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Species-specific coral microbiome assemblages support host bleaching resistance during an extreme marine heatwave

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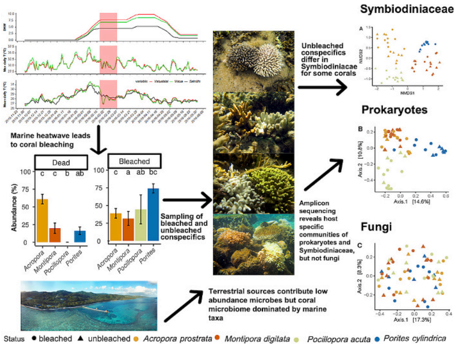
HIGHLIGHTS

- All four species examined hosted unique microbiome assemblages.
- Host bleaching tolerance was driven by photosymbiont assemblages in some coral species, but not others.
- Unbleached *Montipora* had a significantly greater relative abundance of the fungus *Malassezia globosa*.
- Terrestrial fungi and prokaryotes were found in low abundance in coral microbiomes.

GRAPHICAL ABSTRACT



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ABSTRACT

Scleractinian assemblages are threatened by marine heat waves with coral survivorship depending on host genetics and microbiome composition. We documented an extreme marine heat wave in Fiji and the response of corals in two thermally stressed reef flats. Through high-throughput amplicon sequencing of 16S and ITS rDNA phylogenetic markers, we assessed coral microbiomes (Symbiodiniaceae, prokaryotes, fungi, and Apicomplexa) of paired bleached and unbleached colonies of four common coral species representative of dominant genera in the South Pacific. While all coral species exhibited one or more pathways to bleaching resistance, harboring assemblages composed primarily of thermally tolerant photosymbionts did not always result in host bleaching resistance. *Montipora* and *Pocillopora* species, which associate with diverse Symbiodiniaceae and vertically transmit their photosymbionts, fared better than *Acropora*, which acquire their photosymbionts from the environment, and *Porites*, which associate with a narrow photosymbiont assemblage. Prokaryotic and fungal beta diversity did not differ between bleached and unbleached conspecifics, however, the relative abundance of the fungus *Malassezia globosa* was significantly greater in unbleached colonies of *Montipora digitata*. Each coral

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species harbored distinct assemblages of Symbiodiniaceae, prokaryotes, and Apicomplexa, but not fungi, reiterating the importance of host genetics in structuring components of its microbiome. Terrestrial fungal and prokaryotic taxa were detected at low abundance across coral microbiomes, indicating that allochthonous microbial inputs occur, but that coral microbiomes remain dominated by marine microbial taxa. Our study offers valuable insights into the microbiome assemblages associated with coral tolerance to extreme water temperatures.

1. Introduction

Increasing seawater temperatures and marine heat waves associated with global climate change are driving large-scale mass-mortality events of scleractinian corals, the foundation species of coral reef ecosystems (Eakin et al., 2019; Hughes et al., 2018, 2017; Leggat et al., 2019). The survival of scleractinian corals is based on an obligate symbiosis with intracellular photosynthetic dinoflagellates in the family Symbiodiniaceae (LaJeunesse et al., 2018). However, thermal stress can disrupt this symbiosis causing host corals to expel their Symbiodiniaceae, thus losing their normal coloration, a phenomenon referred to as bleaching (Douglas, 2003; Hoegh-Guldberg, 1999; Muller-Parker et al., 2015; Putnam et al., 2017; Rädecker et al., 2021). With Symbiodiniaceae providing most of the energy requirements of their coral hosts, prolonged periods of bleaching can result in coral death as can short pulses of extreme seawater temperatures (Leggat et al., 2019; Lesser and Farrell, 2004). As seawater temperatures continue to increase along with the frequency and duration of marine heat waves (Skirving et al., 2019), coral bleaching events are occurring more frequently bringing into question the ability of coral reef assemblages to persist over coming decades (Hazraty-Kari et al., 2023; Hughes et al., 2018; Keshavmurthy et al., 2022; Oliver et al., 2009).

Symbiodiniaceae have wide-ranging physiological tolerances to temperature and light as well as nutrient preferences that impact the health and resilience of their coral host (Baker, 2003; LaJeunesse et al., 2018; Putnam et al., 2017). Most corals are capable of acclimating to elevated seawater temperatures by rapidly modifying their gene expression (Brener-Raffalli et al., 2022; Palumbi et al., 2014; Torda et al., 2017). However, corals growing in the most stressful habitats may already be close to their thresholds for thermal acclimation through gene regulation (Schoepf et al., 2019, 2015). Some corals are also able to restructure their Symbiodiniaceae communities, which may aid in acclimatizing to thermal and other environmental stressors (Berkelmans and van Oppen, 2006; LaJeunesse et al., 2009; Quigley et al., 2019; Rouzé et al., 2019; Silverstein et al., 2015). For example, at least some species of *Acropora* and *Pocillopora* appear to be flexible in their associations with Symbiodiniaceae and are able to change the lineages they harbor in order to improve their thermal tolerance when challenged with warmer seawater temperatures (Boulotte et al., 2016; Putnam et al., 2012; Rouzé et al., 2019). In contrast, *Porites* and some *Montipora* are more specific in their associations and generally harbor lineages of Symbiodiniaceae that are considered to be thermally tolerant regardless of the thermal environment to which they are acclimated (Fabina et al., 2012; Putnam et al., 2012). Scleractinian corals, however, host a suite of other microorganisms in their microbiome, including bacteria, archaea, fungi, Apicomplexa, and viruses, that may support the colony's survivorship (Bourne et al., 2016; Osman et al., 2020; Putnam et al., 2017; Rosenberg et al., 2007). Ultimately, it is the genetic composition of the coral holobiont - the coral host and its associated microbiome - that determines a coral's thermal tolerance (Chen et al., 2021).

Coral-associated bacterial communities are found in the host's surface mucus layer, tissues, gastrovascular cavity, and skeleton, and can be highly variable and dynamic over the life cycle of the host coral, and in response to environmental variation (Bourne et al., 2016; Pollock et al., 2018; van Oppen and Blackall, 2019). Cophylogeny patterns between some lineages of bacteria such as *Endozoicomonas* sp. with their host indicate co-evolution and likely a tight biological relationship with their hosts (Gardner et al., 2019; Pollock et al., 2018; van Oppen and Blackall, 2019). Studies have shown varying impacts of thermal stress on *Endozoicomonas* abundance depending on the coral host and tissue sampled (Lee et al., 2015; Pogoreutz et al., 2018). Although corals can be flexible in their bacterial associations, corals that regularly experience thermal stress have been found to harbor distinct and more stable bacterial associations than corals acclimated to cooler environments when under thermal stress (Ziegler et al., 2017). While bacteria are known to play a role in coral defense mechanisms, stress tolerance, and nutrient cycling or provisioning within the coral holobiont (Bourne et al., 2016; Torda et al., 2017; Welsh et al., 2017), the roles bacterial communities or specific bacterial lineages may play in supporting coral thermal tolerance are not well understood.

Fungi were once thought to be primarily parasitic on the host coral or other coral symbionts (Bentis et al., 2000; Kendrick et al., 1982; Priess et al., 2000), but more recent studies have found that some coral fungi are involved in metabolic processes that may benefit the coral holobiont, such as nitrogen cycling (Amend et al., 2012; Wegley et al., 2007). Additionally, particular fungal taxa, such as *Malassezia globosa*, appear to be core members of coral microbiomes (Amend et al., 2012; Chavanich et al., 2022). Fungi may also help protect coral tissue from ultraviolet radiation through the production of mycosporine-like amino acids (Dunlap and Malcolm Shick, 1998; Peixoto et al., 2017). As heat stress can lead to altered nutrient cycling within the coral holobiont that ultimately results in bleaching (Rädecker et al., 2021), thermal tolerance may involve specific microbiome components that help stabilize the symbiosis between the host coral and its Symbiodiniaceae, or contribute to Symbiodiniaceae physiology (Maire et al., 2021). Another core component of coral microbiomes are corallicolid Apicomplexa (Kwong et al., 2019). These symbiotic eukaryotes are abundant in corals and may have host-specific associations and functions (Kirk et al., 2013; Kwong et al., 2019). Although Apicomplexa are primarily known as parasites of humans and other animals, their presence has not been linked to coral disease (Keeling et al., 2021). While Apicomplexa are believed to possess non-photosynthetic plastids (Kwong et al., 2019), the interactions between Apicomplexa and their host corals, as well as their impact on host thermal tolerance, remain unknown.

Examining corals that live in extreme environments, such as nearshore reef flats, can provide important insight into the pathways by which coral thermal tolerance can be achieved (Claar et al., 2020; Osman et al., 2020; Schoepf et al., 2015). Between 2013 and 2017, reef systems around the Pacific experienced extreme heat stress that led to consecutive years of bleaching and significant coral loss at many locations (Eakin et al., 2019; Hughes et al., 2018). Coral communities on reef flats along Fiji's Coral Coast experienced a marine heat wave that resulted in severe bleaching and coral death within days of its onset. We documented the bleaching status of reef flat coral communities at two coral-rich, no-take marine protected areas immediately following the peak of this heat wave and evaluated whether putative bleaching-tolerant corals (i.e. *Porites* and *Montipora*) fared better than corals reported to be more susceptible to bleaching (i.e. *Acropora* and *Pocillopora*) during the extreme thermal event. In order to examine relationships between bleaching tolerance and coral microbiome composition (Symbiodiniaceae, prokaryotic, fungi, and corallicolid Apicomplexa), we sampled bleached and unbleached conspecific colonies of four relatively common species of hard corals that each have different life history strategies. We hypothesized that differences in coral-associated microbiomes would be primarily explained by coral host species. Given their role in nutrient cycling within the coral holobiont, we expected to find distinct fungal and prokaryotic communities or lineages associated with bleaching tolerance, and specific Symbiodiniaceae lineages associated with thermal tolerance considering their central role in host function and resilience. Lastly, we compared the prokaryotic and fungal taxa found in the corals to those we detected in soils of the watersheds situated adjacent to the study sites (Benucci et al., 2019a) to gain further insight into terrestrial-aquatic linkages in coral microbiomes.

