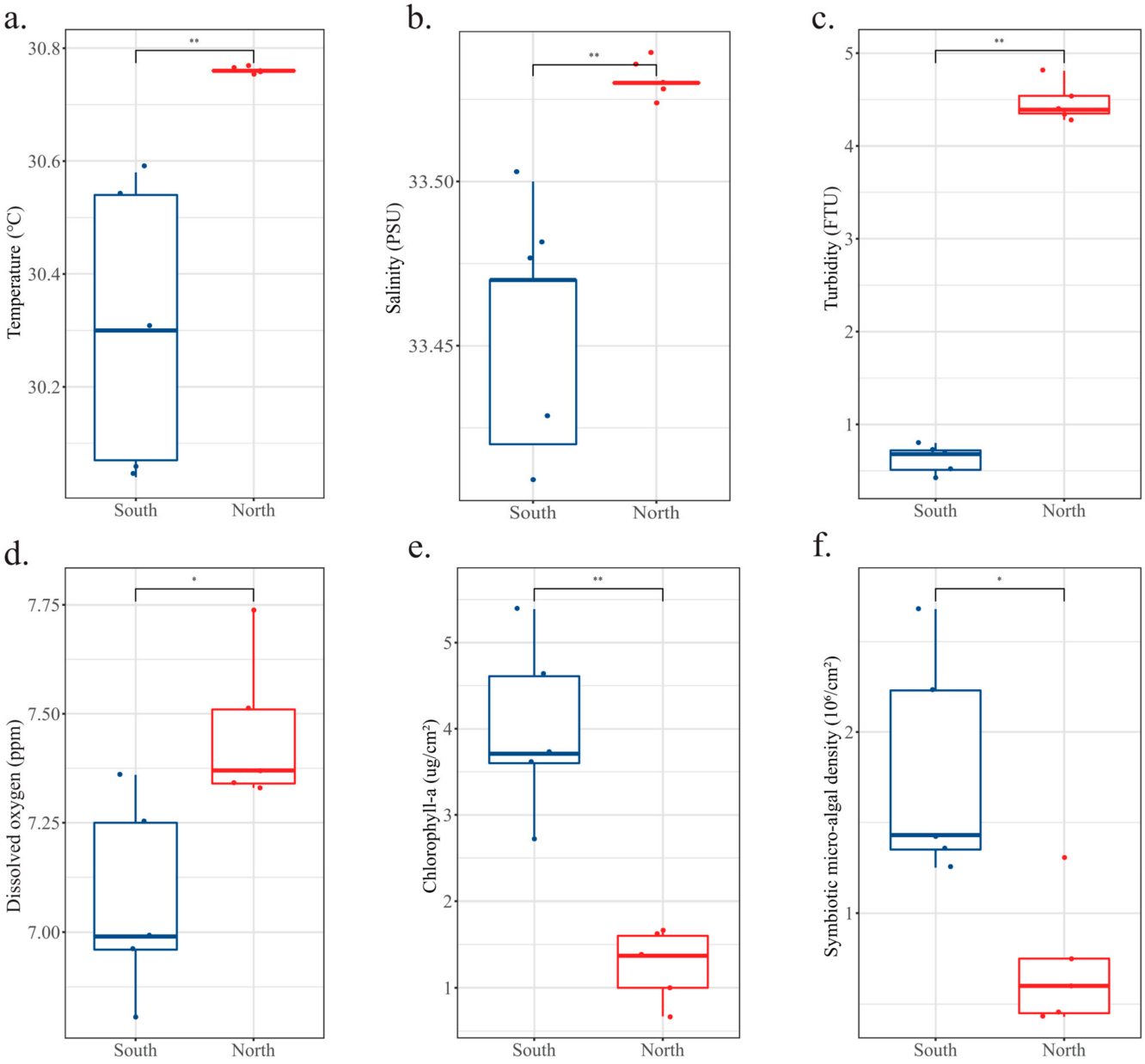

Figure 1. Environmental parameters and photosynthetic indexes of corals. Seawater parameter: (**a**) temperature; (**b**) salinity; (**c**) turbidity; (**d**) dissolved oxygen (DO). The photosynthetic indexes of corals: (**e**) chlorophyll-a; (**f**) symbiotic microalgal density in the corals. The * in each figure represented the *p* value, where * indicated a *p* value less than 0.05, ** indicated a *p* value less than 0.01.

