

Contact with turf algae alters the coral microbiome: contact versus systemic impacts

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Abstract Coral reefs are degrading to algae-dominated reefs worldwide, with alterations of coral microbiomes commonly co-occurring with reef demise. The severe thermal anomaly during the 2016 El Niño event in the South Pacific killed many corals and stressed others. We examined the microbiome of turf algae and of the coral *Porites* sp. in contact with turf during this thermal event to investigate algal turf effects on the coral microbiome during a period of environmental stress. The microbial composition of turf did not differ between coral-contacted and non-contacted turfs. However, microbiomes of corals in direct contact with turf were similar to those of the turf microbiome, but differed significantly from coral portions 5 cm from the point of turf/coral contact and from portions of the coral that looked most healthy, regardless of location. Although the majority of significant differences occurred in coral samples at the point of contact, a small subset of microbial taxa was enriched in coral tissues taken 5 cm from turf contact compared to all other sample types, including samples from areas of the coral that appeared most healthy. These results suggest that the coral microbiome is susceptible to colonization by microbes from turf,

but not vice versa. Results also suggest that algal contact elicits a subtle shift in the coral microbiome just beyond the contact site. The combination of turf microbiome stability and coral microbiome vulnerability at areas of contact may contribute to the continued decline in coral cover and increase in algal cover associated with coral–algae phase shifts.

Keywords *Porites* · Kiritimati · Bacteria · Thermal stress · El Niño

Introduction

Coral reefs provide critical ecosystem services such as protecting coasts from wave surge, producing food, and serving as reservoirs of useful natural products (Cesar 2000). The health and ecosystem services of coral reefs are declining rapidly as overfishing, pollution, and climate change contribute to declines in coral cover and increases in algal cover. This is concerning, as numerous studies indicate that algae can damage coral via abrasion, shading, smothering, formation of hypoxic zones, and allelopathy (Jompa and McCook 2003; Morrow et al. 2011; Rasher et al. 2011; Haas et al. 2013). Furthermore, a switch to algal dominance can produce feedbacks that retard coral recovery (Hughes et al. 2007; Dixson et al. 2014) and enhance algal persistence (Hoey and Bellwood 2011; Dell and Hay 2016), especially under elevated temperatures (Ritson-Williams et al. 2016). However, the mechanisms by which algae affect coral health are incompletely understood and potentially linked to other variables, including the coral-associated microbial community (microbiome).

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It is hypothesized that algae impact corals, in part, by altering the coral microbiome. Numerous recent studies confirm that the coral microbiome is dynamic, sensitive to stressors, and critical to coral health (Rohwer et al. 2002; Kellogg 2004; Bourne and Munn 2005; Koren and Rosenberg 2006; Pantos and Bythell 2006; Littman et al. 2009; Ainsworth et al. 2010). For example, coral-associated microbes, which are most abundant in the surface mucus layer (Ainsworth et al. 2015), are known to contribute to waste removal through nitrogen and sulfur cycling, production of antimicrobial compounds, and pathogen inhibition (Reshef et al. 2006; Ritchie 2006; Lesser et al. 2007; Wegley et al. 2007; Raina et al. 2009; Vega Thurber et al. 2009; Kimes et al. 2010). Disturbances in coral microbial assemblages may therefore compromise coral health.

Coral–algae interactions shift the composition of coral microbiomes in close proximity, or in direct contact, with algae (Vega Thurber et al. 2012; Morrow et al. 2013; Zaneveld et al. 2016). This effect may be due to transfer of algal metabolites or algae-derived microbes that inhibit mutualistic microbes (Lachnit et al. 2010; Persson et al. 2011), exert toxic or pathogenic effects on the coral (Nugues et al. 2004; Dinsdale et al. 2008), or both. Algal release of dissolved organic carbon (DOC) that fuels the growth of resident microbes is hypothesized to induce shifts in the coral microbiome (Smith et al. 2006), potentially affecting local oxygen levels or altering the relative abundances of interacting microbes, including pathogens or microbes that might inhibit pathogens. However, the mechanisms by which algae, corals, and microbes interact to affect coral health remain unclear, as is the extent to which these multi-partite interactions vary depending on the identities of the participants and the environmental context in which they interact.

Various types of algae may differentially affect coral microbiomes and coral health. For example, mat-like algal turf assemblages (“turf algae”) produce DOC at higher rates than other algae (Haas et al. 2011) and consist of complex mixtures of juvenile macroalgae, filamentous microalgae and cyanobacteria (Hester et al. 2016), and a diverse community of other bacteria (Walter et al. 2016). Turf assemblages are ubiquitous on tropical reefs, typically exhibiting rapid growth and providing an important source of primary production (Carpenter 1986) and potentially microorganisms for seeding other local microbiomes (e.g., corals). Additionally, algal turf may harbor coral pathogens (Nugues et al. 2004; Egan et al. 2013). These factors indicate a potential for turf algae to negatively impact corals (Walter et al. 2016), suggesting that characterizing microbial components of the coral–turf interaction may be critical to understanding coral–algae phase shifts.

