Marine Pollution Bulletin 210 (2025) 117331

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Exposure to polypropylene microplastics induces the upregulation of protein digestion-associated genes and microbiome reorganization in the octocoral *Junceella squamata*

Xu Gao [a,1], Junling Chen [b,1], Kefu Yu [b,c,*], Yinyao Bu [a], Limei Wang [a], Xiaopeng Yu [b,*]

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

ARTICLE INFO

Keywords:
Coral holobiont
Gorgonian
Microplastics
Octocorallia
Coral host
Symbiotic bacteria

ABSTRACT

Microplastics, a new type of pollutants found in coral reefs, have attracted increasing attention. However, most of the current research focuses on the scleractinian corals and few reports on Octocorallia. To reveal the impact of microplastic exposure on Octocorallia, we analyzed the transcriptional response of the coral hosts *Junceella squamata* along with changes to the diversity and community structure of its symbiotic bacteria following exposure to polystyrene microplastics. These results suggest that the microplastics have adverse impacts on nutrient metabolism and absorption in *J. squamata*. The symbiotic bacteria of *J. squamata* exhibited a clear response after exposure to microplastics, which may also reflect an adaptation mechanism of corals, and help to maintain the physiological function of coral symbiotic function under the exposure of microplastics. This study has revealed the impact of microplastic exposure on *J. squamata*, providing new insights for coral protection against the background of increased microplastics pollution.

1. Introduction

Microplastics are new pollutants that have attracted increasing attention (Reichert et al., 2019; Tan et al., 2020). Coral reefs develop individual, unique, and and complex three-dimensional structures that can result in their subsistence as long-term microplastic sinks due to complex three-dimensional structures. Notably, plastic particles may adhere to solid substrates that develop during coral growth (Reichert et al., 2018). Variations in coral species morphology and feeding patterns may affect the degree to which microplastics adhere to coral structures (Martin et al., 2019; Lim et al., 2020). Accumulation of microplastic particles has been observed in several tropical coral species as well as in the temperate coral *Astrangia poculata*, suggesting that scleractinian corals might become long-term sinks for microplastics (Soares et al., 2020; Hierl et al., 2021).

Abundant microplastics have been found in coral reefs worldwide, including the South China Sea (Ding et al., 2019; Huang et al., 2019; Zhang et al., 2019; Tang et al., 2021), Rameswaram Coral Island (Vidyasakar et al., 2018), the Maldives (Imhof et al., 2017; Saliu et al., 2018), and the Great Barrier Reef (Jensen et al., 2019). Microplastics detected in the coral reef environment of the South China Sea were found to primarily comprise polyethylene, polypropylene, and polyethylene terephthalate, which are mainly transparent or blue. To date, toxicity tests and measurements of the concentrations of microplastics accumulated have been conducted on the following coral species: *Porites pukoensis* (Zhou et al., 2023), *Acropora* spp. and *Seriatopora hystrix* (Mendrik et al., 2021), *Stylophora pistillata* (Lanctôt et al., 2020), *Pseudodiploria clivosa* (Hankins et al., 2021), *Acropora cervicornis* (Hankins et al., 2021), *Tubastrea aurea* (Liao et al., 2021), *Porites porites* (Grillo et al., 2021), *Pomacentrus amboinensis* (Santana et al., 2021), *Pocillopora damicornis* (Tang et al., 2018; Tang et al., 2021), and *Galaxea fascicularis* (Tang et al., 2021). Given their feeding patterns, scleractinian corals tend to readily consume microplastics by mistake (Reichert et al., 2021), which then remain in their digestive tracts (Allen et al., 2017; Rotjan et al., 2019). Current research suggests numerous potential impacts of plastic particles on coral, including including physical damage,

* Corresponding authors at: Guangxi Laboratory on the Study of Coral Reefs in the South China Sea, Guangxi University, Nanning, China.
E-mail addresses: kefuyu@scsio.ac.cn (K. Yu), xiaopengyu@gxu.edu.cn (X. Yu).
[1] Xu Gao and Junling Chen contributed equally to this work, and should be considered joint first author.

https://doi.org/10.1016/j.marpolbul.2024.117331
Received 13 June 2024; Received in revised form 14 September 2024; Accepted 19 November 2024

activation of the immune stress response, delayed growth, decrease in feeding, and necrosis and albinism (Utami et al., 2021). Moreover, microplastics particles can readily combine with some other pathogenic microorganisms and chemicals, which may have toxic effects on corals and even lead to the outbreak of other related diseases (Khan et al., 2024). However, compared with that of other pollutants, research on the impact of microplastic exposure on Octocorallia is limited (Huang et al., 2021; Tang et al., 2021), and the specific mechanisms behind this impact are still unclear.

Within the subclass Octocorallia, species in the order Gorgonacea have been widely screened for valuable chemicals that can be developed as natural marine products, including abundant active secondary metabolites and lead drug compounds. However, at present, research of the impact of microplastics on corals has thus far mostly concentrated on scleractinian corals, with relatively limited understanding of the impacts and response mechanism of Octocorallia, and Gorgonacea, in particular, to microplastic pollution. Given the increasing predominance of high-concentration microplastic environments worldwide, it is particularly important to explore the impact of environmental exposure to microplastics on gorgonian corals. Inaddition, it has been widely recognized that corals are composed of several symbiotic organisms, namely, the cnidarian host, Symbiodiniaceae, and bacterial community (Brener-Raffalli et al., 2018). These partners are involved in stable symbiosis that effectively constitutes the holobiont (Brener-Raffalli et al., 2018). Toward this end, in this study, we selected *Junceella squamata* as a representative gorgonian coral species to explore the stress response to microplastic pollution in a controlled experiment. We further compared the reactions of coral hosts and their symbiotic bacteria. Additionally, we determined the potential molecular regulatory mechanism governing the survival or death of corals under microplastic pollution stress. The objective of this study was not only to lay a foundation to facilitate further clarification of the impact of marine microplastic pollution on corals, but also to provide a new understanding of what harm microplastics bring to marine life and offer provide a theoretical basis for microplastic-related ecotoxicology and coral reef ecological restoration.

2. Materials and methods

2.1. Coral sampling

The coral *J. squamata* used in this study was obtained from the coral reef area of Weizhou Island, China. After domestication and adaptation in seawater at 26 °C for one month, the coral isolates were used for experiments.

2.2. Microplastic exposure experiments

Before the experiments, the *J. squamata* coral was cut into independent samples of 5 cm in length and fixed on numbered brackets. The aquarium used in this study with an aquarium light (250 W metal halogen lamp and 4 T5HO), as its light spectrum is similar to that of natural light (12 h light: 12 h dark cycle). The described lighting and aquarium conditions were maintained throughout the experiment. The newly hatched *Artemia* (brine shrimp) were used as the food source for the coral samples in this study. Polystyrene microplastics (diameter 1.0–1.9 μm, 5 % *w/v*; Aladdin) were used in this study. Based on previous research (Huang et al., 2021; Tang et al., 2021), microplastic exposure is harmful to some coral species, polystyrene microparticle incorporation in the gastrovascular cavity was observed in all of the treatments (1, 10, 100, 1000 mg L^{-1}) (Grillo et al., 2021). The microplastic exposure in a concentration of 0.25 mg L^{-1} caused species-specific effects on coral growth and photosynthetic performance to *Acropora*, *Pocillopora*, *Porites*, and *Heliopora* (Reichert et al., 2019). Polystyrene microplastics was added into the filtered seawater with a final concentration of 1 mg L^{-1} (~2×10^9 particles L^{-1}) corresponding to the experimental (MP) and control group (C), respectively in this study. A total of 30 coral nubbins were used for the stress experiment. Sampling was performed 48 h after treatment.

2.3. Coral host transcriptome analysis

In this study, ten coral samples were used for transcriptome sequencing. Total RNA was extracted after crushing the coral tissues with scissors and a mortar in liquid nitrogen. Qualified RNA was quantified and used for subsequent experiments. After quantification using a TBS-380 fluorometer (Turner Biosystems, Sunnyvale, CA, USA), the paired-terminal RNA-seq library was sequenced using the HiSeq XTen platform (2 × 150 bp reading length; Illumina).