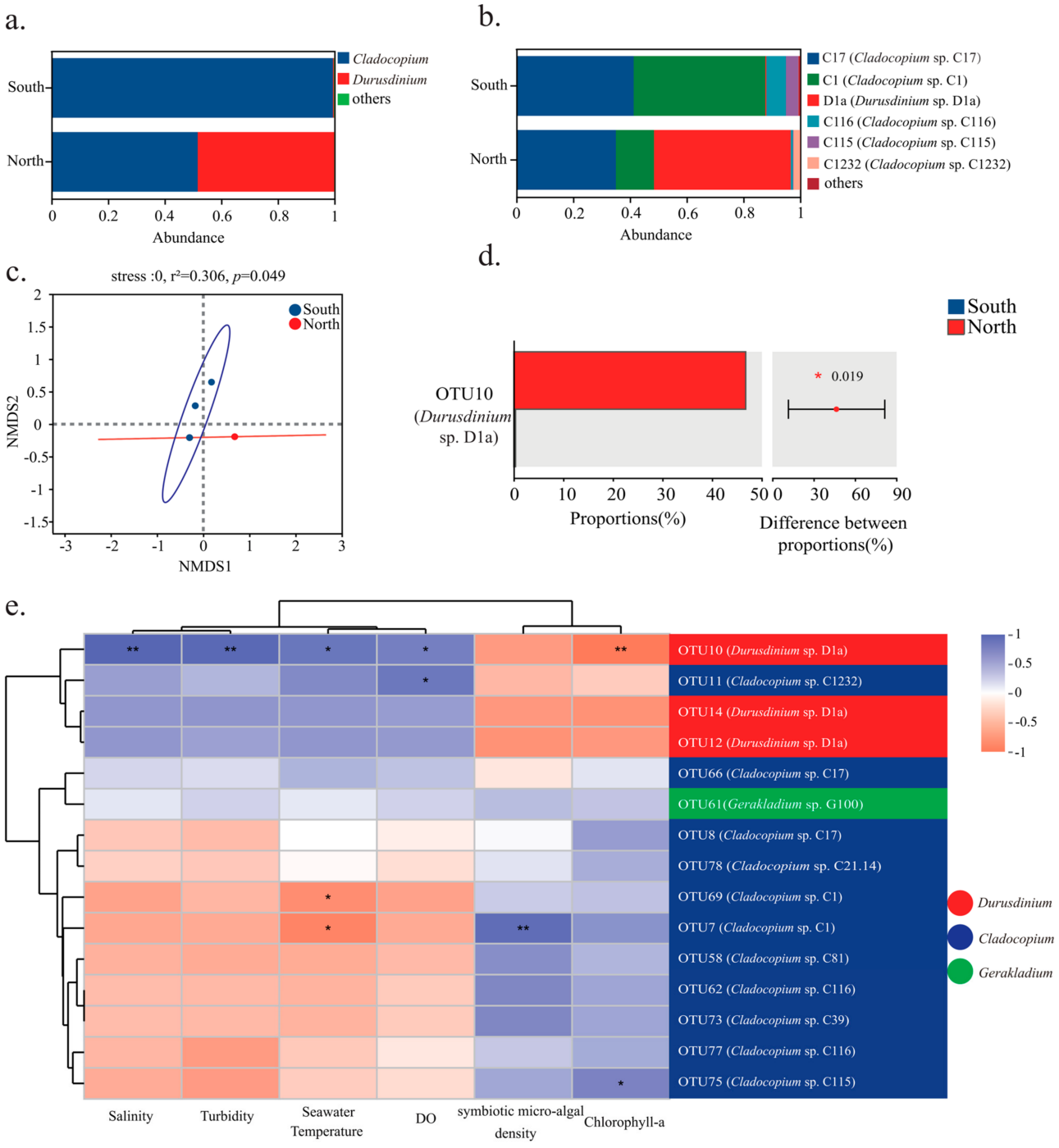

Figure 2. Diversity and community of Symbiodiniaceae from *E. gemmacea* collected from coral to the south and north of WZZ Island. (**a**) Percentage of community abundance at the genus level, south (n = 5), north (n = 4); (**b**) percentage of community abundance on type level, sorted by abundance, 'Others' represents a combination of species with a relative abundance of less than 1%. South (n = 5), north (n = 4); (**c**) NMDS analysis performed on community composition dissimilarities (Bray–Curtis) across *E. gemmacea* in the southern and northern areas; (**d**) Wilcoxon rank-sum analysis performed on significant differences in Symbiodiniaceae abundance between groups based on the OTU level; (**e**) correlation analysis between significant seawater characteristics, physiological indices, and various OTUs. The colors at the bottom level of the OTUs represent the clades to which they belong, as described on the right-hand side. The * in this figure represented the p value, where * indicated a p value less than 0.05, ** indicated a p value less than 0.01.

