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BRIEF REPORT

Microbiome Stability Is Linked to *Acropora* Coral Thermotolerance in Northwestern Philippines

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ABSTRACT

Corals associate with a diverse community of prokaryotic symbionts that provide nutrition, antioxidants and other protective compounds to their host. However, the influence of microbes on coral thermotolerance remains understudied. Here, we examined the prokaryotic microbial communities associated with colonies of *Acropora* cf. *tenuis* that exhibit high or low thermotolerance upon exposure to 33°C (heated) relative to 29°C (control). Using 16S rRNA sequencing, we show that the microbial community structure of all *A.* cf. *tenuis* colonies was similar to each other at control temperature. Thermotolerant colonies, however, had relatively greater abundance of *Endozoicomonas*, *Arcobacter*, *Bifidobacterium* and *Lactobacillus*. At elevated temperature, only thermosensitive colonies showed a distinct shift in their microbiome, with an increase in Flavobacteriales, Rhodobacteraceae and *Vibrio*, accompanying a marked bleaching response. Functional prediction indicated that prokaryotic communities associated with thermotolerant corals were enriched for genes related to metabolism, while microbiomes of thermosensitive colonies were enriched for cell motility and antibiotic compound synthesis. These differences may contribute to the variable performance of thermotolerant and thermosensitive corals under thermal stress. Identification of microbial taxa correlated with thermotolerance provides insights into beneficial bacterial groups that could be used for microbiome engineering to support reef health in a changing climate.

1 | Introduction

Reef-building corals are essential components of coral reef ecosystems, laying the foundations of complex reefs and influencing biogeochemical processes. Corals are holobionts that host Symbiodiniaceae and prokaryotic microbial symbionts, as well as other microorganisms within and on their tissues (Thompson et al. 2015). These symbionts capture and recycle nutrients, provide food for their host and produce antioxidants and protective compounds (Garrido et al. 2020; Matthews et al. 2020). Specifically, the bacterial members of the coral holobiont contribute to coral protection (e.g., antibiotic activity and predation of opportunistic pathogens) and metabolism (e.g., elemental cycling and synthesis of secondary metabolites) (Krediet et al. 2013; McDevitt-Irwin et al. 2017) to support the survival of corals, particularly in shallow oligotrophic waters.

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Unprecedented rates of ocean warming and acidification threaten marine ecosystems and will impact survival of vulnerable marine species (Veron et al. 2009; Doney et al. 2020; Giddens et al. 2022). Rising seawater temperature, in particular, is a major factor that can disrupt mutualistic interactions within the coral holobiont, resulting in bleaching and mortality (Miller et al. 2009; Rädecker et al. 2021). Bleaching refers to the loss of the microalgal endosymbionts and/or their photopigments from the coral tissues (Hoegh-Guldberg 1999; Takahashi et al. 2008; Oakley and Davy 2018; Helgoe et al. 2024). Elevated temperature has been correlated with the spread of coral diseases as a warming ocean may facilitate the proliferation and promote virulence of potential microbial pathogens (Miller and Richardson 2014). When bleaching occurs, the immunity of the coral host is compromised, resulting in changes in coral-associated bacteria and an increase in susceptibility to diseases (Muller, Bartels, and Baums 2018; Kusdianto et al. 2021; Chavanich et al. 2022). In recent decades, many instances of mass coral bleaching during summer heat waves have been reported in Atlantic, Caribbean and Indo-Pacific coral reefs (Neal et al. 2017; Hughes et al. 2018; Lough, Anderson, and Hughes 2018; Sully et al. 2019; Quimpo et al. 2020; Pereira et al. 2022; Henley et al. 2024). These events are predicted to increase in frequency and severity and are the major drivers of continued degradation of coral reefs worldwide (Emslie et al. 2024; Klein, Roch, and Duarte 2024).

Observations of coral communities on reefs experiencing mass bleaching events have revealed intraspecific variation or differences in bleaching susceptibility among individuals of the same species (Penin et al. 2007; Chapron et al. 2022). During a bleaching event, some individuals may die, others within the same population may recover, and yet others may be resistant to thermally induced bleaching (Tilstra et al. 2017; McWilliam et al. 2022; Naugle et al. 2024). This range of intraspecific heat tolerance has been observed in natural populations of acroporid corals, including *Acropora gemmifera*, *A. hyacinthus* and *A. millepora* in American Samoa and the Great Barrier Reef (Morikawa and Palumbi 2019; Thomas et al. 2019; Fuller et al. 2020; Rose et al. 2021; Naugle et al. 2021). These differences could be attributed to various factors, including biophysical conditions (Klepac and Barshis 2020; Leinbach, Speare, and Strader 2022), host genetics (Drury 2020) and associated symbionts (Kemp et al. 2023).

Symbiotic microorganisms contribute to the heat stress response of the coral holobiont. Thermotolerance is often attributed to the microalgal associates of corals (Berkelmans and van Oppen 2006; Jones and Berkelmans 2010; Oliver and Palumbi 2011; Palacio-Castro et al. 2023), with thermotolerant corals or survivors of bleaching events usually hosting members of *Durusdinium* (Jones et al. 2008; Quigley et al. 2022). However, several studies have demonstrated that the taxonomic composition of Symbiodiniaceae does not always correlate with observed heat tolerance of corals (Humanes et al. 2021, 2022, 2024; Naugle et al. 2021).

Prokaryotic symbionts have been shown to influence coral thermotolerance. Coral associated bacteria are known to produce antioxidants, defend against pathogens and play key roles in nutrient cycling, completing metabolic pathways for other holobiont members (Matthews et al. 2023; Doering et al. 2023a; Messer et al. 2024; Voolstra et al. 2024). Stressors can disrupt this complementation, leading to dysbiosis. On the other hand, addition of beneficial bacterial taxa to a coral can reverse dysbiosis and enhance resilience (Peixoto et al. 2017; Santoro et al. 2021; Li et al. 2022; Rosado et al. 2023). Indeed, studies have linked bacterial microbiome community dynamics to coral thermotolerance, with resistant colonies often exhibiting more stable microbiomes compared to susceptible colonies (Palacio-Castro et al. 2022). For instance, in *Acropora hyacinthus* from different reef sites, heat-sensitive individuals observed at a site with a history of moderate temperature fluctuations exhibited changes in their microbiome, while heat-tolerant individuals from a site experiencing high temperature extremes remained unchanged (Ziegler et al. 2017). In addition, metagenomes of mucus-associated microbes from resilient individuals of the coral *Pseudodiploria strigosa*, sampled from both inner and outer reefs exposed to heat stress, showed greater similarity at the level of bacterial genera with an increase in abundance of beneficial taxa, as well as genes related to stress response and metabolism of nitrogen and sulfur when compared to ambient treatments (Lima et al. 2024). Whether prokaryotic community differences underlie variability in thermal tolerance of corals within the same reef habitat remains to be investigated.

Here, we compared the prokaryotic community associated with thermotolerant and thermosensitive colonies of *Acropora* cf. *tenuis* located within the same area in the Bolinao-Anda Reef Complex, Pangasinan, northwestern Luzon, Philippines. We show that, although high and low tolerance corals had similar microbiome structure under ambient conditions, the microbiomes of susceptible colonies were more affected by thermal stress. We also identified microbial taxa that may be indicators of better performance at elevated temperature.

2 | Materials and Methods

2.1 | Thermal Stress Exposure

A total of 30 healthy colonies of *Acropora* cf. *tenuis* from 4 to 5 m depth at Caniogan reef (16.29473 N, 120.01448 E) in Anda, Pangasinan, northwestern Philippines, were selected and tagged. Pieces of the coral colonies were collected with permission from the Philippines Department of Agriculture Bureau of Fisheries and Aquatic Resources (DA-BFAR Gratuitous Permit No. 0169–19 and 0324-24). The coral pieces were transported to the outdoor hatchery of the Bolinao Marine Laboratory on 13 November 2021 where they were held in tanks with flowing seawater at ~29°C for a day. A total of 16 fragments (~1 cm in length) were broken off from each colony and attached to cement nubbin holders using cyanoacrylate glue (Dizon, Edwards, and Gomez 2008). Nubbins (480 in total) were labeled with small plastic tags to enable tracking of parent colonies.

Nubbins were distributed into eight 40-L polycarbonate tanks ($n = 60$ nubbins per tank; 2 from each colony). Tanks were fed with aerated, flow-through (ca. 20 L/h), sand-filtered seawater kept at 29°C. After 3 days of acclimation, the temperature in four tanks was gradually increased to 33°C (+1°C/day) to approximate the maximum summer seawater temperature in the Bolinao-Anda

Reef Complex, while the other four tanks were maintained at 29°C (controls). Upon reaching the treatment temperature, conditions were maintained for another 4 days (Figure A1; Table A1). Temperature was regulated using submersible heaters (Eheim, Germany) controlled by thermostats and additional water circulation was provided by 200 L/h submersible pumps (Atlantis HX-1000) for even distribution of heat. Lighting was provided by cool daylight LEDs (ca. 100 $\mu E\, m^{-2} s^{-1}$). During the experiment, temperature and pH were monitored using a Horiba LAQUAact-PC110 multiparameter meter (HORIBA Advanced Techno Co. Ltd., Japan) and salinity and dissolved oxygen (DO) using a portable Vernier LabQuest 2 (Vernier, USA).

Coral colonies were classified into tolerance categories based on observed bleaching response: (1) thermotolerant if no mortality or signs of bleaching were observed in both 33°C and control tanks, (2) thermosensitive if ≥ 50% of the fragments from the colony showed bleaching in 33°C tanks but not in control tanks. Colonies that showed fragment bleaching or mortality during acclimation, or in ambient tanks during the experiment, were excluded from downstream analysis. To quantify the bleaching response, coral fragments were photographed with the coral health colour reference chart (Siebeck et al. 2006) before and after the experiment and mean grey values (MGV) were estimated (Mclachlan and Grottoli 2021). Images were converted to grey scale and the MGV of each heat-stressed fragment (M_F) was measured using ImageJ. To account for differences in image brightness, the MGV of a 1 cm^2 white standard (M_S) was measured for each photo. Normalised MGV (nMGV) was computed by dividing M_F by M_S multiplied by 100%. Higher nMGV indicates a stronger bleaching response.

2.2 | DNA Extraction and Sequencing

Two fragments were sampled from each of four selected colonies representing thermotolerant and thermosensitive individuals from the 29°C (hereinafter referred to as 'T29' and 'S29', respectively) and 33°C (hereinafter referred to as 'T33' and 'S33', respectively) treatments. All fragments were apparently healthy, except for representatives of S33, which showed bleaching and some tissue sloughing. Coral fragments were flash-frozen in liquid nitrogen and stored at −80°C prior to DNA extraction. Entire fragments were crushed using mortar and pestle, and total genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Winnepenninckx, Backeljau, and De 1993). DNA was resuspended in nuclease-free water and stored at −20°C. The quality of extracted DNA was checked by agarose gel electrophoresis using 1% agarose in 1X Tris/Borate/EDTA buffer. DNA concentration was determined using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). Genomic DNA from fragments derived from the same colony and treatment were pooled together resulting in 16 samples representing 4 colonies per tolerance category and treatment. High quality total genomic DNA was submitted to Macrogen Inc., South Korea, for 16S rRNA gene sequencing. Prokaryotic 16S rRNA gene (V3V4 hypervariable region) amplicon sequence libraries were prepared using Herculase II Fusion DNA Polymerase and the Nextera XT Index V2 Kit (Agilent Technologies, Santa Clara, CA, USA) with primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-3′) (Herlemann et al. 2011). Paired-end sequencing (300 bp) was performed on the MiSeq platform (Illumina Inc., San Diego, CA, USA) following the dual-index sequencing strategy. Symbiodiniaceae ITS2 amplicon sequence libraries were prepared from non-heat stressed samples using primers ITSintfor2 (5′-GAATTGCAGAACTCCGTG-3′) and ITS-reverse (5′-GGG ATCCATATGCTTAAGTTCAGCGGGT-3′) (Coleman, Suarez, and Goff 1994; LaJeunesse 2002).

2.3 | Microbial Community Analysis

Microbial community analysis was conducted using the Quantitative Insights Into Microbial Ecology 2 (QIIME2) package (2021.11) (Bolyen et al. 2019; https://docs.qiime2.org/2021.11/). Raw sequence data were deposited in the NCBI Sequence Read Archive and can be accessed under BioProject accession number PRJNA1033317. Raw data are also available on Figshare (https://doi.org/10.6084/m9.figshare.24715299). Primers were trimmed from the reads using cutadapt. Reads were merged into amplicon sequence variants (ASVs) using the DADA2 package (Callahan et al. 2016) following these parameters: –p-trunc-len-f 260 –p-trunc-len-r 200. Singleton and doubleton ASVs were removed prior to annotation. SILVA version 138.1 (Quast et al. 2013; https://arb-silva.de) was used for taxonomic classification. Mitochondrial and chloroplast sequences were removed from the final set of ASVs. Symbiodiniaceae community analysis from ITS2 sequences was conducted using the Symportal pipeline (Hume et al. 2019).

Rarefaction curves were generated based on Pielou's evenness and Shannon diversity. Alpha diversity metrics, including Faith's phylogenetic diversity (PD), Shannon, observed features, and Chao1 were calculated from microbiome data rarefied to the smallest sequence library. Comparison of alpha diversity among groups was done using Kruskal–Wallis test. Community distance matrix based on Bray–Curtis dissimilarity was visualised using Principal Coordinates Analysis (PCoA) from the vegan 2.6–8 package and hierarchical clustering using average linkage from the stats 4.5.0 package. PERMANOVA using the adonis2 function with 999 permutations was used to test for associations between microbiome composition and heat tolerance classifications or temperature treatments. Differentially abundant ASVs (BH-adjusted p-value < 0.05) were identified using Analysis of Compositions of Microbiomes with Bias Correction (ANCOMBC2) (Lin and Peddada 2020, 2024). Results of pairwise tests for Kruskal–Wallis, PERMANOVA, and ANCOMBC2 were adjusted using Benjamini-Hochberg (BH) false discovery rate correction (Benjamini and Hochberg 1995).

2.4 | Endozoicomonas Phylogeny

Near full-length *Endozoicomonas* 16S rRNA sequences were extracted from RefSeq (Pruitt 2004), aligned using MAFFT (Katoh and Standley 2013) and trimmed using ClipKit (Steenwyk et al. 2020). Reference trees were constructed using maximum likelihood (ML) on IQ-TREE 2.0 (Katoh and Standley 2013;

Minh et al. 2020). Branch support was calculated with 1000 ultrafast bootstrap replicates (UFBS; '-B 1000' command) and Shimodaira–Hasegawa approximate likelihood ratio tests (SH-aLRT; '-alrt 1000' command) (Ota et al. 2000; Minh, Nguyen, and Von Haeseler 2013). *Endozoicomonas* ASVs were extracted from our sequence data and their probable position on the reference tree was estimated by maximum likelihood using EPA-NG (Barbera et al. 2018).

2.5 | Functional Prediction

Functional gene abundance based on ASV taxon affiliations was predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States or PICRUSt2 (Langille et al. 2013). Differentially abundant KEGG orthologs (KO) were identified using ANCOMBC2 (Lin and Peddada 2020, 2024). Differentially abundant KOs (q-value <0.05) with total relative abundance $\geq 0.02\%$ was grouped into functions based on KEGG BRITE hierarchy level 2 for visualisation.

2.6 | Data Visualisation

Data processing and visualisation were done using the packages dplyr 1.1.4, stringr 1.5.1 and ggplot2 3.5.1 on tidyverse (Wickham 2016) (R Core Team, 2021), implemented in RStudio (RStudio Team, 2020).

