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Potential molecular traits underlying environmental tolerance of *Pavona decussata* and *Acropora pruinosa* in Weizhou Island, northern South China Sea

Xiaopeng Yu[a,b,c], Kefu Yu[a,b,c,d,*], Zhiheng Liao[a,b,c], Jiayuan Liang[a,b,c], Chuanqi Deng[a,b,c], Wen Huang[a,b,c], Yanhua Huang[a,b,c]

[a] Guangxi Laboratory on the Study of Coral Reefs in the South China Sea, Guangxi University, Nanning 530004, China
[b] Coral Reef Research Center of China, Guangxi University, Nanning 530004, China
[c] School of Marine Sciences, Guangxi University, Nanning 530004, China
[d] Southern Marine Science and Engineering Guangdong Laboratory, Zhuhai 519000, China

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ABSTRACT

Coral species display varying susceptibilities to biotic or abiotic stress. To address the causes underlying this phenomenon, we profiled the Symbiodiniaceae clade type, bacterial communities and coral transcriptome responses in *Pavona decussata* and *Acropora pruinosa*, two species displaying different environmental tolerances in the Weizhou Island. We found that C1 was the most dominant Symbiodiniaceae subclade, with no difference detected between *A. pruinosa* and *P. decussata*. Nevertheless, *P. decussata* exhibited higher microbial diversity and significantly different community structure compared with that of *A. pruinosa*. Transcriptome analysis revealed that coral genes with significantly high expression in *P. decussata* were mostly related to immune and stress-resistance responses, whereas, those with significantly low expression were metabolism-related. We postulate that the higher tolerance of *P. decussata* as compared with that of *A. pruinosa* is the result of several traits, such as higher microbial diversity, different dominant bacteria, higher immune and stress-resistant response, and lower metabolic rate.

1. Introduction

Tropical reef-building corals constitute the ecological and constructive base of coral reefs, which provide essential ecological goods and services in an oligotrophic environment (West and Salm, 2003; Hoegh-Guldberg et al., 2007). However, coral reefs are degrading rapidly in response to climate change (Grottoli et al., 2006; van Hooidonk et al., 2016; Hughes et al., 2017a, 2017b) and numerous anthropogenic drivers, such as land-use change and overfishing in the Anthropocene (Hughes et al., 2017a, 2017b; Hughes et al., 2020). In particular, severe bleaching events caused by an increase in seawater temperature and ocean acidification have now affected almost every coral reef ecosystem worldwide (Hoegh-Guldberg et al., 2007; Harrison et al., 2019). Several major severe bleaching events have been recorded since 1979 on a global-scale (Keshavmurthy et al., 2019), have resulted in significant coral mortality and destruction of the coral community structure in the Indian (Montefalcone et al., 2018; Head et al., 2019), Pacific (Fox et al., 2019; Raymundo et al., 2019; Vargas-Ángel et al., 2019), and Atlantic Oceans (Smith et al., 2019; Teixeira et al., 2019). Consequently, the imminent threat to the survival of coral reefs from diverse sources raises the urgent question of whether coral reefs will continue to function (Pandolfi and Kiessling, 2014).

However, not all coral species are equally susceptible to bleaching (Loya et al., 2001; Sutthacheep et al., 2013), corals exhibiting branching morphologies being more susceptible to external interference that can lead to blanching than those with massive morphologies (Wooldridge, 2014). For example, numerous unprecedented high temperature events occurred worldwide in 1998 that led to large-scale bleaching and death of corals (Loya et al., 2001). These catastrophic events occurred in three oceans, with over 50 countries reporting local coral bleaching (Loya et al., 2001). Although coral bleaching was extensive, it did not lead to the absolute death of corals, as the degree of bleaching at different locations and in different types of corals was shown to vary markedly (Loya et al., 2001). Similarly, “winners”, “losers”, and unexpected outcomes occurred in 2015/2016 (Loya et al., 2001; Hughes et al., 2017a, 2017b; Eakin et al., 2019), during which

* Office 1111#, Zonghe Shiyan Building, Guangxi University, Nanning City, Guangxi Zhuang autonomous region, China.
E-mail addresses: xiaopengyu@st.gxu.edu.cn (X. Yu), kefuyu@scsio.ac.cn (K. Yu).

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period yet another very strong El Niño–Southern Oscillation occurred (Santoso et al., 2017), which led to widespread coral mortality, particularly in the central to western Pacific and Indian Oceans (Hughes et al., 2017a, 2017b; Stuart-Smith et al., 2018; Riegl et al., 2019; Sheppard et al., 2019). Notably, significant differences in tolerance have been observed among corals in the South China Sea. Tolerant corals still grow well, while sensitive corals have died (Yu, 2012; Tkachenko and Soong, 2017; Yu et al., 2019). Hence, the key to understanding the resilience of the coral reef ecosystem is to conduct in-depth studies regarding the high tolerance mechanism of global coral albinism.

To date, the differences in coral tolerance have been studied to some extent. Despite tentative associations between the traits of the coral host and variable levels of thermal bleaching resistance, some coral species have been shown to adapt to biotic or abiotic stress. The mechanism underlying albinism and mortality of other coral species thus remains unknown (Wooldridge, 2014). From the perspective of coral host, the traditional view is that metabolic rates (Gates and Edmunds, 2015), colony tissue thickness (Loya et al., 2001; Dimond et al., 2012; Qin et al., 2019a, 2019b), host mucus (Fitt et al., 2009; Wooldridge, 2009), host fluorescent pigment concentration (Salih et al., 2000), heterotrophic feeding (Grottoli et al., 2006; Levas et al., 2013), and differential gene expression (Barshis et al., 2013; Zhou et al., 2017; Tang et al., 2018) likely contribute to this dichotomy. It has also been reported that different combinations of symbiotic bacteria (Liang et al., 2017; Ziegler et al., 2017) and Symbiodiniaceae (Abrego et al., 2008; Sampayo et al., 2008; Chen et al., 2019; Qin et al., 2019a; Qin et al., 2019b; Chen et al., 2020; Qin et al., 2020) are the main factors responsible for this difference. Overall, scleractinian corals are mainly composed of several symbiotic organisms; i.e., the cnidarian host, Symbiodiniaceae, and bacterial communities (Brener-Raffalli et al., 2018). These partners are involved in a stable symbiosis and effectively comprise the holobiont (Brener-Raffalli et al., 2018). Therefore, the environmental stress response of scleractinian corals may not derive from a single factor, but rather represents a complex response of symbiotic interactions.

