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Differential physiological and microbial responses of the octocoral *Junceella squamata* to high-temperature and cadmium stress

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ABSTRACT

Global warming and heavy metals have become the major threat to the growth and reproduction of corals. However, unlike scleractinian corals, in the context of widespread coral degradation worldwide, there are few reports on the response of octocorallia corals to high-temperature stress and heavy metals. In the present study, we conducted indoor simulation experiments using *Junceella squamata*. We evaluated the physiological response of these corals under high-temperature stress at 33 °C and cadmium (Cd) stress by comparing the composition and diversity of their symbiotic bacteria and analyzing differences in their transcriptome. The results show that high-temperature stress has more severe adverse effects than cadmium stress. High-temperature stress disrupts coral symbiotic relationships, leading to an increase in alpha diversity associated with disease-causing bacteria, which may increase the risk of infection and potentially contribute to coral mortality. Meanwhile, cadmium stress increases the instability of the coral holobiont, potentially disrupting DNA stability and RNA transcriptional regulation. However, an increase in Cd-tolerant bacteria may help corals respond to cadmium stress. This study reveals the effects of harmful substances on coral and highlights the urgent need for action to protect octocorals in the face of environmental stress.

1. Introduction

Octocorallia a subclass of the phylum Cnidaria and class Anthozoa and plays a vital role in coral reef ecosystems, with approximately 3400 species documented globally (McFadden et al., 2010). As essential contributors to marine biodiversity, octocorals provide critical habitats that support feeding and breeding grounds for economically valuable fish (Fujita et al., 1997). Numerous octocorallia species, such as *Sinularia* sp., contain calcareous spicules, stacked with the calcium carbonate skeletons of other organisms, ultimately forming a massive coral reef (Jeng et al., 2011). They inhabit shallow coastal waters to deep seas, surviving in tropical, temperate, and cold zones (McFadden et al., 2010). Key distribution areas include the Red Sea (Seveso et al., 2016), Weddell Sea in the southern ocean (Ambroso et al., 2017), East and South China Seas in the Western Pacific (Xu and Hao, 2016), Coral Sea (Great Barrier, Tagula, and New Caledonia Reefs; (Summers and Watling, 2021), Southeast Asian atolls and Okinawa (Miyazaki and Reimer, 2015), Florida, the Bahamas, the northeast coast of South America, and the Caribbean Sea in the Atlantic Ocean (Lasker et al., 2020; Paull et al., 2000). Octocorallia's unique distribution and human relevance have

Abbreviations: ACE, Abundance-based coverage estimator; ASV, amplicon sequence variant; AVD, average variability distance; BP, biological process; CC, cellular component; DEGs, differentially expressed genes; FDR, false discovery rate; FPKM, fragments per kilobase of transcript per million mapped reads; GC, Guanine-Cytosine; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, Molecular Function; NMDS, non-metric multidimensional scaling; Nr, non-redundant; PCA, principal component analysis; PCoA, principal coordinate analysis; PCR, polymerase chain reaction; RSEM, RNA-Seq by expectation-maximization; SRA, sequence reads archive.

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increasingly garnered attention for their role in maintaining the structural integrity and biodiversity of marine ecosystem.

The early research on octocorallia mainly focused on separating natural products from soft coral and *Paraplexaura* sp., and screening marine drugs. There have been reports of the isolation of natural products from octocorallia since the 1960s (Modica et al., 2024). Analysis of research progress in the past decade shows that the secondary metabolites of octocorallia play a role in defending against natural predators, toxicity, protecting larvae, preventing the habitat and growth of seaweed and other organisms, natural anti fouling products, and chemical defense against microorganisms (Marrero et al., 2010; Modica et al., 2024). *Paraplexaura* sp., a representative species of octocorallia, is also a key species for studying marine active compounds (Marrero et al., 2010). Many structurally novel and biologically active compounds have been discovered from *Paraplexaura* sp., particularly the discovery of prostaglandins with important physiological activities, making the study of the chemical composition of *Paraplexaura* sp. a hot field in the research of marine natural products (Li et al., 2010). During the 6-year period of concentrated research from 2001 to 2006, more than 400 new compounds were discovered from different species of *Paraplexaura* sp. (Li et al., 2010). These compounds include structural types such as lipids, terpenes, steroids, and alkaloids. Most compounds have undergone corresponding biological activity screening experiments, providing a basis for further structural modification, structure-activity relationship research, and potential pharmaceutical development research (Fu et al., 2023). In recent years, the distribution and classification of *Paraplexaura* sp. have been studied. Previous studies have explored their distribution (Rakka et al., 2021), molecular phylogenetic insights into the evolution of octocorallia(McFadden et al., 2010), new species descriptions (Benayahu et al., 2021; Galván-Villa and Rios-Jara, 2018), and molecular phylogeny of deep-sea octocoral (Xu et al., 2021), conducted extensive research on the spatial patterns and environmental settings of non-reefal coral communities in Taiwan and the isolation and extraction of natural compounds (Chao et al., 2017; Hsieh et al., 2016), and studies on chemical constituents.

With the continuous deterioration of the global climate and intensified human activities, octocorallia is facing a serious degradation crisis. For example, soft coral reefs in the Laksha Islands on the west coast of India were also affected by three temperature induced bleaching events in 1998, 2010, and 2016 owing to the ENSO, resulting in large-scale soft coral bleaching (Narayanankutty and Riyas, 2023). The key environmental stresses affecting octocorallia and scleractinian corals are similar, including temperature anomalies (greenhouse effect and low temperature cold current), ocean acidification, seawater pollution, resource overfishing, natural disasters and so on. Global warming and heavy metal pollution have become the major contemporary threat to almost all ecosystems, particularly coral reef ecosystems (Hughes et al., 2018; Yusfaddillah et al., 2023). High-temperature stress can induce coral bleaching and inhibit the self-repair of coral, posing a serious threat to the survival of coral, causing fundamental changes in the composition and functional characteristics of coral assemblages on coral reefs, transforming large reef systems from mature and diverse combinations to highly degraded systems (Hughes et al., 2018; Yusfaddillah et al., 2023). Cadmium (Cd) pollution is particularly concerning in heavy metal pollution, as it is ubiquitous in the environment and highly toxic (Goering et al., 1995), making it one of the most problematic metals (Clemens, 2019). The cadmium concentration in rivers and lakes reported in natural water bodies varies between 1.120 and 8 μg L^{-1} (Guo et al., 2018). Levels of cadmium are lower in open ocean waters (0.2–60 ng L^{-1}) and coastal waters (1–100 ng L^{-1}). However, cadmium concentrations in estuarine and bay sediments typically range from 0.2 to 10 μg g^{-1} dry weight. In addition to natural enrichment due to upwelling, dissolved cadmium from industrial sources can lead to high Cd concentrations in coastal environments (e.g. up to 1.63 mg•kg^{-1} in surface sediments) (Diop et al., 2014). Many adverse effects caused by cadmium pollution have been reported in marine organisms, including scleractinian corals *Pocillopora damicornis* (Zhou et al., 2018). Heavy metal pollution in the aquatic environment is worsening and spreading worldwide with industry development, posing a serious threat to both the ecological environment and human health (Li et al., 2018). Therefore, similar to high-temperature stress, the threat of cadmium pollution cannot be disregarded. However, research on heat and cadmium stress is mostly focused on scleractinian corals, and research on octocorallia, particularly *Paraplexaura* sp., is scarce. *Junceella squamata*, a unique marine organism that typically inhabits coastal areas, is a typical coral species. However, with the continuous expansion of human activities, its living environment is facing unprecedented challenges, and global warming and nearshore pollution have become one of the main factors threatening its survival. It is a key species for searching for novel and biologically active secondary metabolites (Zhou et al., 2014). We conducted indoor simulation experiments on *J. squamata* to study these effects, evaluating their physiological responses to 33 °C heat and cadmium stress. By comparing the composition and diversity of coral symbiotic bacteria and transcriptome differences, we aim to elucidate the mechanisms of coral responses to acute stress. This study enhances our understanding of octocorallia's adaptation to environmental stress and their co-evolutionary potential with symbionts, which is crucial for evaluating their adaptation to global environmental deterioration.