3 | Results

3.1 | Microbial Community Structure of *Acropora* cf. *tenuis*

Exposure of *A*. cf. *tenuis* colonies to control (29°C) or elevated (33°C) temperature treatments elicited variable responses that indicate inter-individual differences in thermotolerance (Figure 1). While thermosensitive colonies exhibited bleaching and some tissue sloughing at 33°C, thermotolerant colonies remained apparently healthy with no signs of bleaching even after 4 days of sustained exposure to high temperature (Figure 1A;

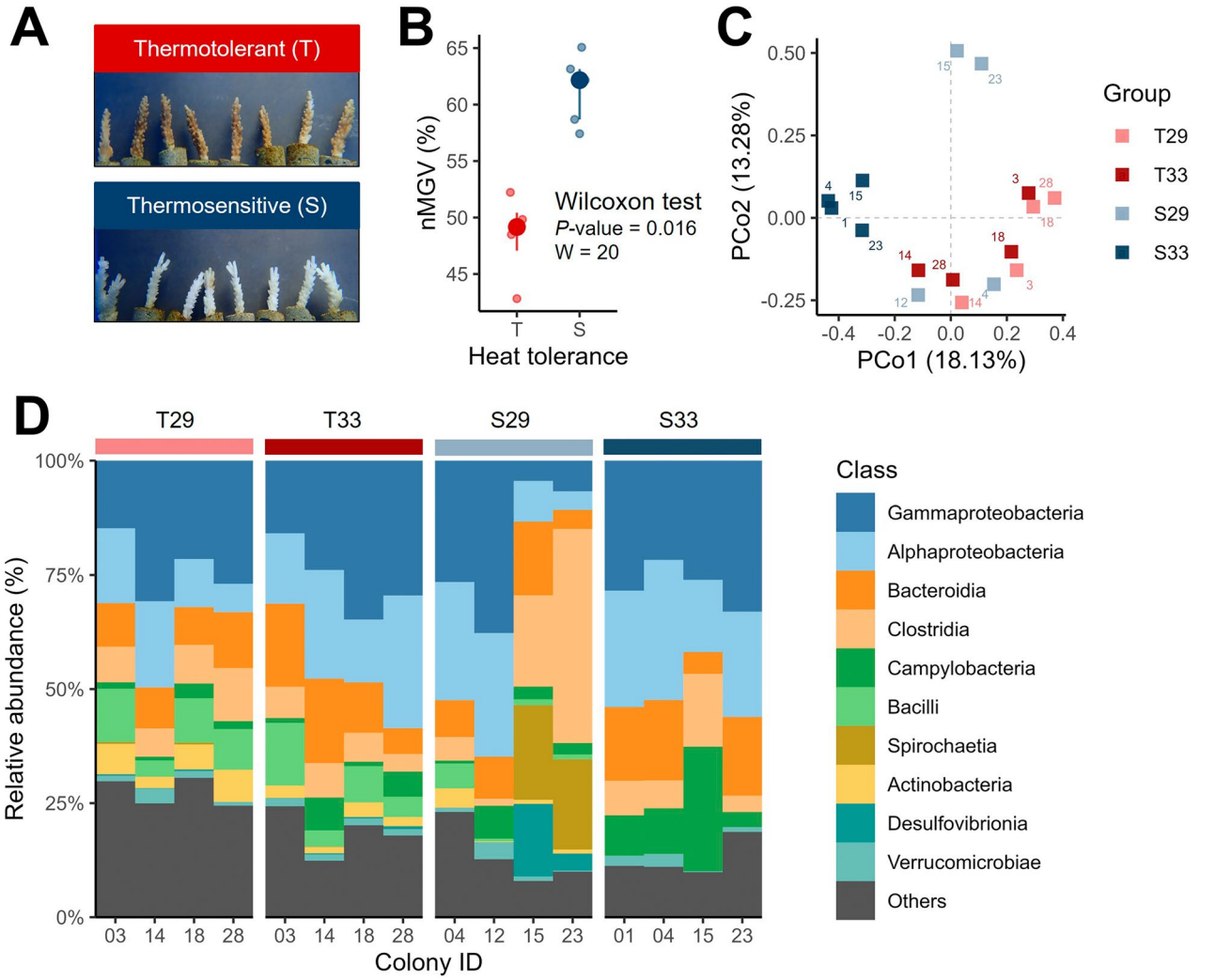

FIGURE 1 | Composition and structure of microbial communities associated with thermotolerant and thermosensitive *Acropora* cf. *tenuis*. (A) Representative image of fragments from thermotolerant (T) and thermosensitive (S) colonies after exposure to 33°C. (B) Normalised mean grey values (nMGV) of fragments from thermotolerant and thermosensitive colonies. Larger circles represent the median value. (C) Principal component analysis of microbial communities in corals subjected to 33°C or 29°C treatments (T29, light red; T33, dark red; S29, light blue; S33, dark blue). Colony IDs are shown beside the markers. (D) Relative abundance of the top 10 microbial classes in the *A*. cf. *tenuis* microbiome.

Figure A2; Table A2). Higher normalised mean grey values (nMGV) in thermosensitive relative to thermotolerant colonies support visual observations of bleaching (Figure 1B).

To examine whether observed differences in bleaching response correlate with associated microbiomes, we characterised the Symbiodiniaceae and prokaryotic communities of the corals. Sequencing of the ITS2 region revealed dominance of *Cladocopium* C3u sequences (78.51%) in all *A*. cf. *tenuis* colonies (Figure A3). No significant difference in Symbiodiniaceae communities was detected among colonies (PERMANOVA, p-value = 0.393, $R^2 = 0.124$). Sequencing of the 16S rRNA gene (V3V4 region) from 16 libraries representing thermotolerant and thermosensitive colonies that had been subjected to control or elevated temperature treatments ($n = 4$) yielded a total of 1,474,619 raw reads. Quality filtering and processing with DADA2 in QIIME2 generated 1,295,805 high-quality reads, with an average of 80,987 ± 9178 (mean ± standard deviation) reads per library. Removal of singletons, doubletons, mitochondrial and chloroplast amplicon sequence variants (ASVs) resulted in 5717 unique ASVs with an average of 51,677 ± 9258 (mean ± standard deviation) ASVs per library. Rarefaction curves based on Pielou's evenness and Shannon diversity reached a plateau at around 5000 reads, indicating that sequencing depth, even for the smallest library (with 35,588 sequences), provided sufficient coverage of the prokaryotic communities in our samples (Figure A4). ASVs associated with the corals were classified into 48 phyla, 113 classes, 273 orders, 466 families and 872 genera (Data File 1).

The *A*. cf. *tenuis*-associated prokaryotic microbiome was dominated by the phyla Proteobacteria (40.64%), Firmicutes (16.72%), Bacteroidota (12.22%), Campylobacterota (5.57%), Actinobacteriota (3.39%) (Figure A5) and the classes Gammaproteobacteria (22.62%), Alphaproteobacteria (18.02%), Bacteroidia (11.6%), Clostridia (11.45%) and Campylobacteria (5.57%) (Figure 1D).

No significant differences in alpha diversity were observed among thermotolerant and thermosensitive corals based on Faith's PD, Shannon, observed ASVs, and Chao1 indices (Figure A6). The core microbiome, which is shared among all groups, comprised 2.41% (138 ASVs) of identified ASVs (Figure A7). Regardless of temperature treatment, 96 and 98 ASVs were present in all thermotolerant and thermosensitive colonies, respectively. Conversely, 69 and 140 ASVs were present in all colonies maintained at ambient or elevated temperature, respectively, regardless of tolerance category. Unique ASVs comprised 16.27%–23.21% of the coral-associated microbial community of each group of samples.

3.2 | Influence of Tolerance Class and Temperature on the Coral Microbiome

Microbial community structure was influenced by tolerance class and temperature treatment. Principal coordinate analysis revealed clustering of all thermotolerant samples, together with some thermosensitive samples from the ambient temperature treatment, whereas all thermosensitive samples from the heated treatment formed an independent cluster (Figure 1C; Figure A8).

At ambient temperature, microbial communities in thermotolerant and thermosensitive colonies showed no significant difference in structure (PERMANOVA, p-value = 0.171; Table 1; Table A3). Only six ASVs were found at significantly greater abundance in thermotolerant relative to thermosensitive corals based on ANCOMBC2 (Data File 2). These ASVs were affiliated with *Arcobacter* (Campylobacteraceae), *Haemophilus* (Pasteurellaceae), *Bifidobacterium* (Bifidobacteraceae), *Lactobacillus* (Lactobacillaceae), *Pyramidobacter* (Synergistaceae) and Sporichthyaceae (Figure 2).

TABLE 1 | Comparison of *Acropora* cf. *tenuis* microbial communities at ASV level between heat tolerance classifications and temperature treatments based on Bray–Curtis dissimilarity.

	PERMANOVA	
Global comparisons	**Pseudo-F**	***p***
Tolerance classification	1.933095	**0.002**
Temperature treatment	2.135664	**0.048**
Tolerance × treatment	1.736775	**0.002**
Pairwise comparisons		
T29 × T33	0.955802	0.550
T29 × S29	1.224929	0.171
T29 × S33	2.929221	**0.034**
T33 × S29	1.158406	0.356
T33 × S33	2.408634	**0.036**
S29 × S33	2.341153	**0.029**

Note: Benjamini–Hochberg adjusted p-values in bold denote statistical significance at $p < 0.05$.

At elevated temperature, the microbiome structure of thermotolerant and thermosensitive colonies behaved differently from one another (PERMANOVA, p-value = 0.036; Table 1). Thermotolerant microbiomes remained similar to controls at ambient temperature (PERMANOVA, p-value = 0.55; Table 1), with only five ASVs (*Salinibacter*, Candidatus *Tenderia*, *Tropicimonas*, *Ferrimonas* and Cryomorphaceae) showing significant enrichment, and four ASVs (Sporichthyaceae, *Haemophilus*, *Arcobacter* and *Bifidobacterium*) showing depletion (Figure 2). In contrast, thermosensitive colonies in the high temperature treatment exhibited a significant shift in microbial structure relative to controls (PERMANOVA, p-value = 0.029; Table 1). Thermosensitive colonies at 33°C were enriched for 49 ASVs, including 17 Bacteroidia, 16 Gammaproteobacteria (12 Pseudomonadales, 2 Vibrionaceae), 4 Alphaproteobacteria (2 Rhodobacteraceae, 1 Terasakiellaceae, 1 unclassified), 4 Bdellovibrionaceae, 3 Desulfuromonadia, 1 Acidomicrobiia (Ilumatobacteraceae), 1 Campylobacteria (Malaciobacter), 1 Phycisphaeraceae and 2 Polyangia, whereas 2 ASVs Endozoicomonaceae (*Endozoicomonas*) and Synergistaceae (*Pyramidobacter*) were significantly depleted (Figure 2).

The core microbiome showed a similar clustering pattern across tolerance categories and treatments as the whole microbiome, but no significant difference in structure was detected for tolerance classification in combination with temperature treatment (Figure A9, Table A4). Moreover, ANCOMBC2 analysis did not reveal significant changes in abundance of core ASVs (Data File 2).

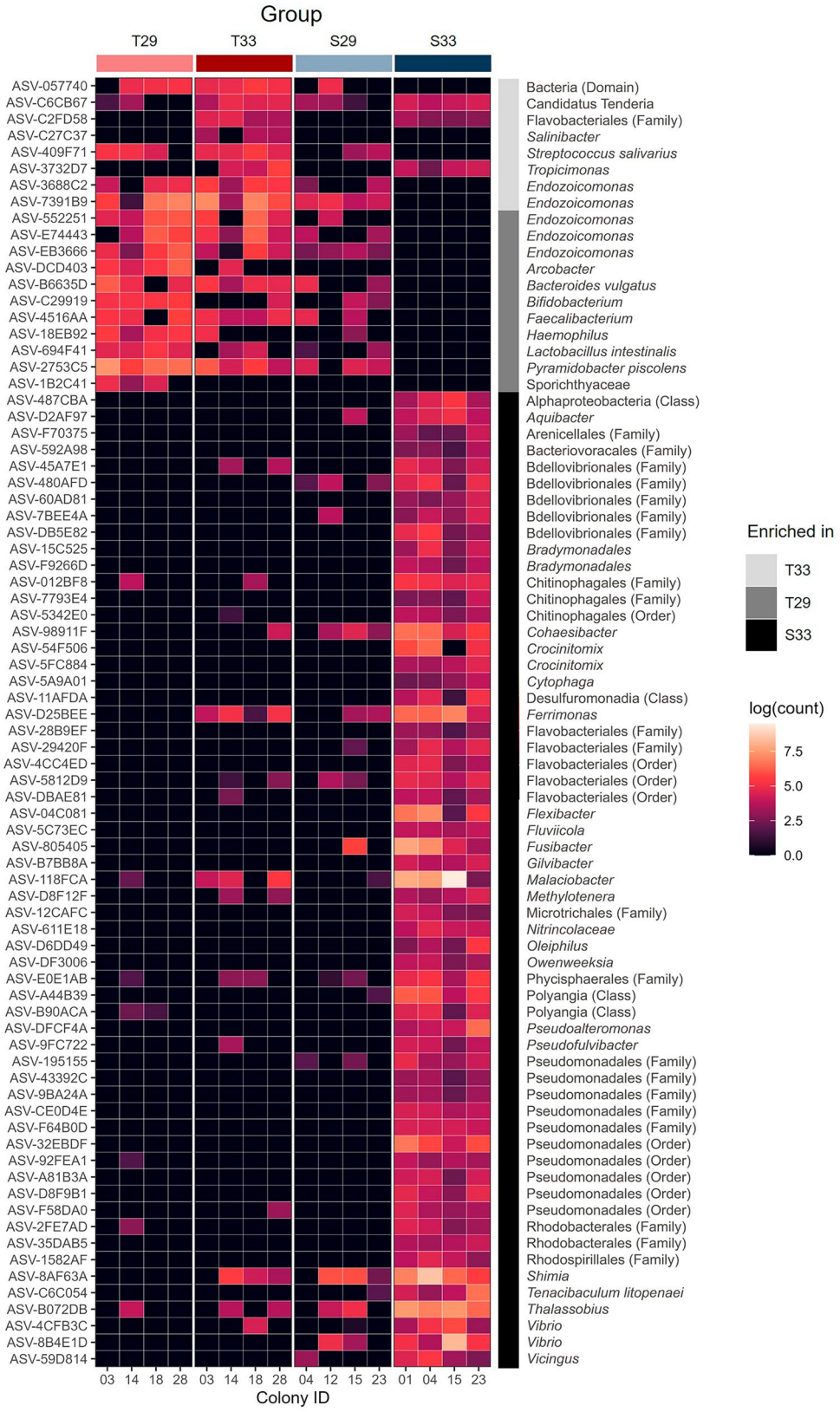

FIGURE 2 | Heatmap of differentially abundant ASVs (p-value < 0.05). Heatmap colours represent log-normalised counts.