2. Methods

2.1. *Site description and seawater temperature measurements*

The study was conducted at two shallow reef flat sites located on the fringing reef along the southwest coast of Viti Levu, Fiji's largest island. During low tides, the circulation of oceanic water across the reef flat can be cut off for several hours allowing sea water temperatures at these reef sites to heat up several degrees Celsius warmer than on the forereef. The studied reefs are situated adjacent to Votua Village (Latitude −18.218°, Longitude 177.715°) and Vatuolalai Village (Latitude −18.206°, Longitude 177.688°) some 3 km apart from each other, and were designated as no-take marine protected areas by the traditional fishing rights owners in 2003 and 2002, respectively.

To assess the historical thermal stress experienced at the study sites relative to the 2016 event, remotely sensed mean-daily Sea Surface Temperature (SST) and Degree Heating Weeks (DHWs) data for the marine-only pixel closest to the Votua reef (Latitude −18.225°, Longitude 177.725°) from January 1985 through December 2019 were obtained from NOAA's Coral Reef Watch program (https://coralreefwatch.noaa.gov/satellite/index.php). These data were complemented with a record of yearly in situ coral bleaching observations recorded for the study sites spanning from the first mass bleaching in 2000 to present (Victor Bonito, unpublished). Onset Hobo Pro V2 temperature loggers with 10-minute sampling intervals were deployed at each of the two study sites in coral-rich reef flat moat areas to document the thermal regime coral communities on the reef flat experienced during the heat-wave event. Daily mean and maximum temperatures along with DHW were calculated using the in-situ data using the same 29.4 °C (DHW threshold as the NOAA data) for the 2015–2016 hot season.

2.2. *Coral community surveys and coral microbiome sampling*

To rapidly assess the condition of the coral communities immediately following the most extreme temperatures of the 2016 marine heat wave, five 50-meter point intercept transects (100 points per transect) were conducted at each study site (March 1, 2016 Vatuolalai; March 5, 2016 Votua) across the same reef flat moat areas where the temperature loggers were deployed. Benthic community components were recorded in categories (crustose coralline algae, turf algae, macroalgae, cyanobacteria, sand, soft coral, hard coral) with hard and soft corals being further recorded to genus level as well as the colony status recorded as either bleached (fully bleached or extremely pale), recently-dead (all coral tissue gone usually with first signs of algal overgrowth), or unbleached (not bleached or still maintaining most of its normal pigmentation). Coral bleaching status data were converted into relative percent of the colonies for the categories examined (*Acropora*, *Montipora*, *Pocillopora*, and *Porites* individually along with all other genera combined), data were checked for normality, and compared with appropriate statistical tests in JMP v16 (ANOVA and Tukey-Kramer post-hoc test or Kruskal-Wallis and Steel-Dwass post-hoc test) (SAS Institute Inc., Cary NC, 1989-2021).

Paired bleached and unbleached colonies of *Acropora prostrata* (Dana, 1846), *Montipora digitata* (Dana, 1846), *Pocillopora acuta* (Lamarck, 1816), and *Porites cylindrica* (Dana, 1846) were sampled opportunistically at the two study sites from the same reef areas where temperature loggers were deployed, and coral community surveys conducted. All sampled colonies were estimated by their size to be at minimum three- to four-years old and thus had survived the bleaching events observed during the 2014 and 2015 hot seasons. Sampling focused on finding unbleached colonies of the targeted species that were not shaded and then sampling those colonies along with a bleached conspecific situated immediately adjacent (<1-m apart) and similarly positioned to the unbleached colony (i.e. not at a different depth or microhabitat) (Fig. 1A-D). In some cases, corals slightly further than 1-m apart from each other were sampled or a nearby bleached conspecific could not be found and only a bleached or unbleached colony was sampled. Sampled colonies were identified in situ based on previous field collections and taxonomic studies conducted by the collector (V. Bonito) at the sites. The type specimens of *A. prostrata*, *P. cylindrica* and *M. digitata* are from Fiji and the entities sampled appeared to be congruent with their respective type specimen. The *P. acuta* colonies were identified using gross morphology in the field and previous examination of skeletal features on specimens of similar morphed colonies (Schmidt-Roach et al., 2014). Each colony was first photographed in situ before two branches were snapped from each of the sampled colonies. The tip of each branch (axial polyp on the *Acropora*) was removed with a metal cutter and a small portion of the top of the remaining branch was cut off and placed into a 2.0 mL Eppendorf tube containing guanidinium chloride. A total of 66 colonies were sampled (33 bleached, 33 unbleached): 19 *Acropora* (10 bleached, 9 unbleached), 18 *Montipora* (7 bleached, 11 unbleached), 15 *Pocillopora* (8 bleached, 7 unbleached), and 14 *Porites* (8 bleached, 6 unbleached). Samples were stored in a freezer until being shipped to Michigan State University (Fiji CITES permit 15FJFD5000314) for processing.

2.3. *Nucleic acids extractions*

Following storage of coral tissue in guanidinium buffer, DNA was extracted from each sample using two methods in order to mitigate known challenges of extracting DNA from corals and to maximize recovery of microbial diversity. Tissue was first ground using the Tissue Lyser II (Qiagen, USA) for 15 min at maximum speed. One extraction method employed CTAB buffer and chloroform/isoamyl alcohol (Mieog et al., 2009), and the other guanidinium (Tkach and Pawlowski, 1999). Full details for DNA extraction methods are available in the Supplementary methods.

2.4. *Polymerase chain reactions and sequencing libraries preparation*

Amplicon sequencing libraries were prepared to target Symbiodiniaceae communities with the nuclear ribosomal Internal Transcribed Spacer 2 (ITS-2 rDNA) region primers ITS_D (Pochon et al., 2001) and ITS2Rev2 (Stat et al., 2009), fungal communities with the ITS rDNA primers ITS1F and 5.8Srev that amplifies the ITS1 region (Gardes and Bruns, 1993), and prokaryotic communities with the 16S rDNA primers 515F and 926R targeting the V4 region (Caporaso et al., 2011). Illumina™ MiSeq libraries were prepared following a three-step protocol as described previously (Benucci et al., 2019b; Chen et al., 2018). Detailed information on PCR amplification is available in the Supplementary methods and Supplementary Tables 1 & 2. Raw sequence reads have been deposited to the NCBI SRA archive under the accession number: PRJNA744218.

2.5. *Coral host sequencing and phylogeny*

To confirm visual identifications of coral host species and to perform phylodiversity analyses, the mitochondrial Cytochrome Oxidase C subunit 1 (COX1) marker was PCR amplified and Sanger sequenced from each coral sample. The COX1 region was targeted using the LCO1 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) primer set (Folmer et al., 1994).

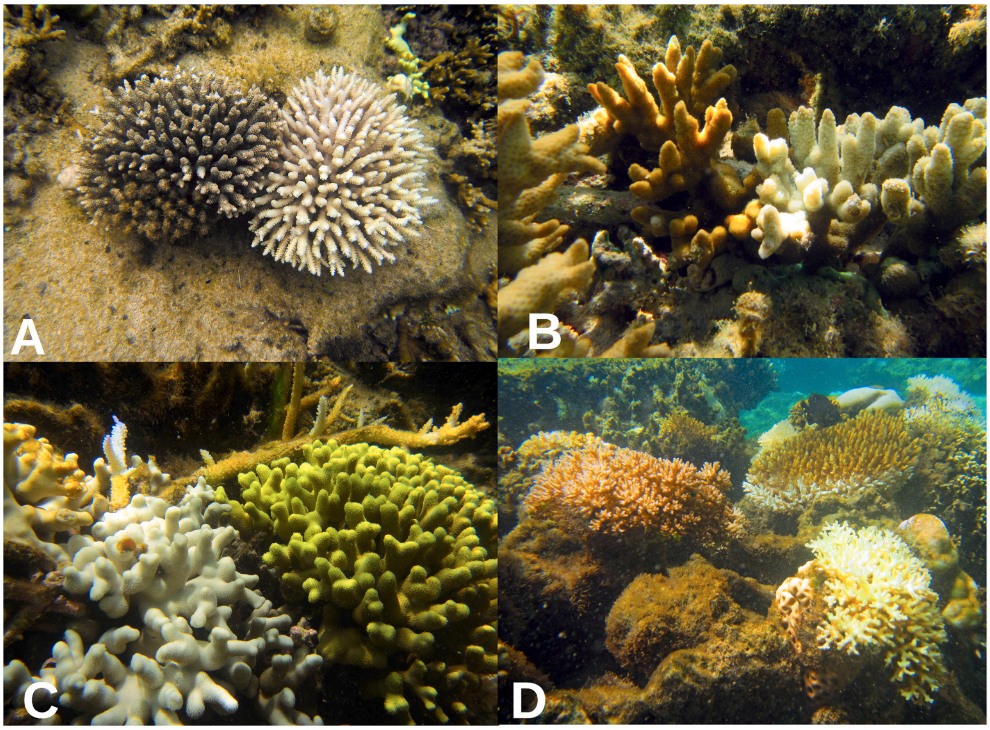

Fig. 1. Examples of bleached and unbleached colonies of (a) *Acropora prostrata*, (b) *Montipora digitata*, (c) *Porites cylindrica*, and (d) Pocillopora acuta sampled for microbiome analyses.

Sequences for each coral sample are available in NCBI's GenBank with the following accession numbers: MZ666434 - MZ666499 (Clark et al., 2016). The region was amplified using cycling conditions previously utilized to amplify the COX1 region of scleractinian corals (Kitahara et al., 2010). Sanger sequences were aligned with Mesquite V 3.70 (Maddison and Maddison, 2009) and phylogenetic trees were created in PAUP* (Swofford, 2003).