3.3. Bacterial Dynamic Composition and Seawater Characteristics Correlation

The OTU data of all bacterial communities were filtered based on three criteria: 1. the OTUs at the domain level were retained, with the domain specified as bacteria; 2. the OTUs aligning with Chloroplast and Mitochondria sequences were removed; and 3. the sequences were rarefied based on the minimum sample sequence count. Finally, each sample retained a total of 23,981 sequences. Among them, there were 40 phyla and 1075 genera. A total of 3073 OTUs were clustered at a similarity threshold of 97%. OTU rarefaction curves indicated that most bacterial communities were nearly fully sampled (Figure S1). The bacterial community was mainly composed of Proteobacteria, Actinobacteriota, Firmicutes, Bacteroidota, Chloroflexi, Cyanobacteria, and Acidibacteriota. Other phyla with a relative abundance of less than 1% were categorized as "others" (Figure 3a). At the phylum level, in the southern *E. gemmacea*, the relative abundance of Proteobacteria, Actinobacteriota, Firmicutes and Bacteroidota, respectively, was 81.07%, 10.57%, 2.28%, and 2.11%, while that of Chloroflexi, Cyanobacteria, and Acidobacteriota was less than 2%. In the northern *E. gemmacea*, the relative abundance of Proteobacteria, Actinobacteriota, Firmicutes, Bacteroidota and Chloroflexi, respectively, was 69.15%, 11.91%, 6.07%, 4.30%, and 2.82%, while that of Cyanobacteria and Acidobacteriota was less than 2%. At the genus level, the bacterial composition of southern *E. gemmacea* mainly consisted of *Achromobacter* (41.40%), *Pseudomonas* (25.23%), *Rhodococcus* (8.34%), unclassified_f_Rhodobacteraceae (3.65%), BD1-7_clade (2.22%), *Ruegeria* (1.29%), and *Acinetobacter* (1.01%), whereas unclassfied_f_Rhodobacteraceae (28.32%), *Achromobacter* (23.08%) *Ruegeria* (2.71%), *Rhodococcus* (2.01%), and *Thermopolypora* (1.43%) were observed in the northern *E. gemmacea* (Figure 3b).

The NMDS analysis showed significant differences in the composition of bacteria at the OTU level between the southern and northern *E. gemmacea* (Figure 3c, stress = 0, $r^2 = 0.427$, $p = 0.009$). The PERMANOVA analysis results indicated reasonable grouping (Table S2, $p < 0.01$, F = 6.95912). The Wilcoxon rank-sum test revealed that the bacteria at the genus level differed between the southern and northern *E. gemmacea* (Figure 3d, $p < 0.05$). The relative abundance of *Pseudomonas* and *Rhodococcus* in the southern *E. gemmacea* was significantly higher than that of the northern *E. gemmacea* ($p = 0.019$). Along with BD1-7_clade (2.22%, $p = 0.024$), *Muricauda* (<1%, $p = 0.037$), *Aquabacterium* (<1%, $p = 0.019$), and *Pelomonas* (<1%, $p = 0.018$) were solely present in the southern *E. gemmacea*. Meanwhile, the relative abundance of unclassfied_f_Rhodobacteraceae in the northern *E. gemmacea* was significantly higher than that of the southern *E. gemmacea* ($p = 0.037$). In addition, *Pseudoxanthomonas* ($p = 0.042$), *Thermomonospora* ($p = 0.042$), *Ureibacillus* ($p = 0.011$), *Thermotunica* ($p = 0.042$), norank_f__Cellvibrionaceae ($p = 0.016$), *Chelativorans* ($p = 0.042$), norank_f__Methyloligellaceae ($p = 0.037$), and *Pseudovibrio* ($p = 0.037$) only exist in the northern *E. gemmacea*, with a relative abundance of less than 1%.

The correlation between seawater characteristics and genera with a top 20 proportion was analyzed (Figure 3e). The major distinct genera, *Pseudomonas* and *Rhodococcus*, had negative correlations with DO and salinity (Spearman, $r > 0.8$, $p < 0.01$), and negative correlations with the seawater temperature and turbidity (Spearman, $r > 0.7$, $p < 0.05$). BD1-7_clade, only seen in the southern *E. gemmacea*, was negatively correlated with the seawater temperature (Spearman, $r = 0.938$, $p < 0.001$), and negatively correlated with the turbidity, DO, and salinity (Spearman, $r > 0.6$, $p < 0.05$). Unclassified_f_Rhodobacteraceae, the abundance of which was significantly higher in the northern *E. gemmacea*, had a positive correlation with all seawater characteristics (Spearman, $r > 0.6$, $p < 0.05$). *Pseudoxanthomonas* and *Thermomonospora* (relative abundance < 1%) displayed increased levels in the northern *E. gemmacea*, with positive correlations with the seawater temperature, salinity, and turbidity (Spearman, $r > 0.6$, $p < 0.05$).

