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Key Points:
- Coral-symbiotic zooxanthellae significantly decreased, and many corals suffered from high thermal stress
- Coral-associated microbial communities changed significantly at different taxonomic levels during GCBE
- Potential pathogens greatly increased, but many beneficial microbes significantly decreased during GCBE

Supporting Information:
- Supporting Information S1
- Table S1
- Table S2
- Table S3
- Table S4
- Table S5

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Significant Changes in Microbial Communities Associated With Reef Corals in the Southern South China Sea During the 2015/2016 Global-Scale Coral Bleaching Event

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Abstract Microbial communities play important roles as coral symbionts, but their changes among coral species in response to thermal bleaching events are not well understood. Therefore, we focused on the microbial communities associated with coral species in the southern South China Sea (SCS) during the 2015/2016 global-scale coral bleaching event (GCBE). Samples of eight typical coral species were collected before and during the GCBE, and the microbial communities, potential gene functions, and zooxanthellae densities (ZDs) were analyzed. We found that ZDs significantly decreased among all coral species during the GCBE. Alpha diversities of the microbial communities also significantly decreased at different taxonomic levels among these coral species. Principal coordinates analysis revealed significant differences in beta diversity among coral specimens, which were divided into two groups before and during the GCBE. Microbial gene functional prediction showed that microbial community physiology significantly changed during the GCBE, with decreased coverage in metabolism, membrane transport, replication and repair, and increased coverage in cell motility and signal transduction. Moreover, the abundance of potential pathogens such as the genus *Vibrio* greatly increased (from ~0.28% to ~52.92%) during the GCBE, whereas the abundance of several beneficial microbes such as *Endozoicomonas* significantly decreased (from ~26.10% to ~0.91%), resulting in an obvious decline in the coral-holobiont physiological functions. Thus, the GCBE greatly affected the health of coral species in the southern SCS, by reducing the biodiversity of associated microbial communities and increasing the abundance of potential pathogens.

1. Introduction

Of late, large-scale use of fossil fuels has increased atmospheric carbon dioxide (CO_2) emissions, resulting in an intensified global greenhouse effect (Abraham et al., 2013; Hansen et al., 2010). The ocean annually absorbs ~25% of humanity's CO_2 emissions to the atmosphere; however, it absorbs ~93% of all the heat trapped by accumulated greenhouse gases (Ciais et al., 2013; Rhein et al., 2013). The directly observed accumulation of heat in the ocean (Abraham et al., 2013; Cheng et al., 2017) and anthropogenic carbon dioxide accumulation (DeVries, 2014; Gruber et al., 2019; Khatiwala et al., 2013) have a significant impact on the ocean, such as ocean warming. First, ocean warming will increase the rates of ocean oxygen consumption, impacting marine life (Brewer, 2019). Moreover, ocean oxygen consumption rates could increase by 29% for every 2°C warming, and by 47% for 3°C warming (Brewer, 2019), driving ocean net oxygen losses. Loss of oxygen solubility from ocean warming will directly affect the gas transfer of organisms' respiratory membrane (Hofmann et al., 2013), which results in organisms with high aerobic demand (e.g., fishes) migrated or suffered (Poertner & Knust, 2007). Second, abnormal high sea surface temperature (SST) caused by ocean warming could have a direct negative impact on marine life. For instance, ocean warming is strongly associated with spreading *vibrio* pathogens, which are responsible for several infections in both humans and animals (Vezzulli et al., 2016).

Coral reefs are critical components of marine ecosystems, with high biodiversity, known as the "tropical rainforest in the ocean," and play important ecological roles in the global ecosystem (Brown, 1997; Hoegh-Guldberg, 1999; Yu, 2012). However, they are facing severe threats from ocean warming (Brown, 1997; Bellwood et al., 2004; Hughes et al., 2017), including carbonate dissolution driven by

diminished carbonate ion concentration, coral reef bleaching driven by ocean warming, invasion of environmental microbial pathogens, and ocean oxygen losses (Brewer, 2019; Vezzulli et al., 2016). In recent decades, abnormal temperatures have frequently been occurring due to global warming, severely threatening the health and survival of coral reefs and creatures living in it. Since the 1990s, coral reefs have frequently experienced global-scale coral bleaching events (GCBEs) driven by ocean warming, including 1998, 2010, and 2015/2016 GCBE (e.g., Hoegh-Guldberg, 1999; Hughes et al., 2018; Loya et al., 2001; Wismer et al., 2019). Having suffered from these GCBEs, most coral reefs worldwide are threatened and have been rapidly degraded. One of main reasons is that coral-microbial holobionts are highly sensitive to abnormally elevated SSTs driven by ocean warming, disrupting the symbiosis under abnormally high temperatures in a short time (Baker et al., 2008).

Coral holobiont comprises of a variety of microorganisms, including dinoflagellate algae, bacteria, archaea, fungi, and viruses, among others (e.g., Reshef et al., 2006; Rohwer et al., 2002). These microorganisms, which live in coral mucus, tissues, and skeletons, interact with their hosts to maintain the holographic biological functions, including those in the biogeochemical cycle, material transformation, antimicrobial defense, nutrient access, and further maintenance of the health of the coral reef ecosystems (Bourne et al., 2008; Mahmoud & Kalendar, 2016; van de Water et al., 2018). Among these microorganisms, zooxanthellae (i.e., Symbiodiniaceae) are the most important symbionts, generally providing more than 95% of energy to the coral host (Brown, 1997; Hoegh-Guldberg, 1999). Large reductions in the abundance of symbiotic zooxanthellae driven by ocean warming will directly affect the coral energy capture and health state, causing coral bleaching (Brown, 1997). Moreover, associated bacteria and archaea are the most diverse and complex microbes among coral holobionts. They are considered essential for coral health and important functions involved in environmental adaptation (Mcfallngai et al., 2013; van de Water et al., 2018). High abundance and diversity of these communities are supposed to maintain holobiont stability and flexibility to deal with a changing environment (Torda et al., 2017). Moreover, associated microbes have an important role in the response and adaptation to thermal stress because of their short reproductive cycle and faster metabolism (Bordenstein, 2016; Pogoreutz et al., 2018; Wegley et al., 2010; Ziegler et al., 2017). It is important to understand the ability of bacteria/archaea in response to environmental stress based on their symbiotic relationship with coral hosts.

