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Increased diversity and concordant shifts in community structure of coral-associated Symbiodiniaceae and bacteria subjected to chronic human disturbance

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Abstract

Both coral-associated bacteria and endosymbiotic algae (Symbiodiniaceae spp.) are vitally important for the biological function of corals. Yet little is known about their co-occurrence within corals, how their diversity varies across coral species, or how they are impacted by anthropogenic disturbances. Here, we sampled coral colonies (n = 472) from seven species, encompassing a range of life history traits, across a gradient of chronic human disturbance (n = 11 sites on Kiritimati [Christmas] atoll) in the central equatorial Pacific, and quantified the sequence assemblages and community structure of their associated Symbiodiniaceae and bacterial communities. Although Symbiodiniaceae alpha diversity did not vary with chronic human disturbance, disturbance was consistently associated with higher bacterial Shannon diversity and richness, with bacterial richness by sample almost doubling from sites with low to very high disturbance. Chronic disturbance was also associated with altered microbial beta diversity for Symbiodiniaceae and bacteria, including changes in community structure for both and increased variation (dispersion) of the Symbiodiniaceae communities. We also found concordance between Symbiodiniaceae and bacterial community structure, when all corals were considered together, and individually for two massive species, *Hydnophora microconos* and *Porites lobata*, implying that symbionts and bacteria respond similarly to human disturbance in these species. Finally, we found that the dominant Symbiodiniaceae ancestral lineage in a coral colony was associated with differential abundances of several distinct bacterial taxa. These results suggest that increased beta diversity of Symbiodiniaceae and bacterial communities may be a reliable indicator of stress in the coral microbiome, and that there may

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be concordant responses to chronic disturbance between these communities at the whole-ecosystem scale.

KEYWORDS

concordance, co-occurrence, coral microbiome, local disturbance, symbiosis

1 | INTRODUCTION

Scleractinian corals function as "meta-organisms," associating with a diverse array of Symbiodiniaceae (prev. *Symbiodinium*; LaJeunesse et al., 2018) (dinoflagellates), bacteria, archaea, viruses and other single-celled eukaryotic organisms (Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007). Extensive study has shown that these microorganisms are fundamentally important for the growth, reproduction and resilience of the coral animal (Ainsworth et al., 2015; Ainsworth, Thurber, & Gates, 2010; Bourne, Morrow, & Webster, 2016; Peixoto, Rosado, Leite, Rosado, & Bourne, 2017). Yet despite research on their individual roles in corals (Ainsworth et al., 2010; Claar et al., 2017; Putnam, Barott, Ainsworth, & Gates, 2017), the interactions between multiple microorganisms and corals are still not well understood.

A vital component of the coral meta-organism is the Symbiodiniaceae, single-celled endosymbiotic dinoflagellates that provide their host with photosynthetic metabolites in exchange for inorganic nutrients and a relatively stable symbiotic environment. Symbiodiniaceae is divided into seven defined genera (LaJeunesse et al., 2018). Some Symbiodiniaceae (e.g., *Cladocopium* C15 and relatives) are more evolutionarily derived for host-specific symbioses, and thus provide their host with a greater proportion of their photosynthetic products. Conversely, other Symbiodiniaceae tend to be opportunistic and flexible, leading to selfish symbiotic interactions that can deviate towards parasitism (Baker, Freeman, Wong, Fogel, & Knowlton, 2018). The sensitivity of coral symbioses to environmental stress is strongly influenced by both the identity and the abundance of their Symbiodiniaceae partners (Cunning & Baker, 2013). Nutrient enrichment can, for example, destabilize Symbiodiniaceae communities and increase symbiont abundance, making corals more susceptible to bleaching and disease (Cunning & Baker, 2013; Vega Thurber et al., 2014; Wiedenmann et al., 2012). There is also emerging evidence that the diversity of Symbiodiniaceae communities may increase with stressors, and be indicative of compromised coral holobiont function, including decreased carbon shared with coral hosts under heat stress in those corals with more diverse Symbiodiniaceae communities (Kenkel & Bay, 2018).

The coral microbiome also includes a diverse range of bacteria, archaea, fungi and viruses (Knowlton & Rohwer, 2003). The most thoroughly studied members of the coral microbiome are the bacteria, which may act both in beneficial roles (Lesser, Mazel, Gorbunov, & Falkowski, 2004) or as pathogens (Ben-Haim, Banim, Kushmaro, Loya, & Rosenberg, 1999). Thermal stress can cause shifts in bacterial community structure (Littman, Bourne, & Willis, 2010), and increase the prevalence of opportunistic pathogens (Ainsworth & Hoegh-Guldberg, 2009; Bourne, Iida, Uthicke, & Smith-Keune, 2008). It can also increase coral production of dimethylsulfoniopropionate (DMSP), which elicits a chemotactic response in pathogenic bacteria (e.g., *Vibrio* sp.) and guides them towards heat-stressed corals (Garren et al., 2014). A variety of stressors can cause increases in the abundance of bacterial genes involved in virulence and stress resistance, as well as a shift from healthy bacterial communities to unhealthy communities often found in association with diseased corals (Vega Thurber et al., 2009). Anthropogenic stressors (e.g., pollution, excess nutrients) can increase coral-associated bacterial taxonomic richness and the abundance of opportunistic bacterial taxa while decreasing the abundance of purported coral bacterial symbionts (Endozoicomonas) (Gignoux-Wolfsohn, Vollmer, & Aronson, 2016; McDevitt-Irwin, Baum, Garren, & Vega Thurber, 2017). More broadly, anthropogenic stressors have been shown to increase both the alpha and the beta diversity of bacterial communities (McDevitt-Irwin, Garren, McMinds, Vega Thurber, & Baum, 2019; Vega Thurber et al., 2009; Zaneveld, McMinds, & Vega Thurber, 2017), suggesting that, as with Symbiodiniaceae, bacterial community diversity may be a reliable indicator of coral resilience.

Because both Symbiodiniaceae and bacteria are crucial components of the coral meta-organism, it is important to understand the interactions among them. Although evidence of bacterial presence within the symbiosome (i.e., the membrane-bound organelle within corals that houses Symbiodiniaceae cells; Emerich & Krishnan, 2014; Yellowlees, Rees, & Leggat, 2008) is limited (Ainsworth et al., 2015; Pernice et al., 2012; Venn et al., 2009), the transfer of metabolic products among coral, Symbiodiniaceae, and bacteria is probably important to symbiotic interactions (Ainsworth et al., 2015). For example, *Pocillopora damicornis* larvae provide their Symbiodiniaceae with nitrogen acquired from bacteria (Ceh et al., 2013). In a coral model system (*Aiptasia*), distinct bacterial communities were found to be associated with symbiotic and aposymbiotic states with direct links to sulphur- and nitrogen-cycling bacterial abundance (Röthig et al., 2016). In coral tissue, bacterial diazotrophs fix nitrogen that Symbiodiniaceae communities need to grow and reproduce (Rädecker, Pogoreutz, Voolstra, Wiedenmann, & Wild, 2015), and the abundance of nitrogen-fixing gammaproteobacteria has been associated with the abundance of Symbiodiniaceae (Olson, Ainsworth, Gates, & Takabayashi, 2009).

There is also some evidence that the interactions between these symbiotic partners can change under stress. For example, in *Acropora tenuis* juveniles, thermal stress caused a dramatic shift in the bacterial community structure of corals dominated by *Durusdinium*, but no observable changes in those of corals dominated by Symbiodiniaceae *Cladocopium* C1 (Littman et al., 2010). During coral bleaching (the

breakdown of the symbiosis between corals and Symbiodiniaceae), coral microbiomes may shift from healthy communities towards opportunistic taxa such as *Vibrio* (Bourne et al., 2008). Furthermore, a recent in vitro experiment showed that warming caused changes in Symbiodiniaceae culture-associated bacterial communities, and that compared to *Cladocopium* C1 and *Breviolum* sp., the heat-tolerant *Durusdinium trenchii* had the most stable bacterial community under heat stress (Camp et al., 2020). Additionally, environmental adaptation of corals probably involves multiple members of the meta-organism (e.g., coral, bacteria, Symbiodiniaceae; van Oppen et al., 2018).

