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Reef environments shape microbial partners in a highly connected coral population

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Evidence is mounting that composition of microorganisms within a host can play an essential role in total holobiont health. In corals, for instance, studies have identified algal and bacterial taxa that can significantly influence coral host function and these communities depend on environmental context. However, few studies have linked host genetics to algal and microbial partners across environments within a single coral population. Here, using 2b-RAD sequencing of corals and metabarcoding of their associated algal (ITS2) and bacterial (16S) communities, we show evidence that reef zones (locales that differ in proximity to shore and other environmental characteristics) structure algal and bacterial communities at different scales in a highly connected coral population (*Acropora hyacinthus*) in French Polynesia. Fore reef (FR) algal communities in Mo'orea were more diverse than back reef (BR) communities, suggesting that these BR conditions constrain diversity. Interestingly, in FR corals, host genetic diversity correlated with bacterial diversity, which could imply genotype by genotype interactions between these holobiont members. Our results illuminate that local reef conditions play an important role in shaping unique host–microbial partner combinations, which may have fitness consequences for dispersive coral populations arriving in novel environments.

1. Introduction

Emerging data showcasing the varied roles that microorganisms play within animal hosts have shifted focus from individuals to holobionts—the conglomeration of host and microbes as an ecological unit. In tropical corals, obligate symbiosis with microalgae in the family Symbiodiniaceae can impact the host in terms of heat tolerance and nutritional input [1,2]. Most recently, insights into the coral bacterial microbiome have revealed putative functional roles, such as nutrient cycling and immunity [3,4]. Indeed, inoculating *Pocillopora damicornis* with a consortium of potentially beneficial bacteria prevented bleaching (the expulsion of corals' algal symbionts) during both heat stress and a pathogen challenge [3]. Clearly, to better understand coral resilience in changing oceans, a holobiont perspective is needed.

Despite the importance of microbial partners for corals, we are still scratching the surface in understanding factors that govern their community assembly. Many studies show algal and bacterial taxa are enriched across habitats due to environmental differences [5,6]. Other work shows that microbial communities vary greatly between coral species and can be conserved across coral phylogeny, implicating host selection and phylosymbiosis [7]. Accordingly, a recent study found that both host environment and genotype structured bacterial communities of clonal colonies of hydrocoral *Millepora* cf. *platyphylla* [8]. However, few studies have examined host genetics in tandem with algal and microbial communities across environments.

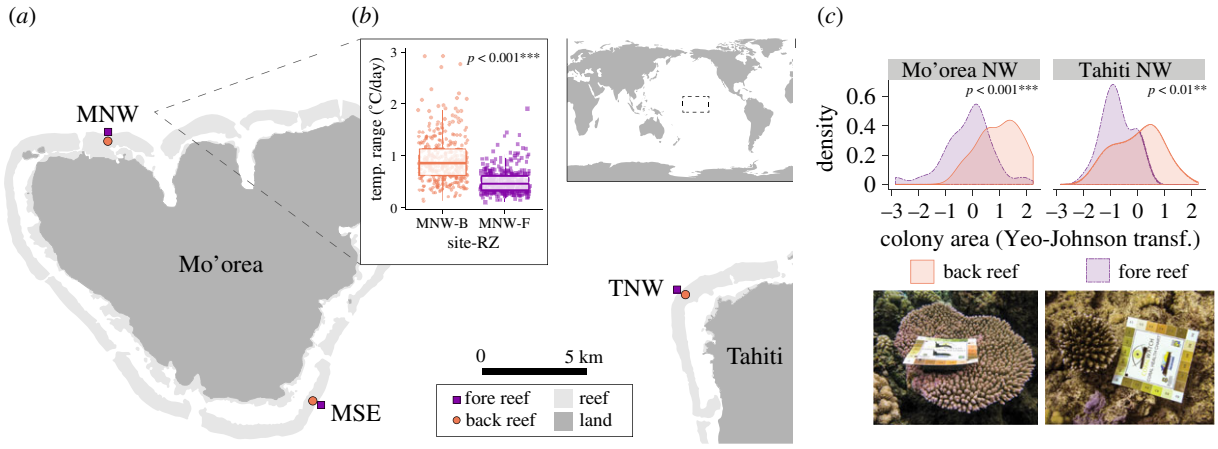

Figure 1. (*a*) Map of French Polynesia study sites: Mo'orea NW (MNW), Mo'orea SE (MSE) and Tahiti NW (TNW) with paired back (B) and fore (F) reef zones. (*b*) Daily temperature ranges (°C) at MNW from June 2013–2014 (data from CRIOBE). (*c*) *Acropora hyacinthus* colony surface area (cm^2; Yeo–Johnson transformed) in MNW and TNW (no MSE data due to inclement weather), where density indicates the proportion of data under the distribution curve, with representative photographs from B (left) and F (right) reef zones. (Online version in colour.)

Reef zones, which are distinguished by a reef's relative location, structure and abiotic factors, provide effective natural systems to study the coral holobiont, as strong variation in temperature, light and nutrient levels are observed even within 1 km [5,6]. Genetic differentiation across reef zones and distinct algal and bacterial communities, have been observed in brooding coral *P. damicornis* [5], highlighting the potential for multiple holobiont members to be structured across short distances. These patterns may differ in a broadcast spawning coral, where genetic differentiation across smaller scales is much less likely [9]. In these cases, associations with unique microbial communities might serve as a mechanism for dispersing corals to acclimatize to novel environments [10–12].