2.6. Amplicon sequence processing

Amplicon sequencing data were processed as previously described; full details of initial amplicon sequencing processing are available in the Supplementary methods (Benucci et al., 2019b). Amplicon sequencing data were used to generate error-corrected and chimera-free zero-radius operational taxonomic OTUs (ZOTUs) which represent exact sequence variants. These ZOTUs are meant to capture all biological sequences present in the samples without clustering at a 97 % OTU threshold (Edgar, 2015). Taxonomy was assigned to ZOTUs representative sequences with CONSTAX (Liber et al., 2021; Gdanetz et al., 2017) based upon the UNITE eukaryotic rDNA sequence database version 04.02.2020 (Abarenkov et al., 2020) for the Fungi and against SILVA v138 (Quast et al., 2013; Yilmaz et al., 2014) sequence database for prokaryotes, respectively. Additionally, due to concerns over fungal contamination due to increased cycling needed to amplify fungi from coral, fungal ZOTUs which were considered to be potential lab contaminants were removed by matching sequences of lab culture strains to ZOTUs at 99–100 % sequence similarity with the BLAST algorithm implemented in CONSTAX (Liber et al., 2021; Gdanetz et al., 2017). Additionally, known macrofungal lab contaminants were removed prior to further processing. The list of fungal ZOTUs removed is shown in Supplementary Table 3. Fungal and prokaryotic ZOTUs were matched to a previous assessment of soil fungi and prokaryotes from the same watershed using the BLAST algorithm implemented in CONSTAX. Taxa were considered to be of terrestrial origin if they matched to a terrestrial ZOTU at an identity level of 99 % or greater. Symbiodiniaceae ITS2 data was processed using SymPortal and the remote SymPortal database (Hume et al., 2019). Non-target ZOTUs were removed following assessment with NCBI BLAST (Johnson et al., 2008). Prokaryote sequences classified as Apicomplexa were retained for separate analysis following Janouškovec et al. (2012).

2.7. Statistical analyses

Preprocessing was performed prior to statistical analysis as previously described, and details are available in the Supplementary methods (Noel et al., 2022). To assess whether DNA extraction method impacted sequence data recovery, data normalized by cumulative sum scaling with the *metagenomeSeq* package in R were used in PERMANOVA analyses to assess if the extraction type had a significant effect on microbial community composition for any of the markers (Oksanen et al., 2019; Paulson et al., 2013). Stacked barplots showing abundances of fungi, prokaryotes, Symbiodiniaceae, and Apicomplexa (extracted from the prokaryotes ZOTUs table) were then generated with the *ggplot2* R package (Wickham, 2009). Prior to analyzing beta diversity, samples with low read counts were removed to avoid biasing beta diversity results based on sequencing depth. Thresholds were chosen based on the average sequencing depth for each marker and to ensure that all samples had at least 10 % of the average sequencing depth for that marker. For Symbiodiniaceae, the threshold was 10,000 reads. For Fungi, the threshold was 750 reads, and for prokaryotes the threshold was 500. Thresholds were determined based upon sequencing depth of each individual library. Next, libraries were normalized with the *metagenomeSeq* package using cumulative sum scaling (Paulson et al., 2013). PCoA plots for fungal and prokaryotic communities and a NMDS plot for Symbiodiniaceae were created using the "ordinate" and "plot_ordination" functions of the *phyloseq* (McMurdie and Holmes, 2013) and *vegan* packages (Oksanen et al., 2019). NMDS was utilized for Symbiodiniaceae due to difficulties visualizing communities with two PCoA axes. Patterns identified from PCoA plots were further analyzed and tested for significance with a PERMANOVA from the "adonis" function in the *vegan* package. Following the PERMANOVA, homogeneity of variance between groups was tested using the "betadisper" function in *vegan*

(Oksanen et al., 2019). Beta diversity analyses were not performed on Apicomplexa due to the low sequencing depth recovered from the bacteria library.

Predictive machine learning random forest models were used to identify which features (ZOTUs) had the highest importance in differentiating coral bleaching status within individual coral hosts and for identifying features which had the highest importance in differentiating coral species. This analysis was performed with the "RandomForest" function in the *randomForest* R package (Liaw and Wiener, 2002). To remove redundancy among ZOTUs we applied recursive feature elimination (backwards selection) available in the package *labgeo* (Fernandes Filho, 2020) that supports a parallel multi-core processing version of the "rfe" function in the *caret* (Kuhn, 2020) R package. ZOTUs are ranked and the less important ones are sequentially eliminated prior to modeling. The smallest number of variables with the highest Kappa in repeated cross-validation (repeats = 5, nfolds = 10) was chosen for building the models. In each model, we optimized i) the number of trees (*ntree*) needed to obtain the lowest stable out of bag (OOB) error estimate, ii) the number of features (ZOTUs) randomly sampled for each tree at each split (*mtry*) with the "tuneRF" function in the randomForest R package. Robustness of model predictions (i.e. the probability produced by random forest voting trees), was assessed with the function "rf. significance" in the *rfUtilities* R package using 999 permutations (i.e. repeating 999 times the random forest models) (Murphy et al., 2010). To graphically show how different samples were classified by the model, a distance matrix of "1 - proximity matrix" was calculated and visualized through a multidimensional scaling (MDS) ordination with the "cmdscale" function in the *stats* package (McMurdie and Holmes, 2013; R Core Team, 2018). Additionally, an indicator species analysis was performed using the *indicspecies* package of R to assess if any taxa were detected as indicator species for particular coral hosts (De Cáceres and Legendre, 2009). In addition to random forest analysis, impacts of bleaching on *Malassezia globosa* and *Endozoicomonas* spp. were specifically assessed due to their abundance in coral communities and their previously characterized tight relationships with coral hosts. Code for R statistical analyses and figure creation is available at: https://github.com/longleyr/Coral-Microbiome-Code.

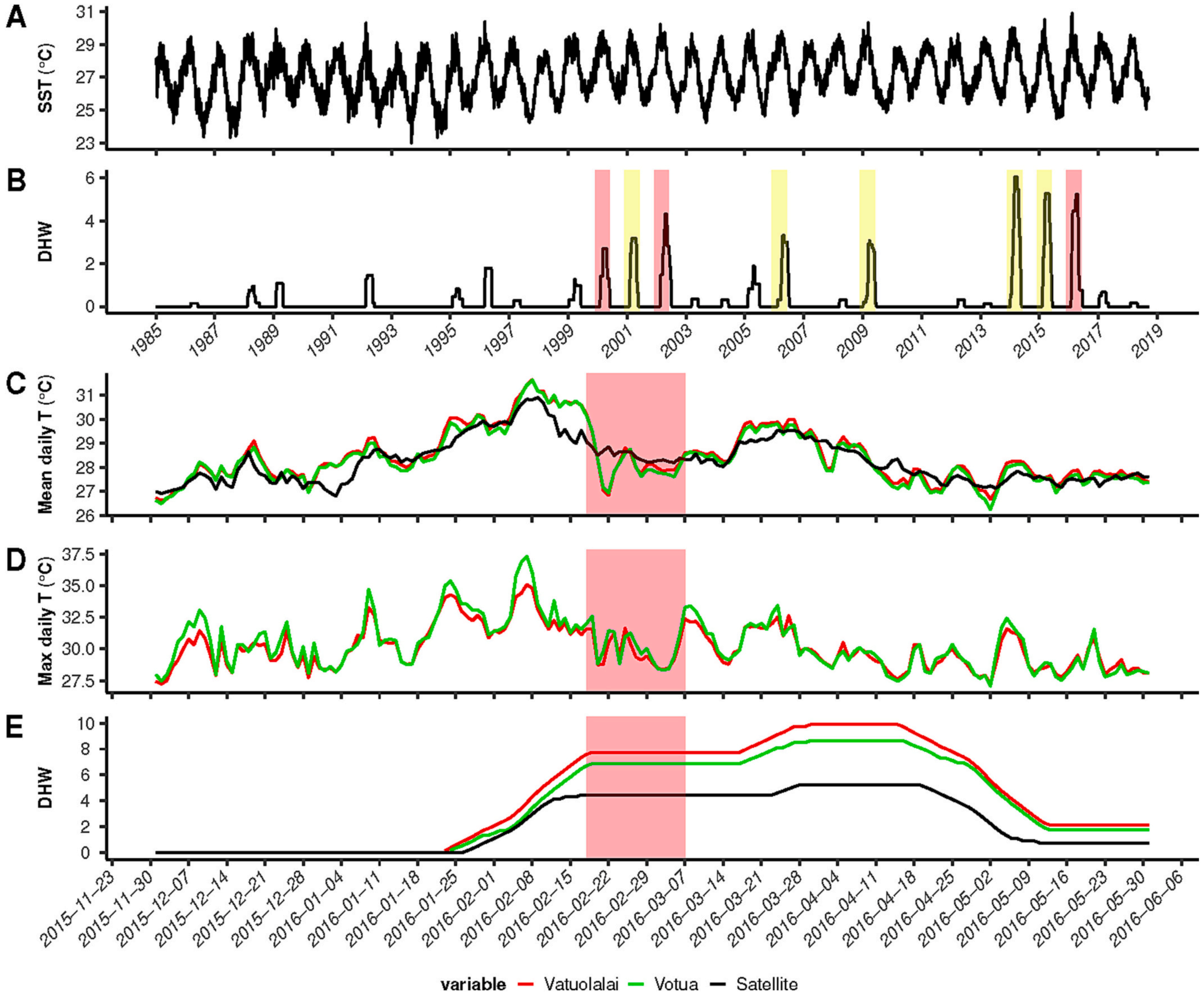

Fig. 2. Mean daily sea-surface temperature (a) and degree heating weeks (b) recorded by satellite between January 1985 and 2020 for the pixel adjacent to the study sites. Shading indicates years bleaching was observed at the study sites, with red indicating events that resulted in significant coral mortality while yellow indicates bleaching without significant coral mortality. Mean daily temperature (c), maximum daily temperatures (d), and Degree Heating Weeks (e) recorded by satellite and in situ temperature loggers at the two study sites. The shading indicates the window during which benthic surveys and coral microbiome sampling were conducted.