Despite the importance of interactions between Symbiodiniaceae and bacteria, their relationship to one another may not be easily discernible, because interactions may be masked by disproportionate variability within the bacterial microbiome compared to algal symbionts (Chen, Tseng, Chen, & Tang, 2011). For example, co-occurrence (i.e., the simultaneous presence of two different microbial taxa in one coral sample) and concordance (i.e., similarity in multidimensional microbial community shapes and response to external forcing) may be difficult to detect, and co-occurrence does not necessarily imply interactions between taxa (Freilich, Wieters, Broitman, Marquet, & Navarrete, 2018). In some instances, bacterial community structure is associated with host species identity and location, regardless of dominant symbiont type (Osman et al., 2017). Additionally, extrinsic factors such as habitat or location may be more influential to the coral bacterial microbiome than intrinsic factors such as host genotype or Symbiodiniaceae (Pantos, Bongaerts, Dennis, Tyson, & Hoegh-Guldberg, 2015). However, our understanding of the interactions between these members of the meta-organism is still in its infancy, and further research is necessary.

Understanding the dynamics among the constituents of the coral meta-organism is vital in the face of increasing threats to coral reefs by a suite of anthropogenic stressors that operate at local and global scales (Hoegh-Guldberg et al., 2007; Hughes et al., 2018). Globally, climate change is impacting corals through gradually increasing stress (e.g., ocean warming and acidification), and through pulse heat stress events (e.g., El Niño) (Ainsworth et al., 2016; Claar, Szostek, McDevitt-Irwin, Schanze, & Baum, 2018; Heron, Maynard, van Hooidonk, & Eakin, 2016). Local stressors can decrease coral reef health by eroding resilience capacity over time, making them more sensitive to climate stressors over the long term (Zaneveld et al., 2016). Stress can weaken the coral meta-organism by harming single components within it (e.g., by increasing pathogenic bacteria within the bacterial community; Vega Thurber et al., 2009), by impacting interactions among components (e.g., by decoupling coral–algal symbioses; Brown, 1997), or both. These impacts increase the probability of coral bleaching (Wiedenmann et al., 2012), disease (Vega Thurber et al., 2014) and consequently mortality.

Our aim here was to advance understanding of how the diversity and community structure of two vital groups of microorganisms residing within corals, Symbiodiniaceae and bacteria, respond to chronic local disturbance in a natural ecosystem. We examined paired communities of coral-associated Symbiodiniaceae and bacteria across a spatial gradient of chronic human disturbance on Kiritimati atoll (Christmas Island, Republic of Kiribati, 01°52′N, 157°24′W; Walsh, 2011; Watson, Claar, & Baum, 2016) for seven different coral species that span a range of life history strategies. Within this natural experimental context, we tested two hypotheses: first, that alpha and beta diversity of both the Symbiodiniaceae and bacterial communities would increase with increasing chronic disturbance (McDevitt-Irwin et al., 2017; Zaneveld et al., 2017); and second, that although bacterial communities would be more sensitive to disturbance and exhibit more site-to-site variability than Symbiodiniaceae communities, chronic human disturbance would still cause qualitatively similar responses in these two groups, resulting in broad concordance (i.e., similarity of multidimensional community shape) across the disturbance gradient, because of coral species-specific associations within both communities.

2 | MATERIALS AND METHODS

2.1 | Study design and sampling

We sampled coral colonies at 11 fore reef sites (all 10–12 m depth) on Kiritimati, in the central equatorial Pacific (Figure S1). The sites span a gradient of chronic local human disturbance (Magel, Burns, Gates, & Baum, 2019; Magel, Dimoff, & Baum, 2020). Sites on the northwest coast of the atoll near the largest villages are exposed to the highest levels of subsistence fishing pressure and sewage runoff, as well as dredging (there is a port at site VH1), while sites at remote ends of the atoll experience virtually no local disturbances. We quantified the intensity of chronic local human disturbance at each site, using spatial data for two separate metrics, human population densities and fishing pressure (Table S1). Specifically, we first generated a geographical buffer (in ARCGIS) to determine human population size within 2 km of each site. Nearly all individuals live in villages, and village location was mapped based on published field surveys (Watson et al., 2016). Population size for each village was extracted from the 2015 Population and Housing Census from the Kiribati National Statistics Office (Ministry of Finance). This population metric incorporates immediate point-source inputs from villages into the marine environment such as pollution and sewage runoff. Second, we generated a kernel density function with 10 steps based on mapped fishing intensity from Watson et al. (2016). This metric accounts for the more diffuse, but still important, effects of subsistence fishing on the reef ecosystem. We weighted each metric equally, and from this combined metric we grouped sites into four distinct disturbance categories, termed very low, low, medium and very high (Figure S1; Magel et al., 2020); note the sites included in this study are a subset of those in our broader ecological monitoring programme, which also includes sites in a high disturbance category. We sampled sites in August 2014, with one additional site collected in January 2015 to

expand the geographical and human disturbance range. All sampling occurred during "normal" nonwarming conditions, prior to the onset of heat stress caused by the 2015–2016 El Niño event.

At each site, we sampled eight to 12 colonies (Table S2) of each of three focal species, *Montipora aequituberculata* (n_{TOTAL} = 124), *Pocillopora grandis* (previously *Pocillopora eydouxi*; n_{TOTAL} = 117) and *Porites lobata* (n_{TOTAL} = 96), plus four additional less abundant species that were sampled opportunistically at each site, *Hydnophora microconos* (n_{TOTAL} = 46), *Platygyra ryukyuensis* (n_{TOTAL} = 39), *Favites pentagona* (n_{TOTAL} = 27) and *Dipsastraea matthaii* (previously *Favia matthaii*; n_{TOTAL} = 23). All sampling was conducted under Republic of Kiribati Scientific Research Permit No. 007/14.

Coral biopsies (<1 cm^3) were collected using a small steel chisel. Samples were stored on ice in individual sterile sample bags until processing. Coral biopsies were scraped with a sterile razor blade and separated with sterile tweezers to remove as much skeleton as possible and then subsampled into two separate tubes for bacterial and Symbiodiniaceae DNA extractions which require different methods. For Symbiodiniaceae internal transcribed spacer 2 (ITS2) sequencing preparation, approximately 50 µl of coral tissue was rinsed with filtered fresh water, preserved in 400 µl of guanidinium buffer (50% w/v guanidinium isothiocyanate; 50 mm Tris pH 7.6; 10 µm EDTA; 4.2% [w/v] sarkosyl; 2.1% [v/v] mercaptoethanol), and stored at 4°C until DNA extraction. For bacterial 16S amplicon sequencing preparation, another 50-µl aliquot of the same piece of coral tissue was immediately frozen at -20°C in the field and subsequently stored at -80°C.

A total of 472 coral colonies were sampled and processed for Symbiodiniaceae identification (Table S2), and a total of 252 coral colonies were sampled and processed for bacterial identification (Table S3). Due to logistical constraints, not every coral colony has data available for both Symbiodiniaceae and bacteria. However, to increase sample size for individual Symbiodiniaceae and bacterial analyses (i.e., alpha and beta diversity), we used all available samples, even if a corresponding sample was not available for the other community. A subset of coral colonies (n = 226) was successfully collected and amplified for both Symbiodiniaceae and bacterial communities, and only this subset of samples was used for all comparisons between the two communities (Table S4).

2.2 | Amplicon sequencing

The ITS2 rRNA gene is commonly used to identify Symbiodiniaceae sequences from coral samples. However, this marker does have intragenomic variants (IGVs); that is, a single Symbiodiniaceae cell might have multiple different copies of the same genetic region (Smith, Ketchum, & Burt, 2017). This can be addressed by either (a) evaluating Symbiodiniaceae sequence diversity (i.e., using exact sequence variants) or (b) analysing IGVs to define ITS2 metahaplotypes (Smith et al., 2017) or ITS2 profiles (Hume et al., 2019). Here, we elected to use the first approach and analyse sequence diversity.