The response of coral microbiomes to algal contact during periods of environmental stress, such as increased

temperature, is unknown but gaining in importance due to the increasing frequency of high temperature events and human disturbance on reefs (Hughes et al. 2003, 2017). Coral microbiomes tend to shift when corals are stressed, particularly when subject to warmer temperatures (Bourne et al. 2009; Vega Thurber et al. 2009). The coral may become more vulnerable to bacterial invasion as fewer bacteria that produce antimicrobials are present in the surface mucus layer of thermally stressed corals (Ritchie 2006; Rypien et al. 2010). In 2014–2016, a severe thermal anomaly (El Niño) coincided with expansive coral bleaching throughout the South Pacific (Hughes et al. 2017). The central Indo-Pacific atoll of Kiritimati (Republic of Kiribati) was particularly affected, with preliminary observations suggesting that less than 10% of Kiritimati corals survived the heating (J.K. Baum pers. comm.). We examined the effect of algal turf contact on the microbiome of the coral *Porites* sp. at Kiritimati during the 2016 thermal anomaly. We assessed microbiome composition in coral tissue in direct contact with turf algae, as well as in coral tissue and in algal turfs away from the contact point, thereby testing the extent to which microbiome responses are localized to the point of contact or systemic throughout the coral colony and associated algal turfs. Understanding coral–algae–microbe relationships, particularly under conditions of environmental stress, may help inform fundamental aspects of coral reef ecology as well as reef conservation and management.

Materials and methods

Collection

In late March 2016, when the Central Pacific was experiencing an anomaly 2–3 °C above normal temperature that persisted for several months (El Niño 2016; Fig. 1), samples were collected from a reef on the west side of Kiritimati Island, Republic of Kiribati (1°50′35.0″N, 157°32′13.9″W) at a depth of 10 m. At the time of sampling, virtually all common branching corals (e.g., *Pocillopora*, *Acropora*, branching *Porites*) were dead and overgrown by filamentous algae; surviving corals consisted almost entirely of massive *Porites* sp. and a few members of the Fungiidae family. Most massive *Porites* sp. also exhibited symptoms of stress (bleaching and partial bleaching), but some colonies did not show such signs and appeared healthy. Heat stress causes slower growth and calcification even in unbleached corals (Lough and Barnes 2000; Marshall and Clode 2004; Cooper et al. 2008; Cantin et al. 2010). Therefore, it seems likely that the unbleached corals that we sampled may also have been experiencing thermal stress, but were more resistant. To assess possible

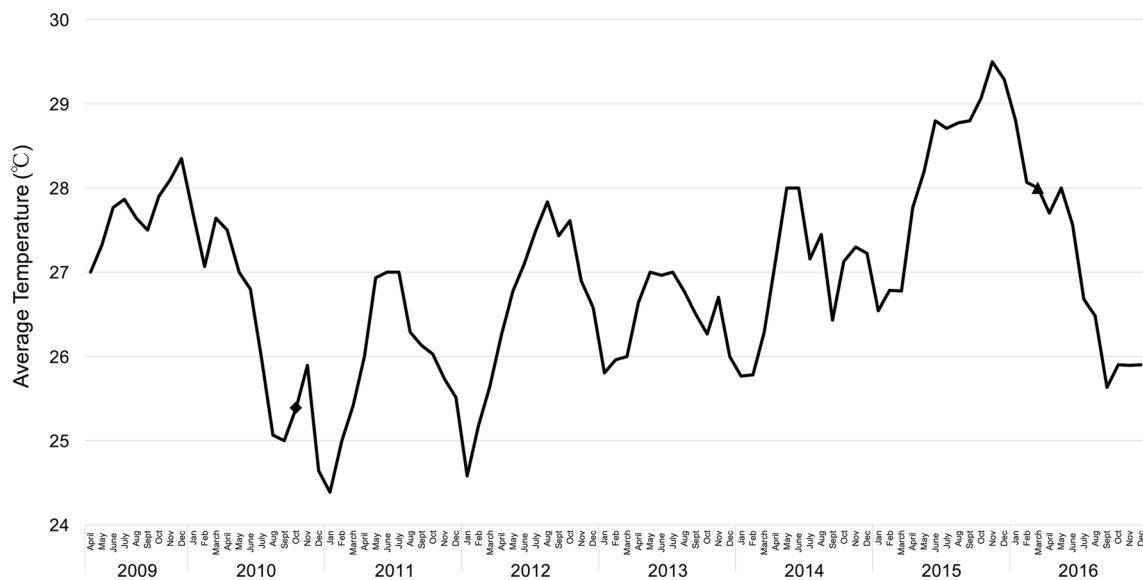


Fig. 1 Average monthly sea surface temperatures from 2009 (partial) to 2016 at Kiritimati. Data were acquired from <http://seatemperature.info>. Sampling time point for this study is indicated by ▲ and sampling time point for Hester et al. (2016) is indicated by ◆

impacts on corals, we performed an analysis of 30 photoquadrats haphazardly taken at the studied reef (50 cm × 50 cm images with 30 points overlaid automatically, photoQuad software; Trygonis and Sini 2012) in a stratified manner to force randomly located points into equal sized portions of the quadrat. We recorded cover of turf, turf overgrowing dead coral, live coral (categorized as bleached or not bleached), and sand. These few categories comprised virtually 100% of benthic cover. The mixed species assemblage of algal turf was 1–2 cm tall and composed of filamentous red algae, with low abundance of macroalgal germlings, filamentous green algae, and filamentous cyanobacteria (see Results).