Here, we leverage reef zones in Mo'orea and Tahiti, French Polynesia, to ask how members of the *Acropora hyacinthus* holobiont are structured across fore reef (FR) (closer to open ocean; higher wave energy) and back reef (BR) (typically more variable in light, temperature and nutrient levels) environments [8,13–15]. Specifically we quantify: (i) coral host genetic structure and loci under selection, (ii) algal community composition, (iii) bacterial community composition, diversity and functional profiles and (iv) correlations of these metrics between the coral and its associated microbes. We found evidence of a single *A. hyacinthus* population across Mo'orea and Tahiti; however, patterns of host–microbe relationships depended on their local reef environment.

2. Material and methods

Detailed materials and methods can be found in electronic supplementary material. All scripts can be found at https://github.com/nicfall/moorea_holobiont_revised.

(a) Site characterization and sampling

From 26 July to 9 August 2013, branch tips of *A. hyacinthus* were collected from one FR and one BR zone ($n = 21–24$/zone) at each of three sites spanning volcanic islands of French Polynesia in the South Pacific: Mo'orea NW (MNW), Mo'orea SE (MSE) and Tahiti NW (TNW) ($N = 126$ colonies total; figure 1*a*; electronic supplementary material, table S1). Collections took place at approximately 3–6 m depth, with approximately 220–370 m between zones. BRs were well-lit lagoons while FRs were approximately 5–10 m past the crest toward the open ocean. Each colony was photographed (except for MSE-F due to inclement weather). Samples were preserved in 96% ethanol and maintained at −20°C until processing.

The Center for Insular Research and Observatory of the Environment (CRIOBE) provided benthic cover data at MNW and MSE and nutrient and temperature data at MNW. Water flow data at MNW were gathered from Cortese *et al*. [15]. To compare nutrients (P_2O_5, NO_3, NO_2, SiO_2, NH_4^+; µM), temperature (daily mean and range; °C) and flow ($m\ s^{-1}$) across reef zones, one-way ANOVAs with raw or log-transformed data were used, unless Shapiro–Wilk and Levene's tests were not passed, in which case Mann–Whitney *U* tests were conducted. Colony surface area (cm^2) was estimated from photographs using ImageJ [16], transformed (Yeo–Johnson) and a nested ANOVA tested for differences between reef zones within sites.

(b) Coral 2b-RAD genotyping

Holobiont DNA was isolated using a phenol–chloroform extraction [17] and 2b-RAD libraries were prepared following [18] with modifications found here: https://github.com/z0on/2bRAD_denovo/. Briefly, DNA (100 ng) was digested with *BcgI* (New England Biolabs) and fragments were multiplexed using custom barcoded ligation adapters. Samples were pooled and sequenced on three lanes of Illumina HiSeq 2500 (76 samples; electronic supplementary material, table S2) or one lane of Illumina HiSeq 4000 (60 samples; electronic supplementary material, table S2).

Sequencing data ($n = 126$) were demultiplexed, de-duplicated when appropriate (see electronic supplementary material, table S2) and filtered using *FASTX-Toolkit* [19]. Filtered reads were mapped to the *Acropora millepora* genome [20] using local alignment with *Bowtie2* [21]. Technical replicates and clones were removed. Samples with an average per-site depth of less than seven reads were removed to improve genotype likelihood confidence (electronic supplementary material, table S2). *ANGSD* [22] calculated genotype likelihoods, pairwise identity-by-state matrices and heterozygosity. *ADMIXTURE* [23] (K 1–5) was run and the lowest cross-validation error determined the optimal 'K'. *PCAngsd* [24] generated a covariance matrix for principal components analysis. Between all sites and reef zones, significance of multivariate dispersion and location were assessed with *vegan* [25] and *hierfstat* [26] calculated global and pairwise F_{ST}. *BayeScan* [27] and *OutFLANK* [28] were used to identify single nucleotide polymorphism (SNP) F_{ST} outliers.

(c) Symbiodiniaceae metabarcoding

From a subset ($n = 96$; 16/reef zone/site; electronic supplementary material, table S1) of samples used in host genotyping, ITS2 PCR amplification was performed using *ITS2-DINO* and *ITS2Rev2* primers [29]. A negative control using water was also prepared; however, it failed to amplify. Samples were dual-barcoded using a second PCR and pooled based on relative concentrations visualized on a 1% agarose gel. Libraries were sequenced on Illumina MiSeq (paired-end 250 bp) at the University of Texas at Austin. Sequencing data were submitted to *SymPortal* to identify ITS2 type profiles [30].

A principal coordinate analysis (PCoA) using Bray–Curtis dissimilarity was conducted on ITS2 type profiles using *phyloseq* [31]. *Vegan* [25] evaluated sample dissimilarity in multivariate space in terms of location and dispersion, contrasting reef zones within each site and *pairwise.adonis* [32] contrasted communities between sites. To verify trends, preceding analyses were conducted both on ITS2 type profiles and post-normalization using the TMM method in *edgeR* [33].