3.3 | Endozoicomonas Are Enriched in High Tolerance Colonies

We identified 28 ASVs affiliated with *Endozoicomonas* in the *A.* cf. *tenuis* microbiome (Figure 3; Table A5). A total of 14 ASVs were affiliated with *Endozoicomonas* sp. P9H01 isolated from coral, 1 ASV with *E. gorgoniicola*, 8 ASVs with *E. euniceicola* and 5 ASVs with *Parendozoicomonas haliclonae* (Figure A10). *Endozoicomonas* ASVs comprised 1.53% of the thermotolerant microbiome and only 0.23% of the thermosensitive microbiome

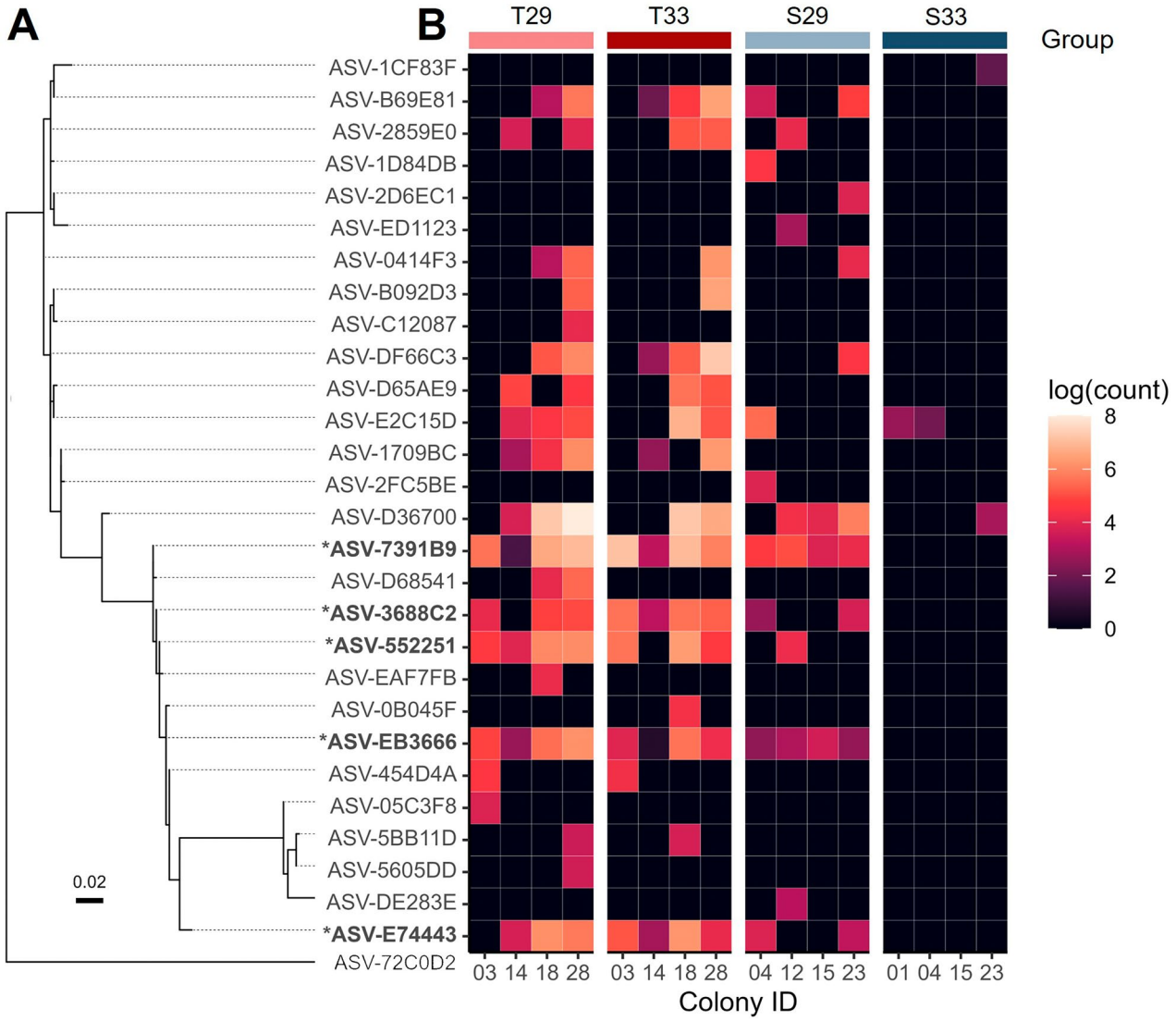

FIGURE 3 | *Endozoicomonas* ASVs in the microbiome of *A*. cf. *tenuis*. (A) Maximum-likelihood tree of *Endozoicomonas* ASVs. The tree is rooted on *Neptunibacter* sp. (ASV-72C0D2). Asterisks indicate differentially abundant ASVs. (B) Heatmap of log-normalised counts of ASVs across thermotolerance categories and treatments (T29, light red; T33, dark red; S29, light blue; S33, dark blue).

under ambient temperature. Only 3 *Endozoicomonas* ASVs (0.0051%) were detected in thermosensitive colonies at 33°C (Data File 1). Five *Endozoicomonas* ASVs, including the 2 most abundant sequences, showed significant depletion in thermosensitive relative to thermotolerant colonies in the heated treatments.

3.4 | Predicted Functions Overrepresented in Coral Microbiomes

Functions associated with the microbiomes of *A*. cf. *tenuis* were predicted using PICRUSt2 (Figure 4; Data File 3). A total of 7731 KEGG ortholog (KO) genes were represented in the microbiomes of *A*. cf. *tenuis* corals. The predicted KO profiles showed no clear differentiation between tolerance classes (PERMANOVA: $R^2 = 0.1$, p-value = 0.195; Figure A11). Thermotolerant corals showed relatively greater abundance of genes involved in metabolism of carbohydrates, lipids and amino acids, and translation, compared to thermosensitive corals. In contrast, thermosensitive corals were enriched for genes involved in cell motility. When subjected to elevated temperature, the microbiome of thermotolerant corals remained similar to the microbiome at ambient temperature, whereas in the thermosensitive corals, functions related to carbohydrate, lipid, and amino acid metabolism were depleted and terpenoid and polyketide metabolism was enriched.

4 | Discussion

4.1 | Indicators of Thermotolerance in the Microbiome of *A*. cf. *tenuis*

Sympatric colonies of *A*. cf. *tenuis* exhibited variable temperature tolerance and could be grouped into thermotolerant and thermosensitive classes based on their bleaching responses upon exposure to elevated temperature. Symbiodiniaceae communities in thermotolerant and thermosensitive colonies showed similar composition and were dominated by *Cladocopium* C3u sequences. This finding suggests that other factors may be

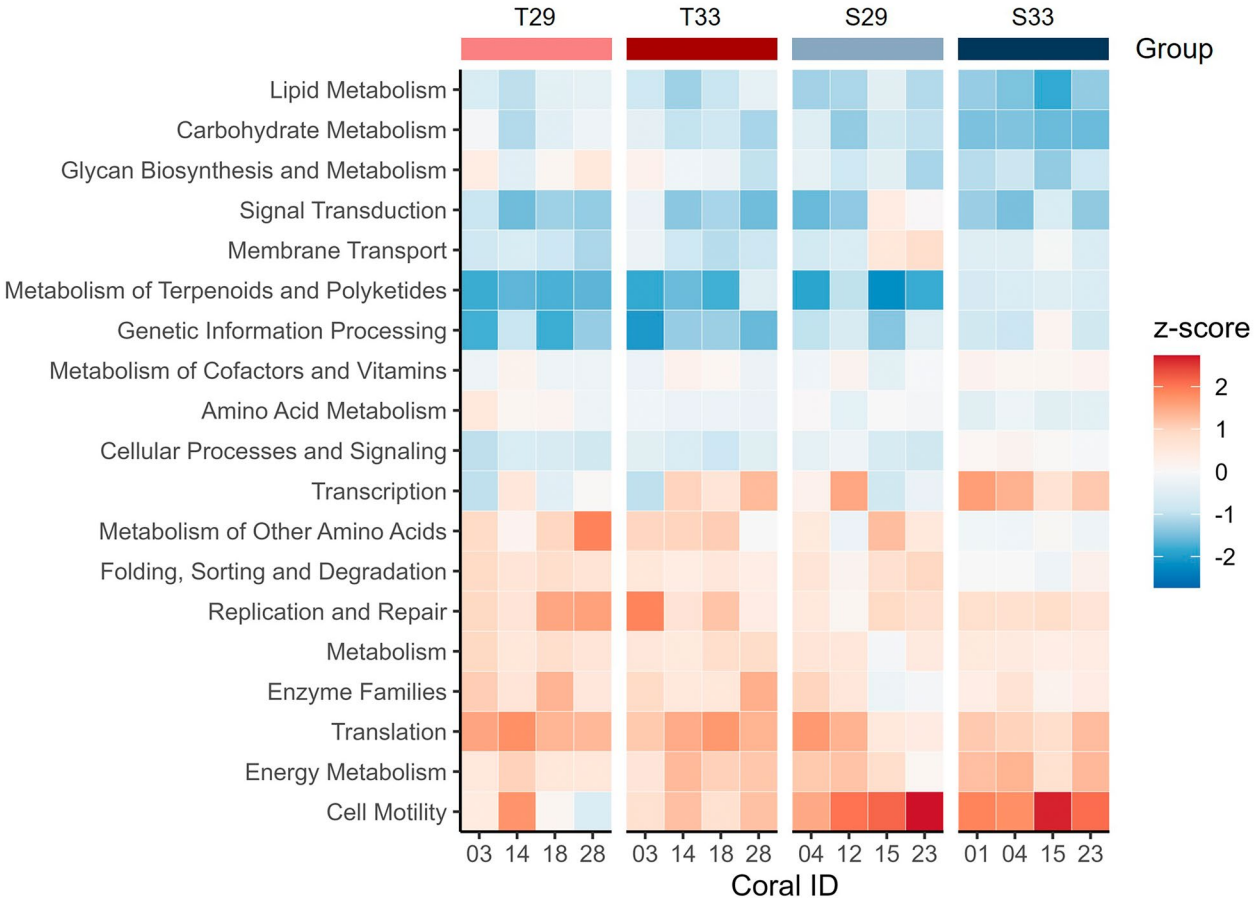

FIGURE 4 | Differentially abundant predicted gene functions in the *A*. cf. *tenuis* microbiome. Only KEGG orthologs (KO) with total relative abundance ≥0.02% and *q*-value <0.05 are represented. KOs were grouped into functions based on KEGG BRITE hierarchy level 2. Heatmap colours represent *z*-scores (red, high; blue, low).

contributing to observed differences in thermotolerance of *A*. cf. *tenuis* individuals.

The bacteria associated with thermotolerant and thermosensitive corals showed similar community structure under ambient conditions. However, distinct microbiome responses were observed when the corals were subjected to elevated temperature. Under these conditions, the microbiomes of thermotolerant corals remained comparable to controls, indicating community stability. Similar observations of microbiome stability in thermotolerant individuals of *Acropora muricata* in Penghu Archipelago, Taiwan (Shiu et al. 2017) and the Luhuitou fringing reef, southern China (Cheng et al. 2023), have been reported.

On the other hand, the microbiomes of thermosensitive corals showed a significant change in community structure, becoming more homogenous in terms of composition. This is likely due to exclusion of heat-susceptible microbes and increased dominance of certain taxa (Sweet et al. 2019). Given the importance of coral-associated bacteria on holobiont performance (Voolstra et al. 2024), microbial community structure and measured responses to external stimuli may offer a useful indicator of coral health and resistance to stressors like elevated temperature (Kusdianto et al. 2021; Palacio-Castro et al. 2022; Chavanich et al. 2022).

A detailed analysis of the microbiomes of *A*. cf. *tenuis* highlighted specific microbial taxa that may relate to thermal tolerance characteristics. *Acropora* cf. *tenuis* is dominated by members of Gammaproteobacteria and Alphaproteobacteria, which concurs with findings by other groups that proteobacteria dominates the core microbiome of *A. tenuis* (Quigley et al. 2019; Marchioro et al. 2020). The microbiome of thermotolerant colonies revealed greater abundance of *Arcobacter, Bifidobacterium*, *Lactobacillus* and Sporichthyaceae compared to thermosensitive colonies. This suggests that members of these microbial groups may be positive predictors of coral thermotolerance. *Arcobacter* has been suggested to be associated with increased protein content reserves potentially benefiting the growth and development of *Pocillopora damicornis* (Zhang et al. 2021). Species belonging to *Bifidobacterium* are known members of the *Porites lutea* core microbiome (Gong et al. 2020). Although not much is known about their roles in corals, members of this group have been reported to aid in human digestive health (Picard et al. 2005) and protect mice from lethal infections by pathogenic microbes (Fukuda et al. 2011). *Lactobacillus*, on the other hand, dominated the *A. cervicornis* microbiome collected from deeper waters (Godoy-Vitorino et al. 2017) and is posited to exhibit antimicrobial activity against deleterious invader microbes (Chen et al. 2021). Sporichthyaceae, which are known to be facultative anaerobes, have been found in microbiomes impacted by

wastewater suggesting that they can withstand stressful conditions (Newton and McLellan 2015; Zárate et al. 2020).

Upon exposure of thermotolerant colonies to elevated temperature, changes in the relative abundance of several microbial taxa were noted. Taxa that increased in abundance include *Tropicimonas, Ferrimonas* and Cryomorphaceae. *Tropicimonas* was previously identified as a bioindicator of microbiome recovery after bleaching in *Montipora peltiformis* (Zou et al. 2024). *Ferrimonas* and Cryomorphaceae, in contrast, are potential microbial pathogens or opportunists and have been reported in the microbiome of diseased *Acropora palmata* (Rosales et al. 2019; Young et al. 2023). Taxa that decreased in abundance in thermotolerant colonies subjected to heat stress include *Haemophilus*, Sporichthyaceae, *Arcobacter* and *Bifidobacterium*, suggesting that these taxa are sensitive to temperature. *Haemophilus* has previously been reported to be less abundant in heat-challenged *Pocillopora damicornis* inoculated with a beneficial actinobacterial strain (SCSIO 13291) (Li et al. 2023).

Specific members of the microbial assemblage of thermosensitive corals showed significant shifts in abundance accompanying the bleaching response at elevated temperature. Under these conditions, heat-susceptible microbes such as Bacilli, Desulfovibrionia, Actinobacteria and the archaea Halobacteria decreased in abundance. This depletion may be attributed to population elimination due to bacterial susceptibility to higher temperature or to competition by other microbiome members. Members of these prokaryotic taxa have previously been reported to be depleted in bleached coral fragments and in corals subjected to warming and acidification conditions (Grottoli et al. 2018; Sun et al. 2022).

Notably, many other microbial taxa in thermosensitive colonies increased in abundance during heat-induced bleaching, including members of Flavobacteria, Rhodobacteraceae, *Malaciobacter, Vibrio* and Pseudomonadales. These groups of copiotrophic bacteria may proliferate in stressed corals due to the overproduction and shedding of carbon-rich mucus from the coral or exudates from degraded Symbiodiniaceae (Fujise et al. 2014; Tout et al. 2014; Lee et al. 2016). Flavobacteriales have been found in corals under stressful conditions and they are known to be initial members of the microbiome associated with stony coral tissue loss disease (McDevitt-Irwin et al. 2017; Rosales et al. 2023). Some Rhodobacteraceae are opportunist microbes that can compromise host health and are known as robust indicator species for thermally stressed corals (Pootakham et al. 2019; Hsieh et al. 2024). *Malaciobacter*, a genus with emerging pathogenic activities for marine hosts, has been found at high abundance in *Drupella*-grazed *Acropora* corals and even in diseased marine bivalves (Auguste et al. 2022; Nguyen et al. 2023). *Vibrio* also includes pathogenic members that have been detected in diseased coral tissues and in stressed microbiomes of other acroporid corals (Gajigan, Diaz, and Conaco 2017; Meyer et al. 2019; Meenatchi et al. 2020; Schul et al. 2023). Metabolites released by certain *Vibrio* species favour the growth of co-pathogens, which can result in tissue necrosis (Rubio-Portillo et al. 2020). Interestingly, Bdellovibrionaceae, which are known predators of *Vibrios*, were also enriched in thermosensitive colonies under elevated temperature suggesting that they may serve as a regulatory agent for *Vibrios* in the *A.* cf. *tenuis* microbiome (Welsh et al. 2017). Finally, the increased abundance of Pseudomonadales in thermosensitive corals may be a mechanism to mitigate the impacts of high temperature, as members of this group are known to exert beneficial effects on corals through their antagonistic activity against disease-causing bacteria (Gignoux-Wolfsohn and Vollmer 2015; Sun et al. 2022; Tiirola et al. 2002).