The sequencing data of all samples were used for assembly. The original double-ended reads were trimmed with SeqPrep and Sickle, and default parameters were used to verify their quality. Trinity was used to assemble clean data from scratch. After assembly, the unigenes were annotated in six databases [Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), non-redundancy (NR), Pfam, Clusters of Orthodoxy Groups (COG), and Swiss-Prot] using the BLAST algorithm (Camacho et al., 2009). Transcription levels were measured using the metric of fragments per kilobases of transcript per million mapped reads.

The RSEM and DESeq2 packages were respectively used to quantify gene abundance (Li and Dewey, 2011) and screen differentially expressed genes (DEGs) according to a fold change (FC) $\geq$ 2. The assembled unigenes were further annotated using the KEGG metabolic pathway database. KEGG annotation and enrichment analyses were performed using the Majorbio cloud platform. In order to reveal the correlation between potential target genes, we conducted association analysis on differentially expressed genes in the selected metabolic pathways.

2.4. Diversity and community structure analysis of symbiotic bacteria

In this study, twenty coral samples were used for sequencing of the symbiotic bacteria. Total genomic DNA was extracted from approximately 100–200 mg coral tissues using the TIANamp Marine Animal DNA Kit (Tiangen Biotechnology Co., Ltd., Beijing, China). Bacterial 16S rRNA V3–V4 was selected as the target site. Polymerase chain reaction (PCR) amplification was performed under previously reported circulating conditions with the primers 338F and 806R (Yu et al., 2020a, 2020b; Chen et al., 2021). Qualified PCR products were quantified and used for subsequent experiments. According to the standard protocol, the purified amplicons were combined at equimolar concentrations using Illumina platform and double-ended sequencing (2 × 300 bp) was performed.

Raw sequences were subject to quality control and merging processes controlled and merged using the "fastp" (v0.19.6) and merged FLASH (v1.2.7) software tools, respectively, under the following criteria: (1) truncate the reads of 300 bp at any point so that the average mass fraction is <20 on a sliding window of 50 bp, discard the truncated reading shorter than 50 bp, and discard the reading containing fuzzy characters; (2) according to their overlapping sequence, only >10 bp readings are assembled, and the maximum mismatch rate of overlapping areas is 0.2, and the readings that cannot be assembled are discarded; (3) differentiate samples according to barcode and primer, adjust the sequence direction, and accurately match barcode. In primer matching, two nucleotides do not match.

The DADA2 plug-in in the Qiime2 platform was used to denoise the optimized sequence after quality control splicing under default parameters. The sequence obtained after DADA2 denoising was used as an amplicon sequence variant (ASV). AVS quantity displayed as mean value ± standard deviation. Based on ASV representative sequences and abundance information, a series of statistical or visual analyses can be conducted, including species taxonomy analysis, community diversity analysis, species difference analysis, correlation analysis, phylogenetic analysis, and functional prediction analysis. The removed chloroplast

and mitochondrial sequences were annotated for all samples. To minimize the influence of sequencing depth on the subsequent analysis of α- and β-diversity data, the sequence numbers of all samples were leveled. Based on the silva138/16s_bacteria database, the naïve Bayes classifier in Qiime2 was used to analyze the species taxonomy of ASVs with a confidence threshold of 0.7.

ASV analysis was performed on the sequences using MOTHUR software, which involved assessing coverage, ACE, and the Shannon and Simpson indices. The Wilcoxon rank-sum test was used to analyze the differences in α-diversity between the groups. The β-diversity of the microbial community was calculated based on the Bray-Curtis distance matrix and visualized using principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). Analysis of similarities (ANOSIM) with 9999 permutations was used to test for differences in microbial communities (Ziegler et al., 2017). Circos-0.67-7 (http://circos.ca/) was used to analyze the relationship between samples and species, it is a visual circle chart that describes the corresponding relationship between samples and species. This chart not only reflects the proportion of dominant species composition in each (or group) sample, but also reflects the distribution ratio of each dominant species in different samples (groups). In order to further screen for potential key symbiotic bacteria that play a regulatory role in coral response to microplastic exposure, we used inter group difference testing method. Based on the obtained community abundance data, we conducted hypothesis testing on the species between the microbial communities of two sample groups using strict statistical methods, evaluated the significance level of species abundance differences, and obtained species information with significant differences between the two groups. The core bacterial microbiome is related to the environmental response and ecological function of coral holobionts, so evaluating the change of core microbial abundance is helpful to clarify the potential molecular mechanism of *J. squamata* exposure to microplastics. QIIME2 was used to identify the bacteria present in >80 % of the samples (Bolyen et al., 2019), which were considered core microbiome bacteria (Hernandez-Agreda et al., 2017; Yu et al., 2021a, 2021b).

3. Results

3.1. Differential transcriptional responses to exposure to microplastics

In this study, a total of 74.73 GB of clean data were obtained from 10 high-quality *J. squamata* transcriptome libraries, and the clean data of each sample reached >6.27 GB; 517,838,734 high-quality clean reads were obtained from 535,002,732 double-ended raw reads, of which the Q30 bases accounting for 91.33 % and Q20 bases accounting for 96.78 %, and the GC content was 40.73 % (Supplementary Table S1). A total of 79,874 single genes were assembled from clean reads after removing ambiguous reads, connectors, and low-quality sequences with an average length of 857.30 bp, the length of N50 was 1814 bp (Supplementary Table S2). Analysis of the expression levels of 79,874 single