Despite numerous microbial functions not being well understood, microbes are known to play important roles as symbionts. Exploring the composition and diversity of coral-associated microbes may help effectively assess the coral health state (Bayer et al., 2013; Bourne et al., 2016; Glasl et al., 2016; Peixoto et al., 2017). In coral-microbial symbiosis, most microbes are beneficial to the coral host. For example, many microbial species of photosymbiont-bearing invertebrates, including Oceanospirillales spp., *Alteromonas* spp., *Pseudomonas* spp., and *Halomonas* spp., are implicated in the metabolism of dimethylsulfoniopropionate (DMSP), which enables the symbionts to attain a more efficient energy cycle (Bourne et al., 2013). Members of Roseobacteriales, which participate in sulfur cycling, are generally identified to be associated with zooxanthellae and promote the growth of algae (Ritchie, 2012). Besides, nitrogen-fixing bacteria can promote the nitrogen cycle through nitrogen fixation, ammoniation, nitrification, and denitrification (Cardini et al., 2015; Wegley et al., 2010). Previous studies indicated that zooxanthellae associated with *Montastrea covernosa* obtained nitrogen from associated nitrogen-fixing cyanobacteria (Lesser et al., 2007; Lesser et al., 2017; Peixoto et al., 2017). Other beneficial bacteria, such as *Endozoicomonas,* have been identified as useful indicators of coral health, and significant loss of these microbes has a severe negative influence on physiological function (Bourne et al., 2016; Meyer et al., 2014; Peixoto et al., 2017). Contrastingly, some microbes are potential pathogens, such as several *Vibrio* species (e.g., *V. shiloi*, *V. coralliilyticus*; Ben-Haim et al., 2003; Jessica et al., 2015; Kushmaro et al., 1996), leading to the invasion of environmental microbial pathogens to coral reefs. A large increase in the abundance of these microbes may affect the health of coral holobionts. Exploring the changes in a coral-associated microbial community provides a basis to assess the coral's health state. However, the physiological functions of a large number of microbes in coral-holobionts remain unknown, especially in natural tropical coral reef areas.

Abnormally high temperatures have many negative effects on coral holobionts, including coral bleaching, microbial community shifts, and increased risk of coral disease (e.g., Frydenborg et al., 2014; Meyer et al., 2015). Thermal stress markedly increases thermal bleaching events, which might result not only in the disintegration of endosymbiotic dinoflagellates but also in coral-microbial symbioses recombination

(Bourne et al., 2008). During a bleaching event, the effects on corals need to be assessed by further studying coral-associated zooxanthellae, microbial community, and their physiological functions. In natural tropical coral reef regions (CRRs), the changes in coral-associated microbial communities and zooxanthellae populations/types, as well as the physiological characteristics of coral holobionts in response to bleaching events, are still poorly understood in a number of coral species. Therefore, it is necessary to investigate the coral-associated zooxanthellae populations, microbial communities and their physiological functions among coral species to analyze the influence and potential risk of disease during thermal bleaching events.

In the Nansha Islands (i.e., the Spratly Islands, southern South China Sea (SCS)), coral bleaching events, such as GCBEs, are one of the main threats to coral health, and lead to coral reef degradation and macroalgae occupation (e.g., Li et al., 2011; Yu et al., 2006). *In situ* observation during an investigation in June 2015 found that corals were in a healthy state such that almost no bleaching corals were found (e.g., Qin et al., 2019). In June 2016, the global El Niño event resulted in high SST anomalies such that corals suffered from high thermal stress. *In situ* observation found that local corals generally showed a light color in June 2016 and that many corals were in the initial stages of bleaching. For example, some branching *Acropora* corals were prebleaching in the southern SCS Xinyi Reef. However, previous studies have insufficiently analyzed the influence of GCBE on coral reefs in tropical islands. For example, only a survey in coral bleaching rate and coral zooxanthellae density (ZD) during the 2007 bleaching event (Li et al., 2011) and a record of bleaching events in *Porites* (Yu et al., 2006) have been reported. The health state and response of southern SCS corals to the 2015/2016 GCBE remain unknown.

In this study, two fundamental questions are being addressed: (i) we currently do not know whether coral bleaching occurred in the southern SCS among coral species as a result of massively reduced zooxanthellae population under the 2015/2016 GCBE; and (ii) the effects of GCBE on associated microbial symbioses among coral species and their potential relationships with coral disease are not well understood. We tested the hypotheses that (i) the GCBE caused a massive loss of zooxanthellae population in some coral species that were in a pre-bleaching/unhealthy state; and (ii) the microbial communities of some corals changed significantly and suffered from potential pathogen disturbance, but that the others were still in a healthy state. To explore these questions, local water quality parameters, ZDs, coral-microbial symbioses compositions, and physiological functions were identified and compared between samples of eight typical coral species collected from the southern SCS before and during the 2015/2016 GCBE. The results indicated that all studied coral species, including heat-tolerant and heat-sensitive corals, suffered from the event, with reduced zooxanthellae population and diversity of their associated microbial communities, and increased abundance of potential pathogens. These results will help further our understanding of corals' health state and their potential susceptibilities to diseases under ocean warming.

2. Materials and Methods

2.1. Study Area and Coral Collection

The study site is located at Xinyi Reef (9°20′–9°21′N, 115°54′–115°58′E), Nansha Islands in the southern SCS (Figures 1a and 1b). The mean annual SST was 28.7°C, and monthly average SSTs ranged between 27.1°C and 30.1°C. In this study, SSTs, Degree Heating Week (DHW) index, chlorophyll content, and particulate organic carbon (POC) concentration were obtained from satellite-derived datasets of NASA, Ocean Color Radiometry, and monthly averaged MODIS-Aqua 9 km, spanning from January 2015 to December 2017 (https://giovanni.gsfc.nasa.gov). Seawater temperature, salinity, dissolved oxygen (DO), transparency, and turbidity were measured during seawater sampling. Seawater samples were collected and immediately filtered (Whatman GF/F; GE Healthcare, Chicago, IL, USA). Dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) were measured using a continuous flow analyzer (SEAL QuAAtro; SEAL Analytical Shanghai, Shanghai, China). Coral species were randomly collected at 6–10 m depths, and in situ photographs were taken during sampling for identification. It is well-known that thermal stress tolerance is different between coral species or morphologies. Generally, massive corals have higher thermal stress tolerance than foliaceous and branching corals under natural conditions, (e.g., Loya et al., 2001; Wooldridge, 2014). Collecting a series of coral species, including those with different morphologies, could be conducive to understand better corals' state in response to thermal stress in the southern SCS. Therefore, a total of 71 sextuplicate samples (six duplicates per sample, 25–30 cm^2 for each sample) across

