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Hawaiian coral holobionts reveal algal and prokaryotic host specificity, intraspecific variability in bleaching resistance, and common interspecific microbial consortia modulating thermal stress responses

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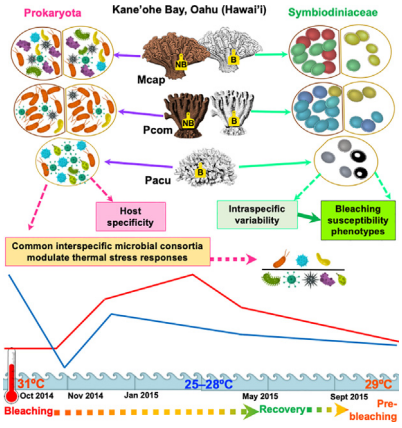
HIGHLIGHTS

- Unprecedented heatwaves in 2014–2015 caused massive coral bleaching in Hawai'i.
- Two species had resistant/susceptible colonies, a third one was fully susceptible.
- Microbial dynamics were tracked with multigene markers and compositional analyses.
- Microbiomes were shaped by host-species, algal profiles by bleaching resistance.
- A taxa consortium orchestrated coral microbiomes bleaching recovery in all hosts.

GRAPHICAL ABSTRACT



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ABSTRACT

Historically, Hawai'i had few massive coral bleaching events, until two consecutive heatwaves in 2014–2015. Consequent mortality and thermal stress were observed in Kāne'ohe Bay (O'ahu). The two most dominant local species exhibited a phenotypic dichotomy of either bleaching resistance or susceptibility (*Montipora capitata* and *Porites compressa*), while the third predominant species (*Pocillopora acuta*) was broadly susceptible to bleaching. In order to survey shifts in coral microbiomes during bleaching and recovery, 50 colonies were tagged and periodically monitored. Metabarcoding of three genetic markers (16S rRNA gene ITS1 and ITS2) followed by compositional approaches for community structure analysis, differential abundance and correlations for longitudinal data were used to temporally compare Bacteria/Archaea, Fungi and Symbiodiniaceae dynamics. *P. compressa* corals recovered faster than

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P. acuta and *Montipora capitata*. Prokaryotic and algal communities were majorly shaped by host species, and had no apparent pattern of temporal acclimatization. Symbiodiniaceae signatures were identified at the colony scale, and were often related to bleaching susceptibility. Bacterial compositions were practically constant between bleaching phenotypes, and more diverse in *P. acuta* and *M. capitata*. *P. compressa*'s prokaryotic community was dominated by a single bacterium. Compositional approaches (via microbial balances) allowed the identification of fine-scale differences in the abundance of a consortium of microbes, driving changes by bleaching susceptibility and time across all hosts. The three major coral reef founder-species in Kāneʻohe Bay revealed different phenotypic and microbiome responses after 2014–2015 heatwaves. It is difficult to forecast, a more successful strategy towards future scenarios of global warming. Differentially abundant microbial taxa across time and/or bleaching susceptibility were broadly shared among all hosts, suggesting that locally, the same microbes may modulate stress responses in sympatric coral species. Our study highlights the potential of investigating microbial balances to identify fine-scale microbiome changes, serving as local diagnostic tools of coral reef fitness.

1. Introduction

Microbial symbioses play critical roles in the ecology and evolution of corals (Ainsworth et al., 2020; Bourne et al., 2016). The majority of research on microbial communities in corals has focused on single celled dinoflagellates in the family Symbiodiniaceae (zooxanthellae), since these symbionts play a large role in coral health and nutrition (Baker, 2003; Sampayo et al., 2008; D'Angelo et al., 2015). Less studied are the populations of Bacteria, Archaea and even Fungi that associate with corals forming the coral holobiont (Bourne et al., 2016). As seawater temperatures increase, coral bleaching is occurring more frequently around the world, which is a stress-induced disruption of symbiosis between the host and symbiotic algae, causing a "bleached" pale-to-white appearance of affected colonies (Douglas, 2003). Bleached corals, depleted of symbiotic algae (Fitt et al., 2001; Jokiel, 2004; Falkowski et al., 1984) may effectively starve until the symbiosis is reestablished (Baker, 2001). Resistance and recovery following bleaching are highly variable both among and within coral species, and may be influenced by environmental factors (e.g., light, temperature, symbiont availability), as well as traits of the host and its associated microbial communities (Edmunds, 1994; Fitt et al., 2001; Baird et al., 2009; Grottoli et al., 2014; Conti-Jerpe et al., 2020; Ainsworth and Gates, 2016). Additionally, the coral animal may be able to switch to heterotrophy to mitigate starvation, and recover faster due to the accumulation of lipids (Grottoli et al., 2006; Hughes and Grottoli, 2013; Wall et al., 2019; Conti-Jerpe et al., 2020); while genetic and epigenetic processes may also promote stress resilience (Edmunds, 1994; Fitt et al., 2001; Grottoli et al., 2014; Baird et al., 2009; Putnam and Gates, 2015).

The diversity of Symbiodiniaceae in relation to coral bleaching has been researched for over 30 years (Rowan and Powers, 1991; Van Oppen and Medina, 2020), as different genotypes have different physiological responses to abiotic conditions (Baker, 2003; Sampayo et al., 2008). For instance, there are thermally tolerant symbionts (e.g., *Cladocopium thermophilum, Durusdinium glynnii, D. trenchii*) that increase bleaching resistance of coral hosts (Baker, 2001; Berkelmans and van Oppen, 2006; Sampayo et al., 2008; Fisher et al., 2012; Hume et al., 2015; Silverstein et al., 2015). The majority of coral species associate with a single species of Symbiodiniaceae (LaJeunesse et al., 2018; Howells et al., 2020), but some are capable of hosting multiple species and/or genera within one coral colony (Rowan et al., 1997; Baker, 2003; Gardner et al., 2019; Hume et al., 2019, 2020). These two strategies are illustrated in three dominant sympatric corals found in Hawaiʻi, with *Porites compressa* only presenting *Cladocopium* C15, *Pocillopora acuta* combining *C. pacificum/C. latusorum* (C1d/C42), and *Montipora capitata* hosting either *Cladocopium* C31or *Durusdinium glynnii*, or both simultaneously in the same colonies (LaJeunesse et al., 2004; Innis et al., 2018; Stat et al., 2013; Turnham et al., 2021). There is some evidence of symbiont shuffling in some coral species (Baker, 2001; Cunning et al., 2015), but this may occur rarely or not at all in others, as reported in *Pocillopora* spp. (McGinley et al., 2012) and *M. capitata* (Cunning et al., 2016). This inflexibility could be intrinsic of those holobionts, or due to the particular conditions of disturbance and recovery not favoring Symbiodiniaceae rearrangements.

Beyond Symbiodiniaceae, patterns of symbiosis with microorganisms forming the coral holobiont are less understood (Amend et al., 2012; Ainsworth and Gates, 2016; Boilard et al., 2020). The coral prokaryotic microbiome is thought to have a core component (Ainsworth et al., 2015; Hernandez-Agreda et al., 2017), as well as a set of unique microbes (Hernandez-Agreda et al., 2018), and rare dynamic taxa that can vary in individuals even within species (Epstein et al., 2019). Stability in coral microbial associations may be beneficial or deleterious, depending on the context (Ainsworth and Gates, 2016). Community shifts involving increases in opportunistic, potentially pathogenic taxa and decreases in beneficial taxa, have been observed during induced and natural bleaching stress (Bourne and Munn, 2005; Littman et al., 2011); including studies on *P. compressa* (Vega Thurber et al., 2009) and *Pocillopora* (Tout et al., 2015). Other work has shown that microbial stability may either promote thermal tolerance (Ziegler et al., 2017; Epstein et al., 2019; Gardner et al., 2019), and/or hamper acclimatization, with deleterious effects on the host (Pogoreutz et al., 2018). As with Symbiodiniaceae, prokaryotic associates may include taxa able to confer stress tolerance to the holobiont (Van Oppen and Medina, 2020; Ainsworth et al., 2020).

In 2014 and 2015, there were repeated massive bleaching episodes in the Hawaiian archipelago (Ritson-Williams and Gates, 2020). These thermal stress events prompted us to survey the fate of coral microbiomes (Symbiodininaceae, Archaea/Bacteria, Fungi) over time during and after the heatwaves in the field. In Kāneʻohe Bay, Oahu, both *Montipora capitata* and *Porites compressa* had bleaching susceptible vs bleaching resistant phenotypes, while only bleaching susceptible colonies of *P. acuta* were observed. These three dominant species were monitored and sampled throughout a year. There has been extensive research on these coral species in Hawaiʻi (e.g., Putnam and Gates, 2015; Cunning et al., 2016; Wall et al., 2019; Matsuda et al., 2020; Ritson-Williams and Gates, 2020; Innis et al., 2018), however, there is little information about their associated microbiota (e.g., Salerno et al., 2011; Shore-Maggio et al., 2015; Epstein et al., 2019). This study quantifies temporal dynamics in coral microbiomes using amplicon sequencing of multiple gene regions for multiple microbial compartments, coupled with compositional data analysis, to track symbiont shifts in multiple bleaching phenotypes within and among coral species. We further inspect for microbial sentinels within the coral holobionts able to diagnose fluctuations from healthy to distressed/diseased states.