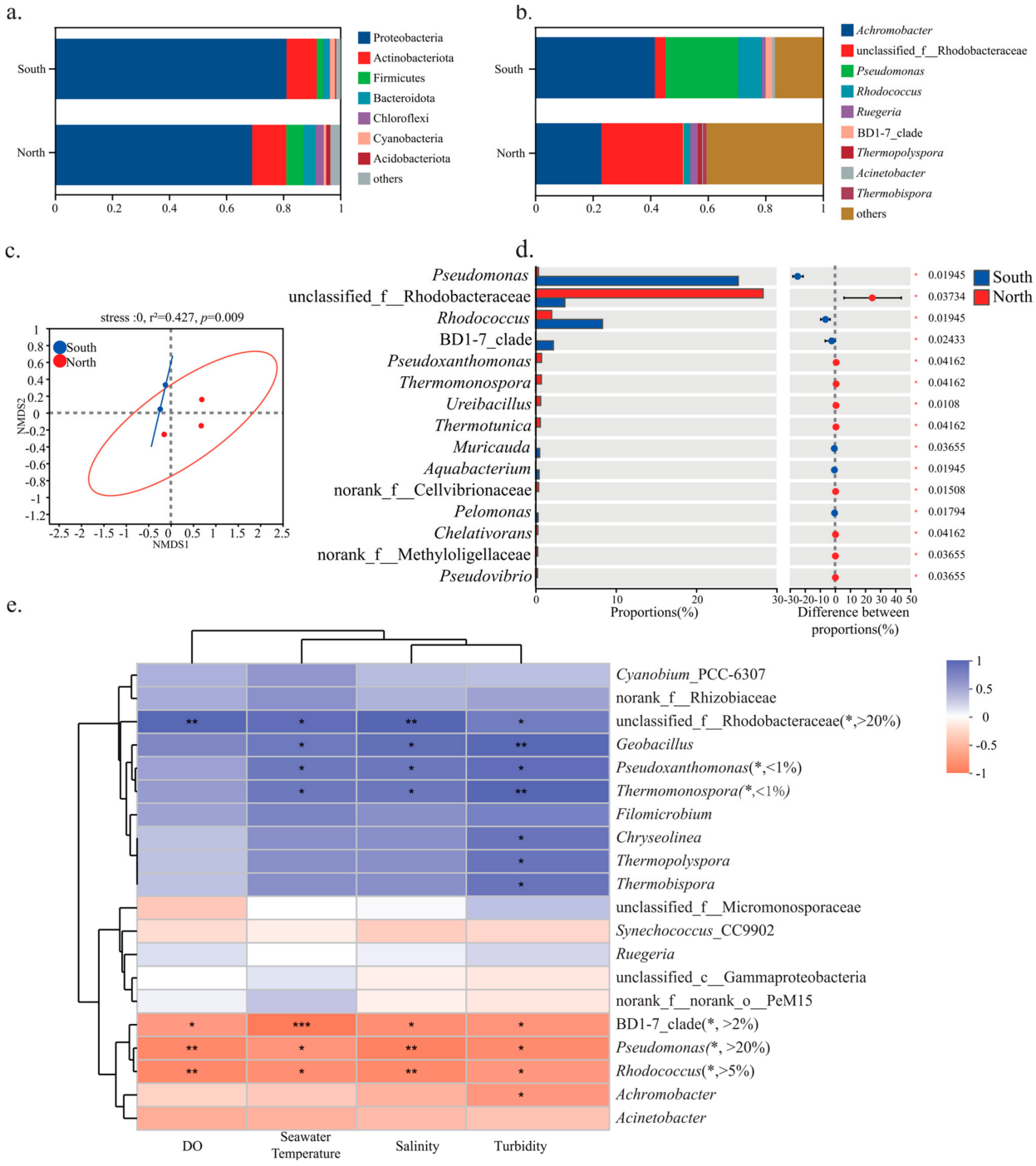

Figure 3. Diversity and community of bacteria from *E. gemmacea* collected from coral to the south and north of WZZ Island. (**a**) Percentage of community abundance at the phylum level, sorted by abundance, 'Others' represents a combination of species with a relative abundance of less than 1%. South (n = 5), north (n = 4); (**b**) percentage of community abundance at the genus level, sorted by abundance, 'Others' represents a combination of species with a relative abundance of less than 1%. South (n = 5), north (n = 4); (**c**) NMDS analysis performed on community composition dissimilarities (Bray–Curtis) across *E. gemmacea* in the southern and northern areas; (**d**) Wilcoxon rank-sum analysis performed on significant differences in bacteria abundance between groups at the genus level; (**e**) correlation analysis between significant environmental parameters indices and various genera. The * in this figure represented the p value, where * indicated a p value less than 0.05, ** indicated a p value less than 0.01, and *** indicates a p value less than 0.001.