2. Materials and methods

2.1. Coral sampling

Junceella. squamata from Weizhou Island were collected for indoor simulation experiments. It belongs to the tropical oceanic monsoon climate, with warm and humid seasons, an average annual temperature of approximately 23 °C, an annual rainfall of approximately 1300 mm, and a salinity of 29–34 ppt, which meets the national seawater quality standards and provides superior natural conditions for the growth of marine organisms. All corals were sampled from the same depth (~5 m). We conducted a careful comparison and collected colonies of *J. squamata* with different morphologies to minimize the opportunity for multiple sampling of the same strain. The selected coral samples are stored within 5 m of each other to ensure consistency. To minimize experimental error, the entire sampling process was completed as quickly as possible. To avoid damage and contamination, the corals were washed with 0.22-μm filtered and sterilized seawater to remove loosely attached microorganisms. The corals were then acclimated in a breeding room for 20 days under a 12-h light/12-h dark cycle using aquarium lamps (250 W metal halogen and 4 T5HO). Water quality conditions were kept stable, and the corals were fed freshly hatched brine shrimp larvae twice a week.

2.2. Indoor simulation experiment

The experimental conditions for this study were established based on previous studies (Mitchelmore et al., 2007; Zhou et al., 2018). Cadmium (Cd) stress ($CdCl_2$, 20 μg L^{-1}) and high-temperature stress (32 °C) conditions were determined through pre-experiments. For research on the response mechanism of coral to high temperature stress, the temperature is commonly set to 32 °C (Yu et al., 2020). The concentration in the cadmium stress experiment was selected based on the concentration used in previous studies, which was close to the cadmium concentration in natural sea areas. Previous studies have revealed that cadmium stress can have a serious impact on corals Pocillopora damicornis (Zhou et al., 2018). Forty coral samples were randomly divided into four groups and subjected to either high-temperature stress or cadmium stress. Samples were collected after 3 days of high-temperature stress and 5 days of cadmium stress. Approximately 0.5 cm of coral tissue was taken from each sample. To minimize experimental error, the sampling process was completed quickly. The corals were washed with 0.22-μm filter-sterilized seawater to remove loosely attached microorganisms,

then photographed and immediately placed into frozen storage tubes, flash-frozen in liquid nitrogen, and stored at −80 °C.

2.3. Analysis of α -diversity and community composition in symbiotic bacteria

Frozen coral tissue was broken with scissors and a mortar. Total genomic DNA was extracted using the TIANamp Marine Animal DNA kit (Beijing Tiangen Biotechnology Co., Ltd., China). DNA concentration and purity were measured for subsequent experiments. Bacterial 16S rRNA V3–V4 regions were amplified by polymerase chain reaction (PCR) using previously reported cycling conditions and primers 338F and 806R. PCR products were extracted from a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using the Illumina Nextseq 2000 platform (Illumina, San Diego, USA) and duplex sequencing.

After demultiplexing, the resulting sequences were quality filtered with fastp (v0.19.6) (Tanja and Steven, 2011) and merged with FLASH (v1.2.7) (Chen et al., 2018). Then the high-quality sequences were de-noised using DADA2 (Edgar, 2013) plugin in the Qiime 2 (version 2020.2) pipeline with recommended parameters, which obtains single nucleotide resolution based on error profiles within samples (Wang et al., 2020). DADA2 denoised sequences are usually called amplicon sequence variants (ASVs). Taxonomic assignment of ASVs was performed using the Naive bayes and the SILVA 16S rRNA database (v138).

ASV analysis of sequences was performed using mothur-1.30, including Coverage, ACE, Shannon Index, and Simpson Index. Microbial community β diversity was calculated based on the Bray–Curtis distance matrix (Zaneveld et al., 2017). The Bray–Curtis distance matrix was constructed using a thin ASV abundance table and visualized using principal coordinate analysis (PCoA) using Vegan v2.5-3 package and Qiime calculates the beta diversity distance matrix and non-metric multidimensional scaling (NMDS) analysis and plotting using R language (version 3.3.1) and Vegan software package (version 2.4.3). To study the similarities and differences in the composition and structure of different sample communities. Based on the sequence corresponding to the classification information at the ASV level, an evolutionary tree is constructed using the Maximum Likelihood (ML) method, and the final result can also be presented in the form of a combination graph of the evolutionary tree and reads abundance. FastTree (version 2.1.3) and R (version 3.3.1) were used to draw graphs and trees. The Pheatmap (1.0.8) package in R (version 3.3.1) was used to draw a Heatmap reflecting the similarities and differences in the community composition of different groups (or samples) at various classification levels. Circos-0.67-7 (http://circos.ca/) was used to describe the correspondence between samples and species, which not only reflects the proportion of dominant species composition for each (or group) sample, but also reflects the distribution proportion of each dominant species in different samples (groups).

2.4. Coral transcriptome sequencing and bioinformatics analysis

Total RNA was extracted from the coral samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed using a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and quantified using ND-2000 (NanoDrop Technologies). Paired-end RNA-seq libraries were sequenced using the HiSeqXTen platform (2 × 150 bp, Illumina).

Sequencing data from all samples were used for assembly. The original paired-end reads were trimmed and quality-validated using SeqPrep and Sickle with default parameters. The clean data were then assembled *de novo* using Trinity (Grabherr et al., 2011). The assembled unigenes were annotated using BLAST in six databases: non-redundant (Nr), Kyoto Encyclopedia of Genes and Genomes (KEGG), Swiss-Prot, Pfam, Gene Ontology (GO), and Clusters of Orthologous Groups. Based on previous studies and reference genomes of *Acropora digitifera*, *Stylophora pistillata*, and *Orbicella faveolata*, the BLASTx pipeline was used to identify contigs that could be confidently assigned to coral transcriptomes (Bay and Palumbi, 2015; Mansour et al., 2016; Yu et al., 2020). These transcripts were used in the subsequent read mapping and function annotation.

Transcript expression levels were measured using the fragments per kilobase transcript per million mapping reads (FPKM) method (Qin et al., 2017). Gene abundance was quantified using RNA-Seq by Expectation-Maximization (RSEM) (http://deweylab.github.io/RSEM). Differentially expressed genes (DEGs) between groups were identified using DESeq2 software (FDR <0.05) (Love et al., 2014). GO and KEGG annotation and enrichment analyses were performed using the Majorbio cloud platform. Overrepresentation analysis (ORA) using Fisher's exact test (Benjamini–Hochberg adjusted $p < 0.01$) was used for KEGG enrichment analysis (Désert et al., 2008). Multiple testing corrections using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995; Désert et al., 2008) was applied to control the error rate in overall inference results. Goatools was used for GO enrichment analysis to identify the main GO functions of the genes. In order to provide a more accurate description of gene function, based on the sample species type, the GO database in this study selected animal categories. A Fisher's exact test was conducted, and significant GO function enrichment was identified when the adjusted p was <0.01.

3. Results

3.1. Differential transcriptional response of corals under high-temperature stress

In order to compare the differential response of corals to high temperature and cadmium stress, we analyzed the phenotypic differences and found that significant tissue shedding occurred under high temperature stress, while coral exhibited tentacle contraction after cadmium stress (Fig. 1). To analyze the response mechanism of *J. squamata* under high-temperature stress, six high-quality Illumina libraries were constructed. Raw reads are stored in the NCBI SRA database: PRJNA1166842. In total, 297,110,102 raw reads were obtained. After removing ambiguous reads, junctions, and low-quality sequences, 283,067,118 high-quality clean reads were assembled into 85,908 unigenes with an N50 length of 1676 bp (GC content of 38.63%) and a mean length of 744.08 bp. The correlation rate of the coral host for each sample was 67.80–85.70% (Tables S1–S3). RSEM was used to analyze the expression levels of unigenes in 85,908 hosts (Table S4).