3. Results

3.1. Thermal exposure

The February 2016 marine heat wave that impacted these study sites in Fiji was the most extreme on record in terms of magnitude and duration, with mean daily Sea Surface Temperatures (SSTs) exceeding 30 °C for eight consecutive days and reaching a maximum of 30.9 °C (Fig. 2A, C). This replaced the 2015 record of five consecutive days exceeding 30 °C and a maximum of 30.5 °C. Sites experienced a record of 6.1° Heating Weeks (DHW) of accumulated heat stress in 2014, and 5.3 DHW in 2015 (calculated using SSTs) with widespread coral bleaching observed, but little to no observable mortality (Fig. 2B). While 5.2 DHW of accumulated heat stress was ultimately recorded in 2016, the onset of widespread coral death began when only 2.6 DHW had been recorded on February 7, 2016 and had only reached 4.6 DHW by the time we conducted the benthic surveys. In situ mean daily temperatures from the two sites largely tracked SSTs, although in situ measurements generally exceeded the remotely acquired data, which consists of only nighttime SST measurements (Fig. 2C). In situ measurements show mean sea water temperature at the two sites began exceeding 30 °C a few days before the observed onset of wide-spread coral bleaching and mass fish death at the sites on February 7, 2016. During the peak of the event, corals experienced mean daily temperatures exceeding 31 °C for five consecutive days with maximum daily temperatures exceeding 37 °C in Votua and 35 °C in Vatuolalai (Fig. 2C & D). While Votua recorded higher maximum daily temperatures, the overall mean daily temperature and thus DHW was higher at Vatuolalai (Fig. 2C, D, E).

3.2. Coral community response to the heat wave

The study sites at Votua and Vatuolalai had relatively high cover of hard corals (55 % and 42 %, respectively) with minimal macroalgae (Fig. 3A). *Acropora*, *Porites*, *Montipora*, and *Pocillopora* species were the most abundant corals found along the transects and together the four genera comprised 87 % (Votua) and 79 % (Vatuolalai) of the total hard coral cover at the two sites (Fig. 3B). A total of 21 genera of corals were recorded during the surveys with *Acropora* and *Porites* being the only genera recorded on all 10 transects, and *Montipora* and *Pocillopora* (represented primarily by a single species, *P. acuta*) present on 9 and 6 transects, respectively. Overall, 17–20 % of the coral cover was unbleached, while 41–52 % was bleached, and 28–42 % was recently dead at the time of sampling (Fig. 3C). Coral genera responded differently to the thermal stress experienced at the sites. *Acropora* cover was the most impacted with no unbleached cover along the transects, and 57–65 % of the cover recently dead. *Montipora* and *Pocillopora* cover were the least affected of the four genera examined with 40–67 % of it unbleached and only 15–25 % of the *Montipora* cover recently dead (Fig. 3C, D). While there were no significant differences between the abundance of bleached and unbleached *Pocillopora*, no recently dead *Pocillopora* colonies were recorded at either site (Fig. 3C). *Porites* cover was largely bleached at both sites with no unbleached cover detected at Votua,

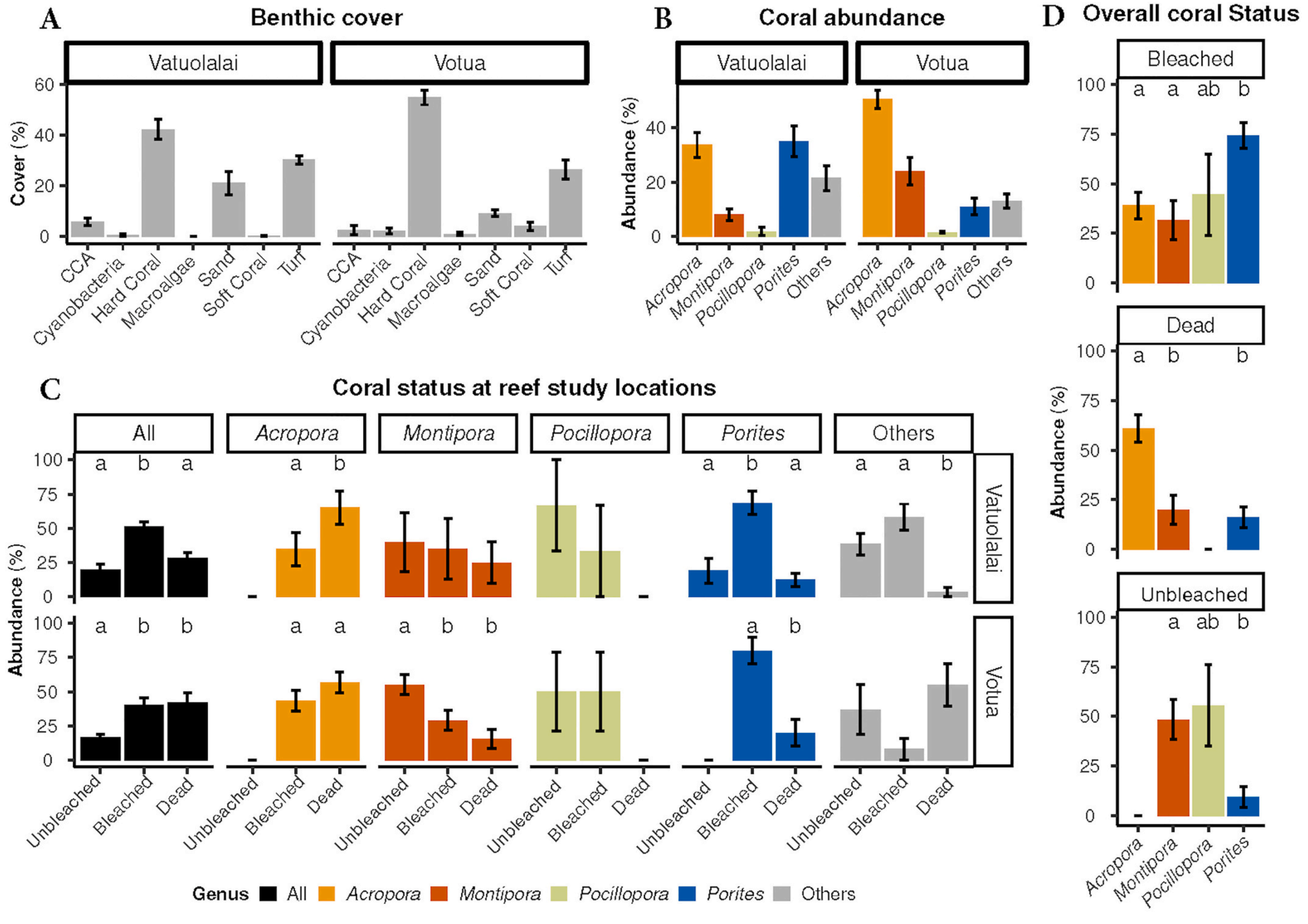

Fig. 3. Benthic community (a) and coral community composition (b) at Votua and Vatuolalai. Coral status by genus (c) at Votua and Vatuolalai and overall (d). Bars represent means ±SE. *N* = 5 for all except for *Pocillopora* in Votua (*n* = 4), and *Montipora* and *Pocillopora* in Vatuolalai (n = 4 and *n* = 2 respectively). Comparison of relative percent unbleached, bleached, and dead cover between the four most abundant coral genera.

which experienced the hottest temperatures, and 12–20 % recently dead (Fig. 3C).

3.3. *Comparison of DNA extraction methods*

PERMANOVA analysis of the 16S and Symbiodiniaceae markers demonstrated that there were no significant differences in communities whether DNA was extracted by CTAB-chloroform or guanidinium thiocyanate (Supplementary Table 4). For the fungal ITS marker, there was a significant difference ($P = 0.013$) between the two extraction methods, but this difference only accounted for 1.4 % of the total variation. Observed and Shannon alpha diversity metrics were not significantly different between the two extraction types for any marker (Supplementary Table 4). Therefore, to maximize the recovery of microbial diversity, results from the two extraction methods were merged.

3.4. *Next generation sequencing*

After merging the two extraction types and prior to further filtering, 66 coral samples were processed, 33 from unbleached colonies and 33 from bleached colonies. Following filtering and removal of samples below sequencing depth thresholds, the Symbiodiniaceae library was composed of 5,195,712 reads with an average sequencing depth of 81,183 reads per sample. Following filtering and removal of a large proportion of host and other non-prokaryotic reads, the prokaryotic community was represented by 168,998 reads with an average depth of 2965 reads per sample. For the fungal community, 441,054 reads remained for an average depth of 6785 reads per sample after filtering and removal of contaminant in controls. Sequences classified as Apicomplexa were extracted from 16S libraries for a total recovery of 16,763 Apicomplexa sequences and average sequencing depth of 278 reads per sample.

3.5. *Symbiodiniaceae community composition*

Amplicon sequencing of the Symbiodiniaceae communities harbored in the four corals revealed that all but one sample were dominated by *Cladocopium* and *Durusdinium* lineages (Fig. 4A). All *M. digitata* and *P. cylindrica colonies* were dominated by *Cladocopium* C15 lineages regardless of bleaching status (70.9 % to 73.6 % relative abundance) (Fig. 4A). However, ITS2 type profiles show that outside of the dominant C15 ITS2 type, rare ITS2 types differed between *M. digitata* and *P. cylindrica* (Supplementary Fig. 1). Particularly, *P. cylindrica* samples contained either the “C15−C15ed−C15pu−C15wc” profile or the “C15−C15cq” profile. However, bleached and unbleached *M. digitata* samples contained various ITS2 type profiles including “C15−C15kl−C15he”, “C15−C15he−C15wb−C15f”, “C15−C15wd−C116bf”, and “C15−C15he−C15f−C15pg”.