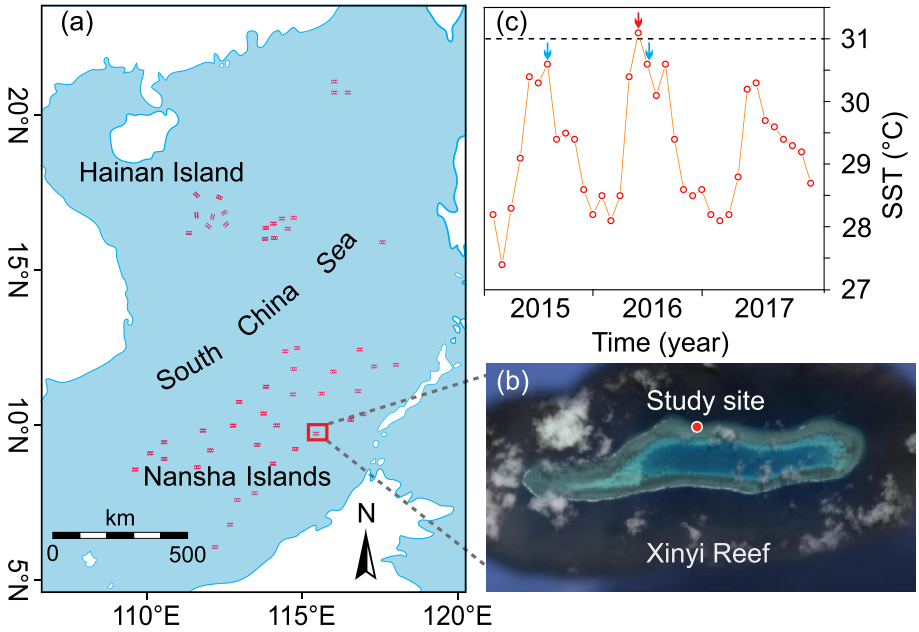

Figure 1. Map of the study area and sea surface temperatures (SSTs). (a and b) Sampling area indicated by red box and circle. (c) SSTs were obtained from NASA satellite-derived datasets, ocean color radiometry, and monthly averaged MODIS-Aqua 9 km from January 2015 to December 2017 (http://oceandata.sci.gsfc.nasa.gov/). The blue arrows indicate the sampling times in June 2015 and June 2016, and the red arrow indicates the period during which corals suffered from high thermal stress in 2016.

eight typical coral species, including massive, foliaceous, branching, and solitary corals, were collected in this study (i.e., 34 sextuplicate samples collected in June 2015 and 37 sextuplicate samples collected in June 2016; Table S1 in the supporting information, *Pocillopora verrucosa*, *Acropora corymbosa*, *Montipora efflorescens*, *Echinopora lamellosa*, *Merulina ampliata*, *Porites lutea*, *Platygyra daedalea*, and *Fungia fungites*). All samples were preserved at −20°C and then immediately transported to the laboratory for DNA extraction.

2.2. ZD Determination

Coral tissue was removed using a Waterpik containing seawater that passed through a 0.45 μm filter. The initial slurry volume was measured in a graduated cylinder. The slurry was homogenized and subsampled into four 3-ml aliquots. Subsamples were centrifuged (5,000 r min^{-1}) for 5 min. After discarding the supernatant, the zooxanthellae in residuum was preserved in 1 ml 5% formaldehyde for 2–4 hr at 4°C until further analysis. ZD was calculated using replicate hemocytometer counts ($n = 8$). Surface areas were determined from the correlations between aluminum foil weight and surface area (Jeffrey & Humphrey, 1975; Li et al., 2011).

2.3. DNA Extraction, PCR Amplification, and Illumina MiSeq Sequencing

Coral nubbins (~50 mg), including tissue, mucus, and skeleton were cut with scissors and then used to extract total genomic DNA using FastDNA® SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) following the manufacturer's protocol provided with the kit. To reduce individual random errors as far as possible, the total genomic DNA of each of the six replicate fragments per coral sample was the same for all coral species. After quality and purity examinations, extracted DNA samples were applied as PCR templates. The V3–V4 region of the 16S rRNA gene for bacteria/archaea was amplified using the barcoded primer 338F (5′-ACTC CTACGGGAGGCAGCAG-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′; Mori et al., 2013; Xu et al., 2016). The ABI GeneAmp® 9700 thermal cycler and TransGen AP221-02 PCR kit (TransStart FastPfu DNA Polymerase, in a 20 μl reaction) were used (Sun et al., 2014). The employed PCR was described in detail by Liang et al. (2017). Triplicate PCR products were pooled for each sample and 420–460 bp fragments were purified and quantified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and QuantiFluor™-ST Fluorescence quantitative system (Promega,

USA). Purified replicated amplicons from the same coral individual were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to the standard protocols (Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA545886).

2.4. Data Analysis

Raw sequences obtained from Illumina MiSeq sequencing were optimized using the Trimmomatic software platform to exclude the reads with homopolymer inserts greater than six base pairs and low-quality tail scores (<20), setting a 50 bp quality window (Bolger et al., 2014). After removing chimeric sequences, all reads were identified and classified using the Ribosomal Database Project (RDP) by setting a bootstrap confidence level of 70% and clustered into operational taxonomic units (OTUs) at a 97% identity threshold. Furthermore, we obtained the representative sequence for each OTU (Edgar, 2010). Based on the clustered OTUs, alpha diversity indices, including ACE, Chao 1, Shannon-Wiener, and Simpson indices, were calculated by Mothur (version v.1.30.1; Schloss et al., 2013). Taxonomy was aligned and compared with the SILVA database using the Qiime platform (Quast et al., 2013). Alpha diversity and microbial genera of coral specimens collected in June 2015 and June 2016 were compared by Wilcoxon rank-sum test. The Kruskal-Wallis test was used to compare specimens among the eight species of corals collected in June 2015 and June 2016. Beta diversity was evaluated by principal coordinates analysis (PCoA) at the OTU level. Welch's t-test was used to compare ZD between coral samples collected in June 2015 and June 2016. Bray-Curtis distance matrices were used to examine additional microbial community patterns. All multidimensional statistical analyses were performed on R software (version 3.1.2) using the package Vegan. A heatmap was plotted using the same R software environment.

Microbial (bacteria and archaea) predicted gene functions were inferred from the 16S rRNA marker gene sequences using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) computational approach from the Clusters of Orthologous Groups of proteins (COG) and Kyoto Encyclopedia of Gene and Genomes (KEGG) catalogs (Langille et al., 2013). OTUs that were closest to the reference were identified using a 97% identity threshold against the Greengenes database by Macqiime prior to the PICRUSt analysis. The newly picked OTUs were then normalized by the known or predicted 16S copy number abundance, and metagenome functions were predicted by the "predict_metagenomes.py" module of PICRUSt. Predicted functional composition profiles were collapsed into the hierarchical categories of Level 2 and Level 3 of KEGG database pathways. The relative abundance of each coral specimen's functional traits was calculated by STAMP software (Hakim et al., 2016). Quality control for PICRUSt was also performed, which gave a percentage of successful reads that were mapped to Greengenes using the closest-to-reference OTUs picked for each coral specimen presented as NSTI (the nearest sequenced taxon index) scores (Table S2). The Welch's *t*-test performed the relative abundance comparison of all metabolic pathways at Levels 2 and 3 in KEGG between corals sampled in June 2015 and June 2016. All results were presented as mean ± standard deviation.

3. Results

3.1. Local Environment and ZD

Average monthly SSTs ranged from 27.7°C to 31.4°C from January 2015 to December 2016, with the highest DHW values being observed during May and June 2016 (Figure 1c, Table S3). SSTs in June 2016 were clearly higher than those in May and June 2015, and corals experienced high thermal stress during May and June 2016 (Figure S1, Table S3). Satellite-derived temperatures suggested that thermal stress reached the highest in June 2016. Other seawater parameters such as salinity, chlorophyll, POC, DO, DIN, SRP, transparency, and turbidity showed no significant differences between June 2015 and June 2016 (Welch's *t*-test, $p > 0.05$). All data suggested that this is an oligotrophic tropical CRR with high transparency (Table S4).

