Coral Reefs
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REPORT

Effect of species, provenance, and coral physiology on the composition of Hawaiian coral-associated microbial communities

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Abstract The resistance of corals to a changing climate has been linked to physiological parameters including heterotrophic capacity and energy reserves. Recently, the potential flexibility and diversity of coral-associated microbial communities have also been related to coral health and resistance to environmental stress. This study uses the island of O'ahu in Hawai'i, USA, as a natural laboratory to explore variability in the microbial community composition of four coral species (*Porites compressa*, *Porites lobata*, *Pocillopora acuta*, and *Pocillopora meandrina*) across a gradient of natural ocean conditions. In addition, we assessed potential relationships between the composition of coral-associated microbial communities with coral physiology. We found that microbial community composition differed among all coral species, as well as among several of the collection sites within species. Microbial community assembly appeared to be governed by a combination of deterministic and stochastic processes, and the composition of these communities was more often related to measurements of coral physiology than environmental parameters among the collection sites. Specifically, coral lipid and protein levels, two components of coral energy reserves, explained significant portions of microbial community composition in *Porites lobata* and *Pocillopora acuta*, respectively. Further, microbial community diversity decreased as the proportionate contribution of heterotrophy relative to photoautotrophy in coral tissues increased in *Porites compressa* and *Pocillopora acuta*, but the opposite was true for *Porites lobata*. These findings suggest that if coral heterotrophy increases with warming oceans, it could co-occur with shifts in microbial community diversity in some coral species, possibly from decreased production of photosynthates and/or changes in the nutritional makeup of the mucus layer. Overall, connections with energy reserves and heterotrophy suggest a role for coral resource use in shaping the composition of coral-associated microbial communities across a range of natural ocean conditions, a relationship that may be important as some corals acclimatize to global climate change.

Keywords Bacteria · *Porites* · *Pocillopora* · Heterotrophy · Microbiome · Temperature

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Introduction

Increasing concentrations of atmospheric carbon dioxide (CO_2) are leading to elevated seawater temperatures and ocean acidification, which threatens the health and long-term survival of corals and the persistence of functional coral reef ecosystems (e.g., Brown 1997; Hoegh-Guldberg et al. 2007). At the current rate of CO_2 emissions, models predict average temperature increases in tropical waters of 2 °C, with a parallel increase in acidity of up to 150% by the year 2100 (IPCC 2019). The resistance of corals to

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stress associated with rapidly changing ocean conditions depends on numerous physiological parameters, including levels of energy reserves (e.g., Grottoli et al. 2006; Anthony et al. 2009; Schoepf et al. 2013), heterotrophic capacity (e.g., Grottoli et al. 2006; Hughes and Grottoli 2013; Levas et al. 2013), and shuffling of algal endosymbiont types (e.g., Abrego et al. 2008; Putnam et al. 2012). More recently, resistance to environmental stress in corals, particularly thermal stress, has also been linked to the structure and function of their microbiome (e.g., Bourne et al. 2016; Peixoto et al. 2017; Grottoli et al. 2018). Indeed, the composition of the coral-associated bacterial and archaeal communities (hereafter referred to collectively as microbial communities) has been connected to disease resistance (e.g., Ritchie 2006; Rosales et al. 2019), nutrient cycling (e.g., Lesser et al. 2007; Rädecker et al. 2015), and potentially to the physiology of the coral host (Glasl et al. 2016; Grottoli et al. 2018). Understanding the connections between corals and their microbial communities is of increasing importance as these relationships may have fundamental roles in the health, acclimatization, and adaptive potential of these organisms, with major implications for the persistence of coral reefs in the face of a changing climate (e.g., Bourne et al. 2016; Torda et al. 2017).

The coral holobiont (i.e., the coral host and its associated algal and microbial communities) is diverse, and the composition of coral-associated microbial communities is often species-specific (e.g., Bourne et al. 2016; Grottoli et al. 2018) and variable across spatial and temporal scales (e.g., Salerno et al. 2016; Wainwright et al. 2019). Specifically, there can be heterogeneity in the coral-associated microbial communities among reefs with disparate environmental conditions including naturally elevated temperatures (e.g., Ziegler et al. 2017; van Oppen et al. 2018), lower pH (Morrow et al. 2015), or higher water flow (Lee et al. 2017). For example, the coral *Porites lobata* in the Hawaiian archipelago is known to host microbial communities that are increasingly dissimilar as geographic separation increases, possibly relating to differences in seawater temperatures among locations (Salerno et al. 2016). It has also been suggested that the microbial communities associated with corals from sites with variable temperature regimes may confer resistance to the corals as seawater temperatures continue to increase (Ziegler et al. 2017; van Oppen et al. 2018). These result mirror studies of coral phenotype, which also show that corals from sites with variable temperature environments can have a higher capacity to tolerate heat stress (e.g., Barshis et al. 2013; Kenkel and Matz 2017; Jury et al. 2019).

However, the relationships between the environment, coral physiology and microbial community composition are only beginning to be understood, particularly in the context of a changing climate. Connections between external environmental factors and the composition of coral-associated microbial communities is often inferred from observed relationships, but it is unclear whether the assembly of these communities is actually governed by deterministic or stochastic processes (e.g., Stegen et al. 2015). The microbial communities associated with benthic organisms on coral reefs throughout the Red Sea appear to be governed by deterministic processes linked to different environmental conditions among sites (Pearman et al. 2019). However, microbial communities associated with corals under experimentally elevated temperatures can exhibit greater dispersion relative to their counterparts at ambient temperatures (Zaneveld et al. 2016, 2017). It is hypothesized that this dispersion occurs because corals typically have some influence over the assembly of their microbial communities, which is diminished in times of stress, giving way to stochastic processes (e.g., random ecological drift) (Zaneveld et al. 2017). Yet, it is unclear whether a deterministic or stochastic influence is dominant when specifically characterizing the microbial communities of natural corals, and what role the physiology of the coral host may play, if any, in controlling their microbial communities.