The Weizhou Island coral reef belongs to the coral suburb of the northern margin of the South China Sea and serves as a potential refuge for corals in the context of global climate change (Yu et al., 2019). Notably, this area has not been studied in depth despite hosting a large number of coral reefs. Ecological investigation of the Weizhou Island coral reef ecosystem over the past 30 years has revealed a significant decline in the coral cover and significant changes in dominant assemblages (Yu et al., 2019). Specifically, to date, areas of taxa with high structural complexity (e.g., *Acropora* and *Montipora*) have been replaced by more tolerant clones (e.g., *Porites*, *Platygyra*, *Goniastrea*, *Favites*, and *Pavona*). In particular, *Acroporids* corals, which are considered "competitive" corals that grow fast and dominate the reefs in productive environments, are also the most sensitive to environmental change. Conversely, the bleaching-tolerant coral *Pavona*, with characteristics of fast growth and high tolerance, has become dominant in the high latitude coral reef area (Yakovleva and Hidaka, 2004; Mezaki et al., 2014; Yu et al., 2019) and may represent a source for broadcasting species during global warming (Glynn and Colley, 2008). Notably, similar to field monitoring results in which corals with different morphologies demonstrate different tolerances to acute heat stress, different Symbiodiniaceae losses may contribute to different tolerances, as observed between *Acropora* and *Pavona*. (Li et al., 2011a, 2011b). Therefore, studying sensitivity differences between *Acropora* and *Pavona* corals in Weizhou Island might provide critical insights regarding the key factors influencing coral tolerance, and potential adaptability of the coral reef ecosystem in the context of future climate change. In this study, a comprehensive approach was used to explore the causes of tolerance differences between *A. pruinosa* and *P. decussata*. Specifically we clarified the potential molecular regulatory mechanisms involved in the survival or death of the scleractinian coral by characterizing the differences with respect three components (Symbiodiniaceae and bacterial communities, and coral host transcriptome response) between the *A. pruinosa* and *P. decussata* holobionts.

2. Materials and methods

2.1. Coral sampling

Weizhou Island is the biggest and youngest island in the Beibu Gulf of the South China Sea (Liu et al., 1991). It provides an ideal habitat for coral growth. The coral here is mainly distributed at a depth of less than 10 m (Yu et al., 2019). The annual variation range of sea surface temperature (SST) is 19–30.35 °C(annual average temperature 24.62 °C), sea water pH ranges from 8.0–8.23, annual average sea water salinity is 31.9‰, and the sea water transparency varies between 3.0 and 10.0 m (Yu et al., 2019). Previous studies concluded that the rapid degeneration of Weizhou Island is the result of global warming and escalating anthropogenic impact, such as seawater pollution, unsustainable tourism activities, and ongoing overfishing, all of which degrade the local ecological environment (Yu et al., 2004; Yu et al., 2019). The average live coral cover declined from 50 to 6.02% (1984–2015) at a rate of 1.42%.y^{-1} (Wang, 2017). In this study, five *A. pruinosa* clones (named Acro1-Acro5) and three *P. decussata* clones (named Pavo1-Pavo3) in good growth condition were collected from coral communities on the north side of Weizhou Island on March 30, 2019. All samples were stored at −80 °C until subsequent analysis.

2.2. Symbiodiniaceae clade type determination

The DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) was used to isolate genomic DNA from 100 to 200 mg frozen coral samples (Acro1-Acro3 and Pavo1- Pavo3) following the manufacturer's instructions. After filtering for quality and purity, high-quality DNA was used as a template for PCR. Based on previous reports (Chen et al., 2019), primers ITS-J1F and ITS-J2R were used to amplify the *ITS2* genes. Prepared amplicons were submitted to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for sequencing using an Illumina MiSeq platform (San Diego, CA, USA) (300 bp × 2). High-throughput sequencing data was submitted to the NCBI Sequence Read Archive (accession number: PRJNA599041). *ITS2* sequence analysis data and operational taxonomic unit (OTU) data were analyzed to determine the type and count the diversity of symbiotic Symbiodiniaceae, as previously described. (Chen et al., 2019). In order to ensure the accuracy of the subsequent analysis, strict quality control and sequence filtering criteria were applied based on previous studies (Chen et al., 2019; Ziegler et al., 2017). The consolidated PEAR data was used to obtain full-length ITS2 rDNA fragments (Zhang et al., 2014), and chimeras were checked using MOTHUR (Chen et al., 2019). Primers sequences were trimmed using CUTADPAT (Ziegler et al., 2017). All sequences were aligned with the sequences deposited in the *ITS2* database using BLASTn, with previously described pipeline and parameter settings (Chen et al., 2019). A minimum cut-off of > 5% was used in this study to allow comparison with the denaturing gradient gel electrophoresis (DGGE) results from previous studies and avoid integrative genomics viewer (IGV) interference (Ziegler et al., 2017). MOTHUR (1000 reads per sample) was used for subsequent subsampling (Ziegler et al., 2017). The threshold of retention length of *ITS2* sequences was 90%, with a similarity threshold > 97% for operational taxonomic units (OTUs) (Arif et al., 2014). OTUs with the most abundant sequences while not containing any non-Symbiodiniaceae OTUs were used for subsequent analysis.

2.3. Microbial diversity and composition analysis

Total genomic DNA was extracted from six frozen coral samples (Acro1- Acro3 and Pavo1- Pavo3) using the TIANamp Marine Animals

DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) following the manufacturer's instructions. The quality of all DNA samples was checked prior to further use. The 16S rRNA V3-V4 hypervariable regions were PCR-amplified using previously described cycling conditions, and primers 338F and 806R (Liang et al., 2017). The high-throughput sequencing mechanism was the same as that used for *ITS2* sequencing as described above. High-throughput sequencing data was deposited in the NCBI Sequence Read Archive (accession number: PRJNA599045). The raw sequences were demultiplexed, quality-filtered by the software platform Trimmomatic, and merged by FLASH, with previously described criteria and parameter settings (Bolger et al., 2014; Yao et al., 2019). After removing the chimeric sequences, OTUs were selected at a 97% similarity cut-off (Edgar, 2010). In this study, alpha diversity estimations of OTU (Schloss et al., 2013), principal coordinates analysis (PCoA), microbial composition, and LEfSe analysis were performed on the free online platform of Majorbio Cloud Platform (www.i-sanger.com) (Liu et al., 2019). The SILVA 119 16S rRNA database (https://www.arb-silva.de) was used for sequence alignment with the RDP Classifier, using a confidence threshold of 70%. PCoA was conducted according to the unweighted UniFrac distance matrix calculated at the OTU level (Yao et al., 2019). Microbial composition at the phylum and genus levels was indicated using a pie chart and heat map. Statistically different biomarkers between *A. pruinosa* and *P. decussata* were searched using LEfSe analysis (Segata et al., 2011).