For Symbiodiniaceae identification, DNA was extracted from guanidinium-preserved samples using a modified guanidinium-based extraction protocol (Cunning, Silverstein, & Baker, 2015; Stat, Loh, LaJeunesse, Hoegh-Guldberg, & Carter, 2009). Extracted DNA was then cleaned following the standard protocol for Zymo Genomic DNA Clean and Concentrator-25 kits (Catalogue Nos. D4064 and D4065). Samples were prepared for ITS2 amplicon sequencing following the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina protocol, Part no. 15,044,223 Rev. B) with modifications to generalize this protocol for ITS sequences. ITS2 primers with Illumina adapters (underlined) were used instead of the 16S primers: ITSintFor2: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTC-3′ (LaJeunesse, 2002) and ITS2Rev2: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCCGCTTACTTATATGCTT-3′ (Michael Stat, Pochon, Cowie, & Gates, 2009). Prepared libraries were sequenced on the Illumina MiSeq platform using 2 × 300 paired-end read chemistry.

Raw sequences were quality controlled, merged, filtered, and assigned taxonomy using the DADA2 ITS pipeline (Callahan et al., 2016). This pipeline produces amplicon sequence variants (ASVs), which are quality-controlled exact sequence variants. The PHYLOSEQ package (McMurdie & Holmes, 2013) in R was used to store and analyse ASV tables, taxonomic information and sample metadata. A total of 472 samples were successfully amplified and sequenced, yielding a final data set with 9,117,607 sequences. Next, a Symbiodiniaceae phylogenetic tree was constructed by aligning sequences from each genus separately using align_seqs.py from QIIME (Caporaso et al., 2010) using the program MUSCLE (Edgar, 2004). After sequences were aligned within each genus, a distance matrix was created using nr28s-rDNA distances (divergence of the D1–D3 region of the 28S; Pochon & Gates, 2010; Putnam, Stat, Pochon, & Gates, 2012) to describe between-genera distances. Using UPGMA—R package PHANGORN (Schliep, 2011) version 2.2.0—a phylogenetic tree was created via hierarchical clustering and the resulting tree was imported into the PHYLOSEQ object before statistical analysis.

For bacteria DNA extraction, library preparation and sequencing were conducted by the Earth Microbiome Project (EMP) following their standard protocols (http://www.earthmicrobiome.org/protocols-and-standards/16s; Caporaso et al., 2011). 16S rRNA amplification was conducted using the EMP's 515fbc/806r primers and PCR (polymerase chain reaction) and clean-up protocols (Caporaso et al., 2012). Sequence data were filtered and trimmed to 90 bp, and singletons were removed using the software DEBLUR (Amir et al., 2017). DEBLUR is an ASV approach that uses error profiles to obtain single-nucleotide resolution clusters. DEBLUR also includes de novo chimera filtering using UCHIME as implemented by VSEARCH (Amir et al., 2017). ASV assignments were done using the 16S rRNA database Greengenes 13.8 (McDonald et al., 2012). Chloroplast and mitochondrial ASVs were removed prior to downstream analysis. Pipeline details and ASV tables are available on GitHub (https://doi.org/10.5281/zenodo.3829987).

All downstream analyses were completed in R (version 3.4.3; R Development Core Team, 2008) using the package PHYLOSEQ

(McMurdie & Holmes, 2013). Code for all analyses is located on GitHub at https://github.com/baumlab/Claar_etal_2020_MolecEcol (https://doi.org/10.5281/zenodo.3829987).

2.3 | Alpha diversity

To estimate alpha diversity of both Symbiodiniaceae and bacterial communities, we rarified each sample to an equal level of sequences (n = 800) and calculated the Shannon diversity index for each sample; we chose this index because it encompasses both richness and evenness, and is suitable in situations where rare species are as important as abundant species (Morris et al., 2014). We selected this level of sequences for rarefaction to strike a balance between having enough sequences for downstream analysis, while including as many samples as possible (because samples with fewer sequences than the cutoff are discarded). To quantify differences in alpha diversity among human disturbance levels and coral species, we transformed the data using a $\log_{10}$ transformation in PHYLOSEQ, then fitted a linear model with the Shannon Index as the response variable and performed backwards stepwise analyses of variance (ANOVAs) to determine the best model fit using the Akaike information criterion corrected for small sample size (AICc). Then, we used the R package LSMEANS to extract the least squares means for the best model to test for significant differences among the levels of the included categorical variables (Lenth, 2016). Next, to assess richness alone, we calculated the Chao1 estimator (Chao, 1984) from the raw ASV tables, for the Symbiodiniaceae and bacteria data sets, in each case for the full data set and for each sample individually. Chao1 estimates "true" diversity based on missing ASVs (Chao, 1984). Chao1 estimates for each sample were then averaged across groups (i.e., human disturbance level; coral species) to compare richness among these groups.

2.4 | Beta diversity

We consider two components of beta diversity: (a) community structure, defined as a difference in multivariate location or structure among sample groups, and (b) variation (dispersion), defined as multivariate spread within each sample group. To quantify Symbiodiniaceae beta diversity, we transformed all ASV counts to proportions and calculated weighted UniFrac dissimilarity, which allowed us to take into account the phylogenetic distance among clades. To quantify bacterial beta diversity, we transformed all sample counts to proportions and calculated Bray–Curtis dissimilarity distances. With these dissimilarity measures, we first assessed community structure using permutational ANOVAs (PERMANOVAs), implemented using the *adonis* function in the R package VEGAN (Oksanen, 2017). For community structure, our models included coral species, as well as site nested within human disturbance category. We then assessed the variation component of beta diversity using PERMDISP, which tests the homogeneity of multivariate variation within groups, implemented as the function *betadisper* in the R package VEGAN (Oksanen, 2017). Because analyses with *betadisper* can only include one grouping variable, we included only human disturbance category as a predictor in beta diversity variation analyses. We conducted this test for each data set (ITS2/Symbiodiniaceae and 16S/bacteria) among coral species and then among local human disturbance categories.

2.5 | Concordance of Symbiodiniaceae and bacterial communities

Concordance represents similarity in multivariate community shape between two communities across locations or sampling units and can indicate co-occurrence or similar responses of both communities in response to environmental drivers. To test for concordance between community structures of Symbiodiniaceae and bacteria, we used Procrustes analysis (functions *procrustes* and *protest* in the R package VEGAN; Oksanen, 2017), using all coral colonies for which we had both Symbiodiniaceae and bacterial sequence data (n = 226). Procrustes (Gower, 1975) is a symmetric canonical analysis method that enables the comparison of multidimensional community shape between two communities. Procrustes analysis works by comparing two community matrices, one "target matrix" that is kept constant, and another "rotated matrix" which is translated to superimpose the centroids of both matrices, scaled so variation is equal, and then rotated to make the matrices match as well as possible. Here, we used Symbiodiniaceae community as the target matrix, and the bacterial community as the rotated matrix. A significant Procrustes result indicates that the two matrices exhibit consistent multivariate community changes (i.e., similar multivariate "shape") in response to environmental drivers. We chose Procrustes analysis over Mantel tests because (a) Procrustes has increased statistical power compared to Mantel (Peres-Neto & Jackson, 2001); (b) Procrustes can be used to compare multiple data matrices (Lisboa et al., 2014); and (c) Procrustes is particularly good when the compared matrices (communities) are equally applicable as explanatory and response variables (Lisboa et al., 2014). We conducted Procrustes analysis for all coral species combined, as well as for each coral species individually.

2.6 | Associations between Symbiodiniaceae lineages and the bacterial microbiome

Finally, we quantified bacterial microbiome members that may be associated with the presence of different ancestral Symbiodiniaceae lineages by testing for differential abundance of bacterial ASVs using DESEQ2 (Love, Huber, & Anders, 2014). Symbiodiniaceae lineage is defined by the major radiations within the family, such as C1, C3, C15, C31, C42 and D1 (Table S6). We chose to investigate the dominant ancestral Symbiodiniaceae lineage within a coral colony,

because these are probably important for the ecological function of the host coral. First, we determined the dominant symbiont lineage (i.e., the most common lineage) for each coral sample that also had bacterial sequences. To do this, each ASV that was dominant in at least one coral colony was blasted with NCBI's nucleotide BLAST to find the most closely related Symbiodiniaceae lineage (Table S5). For each dominant Symbiodiniaceae lineage, we used DESEQ2 to calculate differential bacterial abundances with all coral samples combined. Increased abundance of a bacterial ASV indicates that there is more of that ASV detected if that particular Symbiodiniaceae lineage is dominant. DESEQ2 can be used where sample size is greater than six samples per group (i.e., each lineage), because it is able to flag and remove outlier values when sample size is greater than this cutoff, and thus minimizes false discovery rates. Therefore, we also used DESEQ2 to analyse differential abundance of the bacterial microbiome for the coral species–Symbiodiniaceae lineage combinations that met this criterion (i.e., at least six samples for more than one dominant lineage; Table S6): *Favites pentagona* (*Cladocopium* C3 and *Durusdinium* D1) and *Platygyra ryukyuensis* (*Cladocopium* C3 and *Durusdinium* D1).