The principal component analysis (PCA) map of unigene expression levels showed clear distinctions between the control and high-temperature groups, accounting for 64.68% and 24.15% of the total variance, respectively, indicating greater variation between samples in different groups (Fig. 2A). Compared to the control group, we identified 13,703 differentially expressed coral genes (4967 up-regulated and 8736 down-regulated) (FC ≥ 2, BH-adjusted $p < 0.05$) (Table S5 and Fig. 2B). GO and KEGG enrichment analysis of all DEGs (adjusted $p <$ 0.01) revealed 75 GO terms and 50 KEGG pathways significantly enriched in the high-temperature stress group, including 35 in the molecular function (MF) category, 13 in the cellular component (CC) category, and 27 in the biological process (BP) category (Fig. 3A and Table S6). The most abundant gene in the GO terms was GO:0048856 anatomical structure development, and GO:0050789 regulation of the biological process. Additionally, we found that disease-related pathways were the most abundant in the significantly enriched KEGG pathways (Table S7).

3.2. Differential transcriptional response of corals under cadmium stress

To analyze the response mechanism of *J. squamata* to cadmium stress, 10 high-quality macrotranscriptomic libraries were constructed using Illumina technology. Raw reads are stored in the NCBI SRA database: PRJNA1166920. In total, 524, 895, 922 raw reads were

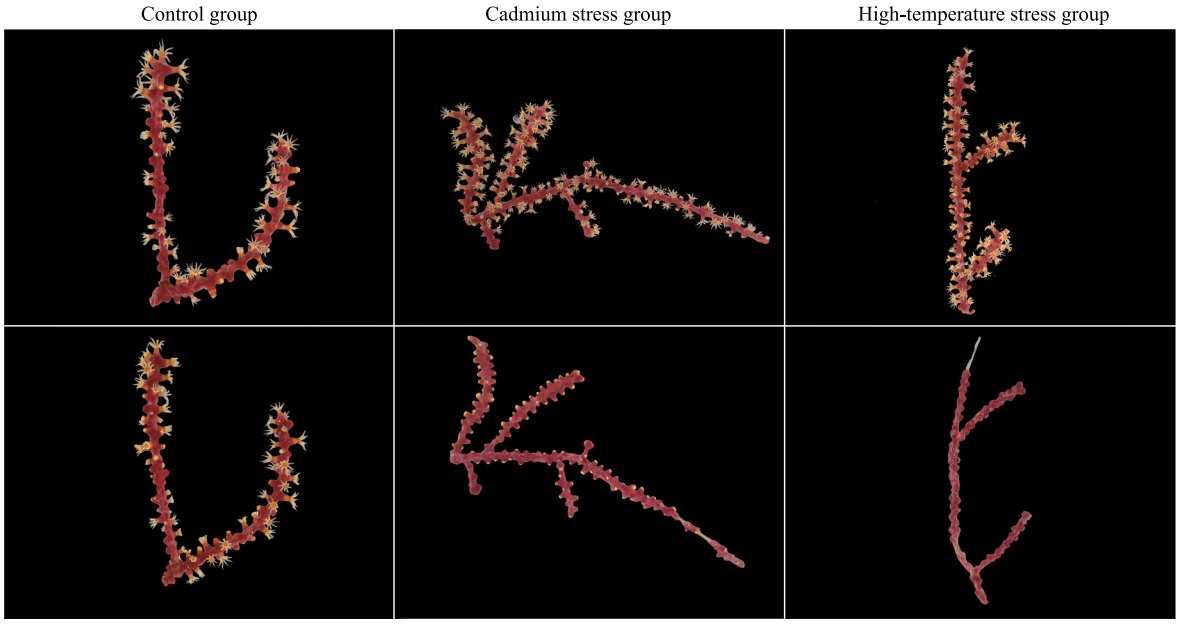

Fig. 1. Differences in phenotypic characteristics of corals under high temperature and cadmium stress. The first and second lines show the phenotypic characteristics of each group of corals before and after stress, respectively.

obtained. After removing ambiguous reads, splices, and low-quality sequences, we assembled 507,956,860 high-quality clean reads into 74,717 unigenes with an N50 length of 1871 bp (GC content 38.37%) and an average length of 876.02 bp. The correlation rates of coral hosts for each sample was 81.82–82.84% (Tables S8–S10). RSEM was used to analyze the expression levels of 74,717 unigenes (Table S11).

The PCA map of unigene expression levels showed clear distinctions between the control and Cd-stressed groups, accounting for 32.46% and 10.02% of the total variance, respectively, indicating greater variations between samples in different groups (Fig. 2C). Compared to the control group, we found 3141 differentially expressed coral genes (2275 up-regulated and 866 down-regulated) (FC $\geq$ 2, BH-adjusted $p < 0.05$) (Table S12 and Fig. 2D). GO enrichment analysis of all DEGs (adjusted $p < 0.01$) identified 16 GO terms with significant enrichment, including 12 in the MF category and four in the CC category (Table S13 and Fig. 3B). The most abundant gene in GO terms was GO: 0005887 integral component of plasma membrane, and GO: 0005576 extracellular region. Interestingly, we found that corals exhibit partially shared responses during high temperature and cadmium stress, and the 16 GO terms identified during cadmium stress were significantly enriched under high temperature stress. The Venn diagram showed the shared stress responses and the distinct mechanisms specific to each stress condition, offering deeper insight into how corals adapt to various environmental stressors (Fig. 2E).

3.3. Diversity and community structure of coral symbiotic bacteria under high-temperature stress

Symbiotic bacteria are vital for scleractinian corals and involved in numerous biological processes. We analyzed the diversity and community composition of symbiotic bacteria under high-temperature stress. Raw reads were deposited in the NCBI SRA database: PRJNA1166931. We compared the α-diversity and community structure of symbiotic bacteria in coral samples from control and high-temperature stress groups. After secondary sampling at the same sequencing depth, 3581 amplicon sequence variants (ASVs) were assigned from 1,146,283 treated bacterial sequences (Tables S14–S15). The average sequence length was 413 bp. The Chao and Ace indices showed no significant difference in ASV abundance between the two groups, while the Shannon and Simpson indices showed significant differences in microbial α-diversity (Table S16 and Fig. 4A and B), which may suggest that the bacterial community in the control samples is dominated by certain bacterial taxa. BD1-7_clade and *Rhodococcus* were the dominant bacterial genus in the control group.

PCoA and NMDS analysis indicated significant differences in the community structure of symbiotic bacteria between control and high-temperature stress groups (Fig. 4C and D). The flora stability index or average variability distance (AVD) showed higher microflora stability in control samples than in high-temperature stress samples (Fig. 4E). Beta diversity analysis showed significant inter-group differences (Fig. 4F). Cicros plot and heatmap were used to present the bacterial community composition in control and high-temperature groups at the genus level (Fig. 5A, Fig. S1). Phylogenetic tree analysis revealed significant changes in the community structure of symbiotic bacteria under high-temperature stress (Fig. 5B). Specifically, the control group was dominated by BD1-7 clade, *Rhodococcus,* and *Endozoicomonas*, while the high-temperature group showed elevations in unclassified Rhodobacteraceae, *Vibrio, Limimaricola, Fusibacter, Cognatishimia, Erythrobacter, Pseudophaeobacter, Nautella*, and *Cohaesibacter* relative abundances.

3.4. Diversity and community structure of coral symbiotic bacteria under cadmium stress

We analyzed the symbiotic bacterial community composition of coral under cadmium stress. Raw reads were deposited in the NCBI SRA database: PRJNA1166945. We compared the diversity and community structure of symbiotic bacteria in coral samples from control and Cd-stressed group. After secondary sampling at the same sequencing depth, 4888 ASVs were assigned from 1,201,521 treated bacterial sequences (Tables S17–S18). The average sequence length was 419 bp. The Chao and Ace indices showed no significant difference in ASV abundance between the two groups, and the Shannon and Simpson indices also showed no significant difference in microbial α-diversity (Table S19).