To evaluate how the microbiome of massive *Porites* sp. may shift due to contact with algal turf, we used a chisel to collect samples of: (1) coral tissue contacting turf algae (~1 cm² of tissue, skeleton, and surface mucus layer; coral contacting turf = C-CT); (2) coral 5 cm beyond the point of contact (inward on the coral colony; = C-5cm); (3) coral further from any contacted edge but also appearing to be among the most healthy looking portion of the coral colony (coral—apparently healthy = C-AH); (4) turf contacting the same coral (turf contacting coral = T-CC); and (5) turf that was visually similar to the turf contacting the coral but free-living, not contacting or within 50 cm of any coral, but within about 50–60 cm of the coral we were sampling (turf—free-living = T-FL). We sampled at nine apparently healthy coral colonies and associated turf (nine blocks), taking the five types of samples from each coral. Each sampled colony was separated from other sampled colonies by ≥10 m. At the time of sampling, a portion of the edge of each sampled coral colony was in contact with algal turf;

the duration of contact prior to sampling is unknown. We sampled only colonies that were not bleached and appeared healthy apart from the coral tissue within 1–3 mm of turf contact. Each sample was placed in a separate sterile Whirl–Pak underwater, including as little water as possible. Two water samples from within the nine-block sampling area were taken from ~0.5 to 1 m above the benthos using two 60-mL syringes for each sample (i.e., 120 mL total volume of each water sample). On return to the surface, water samples were immediately filtered through a 0.2-μm Isopore membrane filter (Millipore), and water was drained from the bags containing the coral and algal samples. All samples (coral fragments, turf, and filters) were held on ice for a maximum of 4 h until returned to shore, placed in vials with RNA stabilizing buffer (25 mM sodium citrate, 10 mM EDTA, 5.3 M ammonium sulfate, pH 5.2), and immediately frozen.

To assess the composition of the turf algae, we placed five of the contact turf samples and five of the free-living turf samples into separate Petri dishes and evaluated five randomly selected fields of view from each of the ten samples under a dissecting scope. Percentage composition of identifiable groups in each field of view was estimated visually, and the five fields of view used to compute a mean for each sample.

DNA processing

We used Illumina sequencing of dual-indexed polymerase chain reaction (PCR) amplicons spanning the taxonomically informative V4 region of the 16S rRNA gene to quantify microbial community composition. Total DNA in coral,

algae, and water samples was extracted with the MoBio PowerSoil DNA isolation kit. Each coral or algal fragment, or filter, was placed directly into a PowerBead tube, and extracted according to the manufacturer's protocol. Amplicons were generated via PCR with V4 primers F515 and R806 (Caporaso et al. 2011), with both forward and reverse primers barcoded and appended with Illumina-specific adapters according to Kozich et al. (2013). PCR reactions included 22 μ L Platinum PCR SuperMix (Life Technologies), 2 μ L template DNA, 0.5 μ L each of forward and reverse primer (for a total concentration of 0.4 nM), and 1 μ L BSA (20 mg mL⁻¹; New England BioLabs Inc.). The thermal cycling protocol involved an initial denaturation at 94 °C (3 min), followed by 30 cycles of denaturation at 94 °C (45 s), primer annealing at 55 °C (45 s) primer extension at 72 °C (90 s), and a final extension at 72 °C (10 min). Amplicons were cleaned using Diffinity Rapid-Tips (Chiral Technologies, Inc), pooled in equimolar concentration, and sequenced on an Illumina MiSeq using a 500 cycle kit with 10% PhiX to increase read diversity.

Sequence data analysis

Barcoded sequence reads were demultiplexed and filtered for quality with Trim Galore!, with the criteria of a minimum length of 100 bp and Phred score ≥ 25 . Paired-end reads were then merged using FLASH (Magoč and Salzberg 2011) with the criteria of a minimum length of 250 bp per input read, minimum length of 300 bp for merged fragments, and maximum fragment standard deviation of 30 bp. Chimeric sequences were detected by reference-based searches using "uchime_ref" in USEARCH against the Greengenes rRNA database (DeSantis et al. 2006) and then removed. Sequence count per sample averaged 25,732 (range 536–123,851; SD: 25,963) after quality filtering.