2.4. Coral transcriptome analysis

Frozen coral samples (Acro1- Acro5 and Pavo1- Pavo3) were submitted to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for total RNA extraction, whole transcriptome library preparation, and RNA sequencing. Equal quantities of high-quality RNA were subsequently sequenced on a HiSeq 4000 instrument (Illumina), with 150 nucleotide long resultant paried-end reads. Prior to de novo assembly, SeqPrep was used to filter the quality of the raw sequences. De novo assembly of all cleaned reads was carried out using the Trinity program with default parameters (Grabherr et al., 2011). All samples had the same sequencing depth. The assembled sequence was used as a reference sequence for subsequent analysis (Tang et al., 2018). Clean reads from each sample were mapped to the assembled unigenes to assess the quality of the assembly (Tanwar et al., 2017). After assembly, annotations for the assembled unigenes were carried out via BLAST in six databases (Non-redundant (NR), Swiss-prot, Pfam, Clusters of Orthologous Groups (COG), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)) (Evalue = 1×10^{-5}) (Li et al., 2019). The BLASTx method, a widely accepted method in transcriptome studies on scleractinian coral, was used to distinguish these coral transcripts (Zhou et al., 2017; Tang et al., 2018; Zhang et al., 2019). Transcripts showing higher similarity to *Acropora digitifera*, *Orbicella faveolata*, *Stylophora pistillata*, *Hydra vulgaris*, *Exaiptasia pallida*, and *Amphimedon queenslandica* were binned as coral host transcripts. The expression levels of genes and transcripts were analyzed by RSEM, with a quantitative index of gene expression in transcripts per million (TPM) (Li and Dewey, 2011). Differentially expressed genes (DEGs) between *A. pruinosa* and *P. decussata* were then obtained used DESeq2 (false discovery rate (FDR) < 0.05 and expression fold change ally (Love et al., 2014). Compared with *A. pruinosa*, up-regulated DEGs in *P. decussata* indicated that the baseline expression of these genes in *P. decussata* is higher than that in *A. pruinosa.* KEGG annotation analysis of all genes was performed using KOBAS 2.1.1 according to default parameters. The method of KEGG enrichment analysis of the DEGs was over-representation analysis (ORA) using the Fisher's exact test ($p < .05$) (Backes et al., 2007). High-throughput sequencing data have been deposited in the NCBI Sequence Read Archive (accession number: PRJNA599447).

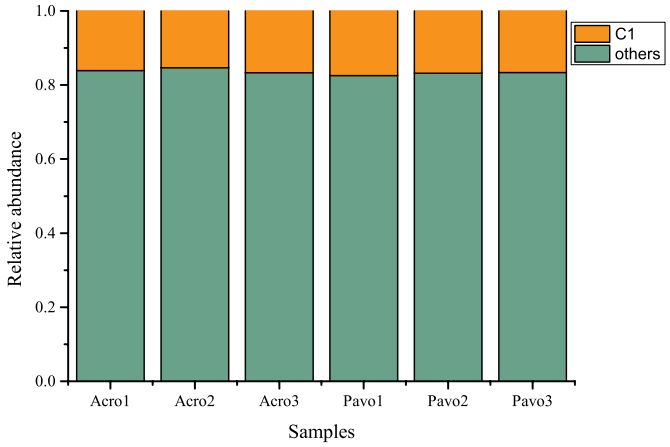

Fig. 1. Bar plot of the relative abundance of different Symbiodiniaceae subclades in coral samples. Each bar represents the relative abundance of different Symbiodiniaceae subclades in one sample.

3. Results

3.1. The Symbiodiniaceae community did not differ between A. pruinosa (Acro) and P. decussata (Pavo)

Symbiodiniaceae compositions in subtropical scleractinian coral *A. pruinosa* and *P. decussata* are shown in Fig. 1. C1 was the most dominant subclade, with a mean relative abundance of 83.49%. With redpect to composition and relative abundance, although some slight differences were observed, the community structure of symbiotic Symbiodiniaceae in all samples remained stable and significant difference were not observed between the two groups.

3.2. Differences in symbiotic bacterial communities between A. pruinosa (Acro) and P. decussata (Pavo)

In the present study a total of 350, 789 processed bacterial sequences were assigned to 6, 695 OTUs at 97% similarity. The near-saturated rarefaction curve indicated that the sequencing results could be used for subsequent analysis. The α diversity index (Chao) showed no significant difference in OTU richness between the two groups. However, the Shannon ($p = .005$) and Simpson ($p = .02$) indices showed higher microbial diversity in Pavo compared with Acro (Table 1). Subsequent analysis using unweighted UniFrac distances and PCoA revealed stronger clustering of the established microbial communities, as shown in Fig. 2.

A community analysis pie chart was used to illustrate the overall microbial community structures in Acro and Pavo groups (Fig. 3). The most abundance phyla in the two groups included Proteobacteria, Cyanobacteria, Bacteroidetes, Chloroflexi, Actinobacteria, and Deinococcus-Thermus. Differences were also detected between the two groups. The dominant phylum of the Acro group was Cyanobacteria, with a mean relative abundance of 54. 79%, whereas Proteobacteria (44.59%) was the dominant phylum of the Pavo group. At the genus

Table 1
Sequencing data summary and community diversity.

Estimators	Acro-Mean	Acro-Sd	Pavo-Mean	Pavo-Sd	Pvalue
Sobs	592.33	135.4	1453.7	560.78	0.1098
Ace	661.51	114.77	1832.9	892.39	0.1488
Chao	649.17	123.69	1778.1	786.49	0.1275
Shannon	2.3645	0.59559	4.9213	0.50514	0.005162
Simpson	0.29635	0.087572	0.042967	0.027997	0.02835
Coverage	0.99711	0.00048929	0.98948	0.007117	0.2039

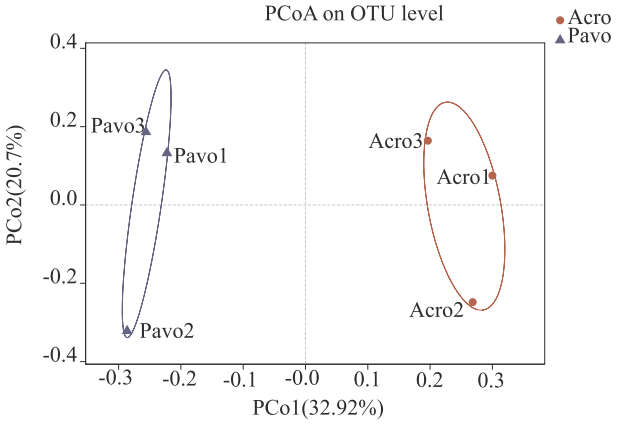

Fig. 2. Principal Coordinates Analysis (PCoA) of the unweighted UniFrac distance matrix representing differences in community structure at the operational taxonomic unit (OTU) level.

level, norank_f_norank_o_Chloroplast was the most abundant genus with a relative abundance of 54.23%. Conversely the abundance of all genera was relatively low in the Pavo group with unclassified_o_Rhizobiales, the most abundant genus, accounting for only 8.38%. Significant differences in community structure existed between the Acro and Pavo groups, as shown in the heat map (Fig. 4).

The LEfSe tool allows the analysis of microbial community data for any clade (Pedamallu et al., 2016)and was used to identify specialized communities in the coral samples, with statistical analysis performed from the phylum to genus level (Fig. 5). A total of 39 taxa were identified as exhibiting significant differences, including 18 in the Acro group, and 21 in the Pavo group. Three phyla, two classes, four orders, five families, and seven genera were significantly higher in the Pavo group. Note that at the genus level, microbes from norank_f_Rhizobiaceae, unclassified_f_Rhizobiaceae, unclassified_o_Rhizobiales, Ruegeria, unclassified_f_Rhodobacteraceae, Sva0996_marine_groupk, and unclassified_f_Flavobacteriaceae were significantly more prominent in the Pavo group. In comparison, the prominent genera in the Acro group included norank_f_norank_o_Chloroplast, MWH_UniP1_aquatic_group, unclassified_c_Alphaproteobacteria, unclassified_p_Prateobacteria, Vibrionimonas, and norank_f_env_OPS_17.