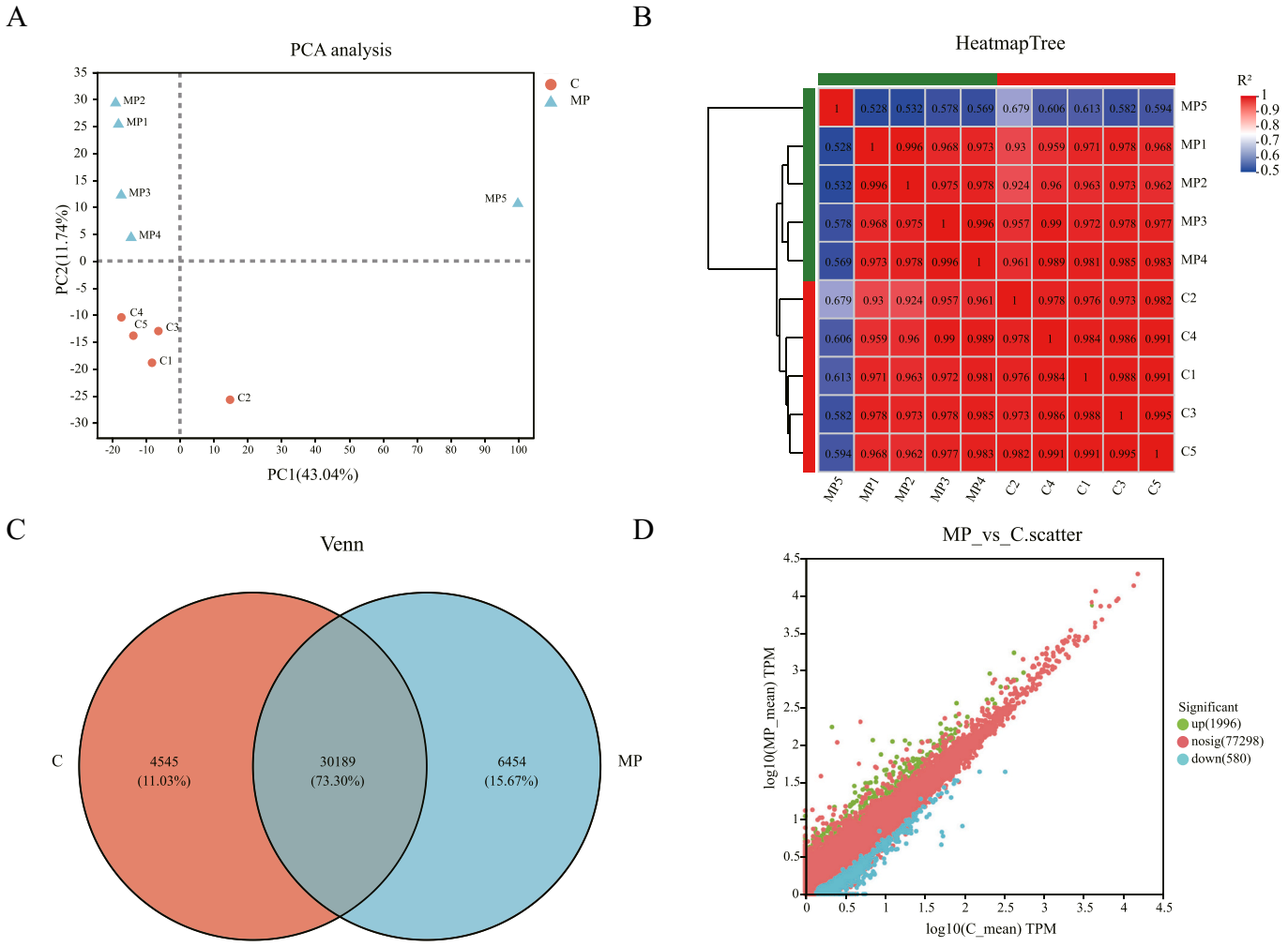

Fig. 1. Comparison between different groups. (A) PCA; (B) Heat map; (C) Venn map of differentially expressed genes; (D) Differentially expressed coral genes (green = upregulated, red = nosig, blue = downregulated). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

genes were analyzed using the RSEM software tool (Supplementary Table S3). The differences between different groups and different samples are analyzed, including principal component analysis and heatmap analysis. The results show that the difference between the experimental group and the control group is obvious, accounting for 43.98 % and 8.53 % of the total variance respectively (Fig. 1A). Thermogram analysis showed that the expression patterns of the five replicates in each group were highly consistent, we also found that the differences between coral samples increased after exposure to microplastics, and there were differences between the experimental group and the control group (Fig. 1B), indicating that our sequencing results were highly reliable. Fourthly, the Venn diagram was used to analyze the number of shared and unique genes between the two groups in Fig. 1C. Fifth, to compare the differential gene expression in coral after exposure to microplastics, we compared the differentially expressed genes in two groups of coral samples. We identified 2576 differentially expressed genes, including

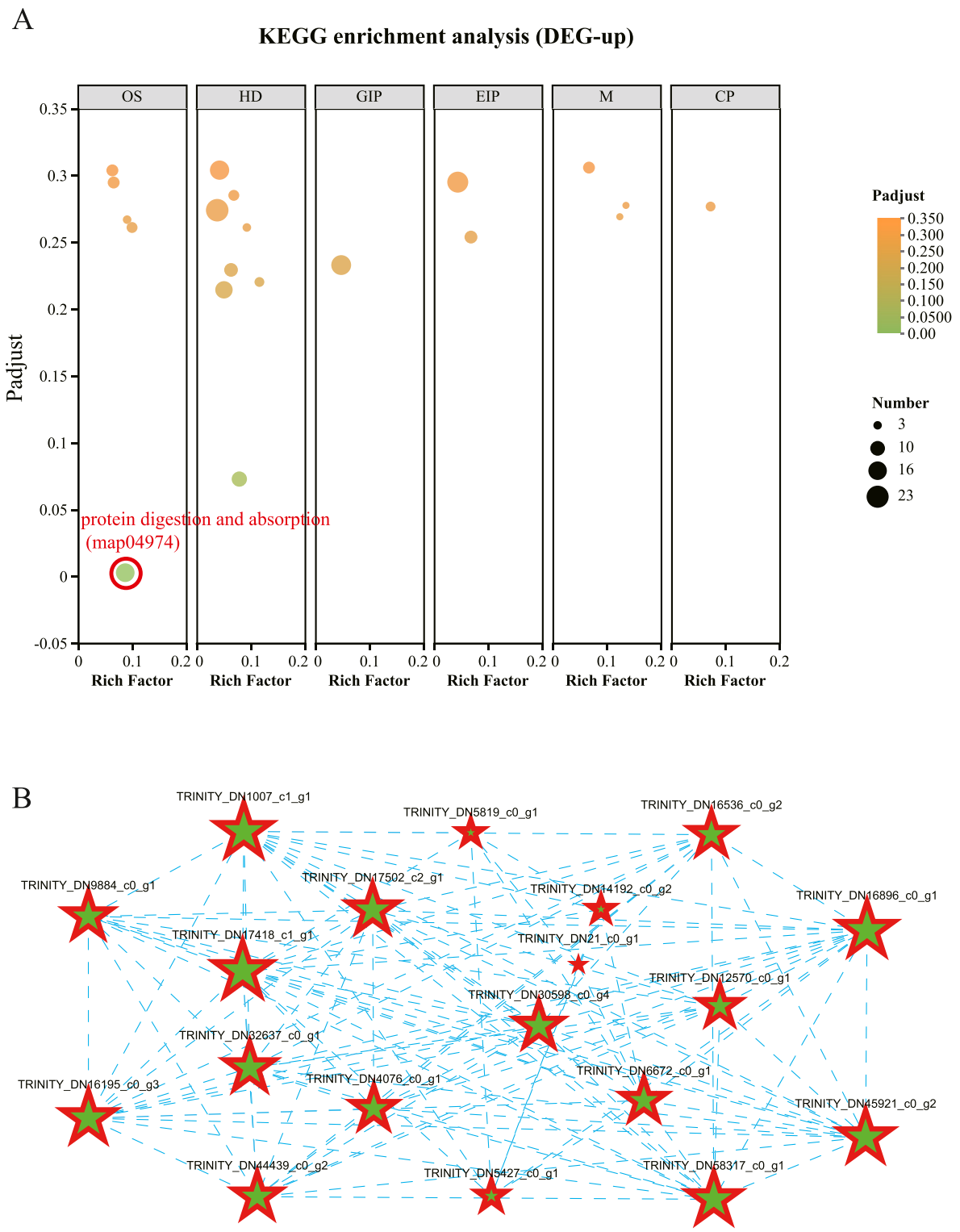

Fig. 2. KEGG pathway enrichment analyses of upregulated coral genes (A) and (B) association analysis of targeted genes.

1996 upregulated genes and 580 downregulated genes (FC ≥ 2, $p < 0.05$) in the experimental group (Fig. 1D, Supplementary Table S4). To further understand the transcriptional reaction process, all DEGs identified in the previous step were subjected to KEGG enrichment analysis (adjusted p-values < 0.05). We found that the significantly upregulated coral genes were significantly enriched in one KEGG pathway protein digestion and absorption (map04974) (Fig. 2A, Supplementary Table S5), indicated the protein digestion and absorption pathways of the coral hosts increased after microplastic exposure. No pathways related to oxidative stress and immunity were found. The significantly downregulated genes do not have significantly enriched pathways. The association analysis of target genes revealed a strong correlation between genes, indicating their coordinated function in response to microplastic exposure stress (Fig. 2B, Supplementary Table S6). The strongest correlation among them is RINITY_DN17418_c1_g1 chymotrypsin-like protease CTRL-1. Key genes also includes TRINITY_DN16896_c0_g1, TRINITY_DN1007_c1_g1, TRINITY_DN45921_c0_g2, TRINITY_DN14192_c0_g2 monocarboxylate transporter 4-like, TRINITY_DN4076_c0_g1 cochlin-like isoform X2, TRINITY_DN21_c0_g1 monocarboxylate transporter 10-like, TRINITY_DN12570_c0_g1 mucin-1-like, TRINITY_DN6672_c0_g1 MAM and LDL-receptor class A domain-containing protein 1-like, TRINITY_DN44439_c0_g2 zinc metalloproteinase nas-30-like, TRINITY_DN58317_c0_g1 collagen alpha-2 (VI) chain-like, TRINITY_DN9884_c0_g1 uncharacterized protein LOC114532837, TRINITY_DN5819_c0_g1 monocarboxylate transporter 12-like, TRINITY_DN16195_c0_g3 uncharacterized protein LOC114533247, TRINITY_DN45921_c0_g2 hornerin-like isoform X2, TRINITY_DN30598_c0_g4 uncharacterized protein LOC114533247, TRINITY_DN17502_c2_g1 excitatory amino acid transporter 1-like, TRINITY_DN1007_c1_g1 zinc metalloproteinase nas-13-like, partial, TRINITY_DN16896_c0_g1 uncharacterized protein LOC114533086 isoform X3, TRINITY_DN32637_c0_g1 uncharacterized protein LOC114533247, TRINITY_DN16536_c0_g2 uncharacterized protein LOC114533086 isoform X3, TRINITY_DN5427_c0_g1 trypsin-4-like.