PCoA ($R = 0.9224$, $p = 0.001$, PC1: 23.14%, PC2: 8.51%) and NMDS (stress: 0.074, $R = 0.9231$, $p = 0.001$) analyses indicated significant differences in the community structure of symbiotic bacteria in the control and Cd-stressed groups (Fig. 6A and B). Beta diversity analysis

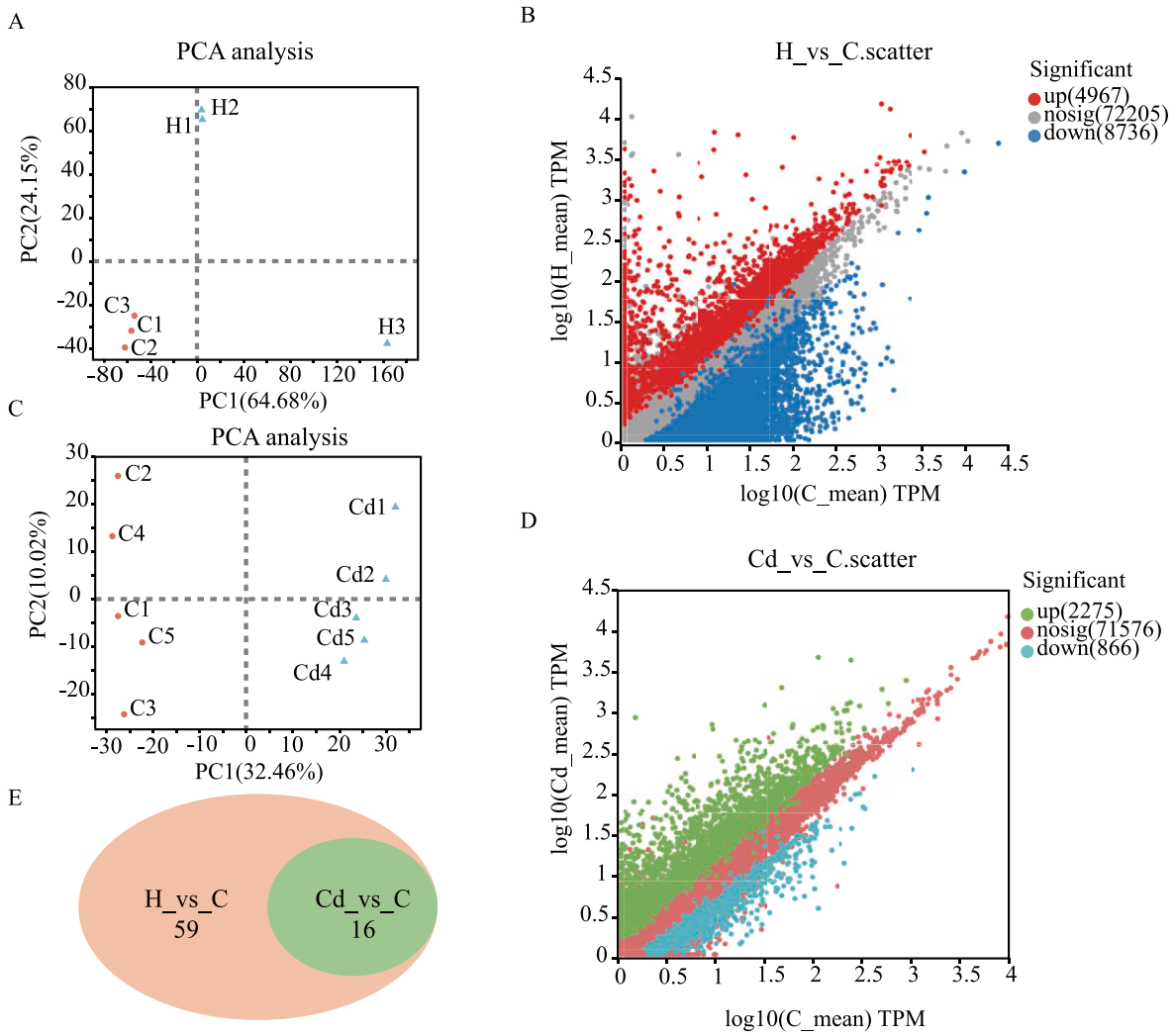

Fig. 2. Transcriptional differences in corals under high temperature and cadmium stress. (A) Principal component analysis of coral between high-temperature group and control group; (B) Differentially expressed coral genes between high-temperature group and control group (red: upregulated, grey: no signal, blue: downregulated)(C) Principal component analysis of coral between Cd-stressed and control group; (D) Differentially expressed coral genes between Cd-stressed group and control group (green: upregulated, pink: no signal, light blue: downregulated); (E) Venn diagram present the overlapping and unique GO terms affected by both Cd and heat stress.

also showed significant differences among samples (Fig. 6C). The AVD revealed that the microflora stability of coral samples in the control group was higher than in the Cd-stressed group (Fig. 6D). Cicros plot and heatmap were used to present the bacterial community composition in the control and Cd-stressed groups at the genus level (Fig. 7A, Fig. S2). Phylogenetic tree analysis (Fig. 7B) indicated that cadmium stress caused significant changes in the community structure of symbiotic bacteria compared with the control. Specifically, in the control group, BD1-7 clade, *Rhodococcus*, and *Endozoicomonas* were dominant, while after Cd-stress treatment, *Achromobacter*, BD1-7 clade, and *Endozoicomonas* were prevalent. The relative abundances of *Achromobacter*, *Delftia*, *Pseudomonas*, and *Ruegeria* were significantly higher in the Cd-stressed group compared to the control group.

4. Discussion

In global coral degradation, scleractinian corals have received special attention. However, the adaptation mechanism of octocorallia to environmental stresses such as high-temperature stress and heavy metals has been rarely reported. In this study, we collected samples of the octocorallia *J. squamata* under high-temperature and cadmium stress. The response mechanism of the coral host and symbiotic bacteria was investigated from the perspective of the coral holobiont using high-throughput sequencing.

4.1. Increased abundance of opportunistic bacteria caused by high-temperature stress increases the risk of disease in coral holobiont

To investigate the response of coral holobiont to high-temperature stress, we compared host transcription characteristics and symbiotic bacterial community changes after high-temperature stress. PCoA and NMDS showed that the control and high-temperature groups samples were separated. The α-diversity of symbiotic bacteria increased significantly after high-temperature stress, and the community structure and relative abundance of symbiotic bacteria differed significantly between the two groups of corals.

Symbiotic bacteria are an important part of the coral holobiont. They can change faster than their coral hosts (Ziegler et al., 2017). Increased alpha diversity may be a way for corals to respond to high temperature stress. In bacterial communities, high alpha diversity may indicate the presence of more bacterial species and more complex interaction networks, thereby increasing the adaptability and flexibility of bacterial communities to environmental changes. High alpha diversity provides bacteria with more choices and possibilities. When bacterial

