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Variability in Microbial Community Composition and Function Between Different Niches Within a Coral Reef

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Abstract To explore how microbial community composition and function varies within a coral reef ecosystem, we performed metagenomic sequencing of seawater from four niches across Heron Island Reef, within the Great Barrier Reef. Metagenomes were sequenced from seawater samples associated with (1) the surface of the coral species *Acropora palifera*, (2) the surface of the coral species *Acropora aspera*, (3) the sandy substrate within the reef lagoon and (4) open water, outside of the reef crest. Microbial composition and metabolic function differed substantially between the four niches. The taxonomic profile showed a clear shift from an oligotroph-dominated community (e.g. SAR11, *Prochlorococcus*, *Synechococcus*) in the open water and sandy substrate niches, to a community characterised by an increased frequency of copiotrophic bacteria (e.g. *Vibrio*, *Pseudoalteromonas*, *Alteromonas*) in the coral seawater niches. The metabolic potential of the four microbial assemblages also displayed significant differences, with the open water and sandy substrate niches dominated by genes associated with core house-keeping processes such as amino acid, carbohydrate and protein metabolism as well as DNA and RNA synthesis and metabolism. In contrast, the coral surface

seawater metagenomes had an enhanced frequency of genes associated with dynamic processes including motility and chemotaxis, regulation and cell signalling. These findings demonstrate that the composition and function of microbial communities are highly variable between niches within coral reef ecosystems and that coral reefs host heterogeneous microbial communities that are likely shaped by habitat structure, presence of animal hosts and local biogeochemical conditions.

Introduction

Microbes play a fundamental role in determining the health and ecology of coral reefs [1, 2]. At the scale of an entire reef, microbes drive chemical cycling processes that ultimately control the productivity and biogeochemical function of the reef ecosystem [3–7]. At finer scales, microbes form both mutualistic and pathogenic relationships with a variety of benthic organisms including corals, sponges, clams, ascidians and macroalgae [8–12]. At the scale of the individual coral colony, the abundance, taxonomic composition and metabolic potential of bacterial communities inhabiting the gastrodermal cavity, skeleton and surface microlayer of a coral differ substantially [13]. However, as a consequence of the complex physical and chemical nature of coral reef ecosystems, we are only just beginning to unravel the spatio-temporal dynamics of coral reef microbial communities [14–17].

In recent years, molecular microbiological techniques have provided important insights into patterns in coral reef microbial diversity and function, enhancing our understanding of how microbes influence overall coral reef ecosystem function and stability [18–20]. Coral colonies have been shown to host diverse and species-specific microbial communities [11, 19, 21] that are typically phylogenetically distinct from those in adjacent waters [2, 11, 21–23]. Clear dissimilarities between

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the microbial assemblages associated with healthy corals, diseased corals and dead coral surfaces have also been demonstrated by 16S rRNA gene sequencing [22]. Furthermore, microbes associated with other benthic organisms on coral reefs have shown similar patterns of host specificity. For instance, sponges harbour diverse and abundant microbial communities, which generally exhibit species specificity regardless of geography [24, 25].

Characterisation of the local spatio-temporal dynamics and regional biogeographical patterns of coral reef microbial communities has recently been assessed using molecular tools such as 16S rRNA sequencing. For instance, Littman et al. [19] revealed that patterns of species specificity among the microbial communities inhabiting three species of Great Barrier Reef (GBR) *Acropora* corals were not conserved according to geographic location. More recently, an examination of the impact of tidal cycles on the spatio-temporal patterns of coral reef bacteria on Heron Island demonstrated relatively homogenous microbial assemblages between sample sites [17]. However, these observations are in contrast to a previous work demonstrating significant differences in microbial community composition between sample sites on coral reefs at Lizard Island (GBR), Magnetic Island (GBR) and Tobago (Caribbean) [14, 16, 26]. Nelson et al. [27] examined the spatio-temporal variations throughout Paopao Bay (French Polynesia) by measuring changes in the community composition between waters from the bay, the reef and the open ocean, revealing distinctly different phylogenetic features of the bacterial communities between sites. The microbial communities inhabiting healthy, diseased and bleached *Acroporas* from American Samoa and the Great Barrier Reef have also recently been shown to demonstrate compositional shifts under natural bleaching and disease events, with the abundance of *Vibrio* bacteria increasing during bleaching [28, 29]. While these studies have begun to reveal the complexities of microbial community ecology on coral reefs, our understanding of how microbial function changes over a coral reef ecosystem is less well developed.

Metagenomic analyses have recently begun to provide insights into the diversity and functional roles of microbes within coral reef ecosystems. For instance, metagenomics has provided snapshots of the structure and function of the microbial community associated with the coral *Porites astreoides* [30], revealing that endolithic algae can be key players in the microbial community and drive important chemical processes such as nitrogen fixation within the coral holobiont. Metagenomics has also been used in manipulative experiments to demonstrate shifts in the composition and metabolic potential of coral-associated microbes under changing environmental conditions such as increased temperature, elevated nutrients, DOC and decreased pH [31]. The work by Vega-Thurber et al. [31] demonstrated that environmental stress led to a shift from a mutualistic to potentially pathogenic

microbial community, as indicated by a substantial increase in genes involved in virulence, stress resistance, chemotaxis and motility.

On a broader scale, metagenomics has been used to assess the effects of natural temperature variability during a bleaching event on Magnetic Island (GBR), revealing a shift in the community composition of microbes associated with a transition from healthy to bleached health state of the coral *Acropora millepora* [32]. This study also reported a shift in metabolism from autotrophy in healthy corals to heterotrophy in bleached corals and a higher proportion of virulence factors in the microbial community associated with bleached corals. Furthermore, metagenomics has shown clear shifts in taxonomic composition and metabolic potential between four coral atolls in the Northern Line Islands (central Pacific Ocean) [18] and the Abrolhos Bank coral reefs off the coast of Brazil (south-western Atlantic Ocean) [33].