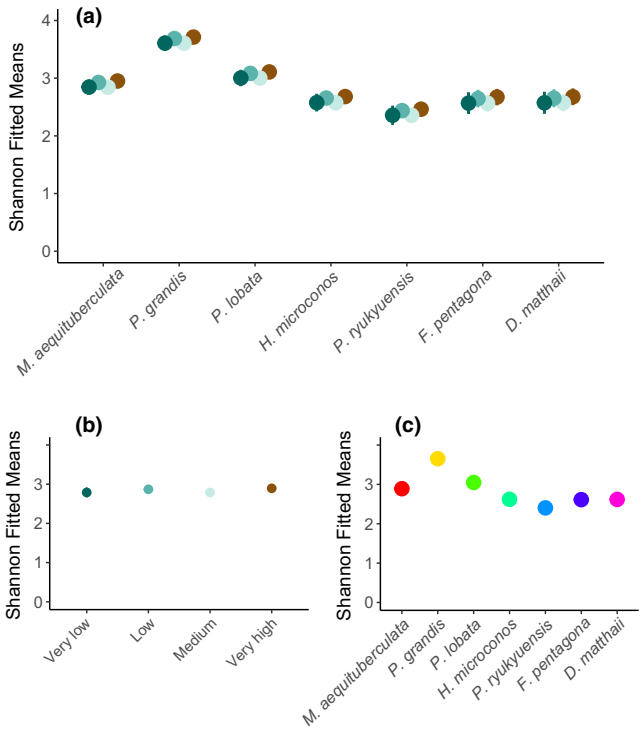

FIGURE 1 Coral-associated Symbiodiniaceae sequence alpha diversity, (a) by coral species and chronic disturbance category (disturbance increases from left to right within each coral species), (b) by chronic disturbance category (all species combined) and (c) by coral species (all sites combined), where each colour represents a single coral species. Coral species in (a) and (c) are ordered by overall sample size (*Montipora aequituberculata*, *n* = 124; *Pocillopora grandis*, *n* = 117; *Porites lobata*, *n* = 96; *Hydnophora microconos*, *n* = 46; *Platygyra ryukyuensis*, *n* = 39; *Favites pentagona*, *n* = 27; *Dipsastraea matthaii*, *n* = 23)

3 | RESULTS

3.1 | Symbiodiniaceae sequence diversity

Symbiodiniaceae sequence alpha diversity was significantly different among coral species (F = 47.1, adj R^2 = 0.62, p < .001; Figure 1a), but not across disturbance levels (Figure 1b). A total of 1,073 ASVs were detected across all samples. Amongst coral species, Symbiodiniaceae sequence Shannon diversity ranged from *Platygyra ryukyuensis* (lowest), followed by *Favites pentagona*, *Dipsastraea matthaii*, *Hydnophora microconos*, *Montipora aequituberculata*, *Porites lobata* and finally *Pocillopora grandis* (highest) (Figure 1c). Symbiodiniaceae sequence estimated richness per sample did not vary consistently across the disturbance gradient, and was lowest in *medium* (Chao1 = 30 ± 17), followed by *very high* (Chao1 = 33 ± 18), *very low* (Chao1 = 35 ± 14) and *low* (Chao1 = 36 ± 17). Symbiodiniaceae sequence estimated richness per sample was lowest in *H. microconos* (Chao1 = 18 ± 6), followed by *Platygyra ryukyuensis* (Chao1 = 19 ± 12), *D. matthaii* (Chao1 = 20 ± 9),

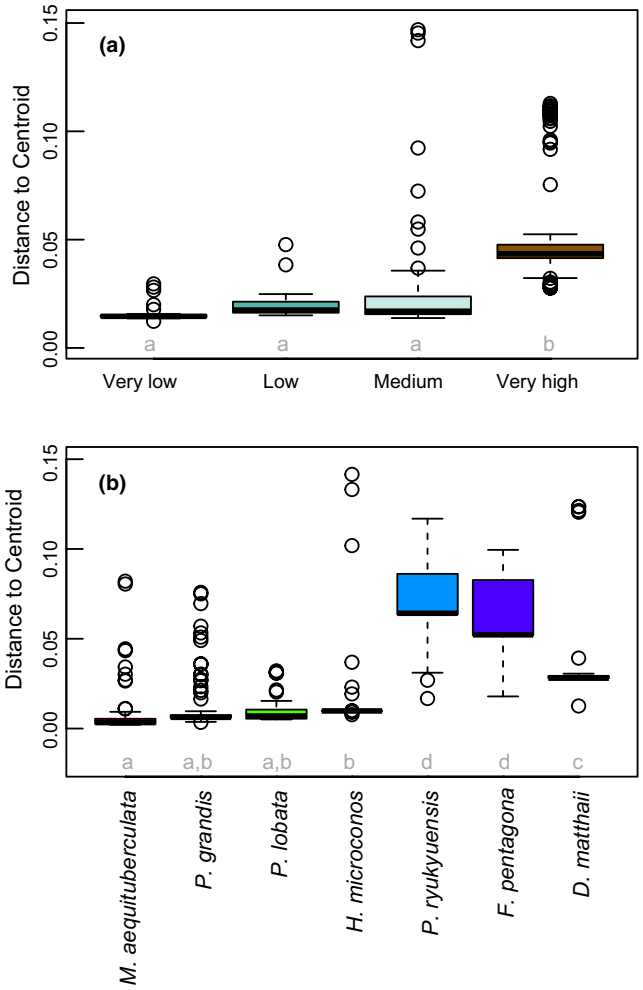

FIGURE 2 Coral-associated *Symbiodiniaceae* beta diversity variation (beta dispersion), (a) by chronic disturbance category (all species combined), and (b) by coral species (all sites combined). Significant groupings and differences among species are shown with lower-case letters

F. pentagona (Chao1 = 20 ± 11), *Porites lobata* (Chao1 = 29 ± 12), *M. aequituberculata* (Chao1 = 31 ± 12) and *Pocillopora grandis* (Chao1 = 52 ± 14).

In contrast, both the variation and the community structure components of Symbiodiniaceae sequence beta diversity varied significantly with chronic human disturbance, as well as across species. Variation was significantly higher at *very high* disturbance than any of the other disturbance categories (all $p < .001$; Figure 2a; Table S7), and also showed significant differences among coral species ($F = 106$, $df = 6$, $p < .001$; Figure 2b; Table S7). When each coral species was analysed separately across the disturbance gradient, there was also significantly more variation at *very high* disturbance compared to the other disturbance levels for *Pocillopora grandis* ($F = 39$, $df = 3$, $p < .001$; all Tukey honest significant difference [HSD] $p < .001$), *H. microconos* ($F = 68$, $df = 3$, $p < .001$; all Tukey HSD $p < .001$), *F. pentagona* ($F = 5$, $df = 2$, $p = .012$) and *D. matthaii* ($F = 76$, $df = 2$, $p < .001$; all Tukey HSD $p < .001$) (Figure S2); the other coral species did not show a consistent change. For community structure, Symbiodiniaceae sequence assemblages were significantly different, both among disturbance categories ($F = 34$, $df = 3$, $R^2 = 0.11$, $p < .001$; *adonis* from the R package VEGAN) and coral species ($F = 58$, $df = 6$, $R^2 = 0.38$, $p < .001$) (Figure S3). There was also a significant interaction of disturbance category and site ($F = 2.7$, $df = 7$, $R^2 = 0.02$, $p = .002$). When each coral species was analysed separately, community structure was significantly different among disturbance categories for five of seven coral species: *Pocillopora grandis* ($F = 15$, $df = 3$, $R^2 = 0.29$, $p < .001$), *H. microconos* ($F = 39$, $df = 3$, $R^2 = 0.39$, $p < .001$), *F. pentagona* ($F = 32$, $df = 2$, $R^2 = 0.76$, $p < .001$), *Platygyra ryukyuensis* ($F = 11$, $df = 1$, $R^2 = 0.39$, $p < .001$) and *D. matthaii* ($F = 12$, $df = 2$, $R^2 = 0.31$, $p = .005$) (Figure S3). There was also a significant interaction between disturbance category and site for three species: *M. aequituberculata* ($F = 2.5$, $df = 7$, $R^2 = 0.13$, $p = .009$), *H. microconos* ($F = 21$, $df = 7$, $R^2 = 0.49$, $p < .001$), and *D. matthaii* ($F = 6.2$, $df = 7$, $R^2 = 0.53$, $p = .01$).