3.3. High-resolution transcriptome profiling identified potential regulators of tolerance differences between P. decussata and A. pruinosa

In the present study, different transcriptional responses of *A. pruinosa* and *P. decussata* were evaluated using the RNA-Seq method. We randomly selected eight samples to construct the transcriptome library. After filtering low-quality sequences and adaptor sequences, 442, 461, 918 clean reads were obtained from all the libraries. The numbers of reads, Q30, GC content, and mapping statistics in each library are shown in Table 2.

After library calibration, the expression of 63,235 coral genes were compared between the Acro and Pavo groups. The two groups could be clearly distinguished through PCoA based on the expression within the total transcriptome (Fig. 6A). In comparison, biological replicates within each group clustered together, as revealed by the heat map (Fig. 6B) and exhibited high Pearson's correlation coefficient values, whereas samples from different groups were separated.

In order to reveal the potential molecular mechanism underlying tolerance difference between *P. decussata* and *A. pruinosa*, we analyzed DEGs between the two groups. We determined that a total of 17,285 unigenes were significantly more highly expressed and 15,187

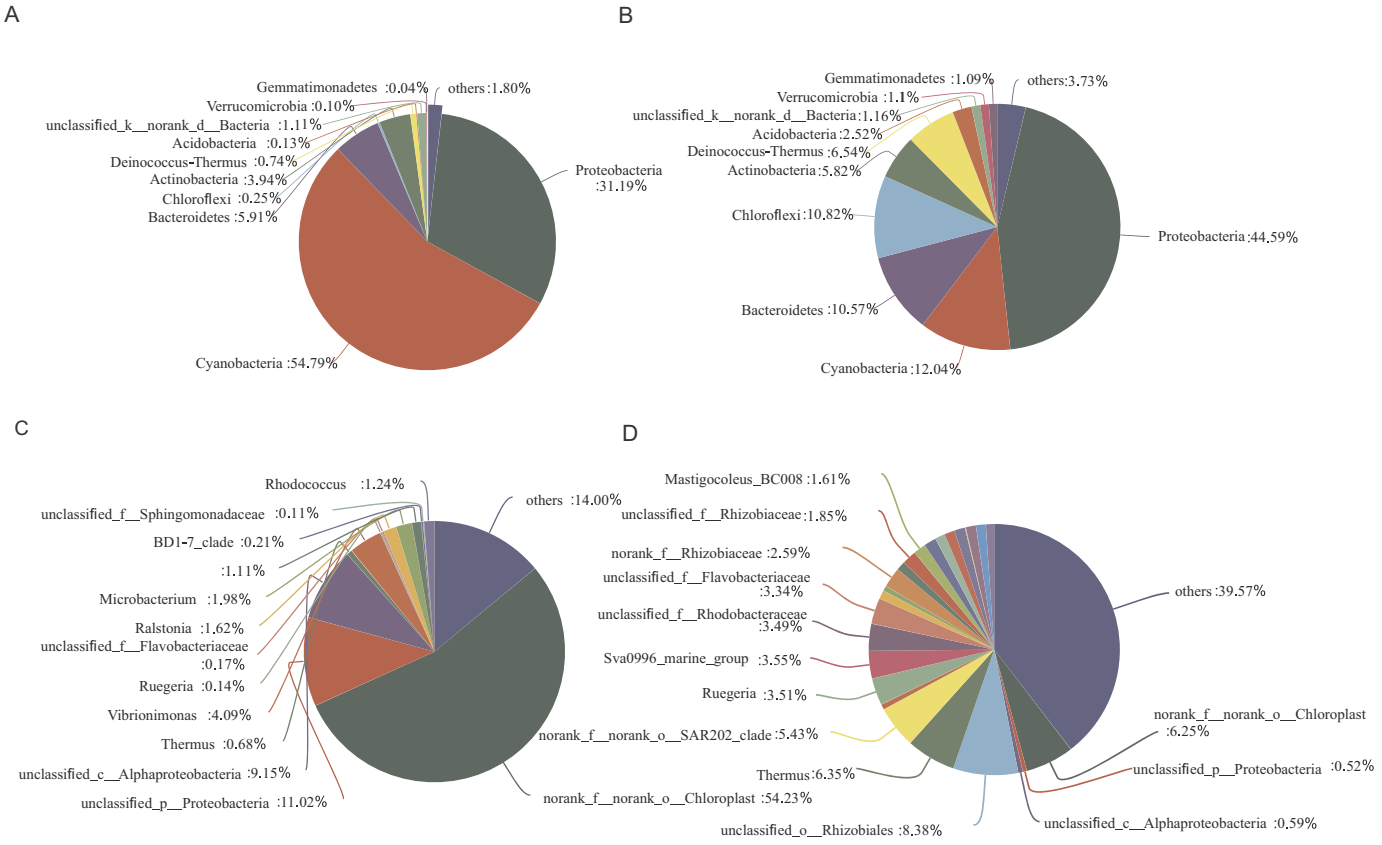

Fig. 3. Microbial composition of each group at the phylum and genus level. (A), Acro group at the phylum level. (B), Pavo group at the phylum level. (C), Acro group at the genus level. (D), Pavo group at the genus level.

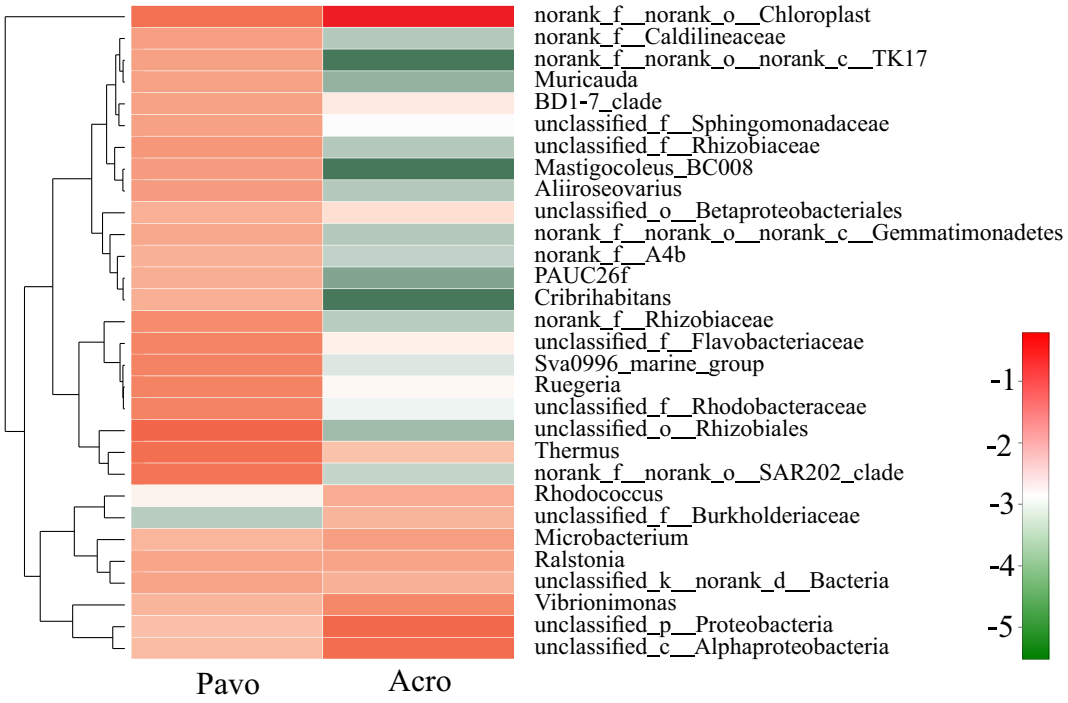

Fig. 4. Heat map showing the relative abundance of bacterial genera in Acro and Pavo groups.