2. Materials and methods

2.1. Study site and sampling

Consecutive coral bleaching events occurred in Hawaiʻi during the late summers of 2014 and 2015 (Ritson-Williams and Gates, 2020). The present study focused on corals from Reef 25 in the central portion of Kāneʻohe Bay, O'ahu Island (N 21.461, W 157.823). In October 2014, 20 colonies of *Montipora capitata* (Mcap) and 20 *Porites compressa* (Pcom) were tagged as adjacent pairs (ten totally bleached and ten non-bleached –fully dark brown, hereinafter referred to as "B" and "NB" colonies respectively for each species); along with ten colonies of fully bleached *Pocillopora acuta*

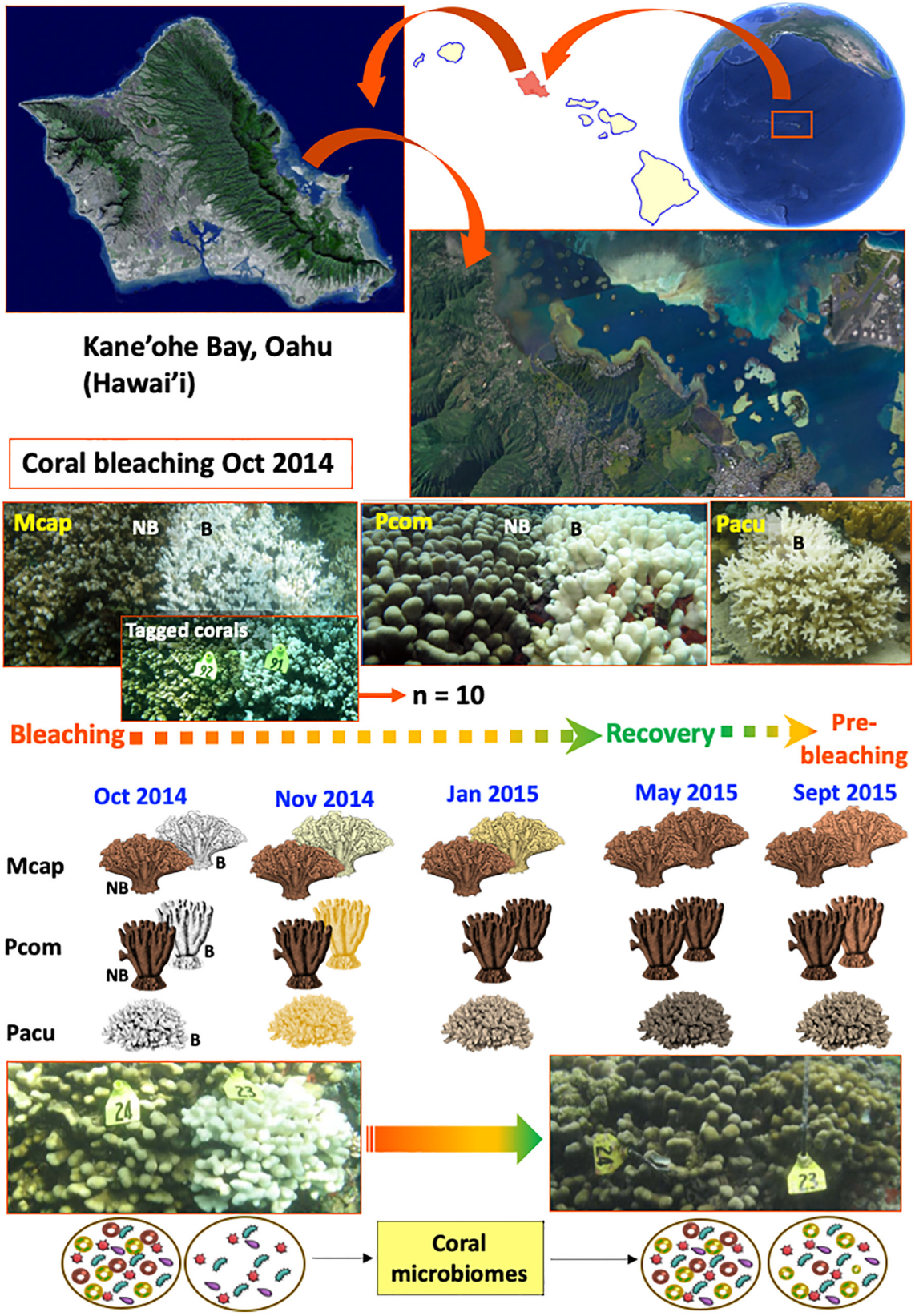


Fig. 1. Map of Kāne'ohe (O'ahu) in the Hawaiian archipelago. Experimental coral sampling scheme from tagged corals in the field, where 1.5 cm fragments were collected for each colony in all sample groups (n = 10): Mcap_B, Mcap_NB, Pcom_B, Pcom_NB, and Pacu _B on five occasions during the recovery after the first beaching event: M0: Oct 2014; M1: Nov 2014; M3: Jan 2015, M6: May 2015, M12: Sept 2015. Species: Mcap: *Montipora capitata*, Pcom:s *Porites compressa*, Pacu: *Pocillopora acuta*. Bleaching susceptibility: B: susceptible colonies; NB: resistant colonies.

(Pacu, there were no individuals of *P. acuta* that did not bleach). All tagged colonies came from 4 to 5 m depth, and were 50 in total (n = 10 for each sample group: Mcap_B, Mcap_NB, Pcom_B, Pcom_NB, and Pacu _B). One coral fragment (1.5 cm long) was repeatedly sub-sampled from every tagged coral on five occasions: M0 = Month 0 – October (24th) 2014; M1 = Month 1 – November (24th) 2014; M3 = Month 3 – January (14th) 2015; M6 = Month 6 – May (6th) 2015; and M12 = Month 12 – September (15th) 2015, yielding a total of 250 coral fragments (Fig. 1). Covariates used in downstream analyses are given in Table S1. Coral fragments (~1 cm^3) were collected at each time point by snorkelers, placed in individual sterile bags and snap-frozen in liquid N within 1 min of collection. All fragments were maintained at −80 °C until processed.

2.2. DNA extraction, library preparation, and sequencing

DNA from coral fragments was extracted using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories) following manufacturer's instructions. Amplicon sequencing of ribosomal RNA (rRNA) target gene markers for three microbial sets: Bacteria/Archaea (16S), Fungi Internal Transcribed Spacer 1 (ITS1) and Symbiodiniaceae (ITS2) was performed in three separate multiplexed runs. Due to technical issues, DNA samples from month M12 (Sept 2015) could not be sequenced for the ITS2 marker. Illumina protocol was applied with a two-PCR approach and two dual-index strategy (Caporaso et al., 2012; Kozich et al., 2013). Primer sets used were: bacterial/archaeal specific primers for V_4 region (*Escherichia coli* position: 515–806) of the small-subunit ribosomal RNA (16S) gene (515F – GTGYCAGCMGCCGCGGTAA Parada et al., 2016 and 806R –GGAC TACNVGGGTWTCTAAT Apprill et al., 2015); ITS-DinoF (GTGAATTGC AGA ACTCCGTG) and ITS2rev2 (CCTCCGCTTACTTATATGCTT (Franklin et al., 2012) targeting the ITS2 for Symbiodiniaceae library; and fungi-specific primers ITS1F (CTTGGTCATTTAGAGGAAGTAA;Gardes and Bruns, 1993) and ITS2R-CoralBetter (GTGARCCAAGAGATCCRTT; designed in the present study) for ITS1. Amplifications were performed in 25 μl reactions with NEBNext® Q5® Hot Start HiFi PCR Master Mix (New England Biolabs, Inc.), 0.8 μl BSA (Bovine Serum Albumin; 20 mg/ml), 1 μl of each 5 μM primer, and 1.5 μl of template. Reactions were under the thermocycling profile: 98 °C for 2 min, then 28 cycles of 98 °C for 15 s, 53 °C for 30 s, 72 °C for 30 s, final extension at 72 °C for 2 min. The second Index PCR to attach dual indexes and Illumina sequencing adapters used forward primers with the 5′-3′ Illumina i5 adapter (AATGATACGGCGACCACC GA GATCTACAC), an 8–10 bp barcode and a primer pad; and reverse fusion primers with 5′-3′ Illumina i7 adapter (CAAGCAGAAGACGGCATACGAGAT), an 8–10 bp barcode, a primer pad. Reactions were made in 25 μl with 0.5 μl of each 5 μM primer, and 1 μl of corresponding products from first amplicon PCR reactions diluted (1:30), and with a temperature regime of: 98 °C for 2 min, then 28 cycles of 98 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s, final extension at 72 °C for 2 min. The PCR products were purified and pooled equimolar on Just-a-Plate ™ 96 PCR Purification and Normalization Kit plates following manufacturer's instructions (Charm Biotec). Paired-end sequencing was performed on an Illumina MiSeq sequencer 2 × 300 flow cell at 10 pM at Core Lab, Hawai'i Institute of marine Biology (USA).

2.3. Bioinformatics analysis

2.3.1. Sequence processing for 16 S V_4 and ITS1 sets

Fastq files containing demultiplexed 16S—V_4 and ITS1paired-end reads were imported into QIIME2 v.2020.11 (Bolyen et al., 2019). DADA2 (Callahan et al., 2016) was used for "denoising" 16S data in paired-end mode. The ITS1 region was first extracted using ITSxpress (Rivers et al., 2018). Only forward reads as in Pauvert et al. (2019) were denoised in single-end mode with DADA2 (Callahan et al., 2016), and filtered from non-fungal ITS sequences (Tables S2A and S2B). Taxonomic annotation was performed using a pre-trained Naïve Bayes classifier (sklearn (Bokulich et al., 2018a, 2018c) against SILVA reference (99 % identity) database v.128 (Quast et al., 2013; Yilmaz et al., 2014) trimmed to span the V_4 region (291 bp) for the 16S data. While for the ITS1 set, UNITE reference database (v. 1.12.2017) was customized adding outgroup metazoan sequences from NCBI to check for host co-amplification (as in McGee et al., 2019; Supplementary Material S1).