To examine the relationships between coral-associated microbial communities and a range of naturally occurring environmental conditions, we characterized the microbial communities associated with four species of Hawaiian corals across six sites surrounding the island of O‘ahu, HI. The sites varied in their environmental conditions providing a natural laboratory for evaluating the potential coral microbial community responses over a small geographic area (Jury et al. 2019). We hypothesized that coral-associated microbial communities differ among coral species and collection sites, and that the composition of those communities would correlate with environmental conditions at each site.

To examine potential relationships between coral-associated microbes and coral physiology, we coupled the microbial community analyses with physiological measurements of the same corals. Previous work by Grottoli et al. (2018) showed that corals with stable microbial communities are also more physiologically tolerant to experimentally induced temperature and pH stress, suggesting connections between the resistance of a coral to environmental stress and the composition of its microbiome. Therefore, we hypothesized that differences in coral-associated microbial community composition would correlate with one or more coral physiological parameters. Although establishing connections between coral physiology and microbial communities may be key to improving our understanding of coral health under future ocean conditions, this study is one of few to directly assess these

potential relationships to date (Grottoli et al. 2018; Marchioro et al. 2020), and the first conducted on naturally occurring corals across multiple local environments. Further, this study introduces ecological null modeling as an approach that can be used to investigate both coral physiology and environmental parameters as potential drivers of coral-associated microbial community composition.

Methods

Study sites

Corals were collected between August 17 and November 13, 2015, from six sites (Moku o Lo‘e, Magic Island, Sampan, Electric Beach, Hale‘iwa, and Waimānalo) surrounding the island of O‘ahu, HI, USA (Fig. 1). The collection sites were chosen to represent a range of environmental conditions around O‘ahu, serving as a natural laboratory (Table 1). Mean annual sea surface temperature (SST), summertime SST (20 June–10 October), annual sea surface chlorophyll *a* concentration, and annual significant wave height were determined for each collection site throughout 2015. Detailed descriptions of the methods used to gather these data are presented in the Supplemental Information.

Coral collection

Samples of four coral species (*Porites compressa*, *Porites lobata*, *Pocillopora acuta*, and *Pocillopora meandrina*) were collected at a depth of 0.5–5 m (Table 2). The vast majority of corals were collected at a depth of 2 ± 1 m, but small differences in reef geomorphology among sites required a few colonies to be collected from slightly shallower (0.5–1 m at Moku o Lo‘e and Sampan) or deeper (3–5 m at Electric Beach and Hale‘iwa) depths. A 5–10 cm ramet (branch or mound) was removed underwater via hammer and chisel from parent colonies separated by at least 5 m to minimize the possibility of selecting corals of the same genet (Baums et al. 2019; Jury et al. 2019). Genets were confirmed by genotyping about half of the colonies using available microsatellite markers (Concepcion et al. 2010), and no clones were identified based on identical multilocus genotypes from the same site, suggesting low probability that any were clonally derived. Corals were only sampled from sites where they were relatively abundant, and therefore not all coral species were sampled at every site. Upon sampling in the field, each coral ramet was bagged in seawater collected adjacent to the colony for subsequent live transport to the Hawai‘i Institute of Marine Biology. Following transport (~ 2 h), each ramet was immediately frozen at –20 °C, and later shipped to The Ohio State University on dry ice where they were stored at –80 °C.

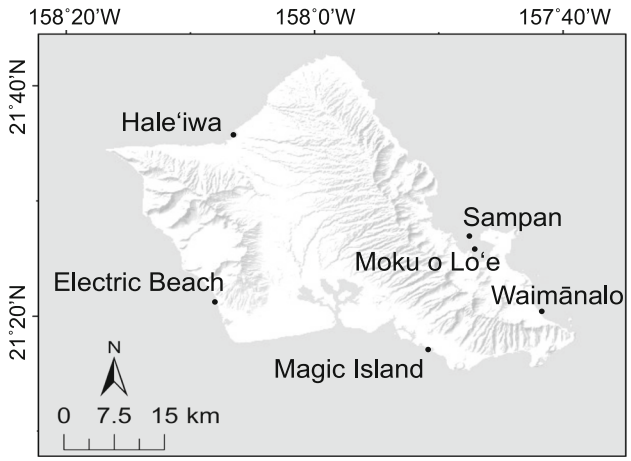

Fig. 1 Coral collection sites surrounding O‘ahu, HI. Specific coordinates of each site are listed in Table 1. Service layer credits: Esri, HERE, Garmin, OpenStreetMap contributors, and the GIS user community