Trimmed and merged sequences were processed through QIIME (Caporaso et al. 2010) to assess taxonomic composition. Sequences sharing 97% nucleotide identity were clustered into operational taxonomic units (OTUs) using the open-reference algorithm (Edgar 2010), with taxonomy assigned to OTUs by comparison to the Greengenes database (DeSantis et al. 2006). All OTUs identified as "chloroplast" at the class level or "other" at the phylum level were removed. Read counts per OTU were determined via rarefaction at a uniform sequence depth of 500 sequences. Using rarefied data, alpha diversity metrics (counts of observed OTUs, Chao1 richness estimates, and Shannon–Weiner diversity indices) were calculated using the R package phyloseq (McMurdie and Holmes 2013). The rarefied OTU table was then imported into PRIMER7 (PRIMER-E Ltd). OTU counts were square-root transformed and a Bray–Curtis dissimilarity matrix created. Water samples were removed from the matrix, and a two-

way crossed permutational analysis of variance (PERMANOVA) was conducted using Monte Carlo sampling to test for significant differences in community composition among sample types (C-AH, C-5cm, C-CT, T-CC, or T-FL) and individual coral/algae replicates (1–9). Any significant differences detected in the two-way crossed PERMANOVA were followed up with pairwise post hoc PERMANOVA tests (PRIMER-E Ltd). An analysis of variance (ANOSIM) was also conducted to obtain a metric of dissimilarity (*R* value) between sample types (Clarke 1993). Non-metric multi-dimensional scaling (NMDS) plots were created to visualize similarity (beta diversity) between sample types (including water samples), based on Bray–Curtis dissimilarity values. Additionally, dispersion among samples of a given sample type was calculated as the average distance from each sample to the centroid of the sample type cluster, with homogeneity of dispersions (among sample types) evaluated using the PERMDISP function in PRIMER7 with the PERMANOVA add-on package. A random forest classification (100 iterations) was conducted using the R package randomForest (Liaw and Wiener 2002) to identify significant predictors (i.e., OTUs) of sample type, with the accuracy of the prediction model cross-validated using a subset of samples (Knights et al. 2011). Finally, DESeq2 (Love et al. 2014) was used to detect microbial genera whose abundance varied significantly among sample types (McMurdie and Holmes 2013). Non-rarefied data (excluding data from water samples) were used for this analysis, as DESeq2 implements an internal normalization technique where the geometric mean of all OTUs is calculated individually across all sample types and the proportions of the calculated geometric means used to standardize sequencing depth.

Comparison to previously published data

To broadly examine the extent to which our 2016 results reflect a departure from past conditions, our sequence data were compared to those of Hester et al. (2016) describing *Porites* sp. and algal turf microbiome composition at six islands of the Line Island chain (including Kiritimati) in October 2010. As in 2016, the October 2010 collection followed an El Niño event, with water temperatures peaking (~ 28 °C) in January 2010 and declining thereafter (Fig. 1). However, temperatures at the time of sampling by Hester et al. (2016) were ~ 2.5 °C below those of our sampling in March 2016. The 2010 data therefore potentially reflect composition during coral recovery after a thermal anomaly. However, in contrast to our data, which were generated via Illumina sequencing of the V4 region of the 16S rRNA molecule, the Hester et al. sequences were produced on the Roche-454 platform and span the V1–V2 region. These data were obtained from MG-RAST

(accession mgm4663447.3), demultiplexed in QIIME, and all *Porites* sp. and turf samples processed using the pipeline described above, with rarefaction to 500 sequences. Owing to the differences in sample collection, primer selection, and sequencing methods, we did not perform analyses using taxonomic classifications below the class level, and OTU tables based on the 2010 and 2016 data were merged at this level. The merged OTU table was imported into PRIMER7 and an NMDS plot created using the parameters previously described.

Results

Filamentous red algae dominated the turf assemblage. Red algae in the genus *Ceramium* comprised 61% of the turf, with other filamentous red algae adding an additional 16%. The remaining assemblage was comprised of 12% red macroalgal germlings, 5% green algal filaments, 5% filamentous cyanobacteria, and 1% branching bryozoans. Benthic cover consisted of 82% algal turfs (of which 30% was clearly overgrowing dead corals), 10% boulder *Porites* sp. coral (of which 30% was bleached), 3% other corals (of which ~35% was bleached), and 5% sand. It should be noted that the majority of the massive *Porites* colonies showing bleaching during our sampling effort had recovered by the following year (K. Cobb, pers. comm.).

The richness and diversity of microbiomes varied significantly among sample types. Based on alpha diversity metrics of observed OTU richness, estimated OTU richness (Chao1), and Shannon–Weiner diversity, microbiomes from the apparently healthy portion of corals, coral tissue 5 cm from turf, and from seawater were ~50% less diverse than turf microbiomes, including both turf contacting coral and free-living turf (Fig. 2). The richness and Shannon–Weiner diversity of microbiomes from coral in contact with turf were between those of turf and of coral not in contact with turf (Fig. 2). These results are supported by PERMANOVA showing significant variation in OTU community composition between sample types (pseudo- $F = 7.141$, $p = 0.001$), but not between individual corals (pseudo- $F = 1.221$, $p = 0.054$; Table 1). Microbiomes from the two coral sample types not in direct contact with algal turf were similar in composition (ANOSIM: $R = 0.134$, $p = 0.06$; PERMANOVA: $t = 1.20$, $p = 0.203$; Table 1) and exhibited only slightly different dispersion (average distance from sample type centroid; PERMDISP: $t = 2.40$, $p = 0.032$; Table 1).