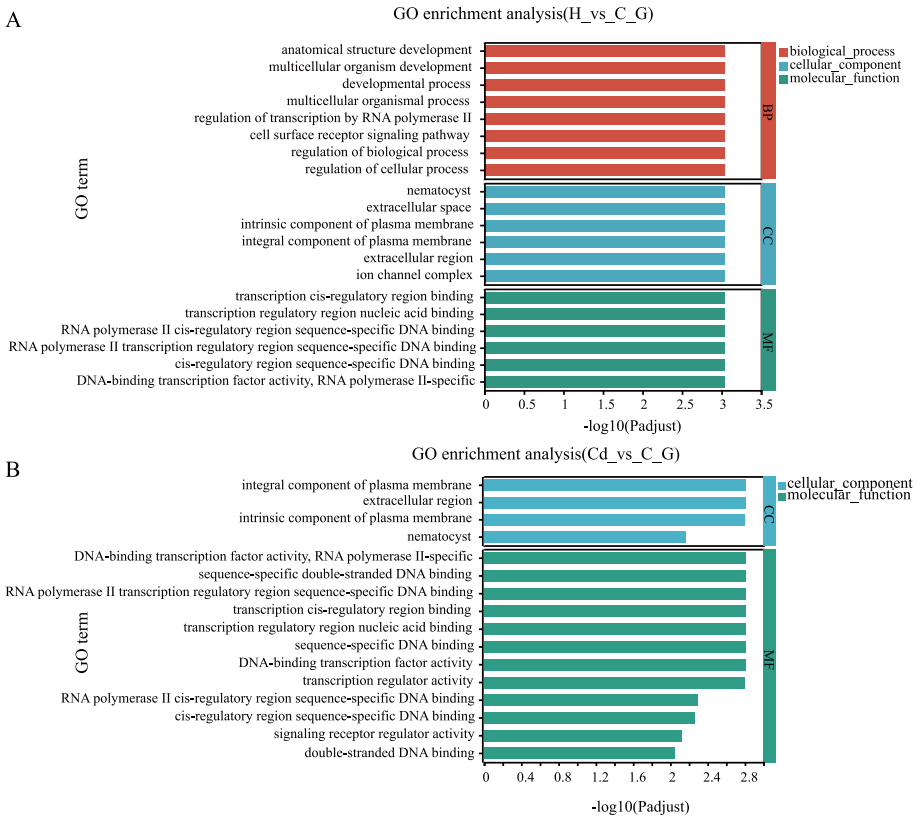

Fig. 3. GO enrichment analyses of the significantly coral genes in high temperature group (A) and Cd-stressed group (B).

communities face external pressures, they may adapt to these changes by altering population structure, switching to more suitable compositions, gene expression, or metabolic pathways. In addition, high alpha diversity helps to increase the stability of ecosystems, which means that bacterial communities are less likely to collapse in the face of external pressures. A stable ecosystem can provide bacteria with a better living environment and more opportunities for adaptation (Prada et al., 2016; Ziegler et al., 2019; Yu et al., 2020a; Yu et al., 2020b). Similar to our study, symbiotic bacteria under adverse conditions such as acidification, eutrophication, overfishing, and coral-algal competition has been found to exhibit a high α-diversity (Prada et al., 2016). In addition, we found that high temperature stress caused changes in the community structure of symbiotic bacteria. The presence of specific bacterial communities may help balance the microbial community and potentially prevent the invasion of harmful bacteria (Canny and McCormick, 2008). High temperature stress may disrupt the stability of the coral holobiont, leading to decreased stability of symbiotic relationships and increased abundance of opportunistic bacteria, and we also found that the relative abundance of dominant bacteria changed and the abundance of many opportunistic bacteria increased. The relative abundance of unclassified Rhodobacteraceae, *Vibrio*, *Limimaricola*, and *Fusibacter* increased after high-temperature stress. Previous coral microbiome studies have successfully characterized the prevalence of Rhodobacteraceae in corals at the community structure level. Rhodobacteraceae was widely present in Florida Reef coral lesions (Meyer et al., 2019) and diseased *Porites* spp. (Rasmussen et al., 2020). A high abundance of *Vibrio* was detected in diseased lesions of the *D. stokesii* colony, whereas a relatively low abundance was observed in apparently healthy tissues (Meyer et al., 2019). In addition to *Roseofilum*, the Black Band Disease consortia in the Florida Keys were dominated by uncultured genera of Rhodobacteraceae and *Bacteroidales*, *Fusibacter*, and *Desulfovibrio* (Meyer et al., 2016). Increased abundance of opportunistic bacteria has been observed in response to high summer temperatures in *Acropora pruinosa* and *Pavona decussata* in Weizhou Island (Yu et al., 2023). An increased abundance of opportunistic bacteria can lead to coral bleaching and an increased risk of disease (Banin et al., 2003).

Additionally, we found that coral hosts exhibit high transcriptional plasticity under environmental stress. GO enrichment analysis revealed many GO terms associated with cellular processes. The pathway with the most enriched genes in our results is GO:0050789 regulation of the biological process, and the most significant pathway for enrichment is GO:0048856 anatomical structure development. Anatomical structure development is a biological process that begins with the formation of structures and ends with their maturation, regardless of their form, including their natural destruction. This is any biological entity that occupies space and distinguishes itself from the surrounding environment, which can be macroscopic or microscopic. Biological processes are regulated in various ways, such as controlling gene expression, modifying proteins, or interacting with substrate molecules. Any process that regulates biological processes' frequency, rate, or range (Chen et al., 2019). This is crucial for responding to high-temperature stress. Similar to our results, it was also found to be involved in disease response (Lu et al., 2018). In addition, many disease-related pathways were found, and genes related to the regulation of cellular and biological processes were significantly enriched in the high-temperature stress group. Therefore, we speculate that high-temperature stress adversely effects coral health, consistent with the observed characteristics of symbiotic bacterial communities. High-temperature stress can make corals feel stressed, thereby damaging their immune systems. Simultaneously, rising ocean temperatures also enhance the reproductive ability of bacteria and other pathogens, increasing the risk of coral infection.

These results indicate that high-temperature stress disrupts the coral holobiont, seriously impacting the health and survival of coral hosts, leading to the invasion of opportunistic bacteria, such as *Vibrio*, and increasing the risk of infection and bleaching.