ZDs were significantly different between corals collected in June 2015 and June 2016 (Table 1, Welch's *t*-test, $p < 0.001$). The ZDs of corals collected in 2016, which were within 0.32 ± 0.26–$0.48 \pm 0.37 \times 10^6$ cells cm^{-2}, were lower than those of corals collected in 2015, which were within 0.81 ± 0.20–$1.16 \pm 0.25 \times 10^6$ cells cm^{-2}. The ZD losses may be divided into two groups using a 70% threshold. The group I included *P. verrucosa*, *A. corymbosa*, *M. efflorescens*, *E. lamellosa*, and *F. fungites* in which ZD losses ranged from

Table 1
Coral-Symbiotic Zooxanthellae Density (ZD) in the Southern SCS Before and During the 2015/2016 Global-Scale Coral Bleaching Event

		ZD ($\times 10^6$ cells cm^{-2})		Welch's *t*-test		
Year	Coral species	Average	±SD	*t*	*df*	*p*
2015	*Pocillopora verrucosa*	0.81	0.20	6.686	52	<0.001
2016		0.36	0.27			
2015	*Acropora corymbosa*	0.85	0.28	7.108	52	<0.001
2016		0.32	0.26			
2015	*Montipora efflorescens*	1.03	0.32	7.523	52	<0.001
2016		0.38	0.30			
2015	*Echinopora lamellosa*	0.89	0.21	5.739	40	<0.001
2016		0.41	0.32			
2015	*Merulina ampliata*	1.03	0.18	8.776	46	<0.001
2016		0.42	0.25			
2015	*Porites lutea*	1.16	0.25	9.417	58	<0.001
2016		0.50	0.29			
2015	*Platygyra daedalea*	0.93	0.28	5.271	58	<0.001
2016		0.48	0.37			
2015	*Fungia fungites*	0.85	0.13	6.861	52	<0.001
2016		0.38	0.34			

Note. ZD = zooxanthellae density; SD = standard deviation.

70.93% to 80.00%, and group II included *P. lutea*, *M. ampliata*, and *P. daedalea* in which ZD losses ranged from 65.52% to 68.63%. In short, ZDs of corals decreased from June 2015 to June 2016 in the southern SCS.

3.2. Alpha Diversity of Coral-Associated Microbial Communities

After quality filtering, the number of recovered reads from each coral sample was not less than 30,460, and these reads were clustered into different microbial OTUs at a 97% identity threshold (Table S5). The average length of these sequences was 440 bp. Good's coverage of each sample library exceeded 99%, indicating that these sequencing results represented real community conditions. The average number of microbial OTUs was significantly higher in June 2015 (782 OTUs) than in June 2016 (609 OTUs).

Specific to coral species, coral-associated microbial OTUs sampled in June 2015 and June 2016 were significantly different (Wilcoxon rank-sum test, $p < 0.05$). Overall, the numbers of microbial OTUs in most coral taxa significantly decreased. For example, the average microbial OTUs in *P. verrucosa*, *M. efflorescens*, *E. lamellosa*, *P. lutea*, and *F. fungites* ranged from 606 to 1,095 in June 2015, compared to 389–825 in June 2016. Diversity was higher in June 2015 than in June 2016 (Table S5). For example, the Shannon-Wiener (H′) index of June 2015 samples was 4.04 ± 1.29, while it was only 3.18 ± 0.89 in June 2016 samples. Moreover, the average H′ index of microbial communities in *P. verrucosa*, *M. efflorescens*, *E. lamellose*, *P. lutea*, *P. daedalea*, and *F. fungites* significantly decreased, which ranged from 2.95 to 4.95 in June 2015 and from 2.52 to 3.81 in June 2016. In contrast, it was almost unchanged in *A. corymbosa* and *M. ampliata*. Other indices including Simpson, Ace, and Chao 1 are displayed in Table S5. These indices were significantly different between June 2015 samples and June 2016 samples. For instance, Ace and Chao 1 indices in *M. efflorescens*, *E. lamellose*, and *F. fungites* corals of June 2015 samples were higher than those in June 2016 samples. In short, these data suggested that the alpha diversity of coral-associated microbial communities was significantly different before and during the 2015/2016 GCBE.

3.3. Taxonomic Compositions of Bacteria and Archaea

Total taxonomic compositions of bacteria/archaea in analyzed samples included 57 phyla, 134 classes, 289 orders, 569 families, and 1,359 genera. Among them, the communities in June-2015 samples consisted of 57 phyla and 1,300 genera, and in June-2016 samples consisted of 44 phyla and 952 genera (Table S5, Figures S2, S3). Kruskal-Wallis test showed that the most dominant microbial genera collected in June 2015, including unknown *Rhodobacteraceae genus*, *Endozoicomonas*, and unknown *Cyanobacteria genus*, showed no significant differences ($p > 0.05$) among coral species. Similarly, the most dominant microbial genera in June 2016, including *Pseudoalteromonas*, *Alteromonas*, and unknown *Rhodobacteraceae genus*, were not significantly different among coral species according to the Kruskal-Wallis test (Figure S3, $p > 0.05$). Taxonomic compositions were further compared between coral specimens collected in June 2015 and June 2016. At the phylum level, Proteobacteria (71.01% ± 23.46%), Bacteroidetes (9.92% ± 12.92%), and Cyanobacteria (5.61% ± 7.41%) were absolutely dominant in all samples (Figures 2 and S2, Table S6). However, their relative abundance was clearly different between the two groups. Proteobacteria showed a significant increase in abundance in June-2016 coral samples, with an average relative increase from 54.16% to 86.49% (Wilcoxon rank-sum test, $p = 0.009$). In contrast, other phyla showed a significant decline in abundances, such as the phyla Bacteroidetes, Cyanobacteria, and Firmicutes (Figure S5, Wilcoxon rank-sum test, $p < 0.05$). All the coral species showed similar trends, but the rates of change in relative abundance varied. For example, the largest increase in Proteobacteria abundance of June-2016 coral samples occurred in *F. fungites* (from 38.29% to 95.41%), while in *M. ampliata*, the increase was smaller (from 77.62% to 91.65%). Most of the other phyla showed decreasing trends among coral species when comparing June-2015 and June-2016 coral samples, but the organisms showing the largest decrease in