significantly lower expressed in coral (Table S1). To further understand the biological functions of unigenes, the KEGG pathway database was employed (Tables S2, S3) to assess the degree of KEGG enrichment based on richness factor, false discovery rate (FDR), and number of genes. The significantly more highly expressed and lower expressed coral genes were mainly enriched in 10 and 7 KEGG pathways, respectively (Fig. 7). The high expression unigenes in the Pavo group were related to immune and stress-resistant responses through KEGG pathway analysis, whereas the low expression unigenes were associated with metabolism. Notably, nitrogen metabolism in the Pavo group was significantly lower than that in the Acro group. The heat map identified 48 DEGs involved in the nitrogen metabolism pathway, including 23 high expression and 25 low expression genes (Fig. 8A). KEGG mapping of the 48 DEGs involved in the nitrogen metabolism pathway is shown in Fig. 8B.

4. Discussion

Ecological investigation of the coral reef ecosystem of Weizhou Island over the past 30 years has demonstrated that resistant biotype scleractinian coral *P. decussata* exhibits higher environmental tolerance than the "competitive" coral *A. pruinosa* (Yu et al., 2019). In the present study, we used a holistic approach to comprehensively survey the molecular characteristics underlying tolerance differences between these coral species. Symbiodiniaceae composition did not differ between the two corals, although the diversity of symbiotic bacteria in *P. decussata* was significantly higher than that in *A. pruinosa* and the community structures of symbiotic bacteria also differed significantly. In particular, the dominant bacteria comprised nitrogen fixing bacteria norank_f_norank_o_Chloroplast and unclassified_o_Rhizobiales. Furthermore, transcriptome analysis revealed that the high expression genes in *P. decussata* were mainly related to immune and stress-resistance responses, whereas the low expression genes were mainly associated with metabolic pathways including nitrogen metabolism.

4.1. The two corals exhibited different environmental tolerances albeit similar Symbiodiniaceae composition

The coral symbiont Symbiodiniaceae, a photosynthetic dinoflagellate, is an important component of coral holobionts and a direct participant in the environmental stress response (Gong et al., 2018). The results of numerous field and indoor experiments on coral bleaching have revealed that different combinations of host and symbionic Symbiodiniaceae could influence the growth rate and response to environmental stress of coral, which might also provide ecological advantages (Baker, 2001; Little et al., 2004). In particular, members of Symbiodiniaceae clade D have attracted considerable attention as increasing evidence suggests that this clade is beneficial to the ability of coral to respond to environmental stress and that they out-compete other, perhaps more functionally beneficial Symbiodiniaceae (Stat et al., 2008; Chakravarti and van Oppen, 2018). Notably, some studies have also found that the relative abundance of D-type Symbiodiniaceae increased significantly after several bleaching events (Baker et al., 2004). Furthermore, Symbiodiniaceae density is also considered to be related to the coral heat tolerance (Qin et al., 2019a, 2019b). For example, massive corals with higher heat tolerance, such as *Porites* and *Favia*, usually exhibit a higher density of Symbiodiniaceae than that in branching corals *Acropora* (Li et al., 2008, 2011a, 2011b).

Moreover, according to the "adaptive bleaching hypothesis", corals may adapt to environmental stress by altering the community structure and density of symbiotic Symbiodiniaceae as a self-protection mechanism (Baker et al., 2004; Fautin and Buddemeier, 2004; Stat et al., 2006). Nevertheless, coral bleaching represents the main malignant result of environmental stress and also represents the main cause of global coral reef ecosystem degradation. Understanding the biological mechanism and process of coral bleaching under environmental stress therefore reflects a primary focus in the field of coral reef research. In particular, with the rapidly deteriorating environment, a central issue to determining whether corals can survive in the future is whether the level of increased tolerance achieved through symbiont density or composition in corals would be sufficient to survive anticipated increases in SST (Berkelmans and van Oppen, 2006).

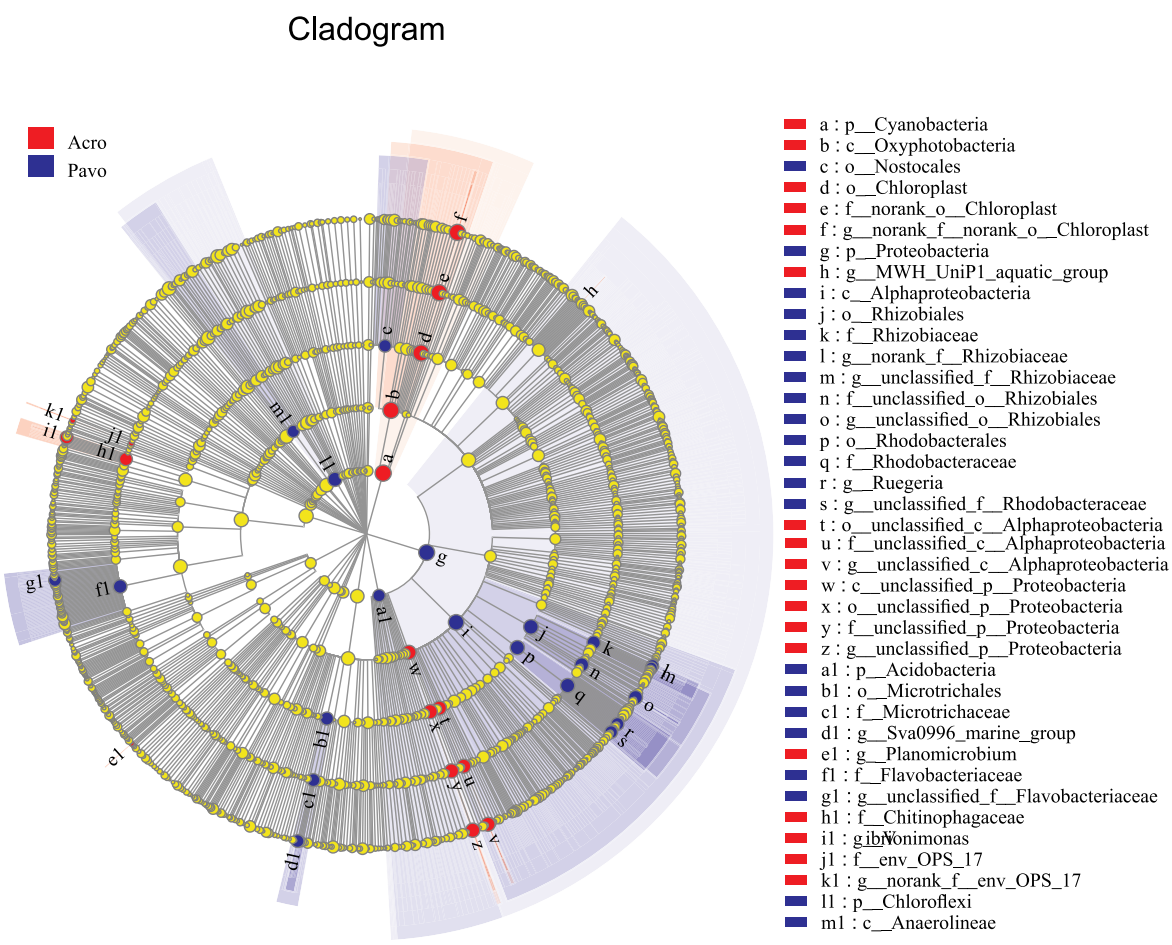

Fig. 5. Microbial communities with statistically significant differences. A cladogram displaying the different microbiota structure from phylum to genus level. Linear Discriminant Analysis score > 4. Different-colored regions represent different constituents (red, Acro; blue, Pavo; yellow: non-significant). The diameter of each circle is proportional to the abundance of the group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Transcriptome mapping statistics.