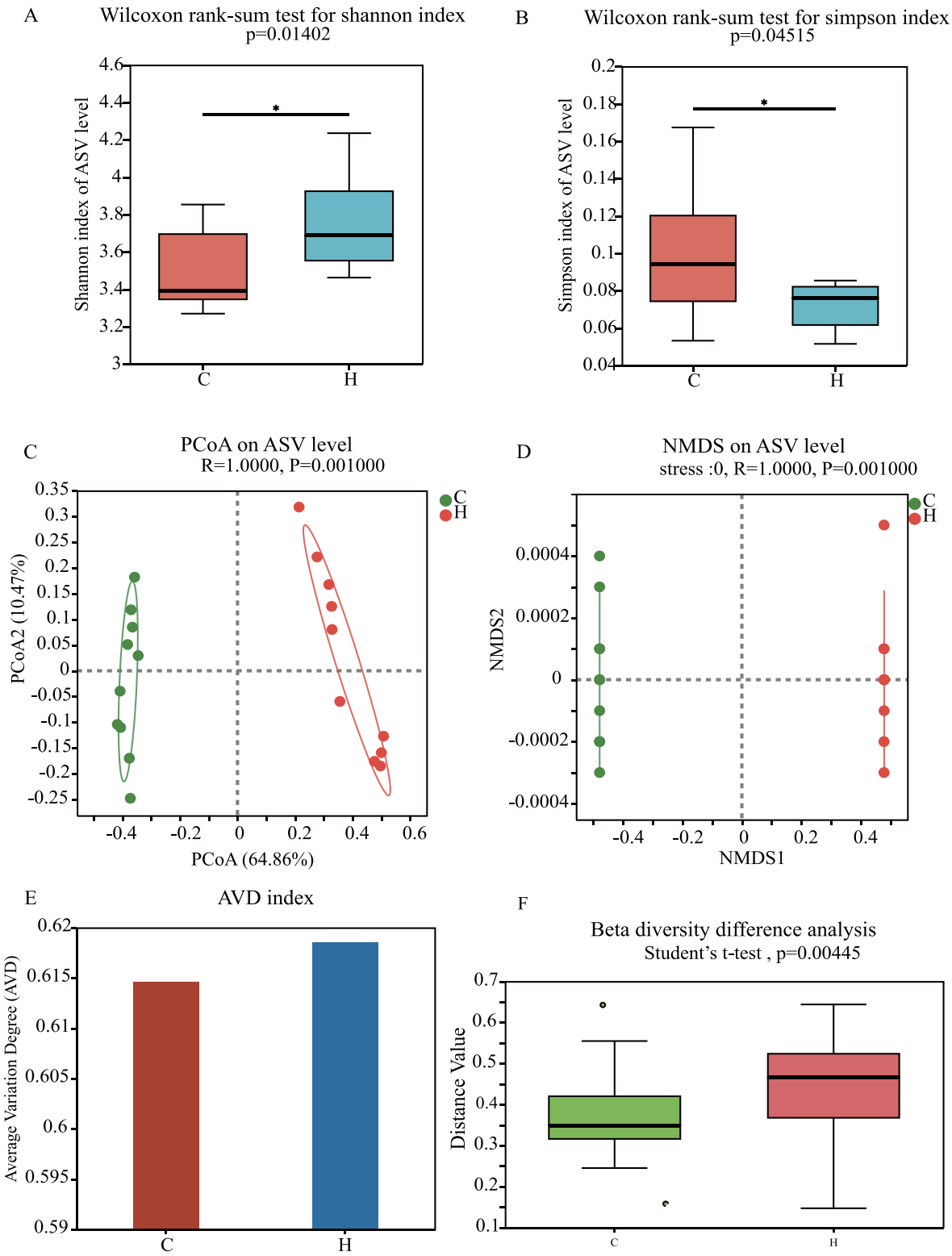

Fig. 4. Comparison of symbiotic bacteria between high-temperature group and control group. (A) Wilcoxon rank-sum test for Shannon index; (B) Wilcoxon rank-sum test for simpson index; (C) PCoA on ASV level; (D) NMDS on ASV level; (E) AVD index; (F) Beta diversity difference analysis.

4.2. Cadmium stress leads to increased instability of coral holobiont, potentially disrupting the stability of coral host DNA and RNA transcriptional regulation

This study compared the coral host transcriptional response and symbiotic bacteria's diversity and community structure in coral samples after cadmium stress. The results showed that the α-diversity of symbiotic bacteria was not significantly different after cadmium stress; however, the β-diversity increased, and the community structure and relative abundance of symbiotic bacteria were significantly differed between the two groups. After Cd-stress treatment, the dominant bacteria changed, significantly increasing the relative abundance of

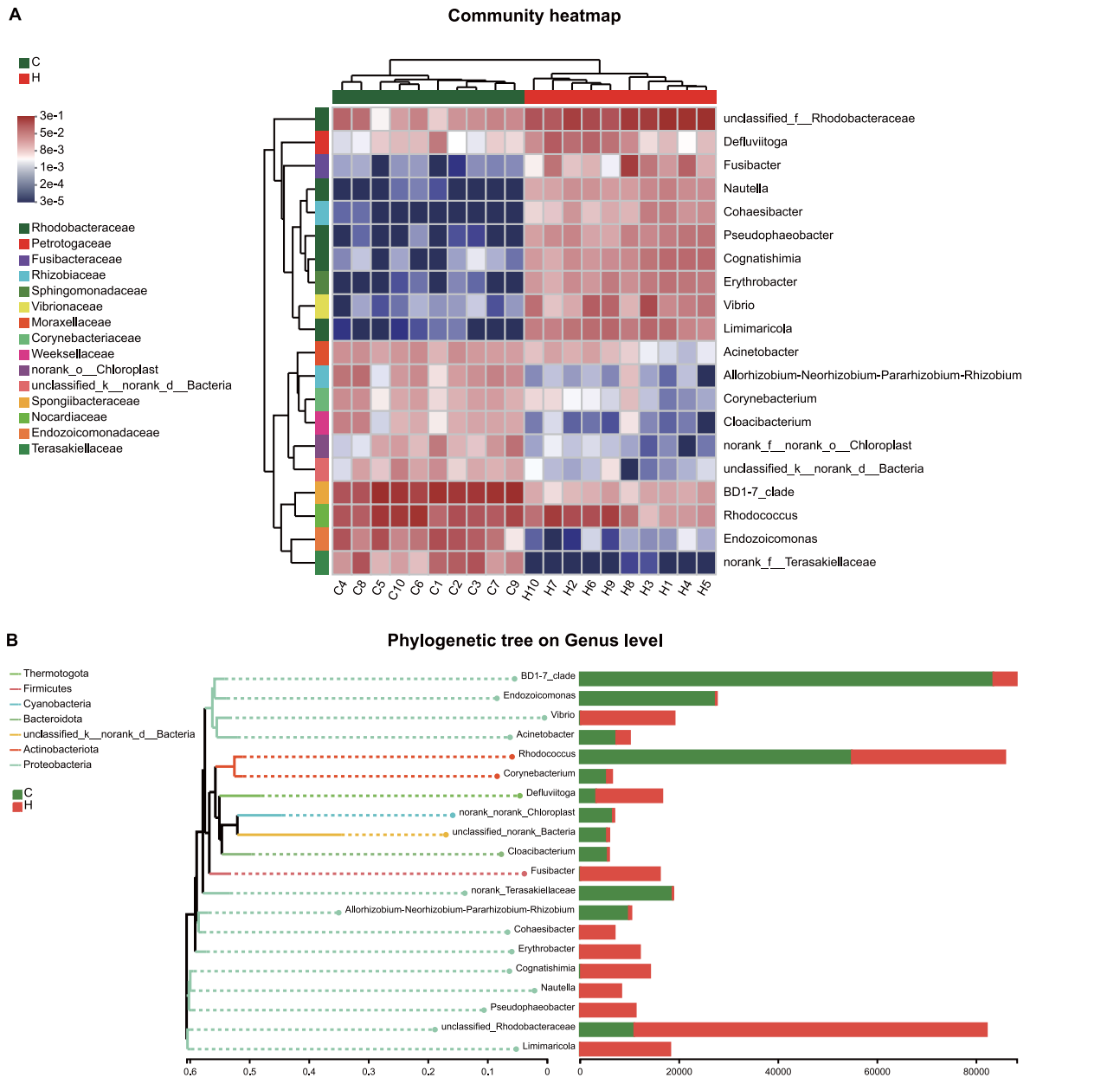

Fig. 5. (A) Community heat maps of symbiotic bacteria in high-temperature group and control group at genus level; (B) Construction of a phylogenetic tree of symbiotic bacteria of high-temperature group and control group. Each branch in the evolutionary tree represents a species, and the length of the branch is the evolutionary distance between two species, which is the degree of difference between the species. The bar chart displays the number of reads for different species in each group.

Achromobacter. Additionally, we observed alterations in host transcription processes.

Symbiotic bacteria are considered an important component of scleractinian corals and are involved in various biological processes (Ziegler et al., 2017). However, while alpha diversity did not significantly change, beta diversity was larger in the Cd-exposed individuals, indicating greater variability among samples. High beta diversity may mean a more diverse bacterial community in the ecosystem, which can be beneficial in some cases as it may increase the stability and resilience of the ecosystem. However, in some cases, high beta diversity may also indicate ecosystem instability or some degree of disturbance (Mori et al., 2018). Similar to our results, previous studies have found that elevations in algal exposure or temperature lead to opportunistic microbial blooms, destabilizing the coral microbiome and increasing β-diversity among coral samples affected by these stressors (Yu et al., 2020; Zaneveld et al., 2016). Stressful conditions may shift the coral microbiome from one stable state to another. Changes in dominant bacteria may be a mechanism by which the coral holobiont resists environmental changes. Symbiotic bacteria can adapt more quickly than their coral hosts, which may help long-lived corals adapt to climate change (Ziegler et al., 2017). According to the coral probiotic hypothesis, there is a dynamic relationship between the coral microbiome and environmental conditions, allowing for the selection of the most favorable symbiotic bacteria for the coral holobiont (Reshef et al., 2006). The significant increase in the relative abundance of cadmium resistant *Achromobacter* may be a positive signal. *Achromobacter* is widely found in soil and water and plays an important role in plant resistance to cadmium stress. Studies have shown that *Achromobacter* has a strong tolerance to heavy metals (Nyoyoko, 2022) and can fix Cd in polluted environments, reducing Cd absorption by plants (Sun et al., 2022). *Achromobacter* can adsorb Cd (Fan et al., 2023), potentially increasing the availability of Cd in the rhizosphere and promoting Cd absorption by plants (Li et al., 2022). This suggests