In *P. acuta* and *A. prostrata*, there were distinct communities found between bleached and unbleached samples. Unbleached *P. acuta* samples were dominated by two *Durusdinium* ITS2 types with *Durusdinium* D6 accounting for 26.4 % of the reads and *Durusdinium* D1 accounting for 35.2 %. However, in bleached *P. acuta* samples, the *Cladocopium* C1d ITS2 type accounted for 70.4 % of the reads. Similarly, there appeared to

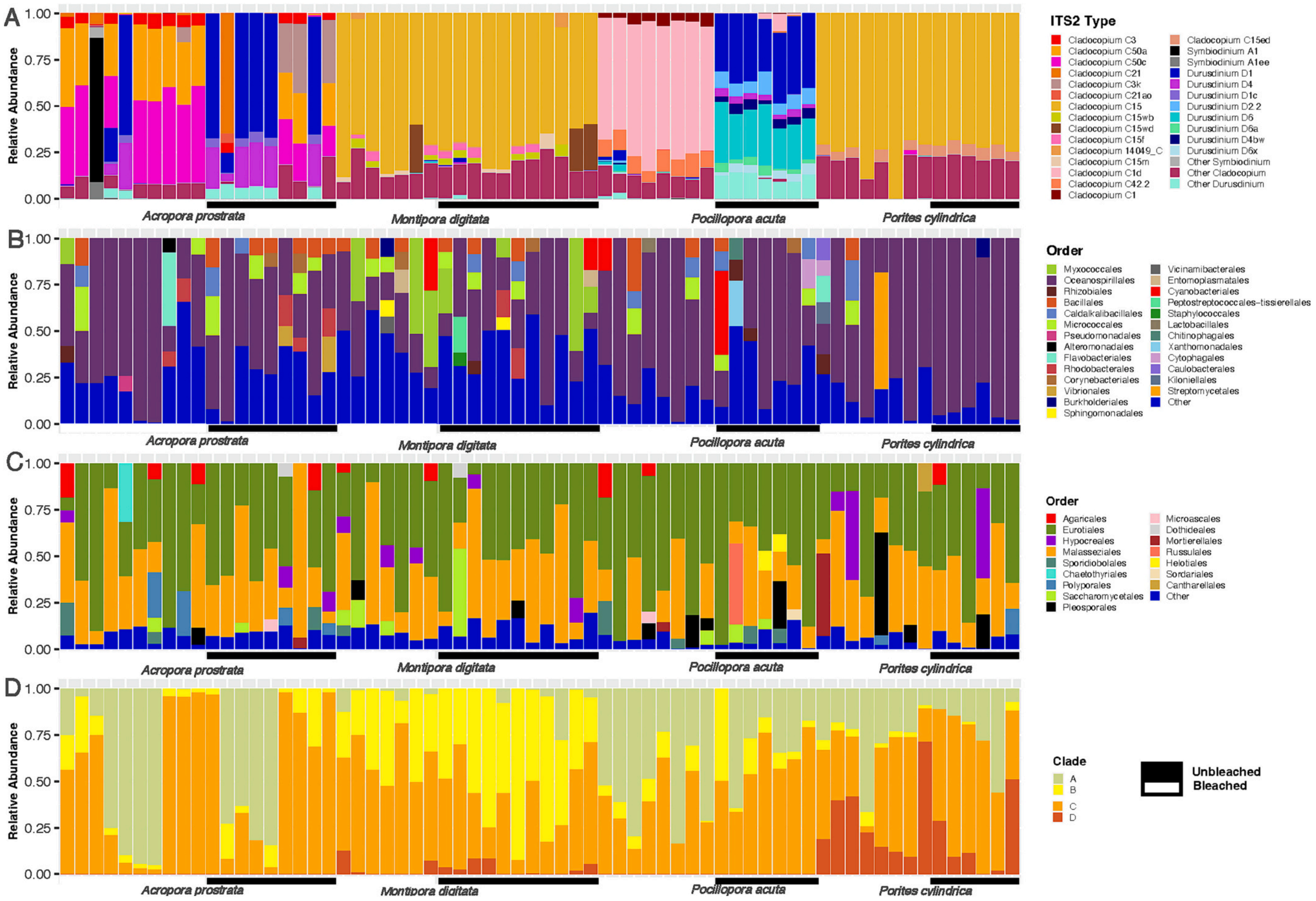

Fig. 4. Bar plots showing relative abundance of Symbiodiniaceae ITS2 types with relative abundance >5 % (a), bar plots showing relative abundance of prokaryotic orders with relative abundance >7 % (b), bar plots showing relative abundance of fungal orders with relative abundance >5 % (c), and bar plots showing relative abundance of Apicomplexa clades based on the phylogeny in Supplementary Fig. 3(d).

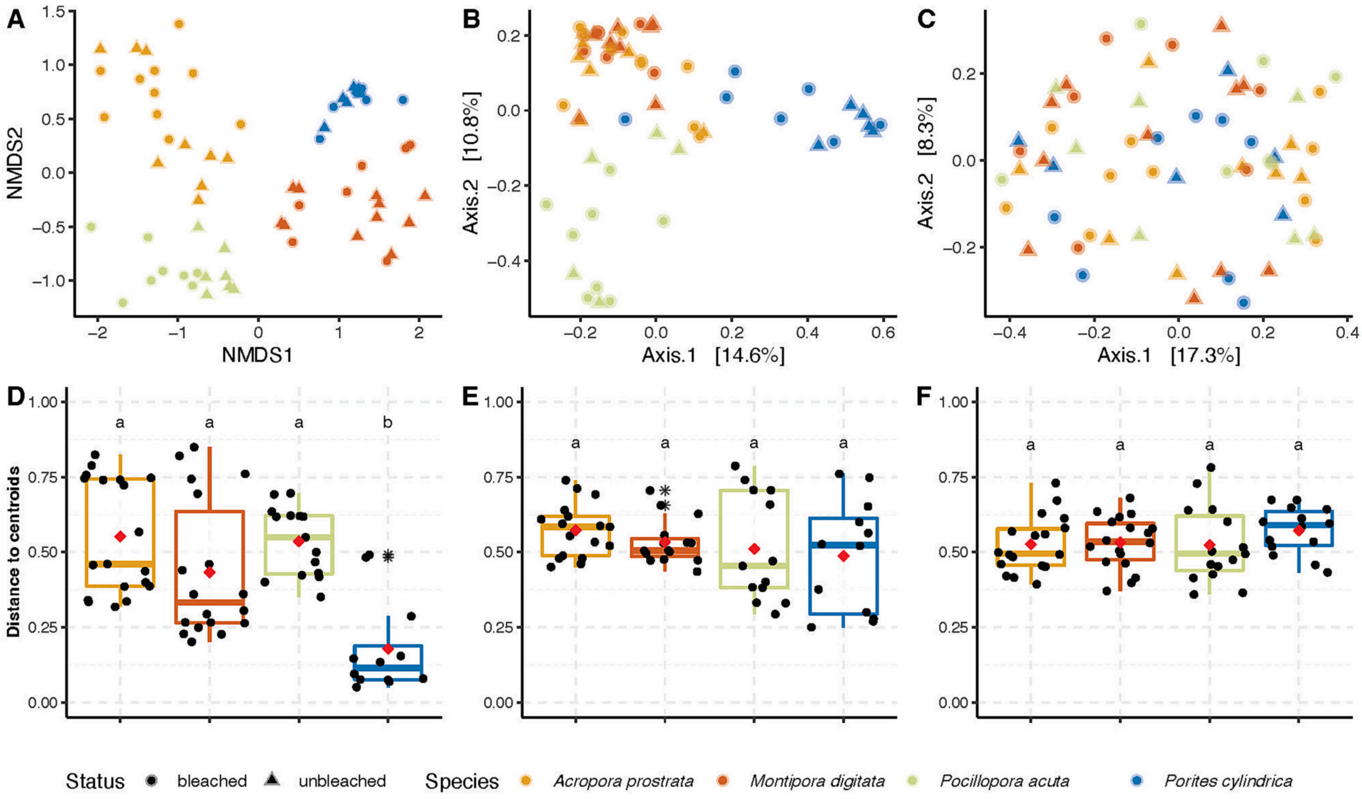

Fig. 5. Non-metric multidimensional scaling (NMDS) plot (stress = 0.14) of Symbiodiniaceae (a), principal coordinates analysis (PCoA) plots of prokaryotes (b), and fungi (c), dispersion around centroids in Symbiodiniaceae ordinations by host species (d), dispersion around centroids in prokaryotic ordinations by host species (e), and dispersion around centroids in fungal ordinations by host species (f).

be distinct Symbiodiniaceae communities between bleached and unbleached samples of *A. prostrata*, but this varied by sample (Fig. 4A). Unbleached *A. prostrata* samples were primarily composed of the *Durusdinium* D1 ITS2 type which accounted for 40.6 % of reads, although four samples were dominated by various *Cladocopium* ITS2 types. Bleached samples were dominated by two *Cladocopium* ITS2 types (C50a and C50c) which accounted for 24.7 and 30.9 % of reads respectively, although one sample was dominated by *Durusdinium* ITS2 types and another by *Symbiodinium* A1. Unbleached *P. acuta* samples primarily contained the following *Durusdinium* ITS2 type profiles: “D1/D6−D2.2−D6a−D4bw−D6x”, “D1−D6−D2.2−D4bw−D6x−D4”, and “D1/D6/D2.2−D4−D6a” (Supplementary Fig. 1). However, bleached samples primarily contained various *Cladocopium* ITS2 type profiles such as “C1d−C42.2−C1nz−C1”, “C1d−C42.2−C1−C1b−C3cg−C3cw”, and “C1d/C42.2/C1/C3cg−C1b−C3cw−C115k−C45c”. The majority of *A. prostrata* samples primarily contained either “D1−D4−D17−D1c” or “C3k/C50a/C50c−C3−C3ba−C50q−C50f”. However, bleached samples which were dominated by *Cladocopium* contained different ITS2 type profiles including “C50c/C50a/C3−C50f−C3b−C50u−C3ad”.