Library	Raw reads	Clean reads	Q30 (%)	GC content (%)	Mapped rate to coral transcripts (%)	Mapped rate to Symbiodiniaceae transcripts (%)
Acro1	53,966,282	53,738,064	96.32	47.56	34.02	24.63
Acro2	57,457,274	57,195,248	96.44	50.07	21.88	38.66
Acro3	54,036,550	53,790,878	96.48	47.24	32.98	24.7
Acro4	51,722,350	51,465,948	96.28	45.79	35.21	19.96
Acro5	58,798,204	58,528,706	96.27	46.62	38.76	18.85
Pavo1	53,182,822	52,910,728	96.34	49.25	20.01	34.2
Pavo2	56,029,808	55,731,492	96.14	48.42	27.27	26.98
Pavo3	59,440,294	59,100,854	96.35	48.89	20.72	33.16

In the present study, the communities of Symbiodiniaceae in *A. pruinosa* and *P. decussata* were dominated by clade C1 Symbiodiniaceae, which is consistent with fast growing small polyp scleractinian coral (Little et al., 2004), and their growth profiles (Huston, 1985). In addition, particular symbiont genotypes may be the outcome of environmental selection and co-evolution (Thornhill et al., 2017). For example, previous studies have shown that the community structure of symbiotic Symbiodiniaceae may be influenced by geographical region rather than coral host (Chen et al., 2019). Weizhou Island is located in the northwestern sector of the South China Sea, within the high-latitude marginal coral reef areas of the Pacific Ocean. Notably, scleractinian coral in high-latitude regions are generally considered more likely to form symbiosis with clade C Symbiodiniaceae (Chen et al., 2019), with subclade C1 especially representing a generalist type associated with most coral species in high-latitude marginal corals (De Palmas et al., 2015). Although it has been reported that change in community structure and density of Symbiodiniaceae may contribute the differences in coral tolerance (Qin et al., 2019a, 2019b), the present study demonstrated the stability of clade C1 Symbiodiniaceae between *A. pruinosa* and *P. decussata* in the subtropical region. Thus, clade C1 Symbiodiniaceae may not constitute the main factor responsible for the tolerance differences observed in these species. Nevertheless, despite the lack of difference in symbiotic Symbiodiniaceae, the possibility remains that Symbiodiniaceae may play a potential role in the tolerance difference of *A. pruinosa* and *P. decussata*. In particular, the "Symbiodiniaceae-rare biosphere" serves as the basis of symbiotic Symbiodiniaceae community plasticity (Shade et al., 2014).

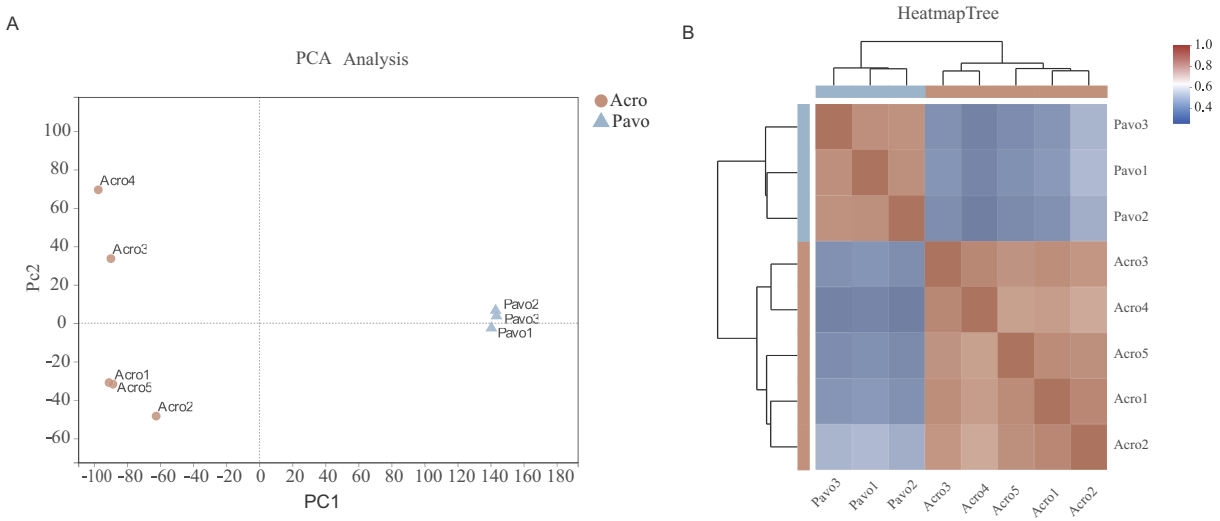

Fig. 6. Analysis of biological replicates of transcriptome datasets. (A), Principal component analysis (PCA) performed on the whole transcriptome of the Pavo group compared with that of the Acro group. (B), Clustering analysis of transcriptome datasets among eight samples.

4.2. Higher bacterial diversity and distinct community structure correlated with high tolerance of P. decussata

Recent studies have revealed that typically 100 s of bacterial taxa are directly related to coral hosts (Ainsworth et al., 2015; Neave et al., 2017), and participate in coral growth and biotic and abiotic stress responses (Reshef et al., 2006). Because of the importance of symbiotic bacteria for tolerance of the coral holobiont (Ziegler et al., 2017), we attempted to explore the differences in the composition of bacterial communities and their potential role in the different tolerance of *A.*