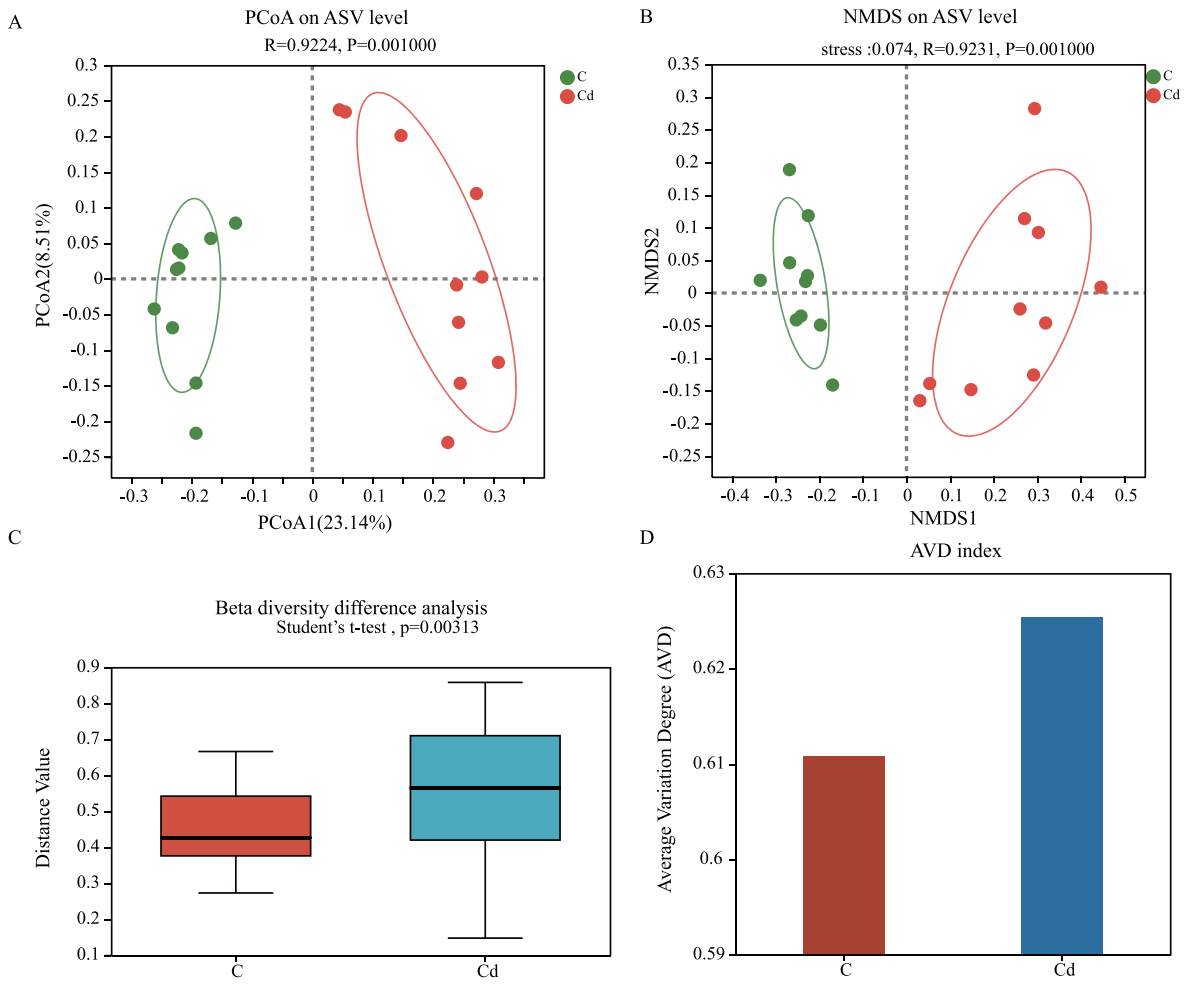

Fig. 6. Comparison of symbiotic bacteria between Cd-stressed group and the control group. (A) PCoA on ASV level; (B) NMDS on ASV level; (C) Beta diversity difference analysis; (D) AVD index.

that the recombination of symbiotic bacterial community structure plays an important role in the environmental adaptation of the coral holobiont. Although alpha diversity did not change, specific bacterial communities shifted, leading to enhanced tolerance to Cd stress. These responses may be part of the holobiont's stress adaptation mechanisms.

Transcriptome sequencing revealed that coral hosts have high transcriptional plasticity under cadmium stress. PCA and cluster heat map analyses showed high agreement between replicates and differences between groups, indicating high confidence in our sequencing results. High-quality RNA-Seq analyses allowed us to identify numerous DEGs involved in differential tolerance. GO enrichment analysis showed that the most abundant genes were related to the integral component of the plasma membrane and extracellular region, which interact with cells and the external environment. Cd has strong biotoxicity and high mobility in living organisms. After absorption, Cd significantly affects the normal growth and development of organisms by entering cells via metal ion transporters, similar to essential nutrients such as Fe, Zn, Ca, and Mn (Zhang et al., 2023). Stress signals can affect the open state of ion channels and thus influence Cd absorption (Ward et al., 2009). Significant enrichment of differential genes in the GO terms related to DNA and RNA transcription regulation was observed in the Cd-stressed corals. RNA transcription and its regulatory mechanisms are crucial for cell function, allowing for precise gene expression regulation (Glisovic et al., 2008). Cadmium stress can increase genomic DNA instability, leading to replication errors and base mismatches during DNA replication (Filipič, 2012). DNA damage can result in increased mismatch repair through translesion synthesis pathways, including base mismatches, DNA oxidation, single and double-strand breaks, and other forms of damage (Hu et al., 2016; Wang et al., 2020; Zhao et al., 2020). Similar phenomena have been observed in other organisms, such as Cd-induced DNA instability in mice (Li et al., 2023). Continuous Cd exposure triggers oxidative stress, causing ongoing damage to DNA, proteins, and cell membranes throughout the organism's life cycle; for example, rice (*Oryza sativa* L.) (Rizwan et al., 2017). Therefore, we speculate that cadmium stress may damage the structure and stability of coral DNA and the regulation of RNA transcription, leading to ongoing oxidative stress and damage.

Our results suggest that cadmium stress increases the instability of the coral holobiont, potentially disrupting the stability of coral host DNA and RNA transcription regulation. However, the increased abundance of Cd-resistant bacteria may be an important adaptive mechanism for the coral holobiont in response to cadmium stress.

4.3. *Differential responses of J. squamata to high-temperature and cadmium stress*

The adverse effects of high-temperature stress and heavy metal stress on coral reefs have been widely recognized (Hughes et al., 2018; Mitchelmore et al., 2007; Zhou et al., 2018). The molecular mechanism of heat and metal exposure is still unclear. Before studying their interactions in the future, we separately discussed the effects of heat and cadmium stress to help us understand the metal toxicity faced by scleractinian corals in the context of global warming.