Sample processing for microbial analyses

In the laboratory at OSU, a small subsample (approximately 2–4 cm^2) of the collected coral ramet was removed aseptically via hammer and a sterile chisel for microbial community characterization. Each sample was then lightly rinsed with sterile water to reduce potential contamination from handling. Bulk coral tissue for each subsample was removed from the skeleton by airbrushing with autoclaved ultrapure 0.22-µm filtered deionized water. DNA was extracted from the resulting slurry using PowerSoil DNA Isolation kits (Qiagen, Hilden, Germany) following the manufacturer protocol. Successful extraction of genomic DNA was confirmed using a Qubit fluorometer prior to amplification of the V5-V6 region of the 16S rRNA gene using the primers CS1_784F and CS2_1061R (forward: 5’-AGGATTAGATACCCTGGTA-3’; reverse: 5’-CRRCAC-GAGCTGACGAC-3’). These primers included CS1 and CS2 linkers to allow the downstream application of adapter sequences and sample-specific barcodes. Polymerase chain reaction (PCR) was completed in two stages. Stage one PCR used Amplitaq Gold 360 DNA polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in 25-µl reaction volumes. Stage-one PCR cycling conditions were as follows: 15 min at 95 °C, followed by 28 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a final extension time of 10 min. Successful amplification was visualized via gel electrophoresis. Stage-two PCR used MyTaq HS mastermix (Bioline, Memphis, Tennessee, USA) in 20-µl reaction volumes and cycling conditions were as follows: 95 °C for 5 min, followed by 8 cycles of

Table 1 Summary of environmental conditions at the six coral collection sites surrounding O'ahu, HI

Collection site	Coordinates	Mean annual SST[a] (°C)	Mean summertime SST (°C)	Mean annual Chl *a*[b] (mg m^{-3})	Mean annual significant wave height[c] (m)
Moku o Lo'e	21.43417° N, 157.78634° W	26.14 ± 2.00	28.44 ± 1.04	4.05 ± 2.77	0.18 ± 0.07
Sampan	21.45239° N, 157.79487° W	26.14 ± 2.00	28.44 ± 1.04	3.31 ± 0.91	0.42 ± 0.10
Magic island	21.28754° N, 157.85037° W	26.19 ± 1.35	27.68 ± 0.75	0.50 ± 0.28	0.80 ± 0.30
Electric beach	21.35321° N, 158.13129° W	26.34 ± 1.40	27.85 ± 0.75	0.37 ± 0.21	0.65 ± 0.30
Hale'iwa	21.59252° N, 158.11034° W	25.80 ± 1.19	27.06 ± 0.79	1.83 ± 1.77	0.98 ± 0.40
Waimānalo	21.32629° N, 157.67460° W	26.00 ± 1.41	27.55 ± 0.89	1.44 ± 0.51	0.86 ± 0.17

Means are shown ± 1 SD. SST = sea surface temperature. Chl = chlorophyll

[a]SST values were calculated using quality-controlled buoy data available from NOAA's National Data Buoy Center and the National Center for Environmental Information

[b]Chlorophyll *a* measurements were composited at a 750-m resolution from the VIIRS instrument by NOAA Coral Reef Watch and NOAA/NESDIS Ocean Color Team

[c]Significant wave heights were extracted from the SWAN hindcast model (Arinaga & Cheung 2012)

Table 2 Number of coral genets analyzed for microbial community composition from each collection site surrounding O'ahu, HI

Collection sites	*Porites compressa*	*Porites lobata*	*Pocillopora acuta*	*Pocillopora meandrina*
Moku o Lo'e	6	–	4	–
Sampan	6	6	5	6
Magic Island	4	6	5	5
Electric Beach	–	6	–	–
Hale'iwa	6	6	6	2
Waimānalo	6	6	6	5
Total	28	30	26	18

Corals were not collected from sites where they were not sufficiently abundant

95 °C for 30 s, 60 °C for 30 s, and 68 °C for 30 s. A final elongation period was performed at 68 °C for 7 min. These amplicons were subsequently prepared for multiplexed sequencing on an Illumina MiniSeq system (2 × 153 base pairs, mid-output). The second stage of the PCR process and the Illumina sequencing were completed by the DNA Services Facility at the University of Illinois at Chicago.

Reads produced by Illumina sequencing were processed using the QIIME software package version 1.9 (Caporaso et al. 2010). Within QIIME, forward and reverse reads were joined and filtered at a quality threshold of 20. Operational taxonomic units (OTUs) were clustered at 97% similarity, and taxonomy was assigned based on release 132 of the Silva ribosomal database (Quast et al. 2013) via UCLUST (Edgar et al. 2010). Chimeric reads associated with these OTUs were removed via USEARCH (Edgar et al. 2010), and OTUs were only retained in the final dataset if present in at least 10% of samples. Any OTUs which were identified as chloroplast, mitochondria, or eukaryotic in origin were removed from further analyses, in addition to a likely contaminant confirmed via a sequenced negative PCR control (Moraxellaceae OTU EF517956.1.1666). All bacteria classified within the order, Halanaerobiales, were also removed because these bacteria are commonly used in the laboratory where work was completed and are not typically found in the habitat associated with these Hawaiian corals. Prior to analysis of the microbial community diversity, three samples (one *Pocillopora acuta* coral from Magic Island and one *Pocillopora meandrina* coral from each of Hale'iwa and Waimānalo) with final read counts below 500 were also removed to limit the consideration of samples with low sequencing depth. The final number of samples included in the analyses are shown in Table 2. All raw unprocessed reads are available on NCBI's Sequence Read Archive under accession number PRJNA645694.

Physiological parameters

In the laboratory at OSU, a suite of physiological analyses were conducted on a subsample of each coral genet using established methods: tissue biomass (McLachlan et al. 2020a), total chlorophyll (chlorophyll *a* and c_2), total soluble lipid concentration (McLachlan et al. 2020b), total

soluble protein concentration (McLachlan et al. 2020c), and the stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of both the coral host and its endosymbiotic algae, Symbiodiniaceae (Price et al. 2020). The difference between both $\delta^{13}C$ and $\delta^{15}N$ of the coral host and its endosymbiotic algae (i.e., $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$) is a proxy for the relative contribution of heterotrophically and photosynthetically acquired carbon and nitrogen to coral tissue (e.g., Muscatine et al. 1989; Rodrigues and Grottoli 2006; Conti-Jerpe et al. 2020). These physiological parameters are explored thoroughly in a companion study (McLachlan et al. 2021) and all coral physiology data is available at the Biological and Chemical Oceanography Data Management Office repository (https://www.bco-dmo.org/dataset/841242).