(d) Bacterial metabarcoding

To characterize the bacterial microbiome, the V4 region of the 16S rRNA gene was PCR amplified using Hyb515F [34] and Hyb806R [35] primers, dual barcoded in a second PCR and pooled in volumes scaled by relative band intensity on a 1% agarose gel ($n = 93$, 15–16/site; electronic supplementary material, tables S1 and S2). Libraries were sequenced on Illumina MiSeq (paired-end 250 bp) at North Carolina State University. Four negative controls were prepared from molecular water in lieu of sample separately but using the same reagents and sequenced at Tufts University.

bbmap [36] was used to pre-process 16S data to retain reads beginning with the primer sequence and *cutadapt* [37] removed primer sequences. *DADA2* [38] conducted quality filtering and inferred 2608 amplicon sequence variants (ASVs). *Decontam* [39] removed 63 contaminant sequences based on negative controls. Taxonomy was assigned via the Silva v. 132 database [40] and National Center for Biotechnology Information's nucleotide database using *blast+* [41]. ASVs matching mitochondria, chloroplasts or non-bacterial kingdoms were removed (341 total).

Using *vegan* [25], counts were rarefied to 6000 per sample, which retained approximately 90% of samples (electronic supplementary material, table S2). *Phyloseq* [31] calculated four diversity metrics: ASV richness, Shannon index, Simpson's index and evenness. Packages *phanghorn* [42], *DECIPHER* [43] and *btools* [44] calculated Faith's D phylogenetic diversity. Of these, richness, Simpson's index and Faith's D were log-transformed and a nested ANOVA compared all diversity metrics across sites and reef zones. Diversity comparisons were repeated on non-rarefied data to verify trends.

MCMC.OTU [45] removed ASVs representing less than 0.01% of counts or those present in only one sample, leaving 223 ASVs. These data were run through *PICRUSt2* to infer metagenomic functions [46], one ASV was removed based on program defaults and *Aldex2* [47] contrasted enrichment across reef zones at each site using Wilcoxon rank tests. *ANCOM* [48] investigated differentially abundant ASVs across reef zones. A PCoA based on Bray–Curtis dissimilarity was conducted using *phyloseq* [31]. *Vegan* [25] evaluated sample dissimilarity in multivariate space in terms of location and dispersion, contrasting sites and reef zones. These analyses were then repeated on 16S data separated by *microbiome* [49] into core (present in greater than 70% of samples) and accessory components. Dissimilarity analyses were repeated with non-rarefied data to ensure trends were consistent.

(e) Links between holobiont members

To explore correlations between coral data and microbial community diversity, linear models with reef zones nested within site were run pairwise between each of the following metrics: host colony size and heterozygosity with five bacterial diversity metrics (Shannon, Simpson, evenness, richness and Faith's D). Within these metrics, Shapiro–Wilk tests were conducted to ensure statistical assumptions were met. If assumptions failed, data were log-transformed or *BestNormalize* [50] determined the best fit transformation. Bacterial diversity metrics, colony size and host heterozygosity were then compared between samples grouped by their most abundant symbiont type. Finally, to explore correlations between overall host, bacterial and algal genetic divergence, pairwise between-sample distance matrices were compared using Mantel tests in *vegan* [25].

3. Results

Detailed results can be found in the electronic supplementary material.

(a) Site and coral colony characterization

At MNW, daily temperature range, nitrate and nitrites were higher and water flow was lower in the BR relative to FR environments while mean daily temperature and concentrations of phosphate, ammonium and silica did not vary between zones (figure 1*b*; electronic supplementary material, figures S1 and S2). Increased coral colony size was observed on the BR relative to the FR at both sites where size data were measured (figure 1*c*). MNW's BR had relatively similar proportions of algae and coral cover, with *Stegastes* turf and *Porites* being the most abundant, respectively (electronic supplementary material, figure S3). MNW's FR had slightly higher algal cover (mostly *Asparagopsis*) than coral cover (mostly *Pocillopora*) (electronic supplementary material, figure S3). MSE's BR was dominated by *Montipora* and *Porites* corals, while the FR was dominated by macroalgae *Halimeda* (electronic supplementary material, figure S3).

(b) Coral host genetics

After quality filtering, average reads per sample were 1.36 ± 0.85 million (mean ± s.d.) and mean mapping efficiency across samples was 81.97 ± 4.03% (electronic supplementary material, table S2). The following samples were removed: 10 technical replicates, two incidental clones and 10 samples having mean read depth less than seven per site (electronic supplementary material, figure S4 and table S2). A total of 114 samples passed quality filtering, which resulted in 3594 SNPs. *ADMIXTURE* [23] determined that the optimal $K = 1$ (electronic supplementary material, figure S5), suggesting that these corals were sourced from the same population. Principal component analysis confirmed this pattern, with sample overlap across sites and reef zones (electronic supplementary material, figure S6). Pairwise F_{ST} values between sites ranged between 0.011 and 0.018 (electronic supplementary material, table S3) and global F_{ST} was 0.001. *BayeScan* [27] and *OutFLANK* [28] found no F_{ST} outliers, indicating that no SNPs were detected to be under selection. Taken together, these results provide strong evidence of a genetically homogeneous population.