However, microbiomes associated with apparently healthy coral and coral 5 cm from the turf were significantly different from microbiomes of coral in contact with the turf (ANOSIM: $R = 0.615$ and 0.759 , $p = 0.001$; PERMANOVA: $t = 2.36$ and 2.42 , $p = 0.005$ and 0.004 ,

respectively) and also exhibited significantly different dispersions between the two sample types (PERMDISP: $t = 5.24$ and $t = 4.04$, $p = 0.002$ and $p = 0.002$, respectively), consistent with NMDS clustering patterns based on Bray–Curtis dissimilarity (Fig. 3). Microbiomes of corals contacting turf were instead more similar to microbiomes of turf in contact with coral (ANOSIM: $R = 0.267$, $p = 0.001$; PERMANOVA: $t = 1.77$, $p = 0.021$), clustering in the NMDS plot between turf samples and coral samples not in turf contact (Fig. 3). In contrast, turf microbiomes (whether free-living or in contact with coral) were similar to one another (ANOSIM: $R = 0.145$; PERMANOVA: $t = 1.43$, $p = 0.070$). This similarity is reflected in the NMDS plot, with turf microbiomes falling in a tight cluster (Fig. 3), and with significantly lower dispersion compared to coral samples (PERMDISP: $t = 0.65$, $p = 0.566$). The two water samples clustered apart from all turf and algal samples.

OTU composition was an effective predictor of sample type. In a random forest analysis of all coral and algal samples, sample type was correctly identified in 75% of predictions based on OTU abundance. All apparently healthy coral and coral 5 cm from turf were distinguishable from turf, whereas coral in contact with turf could be correctly classified only four of nine times, and was misclassified as turf in two of nine predictions (Table 2). This analysis identified the microbial genera whose abundance was predictive of sample type (Fig. 4). The majority (16) of the top 20 most predictive genera, particularly of the Rhodobacteraceae bacterial family, were more abundant in turf than in corals not in direct contact with turf (Fig. 4). However, four of the top 20 most predictive microbial groups—Pseudoalteromonadaceae (genus unknown), Pseudomonadaceae (genus unknown), *Sulfurimonas*, and *Dermacoccus*—were enriched in corals not in direct contact with turf. Interestingly, coral samples 5 cm from the turf were distinguished from all other samples, including apparently healthy coral samples, by enrichment of the predictive *Dermacoccus* genus and an unclassified Pseudomonadaceae genus. Notably, *Dermacoccus* was detected in eight of the nine coral samples 5 cm from the turf, but was absent from all apparently healthy coral samples, and present in only two of nine coral samples in contact with turf, one of nine free-living turf samples, and one of nine samples of turf in contact with coral (Fig. 4; Electronic supplementary material, ESM, Fig. S1).

DESeq2 analysis identified diverse genera whose abundance differed significantly among sample types (Table 1; ESM Table S1). These included the predictive genera also identified by random forest classification, as well as numerous other groups, with the number of significantly differing genera ranging from zero in the turf contacting coral versus free-living turf comparison to 196

Fig. 2 A comparison of alpha diversity among sample types. Each dot represents an individual sample. Metrics are total number of OTUs (a), Chao1 estimates of OTU richness (b, with standard error bars) and Shannon–Wiener diversity (c). Data have been rarefied to 500 sequences. C-AH apparently healthy coral, C-5cm coral 5 cm from turf, C-CT coral contacting turf, T-CC turf contacting coral, T-FL free-living turf