The coral reef metagenomic studies performed to date have collectively enhanced our understanding of coral reef microbiology by describing patterns in microbial structure and function in specific coral species [30–33], across different coral reef ecosystems [18, 33] and under changing environmental conditions and stress levels [31, 32]. However, we still have little insight into how the composition and function of microbial assemblages varies within a single reef ecosystem. Consequently, we lack a full appreciation of the way in which within-reef heterogeneity influences microbial survival, adaptation or niche exploitation. Here, we present a metagenomic study aimed at determining microbial function and composition across different niches within a single reef ecosystem, as a first step towards dissecting how within-reef variability influences the associated microbial communities.

Methods

Niches and Water Collection

Sampling was conducted on Heron Island in the Capricorn Bunker Group on the southern Great Barrier Reef (23°26' 31.20"S, 151°54'50.40"E) during July 2011. Four different seawater niches were selected for sampling based on different reef features [34] and included the following: (1) the sandy substrate, (2) coral surfaces in the lagoon, (3) coral surfaces in the reef crest and (4) the open water. Sample collection occurred on an incoming tide at the same time of day over the course of four consecutive days, approximating the same point within the tidal cycle, where one sample was collected per day.

Coral surface seawater samples were collected adjacent to the surface of a colony of *Acropora palifera* situated in the lagoon (23°26'41"S, 151°54'47"E) and a colony of *Acropora aspera* located on the reef crest (23°26'41"S, 151°54'47"E) (ESM Fig. 1). These samples are henceforth referred to as

lagoon-coral and reef crest-coral, respectively. These coral species were chosen because they represent the dominant species in the respective zones of the Heron Island Reef [35]. Coral-associated seawater samples were collected by placing the mouth of a sterile 10-L Schott bottle immediately over (within 1 cm) the surface of the coral.

The sandy substrate seawater sample was collected immediately above (1 cm) the sandy substrate within the Heron Island lagoon (23°26'36"S, 151°54'47"E), in a location where the water depth was 40 cm and no corals were present within a radius of 10 m (ESM Fig. 1). Finally, an open water sample was taken from the water surface at a point approximately 3 km outside of Heron Island's north-western fore-reef slope (23°24'58"S, 151°53'12"E), where the water depth was 40 m (ESM Fig. 1).

Microbial Cell Counts

Four 500- μ L water samples were collected from each of the niches, fixed with glutaraldehyde (1 % final concentration) and frozen in liquid nitrogen before being stored at -80°C . Prior to flow cytometry (FCM), samples were quick-thawed and stained with SYBR Green I [1:10,000] (Invitrogen Molecular Probes USA) and 1 μm diameter fluorescent microspheres (Invitrogen Molecular Probes USA) were added as an internal reference [36]. Samples were analysed using a Becton Dickinson LSR II flow cytometer (BD Biosciences) and bacterioplankton populations were discriminated according to SYBR Green fluorescence and side-scatter [36]. FCM data was analysed using Cell-Quest Pro software (BD Biosciences) and bacterioplankton abundances were compared between niches using one-way analysis of variance (ANOVA), where normal distribution of data was ensured using Levene's homogeneity of variance test. All analyses were performed using Minitab statistical software (Version 15.1.0.0 2006, PA, USA).

DNA Collection and Extraction

In each of the four niches, 10 L of seawater was collected and immediately returned to the Heron Island Research Station Laboratories, where it was filtered onto 0.2 μm polycarbonate membrane filters (Millipore) within 10 min of collection. The filters were frozen at -80°C until DNA extraction was conducted using the MO BIO PowerWater DNA isolation kit (Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA concentrations were measured using a Qubit 2.0 fluorometer (Invitrogen).

Sequencing and Bioinformatics

A shotgun metagenomic library was prepared and sequenced for each of the four samples using the 454 GS-FLX

pyrosequencing platform (Roche) at the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales. Sequences were subsequently analysed using the *Meta Genome Rapid Annotation using Subsystems Technology* (MGRAS; Version 3 pipeline [37, 38]). Quality control was performed by removing reads with >10 ambiguous bases per read and de-replicating artificial duplicates in which the first 50 bp of the read were identical (Table 1). Within MG-RAST, metabolic assignments were annotated to the SEED subsystems database [37], and taxonomic identification was determined based on the top BLAST hits to the SEED taxonomy. The SEED is organised into three hierarchical levels for metabolism and six levels for taxonomy [37–39]. Matches with a *E*-value of <0.05 were considered significant with a minimum alignment of 50 bp [31, 39–46]. All data was normalised to sequencing effort by dividing by the total number of hits. The metagenomes can be accessed through MG-RAST (<http://metagenomics.anl.gov/>) under the project numbers 4483104.3 (lagoon-coral), 4483105.3 (reef crest-coral), 4483106.3 (sandy substrate) and 4483107.3 (open water).

Taxonomic and metabolic reconstructions generated using MG-RAST were imported into the Statistical Analysis of Metagenomic Profiles (STAMP) package to test for statistically significant differences between the four metagenomes. Fisher's exact test was used to determine significant differences between samples with a Benjamini FDR multiple test correction applied [47]. We used Fisher's exact test [48, 49] to identify the most significantly different categories (taxonomic or metabolic categories) between two samples, as suggested in the STAMP user manual [50] and as has been recommended [49, 50] and routinely applied in this fashion [39, 45, 51–53]. Hence, all quoted *q*-values represent corrected values (equating to *q*), with only values <0.05 reported [50]. Confidence intervals (95 %) were determined using the Newcombe–Wilson method. Using this approach, we compared the relative frequency of taxonomic groups at the class level for Archaea, at the order level for Eukaryota, the genus level for bacteria and the species level for viruses. These taxonomic levels were chosen for the different domains to display the taxonomic differences between the niches at the highest resolution without compromising the abundance of sequences per taxa generated at each level. The metabolic capacity of the communities was examined using the SEED level 1, 2 and 3 groupings [50].