3.2. Bacterial community structure

A total of 3618 ASVs (Supplementary Tables S7 and S8) were identified from 1,079,379 processed bacterial sequences after secondary sampling at the same sequencing depth. The average sequence length was 418 bp (Supplementary Table S7). Good's coverage accounted for ≥99.96 % of the diversity with near saturation of sparse curves, indicating that the sequencing results represented the true situation of symbiotic bacteria in the coral samples (Supplementary Table S9). The average number of ASV in the experimental and control groups were 239 ± 116 and 567 ± 104, respectively. The Chao and ACE indices showed that the richness of ASVs was significantly different between the two groups. The Shannon and Simpson indices showed that the α-diversity of *J. squamata* samples increased significantly after microplastic exposure ($p < 0.05$) (Table 1).

The results of PCoA (unweighted_unifrac, ANOSIM, $R = 0.9758$, $p = 0.001$; Fig. 3A) and NMDS (unweighted_unifrac, ANOSIM, stress = 0.074, R = 0.9758, $p = 0.001$; Fig. 3B) analyses revealed significant differences in the coral symbiotic bacterial community structure between the two groups. Fig. 4 presents the circos diagram to illustrate the species composition of genera identified in the different samples. *Delftia*, *BD1-7_clade*, *Rhodococcus*, *Endozoicomonas*, *Ruegeria*, and *Pseudomonas* were the most abundant genera found in the coral samples. In addition, we analyzed the top 15 differential microbial groups (Wilcoxon rank-sum test, $p < 0.05$; Fig. 5A). *Delftia*, *Pseudomonas*, *norank_f_Terasakillaceae*, and *Achromobacter* were abundant in the control group whereas *BD1-7_clade*, *Endozoicomonas*, *Ruegeria*, *unclassified_f_Rhodobacteraceae*, *norank_f_Methyloligellaceae*, *unclassified_k_norank_d_Bacteria*, *unclassified_o_Thermosynecochocales*, *Spirochaeta_2*, *norank_f_Sapopiraceae*, *Neptuniibacter*, and *unclassified_f_Sphingomonadaceae* were relatively more abundant in the corals exposed to microplastics.

To further explore the role of symbiotic bacteria in the response to microplastic exposure, we analyzed the differences in the core microbiome. The relative abundance of the core microbiome in the experimental and control groups was 28.18 % and 47.37 %, respectively (Fig. 5B, Supplementary Table S10). Notably, we found that the dominant core ASVs in the two groups were the same, although their relative abundances were significantly different. Among the core microbiomes, *Rhodococcus* (ASV1), *BD1-7_clade* (ASV2), and *Endozoicomonas* (ASV4) were dominant, accounting for 45.96 %, 20.39 %, and 16.88 %, respectively, of the core microbial community in the control group, and 25.27 %, 27.52 %, and 26 %, respectively, after microplastic exposure (Fig. 5B).

Table 1
A 16S rRNA gene sequencing data.

Estimators	C-mean	C-sd	MP-mean	MP-sd	Pvalue (C-MP)	Qvalue (C-MP)
ACE	240.07	117.03	572.88	103.23	0.0003	0.0005
Chao	239.48	116.81	570.51	103.96	0.0003	0.0005
Sobs	238.90	116.26	566.70	103.94	0.0003	0.0005
Shannon	2.25	0.82	3.90	0.46	0.0008	0.0009
Coverage	1.00	0.00	1.00	0.00	0.0011	0.0011
Simpson	0.31	0.14	0.07	0.02	0.0003	0.0005

4. Discussion

To reveal the impact of microplastic exposure on Octocorallia corals, we analyzed the transcriptional response of coral hosts after microplastic exposure and the impact on the diversity and community structure of symbiotic bacteria in willow corals. Function enrichment analysis of the DEGs showed that the protein digestion and absorption pathways of the coral hosts increased. In addition, the microbial α-diversity of *J. squamata* samples increased significantly, and the community structure of the dominant bacteria and core microbiome were reorganized after microplastic exposure.

4.1. Microplastic stress interfere the nutrient digestion and absorption processes of corals

To reveal the impact of microplastic exposure on coral hosts, we analyzed the differential transcriptional responses of 10 *J. squamata* samples. Transcriptome analysis revealed that the differences between coral samples increased after exposure to microplastics, and there were differences between the experimental group and the control group. Based on KEGG functional enrichment analysis of coral differentially expressed genes after exposure to microplastics, we found that microplastic stress increased protein digestion and absorption by the coral hosts. The strongest correlation gene is chymotrypsin-like protease CTRL-1. No pathways related to oxidative stress and immunity were found.

The protein digestion and absorption (map04974) is a crucial step in obtaining necessary nutrients, ensuring the normal functioning and healthy development of animals. It is important to respond to changes in nutrient intake, and is a dietary component essential for nutritional homeostasis in animals. CTRL-1 is an important member of the peptidase S1 family, and as a serine protease, it catalyzes the hydrolysis of peptide bonds. Specifically, the biological function of CTRL-1 protein may involve the degradation of intracellular and extracellular proteins and the regulation of signaling pathways, which are crucial for maintaining normal cellular function and responding to external stimuli (Németh et al., 2023). The screening of protein digestion and absorption pathways based on transcriptome results suggests that exposure to microplastics may interfere with nutrient digestion and absorption in *J. squamata*. This may be related to the unique dietary habits of corals. *J. squamata* have a broad and heterogeneous diet that ranges from 3.8 μm (nanoeukaryotes) to 700 μm (copepods) (Marta et al., 1999). Based

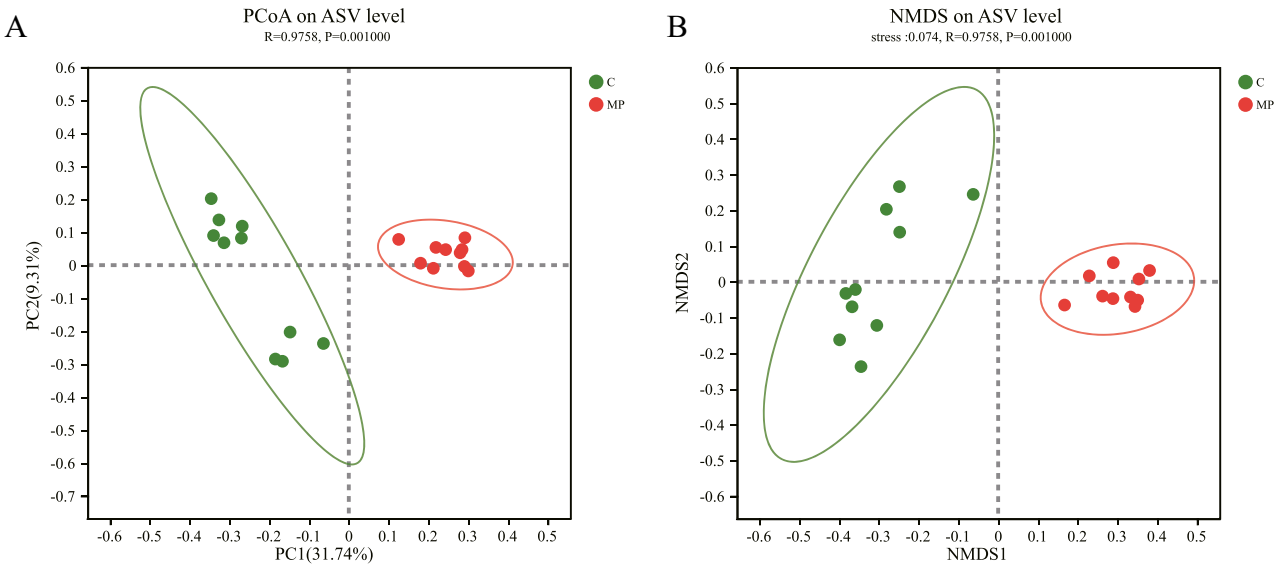

Fig. 3. Principal Coordinates Analysis (PCoA) and NMDS representing differences in community structure at the asv level.