4.2 | Endozoicomonas Abundance Correlates With Thermotolerance

Endozoicomonas comprised a substantial group within the *A.* cf. *tenuis* microbiome. Diverse *Endozoicomonas* ASVs were detected in thermotolerant *A.* cf. *tenuis* colonies at both ambient and elevated temperature conditions, with fewer in the thermosensitive group, especially in colonies subjected to high temperature. These results suggest that diversity and abundance of *Endozoicomonas* within the *A.* cf. *tenuis* microbial community may be an indicator of thermotolerance. Our findings mirror reports showing that *Endozoicomonas* are depleted in stressed, diseased and bleached corals (Tandon et al. 2020; Botté et al. 2022). *Endozoicomonas* are known coral symbionts, contributing beneficial functions such as dimethylsulfoniopropionate (DMSP) transformation, amino acid and carbohydrate metabolism and nutrient cycling (Tandon et al. 2020; Sweet et al. 2019; Pogoreutz et al. 2022). These bacteria can also convert cholesterol derived from the coral host into steroids that may inhibit pathogenic bacteria and fungi (Ochsenkühn et al. 2023). The abundance of *Endozoicomonas* may be considered as a gauge of coral health and may reflect coral recovery after a severe bleaching event (Chuang et al. 2024). However, other studies suggest that certain *Endozoicomonas* taxa may act as opportunistic microbes, switching between parasitism and pathogenicity depending on holobiont status (Pogoreutz and Ziegler 2024). Further studies are therefore warranted to determine the exact functional roles and contributions of this group to coral thermotolerance.

4.3 | Microbiome Functions Mirror Coral Microbial Community Dynamics

Shifts in coral microbial community composition predict changes in the functions of the microbiome (Reigel, Paz-García, and Hellberg 2021; Bergman et al. 2023). The maintenance of metabolic functions for lipids, carbohydrates, glycans and amino acids in the microbiome of thermotolerant *A.* cf. *tenuis* even under thermal stress may help maintain homeostasis, contributing to holobiont heat resistance (Zhang et al. 2015; Ziegler et al. 2017). In contrast, decreased abundance of metabolism-related genes in thermosensitive corals indicates weaker regulation of energy reserves and lowered protection against free radicals (Su et al. 2021). Moreover, enrichment of functions related to chemotaxis and production of chemicals with potential antimicrobial properties, including terpenoids and polyketides, in thermosensitive corals suggests the presence and activity of opportunistic bacteria (Raimundo et al. 2018). Chemotaxis is associated with virulence of pathogenic bacteria and flagellar proteins are abundant in known pathogens like *Vibrio shilonii* (Li, Azam, and Zhang 2016). *Vibrio coralliilyticus* also

uses chemotaxis elicited by increased DMSP concentrations to locate the mucus of its heat-stressed host (Garren et al. 2013; Gao et al. 2021) and its flagellar proteins have been shown to be important for adhesion to the coral during infection (Ramos, Rumbo, and Sirard 2004; Meron et al. 2009; Akahoshi and Bevins 2022). Terpenoids produced by microorganisms are associated with microbial competition, defence and quorum sensing for opportunistically pathogenic microbes and can play a role in their adaptation to common stressors (Avalos et al. 2022). Bacteria have also been observed to use polyketides to defeat other microbial competitors and to enable pathogenesis by suppressing the immune response of the host (Ridley, Lee, and Khosla 2008). It is important to note, however, that the PICRUSt2 method that was used to derive microbiome functions is purely predictive. Nevertheless, it provides a starting point for understanding gene functions potentially represented within a microbial community. Metagenome or metatranscriptome studies would be critical to provide further insights into functions that may differ in microbiomes associated with coral tolerance or susceptibility to thermal stress.

4.4 | Microbiome Responses May Be Regulated by the Host

Microbiome responses in the coral holobiont are driven not only by interactions within the microbial community, but also by interactions with the host. The difference in the microbiome response of thermotolerant and thermosensitive *A*. cf. *tenuis* colonies suggests that host regulation is a major driver of microbiome structure, especially under conditions of stress. Corals can regulate their microbiome through physical barriers such as the mucus layer (Ritchie 2006), manipulation of symbiont physiology (Voolstra et al. 2024; Wilde, Slack, and Foster 2024), and innate immune functions that mediate symbiont recognition, maintenance and dysbiosis (Weis 2008; Posadas et al. 2022; Mohamed et al. 2023). For instance, it has been observed that the coral host can distinguish beneficial versus harmful microbes through pattern recognition receptors (PRRs) on phagocyte cell surfaces that recognise microbial-associated molecular patterns (MAMPs) on microbial cell membranes (Weis 2008). A diversity of cnidarian-associated PRR families have been found in corals, including Toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors (RLRs), nucleotide-binding oligomerization domain-like receptors (NLRs) and C-type lectins (CTLs) (Emery, Dimos, and Mydlarz 2021). Although we did not determine host genotypes in this study, it is likely that presence of a disparate repertoire of PRRs across genotypes may contribute to inter-individual differences in the stress response of the coral microbial community (Glasl et al. 2019). Further investigations are needed to assess host genotype–phenotype connection to microbiome structure, and to elucidate the mechanisms regulating microbiome structure in thermotolerant and thermosensitive coral hosts.

4.5 | Harnessing the Microbiome to Promote Thermotolerance

With increasing interest in harnessing beneficial microorganisms for corals (BMCs) to enhance coral tolerance and mitigate temperature stress (Epstein et al. 2019; Rosado et al. 2019; Santoro et al. 2021; Doering et al. 2023b), understanding the factors that drive coral microbial community structure is crucial. Studies that have tested the efficacy of BMCs on the performance of corals under thermal stress have so far been promising (Peixoto et al. 2017; Santoro et al. 2021; Li et al. 2022; Delgadillo-Ordoñez et al. 2024). However, a major challenge for this approach is that inoculated microbes are not stably integrated or retained by the recipient colony, likely due to a combination of microbiome and host-mediated regulation (Rosado et al. 2019; Santoro et al. 2021; Zhang et al. 2021; Delgadillo-Ordoñez et al. 2024). By comparing microbiomes of thermotolerant and thermosensitive corals, we identified potential BMCs that are ubiquitous and present at greater abundance in thermotolerant colonies. We infer that supplementing thermosensitive colonies with these 'native' beneficial bacteria may improve the efficacy of this approach.

Some potential BMCs in the *A*. cf. *tenuis* microbiome include *Endozoicomonas*, *Pseudoaltermonas* and *Bdellovibrio*. Inoculation of *Pseudoalteromonas* strains and *Halomonas* have been reported to mitigate coral bleaching due to thermal and pathogen stresses in *P. damicornis* (Rosado et al. 2019). Consistent with this, transplantation of the *Endozoicomonas*-dominated microbiome of heat-tolerant *Porites* sp. and the predatory *Bdellovibrio*-containing microbiome of heat-tolerant *Pocillopora* into heat-sensitive conspecific recipients alleviated bleaching from short-term thermal stress exposure (Doering et al. 2021). Culturing of BMCs from heat-tolerant *A*. cf. *tenuis* will enable future experiments to test their ability to boost thermotolerance of sensitive colonies.

5 | Conclusion

The results of this study demonstrate that although microbial community structure does not directly correlate with coral thermotolerance under ambient temperature, microbiome responses to thermal stress varied. Thermotolerant individuals maintained their community structure, whereas thermosensitive colonies exhibited a marked shift in community structure that coincided with bleaching. The presence of specific microbial taxa in the coral community may serve as a predictor of performance under stress. Thermotolerant colonies contained diverse and abundant microbes belonging to potentially beneficial taxa such as *Endozoicomonas*, *Arcobacter*, *Bifidobacterium* and *Lactobacillus*. Thermosensitive colonies, in contrast, exhibited depletion of these potentially beneficial microbes and an increase in opportunistic pathogens (e.g., Flavobacteriales, Rhodobacteraceae, *Vibrio*) when subjected to elevated temperature. This information on microbial signatures associated with coral thermotolerance may be harnessed for applications that aim to increase the vigour of reef-building corals and maintain reef health through a healthy microbiome.

Author Contributions

Jake Ivan P. Baquiran: conceptualization, data curation, methodology, formal analysis, writing – original draft, writing – review and editing, investigation. **John Bennedick Quijano:** methodology, data

curation, formal analysis, writing – original draft, writing – review and editing, visualization, software. **Madeleine J. H. van Oppen:** funding acquisition, writing – original draft, writing – review and editing. **Patrick C. Cabaitan:** funding acquisition, writing – original draft, writing – review and editing. **Peter L. Harrison:** funding acquisition, writing – original draft, writing – review and editing. **Cecilia Conaco:** conceptualization, methodology, supervision, funding acquisition, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.24715299.

References

Akahoshi, D. T., and C. L. Bevins. 2022. "Flagella at the Host-Microbe Interface: Key Functions Intersect With Redundant Responses." *Frontiers in Immunology* 13: 828758. https://doi.org/10.3389/fimmu.2022.828758.

Auguste, M., F. U. Rahman, T. Balbi, et al. 2022. "Responses of Mytilus galloprovincialis to Challenge With Environmental Isolates of the Potential Emerging Pathogen *Malaciobacter marinus*." *Fish & Shellfish Immunology* 131: 1–9. https://doi.org/10.1016/j.fsi.2022.09.048.

Avalos, M., P. Garbeva, L. Vader, G. P. Van Wezel, J. S. Dickschat, and D. Ulanova. 2022. "Biosynthesis, Evolution and Ecology of Microbial Terpenoids." *Natural Product Reports* 39, no. 2: 249–272. https://doi.org/10.1039/d1np00047k.

Barbera, P., A. M. Kozlov, L. Czech, et al. 2018. "EPA-Ng: Massively Parallel Evolutionary Placement of Genetic Sequences." *Systematic Biology* 68, no. 2: 365–369. https://doi.org/10.1093/sysbio/syy054.

Benjamini, Y., and Y. Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society: Series B (Methodological)* 57, no. 1: 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bergman, J. L., F. Ricci, W. Leggat, and T. D. Ainsworth. 2023. "Characteristics of the Bleached Microbiome of the Generalist Coral *Pocillopora damicornis* From Two Distinct Reef Habitats." *Integrative Organismal Biology* 5, no. 1: obad012. https://doi.org/10.1093/iob/obad012.

Berkelmans, R., and M. J. Van Oppen. 2006. "The Role of Zooxanthellae in the Thermal Tolerance of Corals: A 'Nugget of Hope' for Coral Reefs in an Era of Climate Change." *Proceedings of the Royal Society B: Biological Sciences* 273, no. 1599: 2305–2312. https://doi.org/10.1098/rspb.2006.3567.

Bolyen, E., J. R. Rideout, M. R. Dillon, et al. 2019. "Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2." *Nature Biotechnology* 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Botté, E. S., N. E. Cantin, V. J. Mocellin, et al. 2022. "Reef Location Has a Greater Impact Than Coral Bleaching Severity on the Microbiome of *Pocillopora acuta*." *Coral Reefs* 41, no. 1: 63–79. https://doi.org/10.1007/s00338-021-02201-y.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13, no. 7: 581–583. https://doi.org/10.1038/nmeth.3869.

Chapron, L., V. Schoepf, S. J. Levas, M. D. Aschaffenburg, M. E. Warner, and A. G. Grottoli. 2022. "Natural Variability in Caribbean Coral Physiology and Implications for Coral Bleaching Resilience." *Frontiers in Marine Science* 8: 811055. https://doi.org/10.3389/fmars.2021.811055.

Chavanich, S., H. Kusdianto, C. Kullapanich, et al. 2022. "Microbiomes of Healthy and Bleached Corals During a 2016 Thermal Bleaching Event in the Andaman Sea of Thailand." *Frontiers in Marine Science* 9: 763421. https://doi.org/10.3389/fmars.2022.763421.

Chen, C., C. Lai, H. Huang, et al. 2021. "Antimicrobial Ability and Mechanism Analysis of Lactobacillus Species Against Carbapenemase-Producing Enterobacteriaceae." *Journal of Microbiology, Immunology and Infection* 54, no. 3: 447–456. https://doi.org/10.1016/j.jmii.2020.01.005.

Cheng, K., X. Li, M. Tong, et al. 2023. "Integrated Metagenomic and Metaproteomic Analyses Reveal Bacterial Micro-Ecological Mechanisms in Coral Bleaching." *mSystems* 8, no. 6: e0050523. https://doi.org/10.1128/msystems.00505-23.

Chuang, P., S. Yu, P. Liu, et al. 2024. "A Gauge of Coral Physiology: Re-Examining Temporal Changes in Endozoicomonas Abundance Correlated With Natural Coral Bleaching." ISME *Communications* 4, no. 1: ycae001. https://doi.org/10.1093/ismeco/ycae001.

Coleman, A. W., A. Suarez, and L. J. Goff. 1994. "Molecular Delineation of Species and Syngens in Volvocacean Green Algae (Chlorophyta)." *Journal of Phycology* 30, no. 1: 80–90. https://doi.org/10.1111/j.0022-3646.1994.00080.x.

Delgadillo-Ordoñez, N., N. Garcias-Bonet, I. Raimundo, et al. 2024. "Probiotics Reshape the Coral Microbiome in Situ Without Detectable Off-Target Effects in the Surrounding Environment." *Communications Biology* 7, no. 1: 434. https://doi.org/10.1038/s42003-024-06135-3.

Dizon, R. M., A. J. Edwards, and E. D. Gomez. 2008. "Comparison of Three Types of Adhesives in Attaching Coral Transplants to Clam Shell Substrates." *Aquatic Conservation: Marine and Freshwater Ecosystems* 18, no. 7: 1140–1148. https://doi.org/10.1002/aqc.944.

Doering, T., J. Maire, M. J. Van Oppen, and L. L. Blackall. 2023a. "Advancing Coral Microbiome Manipulation to Build Long-Term Climate Resilience." *Microbiology Australia* 44, no. 1: 36–40. https://doi.org/10.1071/ma23009.

Doering, T., K. Tandon, S. H. Topa, S. J. Pidot, L. L. Blackall, and M. J. Van Oppen. 2023b. "Genomic Exploration of Coral-Associated Bacteria: Identifying Probiotic Candidates to Increase Coral Bleaching Resilience in *Galaxea fascicularis*." *Microbiome* 11, no. 1: 185. https://doi.org/10.1186/s40168-023-01622-x.

Doering, T., M. Wall, L. Putchim, et al. 2021. "Towards Enhancing Coral Heat Tolerance: A "Microbiome Transplantation" Treatment Using Inoculations of Homogenized Coral Tissues." *Microbiome* 9, no. 1: 102. https://doi.org/10.1186/s40168-021-01053-6.

Doney, S. C., D. S. Busch, S. R. Cooley, and K. J. Kroeker. 2020. "The Impacts of Ocean Acidification on Marine Ecosystems and Reliant Human Communities." *Annual Review of Environment and Resources* 45, no. 1: 83–112. https://doi.org/10.1146/annurev-environ-012320-083019.

Drury, C. 2020. "Resilience in Reef-Building Corals: The Ecological and Evolutionary Importance of the Host Response to Thermal Stress." *Molecular Ecology* 29, no. 3: 448–465. https://doi.org/10.1111/mec.15337.

Emery, M. A., B. A. Dimos, and L. D. Mydlarz. 2021. "Cnidarian Pattern Recognition Receptor Repertoires Reflect Both Phylogeny and Life History Traits." *Frontiers in Immunology* 12: 689463. https://doi.org/10.3389/fimmu.2021.689463.

Emslie, M. J., M. Logan, P. Bray, et al. 2024. "Increasing Disturbance Frequency Undermines Coral Reef Recovery." *Ecological Monographs* 94, no. 3: e1619. https://doi.org/10.1002/ecm.1619.

Epstein, H. E., H. A. Smith, G. Torda, and M. J. Van Oppen. 2019. "Microbiome Engineering: Enhancing Climate Resilience in Corals." *Frontiers in Ecology and the Environment* 17, no. 2: 100–108. https://doi.org/10.1002/fee.2001.

Fujise, L., H. Yamashita, G. Suzuki, K. Sasaki, L. M. Liao, and K. Koike. 2014. "Moderate Thermal Stress Causes Active and Immediate Expulsion of Photosynthetically Damaged Zooxanthellae (*Symbiodinium*) From Corals." *PLoS One* 9, no. 12: e114321. https://doi.org/10.1371/journal.pone.0114321.