2.3.2. Sequence processing for ITS2 set

Demultiplexed paired-end reads from the ITS2 Symbiodiniaceae marker were submitted to SymPortal (SymPortal.org; Hume et al., 2019) to obtain ITS2 type profile predictions, reflecting the "defining intragenomic [sequence] variants" (DIVs) in order of their relative abundance. Absolute abundance counts tables for ITS2 type profiles and underlying ITS2 sequences were formatted and imported into QIIME2 v.2020.11 (Bolyen et al., 2019) for downstream analyses (Supplementary Material S1; and Table S2C).

2.3.3. Microbial community analysis

16S ASVs and ITS2 sequence compositions were analyzed using DEICODE (https://library.qiime2.org/plugins/deicode/19/) diversity method based on Aitchison distances and robust principal component analysis (RPCA) for compositional data (Aitchison, 1982; Martino et al., 2019). Standard diversity distance metrics that do not account for compositionality of data were also computed on QIIME2 v.2020.11 (Bolyen et al., 2019). Statistics were calculated using q2-diversity adonis for multi-factor permutational multivariate analysis of variance (PERMANOVA). The most informative formula in the model for the 16S data was "Species*TimePoint + Bleaching", while "Species*Bleaching" was the most explicative for ITS2. Pairwise comparisons for single covariates were run with q2-beta-group-significance. In all cases permutations were set to 999, and tests corrections significance to q value >0.05 (i.e., FDR adjusted p value; Supplementary Material S1).

2.3.4. Longitudinal, differential abundance and co-occurrence cross networks analyses

By simultaneously analyzing our samples across all time points, meaningful signals may be lost at a particular time point. Also, having more than one measurement per subject in temporal/longitudinal or paired samples experiments violates independency assumptions between samples of Kruskal-Wallis tests. Therefore, pairwise PERMANOVA comparisons were run for each timepoint by species. Further pertinent methods for differential abundance (Morton et al., 2019; Fedarko et al., 2020), longitudinal analyses –including pairwise differences/distances, linear-mixed-effects (LME) (Bokulich et al., 2018b), and co-occurrence cross network analyses that take into account repeated measurements and data compositionality (Shaffer, 2020; Shannon et al., 2003) were performed as described in Supplementary Material S1.

R (RStudio) was applied for additional statistics and plotting (R Core Team, 2014; http://www.r-project.org).

3. Results

3.1. Bacteria/Archaea composition based on 16S rRNA gene data

3.1.1. Alpha and beta diversity

Pacu and Mcap corals reported higher bacterial diversity, richness and evenness (Shannon, Observed and Pielou's evenness) indexes compared to Pcom (Figs. 2, S3.1; Kruskal–Wallis H = 137.94, $p < 0.001$, $p = 5.56$ e-18). Alpha diversity did not yield significant differences within species between B vs NB colonies in Mcap and Pcom, or across time points in any species (Tables S4; Supplementary Material S2 and S3).

Differences in beta diversity were found by Species, reporting different microbial communities in the three host species, and in the interaction Species*TimePoint; while Mcap and Pacu were more diverse from Pcom for all metrics (PERMANOVA 999 permutations, significance set to $p < 0.05$; Tables S5). Since Bacterial composition was mostly determined by host species, according to all alpha and beta diversity indexes, downstream analyses were performed within species, to test for changes over time in all three species, and between bleached and non-bleached colonies

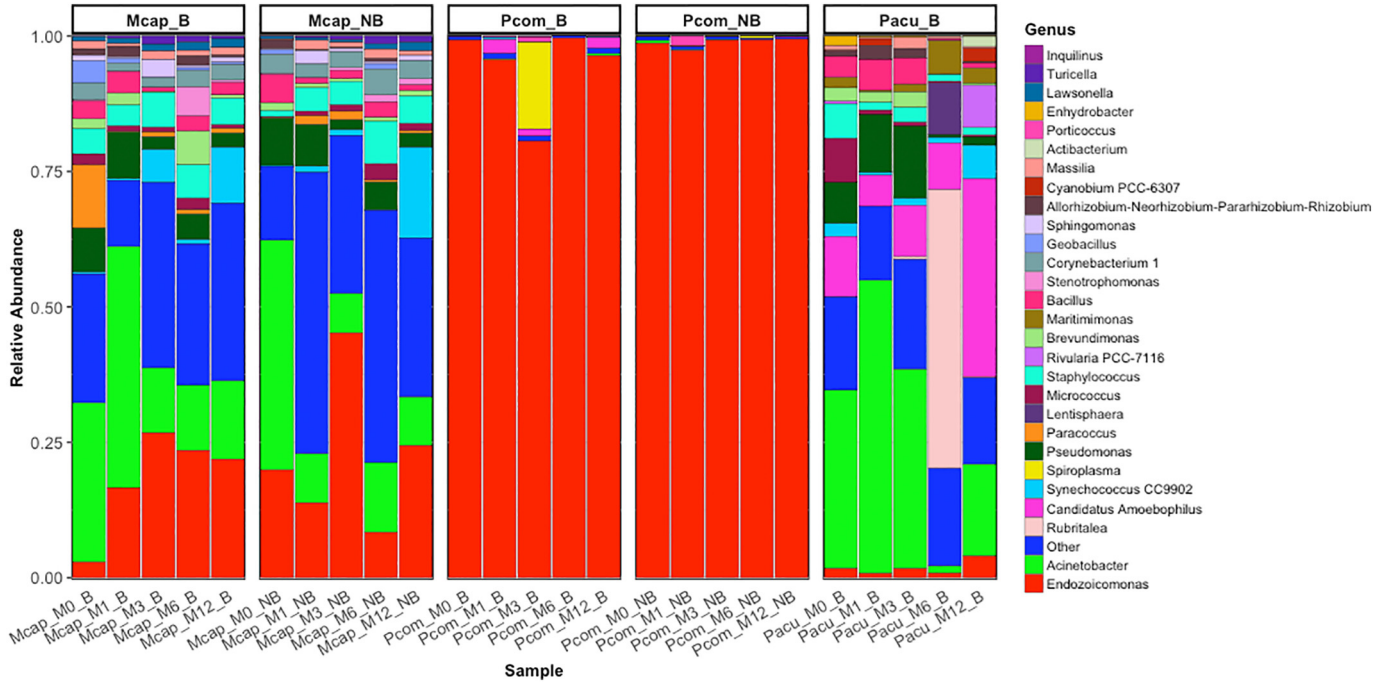


Fig. 2. Prokaryotic composition at the genus level (>0.1 % detection) for the three coral species. Bars are collapsed by species, month after bleaching event and bleaching susceptibility phenotypes; and grouped for species and bleaching susceptibility. Species: Mcap: *Montipora capitata*, Pcom:s *Porites compressa*, Pacu: *Pocillopora acuta*. Bleaching susceptibility: B: susceptible colonies; NB: resistant colonies. Month after bleaching event: M0: Oct 2014; M1: Nov 2014; M3: Jan 2015, M6: May 2015, M12: Aug 2015 (Table S1).

in Mcap and Pcom. Based on Aitchison distances, bacterial composition varied in Mcap NB between M12 and M0, and from M12 with respect to the other months according to Jaccard (Supplementary Material S2 and S3; Tables S6, S7). No significant longitudinal trend was found in beta diversity across nor between timepoints in any B vs NB corals (Tables S6, S7, Supplementary Material S2 and S3).

3.1.2. Bacterial/archaeal community compositions

A total of 1257 ASVs were distributed in 979 Mcap, 523 Pcom, and 737 Pacu associated taxa. Taxonomy annotation at the genus level yielded 331, 211 and 279 bacterial and archaeal genera; this was out of a total of 93, 99 and 40 coral colony fragments belonging to Mcap, Pcom and Pacu respectively. In Mcap corals a bacterial strain within order Myxococcales made up >50 % relative abundance in 35 % of the samples. Other dominant genera were *Acinetobacter* –with preponderance of *A. calcoaceticus*, and *Endozoicomonas*. The least diverse bacterial communities were found in Pcom, predominantly composed of *Endozoicomonas*. A single phylotype in this genus accounted for >90 % in relative abundance in 65 % of Pcom samples. Other representative taxa were *Acinetobacter, Candidatus Amoebophylus,* and order Myxococcales. Pacu was dominated by Proteobacteria, with one strain covering >50 % relative abundance in 43 % of the samples. Most contributing genera included *Acinetobacter* (chiefly *A. calcoaceticus*) and *Candidatus Amoebophylus*, and there was a large proportion of unclassified taxa. In variable abundances, *Pseudomonas, Bacillus, Staphylococcus, Synechococcus, Lawsonella* and unidentified strains in Myxococcales were found in all three species. While, *Micrococcus*, *Corynebacteria, Turicella, Cyanobium, Brevundimonas, Maritimonas, Aerococcus* and *Geobacillus* were more linked to Mcap and Pdam (Fig. 2; Supplementary Material S2 and S3). All coral species shared 237 taxa (19 %), with Mcap sharing more taxa with Pacu (542; 43 %), than with Pcom (395; 31 %), and Pcom and Pacu sharing the least proportion of phylotypes (282; 22 %). The largest number of unique taxa was recorded in Mcap (279), followed by Pacu (149) and Pcom (83).

3.1.3. Phylotype-wise differential abundance analysis of 16S rRNA gene data

The importance (i.e., fold change) of each ASV in relation to the covariates TimePoint (month after bleaching, M0–M12) and Bleaching susceptibility (B vs NB) was calculated in separate analyses within species to create microbial balances.

In Mcap the most informative balance defining longitudinal changes in B vs NB microbiomes consisted of fifteen ASVs in genera: *Endozoicomonas, Acinetobacter, Pseudomonas* in the numerator; and *Micrococcus, Synechococcus, Staphylococcus, Lawsonella, Bacillus* and order Myxococcales, in the denominator (91 out of 93 samples retained). In M0 and M6 NB colonies (ranked to numerator taxa) exhibited significantly higher log-ratios than B (associated to denominator phylotypes; Welch's tests, $p < 0.05$). In M1 and M3, NB had lower log-ratios than B colonies, but the differences were not significant. In M1 and M3, *Bacillus* was not detected as differential taxa, and *Synechoccocus* lost relevance in M6 (Material S4, Tables S8). Longitudinally, for this microbial balance, Mcap_B had higher log-ratio rankings in M3 compared with M6 and M12; whereas Mcap_NB displayed lower log-ratios in all time points with respect to M0 (LME; $p < 0.05$; Fig. 3).