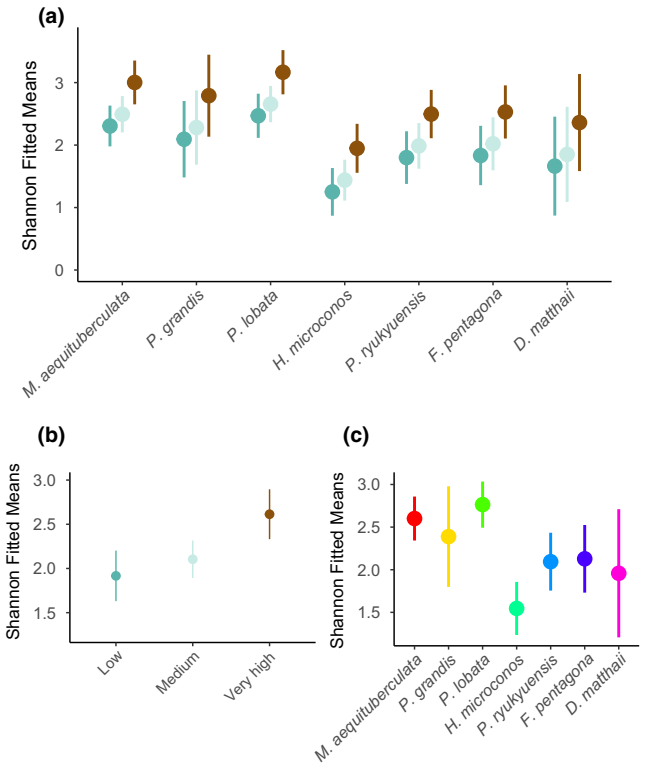

FIGURE 3 Coral-associated bacterial alpha diversity: (a) by coral species and chronic disturbance category—human disturbance increases from left to right within each coral species (see Figure 1b for colour legend by human disturbance level), (b) by chronic disturbance category (all species combined), and (c) by coral species (all sites combined), where each colour represents a single coral species. Coral species in (a) and (c) are ordered the same as in Figure 1

3.2 | Coral-associated bacterial diversity

Unlike Symbiodiniaceae, bacterial alpha diversity was significantly different among coral species and disturbance categories ($F = 5.2$, adj $R^2 = 0.22$, $p < .001$; Figure 3a). Bacterial Shannon alpha diversity was significantly higher at *very high* disturbance sites than at *medium* ($p = .007$) or *low* disturbance ($p = .001$) ones (Figure 3b). Bacterial richness by sample (measured using the Chao1 estimator) also increased consistently across the disturbance gradient, on average almost doubling from sites with low (57 ± 43) and medium (69 ± 62) disturbance to those with very high (118 ± 118). Bacterial estimated richness per sample was lowest in *Pocillopora grandis* (Chao1 = 34 ± 13), followed by *D. matthaii* (Chao1 = 44 ± 24), *M. aequituberculata* (Chao1 = 59 ± 51), *F. pentagona* (Chao1 = 87 ± 80), *H. microconos* (Chao1 = 94 ± 63), *Platygyra ryukyuensis* (Chao1 = 95 ± 120) and *Porites lobata* (Chao1 = 101 ± 99). Bacterial alpha diversity (measured using the Shannon Index) was lowest in *H. microconos* and highest in *Porites lobata* (Figure 3c).

The variation component of bacterial beta diversity was not significantly different among human disturbance categories. Beta diversity variation was, however, significantly different among coral species ($F = 7.9$, $df = 6$, $p < .001$, Figure 4b; Table S8). When each species was analysed separately, there were also no significant differences in beta diversity among human disturbance categories (Figure S4).

Community structure was significantly different among human disturbance categories ($F = 3.3$, $df = 2$, $R^2 = 0.023$, $p < .001$, Figure S5; *adonis* from the R package VEGAN), and coral species ($F = 4.3$, $df = 10$, $R^2 = 0.14$, $p < .001$), and there was a significant interaction of human disturbance category and site ($F = 1.9$, $df = 6$, $R^2 = 0.039$, $p < .001$). When each coral species was analysed separately, community structure was significantly different among disturbance categories for *M. aequituberculata* ($F = 2.1$, $df = 2$, $R^2 = 0.06$, $p < .001$; Figure S5), *Porites lobata* ($F = 1.7$, $df = 2$, $R^2 = 0.06$, $p < .001$; Figure S5), *F. pentagona* ($F = 2.3$, $df = 1$, $R^2 = 0.11$, $p = .02$; Figure S5) and *D. matthaii* ($F = 2.0$, $df = 1$, $R^2 = 0.18$, $p = .041$; Figure S5). Community structure was not significantly different among disturbance categories for *Platygyra ryukyuensis* or *H. microconos*. There was also a significant interaction between disturbance category and site for two of these species, *M.*

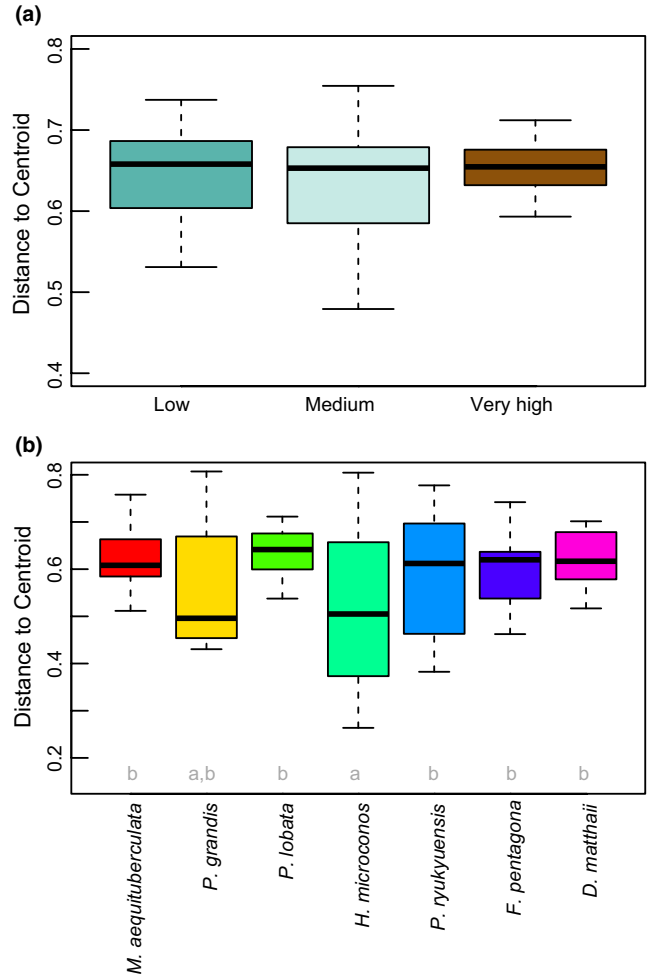

FIGURE 4 Coral-associated bacterial beta diversity variation (beta dispersion), (a) by chronic disturbance category (all species combined), and (b) by coral species (all sites combined). Significant groupings and differences among species are shown with lowercase letters

aequituberculata ($F = 1.5$, $df = 6$, $R^2 = 0.13$, $p < .001$) and *Porites lobata* ($F = 1.5$, $df = 6$, $R^2 = 0.15$, $p < .001$).