Multivariate statistical software (PRIMER v6) was used to measure the degree of similarity between metagenomes [54]. Data was square-root-transformed and the Bray–Curtis similarity was calculated between samples. Similarity percentage (SIMPER) analysis [55] was used to identify the phylogenetic groups and metabolic categories contributing most to the dissimilarity between the metagenomes.

Table 1 Environmental data and metagenome statistics for the four Heron Island niches

Meta data	Sandy substrate	Lagoon-coral	Reef crest-coral	Open water
DNA sequences	233,026	201,910	182,182	103,900
Sequences fail QC	23,899	20,691	18,818	10,573
Mean sequence length of DNA base pairs (post-QC)	457	450	449	454
Known annotated proteins % (SEED)	54	42	50	59
% of matches to Bacteria	88.4 (58.3 % to Proteobacteria)	84 (56.3 % to Proteobacteria)	84.6 (60.9 % to Proteobacteria)	89.5 (55 % to Proteobacteria)
% of matches to Eukaryota	5.2	9	7.3	5.2
% of matches to viruses	4.4	5.3	6.6	3.3
% of matches to Archaea	1.2	0.9	0.8	1.1
Bulk counts of bacteria per mL \pm SD	$9.4 \times 10^4 \pm 1.2 \times 10^4$	$3.7 \times 10^5 \pm 6.7 \times 10^4$	$1.0 \times 10^6 \pm 1.6 \times 10^5$	$3.5 \times 10^6 \pm 4.5 \times 10^5$
Sea water temperature ($^{\circ}$ C)	19.7	19.7	19.6	19.7

Results

Metagenomic sequencing was used to assess variability in microbial community composition and functional potential across four different reef niches on Heron Island. FCM counts showed the highest bacterial abundance in the open water niche ($P < 0.05$), with bacterial abundance decreasing progressively from the reef crest-coral to lagoon-coral and sandy substrate niche (Table 1). There were between 103,900 to 233,026 sequences across the metagenomes, with average read lengths of between 449 to 457 bp (Table 1). When annotated and compared to the SEED database, the metagenomic data revealed significant changes in microbial community composition and metabolic potential across the different coral reef niches.

Taxonomy

By comparing sequences in the four metagenomes to the SEED database, between 42 and 59 % of sequences could be matched to known phylogenies (Table 1), which is a level of phylogenetic assignment consistent with previous ocean metagenomes [56]. All samples were dominated by bacteria, with between 84 and 89 % of sequences matching bacteria (Table 1), and of these Proteobacteria made up an average of 57 % of bacterial matches. Matches to Eukaryota comprised between 5.2 to 9 % of sequences, 3.3 to 6.6 % of sequences matched viruses and 0.8 to 1.2 % of sequences matched Archaea.

Shifts in microbial community composition were observed between the four niches (Fig. 1) and were found to be statistically significant ($q < 0.05$) using Fisher's exact test (Fig. 2). SAR11 was the most abundant bacterial group in all samples (Fig. 1). Statistically significant shifts in the relative abundance of SAR11 were observed between different Heron Island Reef niches ($q < 0.05$), where SAR11 was significantly

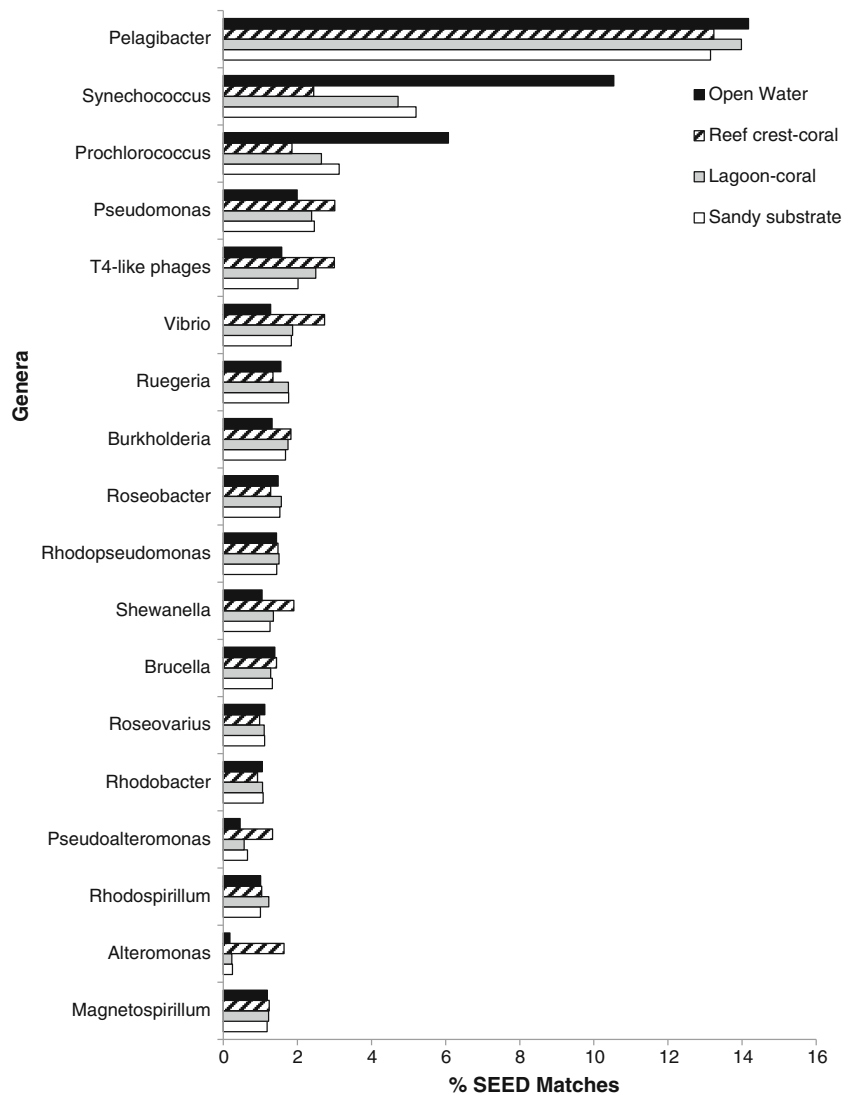
less abundant in the reef crest-coral niche than in all other niches. However, SIMPER analysis revealed that this clade was not a significant driver of the overall variation in community composition (Fig. 1, Online Resource Table 1). Other microbes including the marine cyanobacteria *Synechococcus* and *Prochlorococcus* were also abundant in all niches sampled, but made up a significantly greater proportion of the open water community than within the other niches ($q < 0.05$) (Figs. 1 and 2). In contrast to SAR11, SIMPER analysis revealed that *Synechococcus* and *Prochlorococcus* were responsible for driving statistically significant differences in community composition between the sea water niches and were the most discriminating genera of the non-coral niches (open water and sandy substrate) (Online Resource Table 1). *Synechococcus* and *Prochlorococcus* together accounted for 5.7 % of the dissimilarity between metagenomes.