3.4. Correlation Network of Bacteria Community

To assess bacterial coexistence in the southern and northern corals, the correlation networks were constructed by using the 200 most abundant genera (Figure 4a) and the 100 most abundant species of the bacteria (Figure 4b) in both zones of coral. The modularity index of the microbial community network on the southern side was 0.639 (Figure 4a) and 0.614 (Figure 4b). Meanwhile, the modularity index of the microbial community network on the northern side was 0.575 (Figure 4a) and 0.442 (Figure 4b). These values demonstrate a successful representation of underlying functional interactions and relationships between bacteria in both networks.

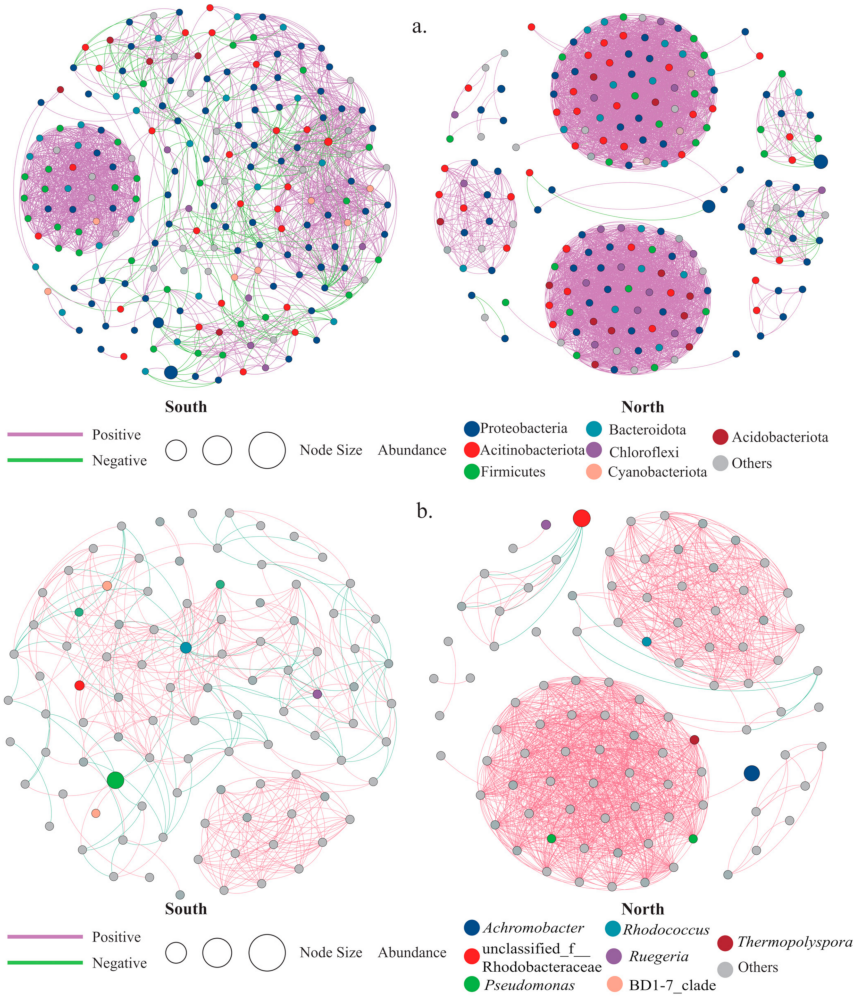

Figure 4. Single-factor correlation network of the bacterial community. (**a**) The nodes are colored based on the phylum level for coral-associated bacteria. The size of each node is proportional to the relative abundance of genera. The edges in the correlation network represent the correlation between two genera of bacteria, which is undirected. The nodes represent a genus, node colors are shown at the phylum level, and node sizes represent the proportion of the genus of bacteria in the community; (**b**) the nodes are colored based on the genus level for coral-associated bacteria. The size of each node is proportional to the relative abundance of species. The edges in the correlation network represent the correlation between two species of bacteria, which is undirected. The nodes represent a species, node colors are shown at the genus level, and node sizes represent the proportion of the species of bacteria in the community.