(c) Symbiodiniaceae community composition

Post-minimum entropy decomposition processing by *SymPortal* [30], ITS2 counts were 3591 ± 1933 per sample (electronic supplementary material, table S2). Most samples were

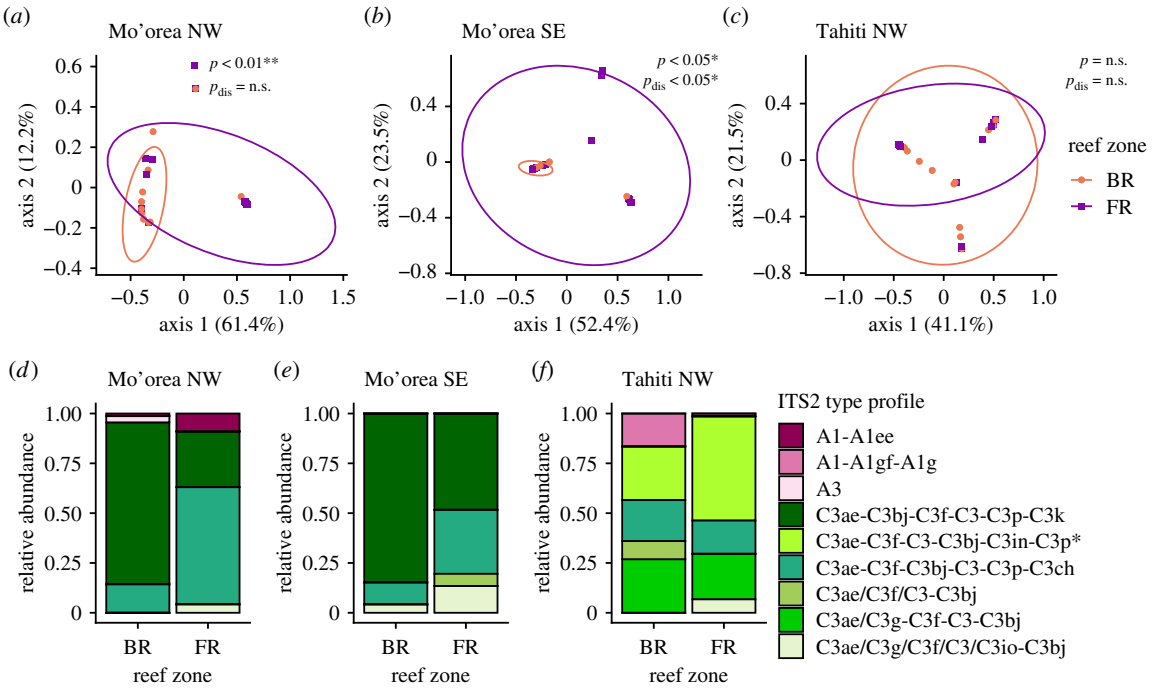

Figure 2. Symbiodiniaceae (ITS2) communities in *Acropora hyacinthus* across reef zones (BR: back reef, FR: fore reef) at (*a* and *d*) Mo'orea NW, (*b* and *e*) Mo'orea SE, and (*c* and *f*) Tahiti NW. Top panels show multivariate ordination plots (PCoA) of Bray–Curtis dissimilarity based on between-sample ITS2 type profiles. Ellipses show 95% confidence intervals and *p*-values indicate significance levels comparing multivariate location, while p_{dis}-values indicate dispersion. Bottom panels show the relative abundances of host-associated ITS2 type profiles summed by site and zone. (*Full name of C3ae-C3f-C3-C3bj-C3in-C3p-C3il-C3im shortened in legend to fit figure margins.) (Online version in colour.)

dominated by ITS2 type profiles belonging to *Cladocopium* C3, with the exception of a few samples being dominated by *Symbiodinium* type profiles A1 or A3 or mixed-genera communities of A1 with C3 (figure 2*d–f*; electronic supplementary material, figure S7). Between sites, type profiles at TNW were significantly different from those at MSE and MNW (electronic supplementary material, figure S8). Data were not significantly dispersed in multivariate space, with the exception of MSE, where FR data were more dispersed than BR (figure 2*b*). Within Mo'orea sites, FR and BR communities were significantly different (figure 2*a*,*b*), though this was likely due to dispersion of samples at MSE. In Mo'orea, the majority of samples from the BR hosted the same type profile C3ae-C3bj × (78.6% and 81.3% of samples at MNW and MSE, respectively) (figure 2*d*,*e*; electronic supplementary material, figure S7). By contrast, FR samples hosted a variety of profiles: C3ae-C3bj × (25% at MNW, 43.8% at MSE), C3ae-C3f-C3bj × (56.3% at MNW, 31.3% at MSE) and C3ae/C3 g/C3f × (6.3% at MNW, 18.8% at MSE) (*full type profile names available in figure 2*d–f*; figure S7).

(d) Bacterial community composition

Prior to rarefaction, mean bacterial reads were 15 115 ± 8839 per sample. Post-rarefaction, average ASV richness was 59 ± 24 and evenness was 0.44 ± 0.11 per sample. ASV richness, Shannon index, Simpson's index, evenness and Faith's D were statistically indistinguishable across reef zones and sites, with the exceptions of lower richness and phylogenetic diversity at Tahiti compared to Mo'orea and lower richness at MSE's BR compared to FR (electronic supplementary material, figure S9).