Fukuda, S., H. Toh, K. Hase, et al. 2011. "Bifidobacteria Can Protect From Enteropathogenic Infection Through Production of Acetate." *Nature* 469, no. 7331: 543–547. https://doi.org/10.1038/nature09646.

Fuller, Z. L., V. J. L. Mocellin, L. A. Morris, et al. 2020. "Population Genetics of the Coral *Acropora millepora*: Toward Genomic Prediction of Bleaching." *Science* 369, no. 6501: eaba4674. https://doi.org/10.1126/science.aba4674.

Gajigan, A. P., L. A. Diaz, and C. Conaco. 2017. "Resilience of the Prokaryotic Microbial Community of *Acropora digitifera* to Elevated Temperature." *MicrobiologyOpen* 6, no. 4: e00478. https://doi.org/10.1002/mbo3.478.

Gao, C., M. Garren, K. Penn, et al. 2021. "Coral Mucus Rapidly Induces Chemokinesis and Genome-Wide Transcriptional Shifts Toward Early Pathogenesis in a Bacterial Coral Pathogen." *ISME Journal* 15, no. 12: 3668–3682. https://doi.org/10.1038/s41396-021-01024-7.

Garren, M., K. Son, J. Raina, et al. 2013. "A Bacterial Pathogen Uses Dimethylsulfoniopropionate as a Cue to Target Heat-Stressed Corals." *ISME Journal* 8, no. 5: 999–1007. https://doi.org/10.1038/ismej.2013.210.

Garrido, A. G., L. F. Machado, C. Zilberberg, and D. C. Leite. 2020. "Insights Into '*Symbiodiniaceae phycosphere*' in a Coral Holobiont." *Symbiosis* 83, no. 1: 25–39. https://doi.org/10.1007/s13199-020-00735-3.

Giddens, J., D. R. Kobayashi, G. N. Mukai, et al. 2022. "Assessing the Vulnerability of Marine Life to Climate Change in the Pacific Islands Region." *PLoS One* 17, no. 7: e0270930. https://doi.org/10.1371/journal.pone.0270930.

Gignoux-Wolfsohn, S. A., and S. V. Vollmer. 2015. "Correction: Identification of Candidate Coral Pathogens on White Band Disease-Infected Staghorn Coral." *PLoS One* 10, no. 11: e0142760. https://doi.org/10.1371/journal.pone.0142760.

Glasl, B., C. E. Smith, D. G. Bourne, and N. S. Webster. 2019. "Disentangling the Effect of Host-Genotype and Environment on the Microbiome of the Coral *Acropora tenuis*." *PeerJ* 7: e6377. https://doi.org/10.7717/peerj.6377.

Godoy-Vitorino, F., C. P. Ruiz-Diaz, A. Rivera-Seda, J. S. Ramírez-Lugo, and C. Toledo-Hernández. 2017. "The Microbial Biosphere of the Coral *Acropora cervicornis* in Northeastern Puerto Rico." *PeerJ* 5: e3717. https://doi.org/10.7717/peerj.3717.

Gong, S., X. Jin, L. Ren, Y. Tan, and X. Xia. 2020. "Unraveling Heterogeneity of Coral Microbiome Assemblages in Tropical and Subtropical Corals in the South China Sea." *Microorganisms* 8, no. 4: 604. https://doi.org/10.3390/microorganisms8040604.

Grottoli, A. G., P. Dalcin Martins, M. J. Wilkins, et al. 2018. "Coral Physiology and Microbiome Dynamics Under Combined Warming and Ocean Acidification." *PLoS One* 13, no. 1: e0191156. https://doi.org/10.1371/journal.pone.0191156.

Helgoe, J., S. K. Davy, V. M. Weis, and M. Rodriguez-Lanetty. 2024. "Triggers, Cascades, and Endpoints: Connecting the Dots of Coral Bleaching Mechanisms." *Biological Reviews* 99, no. 3: 715–752. https://doi.org/10.1111/brv.13042.

Henley, B. J., H. V. McGregor, A. D. King, et al. 2024. "Highest Ocean Heat in Four Centuries Places Great Barrier Reef in Danger." *Nature* 632, no. 8024: 320–326. https://doi.org/10.1038/s41586-024-07672-x.

Herlemann, D. P., M. Labrenz, K. Jürgens, S. Bertilsson, J. J. Waniek, and A. F. Andersson. 2011. "Transitions in Bacterial Communities Along the 2000 km Salinity Gradient of the Baltic Sea." *ISME Journal* 5, no. 10: 1571–1579. https://doi.org/10.1038/ismej.2011.41.

Hoegh-Guldberg, O. 1999. "Climate Change, Coral Bleaching and the Future of the World's Coral Reefs." *Marine and Freshwater Research* 50, no. 8: 839–866. https://doi.org/10.1071/mf99078.

Hsieh, Y. E., L. Chih-Ying, P. Y. Liu, et al. 2024. "Successive Responses of Three Coral holobiont Components (coral Hosts, Symbiotic Algae, and Bacteria) to Daily Temperature Fluctuations." *Ecological Indicators* 158: 111515. https://doi.org/10.1016/j.ecolind.2023.111515.

Hughes, T. P., K. D. Anderson, S. R. Connolly, et al. 2018. "Spatial and Temporal Patterns of Mass Bleaching of Corals in the Anthropocene." *Science* 359, no. 6371: 80–83. https://doi.org/10.1126/science.aan8048.

Humanes, A., E. A. Beauchamp, J. C. Bythell, et al. 2021. "An Experimental Framework for Selectively Breeding Corals for Assisted Evolution." *Frontiers in Marine Science* 8: 669995. https://www.frontiersin.org/articles/10.3389/fmars.2021.669995.

Humanes, A., L. Lachs, E. Beauchamp, et al. 2024. "Selective Breeding Enhances Coral Heat Tolerance to Marine Heatwaves." *Nature Communications* 15, no. 1: 8703. https://doi.org/10.1038/s41467-024-52895-1.

Humanes, A., L. Lachs, E. A. Beauchamp, et al. 2022. "Within-Population Variability in Coral Heat Tolerance Indicates Climate Adaptation Potential." *Proceedings of the Royal Society B: Biological Sciences* 289, no. 1981: 20220872. https://doi.org/10.1098/rspb.2022.0872.

Hume, B. C. C., E. G. Smith, M. Ziegler, et al. 2019. "SymPortal: A Novel Analytical Framework and Platform for Coral Algal Symbiont Next-Generation Sequencing ITS2 Profiling." *Molecular Ecology Resources* 19, no. 4: 1063–1080. https://doi.org/10.1111/1755-0998.13004.

Jones, A., and R. Berkelmans. 2010. "Potential Costs of Acclimatization to a Warmer Climate: Growth of a Reef Coral With Heat Tolerant vs. Sensitive Symbiont Types." *PLoS One* 5, no. 5: e10437. https://doi.org/10.1371/journal.pone.0010437.

Jones, A., R. Berkelmans, M. Van Oppen, J. Mieog, and W. Sinclair. 2008. "A Community Change in the Algal Endosymbionts of a Scleractinian Coral Following a Natural Bleaching Event: Field Evidence of Acclimatization." *Proceedings of the Royal Society B: Biological Sciences* 275, no. 1641: 1359–1365. https://doi.org/10.1098/rspb.2008.0069.

Katoh, K., and D. M. Standley. 2013. "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability." *Molecular Biology and Evolution* 30, no. 4: 772–780. https://doi.org/10.1093/molbev/mst010.

Kemp, D. W., K. D. Hoadley, A. M. Lewis, et al. 2023. "Thermotolerant Coral–Algal Mutualisms Maintain High Rates of Nutrient Transfer While Exposed to Heat Stress." *Proceedings of the Royal Society B: Biological Sciences* 290, no. 2007: 20231403. https://doi.org/10.1098/rspb.2023.1403.

Klein, S. G., C. Roch, and C. M. Duarte. 2024. "Systematic Review of the Uncertainty of Coral Reef Futures Under Climate Change." *Nature Communications* 15, no. 1: 2224. https://doi.org/10.1038/s41467-024-46255-2.

Klepac, C. N., and D. J. Barshis. 2020. "Reduced Thermal Tolerance of Massive Coral Species in a Highly Variable Environment." *Proceedings of the Royal Society B: Biological Sciences* 287, no. 1933: 20201379. https://doi.org/10.1098/rspb.2020.1379.

Krediet, C. J., K. B. Ritchie, V. J. Paul, and M. Teplitski. 2013. "Coral-Associated Micro-Organisms and Their Roles in Promoting Coral Health and Thwarting Diseases." *Proceedings of the Royal Society B: Biological Sciences* 280, no. 1755: 20122328. https://doi.org/10.1098/rspb.2012.2328.

Kusdianto, H., C. Kullapanich, M. Palasuk, et al. 2021. "Microbiomes of Healthy and Bleached Corals During a 2016 Thermal Bleaching Event in the Upper Gulf of Thailand." *Frontiers in Marine Science* 8: 643962. https://doi.org/10.3389/fmars.2021.643962.

LaJeunesse, T. 2002. "Diversity and Community Structure of Symbiotic Dinoflagellates From Caribbean Coral Reefs." *Marine Biology* 141, no. 2: 387–400. https://doi.org/10.1007/s00227-002-0829-2.

Langille, M. G., J. Zaneveld, J. G. Caporaso, et al. 2013. "Predictive Functional Profiling of Microbial Communities Using 16S rRNA Marker Gene Sequences." *Nature Biotechnology* 31, no. 9: 814–821. https://doi.org/10.1038/nbt.2676.

Lee, S. T., S. K. Davy, S. Tang, and P. S. Kench. 2016. "Mucus Sugar Content Shapes the Bacterial Community Structure in Thermally Stressed Acropora muricata." *Frontiers in Microbiology* 7: 371. https://doi.org/10.3389/fmicb.2016.00371.

Leinbach, S. E., K. E. Speare, and M. E. Strader. 2022. "Reef Habitats Structure Symbiotic Microalgal Assemblages in Corals and Contribute to Differential Heat Stress Responses." *Coral Reefs* 42, no. 1: 205–217. https://doi.org/10.1007/s00338-022-02316-w.

Li, J., F. Azam, and S. Zhang. 2016. "Outer Membrane Vesicles Containing Signalling Molecules and Active Hydrolytic Enzymes Released by a Coral Pathogen *Vibrio shilonii* ak1." *Environmental Microbiology* 1811: 3850–3866. https://doi.org/10.1111/1462-2920.13344.

Li, J., Q. Yang, J. Dong, et al. 2022. "Microbiome Engineering: A Promising Approach to Improve Coral Health." *Engineering* 28: 105–116. https://doi.org/10.1016/j.eng.2022.07.010.

Li, J., Y. Zou, Q. Li, et al. 2023. "A Coral-Associated Actinobacterium Mitigates Coral Bleaching Under Heat Stress." *Environmental Microbiomes* 18, no. 1: 83. https://doi.org/10.1186/s40793-023-00540-7.

Lima, L. F., A. T. Alker, M. M. Morris, R. A. Edwards, S. J. De Putron, and E. A. Dinsdale. 2024. "Pre-Bleaching Coral Microbiome Is Enriched in Beneficial Taxa and Functions." *Microorganisms* 12, no. 5: 1005. https://doi.org/10.3390/microorganisms12051005.

Lin, H., and S. D. Peddada. 2020. "Analysis of Compositions of Microbiomes With Bias Correction." *Nature Communications* 11, no. 1: 3514. https://doi.org/10.1038/s41467-020-17041-7.

Lin, H., and S. D. Peddada. 2024. "Multigroup Analysis of Compositions of Microbiomes With Covariate Adjustments and Repeated Measures." *Nature Methods* 21, no. 1: 83–91. https://doi.org/10.1038/s41592-023-02092-7.

Lough, J. M., K. D. Anderson, and T. P. Hughes. 2018. "Increasing Thermal Stress for Tropical Coral Reefs: 1871–2017." *Scientific Reports* 8, no. 1: 6079. https://doi.org/10.1038/s41598-018-24530-9.

Marchioro, G. M., B. Glasl, A. H. Engelen, et al. 2020. "Microbiome Dynamics in the Tissue and Mucus of Acroporid Corals Differ in Relation to Host and Environmental Parameters." *PeerJ* 8: e9644. https://doi.org/10.7717/peerj.9644.

Matthews, J. L., A. Khalil, N. Siboni, et al. 2023. "Coral Endosymbiont Growth Is Enhanced by Metabolic Interactions With Bacteria." *Nature Communications* 14, no. 1: 6864. https://doi.org/10.1038/s41467-023-42663-y.

Matthews, J. L., J. Raina, T. Kahlke, J. R. Seymour, M. J. Oppen, and D. J. Suggett. 2020. "Symbiodiniaceae–Bacteria Interactions: Rethinking Metabolite Exchange in Reef-Building Corals as Multi-Partner Metabolic Networks." *Environmental Microbiology* 22, no. 5: 1675–1687. https://doi.org/10.1111/1462-2920.14918.

McDevitt-Irwin, J. M., J. K. Baum, M. Garren, and R. L. Vega Thurber. 2017. "Responses of Coral-Associated Bacterial Communities to Local and Global Stressors." *Frontiers in Marine Science* 4: 262. https://doi.org/10.3389/fmars.2017.00262.

Mclachlan, R., and A. Grottoli. 2021. "Image Analysis to Quantify Coral Bleaching Using Greyscale Model." Protocols.io. https://www.protocols.io/view/image-analysis-to-quantify-coral-bleaching-using-g-bx8wprxe.

McWilliam, M., J. S. Madin, T. J. Chase, M. O. Hoogenboom, and T. C. L. Bridge. 2022. "Intraspecific Variation Reshapes Coral Assemblages Under Elevated Temperature and Acidity." *Ecology Letters* 25, no. 11: 2513–2524. https://doi.org/10.1111/ele.14114.

Meenatchi, R., T. Thinesh, P. Brindangnanam, S. Hassan, G. S. Kiran, and J. Selvin. 2020. "Revealing the Impact of Global Mass Bleaching on Coral Microbiome Through 16S rRNA Gene-Based Metagenomic Analysis." *Microbiological Research* 233: 126408. https://doi.org/10.1016/j.micres.2019.126408.

Meron, D., R. Efrony, W. R. Johnson, et al. 2009. "Role of Flagella in Virulence of the Coral Pathogen *Vibrio coralliilyticus*." *Applied and Environmental Microbiology* 75, no. 17: 5704–5707. https://doi.org/10.1128/aem.00198-09.

Messer, L. F., D. G. Bourne, S. J. Robbins, et al. 2024. "A Genome-Centric View of the Role of the *Acropora kenti* Microbiome in Coral Health and Resilience." *Nature Communications* 15, no. 1: 2902. https://doi.org/10.1038/s41467-024-46905-5.

Meyer, J. L., J. Castellanos-Gell, G. S. Aeby, C. C. Häse, B. Ushijima, and V. J. Paul. 2019. "Microbial Community Shifts Associated With the Ongoing Stony Coral Tissue Loss Disease Outbreak on the Florida Reef Tract." *Frontiers in Microbiology* 10: 2244. https://doi.org/10.3389/fmicb.2019.02244.

Miller, A. W., and L. L. Richardson. 2014. "Emerging Coral Diseases: A Temperature-Driven Process?" *Marine Ecology* 36, no. 3: 278–291. https://doi.org/10.1111/maec.12142.

Miller, J., E. Muller, C. Rogers, et al. 2009. "Coral Disease Following Massive Bleaching in 2005 Causes 60% Decline in Coral Cover on Reefs in the US Virgin Islands." *Coral Reefs* 28, no. 4: 925–937. https://doi.org/10.1007/s00338-009-0531-7.

Minh, B. Q., M. A. Nguyen, and A. Von Haeseler. 2013. "Ultrafast Approximation for Phylogenetic Bootstrap." *Molecular Biology and Evolution* 30, no. 5: 1188–1195. https://doi.org/10.1093/molbev/mst024.