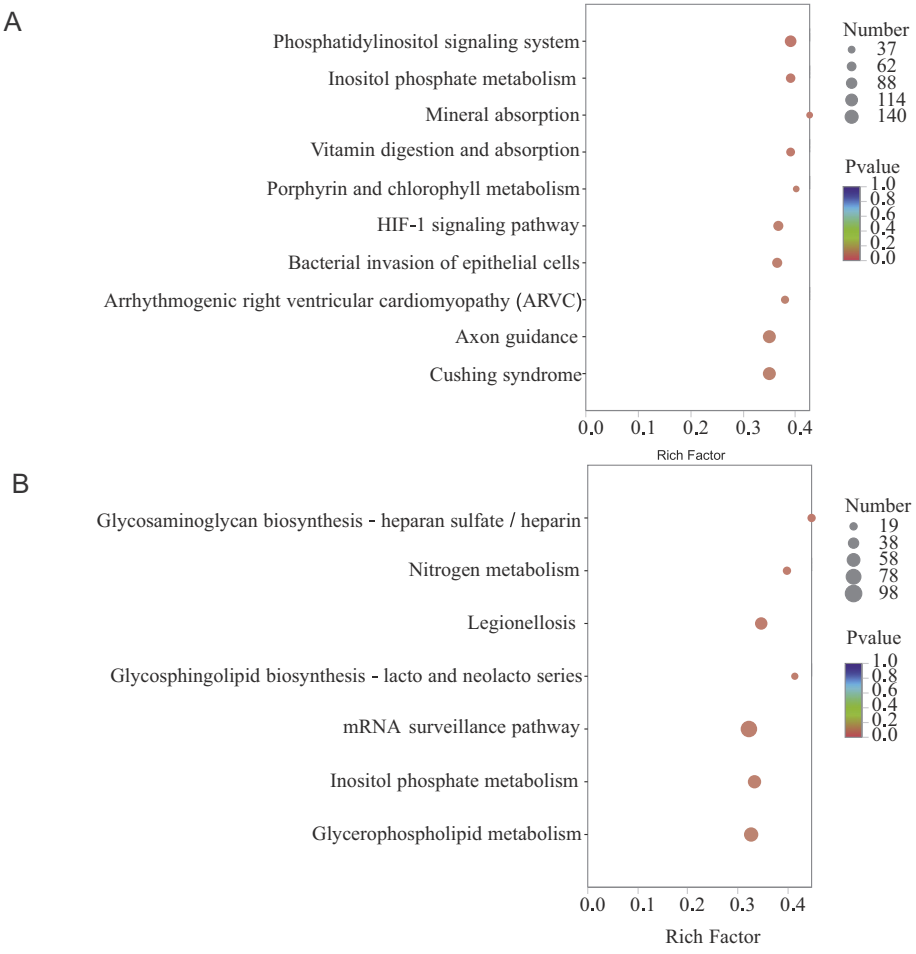

Fig. 7. KEGG pathway enrichment analyses of differentially expressed coral genes between Acro and Pavo groups. The size of the point indicates the number of DEGs enriched in the pathway, and the color indicates the significance of enrichment, FDR $<$ 0.05. (A), High expression coral DEGs. (B), Low expression coral DEGs.

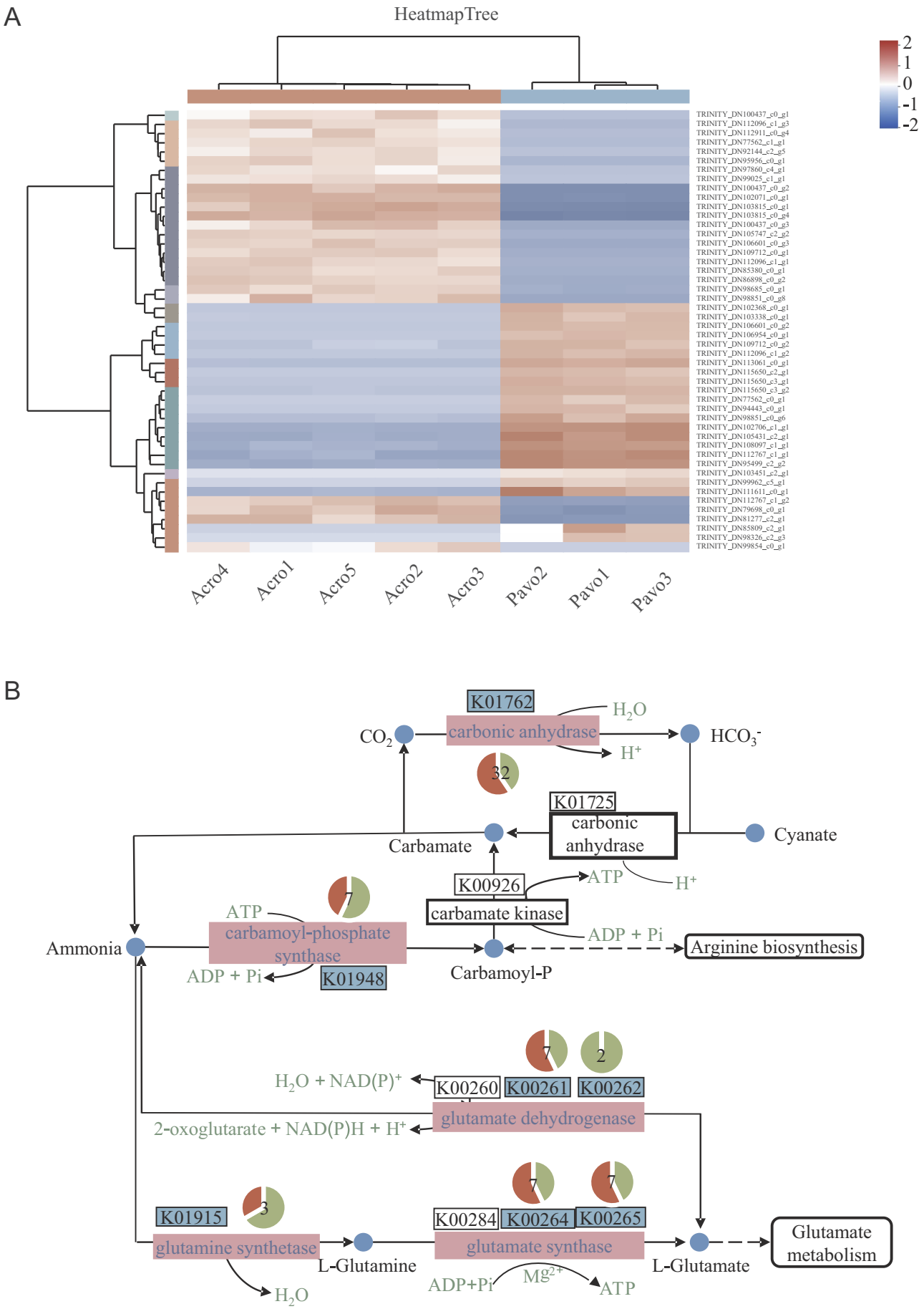

Fig. 8. (A) Cluster analyses and (B) KEGG mappings of DEGs involved in the nitrogen metabolism pathway.

pruinosa and *P. decussata*. Notably, although we observed stable community structure of Symbiodiniaceae, the symbiotic bacteria differed significantly between *A. pruinosa* and *P. decussata,* suggesting a changeable coral-prokaryotic partnership (Ziegler et al., 2019). Symbiotic bacteria of coral are known to change rapidly under environmental stress, which may promote the adaptation of the coral host to environmental stress (Ziegler et al., 2017). PCoA clustering analysis revealed that the structure of the symbiotic bacteria of *A. pruinosa* consistently differed from that of *P. decussata*. This parallels the findings of previous studies that demonstrated variations in the communities of prokaryotic partners across different corals or different regions (Liang et al., 2017; Ziegler et al., 2017). In the present study, compared with *A. pruinosa*, we observed higher microbial diversity in *P. decussata*, which might allow the host to change its main symbiont bacteria to a related species that performs better under certain conditions to maintain the physiological functions of the holobiont. At the microbial level, higher diversity facilitates the ability of symbionts to resist infection, absorb nutrients, and maintain the aggregate function of a healthy microbiome (Flanagan et al., 2007; Pollock et al., 2019). At the macroscopic scale, lower diversity within *A. pruinosa* may increase the risk of sudden and potentially irreversible ecosystem collapse (Hooper et al., 2012), which may also affect the overall biological resilience of the corals (Zaneveld et al., 2017).