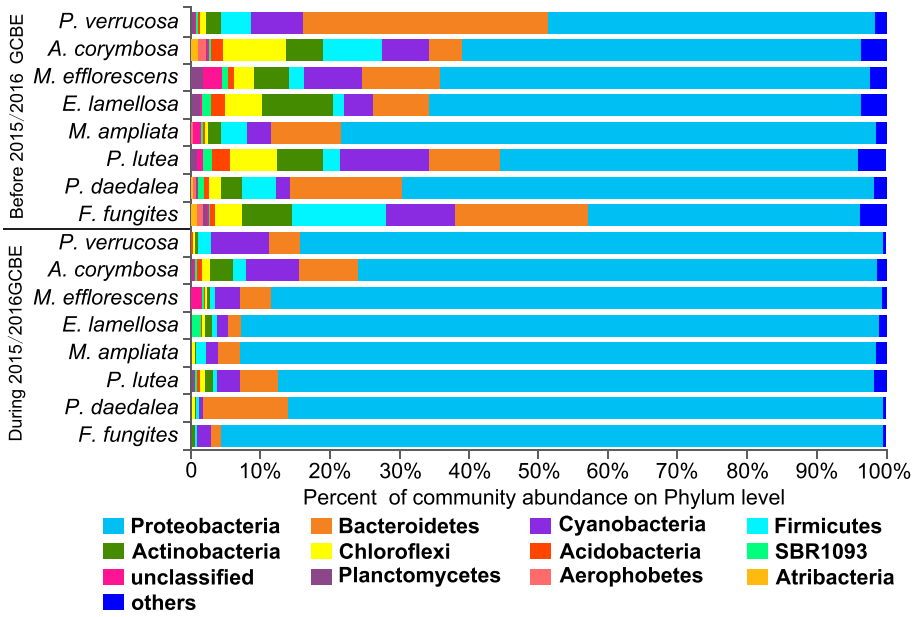

Figure 2. Coral-associated microbial community relative abundance for each coral species sampled before and during the 2015/2016 global-scale coral bleaching event (GCBE) at the phylum level using RDP classifier. The horizontal axis represents the percentage of each bacterial phylum, and each bar represents the community of a coral species with 3–6 replicate coral samples. The "others" denote those phyla whose abundances were less than 0.01%.

abundance differed. For example, the largest decrease in Bacteroidetes abundance occurred in *P. verrucosa* (29.66%) and *F. fungites* (16.43%), whereas Firmicutes abundance showed the largest decrease in *A. corymbosa* (17.10%) and *P. daedalea* (6.25%).

At the bacteria/archaea genus level, more information was obtained (Figures 3 and S3, Table S7). The average abundances of *Pseudoalteromonas*, *Vibrio*, *Alteromonas,* and *Photobacterium* in June-2015 coral samples were significantly higher than those in June-2016 samples (Figure 4, Wilcoxon rank-sum test, $p < 0.001$). By contrast, many genera, including *Endozoicomonas*, *Cyanobacteria*, *Rhodobacteraceae*, and unknown genus of Oceanospirillales, showed a decrease in abundance. Depending on coral taxa, although all species showed similar trends, there were different relative abundance shift ratios (Figure S3). For example, the largest increase in *Vibrio* abundance happened in *F. fungites* (1.61% to 52.92%) in June-2016 coral samples, with the smallest increase occurring in *P. lutea* (from 0.28% to 6.29%). However, most microbial genera showed decreasing trends. For example, *Amoebophilus* abundance in *P. verrucosa* decreased from 26.10% to 1.97%, and *Endozoicomonas* abundance in *A. corymbosa* decreased from 11.97% to 0.91%. In summary, all coral-associated microbial communities were significantly different at all taxonomic levels before and during the 2015/2016 GCBE.

3.4. Beta Diversity

PCoA was used to assess the similarity between microbial communities associated with these studied coral specimens at the OTU level. PCoA analysis using Bray-Curtis metrics showed that coral specimens could be divided into two groups (Figure 5). Group I contained most of the samples collected in June 2015, and Group II contained most of the samples collected in June 2016. PCoA showed that the coral-associated microbial community compositions were different between sampling times.

3.5. Microbial Gene Function Predictions

PICRUSt was used to predict the metagenomic functional capacity of coral-associated bacteria/archaea from 16S rRNA sequencing data. Twenty-seven predicted functional categories were analyzed from Level 2 of KEGG (Figure 6). Most functional categories showed a significant decline in the average relative abundance of the metabolic repertoire, including carbohydrate metabolism, energy metabolism, and amino acid metabolism (Table S8, Welch's *t*-test, $t = 40.782$, $p < 0.001$; $t = 30.083$, $p < 0.001$; $t = 4.720$, $p = 0.033$, respectively). Specific to coral species, the largest decline occurred in *F. fungites* (from 51.09% to 44.96%), and other corals

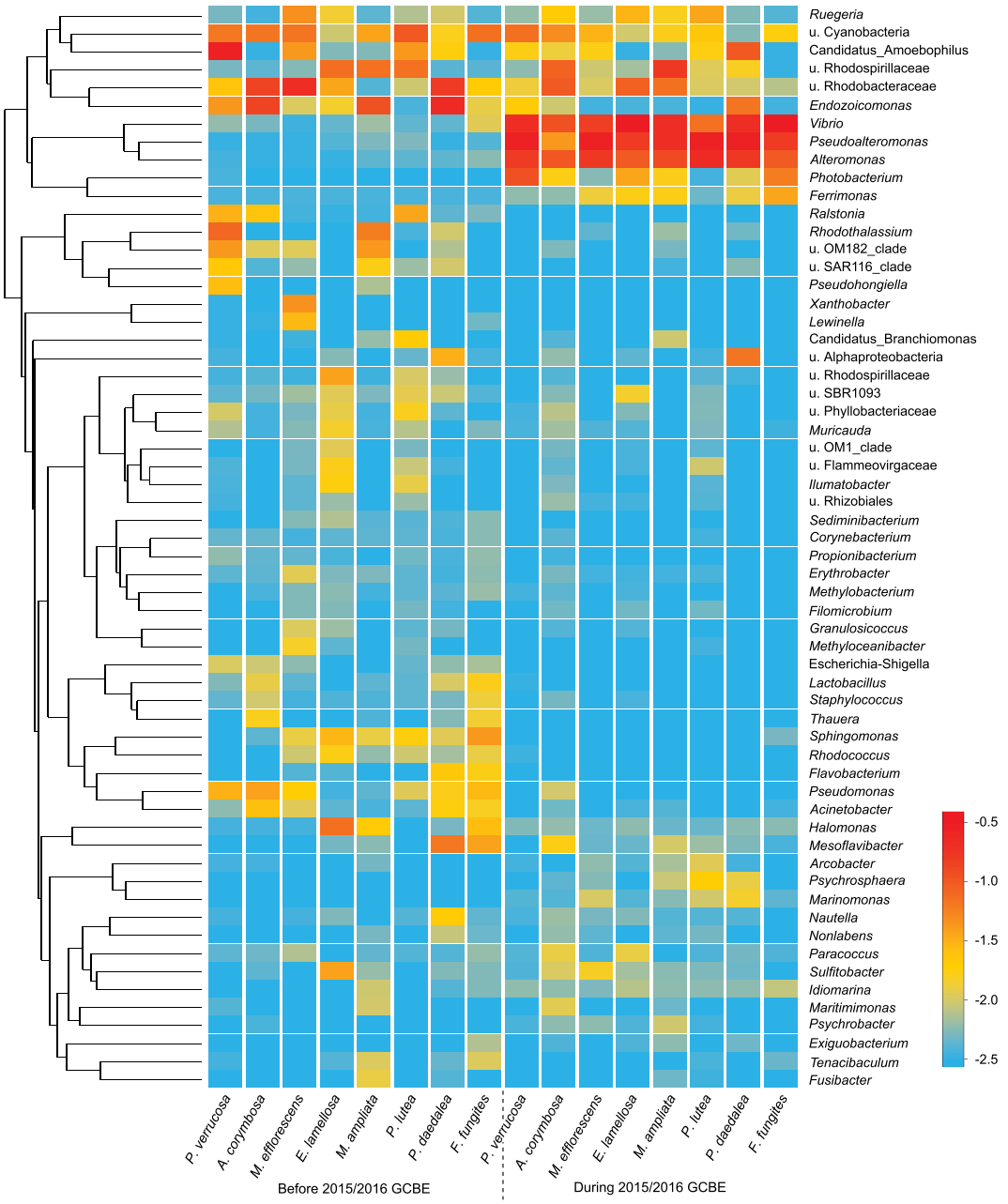

Figure 3. Log-scale percentage heatmap of the 60 most abundant associated microbial genera. The scale "−2.5, −2.0, −1.5, −1.0, −0.5" shows the relative abundance at "0%, 1%, 3%, 10%, and 31%," respectively. Some microbes such as u. Cyanobacteria, u. Rhodospirillaceae, u. Rhodobacteraceae represent unclassified coral-associated microbial genera of Cyanobacteria, Rhodospirillaceae, and Rhodobacteraceae, respectively.