In this study, there was a closer network led by Proteobacteria and Firmicutes in the southern *E. gemmacea*, whereas in the northern *E. gemmacea*, Proteobacteria and Actinobacteria dominated the network (Figure 4a). There were 2119 fewer edges in the network

graph of the southern *E. gemmacea* than in the northern *E. gemmacea* (Table S3). The average degree of the bacterial network was 17.61 in the southern *E. gemmacea,* whereas there were more connections in the northern *E. gemmacea* (40.23) (Table S4). The average path length in the southern *E. gemmacea* (3.364) was longer than that in the northern *E. gemmacea* (1). The correlation results highlight that the proportion of positive correlations was 84.68% for the southern *E. gemmacea*, which was significantly lower than the 97.44% observed in the northern *E. gemmacea*. Meanwhile, the proportion of negative correlations was 15.32% in the southern *E. gemmacea*, which was much higher than that in the north (2.56%). Overall, the networks showed that the bacterial communities of the southern *E. gemmacea* had fewer convergent interactions than those of the northern *E. gemmacea.*

The co-occurrence network involving bacteria of various genera based on species level was investigated (Figure 4b). In the bacterial network of the southern *E. gemmacea*, the number of edges was 427, with an average degree of 8.804, which was less than that the northern *E. gemmacea*, which has 1233 edges with an average degree of 25.423 (Tables S3 and S4). The average path length in the southern *E. gemmacea* (3.446) was longer than that in the northern *E. gemmacea* (1). The correlation results showed that the proportion of positive correlations was 79.39% in the southern *E. gemmacea,* which was much less than that in the northern *E. gemmacea* (99.19%). The proportion of negative correlations was significantly higher in the southern *E. gemmacea*, at 20.61%, as opposed to the northern *E. gemmacea*, at just 0.81%. Remarkably, the unclassified_f_Rhodobacteraceae demonstrated more positive correlations in the southern *E. gemmacea*, but more negative correlations in the northern *E. gemmacea*. Furthermore, the proportion of positive correlations of *Pseudomonas* and *Rhodococcus* was higher in the northern *E. gemmacea* (3.33%, 2.03%) than in the southern *E. gemmacea* (0.47%, 0.23%).

4. Discussion

4.1. The Density, Chlorophyll-a, and Types of Symbiodiniaceae in E. gemmacea Influenced by Integrated Seawater Characteristics

Recent research showed that the seawater characteristics of the north zone of WZZ Island are generally worse than those of the south zone. Compared to the south zone, the north zone has significantly higher turbidity, seawater temperature, and dissolved inorganic nitrogen levels [33,34]. This could be attributed to the continuous influx of visitors over a prolonged period of time [44]. The general seawater characteristics are consistent with our study. Similarly, higher seawater temperature was seen in the north zone, and the seawater temperature on both sides of WZZ Island in August were slightly higher than the coral bleaching threshold (30 °C) [45]. A significant difference was observed in turbidity, in which the north zone exhibited a value seven times higher than that of the south zone. Apart from dissolved inorganic nitrogen, other seawater characteristics were also noticeably higher in the north zone compared to the south zone of WZZ Island, although the discrepancy in the respective mean values stayed relatively small.

Previous studies on *E. gemmacea* have largely focused on gamete cell development and phylogenetic research [46–48], with limited discourse surrounding the variations in environmental conditions and the transitions in Symbiodiniaceae types. This study reveals, for the first time, that the composition of Symbiodiniaceae in *E. gemmacea* predominantly revolves around *Cladocopium* sp. C17, known for its certain level of bleach resistance [49]. This was corroborated by our research, wherein *Cladocopium* sp. C17 was ubiquitously present in both zones of *E. gemmacea*. Considering the impacted seawater characteristics of the north zone, this suggests that environmental stress could induce the conversion of Symbiodiniaceae types within *E. gemmacea* from *Cladocopium* (including *Cladocopium* sp. C1 and *Cladocopium* sp. C17) to *Durusdinium* (specifically *Durusdinium* sp. D1a). Alterations in the living environment can trigger shifts in the proportions of various Symbiodiniaceae types within the symbiotic system [50]. Past research has shown that an increased occurrence of *Durusdinium* can enhance the survival rate of coral hosts [51],

and hence, in areas enduring sustained coral growth stress, *Durusdinium* often assumes dominance, while *Cladocopium* experiences a relative decrease in its prevalence [52].