on our transcriptome analysis, we did not find the existence of symbiotic Symbiodiniaceae; therefore, *J. squamata* in this study was an asymbiotic gorgonian. Heterotrophic feeding is a more important nutrient input method for *J. squamata*, which leads to easier intake of microplastics. *J. squamata* mistake microplastics for prey and feed on them, similar to their consumption of plankton and *Artemia nauplii* (Hall et al., 2015), and after eating microplastics by mistake, they refuse to provide brine shrimp eggs with the same diameter, which will inhibit food intake (Rotjan et al., 2019). More importantly, it is difficult to be completely excreted by organisms because of the particularity of microplastics, so once ingested by organisms, most of it will exist in the intestine, which will cause intestinal dysfunction (Hall et al., 2015). Similar to our results, tourists analyzed the concentration of microplastics in the wild capture community in Rhode Island, and the results showed that there were >100 microplastics particles per polyp (Rotjan et al., 2019). Polystyrene microparticle incorporation in the gastrovascular cavity of scleractinian coral *Porites porites* was observed in all of the treatments (1, 10, 100, 1000 mg L^{-1}) (Grillo et al., 2021). Microplastics damage the small intestinal structure and thus impair digestion and nutrient absorption functions, which was found to disrupt the growth of *animal* (Mbugani et al., 2022). Therefore, we speculate that microplastic stress can lead to coral ingestion of microplastics and interfere with nutrient digestion and absorption of *J. squamata* (Hall et al., 2015).

In addition, we identified only a small number of antioxidant- and immune-related genes among the DEGs, and no pathways related to oxidative stress and immunity were found. This means that the exposure of microplastics in this study did not have a serious impact on *J. squamata*. Different from the present study, previous research has suggested that the negative effects of microplastics on coral mainly include affecting coral nutrition and development, causing coral to refuse to eat, reducing metabolic rate and photosynthetic efficiency, affecting calcification process and even leading to tissue necrosis (Rahman et al., 2023), and microplastics may interfere with the normal function of coral reef ecosystem by affecting the nutrient cycle and changing the food web (Zhang et al., 2023). We speculate that the reason for this difference may be due to differences in coral species, microplastic concentration, and stress time. Similar results were found for *P. porites*, which showed no evident toxic effects when exposed to 1, 10, 100, or 1000 mg L^{-1} (~9.2×10^7, 9.2×10^8, 9.2×10^9, 9.2×10^{10}) of polystyrene microplastics over a period of 96 h (Grillo et al., 2021). *P. damicornis* significant increase in catalase enzymatic activity was followed by a decrease in enzymatic activity, returning to control levels after 24 h, suggesting the coral's ability to regulate the antioxidant response after longer 50 mg L^{-1} exposure times (Tang et al., 2018). Additionally, Tang et al. (2018) did not find a significant alteration in the melanin synthesis pathway, similar to our results, which may indicate that although corals may activate the antioxidant defense system in response to an acute MP exposure, the immune system may not play a role in the coral's response mechanism.

Based on the above results, we speculate that the exposure of microplastics in this study did not have a serious impact on *J. squamata*, the main adverse effects is on their nutrient metabolism and absorption, which is mainly due to the characteristics of microplastics and the particularity of coral predation.

4.2. *Increased α-diversity and community structure recombination of symbiotic bacteria in J. squamata in response to microplastic exposure*

To reveal the influence of microplastics on the symbiotic bacterial community in coral and explore the molecular mechanisms by which symbiotic bacteria participate in the response to microplastic exposure, we analyzed differences in α-diversity, community structure, and the core microbiome of *J. squamata* samples exposed to microplastics. The results showed that the microbial *α*-diversity of *J. squamata* samples increased significantly after microplastic exposure, and the community structures of the dominant bacteria and core microbiome were reorganized.

This result further confirmed the plasticity of symbiotic bacteria in coral. Previous studies have reported a similar phenomenon. Different hosts (Yu et al., 2020a, 2020b), regions (Chen et al., 2021), seasons (Yu et al., 2021a, 2021b), and environmental pressures (Yu et al., 2021a, 2021b) can accompany the recombination of symbiotic bacteria. Compared to their coral hosts, symbiotic bacteria have a faster ability to change, which may help long-lived corals adapt to climate change (Ziegler et al., 2017). Therefore, adaptive recombination of microorganisms is a possible mechanism of coral symbiotic function to resist the influence of environmental changes. According to the hypothesis of coral probiotics, coral symbiotic microorganisms are disturbed by the external environment, and there is a certain correlation between them; therefore, the most favorable symbiotic bacteria can be selected for coral symbiotic function. In addition, we found that the α-diversity of unbleached corals was significantly higher than that of bleached corals (Yu et al., 2021a, 2021b). Other studies have also found high α-diversity of coral symbiotic bacteria under various unfavorable conditions such as

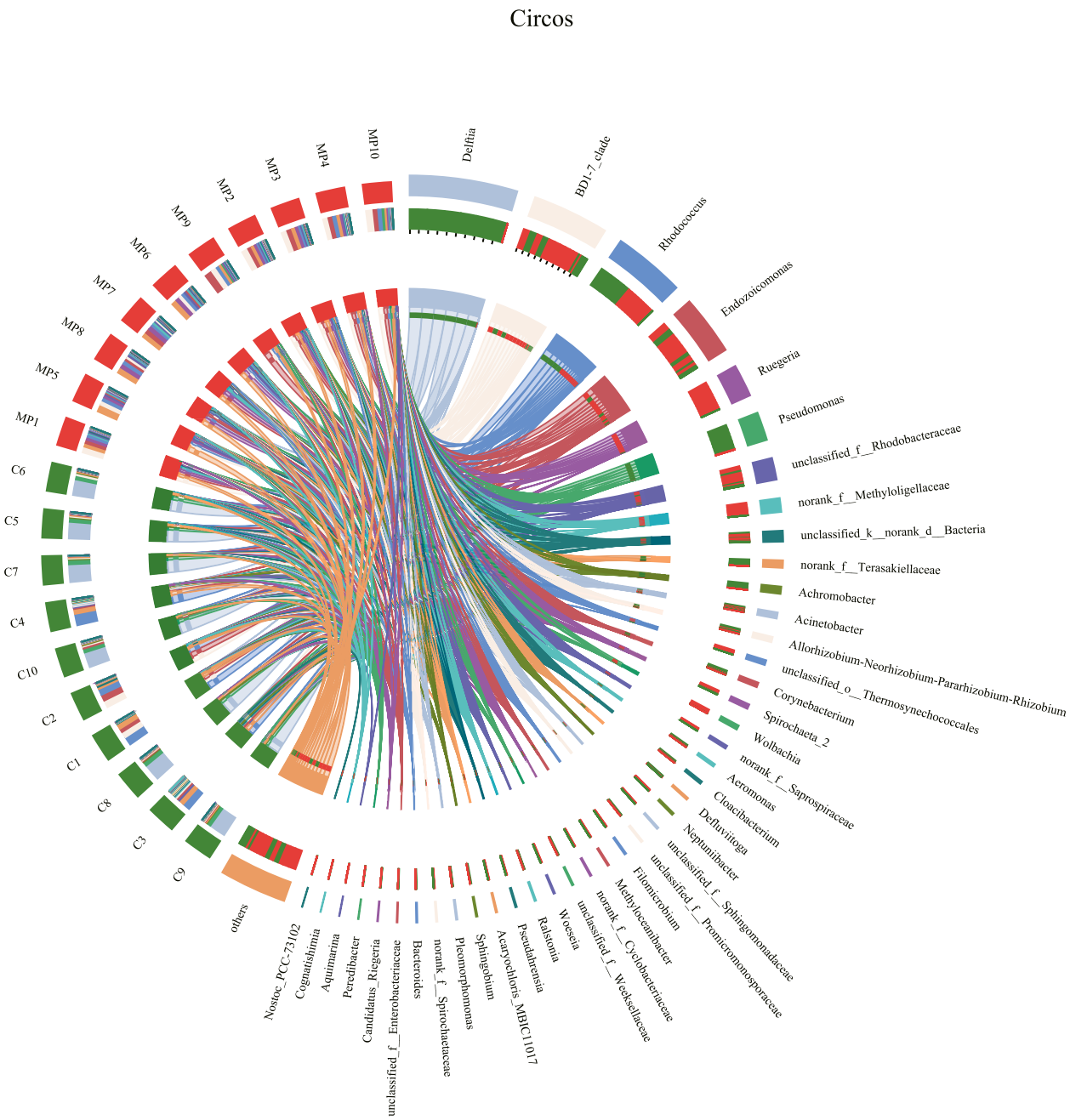

Fig. 4. Microbial community distribution at the genus level of each sample.