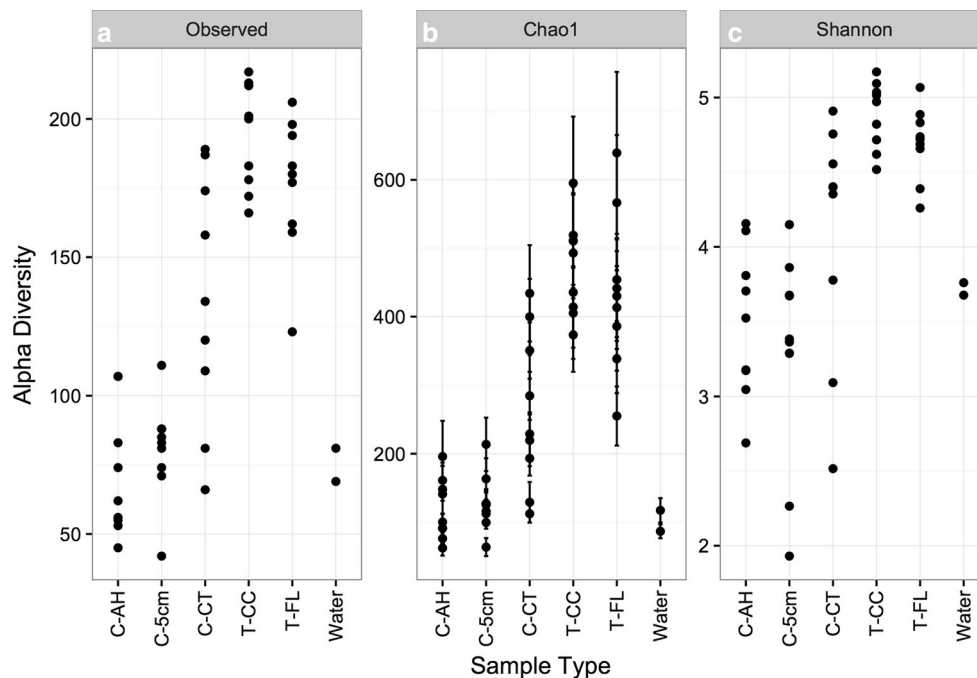


Table 1 Similarity tests between microbiomes

Groups ^a	PERMANOVA ^b		PERMDISP ^c		ANOSIM ^d		DESeq2 ^e Genera
	<i>t</i> value	<i>p</i> value	<i>t</i> value	<i>p</i> value	<i>R</i> value	<i>p</i> value	
C-AH versus C-5cm	1.200	0.203	2.402	0.032	0.134	0.060	9
C-AH versus C-CT	2.359	0.005	5.246	0.002	0.615	0.001	127
C-AH versus T-CC	3.334	0.001	18.87	0.001	0.710	0.001	174
C-AH versus T-FL	3.345	0.001	18.307	0.001	0.727	0.001	169
C-5cm versus C-CT	2.423	0.003	4.036	0.002	0.759	0.001	156
C-5cm versus T-CC	3.498	0.001	17.61	0.001	0.876	0.001	196
C-5cm versus T-FL	3.591	0.001	17.04	0.001	0.903	0.001	184
C-CT versus T-CC	1.770	0.021	5.651	0.001	0.267	0.001	14
C-CT versus T-FL	1.908	0.009	5.918	0.001	0.318	0.001	30
T-CC versus T-FL	1.429	0.07	0.6452	0.566	0.145	0.017	0

^a C-AH apparently healthy coral, C-5cm coral 5 cm from turf, C-CT coral contacting turf, T-CC turf contacting coral, T-FL free-living turf

^b Pairwise tests of a two-way crossed PERMANOVA comparing all treatment groups. Significant differences were detected between sample type (Pseudo-*F* = 7.141, *p* = 0.001), but not individual corals (Pseudo-*F* = 1.221, *p* = 0.054)

^c Pairwise PERMDISP results assessing dispersion by calculating averaged distance from the sample type centroid (*F* = 91.47 and *p* = 0.001)

^d Results of a one-way ANOSIM (*R* = 0.468 and *p* = 0.001)

^e DESeq2 results showing the number of significantly different genera detected between treatment groups. Full results in ESM Table S1

in the coral 5 cm from turf versus turf in contact with coral comparison. Only 14 genera differed in abundance between coral contacting turf and turf contacting coral microbiomes. This number jumped to 127 and 156 when coral contacting turf microbiomes were compared to the apparently healthy coral and coral 5 cm from turf microbiomes, respectively, confirming a significant effect of direct algal

contact on coral microbiome composition, with this impact largely confined to the area of direct contact.

Comparison with data from Hester et al. (2016) revealed broad differences between 2010 and 2016 microbiomes, with samples clustering according to both sample type (coral vs. turf) and study (2010 vs. 2016; Fig. 5). For example, while the relative abundances of Alpha- and