While SAR11, *Prochlorococcus* and *Synechococcus* were generally the most abundant bacteria in all niches, several bacteria occurred in increased frequency in the reef crest-coral niche than in the open water or sandy substrate niches ($q < 0.05$) (Figs. 1 and 2). These included *Pseudomonas*, *Vibrio*, *Shewanella*, *Pseudoalteromonas*, *Mycobacterium* and *Alteromonas* (Figs. 1 and 2) with all but *Shewanella* and *Mycobacterium* significantly more abundant in the lagoon-coral niche than the open water niche (Fig. 1, Online Resource Table 2).

SIMPER analysis revealed that these genera were responsible for driving statistically significant differences in community composition between the seawater samples (Fig. 2, Online Resource Table 1), with *Alteromonas*, *Mycobacterium* and *Vibrio* identified as discriminating genera of the coral-associated (reef crest-coral and lagoon-coral) niches. Combined, these groups accounted for 3.6 % of the dissimilarity between metagenomes (Online Resource Table 1).

Archaea comprised between 0.8 and 1.2 % of sequences across all niches. Hits to the Euryarchaeota phylum (1.45 %)

Fig. 1 Top 18 microbial genera (relative percentage of microbial SEED matches) in each seawater niche metagenome: sandy substrate, lagoon-coral, reef crest-coral and open water (ordered according to frequency of occurrence in each niche)



comprised the greatest number of sequences across all metagenomes, followed by Thaumarchaeota (0.53 %) and Crenarchaeota (0.18 %) (Online Resource Fig. 2). Sequence

matches to an unclassified Thaumarchaeota class were higher than matches to any other class found across the metagenomes (Online Resource Fig. 2) and were significantly more

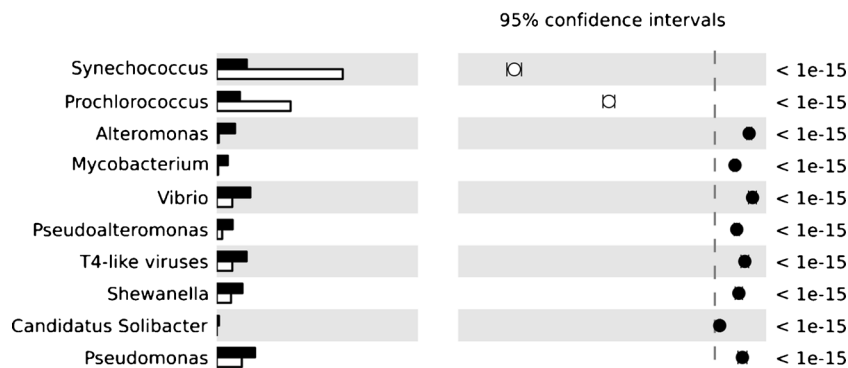


Fig. 2 Fisher's exact test was used to statistically test for significant differences in the relative representation of microbial genera between the reef crest-coral community and open water community. The top 10 most significant differences are displayed here out of the 149 genera that were found to be significantly different between the reef crest-coral seawater

niche and the open water niche. **a** Groups over-represented in the reef crest-coral seawater community (*black*) correspond to positive differences between proportions. Groups over-represented in the open seawater community (*white*) correspond to negative differences between proportions

abundant in the sandy substrate niche than any other niche (Fisher's exact test, $q < 0.05$) (Online Resource Fig. 2). The Euryarchaeota populations were mainly comprised of the following classes: Methanomicrobia which was less abundant in the open water niche than any other niche ($q < 0.05$), Halobacteria showed no differences in abundance across the niches, Thermoprotei which was more abundant in the open water than in the lagoon-coral niche only ($q < 0.05$), Archaeoglobi was more abundant in the coral niches than the non-coral niches ($q < 0.05$) and Methanopyri which was more abundant in the reef crest than any other niche (Online Resource Fig. 2).

We also observed an increase in sequences matching T4-like phages in the two coral-associated metagenomes compared to the non-coral niches (open water and sandy substrate niches) (Figs. 1 and 2, Online Resource Tables 2 and 3). Phages belonging to T4-like phages included *Prochlorococcus* phages P-SSM2 and P-SSM4 and *Synechococcus* phage S-PM2, which dominated all samples, comprising between 95 and 98 % of all T4-like phage sequences retrieved at each niche (Online Resource Fig. 3). *Prochlorococcus* and *Synechococcus* phages were significantly more abundant ($q < 0.05$) in the reef crest-coral and lagoon-coral niches than the open water, and SIMPER analysis revealed that these groups contributed to significant differences between coral (reef crest-coral and lagoon-coral) and non-coral (open water and sandy substrate) niches.

While the sequences matching *Brassicales* from Eukaryota represented only 0.45 % of all sequences, this group comprised the greatest number of eukaryotic sequences across all niches (Online Resource Fig. 4) and was more abundant in the reef crest-coral niche than the sandy substrate niche ($q < 0.05$). Matches to other less abundant Eukaryota such as *Scleractinia* were significantly more abundant in the coral niches than the non-coral niches (Online Resource Fig. 4) ($q < 0.05$). *Chlorellales* was more abundant in the reef crest-coral niche ($q < 0.05$), *Actiniaria* was more abundant in the lagoon-coral niche ($q < 0.05$) and *Cypriniformes* was more abundant in the sandy substrate than in any of the other niches ($q < 0.05$) (Online Resource Fig. 4).