acidification and eutrophication stress, overfishing, and coral–algae competition (Prada et al., 2016). High abundance and α-diversity provide more opportunity for the host microbiome to transform its main symbiotic bacteria into species that perform better under specific conditions. Indeed, higher α-diversity helps coral holobionts resist infection, absorb nutrients, and maintain the aggregation of healthy microorganisms (Pollock et al., 2019). This suggests that higher α-diversity is helpful in maintaining the physiological function of the whole organism, which may be related to higher stress tolerance.

Moreover, we observed a restructuring of the symbiotic bacterial community, which may be a response of coral holobionts to external stress (Yu et al., 2021a; Yu et al., 2021b). *BD1-7_clade*, a member of Spongiibacteraceae, is a common group of coral symbiotic bacteria (Doering et al., 2021) and is relatively abundant in both albino and non-albino *Acropora muricata* samples. Notably, members of Spongiibacteraceae have been found to play an increasingly important role in the global ocean carbon cycle and energy metabolism (Kolber et al., 2001). *Endozoicomonas* is another common and rich bacterial partner of corals and is considered to play an important role in the entire biological nutrient cycle, contributing to coral health, and environmental adaptation (Pogoreutz et al., 2022). Although *Endozoicomonas* is a recognized core coral bacterial group highly related to coral bleaching, the abundance of endophytic bacteria was found to decrease during coral bleaching and increase after coral restoration. However, *Endozoicomonas* can be independent of Symbiodiniaceae in coral organisms (Shiu et al., 2020). *Endozoicomonas* can form the cell-associated microbial aggregates of *Pocillopora acutas*, which may be beneficial not only to their coral host, but also to the Simkaniaceae present in adjacent cell-associated microbial aggregates (Maire et al., 2023). Notably, we found that the key bacteria mentioned above are all coral related probiotics. This means that the exposure of microplastics leads to an increase in the relative abundance of coral related probiotics, which may

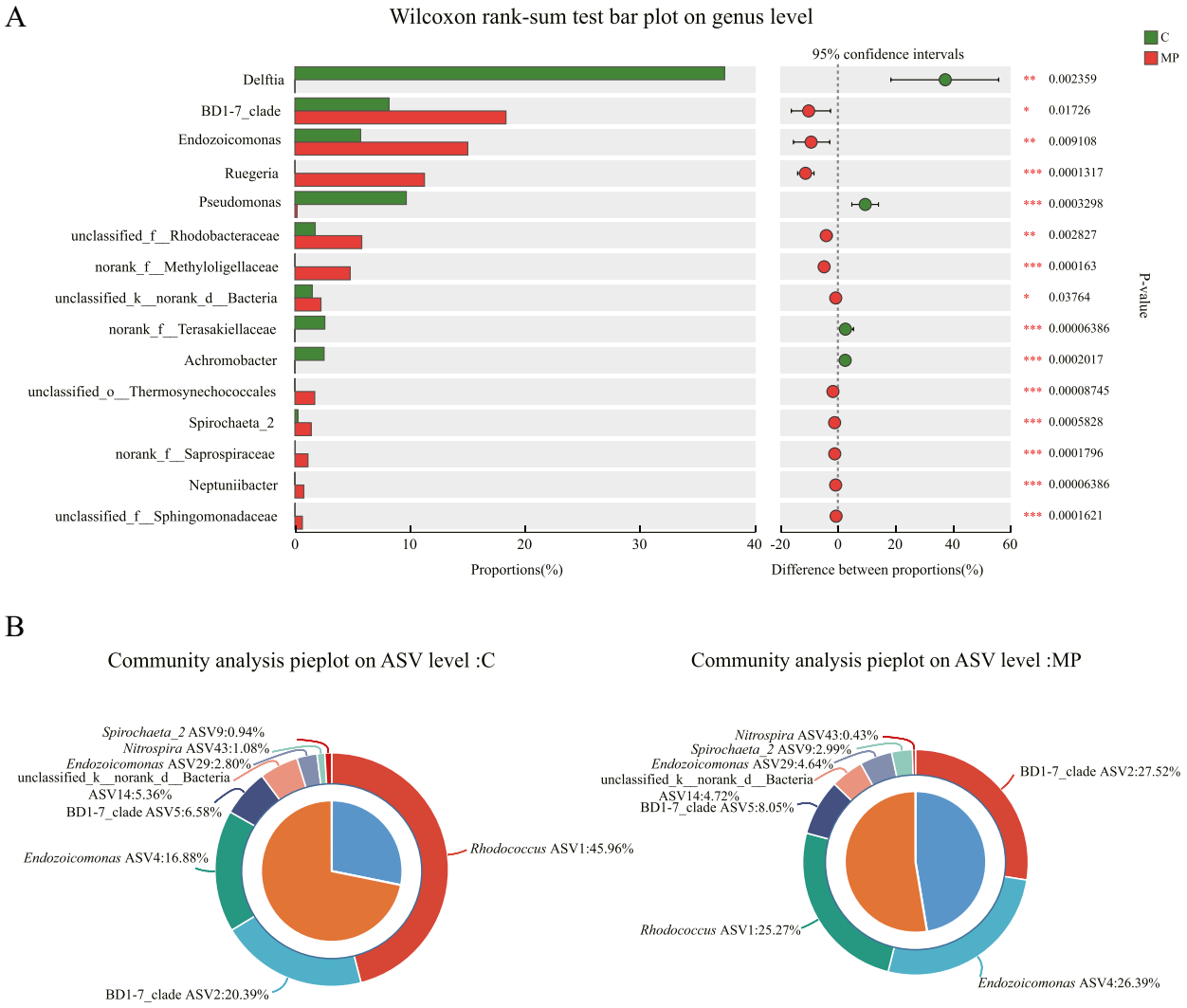

Fig. 5. Microbial communities with statistically significant differences. (A) Differences in relative abundance of species between different groups; (B) The relative abundance and composition of the core bacterial microbiome. Internal pie charts demonstrating the proportion of core microbiome in coral bacterial community composition (blue: core ASV, Orange: non-core ASVs). The outer pie chart illustrates the core bacterial microbiome composition in the coral. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

facilitate adaptation of coral to microplastic exposure. This is also consistent with our transcriptome sequencing results. In this study, microplastic exposure may not cause serious damage to corals.

Overall, we conclude that the symbiotic bacteria of *J. squamata* exhibit a certain response after microplastic exposure, which may represent an an adaptation mechanism of corals. High α-diversity and recombination of the symbiotic bacterial community structure, especially the increase in the relative abundance of probiotics, would help to maintain the physiological function of coral holobionts function under the exposure of microplastic exposure.

5. Conclusion

In the increasingly high-concentration microplastic environment worldwide, it is particularly important to explore the impact of environmental exposure to microplastics on gorgonian corals. To reveal the impact of microplastic exposure on the Octocorallia corals, we selected *J. squamata* as the research object and adopted a comprehensive method to explore its stress response to microplastic pollution. We compared the reactions of coral hosts and symbiotic bacteria. The results revealed that: (i) the exposure of microplastics in this study did not have a serious impact on *J. squamata*, significantly upregulated genes in the *J. squamata* after microplastic exposure were primarily associated with protein digestion and absorption pathways, (ii) the microbial α-diversity in *J. squamata* samples increased significantly, and (iii) the community structures of the dominant bacteria and core microbiome, especially the increase of relative abundance of probiotics, reorganized after microplastic exposure. The results suggest that the exposure of microplastics in this study did not have a serious impact on *J. squamata*, the main adverse effects is on their nutrient metabolism and absorption, which is mainly due to the characteristics of microplastics and the particularity of coral predation. The symbiotic bacteria of *J. squamata* have a certain response after exposure to microplastics, which may also be an adaptation mechanism of corals. High α-diversity and the recombination of symbiotic bacterial community structure, especially the increase of relative abundance of probiotics, help to maintain the physiological function of coral symbiotic function under the exposure of microplastics. This study aimed to not only lay a foundation for further clarification of the impact of marine microplastic pollution on corals, but also generate a new understanding of what harm microplastics bring to marine life and provide a theoretical basis for microplastic-related ecotoxicology and coral reef ecological restoration.