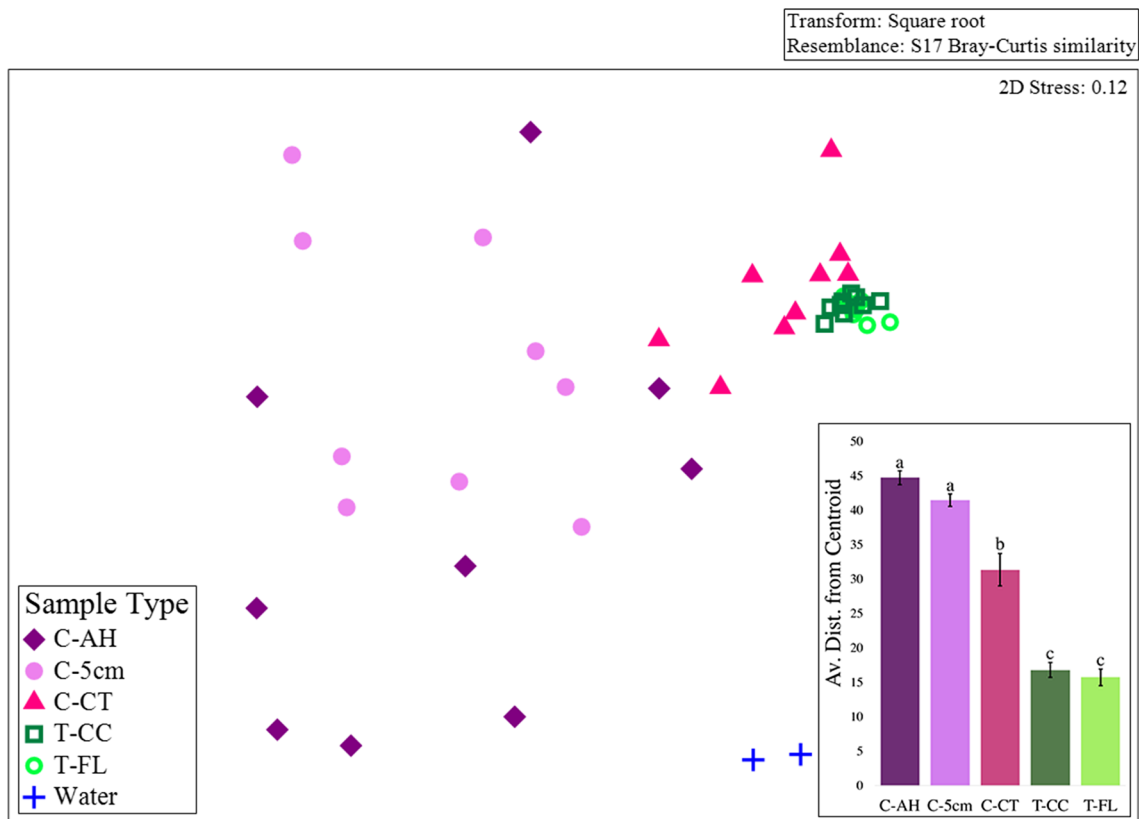


Fig. 3 Community taxonomic relatedness among sample types. Clustering reflects a non-metric multi-dimensional (NMDS) analysis of all sample types based on Bray–Curtis dissimilarities. *C-AH* apparently healthy coral, *C-5cm* coral 5 cm from turf, *C-CT* coral

contacting turf, *T-CC* turf contacting coral, *T-FL* free-living turf. Inset demonstrates the average distance from each sample type centroid, with letters above standard deviation bars indicating significant differences ($p \leq 0.01$)

Table 2 Errors^a in predicted treatment group classification^b determined by random forest analysis

Actual classification	Predicted classification					Classification error (%)
	C-AH	C-5cm	C-CT	T-CC	T-FL	
C-AH	9	0	0	0	0	0
C-5cm	1	8	0	0	0	11
C-CT	0	2	5	2	0	44
T-CC	0	0	2	4	3	56
T-FL	0	0	0	1	8	11

^a Overall bagging error rate was 25%, meaning sample type was correctly identified 75% of the time based on OTU abundance. OTUs that are the strongest predictors of sample type are in Fig. 4

^b *C-AH* apparently healthy coral, *C-5cm* coral 5 cm from turf, *C-CT* coral contacting turf, *T-CC* turf contacting coral, *T-FL* free-living turf

Gammaproteobacteria (in both coral and turf microbiomes) were comparable between studies, Betaproteobacteria were notably enriched in 2010 coral microbiomes (Fig. 6), particularly at Fanning Atoll. However, the bacterial genus *Endozoicomonas*, which has been proposed as an indicator of coral health (Neave et al. 2017), was at low abundance in both datasets, comprising less than 1% of total sequences in 88% (29 of 33) and 89% (24 of 27) of 2010 and 2016 *Porites* samples, respectively.

Discussion

Contact with algal turf significantly alters the microbiota of *Porites* sp., whereas the turf microbiome remains largely unaffected by coral contact. Microbiomes of the apparently healthy portions of corals and of coral portions 5 cm from algal contact were compositionally distinct from those of coral portions contacting turf and of turf (Figs. 3, 4; Table 1). These groupings also differed substantially in