Minh, B. Q., H. A. Schmidt, O. Chernomor, et al. 2020. "IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era." *Molecular Biology and Evolution* 37, no. 5: 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Mohamed, A. R., M. A. Ochsenkühn, A. M. Kazlak, A. Moustafa, and S. A. Amin. 2023. "The Coral Microbiome: Towards an Understanding of the Molecular Mechanisms of Coral–Microbiota Interactions." *FEMS Microbiology Reviews* 47, no. 2: fuad005. https://doi.org/10.1093/femsre/fuad005.

Morikawa, M. K., and S. R. Palumbi. 2019. "Using Naturally Occurring Climate Resilient Corals to Construct Bleaching-Resistant Nurseries." *Proceedings of the National Academy of Sciences* 116, no. 21: 10586–10591. https://doi.org/10.1073/pnas.1721415116.

Muller, E. M., E. Bartels, and I. B. Baums. 2018. "Bleaching Causes Loss of Disease Resistance Within the Threatened Coral Species *Acropora cervicornis*." *eLife* 7: e35066. https://doi.org/10.7554/elife.35066.

Naugle, M. S., H. Denis, V. J. Mocellin, et al. 2024. "Heat Tolerance Varies Considerably Within a Reef-Building Coral Species on the Great Barrier Reef." *Communications Earth & Environment* 5, no. 1: 525. https://doi.org/10.1038/s43247-024-01649-4.

Naugle, M. S., T. A. Oliver, D. J. Barshis, R. D. Gates, and C. A. Logan. 2021. "Variation in Coral Thermotolerance Across a Pollution Gradient Erodes as Coral Symbionts Shift to More Heat-Tolerant Genera." *Frontiers in Marine Science* 8: 760891. https://doi.org/10.3389/fmars.2021.760891.

Neal, B. P., A. Khen, T. Treibitz, et al. 2017. "Caribbean Massive Corals Not Recovering From Repeated Thermal Stress Events During 2005–2013." *Ecology and Evolution* 7, no. 5: 1339–1353. https://doi.org/10.1002/ece3.2706.

Newton, R. J., and S. L. McLellan. 2015. "A Unique Assemblage of Cosmopolitan Freshwater Bacteria and Higher Community Diversity Differentiate an Urbanized Estuary From Oligotrophic Lake Michigan." *Frontiers in Microbiology* 6: 1028. https://doi.org/10.3389/fmicb.2015.01028.

Nguyen, D. H., N. H. Chu, Y. Bettarel, et al. 2023. "Metagenomic Data of Bacterial Communities Associated With *Acropora* Species From Phu Quoc Islands, Vietnam." *Data in Brief* 47: 108977. https://doi.org/10.1016/j.dib.2023.108977.

Oakley, C. A., and S. K. Davy. 2018. "Cell Biology of Coral Bleaching." In *Coral Bleaching. Ecological Studies*, edited by M. van Oppen and J. Lough, vol. 233. Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-319-75393-5_8.

Ochsenkühn, M. A., A. R. Mohamed, T. D. Haydon, L. S. Coe, D. Abrego, and S. A. Amin. 2023. "Endozoicomonas Provides Corals With Steroid Hormones During Thermal Stress." *bioRxiv* 2023.09.19.558257. https://doi.org/10.1101/2023.09.19.558257.

Oliver, T. A., and S. R. Palumbi. 2011. "Do Fluctuating Temperature Environments Elevate Coral Thermal Tolerance?" *Coral Reefs* 30, no. 2: 429–440. https://doi.org/10.1007/s00338-011-0721-y.

Ota, R., P. J. Waddell, M. Hasegawa, H. Shimodaira, and H. Kishino. 2000. "Appropriate Likelihood Ratio Tests and Marginal Distributions for Evolutionary Tree Models With Constraints on Parameters." *Molecular Biology and Evolution* 17, no. 5: 798–803. https://doi.org/10.1093/oxfordjournals.molbev.a026358.

Palacio-Castro, A. M., S. M. Rosales, C. E. Dennison, and A. C. Baker. 2022. "Microbiome Signatures in *Acropora cervicornis* Are Associated With Genotypic Resistance to Elevated Nutrients and Heat Stress." *Coral Reefs* 41: 1389–1403. https://doi.org/10.1101/2022.05.02.490297.

Palacio-Castro, A. M., T. B. Smith, V. Brandtneris, et al. 2023. "Increased Dominance of Heat-Tolerant Symbionts Creates Resilient Coral Reefs in Near-Term Ocean Warming." *Proceedings of the National Academy of Sciences* 120, no. 8: e2202388120. https://doi.org/10.1073/pnas.2202388120.

Peixoto, R. S., P. M. Rosado, D. C. Leite, A. S. Rosado, and D. G. Bourne. 2017. "Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience." *Frontiers in Microbiology* 8: 341. https://doi.org/10.3389/fmicb.2017.00341.

Penin, L., M. Adjeroud, M. Schrimm, and H. S. Lenihan. 2007. "High Spatial Variability in Coral Bleaching Around Moorea (French Polynesia): Patterns Across Locations and Water Depths." *Comptes Rendus Biologies* 330, no. 2: 171–181. https://doi.org/10.1016/j.crvi.2006.12.003.

Pereira, P. H., G. V. Lima, A. V. Pontes, et al. 2022. "Unprecedented Coral Mortality on Southwestern Atlantic Coral Reefs Following Major Thermal Stress." *Frontiers in Marine Science* 9: 725778. https://doi.org/10.3389/fmars.2022.725778.

Picard, C., J. Fioramonti, A. Francois, T. Robinson, F. Neant, and C. Matuchnasky. 2005. "Review Article: Bifidobacteria as Probiotic Agents—Physiological Effects and Clinical Benefits." *Alimentary Pharmacology & Therapeutics* 22: 495–512. https://doi.org/10.1111/j.1365-2036.2005.02615.x.

Pogoreutz, C., C. A. Oakley, N. Rädecker, et al. 2022. "Coral Holobiont Cues Prime *Endozoicomonas* for a Symbiotic Lifestyle." *ISME Journal* 16, no. 8: 1883–1895. https://doi.org/10.1038/s41396-022-01226-7.

Pogoreutz, C., and M. Ziegler. 2024. "Frenemies on the Reef? Resolving the Coral–*Endozoicomonas* Association." *Trends in Microbiology* 32, no. 5: 422–434. https://doi.org/10.1016/j.tim.2023.11.006.

Pootakham, W., W. Mhuantong, T. Yoocha, et al. 2019. "Heat-Induced Shift in Coral Microbiome Reveals Several Members of the Rhodobacteraceae Family as Indicator Species for Thermal Stress in *Porites lutea*." *MicrobiologyOpen* 8, no. 12: e935. https://doi.org/10.1002/mbo3.935.

Posadas, N., P. Jake Ivan, M. A. Baquiran, L. Nada, M. Kelly, and C. Conaco. 2022. "Microbiome Diversity and Host Immune Functions Influence Survivorship of Sponge holobionts Under Future Ocean Conditions." *ISME Journal* 16, no. 1: 58–67. https://doi.org/10.1038/s41396-021-01050-5.

Pruitt, K. D. 2004. "NCBI Reference Sequence (RefSeq): A Curated Non-Redundant Sequence Database of Genomes, Transcripts and Proteins." *Nucleic Acids Research* 33, no. Database issue: D501–D504. https://doi.org/10.1093/nar/gki025.

Quast, C., E. Pruesse, P. Yilmaz, et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41, no. Database issue: D590–D596. https://doi.org/10.1093/nar/gks1219.

Quigley, K. M., C. Alvarez Roa, G. Torda, D. G. Bourne, and B. L. Willis. 2019. "Co-Dynamics of Symbiodiniaceae and Bacterial Populations During the First Year of Symbiosis With Acroporatenuis Juveniles." *MicrobiologyOpen* 9, no. 2: e959. https://doi.org/10.1002/mbo3.959.

Quigley, K. M., B. Ramsby, P. Laffy, J. Harris, V. J. Mocellin, and L. K. Bay. 2022. "Symbioses Are Restructured by Repeated Mass Coral Bleaching. Science." *Advances* 8, no. 49: eabq8349. https://doi.org/10.1126/sciadv.abq8349.

Quimpo, T. J., J. N. Requilme, E. J. Gomez, S. L. Sayco, M. P. Tolentino, and P. C. Cabaitan. 2020. "Low Coral Bleaching Prevalence at the Bolinao-Anda Reef Complex, Northwestern Philippines During the 2016 Thermal Stress Event." *Marine Pollution Bulletin* 160: 111567. https://doi.org/10.1016/j.marpolbul.2020.111567.

Rädecker, N., C. Pogoreutz, H. M. Gegner, et al. 2021. "Heat Stress Destabilizes Symbiotic Nutrient Cycling in Corals." *Proceedings of the National Academy of Sciences* 118, no. 5: e2022653118. https://doi.org/10.1073/pnas.2022653118.

Raimundo, I., S. G. Silva, R. Costa, and T. Keller-Costa. 2018. "Bioactive Secondary Metabolites From Octocoral-Associated Microbes—New Chances for Blue Growth." *Marine Drugs* 16, no. 12: 485. https://doi.org/10.3390/md16120485.

Ramos, H. C., M. Rumbo, and J. Sirard. 2004. "Bacterial Flagellins: Mediators of Pathogenicity and Host Immune Responses in Mucosa." *Trends in Microbiology* 12, no. 11: 509–517. https://doi.org/10.1016/j.tim.2004.09.002.

Reigel, A. M., D. A. Paz-García, and M. E. Hellberg. 2021. "Microbiome of a Reef-Building Coral Displays Signs of Acclimation to a Stressful Shallow Hydrothermal Vent Habitat." *Frontiers in Marine Science* 8: 652633. https://doi.org/10.3389/fmars.2021.652633.

Ridley, C. P., H. Y. Lee, and C. Khosla. 2008. "Evolution of Polyketide Synthases in Bacteria." *Proceedings of the National Academy of Sciences* 105, no. 12: 4595–4600. https://doi.org/10.1073/pnas.0710107105.

Ritchie, K. 2006. "Regulation of Microbial Populations by Coral Surface Mucus and Mucus-Associated Bacteria." *Marine Ecology Progress Series* 322: 1–14. https://doi.org/10.3354/meps322001.

Rosado, P. M., P. M. Cardoso, J. G. Rosado, et al. 2023. "Exploring the Potential Molecular Mechanisms of Interactions Between a Probiotic Consortium and Its Coral Host." *mSystems* 8, no. 1: e0092122. https://doi.org/10.1128/msystems.00921-22.

Rosado, P. M., D. C. Leite, G. A. Duarte, et al. 2019. "Marine Probiotics: Increasing Coral Resistance to Bleaching Through Microbiome Manipulation." *ISME Journal* 13, no. 4: 921–936. https://doi.org/10.1038/s41396-018-0323-6.

Rosales, S. M., L. K. Huebner, J. S. Evans, et al. 2023. "A Meta-Analysis of the Stony Coral Tissue Loss Disease Microbiome Finds Key Bacteria in Unaffected and Lesion Tissue in Diseased Colonies." *ISME Communications* 3, no. 1: 19. https://doi.org/10.1038/s43705-023-00220-0.

Rosales, S. M., M. W. Miller, D. E. Williams, N. Traylor-Knowles, B. Young, and X. M. Serrano. 2019. "Microbiome Differences in Disease-Resistant vs. Susceptible Acropora Corals Subjected to Disease Challenge Assays." *Scientific Reports* 9, no. 1: 18279. https://doi.org/10.1038/s41598-019-54855-y.

Rose, N. H., R. A. Bay, M. K. Morikawa, L. Thomas, E. A. Sheets, and S. R. Palumbi. 2021. "Genomic Analysis of Distinct Bleaching Tolerances Among Cryptic Coral Species." *Proceedings of the Royal Society B: Biological Sciences* 288, no. 1960: 20210678. https://doi.org/10.1098/rspb.2021.0678.

Rubio-Portillo, E., A. B. Martin-Cuadrado, A. M. Caraballo-Rodríguez, F. Rohwer, P. C. Dorrestein, and J. Antón. 2020. "Virulence as a Side Effect of Interspecies Interaction in Vibrio Coral Pathogens." *MBio* 11, no. 4: e00201-20. https://doi.org/10.1128/mbio.00201-20.

Santoro, E. P., R. M. Borges, J. L. Espinoza, et al. 2021. "Coral Microbiome Manipulation Elicits Metabolic and Genetic Restructuring to Mitigate Heat Stress and Evade Mortality." *Science Advances* 7, no. 33: eabg3088. https://doi.org/10.1126/sciadv.abg3088.

Schul, M. D., D. Anastasious, L. J. Spiers, J. L. Meyer, T. K. Frazer, and A. L. Brown. 2023. "Concordance of Microbial and Visual Health Indicators of White-Band Disease in Nursery Reared Caribbean Coral *Acropora cervicornis*." *PeerJ* 11: e15170. https://doi.org/10.7717/peerj.15170.

Shiu, J., S. Keshavmurthy, P. Chiang, et al. 2017. "Dynamics of Coral-Associated Bacterial Communities Acclimated to Temperature Stress Based on Recent Thermal History." *Scientific Reports* 7, no. 1: 14933. https://doi.org/10.1038/s41598-017-14927-3.

Siebeck, U. E., N. J. Marshall, A. Klüter, and O. Hoegh-Guldberg. 2006. "Monitoring Coral Bleaching Using a Colour Reference Card." *Coral Reefs* 25, no. 3: 453–460. https://doi.org/10.1007/s00338-006-0123-8.

Steenwyk, J. L., T. J. Buida, Y. Li, X. Shen, and A. Rokas. 2020. "ClipKIT: A Multiple Sequence Alignment Trimming Software for Accurate Phylogenomic Inference." *PLoS Biology* 18, no. 12: e3001007. https://doi.org/10.1371/journal.pbio.3001007.

Su, H., Z. Xiao, K. Yu, et al. 2021. "High Diversity of β-Glucosidase-Producing Bacteria and Their Genes Associated With Scleractinian Corals." *International Journal of Molecular Sciences* 22, no. 7: 3523. https://doi.org/10.3390/ijms22073523.

Sully, S., D. E. Burkepile, M. K. Donovan, G. Hodgson, and R. Van Woesik. 2019. "A Global Analysis of Coral Bleaching Over the Past Two Decades." *Nature Communications* 10, no. 1: 1264. https://doi.org/10.1038/s41467-019-09238-2.

Sun, F., H. Yang, X. Zhang, F. Tan, and Q. Shi. 2022. "Response Characteristics of Bacterial Communities in Multiple Coral Genera at the Early Stages of Coral Bleaching During El Nino." *Ecological Indicators* 144: 109569. https://doi.org/10.1016/j.ecolind.2022.109569.

Sweet, M., A. Burian, J. Fifer, M. Bulling, D. Elliott, and L. Raymundo. 2019. "Compositional Homogeneity in the Pathobiome of a New, Slow-Spreading Coral Disease." *Microbiome* 7, no. 1: 139. https://doi.org/10.1186/s40168-019-0759-6.

Takahashi, S., S. Whitney, S. Itoh, T. Maruyama, and M. Badger. 2008. "Heat Stress Causes Inhibition of the de Novo Synthesis of Antenna Proteins and Photobleaching in Cultured *Symbiodinium*." *Proceedings of the National Academy of Sciences* 105, no. 11: 4203–4208. https://doi.org/10.1073/pnas.0708554105.

Tandon, K., C. Lu, P. Chiang, et al. 2020. "Comparative Genomics: Dominant Coral-Bacterium *Endozoicomonas acroporae*." *ISME Journal* 14, no. 5: 1290–1303. https://doi.org/10.1038/s41396-020-0610-x.