In this study, although we also found that these two environmental

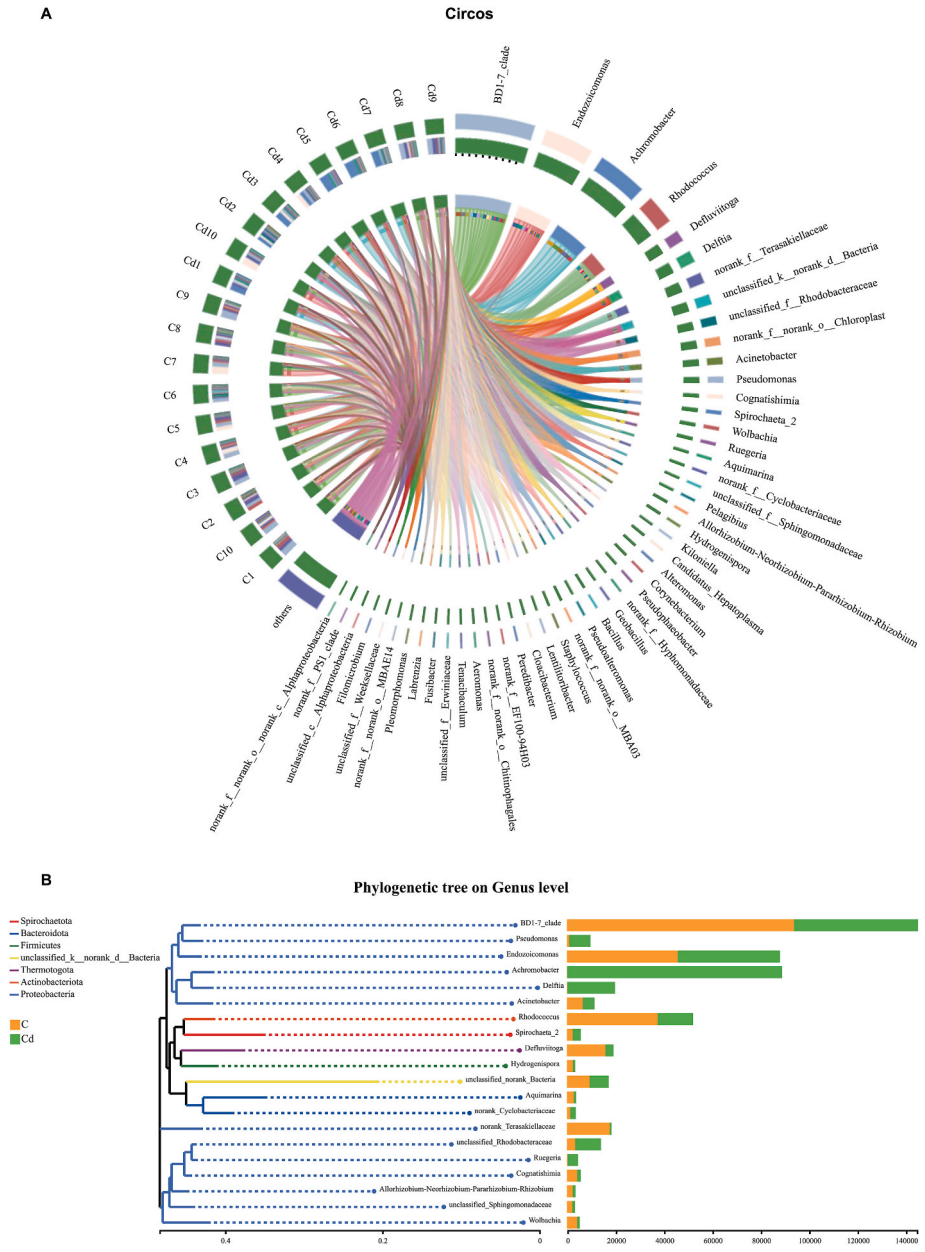

Fig. 7. (A) Microbial community distribution at the genus level of each sample in Cd-stressed group and control group; (B) Construction of a phylogenetic tree of symbiotic bacteria of Cd-stressed group and control group. Each branch in the evolutionary tree represents a species, and the length of the branch is the evolutionary distance between two species, which is the degree of difference between the species. The bar chart displays the number of reads for different species in each group.

pressures have similar adverse effects on corals, high temperature stress has had more severe adverse effects on corals, showing more obvious tissue damage and there are still significant differences in response processes. Under stress conditions, symbiotic bacteria can quickly respond, adjust diversity, stability, and community structure composition, which is beneficial for corals to quickly respond to environmental pressure. Both heat and cadmium stress can reduce the stability of symbiotic bacterial community structure and lead to bacterial community restructuring, but after high-temperature stress, the alpha diversity of coral symbiotic bacteria significantly increases. However, it may break the stability of coral symbiotic relationships, leading to a decrease in symbiotic relationship stability and we also found that the abundance of dominant bacteria changed, and the abundance of a large number of opportunistic bacteria increased. In contrast, there was no significant difference in the alpha diversity of symbiotic bacteria after cadmium stress, and no significant increase in the abundance of opportunistic bacteria was observed. Even after cadmium stress, the relative abundance of cadmium tolerant *Achromobacter* increased significantly. Similarly, there were differences in host responses. After high-temperature stress, coral hosts showed significant changes in gene expression related to physiological and molecular processes, and a large number of immune and disease-related pathways were screened, indicating that high-temperature stress can damage coral's physiological processes and immune system. High-temperature stress also enhances the reproductive ability of pathogens such as fungi and bacteria, thereby increasing the risk of coral infection. However, under cadmium stress, the main manifestation is the disruption of coral host DNA stability and RNA transcription regulation.

In general, we found that both heat and cadmium stress can have adverse effects on octocorallia *J. squamata*, and even disrupt the stability of symbiotic relationships. High-temperature stress has more severe adverse effects than cadmium stress. However, the impact of the two

environmental stresses on corals is different, therefore, in future research, more and differentiated attention should be paid to these two key environmental stresses.

5. Conclusions and limitations

In the context of widespread coral degradation worldwide, reports on the adaptation mechanisms of octocorallia corals in the face of environmental stresses such as high-temperature stress and heavy metals are few. In this study data from octocorallia *J. squamata* were collected under heat and cadmium stress. The current study using high-throughput sequencing methods, investigate the response mechanisms of coral hosts and symbiotic bacteria from the perspective of coral whole organism. The result show that high-temperature stress has more serious adverse effects than cadmium stress. High-temperature stress leads to the destruction of coral symbiotic relationship, which leads to the invasion of opportunistic bacteria, and increases the risk of infection and death of coral hosts. Cadmium stress leads to increased instability of coral symbiotes, which may disrupt the DNA stability and RNA transcriptional regulation of coral hosts. However, an increase in the abundance of cadmium tolerant bacteria may be an important way for coral symbiotes to respond to cadmium stress. This is of great significance for the protection of coral ecosystems in the context of sustained global environmental degradation.

However, some limitations should be noted. First, we only set a single concentration, which may have led to limitations in the results. Different temperatures, concentrations of cadmium stress, and differences in exposure time may all have varying impacts on corals (Mitchelmore et al., 2007; Zhou et al., 2018). In addition, owing to inter species differences in coral's response to environmental stress (Yu et al., 2023), stress conditions and coral species should be included in future research. The accumulation of cadmium in coral tissue should also be of concern, as it is important for evaluating the toxicity of cadmium in coral. Finally, indoor simulation experiments are conducted to better reveal the adaptation mechanisms of corals in natural environments. In the natural environment, cadmium exposure is not a single stressor and may occur simultaneously with high-temperature stress (Guo et al., 2018). There may be synergy effects between different environmental stresses, such as ammonia enrichment stress alleviating the adverse effects of high-temperature stresshigh-temperature stress on corals (Zhou et al., 2017), or high-temperature stress exacerbating cadmium toxicity. Therefore, in future research, it is necessary to consider the synergistic effects of various stressors on corals.

CRediT authorship contribution statement

Xu Gao: Writing – original draft, Validation, Resources, Methodology, Data curation, Conceptualization. **Junling Chen:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Data curation. **Yuling Ma:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation. **Yue Zheng:** Writing – review & editing, Validation, Data curation, Conceptualization. **Yinyao Bu:** Writing – review & editing, Resources, Methodology, Investigation. **Xiaopeng Yu:** Writing – review & editing, Project administration, Formal analysis. **Kefu Yu:** Validation, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Data statement

Sequencing raw reads in this study were deposited into the NCBI sequence reads archive (SRA) database: PRJNA1166842, PRJNA1166920, PRJNA1166931 and PRJNA1166945.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106865.

Data availability

Data will be made available on request.

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