Of 223 ASVs detected post-rarefaction, nine comprised the core microbiome (electronic supplementary material, table S4). The most prominent core microbiome member was the genus *Burkholderia-Caballeronia-Paraburkholderia* (average ASV relative abundance of 41.9%). The next two most abundant ASVs assigned to the genus *Endozoicomonas* (22.1%) and family Simkaniaceae (8.2%). The remaining core and accessory ASVs averaged less than 2.4%. Multivariate location of bacterial communities was significantly different between all three pairs of sites (electronic supplementary material, figure S10). There were no significant differences in dispersion, with the exceptions of higher dispersion of TNW's BR compared to FR (figure 3*c*) and higher dispersion of MSE than TNW (electronic supplementary material, figure S10). Bacterial communities were significantly different between the FR and BR at MSE and TNW, but not MNW (figure 3*a–c*; electronic supplementary material, figure S11). Patterns were similar when examining the core microbiome (electronic supplementary material, table S4 and figure S12). However, when examining the accessory microbiome, reef zone comparisons within all sites displayed significantly different multivariate locations and there was higher dispersion at MSE's FR than BR (electronic supplementary material, figure S13). Patterns from all preceding 16S analyses were consistent without rarefaction.

Differentially abundant ASVs across reef zones varied between sites (figure 3*d–f*). Only one BR-enriched ASV was detected for MNW (*Pseudomonas* sp.) and none were detected for the FR. Two BR-enriched ASVs were significant for MSE (family Simkaniaceae and order Entomoplasmatales) and seven for the FR (*Endozoicomonas* sp., *Lacibacter* sp., *Methylobacterium* sp., *Phreatobacter oligotrophus*, *Curvibacter* sp., order Obscuribacterales, *Rubrobacter* sp.). There were four BR-enriched ASVs for TNW (*Endozoicomonas* sp., *Enhydrobacter aerosaccus*, *Lacibacter* sp. and *Phreatobacter oligtrophus*) and none for the FR. Inferred functional profiles significantly enriched across reef zones were largely site-specific (electronic supplementary material, figure S14). Exceptions included: sucrose-phosphate synthase enriched in the FR of MNW and MSE, as well as