Function

Sequence matches to genes fundamental to basic microbial function, often referred to as "core" or "house-keeping" genes, comprised the most abundant hits in all metagenomes. These included genes involved in carbohydrate, amino acid, protein, RNA and DNA metabolism, respiration and nucleoside synthesis (Fig. 3). Despite their predominance across all niches, SIMPER analysis revealed that variations in the occurrence of these house-keeping genes did not drive significant differences between metagenomes (Figs. 3 and 4, Online Resource Table 4), although Fisher's exact test revealed that many of

these gene groups were significantly more abundant in the open water metagenome compared to the reef crest-coral metagenome ($q < 0.05$) (Fig. 4a). In contrast, metabolic categories with comparatively lower abundance were responsible for the greatest variation in functional potential between the microbial communities. In particular, genes associated with bacterial motility and chemotaxis, regulation and cell signalling varied significantly between niches ($q < 0.05$) (Fig. 4a–d), and SIMPER analysis revealed genes associated with these categories were responsible for significant separation between the four metagenomes (Online Resource Table 4). These gene groups were also significantly more abundant within the reef crest-coral metagenome than at any other niche ($q < 0.05$) (Fig. 4).

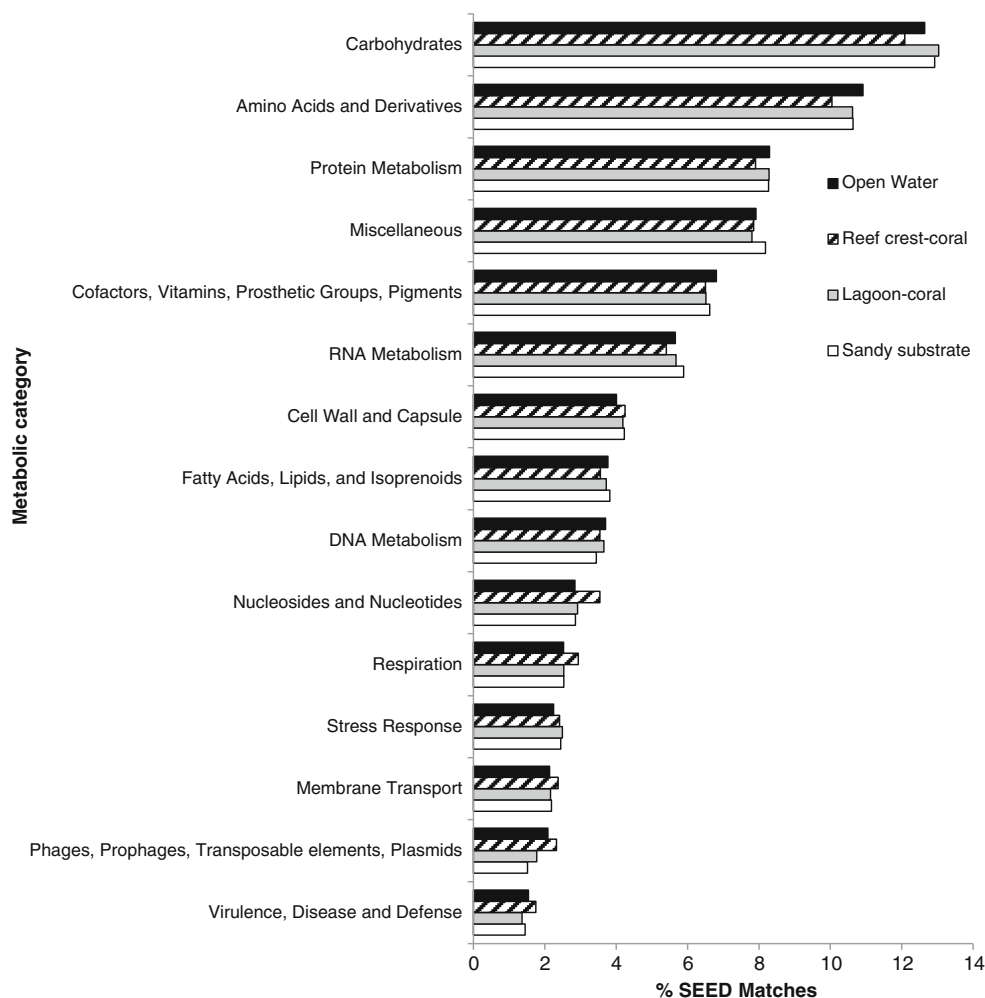
Phage-associated genes (in particular genes affiliated with phage tail proteins, phage integration and phage excision) also varied significantly between niches ($q < 0.05$) (Fig. 4a–d). Overall, the reef crest-coral niche contained a greater abundance of phage-associated genes than the open water niche ($q < 0.05$) (Fig. 4a).

Several other metabolic categories typically related to more specialised processes also occurred in significantly higher proportions in the reef crest-coral niche metagenome than in the open water niche. These included membrane transport, iron acquisition and metabolism, potassium metabolism, metabolism of aromatic compounds, cell wall and cell capsule synthesis and stress response (Fig. 4a). In contrast, other metabolic categories including photosynthesis and respiration were more abundant in the open water niche ($q < 0.05$) (Fig. 4a, b). Photosynthetic genes, specifically genes associated with light-harvesting complexes and photosystem I, were most abundant in the open water niche ($q < 0.05$), whereas genes associated with photosystem II were higher in the reef crest-coral niche ($q < 0.05$).

Discussion

In this study, metagenomic sequencing revealed substantial variability between the four metagenomes despite the close proximity of the samples (<500 m between the sandy substrate, lagoon-coral and reef crest-coral). These data are indicative of substantial heterogeneity in microbial community composition and metabolic potential across different niches within a single coral reef ecosystem. Previous research has used metagenomics to reveal large changes in microbial community composition and function between different coral reefs [18, 19]. However, while 16S rRNA-based approaches have shown that microbial community composition can shift within reef systems [14, 16, 26, 27], patterns in microbial functional potential across different niches within a single coral reef ecosystem had not previously been documented.