Differentially abundant taxa in the balance of Pcom comprised two *Endozoicomonas* strains in the numerator, along with fluctuating taxa in *Candidatus Amoebophilus, Acinetobacter calcoaceticus, Pseudomonas stutzeri, Synechococcus, Roseitalea*; over *Staphylococcus*, *Micrococcus,* Neisseriaceae and five *Endozoicomonas* in the denominator (Material S4, Tables S8). Pcom_B corals revealed higher log-ratios in M1, with respect to Pcom_NB (Welch's tests, $p < 0.05$; Material S4, Tables S8). Longitudinally, Pcom_B displayed higher log-ratios in M1 in comparison to M0 and M6 (LME; $p < 0.05$), instead Pcom_NB showed stability across time points (LME; $p > 0.05$; Fig. 3, Material S4, Tables S8). Further longitudinal analyses can be found in Supplementary Material S2.

The most discriminative microbial balance of Pacu comprised fifteen taxa assigned to: Endozoicomonas, Cyanobium, Acinetobacter, Pseudomonas, Neisseriaceae in the numerator; and Micrococcus, Lawsonella, Synechococcus, Bacillus, Staphylococcus in the denominator (39 out of

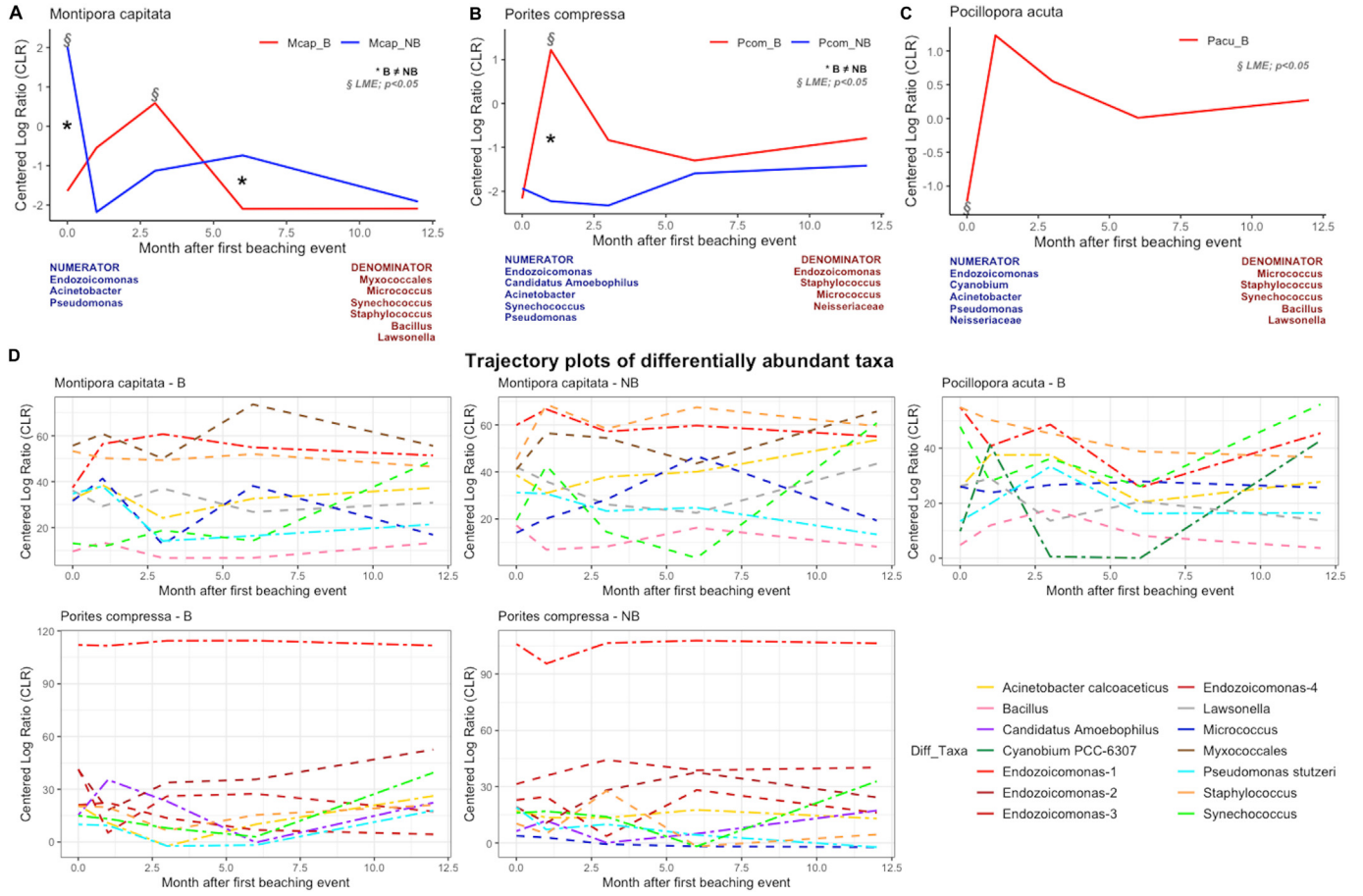


Fig. 3. Volatility of the log-ratios of the microbial/bacterial balance representing the differential taxa across time for the three coral species, by bleaching susceptibility phenotypes. A) *Montipora capitata*; B) *Pocillopora acuta*; and C) *Porites compressa* corals. Numerator and denominator taxa forming each balance are shown in each plot grouped at the genus level, or the next lowest taxonomic annotation. *Represents time points with significant dissimilarities between B and NB colonies (Welch tests $p < 0.05$). §Indicates significant divergence on log-ratio longitudinally over time (LME; $P > [z] < 0.05$). D) Trajectory plots over time of differentially abundant bacterial taxa at the ASV level for the three coral species, by bleaching susceptibility phenotypes. Taxa are named by the lowest taxonomical annotation. Double dashed lines represent selected numerator taxa, while single dashed lines represent denominator taxa within each coral subset. Mcap: *Montipora capitata*, Pcom *Porites compressa*, Pacu: *Pocillopora acuta*; NB: Bleaching resistant colonies, B: Bleaching susceptible colonies; Months after the bleaching event: M0: Oct 2014; M1: Nov 2014; M3: Jan 2015, M6: May 2015, M12: Sept 2015.

40 samples kept). M0 colonies had lower log-ratios with respect to all the other time points (LME; $p < 0.05$; Tables S8; Fig. 3).

The longitudinal behavior (over time) of bacterial genera represented in the above microbial balances for the three host species were inspected in trajectory plots using centered log ratio (CLR) abundances. The investigated genera included: *Endozoicomonas, Acinetobacter, Bacillus, Candidatus Amoebophilus*, *Cyanobium*, *Lawsonella, Micrococcus, Pseudomonas, Staphylococcus, Synechococcus* and *Roseitalea*. Phylotypes included in the differential balances appertaining to family Neisseriaceae and order Myxococcales, but not assigned to genus level, were not included in this analysis (see Fig. 3, and Supplementary Material S2 for detailed interpretations).

3.2. Fungi composition based on ITS1 data

Untargeted host co-amplification was a major constraint in characterizing fungal communities, despite the new primer designed to bypass metazoan DNA. We found 94.8 % coral co-amplification, retrieving only 5.2 % overall fungal sequences. The rate of co-amplification varied among species, with *P. compressa* displaying the largest untargeted co-amplification (98.4 %), followed by *M. capitata* (95.6 %), and *P. acuta* (56 %) (Supplementary Material S2). The most represented fungal species retrieved were *Malassezia restricta, M. globosa, Hortaea_werneckii, Aspergillus penicillioides, Phellinus gilvus*. No further statistical analysis was performed due to insufficient/uneven diversity coverage.

3.3. Symbiodiniaceae composition based on ITS2 data

3.3.1. Symbiodiniaceae ITS2 type profiles

ITS2 type profiles were 29 in total, 27 belonging to the genus *Cladocopium* and 2 to *Durusdinium*. Their associations with corals depended on host species, bleaching susceptibility, and their interaction (PERMANOVA 999 permutations, $p < 0.05$). Certain coral colonies were stable over time in Symbiodiniaceae composition, but others experienced temporal shifts without a clear pattern (Tables S9). *Cladocopium* profiles were dominant in the

Fig. 4. Symbiodiniaceae community composition of the three coral species across time points − 0 (M0), 1 (M1), 3 (M3) and 6 (M6) months after the bleaching event, and beaching susceptibility phenotypes –Bleaching susceptible (B) and resistant (NB) colonies. Each column represents a coral fragment/sample at each collection point. Microalgal IDs are depicted by the relative abundance of ITS2 sequences (> 3 % detection) plotted in the upper bars, and predicted ITS2 profiles plotted in the bars below (normalized to 1). A) *Montipora capitata* (Mcap); B) *Porites compressa* (Pcom); C) *Pocillopora acuta* (Pacu).