3.6. Prokaryotic community composition

Oceanospirillales were part of the coral prokaryotic microbiome across all coral hosts (Fig. 4B). On average, the relative abundance of Oceanospirillales ranged from 17.2 % in bleached *M. digitata* samples to 95.9 % in unbleached *P. cylindrica* samples. In most hosts, the majority of sequences coming from Oceanospirillales were *Endozoicomonas* (Supplementary Fig. 2A) with each coral species containing one or more species-specific *Endozoicomonas* ZOTUs. The relative abundance of Endozoicomonas was significantly higher in *P. cylindrica* and *P. acuta* compared to *M. digitata*. Relative abundances of *Endozoicomonas* showed no clear pattern by coral host status and differences were not significant (Supplementary Fig. 2A). Overall, Endozoicomonas accounted for an average of 44 % of Oceanospirillales reads with a range of 1.3 % - 99.3 %. Other Oceanospirillales taxa such as unidentified Oceanospirillales sp. and *Halomonas* were also abundant. Additionally, in *M. digitata* samples, Myxococcales were highly abundant, accounting for 25.6 % of the reads among bleached samples. Potentially disease-causing *Vibrio* spp. were detected at low levels. Twenty-four coral samples across all four hosts had two or more *Vibrio* reads (11 samples from *A. prostrata*) *Vibrio* ZOTUs were most abundant and prevalent in *A. prostrata*; in two *A. prostrata* unbleached samples the *Vibrio* genus accounted for 9 % and 18 % of reads, but no other samples had >5 %. Overall, 5 % of ZOTUs in the prokaryotic community shared 99 % or greater sequence similarity to those of terrestrial taxa reported previously from the adjacent watershed (Supplementary Table 5). These taxa accounted for 6 % of total reads, and three ZOTUs identified from the terrestrial dataset were detected in most coral samples. However, these ZOTUs (ZOTU 62, ZOTU 89, ZOTU 106) are from halotolerant *Halomonas* and *Nesterenkonia*. Other terrestrial taxa were detected sporadically.

3.7. Fungal community composition

Coral-associated fungal communities were dominated by Eurotiales and Malasseziales, independent of coral host or bleaching status (Fig. 4C). In every coral species sampled, Eurotiales had the highest relative abundance, accounting for between 29.2 % and 56.1 %. Within Eurotiales, a large portion of reads were not classified at the genus level, but *Aspergillus* was the most common classified genus. Following Eurotiales, Malasseziales had the second highest relative abundance accounting for between 19.7 % and 32.5 % of the total reads. Within Malasseziales, *Malassezia* accounted for between 43.3 % and 51.9 % of the reads classified to a genus. No clear patterns of fungal community

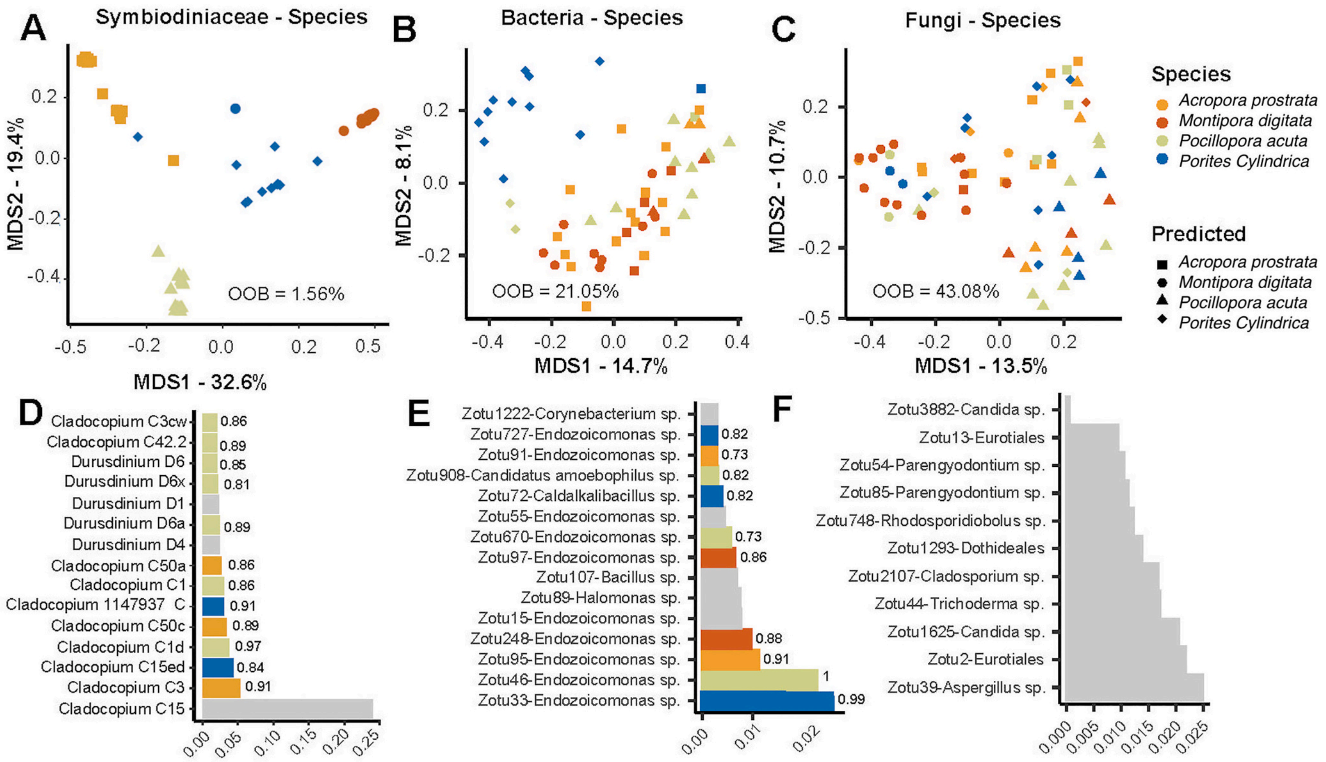

Fig. 6. Metric multidimensional scaling (MDS) ordination showing the "1 - proximity matrix" distances between samples obtained from the random forest models for Symbiodiniaceae (a), Prokaryotic (b) and fungal (c) communities in each species. The shape represents the coral host predicted by random forest modelling and the color represents the actual coral host for that sample. Each ordination is labeled with the out of bag error (OOB) which indicates the percent of samples classified to an incorrect coral host. Model accuracy is obtained by subtracting OOB % from 100. D to F show ZOTUs (y-axis) with highest value in "mean decrease in accuracy" (x-axis) for each of the random forest model visualized in MDS ordination; colored bars reflect indicator ZOTU for specific coral species. Association statistics for indicator taxa are shown to the right of the bars in the case of detected indicator taxa (Please see indicator species analysis in the Material & Methods section 2.7).

composition emerged by coral species or bleaching status (Fig. 4C). The relative abundance of *Malassezia globosa* was significantly greater in unbleached *M. digitata* samples, but not in other species (Supplementary Fig. 2B). ZOTU 4 was the most abundant *M. globosa* ZOTU regardless of host bleaching status. Twenty-seven percent (27 %) of the fungal ZOTUs had 99 % or greater sequence similarity with taxa detected previously in a study that sampled in the surrounding terrestrial watershed (Supplementary Table 6). However, these ZOTUs only accounted for 0.4 % of total reads and were detected sporadically with no clear pattern by host or bleaching status.

3.8. Apicomplexa community composition

Due to a lack of clear taxonomic groupings for corallicolid Apicomplexa ZOTUs detected in this dataset, taxonomy was assigned based on phylogenetic clades within detected ZOTUs (Supplementary Fig. 3). While clades A, B, and C were present in all four coral species, clade D was present only in *M. digitata* and *P. cylindrica* but was relatively more abundant in *P. cylindrica* where it accounted for 19.6 % of the sequences (Fig. 4D). While Clade B was present in all four coral species, it was most abundant in *M. digitata* where it had a relative abundance of 42.2 %.

3.9. Beta diversity analyses

For Symbiodiniaceae and Prokaryotic communities, patterns in beta diversity were driven by coral hosts along the X and Y axes (Fig. 5A, B). However, for Fungi (Fig. 5C) there was no distinct clustering of samples by coral host species. PERMANOVA results indicated that coral species accounted for 40.3 % of the variation in Symbiodiniaceae communities and 25.1 % of variation in Prokaryotic communities. Symbiodiniaceae was also the only microbiome component where bleaching status was significant by PERMANOVA, accounting for 4.5 % of variation (Supplementary Table 7). However, the effect of bleaching status was highly dependent on coral species. Symbiodiniaceae communities were significantly different between bleached and unbleached colonies of *P. acuta*, *P. cylindrica* and *A. prostrata* but not *M. digitata* (Supplementary Table 8). When split by host coral species, the impact of bleaching status was not significant for fungi or prokaryotes (Supplementary Table 8).

Analysis of group dispersion demonstrated that dispersion was significantly different by coral species for the Symbiodiniaceae marker. *Porites cylindrica* had the lowest dispersion around the centroid (Fig. 5D). Prokaryotes and fungi did not show significant differences in group dispersion by coral host species (Fig. 5E, F). There was no consistent pattern of differences in dispersion for any marker when analyzing each coral host separately (Supplementary Fig. 4). However, in *P. cylindrica*, dispersion among Symbiodiniaceae and prokaryotic communities was significantly higher among bleached samples compared to unbleached samples (Supplementary Fig. 4A, B).

3.10. Cophylogeny of coral hosts and microbiome components

A clear link between the host lineage and microbial communities were observed for Symbiodiniaceae, Prokaryotes, and Apicomplexa but not for Fungi regardless of bleaching status (Supplementary Figs. 5, 6). The coral phylograms show that COX1 sequences from individual coral samples from each species were 100 % identical to each other, except for three *M. digitata* (two unbleached and one bleached) samples and one *P. cylindrica* (unbleached) sample, which differed by only a single nucleotide base to the dominant haplotype. For unbleached coral samples, the correlation between host phylogeny and community dissimilarity was the strongest for the Symbiodiniaceae community, which had the lowest entanglement score of 0.19 (Supplementary Fig. 5A).