such as *E. lamellosa*, *P. lutea*, and *M. efflorescens* declined in abundance between 1.36% and 5.43%. By contrast, cellular processes in coral specimens were significantly lower in June 2015 than in June 2016 (from 6.97% to 9.48%; Welch's *t*-test, $t = 53.254$, $p < 0.001$). Moreover, the average relative abundance of cell motility genes (Welch's *t*-test, $t = 83.242$, $p < 0.001$), cellular processes and signaling (Welch's *t*-test, $t = 30.083$, $p < 0.001$), and cell growth and death (Welch's *t*-test, $t = 56.734$, $p < 0.001$) increased significantly in June 2015 than in June 2016. According to coral species, the largest increase occurred in *F. fungites* (from 6.93% to 10.62%), while the smallest occurred in *A. corymbosa* (from 7.22% to 7.96%).

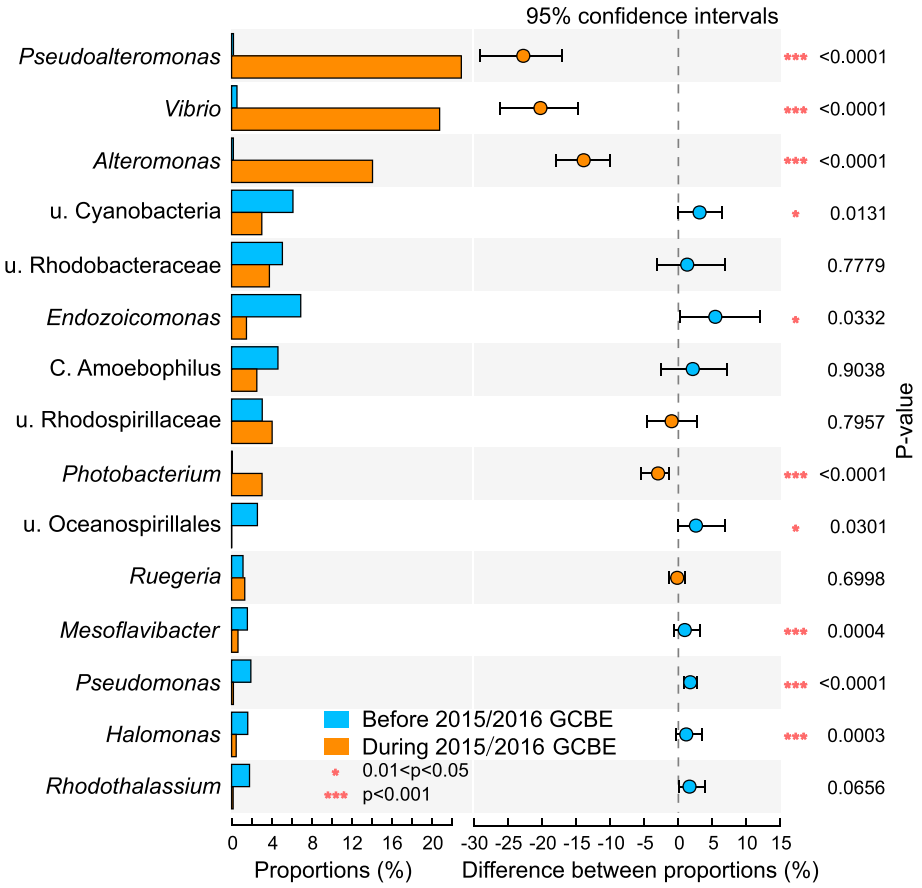

Figure 4. Wilcoxon rank-sum tests at the genus level for both the corals sampled before and during the 2015/2016 GCBE. Extended error bar plot showing the 15 most abundant microbial genera that had significant differences between coral specimens sampled in June 2015 and June 2016. Positive differences (blue dots) in mean relative abundance indicate genera overrepresented in the coral specimens sampled before the GCBE, while negative differences (orange dots) indicate greater abundance in the coral specimens sampled during the GCBE. The red asterisks "*" and "***" represent "$p < 0.05$" and "$p < 0.001$," respectively.

Moreover, a total of 127 functional traits were analyzed using Level 3 KEGG (Table S9). We found that numerous functional characteristics of microbial communities were significantly different between the two groups of specimens (Welch's *t*-test results are shown in Table S9). Metabolism-related functions, such as those of amino acid-related enzymes and metabolism of amino and nucleotide sugar, generally showed downward trends (Figure S6). Their ability to degrade hazardous substances, such as aminobenzoate, chlorocyclohexane, and chlorobenzene, decreased. However, several functional traits significantly increased, such as bacterial secretion system, cell motility and secretion, and bacterial motility proteins (Welch's *t*-test results are shown in Table S9).

4. Discussion

In this study, coral-associated microbial communities significantly changed during thermal stress in the 2015/2016 GCBE. Microbial diversity was generally lower in the coral specimens collected during the GCBE than in those collected before the event. Microbial community composition was subjected to recombination/reshuffling. Before the GCBE, a low thermal stress temperature (average SST, 30.4°C) was observed in the southern SCS. In contrast, high thermal stress may be harmful to coral-associated microbial communities suffering from high thermal stress (average SST 31.2°C in June 2016). Based on the coral-symbiotic zooxanthellae data, the density was low during the GCBE. These data showed that some coral species, such as *Acropora* corals collected during the GCBE, were in a prebleaching state. Therefore, it is important to note that the coral-associated microbial community composition experienced tremendous