3.3 | Concordance between Symbiodiniaceae and bacterial communities

When all coral species were analysed together, there was significant concordance (i.e., similarity in multivariate shape or community structure) between the Symbiodiniaceae and bacterial communities at the island scale (Procrustes $m^2 = 0.82$, $p < .001$; Figure 5a). Because this concordance was probably due to coral species-specific bacterial and Symbiodiniaceae communities (i.e., apparently similar responses within coral species, Figure 5a), we next conducted a Procrustes analysis for each coral species individually, and found significant concordance for *H. microconos* ($m^2 = 0.90$, $p = .037$; Figure 5b) and *Porites lobata* ($m^2 = 0.91$, $p = .04$; Figure 5c) but not for the other coral species.

3.4 | Changes in bacterial communities related to dominant Symbiodiniaceae lineage

Corals were dominated by 15 different ASVs from six Symbiodiniaceae lineages (Tables S5 and S6). We found distinct bacterial associations for each of the six *Symbiodiniaceae* lineages investigated. Across all coral species, dominance by *Cladocopium* C1 was associated with significantly decreased abundance of four bacterial orders from two phyla (Firmicutes, Proteobacteria; DESEQ2 analysis; Figure 6). This included the families Endozoicomonaceae (order Oceanospirillales), Rhodobacteraceae (order Rhodobacterales), Clostridiaceae (order Clostridiales) and Streptococcacaeae (order Lactobacillales). Dominance by *Cladocopium* C15 was associated with significantly decreased abundance of one

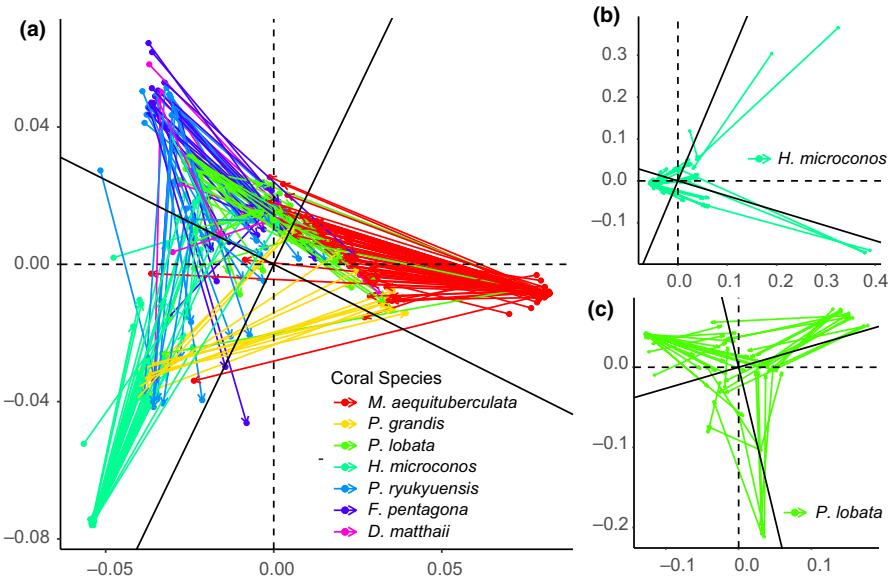

FIGURE 5 Procrustes plots for (a) all coral species together (Procrustes $m^2 = 0.82$, $p < .001$), and each significant coral species-level analysis: (b) *Hydnophora microconos* ($m^2 = 0.90$, $p = .037$), and (c) *Porites lobata* ($m^2 = 0.91$, $p = .044$). Each coral colony is represented by two points, connected by an arrow; the arrow starts at the Symbiodiniaceae community and points toward the bacterial community. All coral colonies that had samples for both Symbiodiniaceae and bacteria are included in these plots

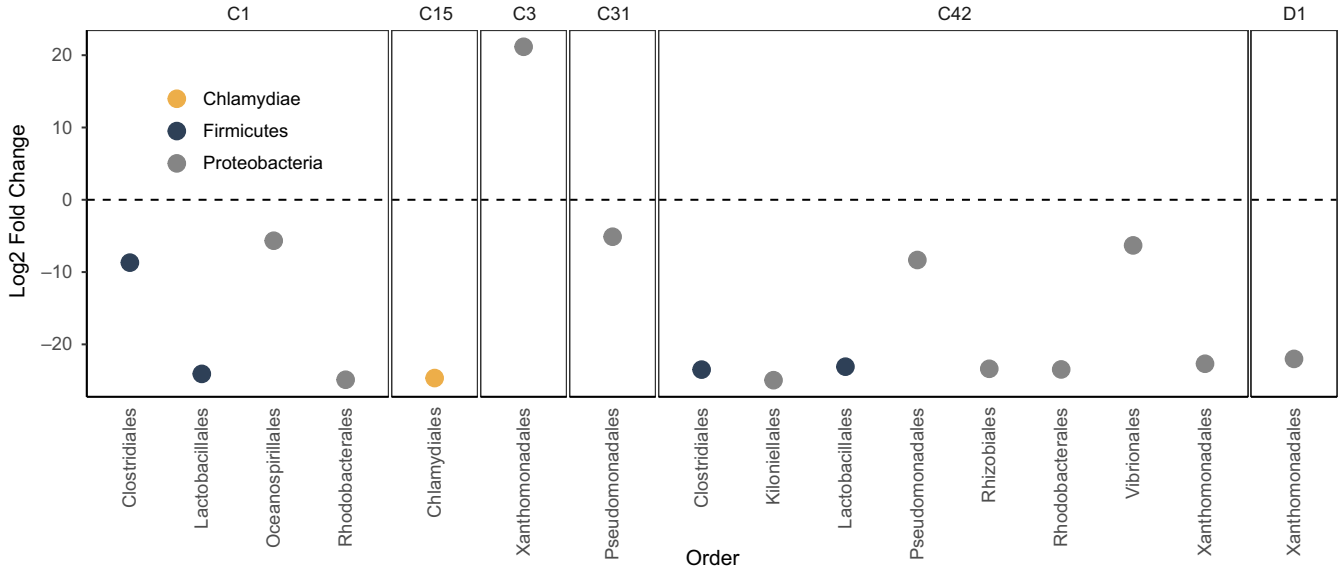

FIGURE 6 Differential abundance of bacterial amplicon sequence variants (ASVs) by dominant Symbiodiniaceae lineage (i.e., *Cladocopium* C1, C15, C3, C31, C42 and *Durusdinium* D1). Each dot represents one ASV, and colours represent bacterial phyla. All coral colonies that had samples for both Symbiodiniaceae and bacterial communities are included in this figure

bacterial ASV (phylum Chlamydiae, order Chlamydiales, family Simkaniaceae; Figure 6). Dominance by *Cladocopium* C3 was associated with significantly increased abundance of one bacterial ASV (phylum Proteobacteria, order Xanthomonadales, family Xanthomonadaceae; Figure 6). Dominance by *Cladocopium* C31 was associated with significantly decreased abundance of one bacterial ASV (phylum Proteobacteria, order Pseudomonadales, family Moraxellaceae; Figure 6). Dominance by *Cladocopium* C42 was associated with significantly decreased abundance of ASVs from eight bacterial orders from two phyla (Figure 6), including Clostridiales (family Clostridiaceae), Kiloniellales, Lactobacillales (family Streptococcacaeae), Pseudomonadales (family Moraxellaceae), Rhizobiales (Rhizobiaceae), Rhodobacterales (Rhodobacteraceae), Vibrionales (Vibrionaceae) and Xanthomonadales (Xanthomonadaceae). Dominance by *Durusdinium* D1 was associated with significantly decreased abundance of one bacterial ASV (phylum Proteobacteria, order Xanthomonadales, family Xanthomonadaceae; Figure 6).