Fig. 3 Frequency distribution (relative percentage of microbial SEED matches) of top 15 microbial metabolic functions in each seawater niche metagenome: sandy substrate, lagoon-coral, reef crest-coral and open water (ordered according to frequency of occurrence in each niche)



Taxonomy

Microbial taxonomy varied significantly between the four niches, with differences often related to the presence or absence of corals. We also found evidence that the trophic strategies of coral-associated microbes were distinct to the non-coral microbial communities. It has previously been demonstrated that marine microbes broadly fall into two trophic categories, defined as copiotrophic and oligotrophic organisms [57]. Oligotrophs are generally highly abundant, but slow-growing microbes that dominate within environments characterised by stable and low nutrient conditions [57]. Conversely, copiotrophs are microbes that are generally less abundant, but capable of bursts of rapid growth in response to intermittent pulses of high nutrient concentrations and often live in association with plant and animal hosts [57]. Our data are indicative of a partitioning of these life strategies between the different coral reef niches sampled.

Consistent with most marine metagenomic studies, sequence matches to the classic marine oligotroph *Candidatus*

pelagibacter (SAR11) dominated all samples [58, 59]. However, the relative importance of SAR11 differed between samples, with sequence matches to SAR11 significantly less abundant in the reef crest-coral niche. Similarly, the two common cyanobacterial genera *Synechococcus* and *Prochlorococcus*, which are also typical of oligotrophic open water niches, were significantly more abundant in the non-coral associated niches (open water and sandy substrate). It has previously been demonstrated that extracts from 100 coral species, including eight *Acropora* species, produce antimicrobial compounds against *Synechococcus* spp. [60]. While our finding that *Synechococcus* sequences occur at lower frequency in the coral-associated metagenomes is consistent with this observation, the simultaneous decrease in sequences matching the other dominant oceanic microbes (SAR11 and *Prochlorococcus*) indicates that more general environmental differences between the open water and coral-associated niches may be responsible.

While oligotrophs dominated the non-coral niches, we observed an increase in the numbers of sequences matching

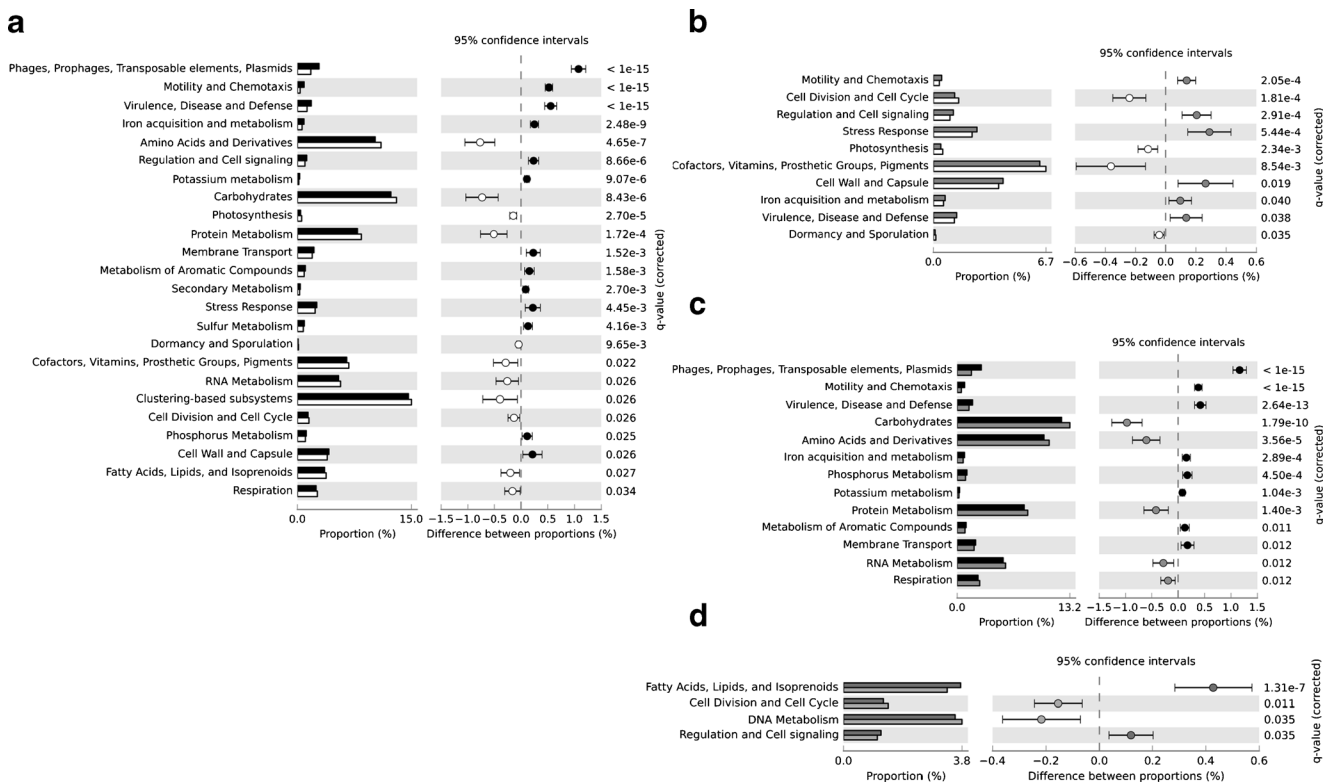


Fig 4 Fisher’s exact test was used to statistically test for significant differences in the relative abundance of functional categories. **a** Groups over-represented in the reef crest-coral community (*black*) correspond to positive differences between proportions. Groups over-represented in the open water community (*white*) correspond to negative differences between proportions. **b** Groups over-represented in the lagoon-coral community (*light grey*) correspond to positive differences between proportions. Groups over-represented in the open water community (*white*)

correspond to negative differences between proportions. **c** Groups over-represented in the reef crest-coral community (*black*) correspond to positive differences between proportions. Groups over-represented in the lagoon community (*light grey*) correspond to negative differences between proportions. **d** Groups over-represented in the lagoon community (*dark grey*) correspond to positive differences between proportions. Groups over-represented in the sandy substrate community (*light grey*) correspond to negative differences between proportions

copiotrophic organisms in the coral-associated samples. This included statistically significant increases in the relative importance of copiotrophic bacteria including *Pseudomonas*, *Vibrio*, *Shewanella*, *Pseudoalteromonas*, *Mycobacterium* and *Alteromonas*. These increases in copiotrophic organisms are consistent with the higher concentrations of organic material typically found near the surfaces of corals compared to the surrounding open water [35, 61–64].