Statistical analyses

All analyses were performed using R software package version 3.5.0 (R Core Team 2015) and PRIMER v6 (Clarke and Gorley 2006). Statistical significance was defined as $\alpha = 0.05$. Alpha diversity of microbial communities among coral species and collection sites was measured via the number of observed OTUs, Shannon's diversity index (Shannon 1948), Pielou's evenness (Pielou 1966), and Faith's phylogenetic diversity (Faith 1992). Normality and homoscedasticity assumptions for parametric analysis of variance could not be met given the data distribution of the alpha diversity metrics; therefore, each metric was compared among species via a Kruskal-Wallis one-way analysis of variance with a Dunn's post hoc test. Beta diversity was visualized using non-metric multidimensional scaling (NMDS) plots calculated with a Bray–Curtis dissimilarity using relative abundances via the R package *vegan* v 2.5–7. Beta diversity data were compared among coral species and collection sites and the interaction of both via a two-way permutational multivariate analysis of variance (PERMANOVA) with up to 9999 permutations. One-way PERMANOVAs were then used to test for pairwise differences among coral species and collection site. Similarity percentage analyses (SIMPER) were used to identify the microbial OTUs that differed most in relative abundance among coral species and collection sites.

To determine if coral physiological parameters and environmental parameters at each site correlated with coral microbial community composition, two sets of analyses were conducted across collection sites within each coral species. First, Spearman's rank-order correlations were used to test for relationships between the previously described alpha diversity metrics and the physiological and environmental measurements associated with each coral ramet. Second, distance-based linear models (DISTLM) were used to test for relationships between coral-associated microbial community composition (via a Bray-Curtis dissimilarity matrix) and each of the parameters of coral physiology and environmental parameters. The proportionate contribution of each variable in explaining variation in the microbial community was investigated using marginal tests with 9999 permutations.

Ecological null models using OTU data were performed to assess relationships between phylogeny of the coral-associated microbes and potential controls on that microbial community composition (i.e., environmental parameters and coral physiology). As outlined in Stegen et al. (2015) the β-mean nearest taxon distance (βMNTD) was calculated for each possible pairwise comparison between samples of the same coral species in order to better understand the potential controls on microbial community composition. Using 999 community randomizations to create null models based on only the microbial OTUs associated with each species, β-nearest taxon index (βNTI) was calculated to determine the deviation of the observed βMNTD from the null βMNTD. The resulting βNTI values were then used to predict whether deterministic (i.e., selection) or stochastic (i.e., random) processes shape the community. If the resulting βNTI value is > 2 or is < -2, a deterministic process is most likely responsible for differences between microbial communities in two samples. Conversely, if a βNTI value is between 2 and -2, a stochastic process better explains observed differences in microbial community composition between two samples.

Results

Overall, there were 1,563 OTUs across the 1,687,072 reads (mean of 16,540 ± 10,663 reads per sample) included in this analysis of 102 microbial communities from the four coral species collected around O'ahu. OTUs affiliated with the orders Oceanospirillales and Rhodobacterales were the most abundant among all corals, but their relative abundance varied among coral species (Fig. 2). The order Oceanospirillales were found to be most abundant among *Porites compressa* and *Porites lobata* corals (60.4% and 29.8%, respectively), with the genus *Endozoicomonas* comprising approximately 99.5% of those observations within the Oceanospirillales. Among *Pocillopora acuta* corals, OTUs affiliated with the order Actinomycetales had the highest relative abundance (18.4%) followed by Lactobacillales (15.5%). Sequences matching taxa within the Rhodobacterales were the most abundant order among *Pocillopora meandrina* corals (18.5%), followed by Oceanospirillales (16.1%).

Closer examination at the OTU level revealed that all four coral species hosted significantly different microbial communities from each other (Fig. 3, Table S2 and S3), though the greatest differences appear to be among the

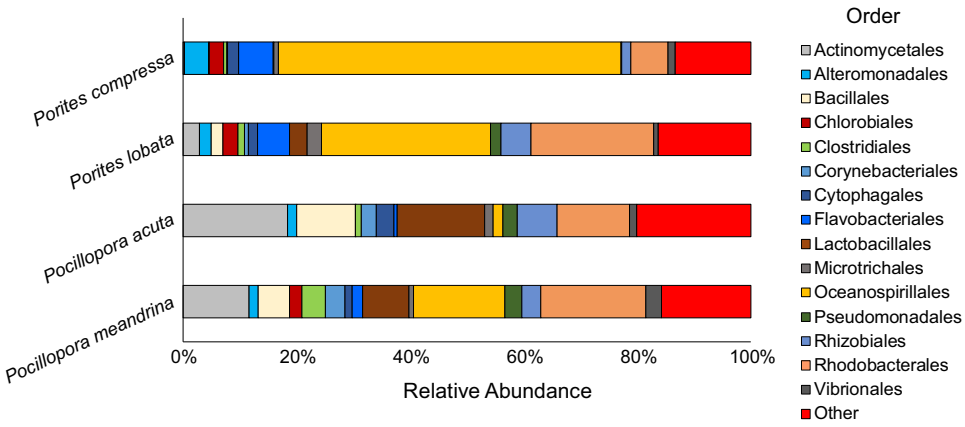

Fig. 2 Relative abundances of microbial community members at the Order level within each coral species. Microbial Orders with less than 2.5% mean relative abundance in at least one coral species are excluded from this plot