Thomas, L., E. H. López, M. K. Morikawa, and S. R. Palumbi. 2019. "Transcriptomic Resilience, Symbiont Shuffling, and Vulnerability to Recurrent Bleaching in Reef-Building Corals." *Molecular Ecology* 28, no. 14: 3371–3382. https://doi.org/10.1111/mec.15143.

Thompson, J. R., H. E. Rivera, C. J. Closek, and M. Medina. 2015. "Microbes in the Coral Holobiont: Partners Through Evolution, Development, and Ecological Interactions." *Frontiers in Cellular and Infection Microbiology* 4: 176. https://doi.org/10.3389/fcimb.2014.00176.

Tiirola, M., E. Valtonen, P. Rintamäki-Kinnunen, and M. Kulomaa. 2002. "Diagnosis of Flavobacteriosis by Direct Amplification of rRNA Genes." *Diseases of Aquatic Organisms* 51: 93–100. https://doi.org/10.3354/dao051093.

Tilstra, A., T. Wijgerde, F. Dini-Andreote, et al. 2017. "Light Induced Intraspecific Variability in Response to Thermal Stress in the Hard Coral *Stylophora pistillata*." *PeerJ* 5: e3802. https://doi.org/10.7717/peerj.3802.

Tout, J., T. C. Jeffries, N. S. Webster, R. Stocker, P. J. Ralph, and J. R. Seymour. 2014. "Variability in Microbial Community Composition and Function Between Different Niches Within a Coral Reef." *Microbial Ecology* 67, no. 3: 540–552. https://doi.org/10.1007/s00248-013-0362-5.

Veron, J., O. Hoegh-Guldberg, T. Lenton, et al. 2009. "The Coral Reef Crisis: The Critical Importance of <350 ppm CO_2." *Marine Pollution Bulletin* 58, no. 10: 1428–1436. https://doi.org/10.1016/j.marpolbul.2009.09.009.

Voolstra, C. R., J. Raina, M. Dörr, et al. 2024. "The Coral Microbiome in Sickness, in Health and in a Changing World." *Nature Reviews Microbiology* 22: 460–475. https://doi.org/10.1038/s41579-024-01015-3.

Weis, V. M. 2008. "Cellular Mechanisms of Cnidarian Bleaching: Stress Causes the Collapse of Symbiosis." *Journal of Experimental Biology* 211, no. 19: 3059–3066. https://doi.org/10.1242/jeb.009597.

Welsh, R. M., S. M. Rosales, J. R. Zaneveld, et al. 2017. "Alien vs. Predator: Bacterial Challenge Alters Coral Microbiomes Unless Controlled by *Halobacteriovorax* Predators." *PeerJ* 5: e3315. https://doi.org/10.7717/peerj.3315.

Wickham, H. 2016. "Create Elegant Data Visualisations Using the Grammar of Graphics. R Package Version 2.2.1," http://ggplot2.tidyverse.org.

Wilde, J., E. Slack, and K. R. Foster. 2024. "Host Control of the Microbiome: Mechanisms, Evolution, and Disease." *Science* 385, no. 6706: eadi3338. https://doi.org/10.1126/science.adi3338.

Winnepenninckx, B., T. Backeljau, and R. W. De. 1993. "Extraction of High Molecular Weight DNA From Molluscs." *Trends in Genetics* 9, no. 12: 407. https://doi.org/10.1016/0168-9525(93)90102-n.

Young, B. D., S. M. Rosales, I. C. Enochs, et al. 2023. "Different Disease Inoculations Cause Common Responses of the Host Immune System and Prokaryotic Component of the Microbiome in *Acropora palmata*." *PLoS One* 18, no. 5: e0286293. https://doi.org/10.1371/journal.pone.0286293.

Zárate, A., C. Dorador, R. Araya, et al. 2020. "Connectivity of Bacterial Assemblages Along the Loa River in the Atacama Desert, Chile." *PeerJ* 8: e9927. https://doi.org/10.7717/peerj.9927.

Zhang, Y., J. Ling, Q. Yang, et al. 2015. "The Functional Gene Composition and Metabolic Potential of Coral-Associated Microbial Communities." *Scientific Reports* 5, no. 1: 16191. https://doi.org/10.1038/srep16191.

Zhang, Y., Q. Yang, J. Ling, et al. 2021. "Shifting the Microbiome of a Coral Holobiont and Improving Host Physiology by Inoculation With a Potentially Beneficial Bacterial Consortium." *BMC Microbiology* 21, no. (1): 130. https://doi.org/10.1186/s12866-021-02167-5.

Zou, Y., J. C. H. Ip, J. Y. Xie, et al. 2024. "Dynamic Changes in Bacterial Communities in Three Species of Corals During the 2017 Bleaching Event in Subtropical Hong Kong Waters." *Marine Pollution Bulletin* 199: 116002. https://doi.org/10.1016/j.marpolbul.2023.116002.

Ziegler, M., F. O. Seneca, L. K. Yum, S. R. Palumbi, and C. R. Voolstra. 2017. "Bacterial Community Dynamics Are Linked to Patterns of Coral Heat Tolerance." *Nature Communications* 8, no. 1: 14213. https://doi.org/10.1038/ncomms14213.

Appendix A

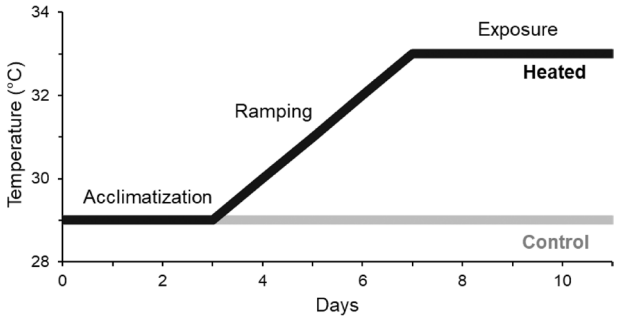

FIGURE A1 | Temperature profile for the short-term thermal stress experiment.

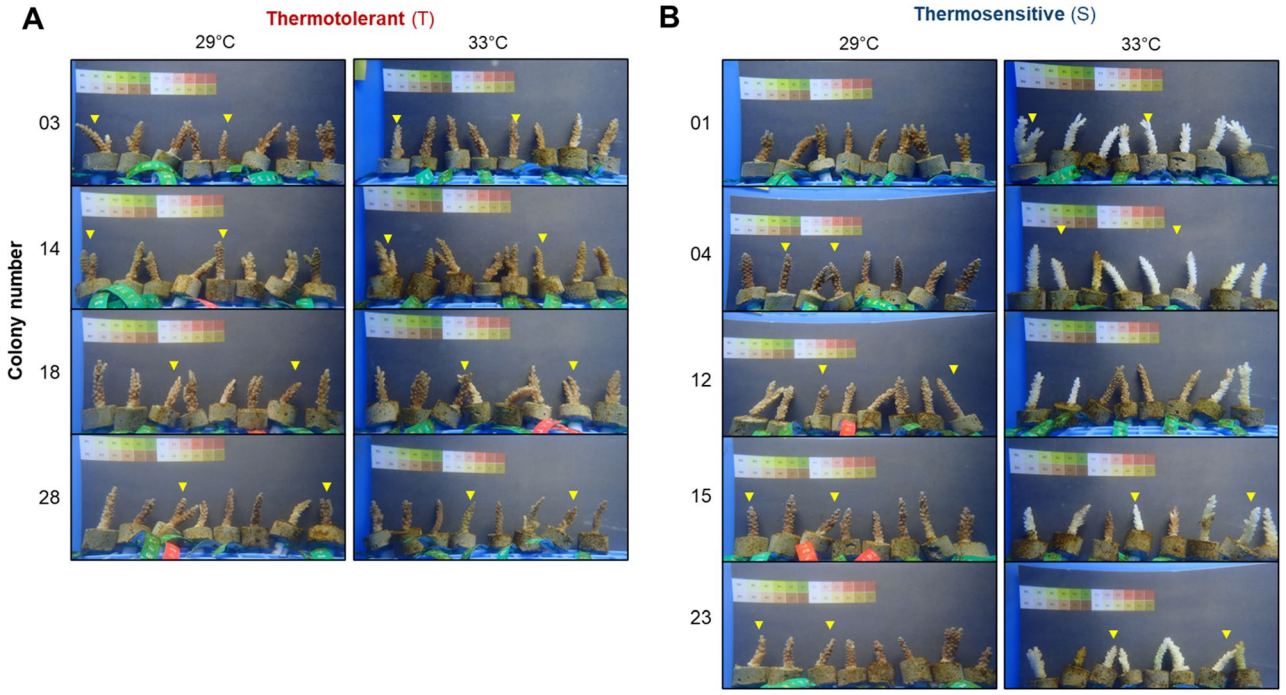

FIGURE A2 | Heat tolerance variation among *A*. cf. *tenuis* colonies selected for microbiome sequencing. Images of fragments from corals classified as (A) thermotolerant and (B) thermosensitive after 4 days of sustained exposure to 29 (ambient) or 33°C (heated). Yellow inverted triangles denote sampled fragments.

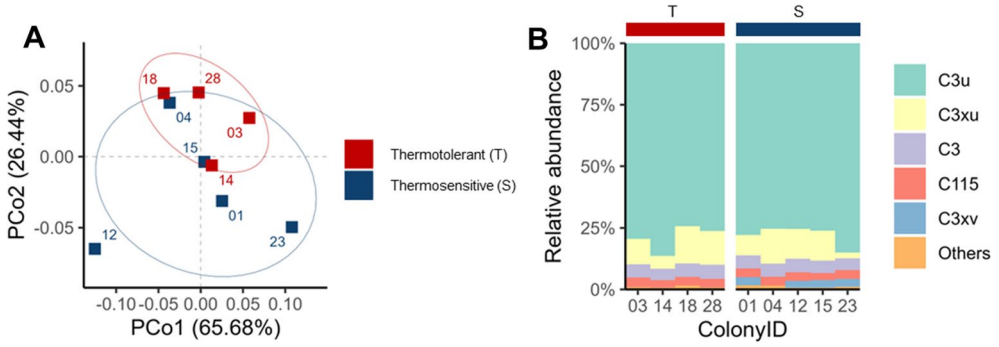

FIGURE A3 | Composition and structure of Symbiodiniaceae communities associated with thermotolerant (T) and thermosensitive (S) colonies of *Acropora* cf. *tenuis*. (A) PCoA plot of Symbiodiniaceae communities based on ITS2 sequencing. Colony IDs are shown beside the markers. Ellipses indicate a 75% confidence interval. (B) Relative abundance (≥ 1%) of ITS2 sequences in thermotolerant and thermosensitive *A*. cf. *tenuis* colonies.

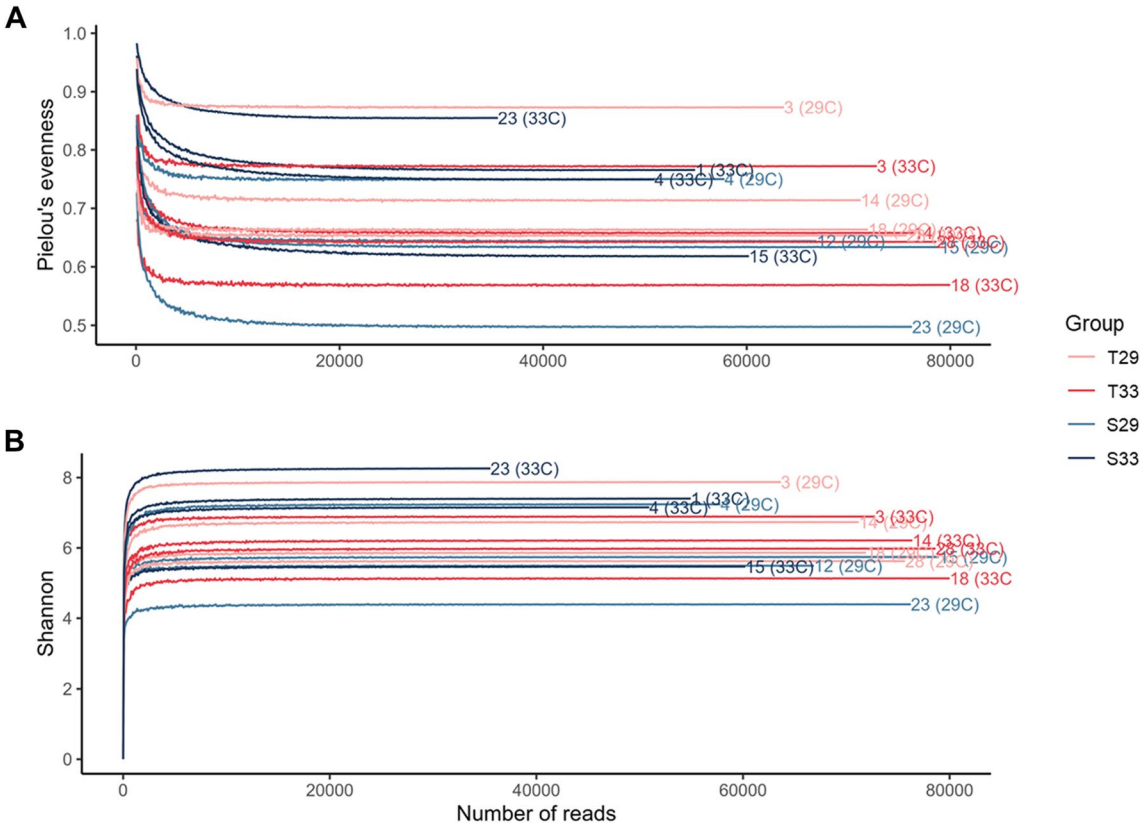

FIGURE A4 | Rarefaction curves based on (A) Pielou's evenness and (B) Shannon index.

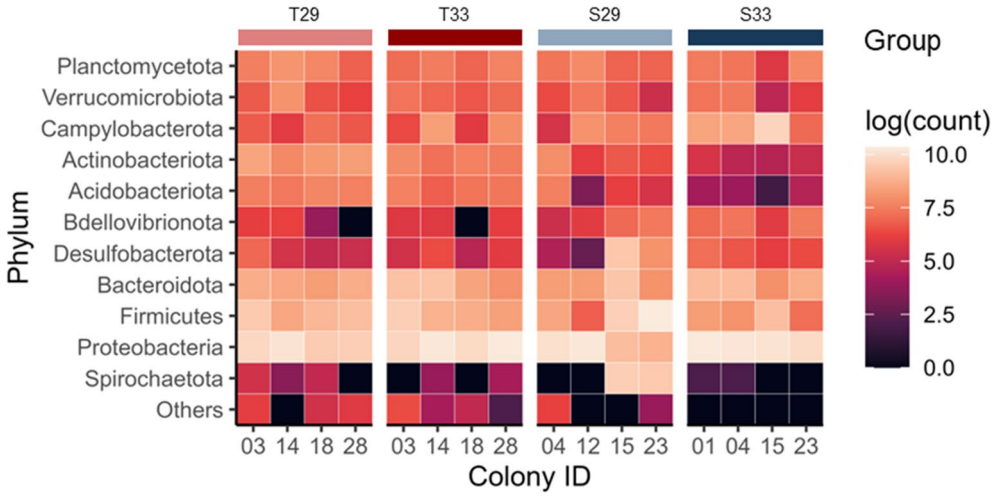

FIGURE A5 | Relative abundance of major (> 1% total relative abundance) microbial phyla in the *A*. cf. *tenuis* microbiome.

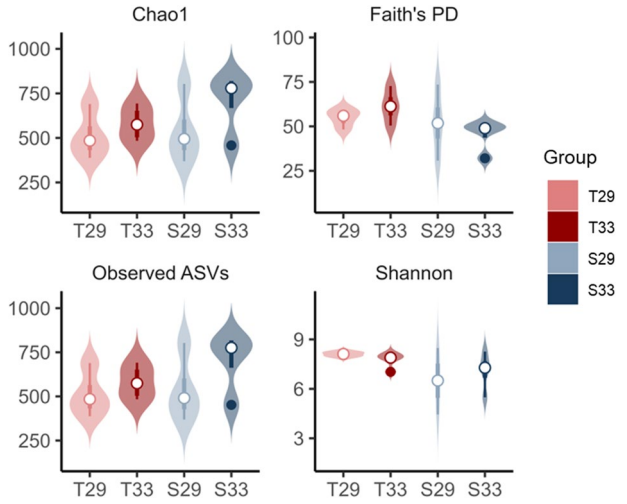

FIGURE A6 | Alpha diversity measures compared among heat tolerance classifications and temperature treatments. No significant differences were observed for all the measures among groups (Kruskal–Wallis, p-value > 0.05).