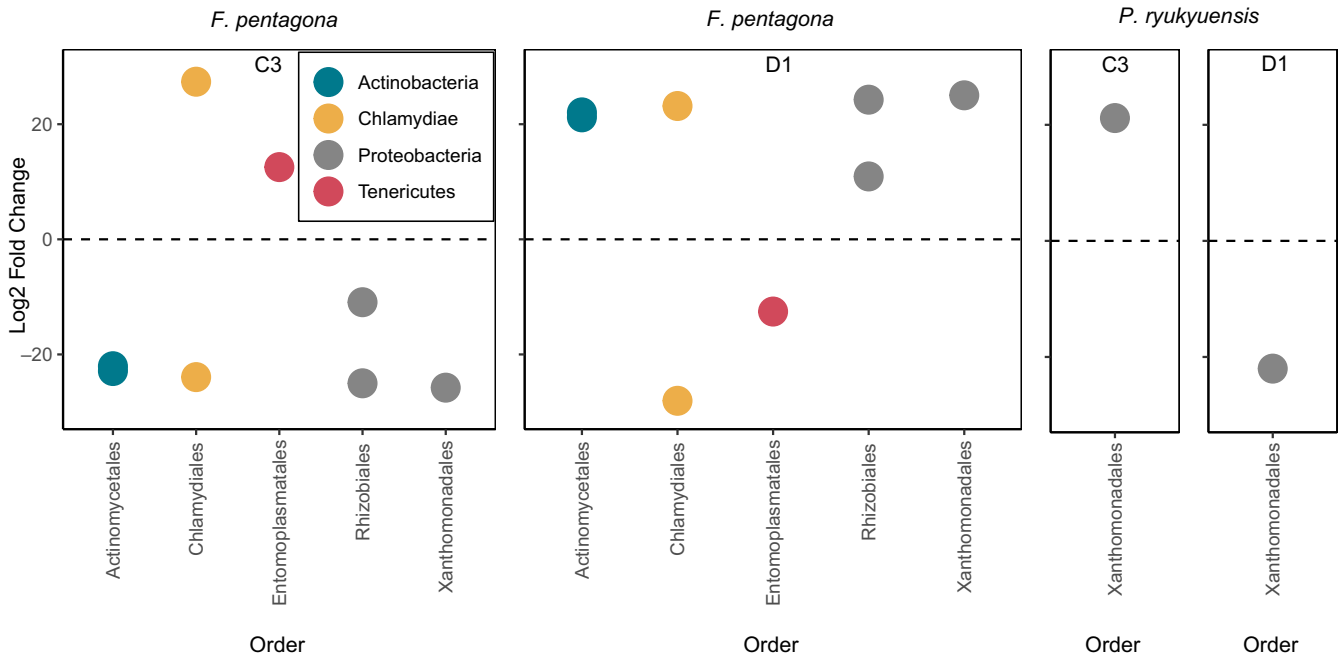

FIGURE 7 Differential abundance of bacterial amplicon sequence variants (ASVs) by dominant Symbiodiniaceae lineage (i.e., *Cladocopium* C3 and *Durusdinium* D1) in *Favites pentagona* and *Platygyra ryukyuensis*. Each dot represents one ASV, and colours represent bacterial phyla

When the only two coral species that were commonly dominated by two different Symbiodiniaceae lineages (*F. pentagona* and *Platygyra ryukyuensis*; *Cladocopium* C3 and *Durusdinium* D1) were analysed separately, we found differences in the microbiome related to the dominant Symbiodiniaceae lineage. In *Platygyra ryukyuensis*, *Cladocopium* C3 followed the same pattern for Xanthomonadales, where corals dominated with *Cladocopium* C3 had an increased abundance of Xanthomonadales, while those dominated by *Durusdinium* D1 had a decreased abundance of the same bacterial ASV. In contrast, in *F. pentagona* colonies, *Cladocopium* C3 was associated with significantly decreased abundance of one ASV from Xanthomonadales. *F. pentagona* colonies dominated by *Cladocopium* C3 also had decreased abundances of ASVs from three additional phyla including Actinomycetales, Chlamydiales, and Rhizobiales, and significantly increased abundances of ASVs from two orders from two bacterial phyla (i.e., Chlamydiales and Entomoplasmatales; Figure 7). The opposite pattern was found in *F. pentagona* dominated by *Durusdinium* D1.

4 | DISCUSSION

Our study reveals that, within a natural ecosystem setting, chronic local disturbance increased the variation of coral-associated Symbiodiniaceae sequence assemblages and the alpha diversity of bacterial communities and altered the structure of both types of microbial communities. These results add to a nascent body of literature demonstrating that disturbance can destabilize vital symbiotic relationships in corals and other symbioses, and increase diversity of the coral microbiome (reviewed by Zaneveld et al., 2017). Furthermore, concordance between Symbiodiniaceae and bacterial communities in two coral species suggests that within these corals, microbial communities respond in a similar manner to environmental disturbances. Although previous research has investigated concordance between metabolites and bacterial and Symbiodiniaceae communities individually (Sogin, Putnam, Nelson, Anderson, & Gates, 2017), we believe our study is the first to investigate concordance between these two vital microbiome components. We also provide novel evidence that the presence of dominant Symbiodiniaceae genera was associated with significant changes in the abundance of several bacterial ASVs. Previous research has shown links between the dominant Symbiodiniaceae hosted by a coral and microbiome sensitivity to warming (Littman et al., 2010; Littman, Willis, & Bourne, 2009) but no such associations have been observed across a disturbance gradient. This finding is significant, because it suggests that changes in one component of the symbiosis may be linked to responses in other important facets of this symbiosis.

4.1 | Chronic disturbance and diversity of the coral microbiome

Our findings are in agreement with previous studies of coral-associated bacterial communities, which have concluded that chronic disturbance may inhibit holobiont control of associated microorganisms, allowing invasion by opportunistic and pathogenic taxa (Krediet, Ritchie, Paul, & Teplitski, 2013; Littman, Willis, & Bourne, 2011; Vega Thurber et al., 2009). We also show that bacterial communities appear to be responsive to incremental increases in disturbance, with bacterial alpha diversity increasing linearly across the disturbance gradient. Conversely, Symbiodiniaceae sequence alpha diversity did not show a significant change across the disturbance gradient. For bacterial communities, because macroalgal release of dissolved organic carbon can cause increased bacterial activity and localized hypoxia (Barott et al., 2009; Rohwer, Youle, & Vosten, 2010), incremental increases could be mediated by an increasing abundance of turf algae and macroalgae across the disturbance gradient. Additionally, direct transfer of algae-associated bacteria to corals through contact (Nugues, Smith, Van Hooidonk, Seabra, & Bak, 2004; Vega Thurber et al., 2012) may instigate changes in microbial community structure at chronically disturbed sites. The community structure component of beta diversity varied across the human disturbance gradient for both Symbiodiniaceae and bacteria, while the variation component only varied across the human disturbance gradient for Symbiodiniaceae. This supports evidence showing that beta diversity of coral microorganisms is affected by local human disturbance (Zaneveld et al., 2017), and is congruent with evidence that symbiont community diversity is more variable in stress-sensitive corals (Howe-Kerr et al., 2020).

Our results suggest that in these coral species, beta diversity, specificity, and flexibility are not tightly correlated between bacterial and Symbiodiniaceae communities. For Symbiodiniaceae, the relationship between coral life history and sequence beta diversity was inconsistent, in contrast to previous work that found congruency between symbiotic flexibility and broad-scale patterns in coral traits (Cunning, Gates, & Edmunds, 2017). First, *Montipora aequituberculata* and *Porites lobata* had no significant difference in their variation (i.e., beta dispersion), even though *M. aequituberculata* is a generalist, and *Porites lobata* is stress tolerant (Darling et al., 2012). Contrary to our expectations, *M. aequituberculata* had the lowest beta diversity variation of all species, while stress-tolerant massive corals *Platygyra ryukyuensis* and *Favites pentagona* had the highest beta diversity (Figure 2b). This is probably because, despite higher overall alpha diversity, *M. aequituberculata* tended to be dominated by only one Symbiodiniaceae lineage (*Cladocopium* C31; n = 120 of 124), whereas *Platygyra ryukyuensis* and *F. pentagona* could be dominated by either *Cladocopium* C3 or *Durusdinium* D1. Variable dominance by multiple Symbiodiniaceae lineages implies greater symbiotic flexibility (Putnam et al., 2012), suggesting that stress-tolerant species (e.g., *Platygyra ryukyuensis* and *F. pentagona*) may not always be less flexible than stress-sensitive species. Because our results focus on Symbiodiniaceae sequence diversity (rather than species diversity), additional research is needed to understand how these patterns in sequence diversity map to Symbiodiniaceae species diversity.