coral genera, *Porites* and *Pocillopora*. Further, SIMPER analyses revealed that bacteria from the genus *Endozoicomonas* (Phylum Proteobacteria, Order Oceanospirillales) were the primary contributor to differences among most coral species' microbial communities, but the genera *Cutibacterium* (Phylum Firmicutes, Order Actinomycetales) and *Endozoicomonas* together contributed the most to the differences between the two *Pocillopora* corals (Table S4). Both *Pocillopora* corals hosted microbial communities with significantly lower numbers of observed OTUs and Faith's PD than both *Porites* corals, but the communities associated with *Porites compressa* showed lower mean evenness than the other coral species due to the high relative abundances of the bacterial genus *Endozoicomonas* (Table 3, S6). Microbial communities associated with *Porites lobata* also had a greater Shannon's Diversity than *Porites compressa* and *Pocillopora meandrina* (Table 3).

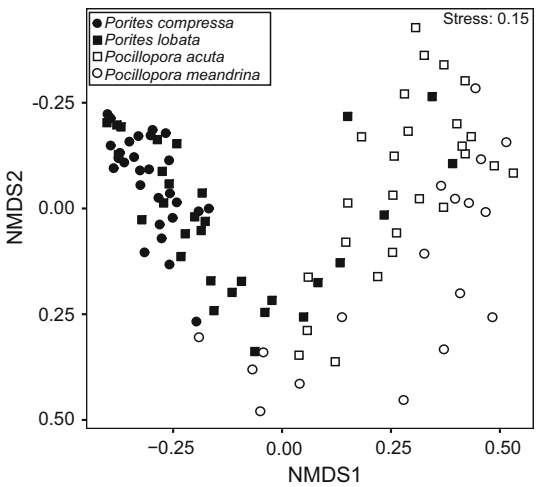

Fig. 3 Non-metric multidimensional scaling (NMDS) plot of microbial community composition of *Porites compressa* (closed circle), *Porites lobata* (closed square), *Pocillopora acuta* (open square), and *Pocillopora meandrina* (open circle) coral collected from sites surrounding O'ahu, HI. See full PERMANOVA results in Table S3

Considering each species individually, *Porites compressa*, *Porites lobata*, and *Pocillopora acuta* all hosted microbial communities that differed between at least two collection sites (Fig. 4 and Table S5a–c). For example, microbial communities associated with *Porites compressa* collected from Magic Island differed from those at all other sites except Hale'iwa. *Porites lobata* corals collected from Magic Island and Hale'iwa hosted microbial communities that differed significantly in composition from all sites except Sampan. *Pocillopora acuta* also hosted distinct microbial communities in Hale'iwa that differed significantly from all collection sites except Moku o Lo'e. Finally, *Pocillopora meandrina* microbial communities did not differ among any sites.

Ecological null modeling using βNTI revealed that the microbial communities of corals surrounding O'ahu were controlled by a combination of variable selection (52.0% of all pairwise comparisons had a $\beta NTI > 2$) and stochastic processes (48.0% of all pairwise comparisons had a βNTI between 2 and −2). Although all four coral species had some microbial community comparisons with βNTI values above the variable selection threshold of 2, only *Pocillopora acuta* and *Porites lobata* had median βNTI values above that threshold (Fig. 5). Indeed, *Pocillopora acuta* had the greatest proportion of βNTI values above 2 (72.0%), while *Porites lobata* (51.5%), *Porites compressa* (42.1%), and *Pocillopora meandrina* (35.3%) all had fewer βNTI values above that threshold (Table S7).

There were several significant relationships between coral physiology and microbial community diversity (Table S8 and S9). The $\delta^{13}C_{h\text{-}e}$ values of both *Porites compressa* and *Pocillopora acuta* were significantly correlated with the alpha diversity of their microbial communities, such that $\delta^{13}C_{h\text{-}e}$ values increased with Shannon's diversity and Pielou's evenness of *Porites compressa* (Table S8a) and Faith's PD of *Pocillopora acuta* (Table S8c). In *P. lobata*, the number of observed OTUs and Faith's PD increased with higher $\delta^{15}N_{h\text{-}e}$

Table 3 Summary of coral-associated microbial community alpha diversity metrics

Species	Observed OTUs	Shannon's H'	Pielou's Evenness	Faith's PD
Porites compressa	528.46 ± 215.72[a]	2.55 ± 1.46[a]	0.40 ± 0.21[a]	35.89 ± 11.86[a]
Porites lobata	330.20 ± 205.19[b]	3.24 ± 1.15[b]	0.58 ± 0.20[b]	23.28 ± 12.80[b]
Pocillopora acuta	119.54 ± 59.03[c]	3.04 ± 0.70[a,b]	0.65 ± 0.11[b]	7.94 ± 4.09[c]
Pocillopora meandrina	106.22 ± 102.04[c]	2.58 ± 0.61[a]	0.60 ± 0.08[b]	8.09 ± 6.94[c]

Significant statistical differences ($p < 0.05$) among groups indicated by letters. The post hoc Dunn's test statistics are presented in Table S6