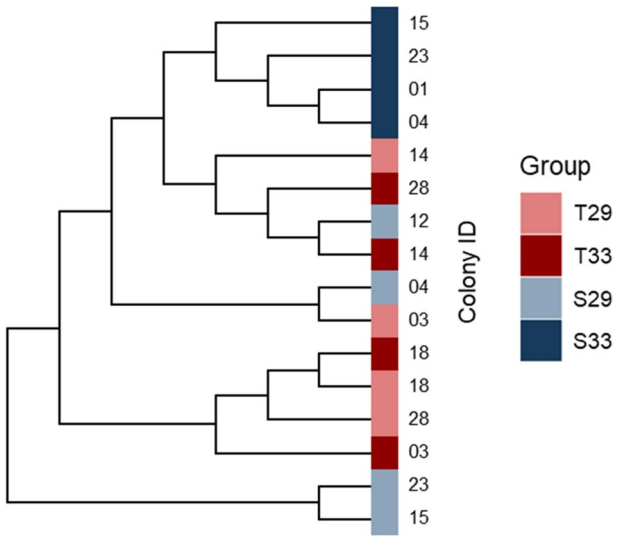

FIGURE A8 | Hierarchical clustering based on Bray–Curtis dissimilarity of microbiome communities among *A*. cf. *tenuis* colonies. Tolerance classifications and treatment groups are indicated by the coloured boxes.

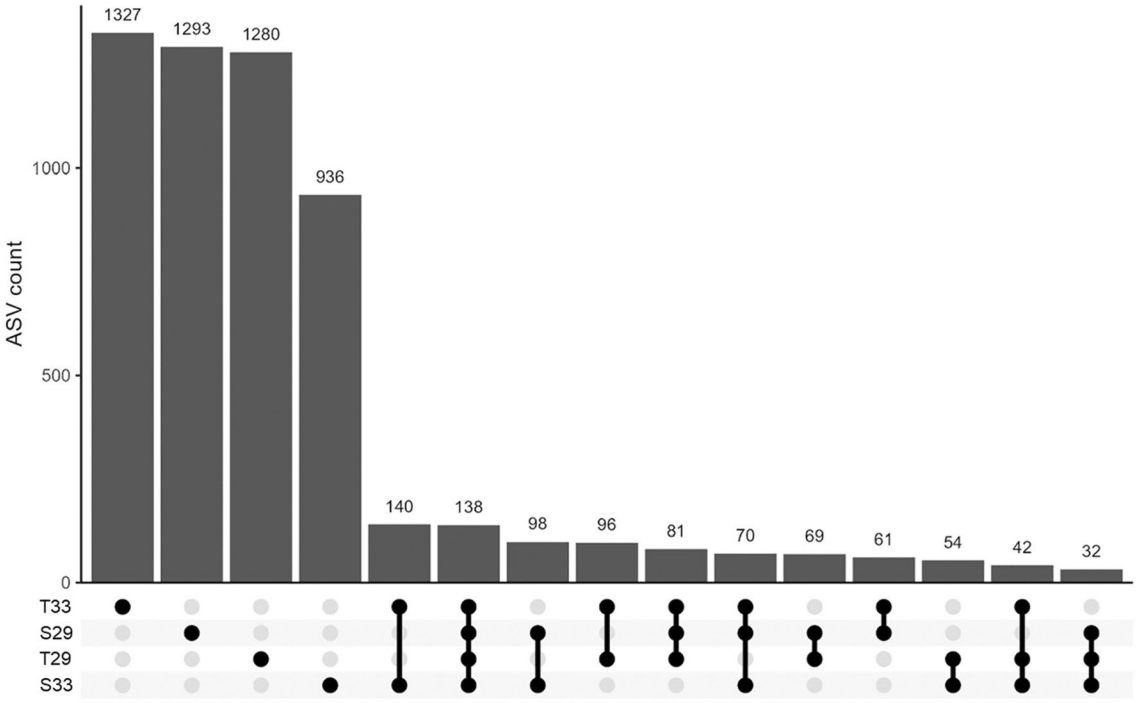

FIGURE A7 | Number of shared and unique ASVs among corals from different heat tolerance classifications and treatment groups.

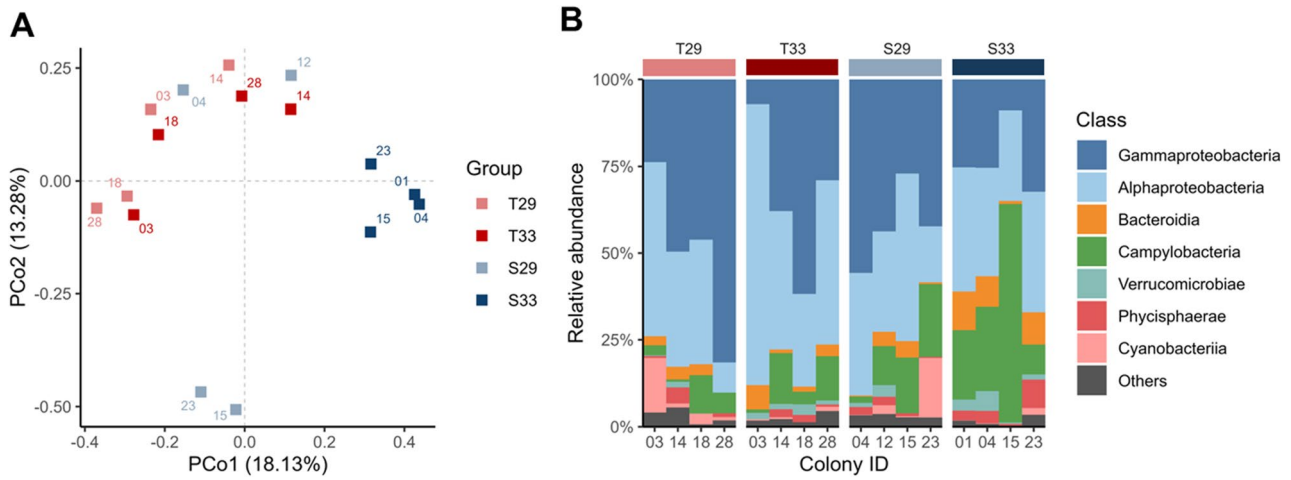

FIGURE A9 | Composition of the *A*. cf. *tenuis* core microbiome based on 16S rRNA sequencing. (A) Principal coordinates analysis (PCoA) of the core microbial communities of thermotolerant (T) or thermosensitive (S) colonies subjected to 33°C or 29°C treatments (T29, light red; T33, dark red; S29, light blue; S33, dark blue). Colony IDs are shown beside the markers. (B) Relative abundance of core ASVs in the *A*. cf. *tenuis* microbiome shown at class level.

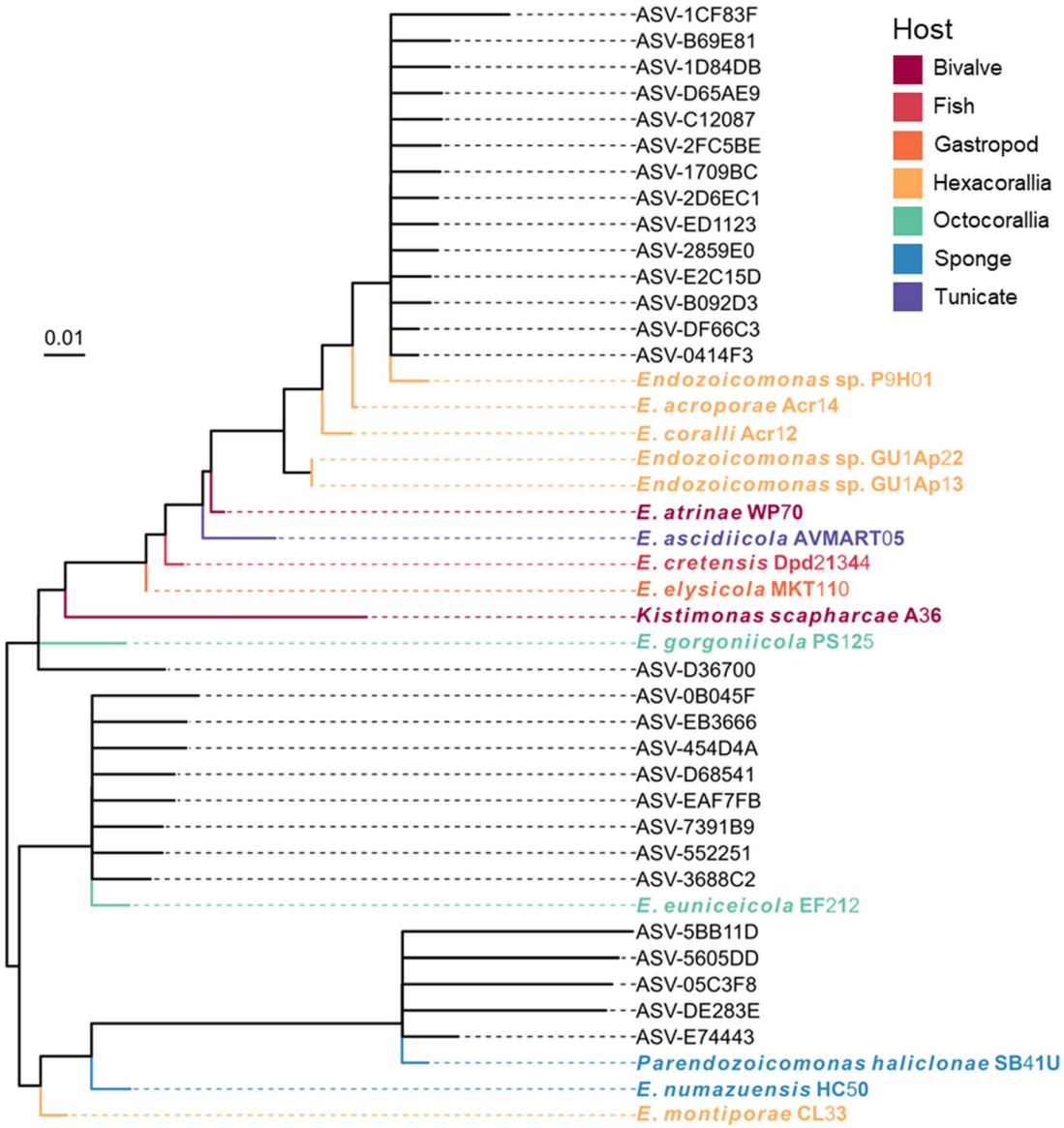

FIGURE A10 | Phylogenetic placement of *Endozoicomonas* ASVs from *A*. cf. *tenuis* (black text) on an ML tree based on near full-length *Endozoicomonas* 16S rRNA sequences from RefSeq. Names in bold represent reference sequences. Colours represent the host group from which the isolates originated.

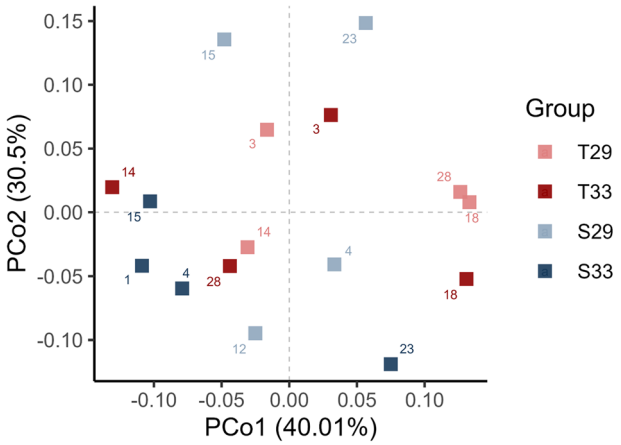

FIGURE A11 | PCoA plot of predicted functions of the *A*. cf. *tenuis* microbiome. Plot is based on Bray–Curtis dissimilarity of PICRUSt2-predicted functions. Colony IDs are shown beside the markers.

TABLE A1 | Physicochemical parameters in control and heated tanks during the sustained exposure stage of the thermal stress experiment (Days 8–11).

Treatment	Temperature (°C)	Salinity (ppt)	Dissolved oxygen (mg/L)	pH
29°C	28.77 ± 0.02	32.85 ± 0.11	8.14 ± 0.05	8.11 ± 0.01
33°C	33.12 ± 0.02	31.93 ± 0.06	7.86 ± 0.05	8.09 ± 0.01

Note: Values represent mean ± standard error.

TABLE A2 | Bleaching proportion of fragments after 4 days of sustained exposure to 33°C.

Colony ID	Category	No bleaching (%)	Partial bleaching (%)	Complete bleaching (%)
3	T	100	0	0
14	T	100	0	0
18	T	100	0	0
28	T	100	0	0
1	S	0	0	100
4	S	0	0	100
12	S	50	0	50
15	S	12.5	12.5	75
23	S	0	0	100

TABLE A3 | Comparison of dispersion or variability of *Acropora* cf. *tenuis* microbial communities at ASV level among tolerance classifications and temperature treatments based on Bray–Curtis dissimilarity.

Group	Parameters	Df	Sum Sq	Mean Sq	*F* value	Pr(>*F*)
Tolerance (T or S)	Groups	1	0.0034	0.0034	0.3288	0.5755
	Residuals	14	0.1433	0.0102	—	—
Treatment (29°C or 33°C)	Groups	1	0.0160	0.0160	1.6808	0.2158
	Residuals	14	0.1337	0.0095	—	—
Tolerance × treatment (S29, T29, S33, T33)	Groups	3	0.1114	0.0371	3.1887	0.0628
	Residuals	12	0.1397	0.0116	—	—

TABLE A4 | Comparison of *Acropora* cf. *tenuis* core microbial communities at ASV level between heat tolerance classifications and temperature treatments based on Bray–Curtis dissimilarity.

	Permanova	
Global comparisons	**Pseudo-F**	***p***
Tolerance classification	2.0881	**0.0020**
Temperature treatment	1.7729	**0.0115**
Tolerance × treatment	1.3494	0.0953
Pairwise comparison		
S29×S33	2.3412	0.0660
S29×T29	1.2249	0.3105
S29×T33	1.1584	0.4164
S33×T29	2.9292	0.0660
S33×T33	2.4086	0.0660
T29×T33	0.9558	0.5610

Note: Benjamini–Hochberg adjusted *p*-values in bold denote statistical significance at $p < 0.05$.

TABLE A5 | Comparison of *Endozoicomonas* ASV abundance in the *Acropora* cf. *tenuis* microbial community for different tolerance classifications and temperature treatments based on Bray–Curtis dissimilarity.

	Permanova	
Comparison	**Pseudo-F**	***p***
T29×T33	0.7619	0.639
T29×S29	0.535	0.883
T29×S33	3.014	**0.048**
T33×S29	0.861	0.496
T33×S33	3.4327	**0.034**
S29×S33	2.1476	0.188

Note: Benjamini–Hochberg adjusted *p*-values in bold denote statistical significance at $p < 0.05$.

Appendix B

Data Files

Data File 1. Abundance table at ASV level with taxon affiliations.

Data File 2. List of differentially abundant ASVs from the general microbiome based on ANCOMBC2 analysis (p-value < 0.05).

Data File 3. Differentially abundant predicted microbial functions based on KEGG ortholog (KO) genes BRITE Level 2.

Data availability statement: The data files that support the findings of this study are available on Figshare: https://doi.org/10.6084/m9.figshare.24715299.