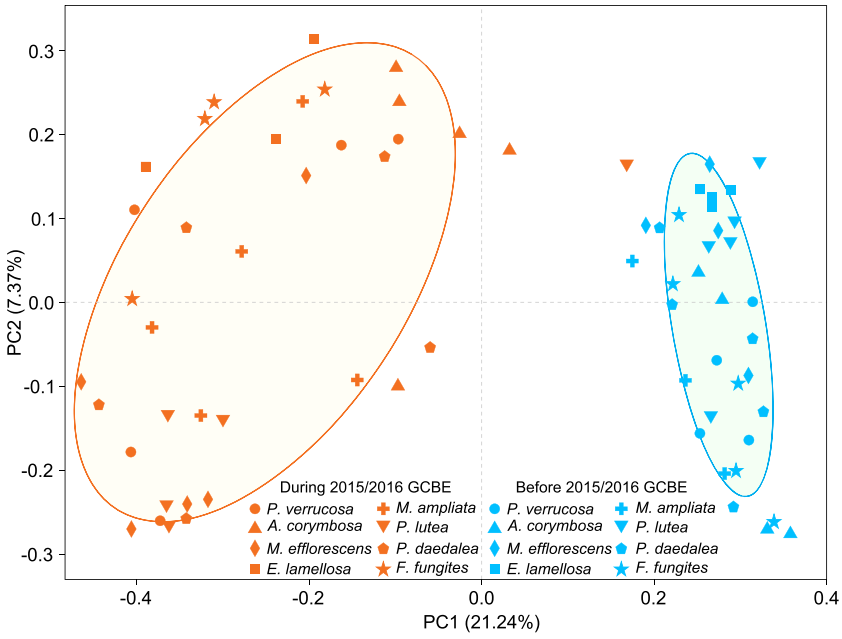

Figure 5. Principal coordinates analysis (PCoA) based on OTUs with grouping based on complete linkage cluster analysis of 60% similarity. PC1 and PC2 explained 21.24% and 7.37% of the total variation, respectively.

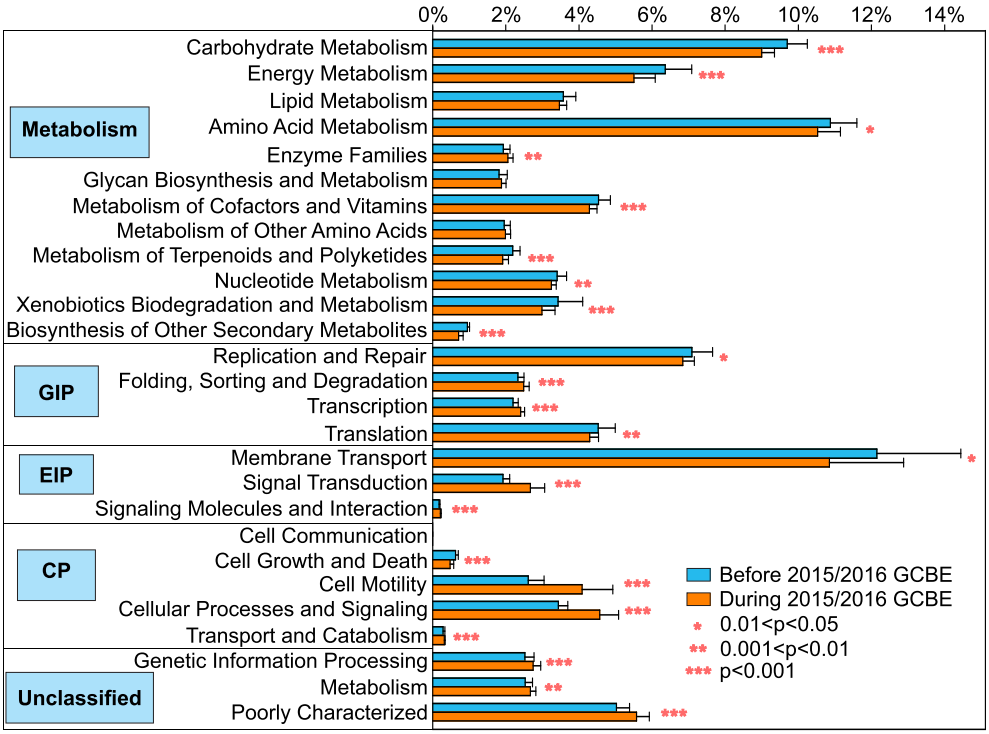

Figure 6. Mean relative abundance of each predicted functional trait in KEGG pathways (level 2) using PICRUSt (V1.0.0) analysis of predicted metagenomes based on the 16S rRNA gene data of coral-associated bacteria before and during the GCBE. GIP: Genetic Information Processing; EIP: Environmental Information Processing; CP: Cellular Processes. The error bars denote the standard deviation. The red asterisks "*," "**," and "***" represent "$p < 0.05$," "$0.001 < p < 0.01$," and "$p < 0.001$," respectively.

shifts regardless of the taxonomic level considered. Corals were harshly threatened by the GCBE, resulting in a reduction of microbes, such as *Endozoicomonas,* and increases of *Vibrio* and *Pseudoalteromonas*.

Two possible causes that may well explain the changes in coral-associated microbial communities and the abundance of *Vibrio* increased significantly in coral holobionts before and during the 2015/2016 GCBE. On the one hand, under the impact of abnormally high temperature caused by the GCBE, the relative abundance of the *Vibrio* increased directly by reproduction, which was original low-abundance in the microbial communities. Previous tank experiments showed that corals are prone to change their microbial communities during acute heat stress (e.g., Zha et al., 2018; Ziegler et al., 2017). During the GCBE, coral-microbial symbioses are seriously disturbed, and the coral host might be unable to fully control and regulate the bacterial reproduction and composition (Ainsworth & Gates, 2016; Muller et al., 2018), resulting in the reduction of beneficial microbes and the relative abundance of the potential pathogens increases. On the other hand, suffering from abnormally high temperatures, coral hosts are in hungry when their symbiotic photosynthetic zooxanthellae are largely discharged or died, or photosynthetic pigments loss. In order to compensate for the energy loss caused by zooxanthellae reduction, these starved Cnidaria would increase the zooplankton predation, such as some chitin-containing copepods. However, these copepods are one of the most important environmental repositories of *Vibrio* in marine ecosystems (Vezzulli et al., 2010). These planktonic crustaceans may be the host's increasing spread of *Vibrio* species associated with ocean warming (Vezzulli et al., 2016). Ingestion of these zooplanktons may increase the relative abundance of *Vibrio* in these coral holobionts. However, it has not yet been determined whether the *Vibrio* largely bred in the coral host under thermal stress or ingested zooplankton with the potential pathogen, or both. In either case, the result, as we have seen, is that coral-associated microbial communities were significantly different between the sampling times considered in this study and significantly increase in the abundance of potential pathogens (e.g., *Vibrio*) in the June-2016 coral samples.

Furthermore, the changes in microbial taxonomic compositions before and during the 2015/2016 GCBE were different among coral species. Many studies have reported that abnormal temperatures have different effects on corals (e.g., Baums et al., 2010; Fitt et al., 2001; Loya et al., 2001). For example, Baird and Marshall (1998) found that, in 1998, the bleaching response of coral taxa was significantly different. Most Acroporids and Pocilloporids corals experienced severe bleaching, but *Cyphastrea*, *Turbinaria*, and *Galaxea* corals were relatively unaffected (Baird & Marshall, 1998). This event showed that different corals varied in thermal susceptibility. In our study, microbial diversity of *P. verrucosa*, *M. efflorescens*, *M. ampliata*, and *F. fungites* decreased dramatically during the 2015/2016 GCBE. The relative abundance of Bacteroidetes decreased significantly, but that of *Pseudoalteromonas* and *Vibrio* genera increased significantly. Resistance to thermal stress varies widely among coral species. *Porites* is generally considered to be a heat-tolerant coral species due to its broad-spectrum defense mechanism and stable symbiotic coral-algae relationship (Krediet et al., 2013; Parkinson et al., 2015; Putnam et al., 2012). However, branching corals, such as *Pocillopora* and *Acropora*, are usually considered to be heat-sensitive and more likely to be affected by thermal stress (Li et al., 2011; Loya et al., 2001; Xu et al., 2017). In this study, the detected lower microbial community stability in *F. fungites*, *P. verrucosa*, and *A. corymbosa* than in other coral species during the 2015/2016 GCBE may cause higher thermal bleaching susceptibility.