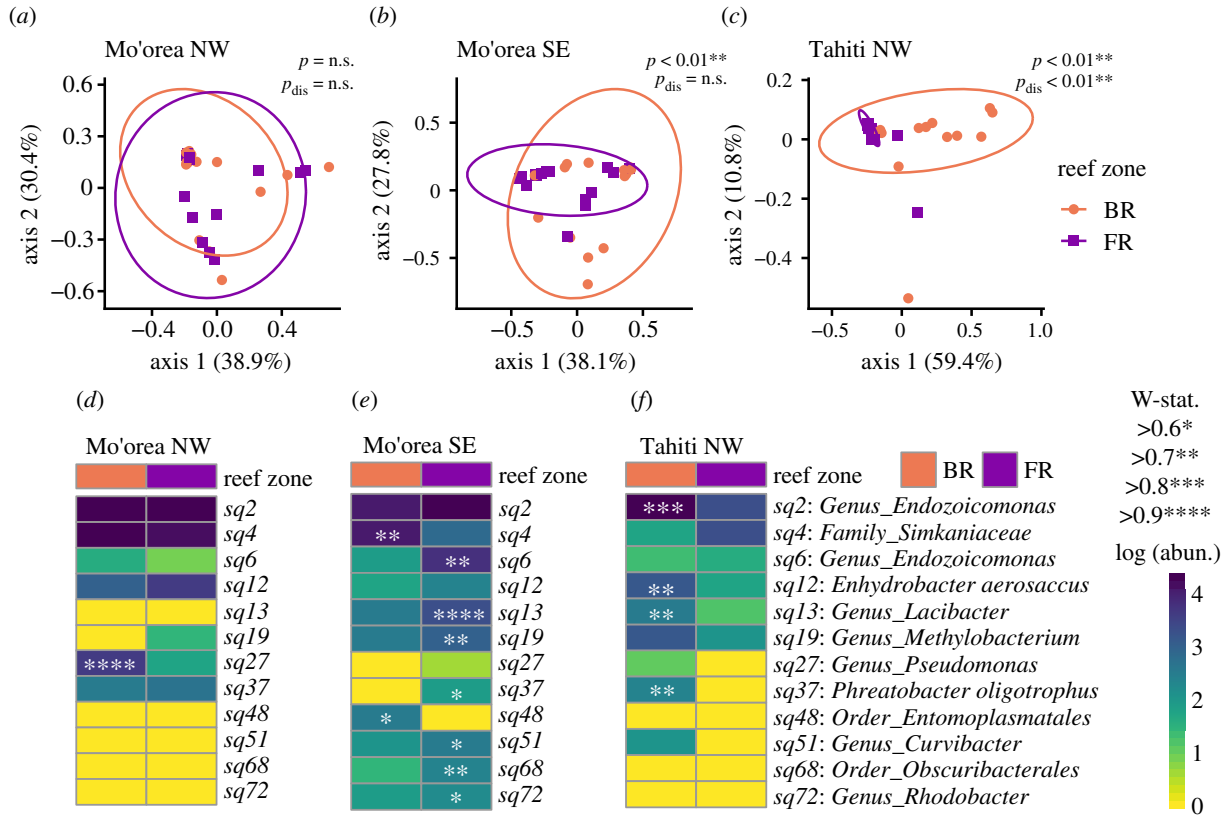

Figure 3. *Acropora hyacinthus* bacterial (16S) communities across reef zones (BR: back reef, FR: fore reef) in (*a* and *d*) Mo'orea NW (*b* and *e*) Mo'orea SE and (*c* and *f*) Tahiti NW. Top panels show multivariate ordination plots (PCoA) of between-sample Bray–Curtis dissimilarity based on rarefied ASVs (relative abundance produced the same patterns). Ellipses show 95% confidence intervals and *p*-values indicate significance levels comparing multivariate location, while p_{dis}-values compare dispersion. Bottom panels show differentially abundant taxa across zones, where level of significance is denoted by ANCOM's W-statistic and heat map colours are log-scaled abundance. (Online version in colour.)

putrescine aminotransferase and multicomponent K^+ : H^+ antiporter subunits A, D and G enriched in the BR of MNW and TNW (electronic supplementary material, figure S14).

(e) Links between holobiont members

Corals hosting type C3ae-C3bj* were significantly larger than those hosting A1-A1gf-A1 g, C3ae-C3f-C3* and C3ae/C3 g/C3f* (*full type profile names available in electronic supplementary material, figure S15). Corals associated with different symbiont type profiles were not significantly different from each other in terms of host heterozygosity or bacterial diversity metrics. In coral colonies living on the FR, bacterial community Shannon index, Simpson's index and evenness all increased with increasing host heterozygosity (figure 4). No significant correlations were found between host heterozygosity with bacterial ASV richness or Faith's D, or colony size with any bacterial diversity metrics (electronic supplementary material, figure S16). In addition, there were no significant correlations between any pairwise genetic divergence comparisons between coral host, bacteria and algal symbiont data.

4. Discussion

(a) Coral host gene flow is pervasive across reef zones and islands

Dispersive marine organisms displaying structured populations and outlier loci across surprisingly small distances are accumulating [5,51]. Reef zones, which can vary dramatically within short distances [5,6], can play notable roles in this structuring. Bongaerts *et al*. [6] and van Oppen *et al*. [5] found higher differentiation across habitats (reef flat versus slope; metres apart) than across sites (kilometres apart). In American Samoa, 114 outlier loci were found in *A. hyacinthus* sampled from neighbouring BR pools, even though no overall genetic structure was detected [51]. Here, however, we observed that *A. hyacinthus* gene flow across habitat types, sites and even islands was pervasive (electronic supplementary material, figures S5 and S6) and no outlier loci were uncovered, suggesting that this population experiences high gene flow. We acknowledge that the genotyping method used here, which only re-sequences approximately 1% of the genome [18], may have missed putative loci under selection. Nevertheless, our results indicate that molecular mechanisms beyond the host genome, namely acclimatory changes such as gene expression, epigenetics or changes in other holobiont components [4,52,53], are more likely responsible for *A. hyacinthus* thriving across these sites and reef zones.

Our findings have broad implications for the persistence of this important reef-building coral in French Polynesia. In our data, *A. hyacinthus* colonies in the FR were smaller than BR colonies (figure 1*c*), suggesting they were more recently established, and *Acropora* were found in higher abundances on the FR (electronic supplementary material, figure S3). These results are consistent with the ability of *A. hyacinthus* to quickly repopulate post-disturbance and also consistent with dual disturbances of crown-of-thorns starfish (*Acanthaster planci*) outbreaks and Cyclone Oli in 2010 (3 years