6. Limitations of the study

This study has revealed the impact of microplastic exposure on *J. squamata*, providing new insights for coral protection against the background of increased microplastics pollution. However, some limitations should be noted. First, we only selected one type of microplastic particle and set only one concentration, which may lead to limitations in the results, and due to the interspecies differences in coral response to microplastic exposure (Reichert et al., 2019), source and characteristics of microplastics vary from different coral reef regions (Huang et al., 2021), stress conditions and coral species should be added in future research. Secondly, we did not detect the effective concentration because adding microplastics would result in a decrease in effective concentration due to sedimentation and adsorption processes (Rocha et al., 2020), which may have contributed to the fact that microplastic exposure in this study did not have a serious impact on *J. squamata*. Third, no detection the uptake of microplastics by corals. In order to have this comparison, the current study would have made efforts to measure the microplastic intake. The impact of microplastics on corals is mainly on their nutrient metabolism and absorption, microplastic intake should be measured in future research to validate our findings and address the limitations of our current methods.

Funding sources

This work was supported by the National Natural Science Foundation of China (Nos. 42090041 and 42030502), the Autonomous Project of Guangxi Laboratory on the Study of Coral Reefs in the South China Sea (No. GXLSCRSCS2022101).

CRediT authorship contribution statement

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

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117331.

Data availability

Sequencing raw reads in this study were deposited into the NCBI sequence reads archive (SRA) database: PRJNA986099 and PRJNA986103.

References

Allen, A.S., Seymour, A.C., Rittschof, D., 2017. Chemoreception drives plastic consumption in a hard coral. Mar. Pollut. Bull. 124 (1), 198–205.
Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., et al., 2019. Author correction: reproducible, interactive, scalable and extensible microbiome data science using qiime 2. Nat. Biotechnol. 37 (9), 1091.
Brener-Raffalli, K., Vidal-Dupiol, J., Adjeroud, M., et al., 2018. Gene expression plasticity and frontloading promote thermotolerance in Pocilloporid corals. bioRxiv 398602. https://doi.org/10.1101/398602.
Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. Blast+: architecture and applications. BMC Bioinformatics 10, 421.
Chen, B., Yu, K., Liao, Z., Yu, X., Qin, Z., Liang, J., Wang, G., Wu, Q., Jiang, L., 2021. Microbiome community and complexity indicate environmental gradient acclimatisation and potential microbial interaction of endemic coral holobionts in the South China Sea. Sci. Total Environ. 765, 142690.
Ding, J., Jiang, F., Li, J., Wang, Z., Sun, C., Wang, Z., Fu, L., Ding, N.X., He, C., 2019. Microplastics in the coral reef systems from xisha islands of South China Sea. Environ. Sci. Technol. 53 (14), 8036–8046.
Doering, T., Wall, M., Putchim, L., Rattanawongwan, T., Schroeder, R., Hentschel, U., Roik, A., 2021. Towards enhancing coral heat tolerance: a "microbiome transplantation" treatment using inoculations of homogenized coral tissues. Microbiome 9 (1), 102.
Grillo, J.F., Sabino, M.A., Ramos, R., 2021. Short-term ingestion and tissue incorporation of polystyrene microplastic in the scleractinian coral porites porites. Reg. Stud. Mar. Sci. 43, 101697.
Hall, N.M., Berry, K.L.E., Rintoul, L., Hoogenboom, M.O., 2015. Microplastic ingestion by scleractinian corals. Mar. Biol. 162 (3), 725–732.
Hankins, C., Moso, E., Lasseigne, D., 2021. Microplastics impair growth in two Atlantic scleractinian coral species, pseudodiploria clivosa and acropora cervicornis. Environ. Pollut. 275, 116649.
Hernandez-Agreda, A., Gates, R.D., Ainsworth, T.D., 2017. Defining the core microbiome in corals' microbial soup. Trends Microbiol. 25 (2), 125–140.
Hierl, F., Wu, H.C., Westphal, H., 2021. Scleractinian corals incorporate microplastic particles: identification from a laboratory study. Environ. Sci. Pollut. Res. 28 (28), 37882–37893.
Huang, Y., Yan, M., Xu, K., Nie, H., Gong, H., Wang, J., 2019. Distribution characteristics of microplastics in zhubi reef from South China Sea. Environ. Pollut. 255, 113133.
Huang, W., Chen, M., Song, B., Deng, J., Shen, M., Chen, Q., Zeng, G., Liang, J., 2021. Microplastics in the coral reefs and their potential impacts on corals: a mini-review. Sci. Total Environ. 762, 143112.
Imhof, H.K., Sigl, R., Brauer, E., Feyl, S., Giesemann, P., Klink, S., Leupolz, K., Löder, M. G.J., Löschel, L.A., Missun, J., et al., 2017. Spatial and temporal variation of macro-, meso- and microplastic abundance on a remote coral island of the Maldives, Indian Ocean. Mar. Pollut. Bull. 116 (1–2), 340–347.
Jensen, L.H., Motti, C.A., Garm, A.L., Tonin, H., Kroon, F.J., 2019. Sources, distribution and fate of microfibres on the Great Barrier Reef, Australia. Sci. Rep. 9 (1), 9021.
Khan, Saimah, Ali, Mumtaj Zeba, Rahman, Khan Abdul, Qablan, Alkahtani Meshel, Enis, Aleya, Maxime, Louzon, Lotfi, Aleya, 2024. Reviewing the role of microplastics as carriers for microorganisms in absorbing toxic trace elements. Environ. Sci. Pollut. Res. 31 (34), 46806–46819.
Kolber, Z.S., Gerald, F., Lang, A.S., Beatty, J.T., Blankenship, R.E., Vandover, C.L., Vetriani, C., Koblizek, M., Rathgeber, C., Falkowski, P.G., 2001. Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science 292 (5526), 2492–2495.
Lanctôt, C.M., Bednarz, V.N., Melvin, S., Jacob, H., Oberhaensli, F., Swarzenski, P.W., Ferrier-Pagès, C., Carroll, A.R., Metian, M., 2020. Physiological stress response of the scleractinian coral stylophora pistillata exposed to polyethylene microplastics. Environ. Pollut. 263, 114559.
Li, B., Dewey, C.N., 2011. Rsem: accurate transcript quantification from rna-seq data with or without a reference genome. BMC Bioinformatics 12, 323.
Liao, B., Wang, J., Xiao, B., Yang, X., Xie, Z., Li, D., Li, C., 2021. Effects of acute microplastic exposure on physiological parameters in tubastrea aurea corals. Mar. Pollut. Bull. 165, 112173.
Lim, H.S., Fraser, A., Knights, A.M., 2020. Spatial arrangement of biogenic reefs alters boundary layer characteristics to increase risk of microplastic bioaccumulation. Environ. Res. Lett. 15 (6), 64024.
Maire, J., Tandon, K., Collingro, A., van de Meene, A., Damjanovic, K., Gotze, C.R., Stephenson, S., Philip, G.K., Horn, M., Cantin, N.E., et al., 2023. Colocalization and potential interactions of endozoicomonas and chlamydiae in microbial aggregates of the coral pocillopora acuta. Sci. Adv. 9 (20), g773.
Marta, R., Rafel, C., Josep-Maria, G., 1999. Heterogeneous feeding in benthic suspension feeders: the natural diet and grazing rate of the temperate gorgonian paramuricea clavata (cnidaria: octocorallia) over a year cycle. Mar. Ecol.-Prog. Ser. 183, 125–137.
Martin, C., Corona, E., Mahadik, G.A., Duarte, C.M., 2019. Adhesion to coral surface as a potential sink for marine microplastics. Environ. Pollut. 255, 113281.
Mbugani, J.J., Machiwa, J.F., Shilla, D.A., Joseph, D., Kimaro, W.H., Khan, F.R., 2022. Microplastics 334–345.
Mendrik, F.M., Henry, T.B., Burdett, H., Hackney, C.R., Waller, C., Parsons, D.R., Hennige, S.J., 2021. Species-specific impact of microplastics on coral physiology. Environ. Pollut. 269, 116238.
Németh, B., Nagy, Z., Kiss, B., Gellen, G., Schlosser, G., Demcsak, A., Geisz, A., Hegyi, E., Sahin-Tóth, M., Pál, G., 2023. Substrate specificity of human chymotrypsin-like protease (CTRL) characterized by phage display-selected small-protein inhibitors. Pancreatology 23.
Pogoreutz, C., Oakley, C.A., Rädecker, N., Cárdenas, A., Perna, G., Xiang, N., Peng, L., Davy, S.K., Ngugi, D.K., Voolstra, C.R., 2022. Coral holobiont cues prime endozoicomonas for a symbiotic lifestyle. ISME J. 16 (8), 1883–1895.
Pollock, F.J., Lamb, J.B., van de Water, J.A.J.M., Smith, H.A., Schaffelke, B., Willis, B.L., Bourne, D.G., 2019. Reduced diversity and stability of coral-associated bacterial