A
B M0
NB M0
B M1
NB M1
B M3
NB M3
B M6
NB M6
ITS2 sequences
Profiles
ITS2 sequences
ITS2 type profiles
Mcap
B
Pcom
C
Pacu

three host species. Only one type profile was shared between Mcap and Pacu (C1d), the rest (95 %) were only found in single host species. Mcap had the most varied profiling −10 *Cladocopium*, 2 *Durusdinium*, and were the only corals harboring *Durusdinium* types. Pcom and Pacu reported 10 and 7 distinct unshared *Cladocopium* profiles respectively (Fig. 4). The resistant phenotypes Mcap_NB reported 9 *Cladocopium* and 2 *Durusdinium* profiles, as compared to Mcap_B with 5 and 1 respectively. *Durusdinium* profiles always occurred mixed with *Cladocopium* in 4–5 Mcap_NB colonies per time point. Pcom_B displayed more assorted type profiles across individuals within each time point than Pcom_NB. Mcap_B corals acquired more varied ITS2 profiling with time –including a *Durusdinium* profile acquired in one Mcap_B colony in M6, but this effect was not statistically supported. With the exception of one sample in Mcap_B and one in Pcom_N both in M6, the presence of mixed ITS2 type profiles was only ascertained in Mcap_NB colonies with an incidence of 48 % (Fig. 4).

3.3.2. Underlying Symbiodiniaceae ITS2 sequence composition

Our corals contained 173 *Cladocopium* and 28 *Durusdinium* ITS2 sequences (≥1 % abundance). By coral species and bleaching susceptibility the number of different *Cladocopium/Durusdinium* sequences was higher in NB colonies in Mcap (Mcap_B 51/20 vs Mcap_NB 80/28), as opposed to Pcom that showed more sequence variability in B (Pcom_B 82/3 vs Pcom_NB 62/3); while Pacu reported 19 / 5.

Within the same coral species, ITS2 profiles shared common major sequences (predominant DIVs within type profiles), and were distinguished by other major and minor sequences (nonmajor DIVs, Fig. 4). Across different host species, ITS2 profiles did not share major sequences. Major *Cladocopium* DIVs designating profiles in *M. capitata* were C31 and C17d, in *P. compressa* C15, and in *P. acuta* C1d. *Durusdinium* major DIVs were D4 and D1, followed by D6, only represented as major sequences in *M. capitata*. Variations in ITS2 profiles within the same colonies across time points were therefore due to the loss, gain or substitution of minor sequences prompting a shift in profile assignment (Fig. 4).

ITS2 sequence compositions confirmed the observed pattern of ITS2 profiles, highly structured by host species and bleaching susceptibility, with no consistent temporal shifts. In the RPCA biplots Mcap showed differences in B vs NB colonies at M0, M1 and M3, being D1, D4 and D6 the most correlated DIVs with NB. Pcom revealed dissimilarity between B vs NB colonies at M0, and here D6, C15cc, C3, 70890_C and C3dg were major drivers of NB clustering, versus C15id and 70894_C associated to B (Fig. 5 and PERMANOVA 999 permutations, $p < 0.05$, Tables S10, S11, S12; Supplementary Material S2 and S3).

3.3.3. Phylotype-wise differential abundance analysis of ITS2 data

Selection of the 43 % most differentially abundant ITS2 sequences in relation to covariates TimePoint (M0–M12) and Bleaching susceptibility (B vs NB) in *M. capitata* yielded 23 numerator and 23 denominator phylotypes (keeping 90.78 % samples). This balance discriminated Mcap_B with higher log-ratios from Mcap_NB corals in all time points. Numerator DIVs associated to Mcap_B included some C31, a few C17, C21 and other *Cladocopium* DIVs; denominator DIVs correlated to Mcap_NB comprised 55 % *Durusdinium* DIVs (D4, D1, D6, D1ab, D3h) along with a few C17 and C21 among other *Cladocopium* DIVs (Fig. 5; Tables S13).

In *P. compressa*, the top 25 % differential ITS2 sequences (maintaining 91.25 % samples) resulted in 12 phylotypes (C3 and other *Cladocopium* DIVs) in the numerator correlated Pcom_B colonies, and 12 denominator phylotypes (several C15 and other *Cladocopium* DIVs) associated to Pcom_NB in M0 and M1. In M3 and M6, when corals recovered colouration, Pcom_B and Pcom_NB corals recorded similar log-ratios (Fig. 5; Tables S13).

In *P. acuta* the model with the co-variate "TimePoint" was uninformative with respect to the null model (adding "1" in the formula), indicating no response of Symbiodiniaceae across time.

Log-ratios in the balances of differentially abundant ITS2 sequences tracked longitudinally over time had no significant shifts in any species (LME; $P < [z] < 0.05$; Supplementary Material S5, Tables S13).

3.4. Cross networks between 16S rRNA gene ASVs and ITS2 profiles

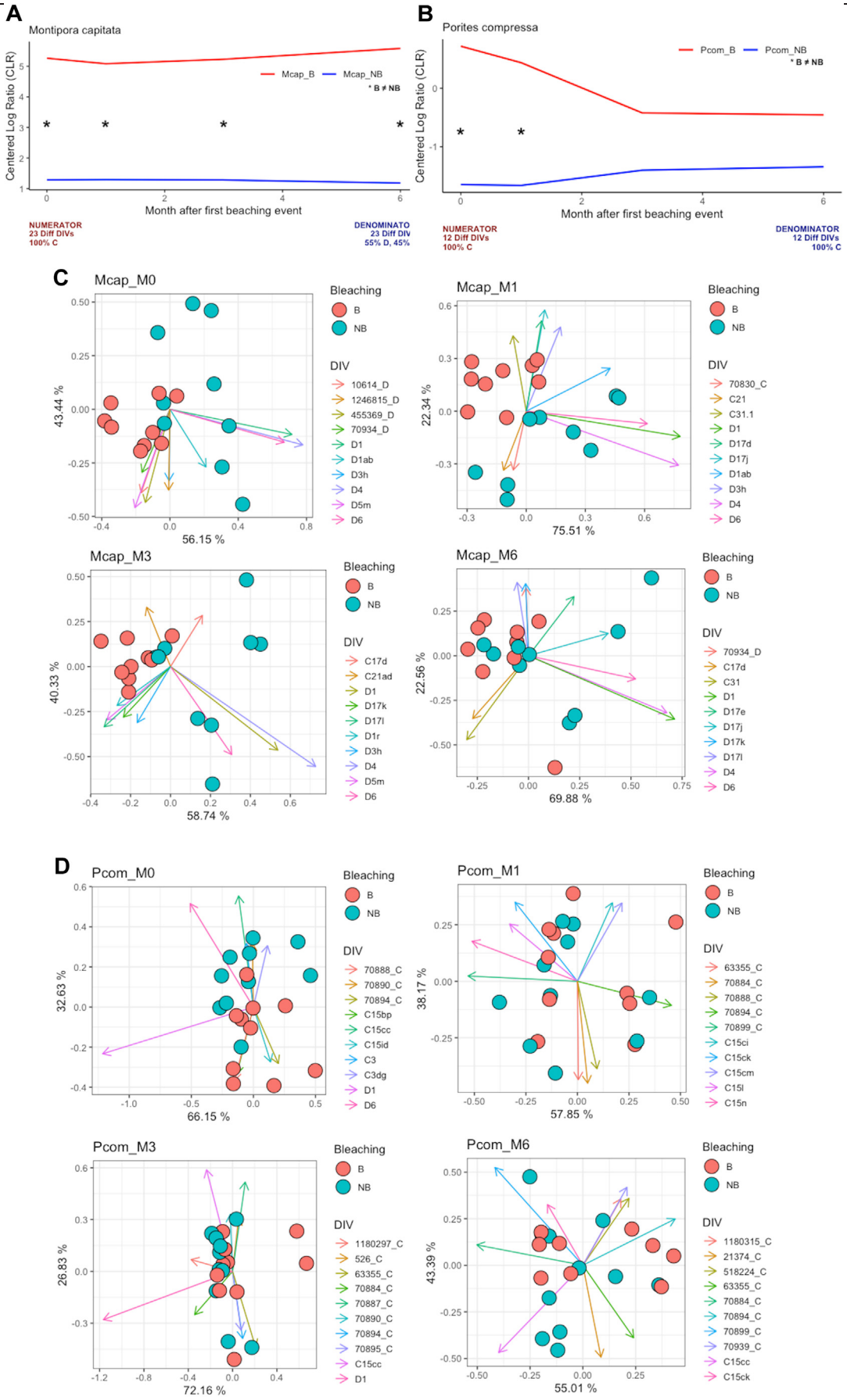
Co-occurrence cross networks illustrated potential interaction patterns among bacteria and Symbiodiniaceae, allowing the detection of changes in coral microbiomes' structure. In Mcap_B a simple network in M0 formed by two C31 *Cladocopium* ITS2 profiles and few bacteria, increased conspicuously in bacterial nodes from M1 to M6, together with the addition of another C31 and a *Durusdinium* D4/D1 nodes in M3 and M6. Mcap_NB started with a complex network composed by four C31, two C17d/C31 and two D4/D1 profiles connected with dense agglomerations of bacteria. The network became less complex in M1 and M3, with the exclusion of two C31 profiles; and acquired more bacterial nodes again over M6, with the re-inclusion of C31 nodes and exclusion of a C17d/C31 node. Pcom_B started with three C15 profiles connected to few bacteria. Bacterial nodes increased over M1 with the addition of a C15 profile, and declined in M6 with the removal of a C15 node. Pcom_NB networks maintained three C15 profiles and few bacteria nodes over M0–M3. In M6 a C1d profile was added, but bacterial nodes and edges diminished. Network complexity increased in Pacu_B from M0–M1, with the increase of C1d profiles from three to four nodes, and with a progressive increment of bacterial nodes over M1–M6. Co-occurrence interconnections were predominant over co-exclusion, except in Mcap_B at M1 and Pacu_B at M3. Networks in susceptible-B colonies in the three species displayed increased positive interactions during bleaching recovery. Whereas, in resistant-NB corals the number of interactions decreased in Mcap_NB, or fluctuated in Pcom_NB. Consistently, Pcom_B and Pcom_NB maintained smaller networks (fewer nodes and edges) than the rest, with the punctual exception of Mcap_B in M0 (Fig. 6; Supplementary Material S2 for detailed results).