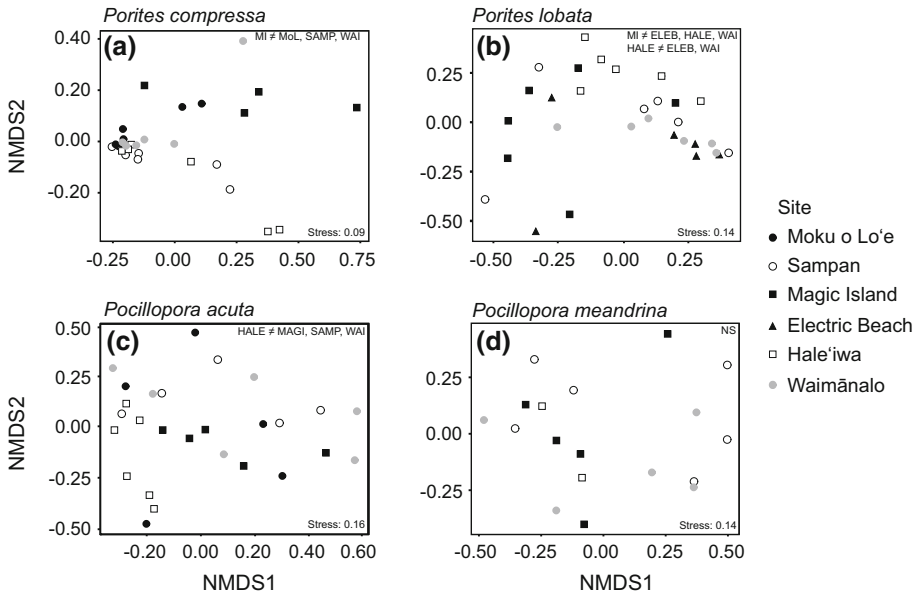

Fig. 4 Non-metric multidimensional scaling (NMDS) plot of microbial community composition of **a** *Porites compressa*, **b** *Porites lobata*, **c** *Pocillopora acuta*, and **d** *Pocillopora meandrina* coral collected from sites surrounding O'ahu, HI. Significant differences among sites ($p < 0.05$) are noted within each plot. See full PERMANOVA results in Table S5

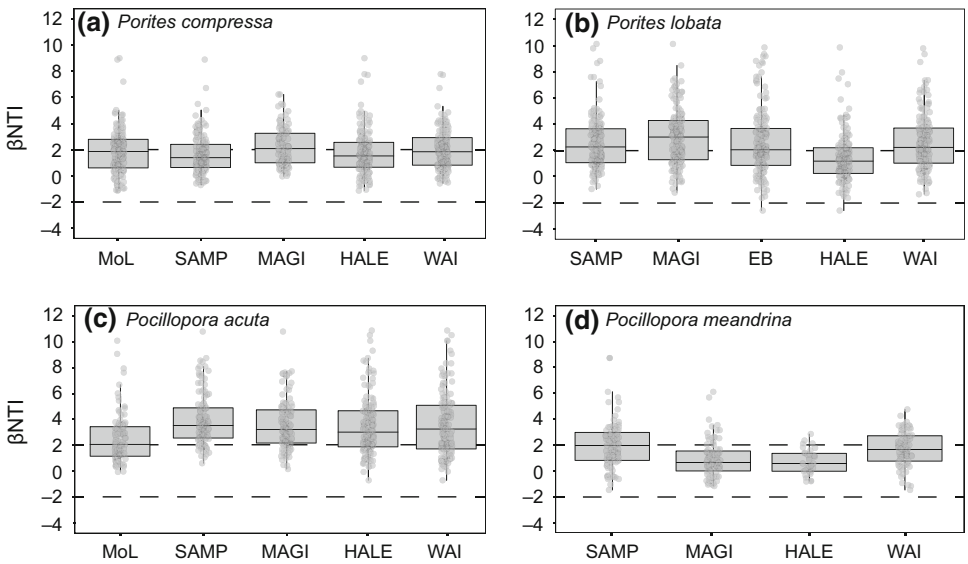

Fig. 5 Boxplot of nearest taxon index (βNTI) of microbial communities for each collection site for **a** *Porites compressa*, **b** *Porites lobata*, **c** *Pocillopora acuta*, and **d** *Pocillopora meandrina*. Dashed lines represent βNTI thresholds of 2 and −2. Collection sites are abbreviated as follows: MoL = Moku o Lo'e, SAMP = Sampan, MAGI = Magic Island, EB = Electric Beach, HALE = Hale'iwa, and WAI = Waimānalo

(Table S8b) and microbial community evenness increased with lipid levels. Coral chlorophyll levels also correlated with several alpha diversity metrics in the microbial communities of *Pocillopora acuta* and *Pocillopora meandrina*, but these relationships differed as they were negative and positive, respectively (Table S8b and c). However, few consistent patterns were observed when considering the relationships between the beta diversity of these microbial communities (via a Bray-Curtis dissimilarity matrix) and coral physiology. Unlike with alpha diversity, the isotopic

proxies for coral heterotrophy did not explain any significant amount of variation in the composition of the microbial communities for any species. Protein levels were found to explain a significant amount of variation (8.49%) in the composition of microbial communities associated with *Pocillopora acuta* (Table S9c), which did not significantly correlate with any alpha diversity metric. The only patterns that remained consistent were 1) the relationship between the microbial communities of *Porites lobata* and coral lipid levels, as 7.99% of the variation in community composition was explained by this parameter (Table S9b), and 2) the lack of any significant relationships between the microbial community composition and physiology of *Pocillopora meandrina*.

Among the environmental parameters, none correlated significantly with the alpha diversity metrics for any species, although mean significant wave height and summer SST had a positive trend with the number of observed OTUs of *Porites lobata* (Table S8b). However, mean annual chlorophyll at each collection site was found to explain a significant amount of variation in the microbial community composition of *Porites compressa* (9.80%) (Table S9a). No other environmental parameters were found to be significantly related with microbial community composition, as assessed by DISTLM.