Corals provide a niche enriched in dissolved and particulate organic material [6, 15, 35, 61–63, 65–67]. For instance, coral mucus and the exudates of *Symbiodinium* spp. are rich in organic compounds, including amino acids, sugars and dimethylsulfoniopropionate (DMSP) [63, 64, 68–71], meaning that concentrations of these compounds can be two to four orders of magnitude higher near the coral surface than in the surrounding seawater [72]. Diffusion of this dissolved organic material and shedding of coral mucus into the water immediately overlaying coral surfaces is likely to produce a localised plume of organic material available to bacteria in the water column, and the elevated occurrence of copiotrophic bacteria in the coral-associated metagenomes is consistent with this.

Notably, many of the copiotrophic bacteria identified here can represent either beneficial bacteria for the corals or potential pathogens. Several of the microbial genera that were over-represented in the reef crest-coral niche have been identified as potential coral pathogens. For instance, *Vibrio* species have been implicated in several coral diseases. *Vibrio shiloi* and *Vibrio corallilyticus* have been shown to be involved in coral bleaching [2, 73, 74], *Vibrio owensii* was found to cause *Montipora* white syndrome [75] and a consortium of *Vibrio* are believed to be involved in yellow band disease [75–79]. However, while the roles of *V. shiloi* and *V. corallilyticus* in bleaching has been confirmed by several studies [2, 73, 74], it is still not clear whether other *Vibrio* species found in association with diseased corals [75–79] are the disease-causing agent, or opportunistic colonisers that most efficiently exploit already compromised corals [70].

In addition to potential pathogens, some of the bacterial genera over-represented in the reef crest-coral metagenome may have beneficial effects for the coral. Among these, members of the *Pseudomonas* genus have been shown to inhibit the growth of potential pathogens when isolated from the soft

coral *Sinularia polydactyla* [80] and members of the *Pseudoalteromonas* genus have been shown to inhibit the settlement of the coral pathogen *V. shiloi* [81].

Recently, it has been demonstrated that the 16S rRNA sequences of coral-associated Archaea on Heron Island, the Gulf of Eilat and the Virgin Islands are more than 97 % similar [82]. Our results demonstrate that at a finer scale, the distribution and diversity of Archaea is not uniform across Heron Island Reef. Archaea only comprised a maximum of 1.2 % of all sequences, but this is comparable to the relative abundance of archaeal sequences found in other coral reef environments [33].

The most dominant archaeal class across all metagenomes was an unclassified representative from the Thaumarchaeota phylum which was found to be in higher abundance in the sandy substrate niche. Thaumarchaeota have recently been detected in benthic coral reef organisms such as marine sponges [83] and tropical and temperate ascidians [84]. There is some evidence that the Thaumarchaeota may be involved in ammonia oxidisation within sponges and ascidians [83, 84]. There is less evidence for ecological associations between Thaumarchaeota and corals, and the distributional dynamics of this group across different coral reef niches is previously unknown. Our results indicate that the relative importance of Thaumarchaeota is higher in the sandy substrate, lagoon-coral and reef crest-coral niches than within open water, indicating that this group may have greater ecological importance in coral reef ecosystems than the surrounding open waters.

Euryarchaeota were collectively the most dominant Archaea phylum across all metagenomes. Previously, Euryarchaeota have been shown to be present in warm shallow waters associated with coral reefs [23] and can comprise a significant proportion of both the coral and sponge-associated prokaryotic communities [10, 23, 83, 85]. Our results demonstrate for the first time the distribution of Euryarchaeota throughout a single reef ecosystem, where the only class which showed a homogeneous distribution across all niches was Halobacteria, with the remaining eight archaeal classes being found in different abundances throughout the niches. Notably, Archaeoglobi was the only class to be more abundant in the coral niches than the non-coral niches, and Methanopyri was more abundant in the reef crest niche than any other niche. Among the Crenarchaeota, which are often the dominant archaeal group in marine samples, Thermoprotei was the only class represented and accounted for only 0.03 % of sequences. This phylum includes members that have previously been shown to form associations with coral [82]. While our results have shown that the distribution and abundances of Crenarchaeota and Euryarchaeota throughout Heron Island Reef are not uniform, there is still much to be learnt about the roles that Archaea play on coral reefs.

We also observed an increase in sequences matching T4-like phages in the two coral-associated metagenomes. This is consistent with previous observations that the surfaces of corals are often highly enriched in viruses when compared to surrounding seawater [16, 86]. High microbial abundance and activity within the coral holobiont are expected to provide a host-rich environment for viruses relative to open water conditions, and it has been shown that this environment is occupied by a diverse range of viruses including Herpesviridae, Phycodnaviridae, phages and archaeal viruses [87].

Assignment of eukaryotic taxonomy in marine metagenomes is typically problematic due to the scarcity of relevant eukaryotic reference genomes. Our data, which revealed closest matches to organisms not expected to inhabit the coral holobiont, including *Brassicales* (grasses) and *Galliformes* (birds), are consistent with this. However, it is notable that significant increases in matches to *Scleractinia* (hard coral) sequences were observed in our coral-associated samples, which is supportive of our discrimination of sample types into coral- and non-coral-associated seawater niches.