4. Discussion

Historically, massive coral bleaching in Hawaiian ecosystems was unusual, until 1996 (Bahr et al., 2015). The consecutive heatwaves of 2014 and 2015 in Kāne'ohe Bay allowed us to track temporal shifts in bleaching susceptible and resistant coral microbiomes in situ, during and after the bleaching peaks. Pcom_B corals recovered faster (after ~2.5 months) than Pacu_B (~3 months), and Mcap_B (~6 months), according to color scores (Ritson-Williams and Gates, 2020), yet actual Symbiodineaceae densities could have been regained faster (Cunning et al., 2016). Prokaryotes, in turn, were expected to exhibit more rapid responses to stressors, due to their fast generation times (Ziegler et al., 2017; Glasl et al., 2017; Pogoreutz et al., 2018).

Algal and prokaryotic communities in our corals followed a species-specific pattern, frequent in sympatric populations (Gardner et al., 2019;

Fig. 5. Volatility of the log-ratios of the balance formed by the ITS2 sequences representing the top differential phylotypes in two coral species by bleaching susceptibility phenotypes across time. A) *Montipora capitata*: The balance included 23 top ranked numerator DIVs comprising some C31, C17 and C21 and other C DIVs; and 23 top ranked denominator DIVs, formed by D4, D1, D6, D1ab and D3h, and a few C17 and C21 among other C DIVs. B) *Porites compressa*: In the balance 12 top ranked numerator DIVs included C3 and other C DIVs; and the 12 top ranked denominator DIVs comprised several C15 and other C DIVs. *Represents time points with significant dissimilarities between B and NB colonies (Welch tests $p < 0.05$).
RPCA compositional biplot based on Aitchison distances (DEICODE) of the total Symbiodiniaceae ITS2 sequences from coral fragments belonging to two species at four time points − 0 (M0), 1 (M1), 3 (M3) and 6 (M6) months after the bleaching event. Samples (circles) were distinguished by color according to Bleaching susceptibility. C) *Montipora capitata* (Mcap) showed differences in B vs NB colonies at M0, M1 and M3. D) *Porites compressa* (Pcom) revealed divergencies in B vs NB colonies only at t0 (PERMANOVA, $p < 0.05$). Ten most relevant DIVs driving differences in the ordination space are illustrated by the vectors in each plot.


A
Montipora capitata
Centered Log Ratio (CLR)
Mcap_B
Mcap_NB
* B ≠ NB
Month after first beaching event
NUMERATOR
23 Diff DIVs
100% C
DENOMINATO
23 Diff DIV
55% D, 45%
B
Porites compressa
Pcom_B
Pcom_NB
* B ≠ NB
Centered Log Ratio (CLR)
Month after first beaching event
NUMERATOR
12 Diff DIVs
100% C
DENOMINATOR
12 Diff DIVs
100% C
C
Mcap_M0
43.44 %
56.15 %
Bleaching
B
NB
DIV
10614_D
1246815_D
455369_D
70934_D
D1
D1ab
D3h
D4
D5m
D6
Mcap_M1
22.34 %
75.51 %
70830_C
C21
C31.1
D1
D17d
D17j
D1ab
D3h
D4
D6
Mcap_M3
40.33 %
58.74 %
C17d
C21ad
D1
D17k
D17l
D1r
D3h
D4
D5m
D6
Mcap_M6
22.56 %
69.88 %
70934_D
C17d
C31
D1
D17e
D17j
D17k
D17l
D4
D6
D
Pcom_M0
32.63 %
66.15 %
70888_C
70890_C
70894_C
C15bp
C15cc
C15id
C3
C3dg
D1
D6
Pcom_M1
38.17 %
57.85 %
63355_C
70884_C
70888_C
70894_C
70899_C
C15ci
C15ck
C15cm
C15l
C15n
Pcom_M3
26.83 %
72.16 %
1180297_C
526_C
63355_C
70884_C
70887_C
70890_C
70894_C
70895_C
C15cc
D1
Pcom_M6
43.39 %
55.01 %
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70939_C
C15cc
C15ck

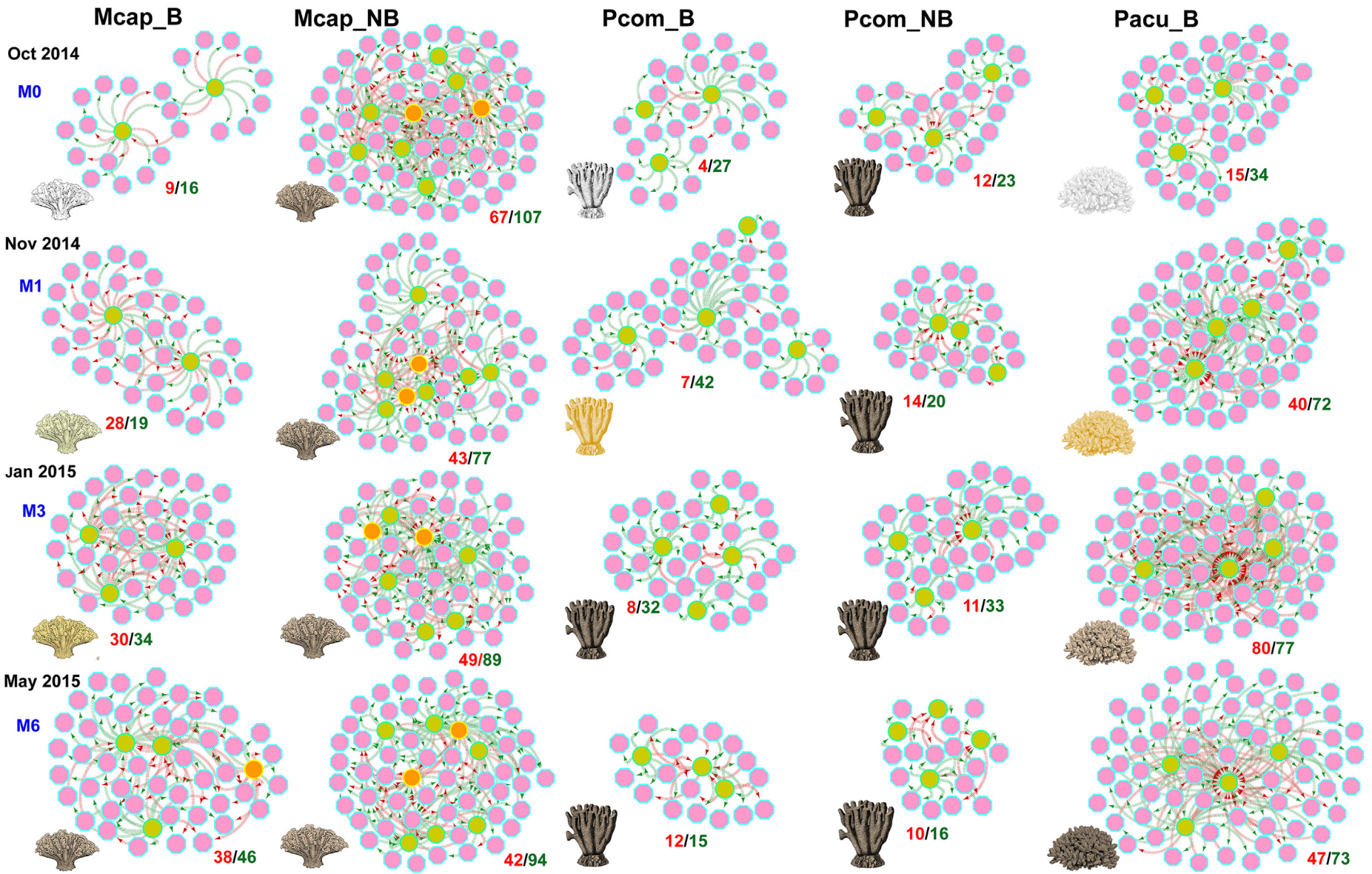

Fig. 6. Cross co-occurrence networks of bacteria phylotypes at the ASV level vs ITS2 type profiles built on SCNIC for the three coral species, by bleaching susceptibility phenotypes 0 (Oct 2014 –M0), 1 (Nov 2014 –M1), 3 (Jan 2015 – M3) and 6 (May 2015 –M6) months after the bleaching event. Mcap: *Montipora capitata*, Pcom *Porites compressa*, Pacu: *Pocillopora acuta*; B: Bleaching susceptible colonies, NB: Bleaching resistant colonies. In the networks bacterial ASVs are represented by pink hexagons, Symbiodiniaceae type profiles of the *Cladocopium* clade are green circles and *Durusdinium* are orange circles. Negative interactions are depicted by red arrows and quantified as red numbers/ positive interactions by green arrows and green numbers.

Howells et al., 2020), whereas intraspecific Symbiodiniaceae signatures were identified at the colony scale (Rouzé et al., 2019). Mcap had the most variable ITS2 profiling, followed by Pcom and Pacu, while Symbiodiniaceae composition was influenced by bleaching susceptibility. Algal-genotypes conferring different bleaching resistance in conspecific hosts may appertain to the same genus, as in Pcom (Sampayo et al., 2008), or to different ones as in Mcap (Berkelmans and van Oppen, 2006; Gardner et al., 2019). But also, susceptibility can be independent from symbiont-type (Smith et al., 2017; Howells et al., 2020).

Bacterial compositions were more diverse in Mcap and Pacu than in Pcom, and were practically constant between bleaching phenotypes. Microbial stability after natural thermal disturbance has been reported in corals undergoing sub-bleaching (Epstein et al., 2019) and bleaching (Gardner et al., 2019). While, community shifts were documented after induced stress (Bourne and Munn, 2005; Vega Thurber et al., 2009; Littman et al., 2011; Ziegler et al., 2016). In our corals, certain bacterial-ASVs/Symbiodiniaceae-DIVs were differentially abundant across time and/or bleaching susceptibility, highlighting the potential of fine-scale microbiome changes in coral resilience (Glasl et al., 2017; Ziegler et al., 2019; Epstein et al., 2019). Below we discuss the dynamics of coral microbiomes during the process of bleaching and recovery in the different host species.