Discussion

Here, we find that the microbial community composition differed greatly among all four coral species and among some sites within species (Figs. 3 and 4). Further, although several parameters of coral physiology and the environment were correlated with observed differences in coral-associated microbial community composition, few consistent patterns emerged, suggesting that several factors including stochastic processes influence the composition of these microbial communities.

Microbial community composition among coral species

The microbial community composition of the four coral species differed primarily in their relative abundance of the bacterial genus, *Endozoicomonas* (Order Oceanospirillales). Specifically, the low relative abundance of *Endozoicomonas sp.* in *Pocillopora acuta* (Fig. 2) is surprising, given that *Endozoicomonas sp.* is common in many other tropical corals, including other Pocilloporids (Bayer et al. 2013; Pogoreutz et al. 2018; Wainwright et al. 2019) and its congener in this study, *Pocillopora meandrina*. The bacterial genus, *Cutibacterium* (Order Actinomycetales), was relatively abundant in both *Pocillopora* corals (Fig. 2). While not typically found in the high relative abundances seen in this study (Fig. 2), the *Cutibacterium sp.* are generally widespread and consistently found as members of the core coral microbiome (e.g., Ainsworth et al. 2015; Sweet et al. 2017). It is possible that the proximity of the collection sites to community beaches and terrestrial runoff around O'ahu affected the *Pocillopora* corals differently than the *Porites* corals, leading *Pocillopora* corals to host a higher relative abundance of the often human-associated *Cutibacterium* bacteria (Yang et al. 2017). Indeed, the genetic structure of *Porites lobata* holobionts matches runoff gradients on nearshore Hawaiian reefs (Tisthammer et al. 2020), suggesting that similar processes could also affect microbial communities hosted by these corals.

Conversely, the high relative abundance of *Endozoicomonas sp.* in *Porites* corals and the Rhodobacterales in all four coral species is congruent with numerous past studies of coral-associated microbial communities (e.g., Bayer et al. 2013; Glasl et al. 2016; Pogoreutz et al. 2018). In particular, the *Endozoicomonas sp.* are thought to assist with nutrient acquisition via nitrogen and carbon cycling, as well as structuring of the microbial community through regulation of bacterial colonization (Neave et al. 2016). While the exact roles of these bacteria are unclear and may vary among hosts, their high abundance is generally linked with healthy corals (Bayer et al. 2013; Neave et al. 2016; Pogoreutz et al. 2018). Though corals need not have these bacteria to appear healthy (Grottoli et al. 2018), corals with lesions, disease, or those under environmental stress often exhibit low abundances of *Endozoicomonas* (e.g., Vezzulli et al. 2013; Meyer et al. 2014). Hence, *Pocillopora acuta* around O'ahu may be more susceptible to future changes in ocean conditions or other stressors than the other three species given their lower prevalence of these microbes.

Microbial community composition and environmental parameters

While we found significant differences in microbial community composition among coral species, differences also existed among some of the collection sites within three of the four coral species (Fig. 4, Table S5). Indeed, *Porites compressa*, *Porites lobata*, and *Pocillopora acuta* from Magic Island and/or Hale 'iwa hosted microbial communities that differed from most other sites (Table S5a–c). This suggests that either spatial separation among sites or environmental parameters such as the low seawater chlorophyll *a* levels at Magic Island or high significant wave height at Hale'iwa could be influencing microbial community composition. In addition, high nitrogen levels at Hale'iwa (Ellison 2020) or periodic rain-driven effluent from the Ala Wai Canal near Magic Island (Johnson et al. 2013) could cause the coral-associated microbial

communities from these two sites to differ from most others. However, the absence of consistent correlations between microbial community composition and environmental parameters (Table S8 and S9) suggests that the parameters assessed here may not be a major influence on the microbial community composition around O'ahu. While there are known environmental constraints on the benthic cover (Franklin et al. 2013) and physiology of Hawaiian corals from this study (McLachlan et al. 2021), it is also possible that the coral-associated microbial communities respond faster to changes in environmental parameters than the four-month long summer or annual average values used in this study (Glasl et al. 2019). Therefore, these results suggest that (1) the measured environmental parameters surrounding O'ahu were measured at too low of a spatial and/or temporal resolution to effectively detect effects on the composition of the coral-associated microbial communities, (2) the community assembly was controlled by other environmental parameters not included in this study, (3) there are species-specific effects, or (4) some combination of all three options.

We found that the microbial communities associated with all four coral species are likely controlled by a combination of both deterministic and stochastic processes, and that the dominant process is species-specific. The median βNTI value of *Porites lobata* and *Pocillopora acuta* was greater than 2 (Fig. 5b and c), suggesting that variable selection influences the microbial communities among the collection sites in these two coral species. Stochastic processes were more common in *Porites compressa* and *Pocillopora meandrina*, as the median βNTI value was below 2 (Fig. 5a and d). This may indicate that the microbial communities associated with *Porites lobata* and *Pocillopora acuta* are more susceptible to selective pressures than the microbial communities of *Porites compressa* and *Pocillopora meandrina*. However, the proportion of pairwise microbial community comparisons influenced by variable selection ranged from 35.3 to 72.0% (Table S7), suggesting that regardless of species, both variable selection and stochastic processes have an important role in microbial community assembly to some degree. Stochastic processes are known to affect coral-associated microbial communities when under stress (Adair and Douglas 2017; Zaneveld et al. 2017), but here we observed varying levels of stochastic influence even when the corals are presumably healthy. Stochastic processes, like ecological drift, occurring over time in these coral-associated microbial communities could explain some of the divergence among sites and the lack of consistent correlations with any specific environmental variable.