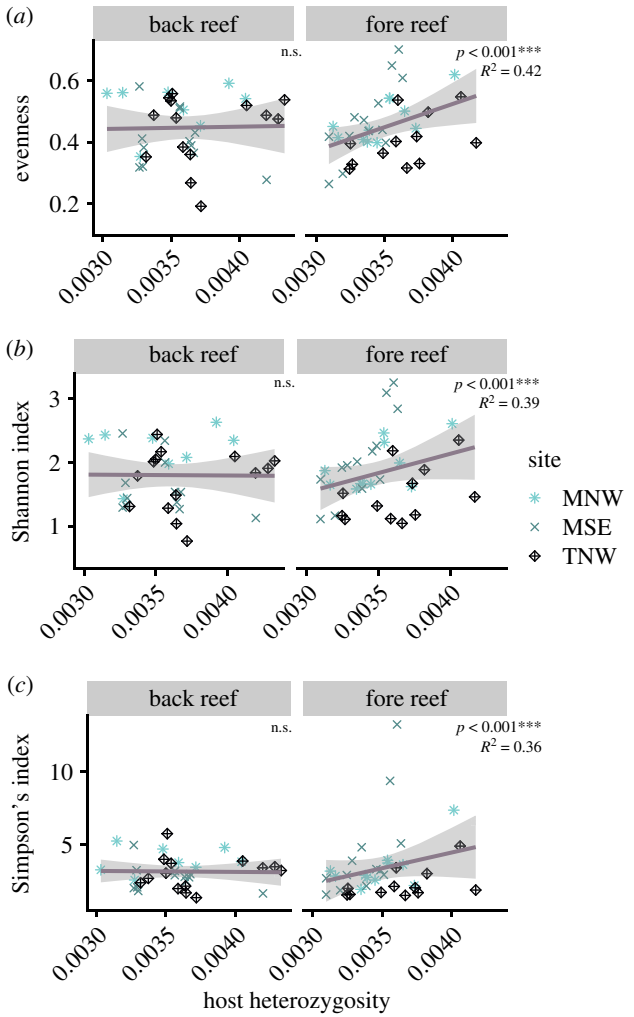

Figure 4. Correlations between *Acropora hyacinthus* coral host heterozygosity (metric of genetic diversity; *x*-axes) and associated bacterial diversity metrics (*y*-axes) in terms of: (*a*) evenness, (*b*) Shannon index and (*c*) Simpson's index (inverted to reflect increasing diversity as *y*-axis increases) across reef zones and sites (MNW: Mo'orea NW, MSE: Mo'orea SE, TNW: Tahiti NW). (Online version in colour.)

prior), both of which more severely damaged ocean-exposed areas [54–56]. Size class and abundance dissimilarities could also be explained by environmental differences across reef zones, (e.g. higher nutrients (electronic supplementary material, figure S1) and light [8] at MNW-BR), which can alter coral growth rates. Regardless, gene flow between sites and zones would facilitate re-seeding of areas impacted by disturbance [56] but it remains to be seen whether the pace of climate change will overcome corals on these historically resilient reefs [54,56]. Significant shifts in abundance from Acroporidae to Pocilloporidae and algae have been observed in Mo'orea and Tahiti over the past decade [54,55] and were apparent in benthic cover data presented here (electronic supplementary material, figure S3), highlighting the urgent need to continue monitoring this highly connected *A. hyacinthus* population [56].

(b) Higher diversity Symbiodiniaceae communities in fore reef environments

Symbiodiniaceae type profiles were largely made up of C3 variants (*Cladocopium* genus) along with a few corals hosting A1 and A3 (*Symbiodinium* genus) (figure 2*d–f*; electronic supplementary material, figure S7), consistent with compositions found by Putnam *et al*. [57]. Interestingly, Howe-Kerr *et al*. [58] found higher frequencies of *Symbiodinium* and *Durusdinium* genera than *Cladocopium* in their 2019 collections, despite their sampling from adjacent reefs in Mo'orea. This may reflect a shift in *A. hyacinthus* Symbiodiniaceae communities due to climate change stressors, given that collections from Putnam *et al*. [57] and our study occurred prior to 2014 and a devastating bleaching event occurred in Mo'orea in 2019. Accordingly, Putnam *et al*. [57] characterized *Acropora* as one of the most sensitive genera to coral bleaching, and demonstrated that this sensitivity correlated with symbiont community flexibility.

Interestingly, we detected differences in the degree of flexibility with symbiont associations across reef zones (figure 2*d*,*e*). In both MNW and MSE, corals from the BR were dominated by type C3ae-C3bj* (greater than 75% of all samples), while the two most common types in the FR (C3ae-C3bj* and C3ae-C3f-C3bj*) ranged between 25 and 56% of samples (figure 2*d*,*e*; electronic supplementary material, figure S7). Such differences can have important implications for the coral holobiont, as hosting different Symbiodiniaceae communities and populations can impact host fitness [11,59]. For instance, Howe-Kerr *et al*. [59] found that *A. millepora* hosting more diverse symbiont communities performed less well under stress. Unfortunately, Cowles *et al*. [56] reports that FR corals were in fact particularly susceptible to bleaching in 2019. Resolving relationships between symbiont flexibility and holobiont fitness is a critical area for follow-up investigations, as consequences for corals are dire.

Selective environmental pressures in Mo'orea BRs (e.g. higher daily temperature range, nitrates and nitrites, and lower wave action in the BR than the FR at MNW; figure 1*b*; electronic supplementary material, figures S1 and S2) may have constrained the diversity of symbiont variants. As an extreme example of habitats constraining symbionts, Bay & Palumbi [51] found that nearly all *A. hyacinthus* in a highly thermally variable BR pool were dominated by *Durisdinium* while corals in the moderately variable pool hosted a mix of *Cladocopium* and *Durisdinium*. By contrast, van Oppen *et al*. [5] found higher symbiont diversity in *P. damicornis* living in more thermally variable reef flats, highlighting that diversity patterns are likely location and species specific. Also, such differences may arise from environmental structuring of free-living Symbiodinaceae sources [60,61]. Fine-scale environmental data collection, including sequencing of Symbiodiniaceae sources, would help disentangle these factors and help explain why Tahiti's FR did not exhibit similar patterns.