4.1. Pocillopora acuta

Pacu had the highest bleaching incidence, and was associated with eight fluctuating Symbiodiniaceae C1d-profiles. This agreed with the C1d-dominance described for this species in Hawai'i (LaJeunesse et al., 2004). Predicted profiles dominated by C1d and C42.2 likely reflect the preponderance of mixed *Cladocopium pacificum* and *C. latusorum* (Turnham et al., 2021). Lack of acclimatization patterns agrees with stabilities of dominant symbionts in pocilloporids under thermal stress. While, profile shifting driven by minor ITS2-sequences shifts, is presumably matching with background genotype variability reported in previous studies (Stat et al., 2009; McGinley et al., 2012; Epstein et al., 2019). In other geographies, higher bleaching thresholds have been reported in populations harboring *Durusdinium glynnii* (previously D1) (Glynn et al., 2001; Wham et al., 2017; Brener-Raffalli et al., 2018; Li et al., 2021; Zhou et al., 2021), or in chunky (versus fine) morphotypes, even when presenting C1d (Smith et al., 2017; Epstein et al., 2019). Therefore, the bleaching incidence observed in Pacu could rely on a combination of having fine morphology and *Cladocopium*–profiles, both correlated to higher susceptibilities (Smith et al., 2017).

Bacterial communities were dominated by phylum Proteobacteria, followed by Bacteroidetes, Actinobacteria, Firmicutes and Cyanobacteria, similar to pocilloporids from other regions; whereas, Family Amoebophilaceae (mostly *Candidatus Amoebophylus*) and genus *Acinetobacter* (largely *A. calcoaceticus*) were more preeminent, and *Endozoicomonas* less abundant in Pacu (Bourne and Munn, 2005; Tout et al., 2015; Brener-Raffalli et al., 2018; Li et al., 2021; Zhou et al., 2021, but see Epstein et al., 2019; Osman et al., 2020). Prokaryotic community, in terms of overall alpha and beta diversity, did not show significant changes over time, as in other surveys involving coral bleaching (Pogoreutz et al., 2018; Gardner et al., 2019). Nonetheless, microbial rearrangements could be detected via balances of differentially abundant taxa, revealing lower log-ratios in corals at the bleaching peak M0. Upon recovery (M1–M12) Pacu was correlated to *Endozoicomonas, Cyanobium, Acinetobacter, Pseudomonas* and *Neisseriaceae*, whereas bleached colonies in M0 were associated to *Micrococcus, Lawsonella, Synechococcus, Bacillus* and *Staphylococcus*. Likewise, cross co-occurrence networks showed an increase in node complexity and positive interconnections from M1. This implied that sparse interactions between bacteria and Symbiodiniaceae during thermal stress, increased in number as algal cells repopulated in the recovery process after M0, yielding larger networks.

Recovery in Pacu happened after 2–3 months (Ritson-Williams and Gates, 2020); probably thanks to heterotrophic feeding (Lyndby et al., 2020; Dobson et al., 2021) and, microbiome rearrangements in early recovery phases (Santos et al., 2014; Ziegler et al., 2017).

4.2. Montipora capitata

Mcap colonies were associated with *Cladocopium* and *Durusdinium* symbionts. At the DIV level C31, C17 and C21 were predominant genotypes in both B and NB corals, while D4, D1, D6, D1ab and D3h characterized NB colonies, in agreement with recent studies (Matsuda, 2021). Bleaching resistant Mcap_NB colonies contained either pure C or mixed D/C profiles (50 % of the times), and were different from susceptible Mcap_B, which contained basically C-profiles. Adjacent colonies never shared the same ITS2-profile. In both bleaching phenotypes, six colonies (66 %) maintained their corresponding dominant profiles, the remaining (three) experienced temporal shifts, in agreement with Cunning et al. (2016). C31-C17d-C31.1-C31a-C21-C31f-C17e-C31l-C21ac might represent a thermosensitive ITS2-profile, as 8 out of 9 Mcap_B bleached colonies in M0 contained this profile, while its presence in Mcap_NB (1–2 colonies) was always in combination with D-profiles. In purity or mixed, D-genotypes provide thermal resistance in *M. capitata*, but colonies with C-profiling also demonstrated stress-tolerance (Cunning et al., 2016). Our analyses based on ITS2-types (Hume et al., 2019) identified different *Cladocopium* profiles, in comparison to previous surveys reporting solely C31-genotype (LaJeunesse et al., 2004; Stat et al., 2013; Cunning et al., 2016), which could resolve the disparate stress-resistance of Mcap_NB vs Mcap_B. In one exception though, two colonies containing the same profile (C31/C17d-C21-C31.9-C21ac-C17e-C31h-C31i) at M0, one underwent bleaching and the other one not, suggesting multiple factors (including different microenvironments affecting these corals) other than symbiont type regulating thermal tolerance. Mcap_B and Mcap_NB maintained different Symbiodiniaceae compositions, based on profiles and underlying ITS2-sequences, while colony heterogeneity in bleached Mcap_B increased with time, with no clear stabilization pattern. Actually, only one colony acquired a partial *Durusdinium* profile at M6, supporting the low prevalence of symbiont shuffling described in this species (Cunning et al., 2016).

Prokaryotic communities were dominated by Proteobacteria (Family P3OB-24, Order Myxococcales), and by genera *Acinetobacter* (largely *A. calcoaceticus*) and *Endozoicomonas*. In general, they matched with *M. capitata* microbiomes, characterized by the presence of Cyanobacteria and Deinococcus-Thermus, and low abundance of *Vibrio* (Shore-Maggio et al., 2015; Beurmann et al., 2018). Even if non statistically significant, increased alpha diversities observed in Mcap_B at M1 and M3 may suggest microbial rearrangements after thermal-stress (Vega Thurber et al., 2009; Tout et al., 2015; McDevitt-Irwin et al., 2017), or seasonal fluctuations in Mcap_NB at M6 (Cunning et al., 2016). Log-ratio rankings of differentially abundant taxa were higher in Mcap_NB with respect to Mcap_B at M0 and M6. At these two time points of symbiont depletion: bleaching peak (M0) and seasonal algal downturn (M6; as in Cunning et al., 2016), Mcap_NB was ranked to numerator taxa –*Endozoicomonas, Acinetobacter and Pseudomonas*; whereas bleached Mcap_B were correlated to denominator taxa –Myxococcales, *Lawsonella, Micrococcus, Synechococcus, Bacillus* and *Staphylococcus*. Cross networks became more complex in Mcap_B from M1 to M6, as algal densities recovered (M1–M3), and bacteria established interactions with Symbiodiniaceae. Instead, Mcap_NB showed higher network complexity in M0 compared to bleached Mcap_B colonies, reflecting stress response rearrangements between thermo-tolerant algal and prokaryotic symbionts during the heatwave.

M. capitata was found to rely on heterotrophy to compensate for energy losses when experimentally bleached (Grottoli et al., 2006). Mcap did not evidence such trophic plasticity, and would have regained symbiont populations at expense of biomass resources by January 2015 (Wall et al., 2019; Ritson-Williams and Gates, 2020), in agreement with the microbial outcomes.

4.3. Porites compressa

ITS2-profiling in Pcom revealed C15-dominance, in accordance with older surveys on *Porites compressa* (LaJeunesse et al., 2004). Pcom_NB and Pcom_B corals held distinct Symbiodiniaceae patterns 70–90 % of the

times, across M0–M12. While, other characteristics in the holobiont or microenvironmental variabilities causing different stress conditions, should explain why 20 % adjacent Pcom_B and Pcom_NB colonies sharing the same profiles had different susceptibilities in M0. During the peak of the heatwave in Oct-2014 (M0) Pcom_NB associated to DIVs C15cc and D6, and more often to the ITS2 profile C15-C5ci-C15cc-C15cl-C15n-C15cj-C15l, which could represent a thermotolerant symbiont-type found in 7 out of 10 resistant colonies, and in only one susceptible Pcom_B. Accordingly, this profile was less prevailing in M6 (May 2015), coinciding with a period of minor thermal disturbances and lower symbiont abundances (Brown et al., 1999; Cunning et al., 2015). C15-genotypes with higher temperature tolerance were already described associating to *Porites* spp. from the Great Barrier Reef (Fisher et al., 2012). Dissimilarities in ITS2-sequences between Pcom_B and Pcom_NB tended to vanish after M1, reflecting algal rearrangements linked to recovery from this time point. This concurs with coral photo-physiology data supporting intense symbiont repopulation (elevated cell mitosis and photopigment synthesis) from Nov 2014 (Wall et al., 2019; Matsuda et al., 2020; Ritson-Williams and Gates, 2020).

Bacterial communities in Pcom were less diverse than in the other hosts, accounting for many low abundance taxa, and ~ 90 % predominance of a single *Endozoicomonas* microbe. The bacterial community structures were relatively constant, across bleaching phenotypes and time. Salerno et al. (2011) also found stable microbiomes in *P. compressa* under mild thermal treatments; whereas Vega Thurber et al. (2009) observed switches from healthy to pathogenic microbiota after intense high temperature exposures. Both of these thermal stresses were administered in an experimental setting. In our field data the prevalent ASV (694df3c7f8b6b66c922ed51a965d75d0a) matched with a symbiont (Oceanospirillaceae-OTU C7-A01: FJ930289.1; Supplementary Material S2) broadly documented in *Porites* spp. (including *P. compressa* from Maui) and other hermatypic corals from Australia, Hawai'i, and Bermuda (Speck and Donachie, 2012), suggesting a conserved large-scale partnership with corals (Neave et al., 2016). Coral-microbiomes dominated by one or few *Endozoicomonas* phylotypes were described to have microbial inflexibility in stress responses (Pogoreutz et al., 2018). In our susceptible Pcom_B corals dominated by one *Endozoicomonas* strain though, the microbial balance composed by two *Endozoicomonas* (the predominant ASV above and another congeneric strain), *Candidatus Amoebophilus, Acinetobacter calcoaceticus, Pseudosmonas stutzeri, Synechococcus* and *Roseitalea* phylotypes; over five antagonistic *Endozoicomonas* strains, *Micrococcus, Staphylococcus* and Neisseriaceae taxa, pinpointed a longitudinal discontinuity of increased log-ratios in Pcom_B at M1. Microbial communities of bleaching resistant Pcom_NB phenotypes, in contrast, remained stable and dominated by *Endozoicomonas*. Different from Pogoreutz et al. (2018) findings, the relative microbial inflexibility of Pcom and *Endozoicomonas* predominance, could afford benefits in terms of resistance or faster recovery during thermal stress responses.