For bacterial communities, *M. aequituberculata* had the highest beta diversity variation, as we expected, while *Hydnophora microconos* and *Porites lobata* (both massive corals) had the lowest and second lowest beta diversity, respectively (Figure 4b). The remaining

species had similar beta diversity, with fewer differences among species than we expected. For example, bacterial beta diversity of *Pocillopora grandis* (a competitive species) was no different from most of the massive coral species (excluding *H. microconos*, which it was significantly higher than). These apparent mismatches in microbiome flexibility between Symbiodiniaceae and bacterial communities imply that corals interact with, and control, these two microbiome communities variably among different coral species.

4.2 | Concordance between Symbiodiniaceae and bacterial communities

Concordance (i.e., similarity of multidimensional community shape) of Symbiodiniaceae and bacterial communities in *H. microconos* and *Porites lobata* suggests that within these corals, the microbial communities respond similarly to external forcings, such as local human disturbance. We suggest that lack of concordance in the remaining coral species may have arisen because of two distinct mechanisms. Lack of concordance in the other coral species that can switch Symbiodiniaceae when under stress, including Pocillopora, may reflect an "Anna Karenina Principle"-type response, where susceptibility to opportunistic taxa leads to stochastic changes in coral-associated taxa (Zaneveld et al., 2017). Alternatively, for *M. aequituberculata*, a coral species that was consistently dominated by the same Symbiodiniaceae under typical conditions, the lack of concordance probably represents a stable dominant Symbiodiniaceae lineage (*Cladocopium* C31) paired with a higher level of bacterial community variability (Figures 3c and 4b). Therefore, while external forcing may have instigated changes within the bacterial community of this coral species, the Symbiodiniaceae community remained stable. At an island scale, the community structures of Symbiodiniaceae and bacteria were concordant, in accordance with our second hypothesis, probably due to coral host specificity for each of the Symbiodiniaceae (e.g., several coral species are primarily dominated by just one lineage of Symbiodiniaceae) and bacterial communities.

4.3 | Associations between distinct bacterial taxa and dominant Symbiodiniaceae lineage

Our finding that distinct bacteria are associated with the dominance of specific Symbiodiniaceae lineages implies that associations between these components of the coral meta-organism need further study to understand their specific functions. A previous laboratory warming experiment showed that changes in bacterial communities depended on which Symbiodiniaceae genus was dominant: colonies dominated by *Durusdinium* experienced significant bacterial community shifts, including a notable increase in *Vibrio*-affiliated sequences, while those dominated by *Cladocopium* (C1) maintained stable bacterial communities through a brief heat stress (Littman et al., 2010). In our study, we also found differences in bacterial community composition that were associated with the dominant Symbiodiniaceae lineage. At the coral community level, dominance by *Cladocopium* C1 was associated with a decreased abundance of the bacterial orders Clostridiales, Lactobacillales, Oceanospirillales and Rhodobacterales. Whereas a decrease in Endozoicomonas (Oceanospirillales), a purported symbiont (Neave, Apprill, Ferrier-Pagès, & Voolstra, 2016), probably has a negative effect on the coral microbiome, decreases in orders that are pathogenic or increase under stress, namely Clostridiales, Lactobacillales (i.e., family Streptococcaceae), and Rhodobacterales (Kellogg et al., 2014; McDevitt-Irwin et al., 2017; Roder et al., 2014), may suggest improved microbiome condition. The implication of a decreased abundance of a Chlamydiales ASV in *Cladocopium* C15-dominated coral colonies is unclear. This bacterial order includes obligate intracellular species that are well-known pathogens of vertebrates (Everett, Bush, & Andersen, 1999), but is now recognized to also be widely present in the environment (Wagner & Horn, 2006). In corals, Chlamydiales may act as inter- or extracellular symbionts, but their role in these environments remains poorly understood (Apprill, Weber, & Santoro, 2016; Goldsmith et al., 2018; Work & Aeby, 2014). A decreased abundance of an ASV of *Psychrobacter* (order Pseudmonadales) in colonies dominated by *Cladocopium* C31 is probably related to the fact that 120 of 125 colonies dominated by *Cladocopium* C31 were *M. aequituberculata*, and *Psychrobacter* sp. have been previously found in association with *Acropora* and *Porites* colonies (McKew et al., 2012).

Likewise, patterns of Symbiodiniaceae lineage dominance were closely tied to coral species, with *Cladocopium* C1, C15, C31 and C42 each being the primary dominant symbiont for a single coral species (i.e., 93% of colonies dominated by C1 were *H. microconos*, 96% of C15 were *Porites lobata*, 96% of C31 were *M. aequituberculata* and 97% of C42 were *Pocillopora grandis*). Thus, it is possible that the observed bacterial associations with these Symbiodiniaceae lineages may instead reflect coral identity rather than dominant symbiont lineage. However, potential coevolution or codiversification between each coral species with specific Symbiodiniaceae lineages and bacterial taxa does not preclude the presence of mutualism or coevolution between these microbial members of the meta-organism. Potential associations with dominant symbionts should therefore not be discounted and should be investigated further to determine drivers of bacterial associates.

For the two coral species that were commonly dominated by two different Symbiodiniaceae lineages (*F. pentagona* and *Platygyra ryukyuensis*; *Cladocopium* C3 and *Durusdinium* D1), we were able to test differences in the microbiome based on dominant symbiont type within the same coral species. While there were multiple bacterial ASVs that were either upregulated or downregulated in association with the dominant Symbiodiniaceae lineage in *F. pentagona*, only one ASV (order Xanthomonadales) changed in both coral species (Figure 7). The implications of differential abundance of a bacterial ASV of Xanthomonadales is also unclear, as a previous study found that when diseased, one coral species had an increased abundance of Xanthomonadales, while another had a decreased abundance (Cárdenas, Rodriguez-R, Pizarro, Cadavid, & Arévalo-Ferro, 2012).

Further research is needed to better understand the causes and consequences of changing bacterial communities, the function of common bacterial taxa within the meta-organism, and their associations with changes in the symbiotic state.

5 | CONCLUSION

We found that the diversity of both Symbiodiniaceae and bacterial communities varied across an island-scale chronic human disturbance gradient, but in slightly different ways. Beta diversity of both Symbiodiniaceae and bacteria communities varied by disturbance, while alpha diversity of only bacterial communities increased with disturbance. There was significant concordance between Symbiodiniaceae and bacterial communities for two massive coral species (*Hydnophora microconos* and *Porites lobata*), suggesting that these communities respond similarly to external stressors. Differential abundance analysis revealed that the dominant Symbiodiniaceae lineage was associated with changes in the abundance of several distinct bacterial ASVs. Further research is needed to determine whether these differences are consistent and functionally relevant at larger scales. As local and global stressors continue to increase, a better understanding of the connections between the microbial components of the coral meta-organism and their responses to stressors will provide novel insights into the mechanisms underlying coral resilience.

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AUTHOR CONTRIBUTIONS

D.C.C., J.K.B. and R.D.G. designed this research project. D.C.C., J.K.B. and J.M.I. performed the field research and D.C.C. performed the laboratory research. R.D.G. contributed necessary reagents and laboratory equipment. D.C.C. analysed the data, and all authors contributed to data analysis interpretation. D.C.C. wrote the first draft of the manuscript; D.C.C., M.G., R.V.T. and J.K.B. wrote subsequent drafts; all authors reviewed the manuscript for publication.

DATA AVAILABILITY STATEMENT

All data used for this manuscript are available and open access. Next generation sequencing data are available at Dryad https://doi.org/10.5061/dryad.1g30729. All code to reproduce our analyses is available on GitHub at https://github.com/baumlab/Claar_etal_2020_MolecEcol (https://doi.org/10.5281/zenodo.3829987).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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