Entanglement values for Prokaryotes (0.29) and Apicomplexa (0.31) were similar to each other (Supplementary Fig. 5B, D). The fungal community had an entanglement of 0.52 and showed no clear correlation between the host phylogeny and fungal community (Supplementary Fig. 5C). Tanglegrams created from bleached samples were not substantially different from those created from unbleached samples (Supplementary Fig. 6).

3.11. Random forest and indicator species analysis

Random Forest models were developed to determine whether microbial taxa could be used as a predictor of host species or host bleaching status. Significant random forest models to classify coral species, visualized as metric multidimensional scaling of the proximity matrix, and associated barplots of ZOTUs or ITS2 types with highest mean decrease in accuracy for each microbial community are provided in Fig. 6 (details on the models are in Supplementary Table 9). Models used 26 Symbiodiniaceae ITS2 types to classify coral species with 98.4 % accuracy (Fig. 6A). The most important features for classification were in the *Cladocopium* genus with *Cladocopium* C15 and other species-specific *Cladocopium* lineages (Fig. 6D). Prokaryotic (Fig. 6B) and fungal (Fig. 6C) models were less accurate (21.05 % and 43.08 %). Species-specific *Endozoicomonas* and *Aspergillus* (fungal ZOTU39) were the most important ZOTUs for predicting host (Fig. 6E, F).

Regarding bleaching status, four models (*A. prostrata* and *P. acuta* Symbiodiniaceae and *P. cylindrica* and *M. digitata* fungi) were able to predict host bleaching status with OOB below 40 %. The most important ITS2 types for predicting bleaching status in *A. prostrata* included various *Cladocopium* ITS2 types (C3B, C37039_C) and several *Durusdinium* ITS2 types (D1C and D17). The most important ITS2 types in *P. acuta* also included *Cladocopium* types (C1d and C42.2) as well as *Durusdinium* types (3194476_D and D4bw) (Supplementary Fig. 7). The most important fungal ZOTUs for classifying *M. digitata* and *P. cylindrica* by status included several *Malassezia* ZOTUs, other yeasts such as *Rhodosporidiobolus* and *Penicillium adametzioides* (Supplementary Fig. 7).

4. Discussion

4.1. Coral response to the 2016 marine heat wave in Fiji

The February 2016 marine heat wave that our study sites experienced was the most extreme documented since the SST record began in 1985 in terms of both daily-mean temperature and duration (Fig. 2). Within four weeks of the onset of the heat wave, more than a third of the coral cover at our study sites was dead. We attribute this mass coral death to the extreme temperature corals experienced and not accumulated heat stress. Surveyed coral communities had survived at least 50 % higher DHWs of accumulated heat stress during both the 2014 and 2015 hot seasons than they had experienced in 2016 at the time we surveyed. Exposure to extreme temperatures has been shown to result in rapid coral death, but also accelerates the dissolution of dead coral skeletons, and thus loss of reef structure (Leggat et al., 2019).

Coral genera vary in their tolerance for thermal stress (Hoegh-Guldberg and Salvat, 1995; Loya et al., 2001; Marshall and Baird, 2000) and indeed we found coral response to the marine heat wave varied across the most abundant genera (Fig. 3). Interestingly, ‘generalist’ corals that are more flexible with their Symbiodiniaceae associations fared better than the ‘specialist’ *Porites* (Putnam et al., 2012), as long as they were also vertical transmitters of their Symbiodiniaceae symbionts (i.e., *Pocillopora* and *Montipora*). Some ‘generalist’ corals that vertically transmit their Symbiodiniaceae appear to be able to acquire heat tolerant symbionts and then later transfer those same symbionts to their offspring in their eggs (Quigley et al., 2019). This may help explain why *Montipora* and *Pocillopora* communities seem to be better adapted to the repeated heat stress that our study sites have experienced compared to *Porites* and *Acropora* communities. *Acropora*, considered to be photosymbiont generalists, acquire their Symbiodiniaceae communities from the environment and fared the worst of all the genera that we studied, suffering over 50 % mortality at both sites with no unbleached colonies detected under the transects despite comprising a third or more of the coral cover. Contrary to ex situ studies that report that a coral’s response to a short thermal pulse disturbance is a good indicator of that holobiont’s response to extended heat stress (Voolstra et al., 2020), the status of coral communities at our study sites indicate that some corals, such as *Pocillopora* and *Porites*, may respond differently to short periods of extreme temperatures compared to extended periods of accumulated heat stress (DHW) resulting from elevated, though not extreme, temperatures. While *Pocillopora* and *Acropora* are often highly susceptible to bleaching and mortality from heat stress (Hoegh-Guldberg and Salvat, 1995; Loya et al., 2001; Marshall and Baird, 2000), we found that the majority of the *Pocillopora* cover was unbleached, with no dead cover recorded or dead colonies noted despite *Acropora* cover being largely dead or otherwise bleached (Fig. 3D). This suggests that *Pocillopora* may be able to tolerate relatively short periods of extreme temperatures better than extended periods of heat stress driven by warmer than normal but not extreme temperatures (i.e. high DHW). Conversely, while *Montipora* and *Porites* tend to be more bleaching resistant (Marshall and Baird, 2000; van Woesik et al., 2011) and indeed suffered less mortality at our sites during the extreme temperatures than *Acropora*, little to no *Porites* cover remained unbleached despite ~40 % or more of *Montipora* and other coral genera remaining unbleached. In contrast, during the 2014 and 2015 bleaching events during which record high accumulated heat stress (DHWs) was recorded at our study sites, minimal to no bleaching of *Porites* was observed (V. Bonito, personal observation).

In reef flat habitats, coral communities regularly experience temperatures exceeding those of adjacent forereef areas by several degrees Celsius (Oliver and Palumbi, 2011; Smith, 2004). Extensive bleaching events were observed at our study sites during eight of the previous 17 hot seasons. However, we only found that 17 %–20 % of coral cover at our study sites displayed a bleaching-resistant phenotype with highly susceptible *Acropora* being a dominant component of the coral communities at both sites. Thus, bleaching resistant phenotypes may not be the most competitive, even in these thermally stressed reef flats. While corals that harbor primarily thermally tolerant symbionts such as *Durusdinium* spp. usually exhibit strong bleaching resistance during periods of thermal stress, they have been shown to grow slower, representing an ecological tradeoff (Cunning et al., 2015; Little et al., 2004). While we did not track the ultimate outcome of individual colonies once heat stress subsided, most of the coral cover that survived the extreme temperatures in February 2016 ultimately recovered. Some bleached corals are able to survive extended periods of heat stress by increasing heterotrophic feeding (Bessell-Browne et al., 2014; Grottoli et al., 2006; Littman et al., 2011) or utilizing energy reserves (Grottoli et al., 2004), while others that bleach are able to acquire heat tolerant symbionts that aid in survival (Claar et al., 2020).

4.2. Microbiomes of bleached versus unbleached corals

Each of the four coral species sampled here exhibited one or more pathways to bleaching resistance. However, only in *P. acuta* and *A. prostrata* was bleaching resistance associated with harboring different Symbiodiniaceae communities. In *P. acuta*, bleaching resistance was strictly associated with harboring communities composed largely of heat tolerant *Durusdinium* ITS2 types while bleached colonies primarily harbored *Cladocopium* ITS2 types. In *A. prostrata*, however, while five of the nine unbleached colonies we sampled harbored primarily *Durusdinium* ITS2 types, one unbleached colony was dominated by *Cladocopium* (C42.2) and three unbleached samples contained the same *Cladocopium* ITS2 types (C50c and C50a) that most of the bleached samples harbored. However, these ITS2 types were present in lower proportions in unbleached samples and these samples were also enriched in the *Cladocopium* C3K ITS2 type. Additionally, the three unbleached samples contained different ITS2 type profiles indicating differences in Symbiodiniaceae community composition (Supplementary Fig. 1). Some of the bleached *A. prostrata* colonies we sampled contained the same *Durusdinium* ITS2 types and in similar proportions as found in unbleached conspecifics. Bleached and unbleached *A. prostrata* colonies where *Durusdinium* was detected harbored primarily D1 and D4 ITS2 types, while unbleached *P. acuta* conspecifics harbored primarily D3 and D6 ITS2 types, highlighting how host genetics may structure Symbiodiniaceae associations (Brener-Raffalli et al., 2018; Quigley et al., 2018) (Fig. 4A). While we were not able to track the colonies to observe their fate post-sampling, nor resample colonies to evaluate temporal dynamics in symbiont assemblages, it is possible that bleached colonies were beginning to acquire heat tolerant Symbiodiniaceae despite the continued thermal stress (Claar et al., 2020). Alternatively, bleached *A. prostrata* samples dominated by *Durusdinium* may have initially been colonized by *Durusdinium* and *Cladocopium* ITS2 types. However, *Cladocopium* ITS2 types were primarily lost during bleaching, leaving *Durusdinium* ITS2 types dominant. Although harboring *Durusdinium* ITS2 types was a pathway to bleaching resistance, further studies are needed to determine whether unbleached colonies are dominated by *Durusdinium* spp. year-round, or whether these colonies are changing symbiont communities to survive during periods of heat stress. PERMANOVA results also indicated a significant difference between bleached and unbleached *P. cylindrica*, but this result is likely due to a significant difference in dispersion as no differences in Symbiodiniaceae community composition were detected with random forest analyses. The lower dispersion of Symbiodiniaceae communities among unbleached *P. cylindrica* colonies is consistent with the Anna Karenina principle in animal microbiomes which suggests higher dispersion among stressed colonies (Zaneveld et al., 2017).