Pcom was characterized by small cross networks with mild fluctuations between heatwaves, reflecting a much simpler microbial community. Increased edge complexity at M1 in Pcom_B again suggests a rapid recovery response, with reliance on few bacterial ASVs; as compared to Mcap_B and Pacu_B, reflecting larger bacterial consortia participating in the recovery. Reduced trophic plasticity, and intense loss of Symbiodiniaceae and photosynthetic pigments might obligate Pcom_B to regain symbionts faster, at high biomass investment with respect to the other species (Wall et al., 2019; Matsuda et al., 2020). Furthermore, intense algal repopulation in Pcom_B from October–November 2014 was correlated with low symbiont $\partial^{15}N$ (Wall et al., 2019), and assimilation of ^{15}N depleted sources, possibly derived from diazotroph bacteria via N_2 fixation (Lesser et al., 2007; Cardini et al., 2015; Bednarz et al., 2017). Indeed, differentially abundant taxa ranked to recovering Pcom_B included various diazotroph taxa (see below).

4.4. Differentially abundant bacterial taxa defining temporal shifts in bleaching recovery

Coral microbiomes in the present study revealed minor community disruption in response to heatwaves. Similar outcomes were reported previously, together with increases in potentially beneficial microbes (Santos et al., 2014; Epstein et al., 2019). One bacterial group widely associated to corals and documented to display diversified tolerances and/or functional traits to stress conditions is *Endozoicomonas* (Bourne and Munn, 2005; Vega Thurber et al., 2009; Littman et al., 2011; Neave et al., 2016; McDevitt-Irwin et al., 2017; Pogoreutz et al., 2018; Ziegler et al., 2016, 2017, 2019; Epstein et al., 2019). In our corals, saving an initial decline in Mcap_B at M0, this genus displayed preponderance throughout bleaching stress, in agreement with other studies (Ziegler et al., 2016; Pogoreutz et al., 2018; Epstein et al., 2019). *Endozoicomonas* symbionts are proposed to play three kinds of functions: 1) nutrient acquisition/provision –carbon, nitrogen, sulfur, methane recycling, amino acid production, dimethylsulfoniopropionate (DMSP) metabolism; 2) microbiome modulation –quorum-sensing; and 3) promotion of host health –antimicrobial activity, pathogens exclusion (Neave et al., 2016, 2017). DMSP produced by Symbiodiniaceae and sulfur-derivatives from certain prokaryotes (*Endozoicomonas*, *Acinetobacter*, *Pseudomonas*, *Vibrio*) provide a selective environment structuring bacterial populations (Raina et al., 2010). Hence, upon the downturn of DMSP production throughout bleaching/stress episodes, high abundances of *Endozoicomonas* might modulate microbiomes steadiness (Ziegler et al., 2016; Pogoreutz et al., 2018; Epstein et al., 2019). Further, diazotroph bacteria contribute to homeostasis during bleaching and sub-bleaching recovery after thermal stress (Santos et al., 2014; Epstein et al., 2019), by suppling limiting nitrogen to Symbiodiniaceae (Lesser et al., 2007; Olson et al., 2009; Cardini et al., 2015; Bednarz et al., 2017). Indeed, many differentially abundant taxa positively ranked to our recovering corals included diazotrophs and/or DMSP-metabolizing bacteria: e.g., *Endozoicomonas, Acinetobacter calcoaceticus*, *Pseudomonas stutzeri, Cyanobium* (Lalucat et al., 2006; Lesser et al., 2007; Olson et al., 2009; Raina et al., 2010). High occurrence of *Acinetobacter* spp. and *Endozoicomonas* spp. is frequently documented in healthy and bleached Scleractinia, implying synergistic roles in fitness (Cai et al., 2018). Another recently described symbiont in coral holobionts is *Candidatus Amoebophilus*, an intracellular associate of unicellular eukaryotes, like Symbiodiniaceae or amoebae, with undefined role (Huggett and Apprill, 2019; Epstein et al., 2019). Differential phylotypes in this genus were correlated to algal repopulation, particularly in Pcom_B. Bleaching entails loss of major nourishment inputs and photoprotection, and corals therefore implement compensatory strategies (Fitt et al., 2001). For instance, bleached corals have been observed to reinforce feeding on planktonic diazotrophs and preferentially on nitrogen-rich *Synechococcus* cyanobacteria (Meunier et al., 2019). Accordingly, bleached colonies and incipient recovery stages in this study were associated to *Synechococcus*; but interestingly also to differential phylotypes with potential UV-absorbing properties, like *Bacillus*, *Staphylococcus* (Ravindran et al., 2013), *Micrococcus* (Arai et al., 1992), and the already mentioned Cyanobacteria –*Cyanobium, Synechococcus* (Sinha et al., 2001). Notably, *Bacillus* and *Staphylococcus* strains within the coral mucus have demonstrated to increase their UV-absorbance range in response to elevated temperatures, likely protecting bleached colonies from excessive irradiation prior to recovery (Ravindran et al., 2013). *Lawsonella* was another genus frequently associated with bleached corals here. Despite the little information that exists on marine representatives, it could involve opportunistic/transient microbes, as those described in certain human abscesses (Bell et al., 2016). Differentially abundant taxa were broadly shared between Mcap and Pacu, and partially matching with Pcom –this last chiefly influenced by *Endozoicomonas* spp. This outcome is appealing, and suggests that locally the same players may modulate stress responses in different coral species. Thus, understanding the dynamics of differentially abundant microbial consortia in correlation with bleaching and recovery, could provide regional indicators to forecast the fate of sympatric corals to upcoming heatwaves (Glasl et al., 2017). Furthermore, certain strains could be proposed as "probiotics" to improve coral resistance (Peixoto et al., 2017).

5. Conclusions

Prokaryotic and algal microbiomes differed among the three coral species. Despite the recovery of the bleached individuals, there was no apparent pattern of temporal acclimatization. Symbiodiniaceae shifts were found by bleaching phenotype in Mcap and Pcom, probably contributing to resistance. Compared to previous work, ITS2-type profiling (Hume et al., 2019) allowed us to disentangle higher intraspecific resolution within Symbiodiniaceae diversity. Whist compositional analyses (Morton et al., 2019) on the other, permitted the identification of fine-scale differences in the abundance of certain ASVs/DIVs driving changes by bleaching susceptibility and time within each host, despite overall stability of the communities. Fungal associates remain unexplored, until better methods can address host co-amplification and improve taxonomic identifications (Amend et al., 2012).

The three major coral reef founders in Kāne'ohe Bay revealed different responses after 2014–2015 heatwaves. Pacu had thorough bleaching susceptibility and recovered photosynthetic symbionts probably relying on heterotrophy (Lyndby et al., 2020; Dobson et al., 2021), and microbiome rearrangements in the early recovery phases (Santos et al., 2014; Ziegler et al., 2017). Mcap and Pcom displayed two bleaching phenotypes, and the susceptible colonies Mcap_B revealed greater bleaching resistance and slower recovery at low biomass investment. Instead, Pcom_B underwent stronger bleaching (higher pigment loss), faster symbiont repopulation at higher metabolic expenses, but attained better energetic standing (Wall et al., 2019; Ritson-Williams and Gates, 2020).

It is difficult to forecast which of the three strategies will become successful in future scenarios. Yet, after the recent 2019 heatwave in Hawai'i *P. compressa* demonstrated better performance than *M. capitata*, suggesting certain acclimatization (Innis et al., 2018; Matsuda et al., 2020). Similarly, poritid corals from Panamá predicted to be disadvantaged to upcoming climate and anthropogenic disturbances with respect to other co-dominant scleractinians (Aronson et al., 2014), have demonstrated unexpected resilience (O'Dea et al., 2020). Cumulative research demonstrates that coral responses to thermal stress are reliant on host species, geography, and severity/frequency of the events, impeding the elaboration of generalizations. Notwithstanding this limitation, further understanding on microbial balances, may allow for the identification of finer scale taxa dynamics as local indicators of coral reef fitness (Glasl et al., 2017; Peixoto et al., 2017), serving as diagnostic tools for ecosystem stress.

CRediT authorship contribution statement

Laura Núñez-Pons: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **Ross Cunning:** Methodology, Validation, Visualization, Writing – review & editing. **Craig E. Nelson:** Conceptualization, Data curation, Methodology, Resources, Supervision. **Anthony S. Amend:** Conceptualization, Methodology, Resources, Validation. **E. Maggie Sogin:** Methodology, Validation. **Ruth Gates:** Conceptualization, Funding acquisition, Project administration, Resources. **Raphael Ritson-Williams:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Data availability

Sequencing data and associated metadata are available at National Center for Biotechnology Information (NCBI, Genbank) under BioProject PRJNA791513 for 16S rRNA gene, BioProject PRJNA794040 for ITS1, and BioProject PRJNA794042 for ITS2 data. Other data will be made available by contacting the corresponding author.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164040.

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