Increased temperature and turbidity are associated with dynamic changes in the Symbiodiniaceae community, which can result in variations in symbiotic microalgal density and light adaptation capacity [53]. Studies have suggested a direct correlation between the quantity of chlorophyll-a in coral cells and their light adaptation capacity. Corals with lower symbiotic microalgal density are prone to photo-inhibition under comparative light conditions, leading to a reduction in chlorophyll-a content [54,55]. Our results showed that the symbiotic microalgal density was significantly lower in northern corals than in southern corals. In addition, there was a positive correlation between the relative abundance of OTU7 (*Cladocopium* sp. C1) and the symbiotic microalgal density (Figure 2e). This finding can also serve as evidence for the reduction in the relative abundance of *Cladocopium* sp. C1 in northern corals. OTU75 (*Cladocopium* sp. C115) and chlorophyll-a were positively correlated, and OTU10 (*Durusdinium* sp. D1a) and chlorophyll-a content were negatively correlated, suggesting that the decrease in chlorophyll-a content in northern corals may be related to a decrease in *Cladocopium* and an increase in *Durusdinium*. Symbiotic microalgal density in the coral may be positively correlated with light adaptation, and this phenomenon is more evident in communities dominated by *Cladocopium* [18]. Simultaneously, the proportion of *Cladocopium* positively correlates with the maximum relative electron transport rate of the photosystem II and the transfer of carbon to the host [56]. High concentrations of *Durusdinium* are linked to a stunted host growth rate, damage to the photosystem, and a reduction in chlorophyll-a content [57,58]. Nonetheless, several studies have noted an upsurge in the proportion of *Durusdinium* sp. D1a under stressful conditions, thereby enhancing host survival in a compensatory manner [57,59].

4.2. Important, Susceptible, Stress Resistant Bacteria in E. gemmacea

Symbiodiniaceae communities harness light energy for photosynthesis, supplying fixed organic carbon to their coral hosts [60]. In parallel, bacterial communities can bolster host metabolism and play a pivotal role in enhancing the host's ability to recover from disease [61,62]. In this study, we found the α-diversity of the bacterial community in both northern and southern *E. gemmacea* to be largely similar, predominantly composed of Proteobacteria, Actinobacteria, and Bacteroidetes. At the genus level, *Achromobacter* served as an important bacterium in *E. gemmacea*, with a relative abundance of over 20% in both the southern and the northern corals, and it was speculated to be important in the maintenance of coral health. Previous research has indicated that *Achromobacter* aids in biological nitrogen fixation [63], and certain studies have also demonstrated its role in augmenting host biomass [64].

The three genera, *Pseudomonas*, *Rhodococcus*, and BD1-7_Clade, were significantly present in southern *E. gemmacea*, which occupied a less disturbed environment. Moreover, the relative abundance of these genera showed a negative correlation with multiple seawater characteristics. Furthermore, a recent study indicated a negative correlation between *Pseudomonas* and salinity [65]. *Pseudomonas* members are highly genetically adaptable and contribute to the coral's carbon sources [66]. In our study, *Pseudomonas* exhibited a relatively higher abundance (25.23%) in southern *E. gemmacea* but significantly decreased in northern *E. gemmacea*. This suggests that *Pseudomonas* was sensitive to environmental changes. Similarly, *Rhodococcus* and BD1-7_Clade were less abundant in the northern *E. gemmacea*. Although *Rhodococcus* was commonly found in terrestrial soil, it also serves as a beneficial bacterium for corals in the ocean [67]. At the same time, *Rhodococcus* assimilates various γ-butyrolactone molecules with a branched-aliphatic chain, such as GCL (Glutamate cysteine ligase) [68]. *Rhodococcus* can eliminate contaminants in the environment by participating in reducing reactions such as nitric acid reduction [69].

These differences serve as evidence for variations in the composition of the bacterial community of the coral *E. gemmacea* with environmental changes. There was an observed increase in the proportion of bacteria with enhanced resistance in environments subjected

to greater stress. For instance, the abundance of *Endozoicomonas*, generally correlated positively with the health of corals [70], aids in nutrient acquisition through the nitrogen and carbon cycles and contributes to the establishment of microbial communities [71]. Conversely, the levels of *Rhodobacteraceae* rise under conditions of stress [72]. As a family, *Rhodobacteraceae* demonstrates environmental flexibility [73], and healthy corals are characterized by a richer presence of *Rhodobacteraceae* than diseased corals [74]. Their abundance escalates under stress [75], while the recovery rate from coral lesions is inversely proportional to *Rhodobacteraceae* levels [76]. In this research, we observed that a high concentration of unclassified_f_Rhodobacteraceae in deteriorating environmental conditions led to a reduction in the proportion of other beneficial bacteria such as *Achromobacter*, *Pseudomonas*, and *Rhodococcus*. This phenomenon represents a dynamic bacterial composition within the holobiont, responding to varying environmental conditions. Furthermore, resistant bacteria within the host displayed an increase. At the genus level, *Geobacillus*, *Thermomonospora*, *Thermopolyspora*, and *Thermobispora* were found in higher proportions in northern corals due to their notable heat resistance [77,78]. *Chryseolinea* also displayed a significantly positive correlation with turbidity, suggesting its role in mitigating biological contamination under polluted conditions [79]. *Ruegeria*, a type of bacterium closely associated with corals [80], exhibited a higher proportion in northern corals due to its significant role in resisting *Vibrio coralliilyticus*. This bacterium also demonstrates a degree of salt tolerance [81,82], suggesting that it may improve coral conditions in the northern zone.