The bacterial communities of samples of *A. pruinosa* differed significantly from those of *P. decussata*. Because the two kinds of scleractinian coral in this study were collected from the same marine environment, this finding further verified the role of the host in regulating the community structure of symbiotic bacteria (Souza et al., 2016). Notably, we found that the dominant bacteria in both corals were related to nitrogen fixation. As corals usually grow in an oligotrophic marine environment. Symbiotic nitrogen fixing bacteria, potentially provide corals with a supplemental source of fixed nitrogen in nitrogen-poor reef waters (Lema et al., 2014). In particular, Rhizobiaceae were found to constitute key “hub” taxa associated with *P. decussata*. As in terrestrial plants, Rhizobiaceae, have evolved a mutually beneficial mechanism with coral hosts, which may contribute fixed nitrogen to coral symbionts (Lema et al., 2014; Lema et al., 2012) and contribute significantly to host nitrogen cycling (McDevitt-Irwin et al., 2017; Quigley et al., 2019). In comparison, the dominant bacteria of *A. pruinosa* was cyanobacteria, which is associated with photosynthesis-dependent nitrogen fixation in coral reefs (Lesser et al., 2004). Although the different effects of the two diazotrophic bacteria on the nitrogen cycle of the host have not been confirmed, the difference in dominant bacteria may also be related to differences in host tolerance (Liang et al., 2017).

In addition, we found that Vibrionimonas abundance differed significantly between *A. pruinosa* and *P. decussata*. Vibrionimonas is generally recognized as a potentially pathogenic and opportunistic microbial taxa, and may flourish when the coral is stressed and cannot regulate its microbiome (McDevitt-Irwin et al., 2017). Relatively higher levels of this genus may also contribute to increased sensitivity of *A. pruinosa* to environmental stress compared with that of *P. decussata*. In turn, these results potentially suggest higher microbial diversity and different bacterial community are responsible for the higher tolerance of *P. decussata* to environmental stress.

Thus, although our data do not indicate whether these microbial community differences contribute to or result from increased host tolerance of *P. decussata*, the observed patterns concur with the hypothesis that microbial adaptation may play an important role in promoting the acclimatization of coral to environmental changes.

4.3. Immune defense and metabolic regulation may contribute to tolerance differences between A. pruinosa and P. decussata

The transcriptome of *P. decussata* differed significantly from that of *A. pruinosa*. High expression coral genes in the Pavo group were related to immune and stress-resistance responses. The immune system constitutes the core component of the coral host defense system and is considered to be responsible for recognition and clearance of pathogenic organisms or materials (Tang et al., 2018).Enhancement of this system increases the resistance of scleractinian corals to biotic and abiotic stresses (Ben-Haim et al., 2003). For example, the phosphatidylinositol signaling system is important in the response to abiotic stress (Lin et al., 2004), which plays a critical role in modulating the oxidative stress signal from the plasma membrane under heat stress (Wang et al., 2018). It was also reported that heat-tolerant *Pyropia haitanensis* could increase the transduction of phosphatidylinositol signal to resist heat stress (Wang et al., 2018). In *Echinochloa* spp., the resistant biotype has more DEGs involved in porphyrin and chlorophyll metabolism than the sensitive biotype (Gao et al., 2019). For example, hypoxia-inducible factor and NF-κB are considered central transcription factors of the innate immune response (Rius et al., 2008). Moreover, these two factors are interdependent and mutually regulated (Taylor, 2008; D'Ignazio et al., 2016). Specifically, NF-κB modulates the expression of HIF-α (Rius et al., 2008) and HIF-1β (van Uden et al., 2011) in addition to HIF target genes (Rius et al., 2008). Increased HIF-1 expression and activity can mediate NF-κB activity either positively or negatively (D'Ignazio et al., 2016; Taylor, 2008), thereby contributing to maintaining the balance of oxygen homeostasis in animals (Song et al., 2018).

Comparatively, KEGG pathway analysis revealed that low-expression coral genes were associated with metabolism. In particular, nitrogen metabolism of *P. decussata* was significantly lower than that of *A. pruinosa*. According to previous studies, scleractinian coral *Acropora digitifera* larvae and other marine invertebrates enhanced their survival by slowing down their metabolism in response to stress caused due to seawater acidification (Reipschläger and Pörtner, 1996; Basile et al., 2005; Nakamura et al., 2011). It has also been reported that increased stress tolerance tended to be associated with decreased metabolic rate in naked mole-rats *Heterocephalus glaber* (Kirby et al., 2018) and *Drosophila* (Hoffmann and Parsons, 2009), whereas the narrower thermal window of endemic high-Antarctic fish may be due to their higher metabolic rates and energy costs (Sandersfeld et al., 2017). Therefore, we speculated that a lower metabolic rate may be related to the higher tolerance of *P. decussata.* Moreover, the difference in nitrogen metabolism may also be related to the difference in symbiotic nitrogen fixing bacteria. Although the important roles of cyanobacteria and rhizobia in the nitrogen cycle of corals have been reported in previous studies (Lesser et al., 2004; Lema et al., 2012), the extent and ubiquity of this relationship remains unknown (Olson et al., 2009). Notably, this difference may be an indicator of a specific association between coral and diazotrophic microorganisms, suggesting that the symbiotic relationship between coral host and nitrogen fixing bacteria is species specific (Lema et al., 2012).

5. Conclusions

In this study, we compared the differences of the three main components (Symbiodiniaceae communities, bacterial communities, and coral host transcriptome response) of *A. pruinosa* and *P. decussata*. Our findings highlighted that the higher environmental tolerance of *P. decussata* is likely due to a complex biological process involving the coral host, Symbiodiniaceae, and bacteria. We hypothesize that the higher tolerance of *P. decussata* compared with that of *A. pruinosa* might result from a complex biological process caused by higher symbiotic bacterial diversity, different dominant bacteria, higher host immune and stress resistance responses, and lower metabolic rate. Although we found that these differences may lead to higher environmental tolerance, the molecular mechanism underlying the tolerance difference remains uncertain owing to the complexity of coral symbionts. Because of the rapid global climate change in the Anthropocene, the highly tolerant *P. decussata* may provide hope for the ability of coral reefs to acclimatize

to habitat degradation in the future. Further evaluation of the molecular mechanisms underlying the likelihood for coral reef survival or death is therefore warranted.

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CRediT authorship contribution statement

Xiaopeng Yu:Conceptualization, Methodology, Resources, Data curation, Visualization, Validation, Writing - original draft.**Kefu Yu**:Conceptualization, Resources, Methodology, Validation, Writing - review & editing, Project administration, Funding acquisition.**Zhiheng Liao**:Software, Formal analysis, Visualization, Writing - review & editing.**Jiayuan Liang**:Project administration, Investigation, Writing - review & editing.**Chuanqi Deng**:Resources, Investigation, Writing - review & editing.**Wen Huang**:Resources, Supervision.**Yanhua Huang**:Resources, Investigation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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