Host–symbiont interactions are another potential avenue of structuring Symbiodiniaceae communities, however we found no evidence of host genetic variation correlating with symbiont associations. However, we did find that colony size correlated with the most relatively abundant ITS2 type profiles (electronic supplementary material, figure S15). While these data are exploratory (sample sizes ranged from $n = 3$ hosting A1-A1fg-A1 g to $n = 37$ hosting C3ae-C3bj*; electronic supplementary material, figure S15), patterns are consistent with several hypotheses published in the literature. First, identity of the algal symbiont has been shown to play an important role in nutrition and can affect coral growth rates [2,62]. Alternatively, and perhaps more likely, smaller FR corals (figure 1*c*) may be younger due to disturbance

and have not yet winnowed their algal community to a dominant type that matches other adult conspecifics—a process that can take years in *Acropora* [60,63]. On the other hand, colony size is not always a reliable metric for age, especially when fragmentation may be involved [64]. Regardless, our results showcase that reef zones in Mo'orea play an important structuring role in Symbiodiniaceae community dynamics.

(c) Bacterial taxa and functions are structured on smaller scales than other holobiont members

Bacterial communities associated with *A. hyacinthus* were largely composed of the core microbiome players Proteobacteria *Paraburkholderia* (approx. 41.9% average relative abundance) and *Endozoicomonas* spp. (approx. 21%) (electronic supplementary material, table S4). These compositions are consistent with *A. hyacinthus* in Epstein *et al*. [65] from the Great Barrier Reef. By contrast, FR *A. hyacinthus* collected in Mo'orea by Maher *et al*. [66] in 2016 showed higher relative abundances of *Endozoicomonas* relative to our results, which may indicate shifts in microbial partners through time. Even more remarkably, in early 2017 Maher *et al*. [66] found that *Endozoicomonas* increased in relative abundance even further following a significant thermal stress event, which negatively impacted overall within-colony diversity. Here, the ASV belonging to *Endozoicomonas* was present in the core microbiome (electronic supplementary material, figure S12 and table S4), and was also enriched in Tahiti's BR (figure 3*f*). This genus is ubiquitous in marine invertebrates and vertebrates and has been proposed as a beneficial coral symbiont [67]. *Endozoicomonas* members have been implicated in sulfur cycling, and the same is true for genus *Pseudomonas* [4], which was enriched in MNW's BR. While much work remains to empirically connect these two bacteria to host function, they most likely play prominent roles in *A. hyacinthus* microbiomes in French Polynesia.

All three sites displayed differentially enriched bacterial functions across reef zones (electronic supplementary material, figure S14). Of these enriched functions, several were consistent across reef zones at multiple sites (electronic supplementary material, figure S14). The shared function between the FR environment of MNW and MSE was involved in carbohydrate metabolism, while the functions shared between the BR environment of MNW and TNW were involved in amino acid metabolism and electrochemical potential-driven transport. Interestingly, all but one of the functions conserved across multiple sites' reef zones were enriched in BR sites. Our results corroborate Ziegler *et al*. [12], which showed the majority of enriched bacterial functions within *A. hyacinthus* hosts were observed in the more variable environment, perhaps due to environmental constraints. In addition, nitrates and nitrites concentrations are typically higher in the BR around Mo'orea, likely due to anthropogenic influences (electronic supplementary material, figure S1) [13,14]. Such gradients across environments are correlated with different pelagic bacterial communities in Mo'orea [68,69], perhaps explaining our observed enrichment of metabolic functions and taxa across reef zones.

In terms of potential biotic interactions, we found intriguing correlations between coral genetic diversity and bacterial diversity, but only in FR corals (figure 4). This pattern could be due to an environmental covariate in the FR that structures these host–bacteria relationships on a microscale. Alternatively, it may be evidence of genotype by genotype interactions of host and bacteria occurring in the FR and not the BR due to stronger overall environmental pressures experienced closer to shore (figure 1*b*; electronic supplementary material, figures S1 and S2) [5,8,13]. Greater flexibility of microbial associations correlating with greater host genetic diversity hints at functional redundancy in both the host genome and bacterial community [70]. Metabolic supplementation by bacteria is a burgeoning area of inquiry and multiomic studies cross-referencing multiple holobiont members are becoming more common [70] and will continue to lead to new discoveries.

5. Conclusion

Understanding the scale of environmental selection for multiple members of the coral holobiont and their interactions remains critically important as intra- and inter-reef variability can determine how corals respond to environmental stressors [5,51,53]. Our work contributes to this area of investigation by showing flexibility of algal symbiont associations and correlations of host–microbe diversity changing between habitats less than half a kilometre apart despite long-distance dispersal capacity of the host. It is important to note that additional fine-scale environmental data and more in-depth sequencing would help disentangle factors contributing to these patterns. Nevertheless, key findings from this study add baseline context to current events in French Polynesia, including widespread *A. hyacinthus* population connectivity and apparent shifts in their associated symbiont and bacterial taxa over time and space. As oceans continue to warm, understanding the distributions and roles of key microbial partners in the coral holobiont may hold the key to effective reef restoration.

Data accessibility. All raw sequencing data are available on NCBI's SRA (2bRAD: PRJNA779414, ITS2: PRJNA660421, 16S: PRJNA660779). Processed data files and scripts used in analysis are available at https://github.com/Nicfall/moorea_holobiont_revised. Additional information is provided in the electronic supplementary material [71].

Authors' contributions. N.G.K.: formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; G.V.A.: data curation, methodology, project administration, writing—review and editing; M.R.K.: methodology, software, writing—review and editing; S.W.D.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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