4.3. The Interbacterial Competition and Cooperation in E. gemmacea

Host selection, environmental changes, and bacterial species interactions are key factors in the dynamics of bacterial communities [83]. The health of coral and its environmental adaptability can influence the stability of bacterial networks to some degree [84]. The construction of co-occurrence networks is important, as a host may rely on interbacterial interactions to form a suitable bacterial community [85]. In this study, we found that the overall connection density, network complexity, and interconnectivity amongst bacterial nodes were higher in the northern corals, with stronger convergent interactions. More positive correlations and fewer negative correlations were observed among bacterial communities found in the northern coral. This demonstrates that the changes in the external environment can also alter the interactions between bacterial communities. In such networks, a positive relationship implies cooperation, while a negative relationship denotes competition [86,87]. Complex interactions may be attributed to dynamic changes in the holobiont due to external environmental factors. The results of this study indicate the presence of energy differences in *E. gemmacea* between the northern and southern sides of Wuzhizhou Island. These data suggest that the bacterial communities on the northern side of *E. gemmacea* exhibit stronger cooperation. Simultaneously, the bacterial communities demonstrate more convergent interactions, more positive correlations, and more complex relationships.

The interactions between bacterial communities and the environment are sensitive, but they usually do not directly change the physiological functions of corals [88]. Coral reef microbial communities can buffer or exacerbate environmental impacts on coral hosts by altering energy flows in the ecosystem during environmental acclimatization processes [89]. Previous research has shown that the increase in Proteobacteria levels has a detrimental effect on the stability of the bacterial community [90]. In this study, the high proportion of Proteobacteria, reaching 81.07% in the southern region, resulted in less tight inter-bacterial connections. In the northern coral, unclassified_f_Rhodobacteraceae was more abundant, and showed more negative correlations with other bacteria, indicating that it has a competitive advantage for ecological niches within the coral bacterial community. This increase in the coral bacterial community may exacerbate the impact of the environment on hosts in resource-limited situations. Conversely, *Pseudomonas* and *Rhodococcus* showed a significant reduction in relative abundance in the northern coral and showed high tightness but a strong positive correlation with other bacteria. This could be due to nutrient lim-

itations, prompting the bacterial community to enhance interbacterial cooperation and meet trophic interaction needs, potentially protecting the coral host against environmental effects. Nevertheless, co-occurrence network analysis based on topology features is not always representative of the real coexistence of species, and more experimental validations are required to verify these potential relationships [91].

5. Conclusions

In summary, the differences in the composition of symbionts (Symbiodiniaceae and bacteria) between the southern and the northern *E. gemmacea* around WZZ Island were studied. The general seawater characteristics of the north zone were worse than those of the south zone, with relatively higher turbidity, temperature, salinity, and dissolved oxygen content, indicating that the northern corals were under environmental stress. *Cladocopium* sp. C17 was commonly present in both zones of *E. gemmacea*. The different characteristics of seawater result in distinct coral Symbiodiniaceae communities on the north and south sides of the island. *Cladocopium* sp. C1 and *Cladocopium* sp. C17 dominated in the southern *E. gemmacea*, while *Durusdinium* sp. D1a and *Cladocopium* sp. C17 dominated in the northern *E. gemmacea*, resulting in a decrease in the symbiotic microalgal density and chlorophyll-a content in *E. gemmacea*. In addition, *Achromobacter* was commonly found as an important bacterium in both zones of *E. gemmacea*. *Psudomonas*, and *Rhodococcu* were more abundant in southern corals, and showed a positive correlation with relative bacteria, which may enhance interbacterial cooperation and buffer the effects of the environment on the coral host. Unclassfied_f_Rhodobacteraceae, as well as some stress-resistant bacteria such as *Geobacillus*, *Thermomonospora*, *Thermopolyspora*, and *Thermobispora*, were found to be increased in the disturbed northern corals. A negative correlation was observed between unclassified_f_Rhodobacteraceae and other bacteria, indicating greater competition for ecological niches, which may exacerbate the environmental effects on coral hosts in resource-limited situations. The analysis of this study reveals the difference in the symbionts (Symbiodiniaceae and bacteria) in the southern and northern *E. gemmacea*, and lays the groundwork for coral conservation efforts involving *E. gemmacea*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmse11122262/s1. Table S1: Symbiodiniaceae type annotation table; Table S2: PERMANOVA analysis results; Table S3: Edges in the network graph of the southern and the northern *E. gemmacea*; Table S4: The nodes of the bacterial network in the southern and the northern *E. gemmacea*; Figure S1: The geographical location of Wuzhizhou Island and Rarefaction curve analysis results.

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