Despite differences in the phylogenetic patterns between coral and non-coral seawater samples, it is notable that the composition of the microbial communities associated with the two coral species also exhibited some differences to each other. This is in accordance with previous work that has shown that different coral species harbour different microbial communities [11, 19, 21, 22]. Furthermore, previous work has shown that the microbial communities associated with diseased corals can differ substantially to the communities associated with healthy corals [28, 32, 76–78]. Although both samples were retrieved from above corals that appeared healthy, without any visible signs of tissue necrosis, colour loss or disease, there remains the possibility that the health status of the two sampled coral niches was different.

Function

The metagenomic data derived from this study provides new insights into the metabolic roles of microbes occupying different niches within a reef. Genes affiliated with core or house-keeping processes were the most abundant metabolic categories across all metagenomes. This is consistent with these general metabolic functions (such as carbohydrate, protein and amino acid metabolism) being essential for microbial survival [88–90] and is in line with the observations of other metagenomic studies in tropical marine environments [56]. Also in line with these previous studies, we found that substantial differences in less abundant, yet more specialised and dynamic metabolic processes also occurred between the four niches. For example, the two coral seawater metagenomes (lagoon-coral and reef crest-coral) had relatively higher abundances of genes associated with chemotaxis and motility, cell

signalling and regulation, and phages, prophages, transposable elements and plasmids, whereas genes associated with photosynthesis were more common in the open water niche. These findings illustrate that microbes inhabiting coral reefs have a general repertoire of core genes, but specific niches promote variability in the importance of more specific functional genes.

Genes relating to phages, prophages, transposable elements and plasmids were most abundant in the reef crest-coral metagenome and were consistently responsible for the greatest significant differences between niches. The increased occurrence of these genes in the reef crest seawater niche mirrors the increase in sequences affiliated with bacteriophages discussed above and lends support to the importance of viruses within the coral holobiont [87]. However, the other coral seawater metagenome, the lagoon-coral niche, had fewer genes associated with this category. This difference between the two coral seawater niches could be attributed to differences in the coral species sampled, differences in the bacterial communities associated with these different coral species or variability in the health of the corals [11, 19, 21, 22, 90].

Genes associated with bacterial motility and chemotaxis also differed significantly between metagenomes, primarily due to their elevated representation in the reef crest-coral niche. While oligotrophic bacteria like SAR11 and *Prochlorococcus* are not motile, many other marine bacterioplankton are highly motile [91–93] and the copiotrophic bacteria observed in elevated abundance in the reef crest-coral and lagoon-coral niches, including *Pseudomonas*, *Vibrio*, *Shewanella*, *Pseudoalteromonas* and *Alteromonas*, are all motile. It is likely that motility and chemotaxis are particularly important for microbial communities living close to biotic surfaces on coral reefs, where strong chemical gradients are associated with benthic organisms. The chemical products released from corals and algal exudates are often strong chemoattractants for motile marine bacteria [67, 93–95]. Coral mucus and the exudates of *Symbiodinium* are rich in several organic compounds including amino acids, sugars and DMSP [6, 63, 64, 68–70], which are known chemoattractants for marine bacteria [7, 53, 96], and have recently been shown to attract key coral pathogens [95]. Microscale gradients in these compounds in the microenvironment immediately adjacent to the coral surface may promote chemotactic migration of bacterioplankton cells to the coral holobiont [95].

Genes associated with regulation and cell signalling were also significantly more abundant in the reef crest-coral metagenome. Not only does coral mucus provide a nutrient-rich environment for microbes within otherwise oligotrophic surroundings, but it can also comprise chemical signals involved in the microbial communities' behaviour and function [97]. The high abundance of regulation and cell signalling genes in the coral seawater niches suggests that the microbial

community associated with corals engages in higher levels of cell–cell communication than what occurs in open water niches. Regulation and cell signalling are likely to be important functions on coral reefs, where bacteria may use signalling processes, including quorum sensing, to organise cellular functions to colonise host organisms including corals and sponges [97–100]. Chemical signalling also potentially allows bacteria to defend the holobiont from invading pathogens by altering behaviours such as swarming, biofilm formation and the production of anti-microbial compounds [97, 98, 100–104]. On the other hand, quorum sensing can regulate virulence in some bacteria [103], which could enable pathogens to more readily infect the host and outcompete beneficial microbes [100, 104]. The elevated occurrence of regulation and cell signalling genes in the coral seawater niches indicates that these processes, which will affect the interactions between microbes and the coral host, are particularly important in the coral holobiont.

Genes involved in photosynthesis were most abundant in the open water niche with a high proportion of photosynthetic microbes from the genera *Synechococcus* and *Prochlorococcus* underpinning this pattern. Consistent with our findings, Dinsdale et al. [18] observed shifts in the genes associated with photosystem I and II (PSI and PSII) between coral reef ecosystems. A higher abundance of genes associated with PSI was observed in the open water niche, while PSII-associated genes were found to be higher in the reef crest-coral niche. These differences likely reflect subtle changes in the structure of the phototrophic microbial community (e.g. shifts in relative importance of *Synechococcus* and *Prochlorococcus*) across the reef.

Conclusion

Microbial communities of dissimilar composition and metabolic function were found to occupy different niches within a single coral reef ecosystem. While there were some shared traits across all metagenomes, each niche was characterised by a specific microbial community likely shaped by different conditions, reef structure and occurrence of benthic host organisms. Specifically, the taxonomic shift from oligotrophic to copiotrophic bacteria from open water niches to coral seawater niches within a reef supports the assumption that benthic reef inhabitants such as corals provide a niche enriched in organic material. Similarly, an enrichment of genes associated with chemotaxis and motility, as well as regulation and cell signalling in coral seawater niches, indicates the importance of fine-scale chemical gradients emanating from the surfaces of corals in structuring the microbial community. This research has revealed the high level of heterogeneity in species composition and functional capacity of microbial assemblages

across a single coral reef ecosystem, highlighting the heterogeneous nature of microbial communities within coral reefs.

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