We did not find significant differences in Symbiodiniaceae communities of bleached and unbleached *M. digitata* conspecifics. These results again indicate that bleaching tolerance can be achieved through pathways other than harboring communities dominated by more thermally tolerant Symbiodiniaceae in these species. Both *M. digitata* and *P. cylindrica* primarily harbored closely related lineages of *Cladocopium* ITS2 types, predominantly classified as the C15 ITS2 type, although some colonies contained very small amounts of *Durusdinium* ITS2 types (Fig. 4A). It has been proposed that rare Symbiodiniaceae components in the coral microbiome may play an important role in stabilizing the host-symbiont community and supporting environmental resilience of the coral holobiont (Chen et al., 2021; Ziegler et al., 2018a). We did find some rare Symbiodiniaceae lineages in all *M. digitata* and *P. cylindrica* samples that comprised a total of ~25 % or less of the Symbiodiniaceae community of any sample (Fig. 4A), however none of these rare lineages appeared to be strongly associated with heat tolerance. These findings further suggest that the pathway to bleaching tolerance in *M. digitata* and *P. cylindrica* was not strictly driven by Symbiodiniaceae association.

As a whole, neither prokaryotic nor fungal communities were associated with a particular bleaching status in any of the four coral species. However, like *P. cylindrica* Symbiodiniaceae communities, dispersion among the prokaryotic communities of unbleached *P. cylindrica* colonies was significantly lower than bleached colonies, consistent with the ‘Anna Karenina principle’ (Zaneveld et al., 2017). *Endozoicomonas* (order Oceanospirillales) are considered ubiquitous and often dominant bacteria in coral microbiomes and are thought to play important roles in controlling coral pathogens as well as protein provision and carbohydrate cycling (Neave et al., 2017; Peixoto et al., 2017). We found *Endozoicomonas* in all corals sampled, and *Endozoicomonas* were a dominant component of the bacterial community found in all species except *M. digitata* (Fig. 4B). *Endozoicomonas* are putative beneficial microorganisms for corals, as diseased or compromised corals often harbor a lower relative abundance of *Endozoicomonas* compared to healthy corals (Peixoto et al., 2017; van Oppen and Blackall, 2019). While *Endozoicomonas* may indeed be a core microbiome component of corals, and may play important roles in promoting host well-being, from the four coral species we sampled, none had significant differences in the relative abundance of *Endozoicomonas* between bleached and unbleached colonies. It is possible that *Endozoicomonas* abundance is not correlated with bleaching tolerance, or that relic DNA was detected. It is also possible that unbleached samples were compromised to some extent by the environmental stress they experienced. Potentially disease-causing *Vibrio* spp. were most common in *A. prostrata* and were most abundant in two unbleached *A. prostrata* samples indicating that while unbleached, coral holobionts may be compromised and vulnerable to infections (van Oppen and Blackall, 2019).

Fungal communities as a whole did not differ between bleached and unbleached samples of any species. However, fungal ZOTUs from taxa such as *Malassezia globosa* and *Candida* sp. were important in random forest modeling of bleaching status using fungal communities, indicating that these taxa may be impacted by bleaching. *Malassezia* taxa were not consistently associated with either bleached or unbleached conspecifics with ZOTU 20 being more abundant in unbleached *P. cylindrica* conspecifics and ZOTU 3 being more abundant in bleached conspecifics while *M. globosa* ZOTU 4 was more abundant in unbleached *M. digitata* samples. However, none of the ZOTU level differences identified by random forest were significant. Although in *M. digitata*, the overall relative abundance of *M. globosa* was significantly higher in unbleached conspecifics (Supplementary Fig. 2). These results indicate that *Malassezia* associations with bleaching may be species or strain or specific. Chavanich et al. (2022) assessed coral-associated eukaryotic communities using the 18S region and found that *Malassezia* was more abundant in bleached corals than unbleached. *Malassezia* associated with human skin often rely on host lipids for survival (Triana et al., 2017). If this is the case for coral associated *Malassezia*, then *Malassezia* would be expected to have a negative impact on coral bleaching survival as corals rely on stored lipids for bleaching survival (Rodrigues and Grottoli, 2007). However, functional studies such as transcriptomics are needed to determine the role of *Malassezia* in the biology of coral holobionts. *Malassezia globosa* has been suggested as a ‘core’ component of *Acropora hyacinthus* and was found to be associated with samples dominated by bleaching resistant *Durusdinium* spp. Symbiodiniaceae communities (Amend et al., 2012). We found a *M. globosa* ZOTU in every coral sample, regardless of the host species or its Symbiodiniaceae composition, providing further evidence that this taxon is likely a core microbiome component of scleractinian corals (Amend et al., 2012). Further work is needed to determine the role of these fungal taxa in corals and their relationship to host bleaching tolerance.

4.3. Species-specific microbiomes

Host genetics have been shown to be important in structuring Symbiodiniaceae and bacterial assemblages (Pollock et al., 2018; Ziegler et al., 2018b) and indeed we found that all four coral species examined hosted distinct assemblages of Symbiodiniaceae, prokaryotes and Apicomplexa (Supplementary Figs. 5 & 6). Symbiodiniaceae assemblages were the most distinct among the coral species, and each coral species hosted some unique Symbiodiniaceae taxa such as *Durusdinium* ITS2 types found only in *P. acuta* and unique *Cladocopium* ITS2 types found in each of the four coral species. For example, *M. digitata* and *P. cylindrica* were both dominated by the the C15 ITS2 type, but examination of ITS2 type profiles revealed host-specific, lower abundance ITS2 types such as C15ed in *P. cylindrica* and C15he in *M. digitata* (Supplementary Fig. 1). Additionally, when comparing Symbiodiniaceae dispersion by coral species, *P. cylindrica* dispersion was the lowest regardless of bleaching status (Fig. 5D). All coral species hosted species-specific bacteria belonging to *Endozoicomonas*, reiterating the prominence of these bacteria as a core microbiome component of its coral hosts (Pollock et al., 2018). While neither ubiquitous nor nearly as abundant as *Endozoicomonas*, two other potentially beneficial bacterial groups with demonstrated cophylogeny with coral hosts (Pollock et al., 2018), Myxococcales and Kilioniellales, were also detected. Myxococcales were higher in relative abundance in *M. digitata* compared to other coral hosts. Similarly, Apicomplexa in clade B were found most abundantly in *M. digitata* relative to other host species (Fig. 4D).

Unlike with Symbiodiniaceae and prokaryote assemblages, no coral species-specific fungal assemblages or ZOTUs were identified among the samples. However, several of the fungal taxa present in the samples have previously been described as being potential obligate associates of coral due to their high occupancy, being present in >90 % of examined samples (Amend et al., 2012). Among the eleven core fungal taxa Amend et al. (2012) reported from *Acropora hyacinthus* samples in American Samoa, the only genera present in >90 % of Fijian samples (this study) were *Malassezia* and *Aspergillus*. *Malassezia* spp. were detected in all of our samples, with multiple ZOTUs present. *Aspergillus* spp. were detected in 91 % of samples, but none of the ZOTUs belonged to the opportunistic coral pathogen *A. syndowii*. Among other core taxa detected by Amend et al. (2012), ZOTUs in the Polyporales and Marasmiaceae were also detected in our study, but their distribution was uneven. Additionally, Dothideales ZOTUs belonging to *Hortaea*, and others such as *Curvularia* were present in most samples, but not at the 90 % threshold. Just over one-quarter of the ZOTUs identified in fungal microbiomes were matches for terrestrial OTUs identified from the adjacent watershed (Benucci et al., 2019a) (Supplementary Table 6). While this contributed to the relatively high alpha diversity found across all species, matches for terrestrial ZOTU were neither the most abundant nor core components in coral microbiomes. These terrestrial fungal OTUs are likely transient or opportunistic and dispersed from land to sea through run-off and soil erosion.

While the more distantly related *M. digitata* and *P. cylindrica* both harbored similar Symbiodiniaceae assemblages dominated by the C15 ITS2 type, the prokaryote and Apicomplexa assemblages associated with *M. digitata* samples were more similar to confamilial *A. prostrata*. This indicates that the host, rather than Symbiodiniaceae, exerts the most control in structuring assemblages of prokaryote microbiome components. Overall, prokaryotes and fungal assemblages were poor predictors of Symbiodiniaceae. Unlike Amend et al. (2012), we did not find any fungal lineages associated exclusively with corals containing *Durusdinium* spp.

Acropora, *Montipora*, and *Pocillopora* species have been described as having more flexibility in their Symbiodiniaceae symbiont associations compared to *Porites* (Putnam et al., 2012). Indeed, *P. cylindrica* samples had significantly lower variation of Symbiodiniaceae communities compared with the other three species, but this was not the case for prokaryotic or fungal assemblages. The diversity of fungal communities, and to some extent prokaryote assemblages in the corals, was enhanced by the presence of terrestrial taxa, indicating that terrestrial microbes may interact with corals.

5. Conclusions

We evaluated the response of thermally stressed reef flat coral communities in Fiji to the most extreme marine heat wave on record for the study sites. We found that the bleaching responses of the dominant genera in the coral microbiome were linked to coral life-history characteristics. Specifically, coral host flexibility in photosymbiont association coupled with their ability to vertically transmit photosymbionts provides an apparent adaptive advantage to coral communities experiencing repeated and increasing intensity of thermal stress. All four coral species assessed hosted unique assemblages of Symbiodiniaceae, prokaryotes, and Apicomplexa, including host-specific lineages of Symbiodiniaceae and prokaryotes. In contrast, fungal microbiomes were highly variable within coral species and were not structured by the coral host. In summary, all four species exhibited one or more pathways to bleaching resistance, however only two species, *P. acuta* and *A. prostrata*, achieved this by harboring thermally tolerant photosymbionts highlighting the importance of host genetics to thermal tolerance of corals. Additional studies are needed to evaluate the role of host genetics and microbiome inheritance in thermal tolerance as well as the flexibility and transmission of coral microbiome components that may be associated with host thermal tolerance.

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CRediT authorship contribution statement

Reid Longley: Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Gian Maria Niccolò Benucci:** Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Xavier Pochon:** Validation, Writing - original draft, Writing - review & editing. **Gregory Bonito:** Validation, Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision. **Victor Bonito:** Conceptualization, Investigation, Resources, Supervision, Formal analysis.

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.

Data availability

Data and code are linked in the manuscript text

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