Microbial community composition and coral physiology

Associations between coral-associated microbial community composition and physiological parameters were more common than those found with environmental conditions, but we still did not identify consistent patterns between those communities and coral physiological parameters. For both *Porites* species and *Pocillopora acuta*, we found significant relationships between microbial community alpha diversity and $\delta^{13}C_{h\text{-}e}$ or $\delta^{15}N_{h\text{-}e}$, which are proxies for the proportionate contribution of heterotrophically and photoautotrophically derived organic matter to coral tissues (e.g., Muscatine et al. 1989; Rodrigues and Grottoli 2006; Conti-Jerpe et al. 2020). $\delta^{13}C_{h\text{-}e}$ positively correlated with Shannon's diversity and Pielou's evenness of the microbial communities associated with *Porites compressa* and with Faith's PD of those communities associated with *Pocillopora acuta* (Table S8a and c). These results suggest that microbial diversity decreased as $\delta^{13}C_{h\text{-}e}$ decreases (i.e., as the proportionate contribution of heterotrophy to coral tissues increases). Relationships with this proxy indicate that nutritional sources to the coral and nutrient cycling within the coral are potentially important factors in the composition of the microbial communities. This finding could be due to less reliance on photosynthesis at higher heterotrophic rates, leading to a lower release of photosynthates by the endosymbiotic algae, which can be metabolized by microbes (e.g., *Endozoicomonas sp.* and *Alteromonas sp.*) resulting in less diverse microbial communities (Bourne et al. 2013; Neave et al. 2016). Similar connections between microbial communities and potential energetic resource use were found in *Acropora tenuis,* where a greater density of endosymbiotic algae was linked to a greater relative abundance of the bacterial family Endozoicimonaceae (Marchioro et al. 2020). In addition, because photosynthesis and heterotrophy both contribute to the production of coral mucus, changes in resource use by the coral can affect the composition of the coral mucus and/or the amount of mucus released (Naumann et al. 2010; Levas et al. 2013), which could affect microbial community diversity (Glasl et al. 2016).

In contrast, the alpha diversity of the microbial communities of *Porites lobata* increased with greater $\delta^{15}N_{h\text{-}e}$ values (i.e., greater microbial diversity with a higher proportionate contribution of nitrogen to tissues from heterotrophic sources, see Conti-Jerpe et al. 2020). Interestingly, *Porites lobata* hosted the greatest relative abundances of bacteria from the family Rhodobacteraceae, which are often associated with nitrogen fixation in corals (Lesser et al. 2018). The relationship between coral microbial diversity and $\delta^{15}N_{h\text{-}e}$ values suggests that nitrogen incorporation by *Porites lobata* affects its microbial community

composition. However, $\delta^{15}N_{h\text{-}e}$ may be sensitive to differences or changes in seawater nutrient concentrations and isotopic composition. For instance, nitrate concentrations near the beachfront of Hale'iwa can be higher and with a higher $\delta^{15}N$ signature than other areas around O'ahu during baseflow conditions due to the large watersheds and water treatment facilities that feed into this area (Hoover et al. 2009; Wall et al. 2019; Ellison 2020). Thus, it is possible that the relationship between microbial community diversity and $\delta^{15}N_{h\text{-}e}$ is actually indicative of changes in nutrient source and not of coral heterotrophy. While these findings are interesting, further study is needed to decouple the effect of seawater $\delta^{15}N$ values and coral $\delta^{15}N_{h\text{-}e}$ as a proxy for heterotrophy.

Levels of coral lipid and protein, two coral energy reserves, were also found to explain significant portions of the microbial community composition in *Porites lobata* and *Pocillopora acuta*, respectively (Table S9). In *Porites lobata*, a significant relationship between evenness and lipid levels was also observed. These relationships together suggest that the amount of lipid and/or protein reserves maintained by some coral species could also influence the microbial community composition. Considering heat-stressed corals can release mucus with higher lipid and protein levels than corals in control conditions, and that this mucus was found to have enhanced antibacterial properties (Wright et al. 2019), it is not surprising that these physiological parameters would relate to microbial community composition. Interestingly, lipid levels in *Porites lobata* were relatively consistent around the island and were not found to correlate with these measured environmental parameters (McLachlan et al. 2021), suggesting that co-occurring changes in lipid and microbial community composition may be related to other environmental or colony-specific factors.

Implications

Overall, we found that the four Hawaiian corals in this study host distinct microbial communities. While the coral-associated microbial communities also differed among collection sites around the island of O'ahu, our results suggest that these differences are not consistently driven by local variation in any one environmental parameter, but rather by the geographic isolation of corals at each site, with a combination of stochastic and selective processes influencing community composition over time. Within each coral species, we further discovered that the nutritional sources accessed by a coral (i.e., photoautotrophic vs. heterotrophic) may partially influence the diversity of its microbial community. This structuring has important implications for corals in a changing climate, as heterotrophic capacity and plasticity are posited as key factors in the potential resilience of corals to rising seawater temperatures (e.g., Grottoli et al. 2006, 2017). As some coral species increase heterotrophy to support reduced photosynthesis rates or higher energy demands in times of thermal stress (e.g., Grottoli et al. 2006, 2017; Hughes and Grottoli 2013), our study suggests that any change in observed microbial diversity may simply be an artifact of that shift in resource use. Therefore, changes in microbial diversity are not necessarily an indication that the coral is stressed, but instead may be a consequence of other physiological acclamatory or compensatory processes. However, not all coral species shared the same relationship among microbial community composition, environmental conditions, and coral physiology, indicating a high degree of species specificity in how coral-associated microbial communities could respond to the chronic pressures of climate change.

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