Large changes in the coral-associated microbial community resulted in physiological function changes of the holobiont. Beneficial microbial communities were dominant in coral-microbial symbioses before the GCBE. These microbes play important roles in maintaining the physiological activities of normal holobionts (Bourne et al., 2016; Neave et al., 2017; Peixoto et al., 2017). For example, *Endozoicomonas* genera have metabolic versatility and are generally considered to play important roles in coral holobiont function (Neave et al., 2017; Pogoreutz et al., 2018; Wegley et al., 2010). Their potential biological role is to transfer, transmit, and regulate coral hosts and contribute to protein supply and carbohydrate cycling (Ding et al., 2016; Neave et al., 2017). Roseobacteriales that are involved in the sulfur cycle are generally considered to be obligate partners in symbiotic algae cultures, increasing the growth rate of dinoflagellates (Peixoto et al., 2017; Ritchie, 2012). During the 2015/2016 GCBE, large loses of beneficial associated microbe impaired coral physiology. The microbial functional predictions showed that the microbial metabolic capacities were significantly decreased when comparing June-2015 and June-2016 coral samples, including amino acid metabolism, biosynthesis of other secondary metabolites, and carbohydrate metabolism. However, several traits of environmental information processing and cellular processes increased when

comparing June-2015 and June-2016 coral samples, including bacterial secretion system, cell motility and secretion, and bacterial chemotaxis, and peroxisome. The physiological functions of bacterial communities significantly decreased during the 2015/2016 GCBE. The main reason was that the relative abundance of beneficial bacteria including *Cyanobacteria*, *Endozoicomonas*, Oceanospirillales, Mesoflavibacter, *Pseudomonas*, and *Halomonas* significantly decreased during the bleaching event. The balanced state in coral-microbial symbioses may be destroyed, leading to physiological function declines in the microbial community.

Functional predictions indicated that the health state differed between sampling periods. Corals collected before the 2015/2016 GCBE showed normal physiological activities and were healthy because of the highly complex and diverse microbial communities and better physiological and metabolic activities. By contrast, during the GCBE, corals were threatened by thermal stress and were in a subhealthy or prebleaching state, characterized by changes under thermal stress at the level of coral-associated microbial metabolism, such as carbohydrate composition (Rubio-Portillo et al., 2016; Ziegler et al., 2017). For example, Ziegler et al. (2017) found different functional characteristics in microbial communities of highly variable and moderately variable corals, with the highly variable coral microbiome being rich in several protein functions associated with carbohydrate metabolism. Our study suggested that metabolism was the most obviously affected physiological characteristic when experiencing a large-scale coral bleaching event. All the studied coral species during GCBE that were generally suppressed in carbohydrate metabolism, energy metabolism, and amino acid properties significantly declined and may have been in a thermal-stress state and vulnerable to be invaded and threatened by pathogens.

Many types of coral diseases have been reported, including black band, black spot, white band, white pox, and yellow band disease, white plague, and bacterial bleaching (Stachowitsch, 2010). The occurrence of these diseases is mainly caused by pathogenic bacteria invasion or propagation in the coral host (Krediet et al., 2013). Among these microbes, the *Vibrio* group is the most common pathogen (Kline & Vollmer, 2011; Weil & Rogers, 2011). Members of the Vibrionaceae are associated with a range of coral diseases, such as white band disease; bacterial bleaching is caused by *Vibrio* spp., and *V. coralliilyticus* has been shown to be a causative agent for some types of white syndromes in the Indo-Pacific, as well as the etiological agent for bleaching and tissue lysis in *Pocillopora damicornis* (Ben-Haim et al., 2003; Littman et al., 2010; Sussman et al., 2008). Under temperature abnormalities, the significant increase in the abundance of *Vibrio* spp. in corals has also been shown to be closely related to bacterial diseases. In the Caribbean, *V. shiloi* has become a causative agent of coral bleaching during periods of anomalous SST rise (Cervino et al., 2004; Kushmaro et al., 2001). Our results showed that the relative abundance of *Vibrio*, *Pseudoalteromonas*, and *Alteromonas* was significantly increased in all coral species during the 2015/2016 GCBE. However, significant differences in community shifts were found among coral species. For instance, *F. fungites*, *P. verrucosa*, and *E. lamellosa* had large increases in *Vibrio* relative abundance, while *P. lutea* showed a smaller increase. A large increase in the abundance of potential pathogens increases the risk of coral diseases. The abundance of potential pathogens such as *Vibrio* spp. and *Alteromonas* spp. increased in the coral holobiont, and these became the dominant microbial composition during the 2015/2016 GCBE. These potential pathogens began to occupy the niche through mass reproduction and breaking the steady symbiotic state. As a result, microbial community metabolism declined, thereby reducing the holobiont resistance to thermal stress and increasing the risk of coral diseases.

Due to their short outbreak time and huge destructive power, coral diseases are extremely harmful to coral community health. Disease outbreaks always result in numerous coral deaths and declines in live coral cover and diversity (Miller et al., 2009; Randall & Woesik, 2015). In the Caribbean Sea, coral white plague affects nearly half of reef-building corals, killing small corals in a few days and large corals in a few weeks (Randall & Woesik, 2015). During bleaching stress, the white plague-II spread throughout the Caribbean, with an average prevalence of more than 33% of coral infection (Remily & Richardson, 2006; Richardson & Voss, 2005). It is the most prevalent coral disease-causing coral tissue lysis and community shifts (Remily & Richardson, 2006). Pathogens show preference when infecting corals (Aronson et al., 2001; Ben-Haim et al., 2003; Rosenberg et al., 2007). For example, *V. shiloi*, which causes white band disease, is more likely to infect *Acropora*, and *V. coralliilyticus* is generally reported to infect *Pocillopora*. Moreover, the ability to resist pathogen infection is different between coral species (Ben-Haim et al., 2003; Lee et al., 2012). Our study showed that susceptible corals such as *F. fungites*, *P. verrucosa*, and *A. corymbosa* may be susceptible to

GCBE and death because of being threatened by potential pathogens. In short, as a result of the relatively weak resistance of these corals to infection by potential pathogens, bleaching events may result in coral disease with subsequent bleaching events.

Data Availability Statement

The data used in this paper are available from supporting information (SI), and the data can be available from the Data Sharing Infrastructure of Earth System Science (10.12041/geodata.225876591200175.ver1.db).

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