communities and suppressed immune function precedes disease onset in corals. R. Soc. Open Sci. 6 (6), 190355.
Prada, C., Hanna, B., Budd, A.F., Woodley, C.M., Schmutz, J., Grimwood, J., Iglesias-Prieto, R., Pandolfi, J.M., Levitan, D., Johnson, K.G., et al., 2016. Empty niches after extinctions increase population sizes of modern corals. Curr. Biol. 26 (23), 3190–3194.
Rahman, M.N., Shozib, S.H., Akter, M.Y., Islam, A.R.M.T., Islam, M.S., Sohel, M.S., Kamaraj, C., Rakib, M.R.J., Idris, A.M., Sarker, A., et al., 2023. Microplastic as an invisible threat to the coral reefs: sources, toxicity mechanisms, policy intervention, and the way forward. J. Hazard. Mater. 454, 131522.
Reichert, J., Schellenberg, J., Schubert, P., Wilke, T., 2018. Responses of reef building corals to microplastic exposure. Environ. Pollut. 237, 955–960.
Reichert, J., Arnold, A.L., Hoogenboom, M.O., Schubert, P., Wilke, T., 2019. Impacts of microplastics on growth and health of hermatypic corals are species-specific. Environ. Pollut. 254, 113074.
Reichert, J., Arnold, A.L., Hammer, N., Miller, I.B., Rades, M., Schubert, P., Ziegler, M., Wilke, T., 2021. Reef-building corals act as long-term sink for microplastic. Glob. Change Biol. 28, 33–45.
Rocha, R.J.M., Rodrigues, A.C.M., Campos, D., Cícero, L.H., Costa, A.P.L., Silva, D.A.M., Oliveira, M., Soares, A.M.V.M., Patrício Silva, A.L., 2020. Do microplastics affect the zoanthid zoanthus sociatus? Sci. Total Environ. 713, 136659.
Rotjan, R.D., Sharp, K.H., Gauthier, A.E., Yelton, R., Lopez, E.M.B., Carilli, J., Kagan, J. C., Urban-Rich, J., 2019. Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, astrangia poculata. Proc. R. Soc. B Biol. Sci. 286 (1905), 20190726.
Saliu, F., Montano, S., Garavaglia, M.G., Lasagni, M., Seveso, D., Galli, P., 2018. Microplastic and charred microplastic in the Faafu Atoll, Maldives. Mar. Pollut. Bull. 136, 464–471.
Santana, M.F.M., Dawson, A.L., Motti, C.A., van Herwerden, L., Lefevre, C., Kroon, F.J., 2021. Ingestion and depuration of microplastics by a planktivorous coral reef fish, pomacentrus amboinensis. Front. Environ. Sci. 9, 79.
Shiu, J., Yu, S., Fong, C., Ding, J., Tan, C., Fan, T., Lu, C., Tang, S., 2020. Shifting in the dominant bacterial group endozoicomonas is independent of the dissociation with coral symbiont algae. Front. Microbiol. 11.
Soares, M.D.O., Matos, E., Lucas, C., Rizzo, L., Allcock, L., Rossi, S., 2020. Microplastics in corals: an emergent threat. Mar. Pollut. Bull. 161, 111810.
Tan, F., Yang, H., Xu, X., Fang, Z., Xu, H., Shi, Q., Zhang, X., Wang, G., Lin, L., Zhou, S., et al., 2020. Microplastic pollution around remote uninhabited coral reefs of nansha islands, South China Sea. Sci. Total Environ. 725, 138383.
Tang, J., Ni, X., Zhou, Z., Wang, L., Lin, S., 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral pocillopora damicornis. Environ. Pollut. 243, 66–74.
Tang, J., Wu, Z., Wan, L., Cai, W., Chen, S., Wang, X., Luo, J., Zhou, Z., Zhao, J., Lin, S., 2021. Differential enrichment and physiological impacts of ingested microplastics in scleractinian corals in situ. J. Hazard. Mater. 404, 124205.
Utami, D.A., Reuning, L., Konechnaya, O., Schwarzbauer, J., 2021. Microplastics as a sedimentary component in reef systems: a case study from the java sea. Sedimentology 68 (6), 2270–2292.
Vidyasakar, A., Neelavannan, K., Krishnakumar, S., Prabaharan, G., Sathiyabama Alias Priyanka, T., Magesh, N.S., Godson, P.S., Srinivasalu, S., 2018. Macrodebris and microplastic distribution in the beaches of Rameswaram Coral Island, Gulf of Mannar, southeast coast of India: a first report. Mar. Pollut. Bull. 137, 610–616.
Yu, X., Yu, K., Huang, W., Liang, J., Qin, Z., Chen, B., Yao, Q., Liao, Z., 2020a. Thermal acclimation increases heat tolerance of the scleractinian coral acropora pruinosa. Sci. Total Environ. 733, 139319.
Yu, X., Yu, K., Liao, Z., Liang, J., Deng, C., Huang, W., Huang, Y., 2020b. Potential molecular traits underlying environmental tolerance of pavona decussata and acropora pruinosa in weizhou island, northern South China Sea. Mar. Pollut. Bull. 156, 111199.
Yu, X., Yu, K., Chen, B., Liao, Z., Liang, J., Yao, Q., Qin, Z., Wang, H., Yu, J., 2021a. Different responses of scleractinian coral acropora pruinosa from weizhou island during extreme high temperature events. Coral Reefs 40 (6), 1697–1711.
Yu, X., Yu, K., Liao, Z., Chen, B., Deng, C., Yu, J., Yao, Q., Qin, Z., Liang, J., 2021b. Seasonal fluctuations in symbiotic bacteria and their role in environmental adaptation of the scleractinian coral acropora pruinosa in high-latitude coral reef area of the South China Sea. Sci. Total Environ. 792, 148438.
Zhang, L., Zhang, S., Wang, Y., Yu, K., Li, R., 2019. The spatial distribution of microplastic in the sands of a coral reef island in the South China Sea: comparisons of the fringing reef and atoll. Sci. Total Environ. 688, 780–786.
Zhang, W., Sik Ok, Y., Bank, M.S., Sonne, C., 2023. Macro- and microplastics as complex threats to coral reef ecosystems. Environ. Int. 174, 107914.
Zhou, Z., Tang, J., Cao, X., Wu, C., Cai, W., Lin, S., 2023. High heterotrophic plasticity of massive coral porites pukoensis contributes to its tolerance to bioaccumulated microplastics. Environ. Sci. Technol. 57 (8), 3391–3401.
Ziegler, M., Seneca, F.O., Yum, L.K., Palumbi, S.R., Voolstra, C.R., 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat. Commun. 8, 14213.