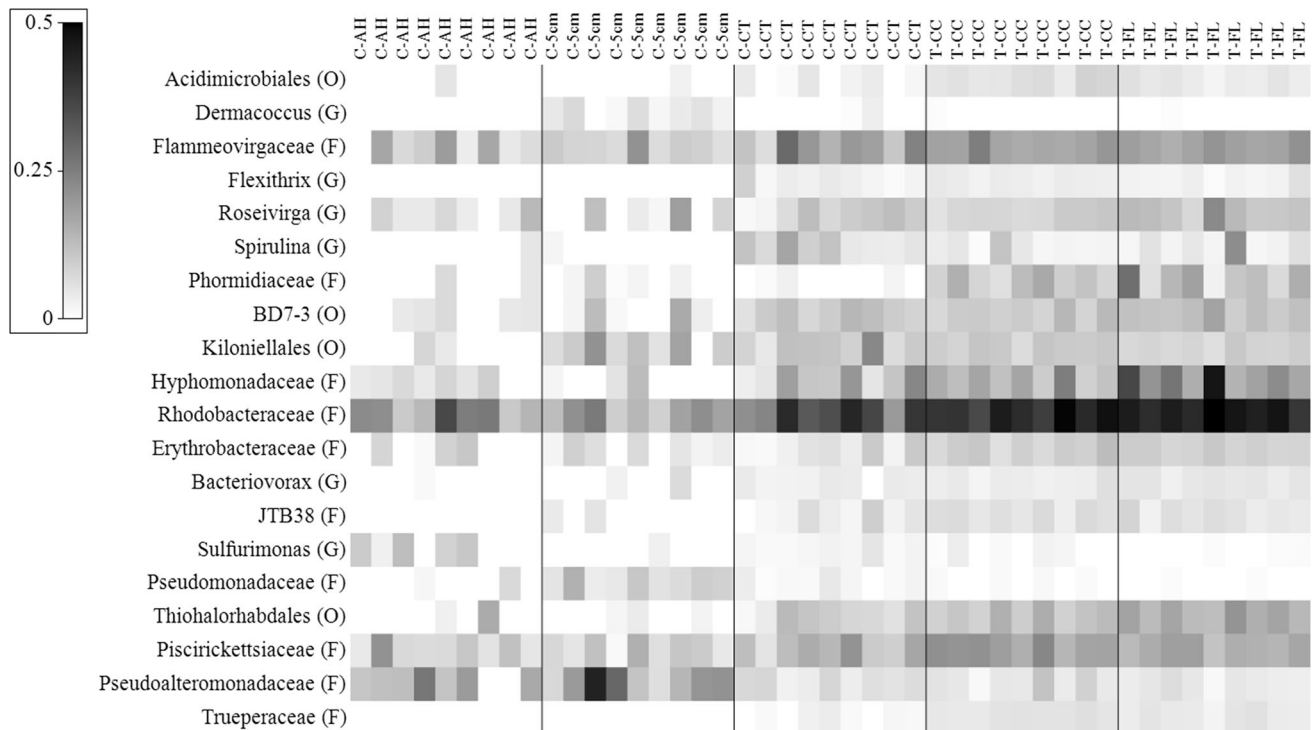


Fig. 4 Twenty microbial genera most predictive of sample type. Genera were identified based on the mean decrease in Gini coefficient in the random forest classifier. Relative abundances have been square-root transformed for plotting. Predictive genera (y axis) that were not

identifiable at the genus level (G) are named to the nearest identified family (F) or order (O). *C-AH* apparently healthy coral, *C-5cm* coral 5 cm from turf, *C-CT* coral contacting turf, *T-CC* turf contacting coral, *T-FL* free-living turf

OTU diversity, with non-contact coral portions being less diverse than coral in contact with turf (Fig. 2). At the point of turf contact, the coral microbiome becomes more like that of turf, with increased diversity (Fig. 2) and similarity in OTU composition (C-CT vs. T-CC comparison; Tables 1, 2), indicating “sharing” of microbes between the contact communities. The similarity between areas of the coral not contacted by turf and the difference between these coral portions and those in contact with turf suggests that sharing primarily involves transfer of turf microbes to the coral, and not vice versa, and that this occurs on a spatial scale of <5 cm. This is validated by a nine-fold increase in the number of significantly varying genera between the apparently healthy coral and coral contacting turf (127 genera) relative to the coral in contact with turf versus turf in contact with coral (14 genera), as well as by the random forest classification, showing that the vast majority of predictive genera were less abundant in apparently healthy portions of the coral, relatively more abundant in areas of turf contact, and highly abundant in the turf microbiomes. Together, these results indicate that *Porites* sp. at the time of sampling was highly vulnerable to invasion from turf-associated microbes, and that invasion was restricted to points of turf contact.

Notably, bacteria of the Rhodobacteraceae family were highly abundant in algal turf and in coral microbiomes in

contact with turf, but less abundant in coral sections not contacting turf (Fig. 4). This ubiquitous bacterial family contains metabolically diverse members whose roles in coral health remain unclear. However, rhodobacters have been associated with coral disease and bleaching (Pantos and Bythell 2006; Mouchka et al. 2010; Gray et al. 2013; Roder et al. 2014) and may include coral pathogens (Pratte and Richardson 2016). The high abundances of this group in turf-contacted coral sections, presumably via transfer of microbes from the turf, may indicate a decline in coral health at the point of contact.

Contact with turf may also elicit some changes in coral microbiomes away from the contact site. Although coral microbiome shifts were most pronounced at the contact site (i.e., C-CT vs. C-5cm or C-AH samples), a small number of microbial taxa (nine) varied significantly in representation between the apparently healthy parts of the corals and coral tissue 5 cm from turf contact (C-AH vs. C-5cm). These included unclassified genera of the Pseudomonadaceae and Alteromonadaceae (ESM Table S1; DESeq2 analysis). These ubiquitous bacterial families have been recovered in both healthy and diseased corals (Raina et al. 2009; Sunagawa et al. 2009) and are typically associated with copiotrophic lifestyles, suggesting that they may be among the first responders to changes in the local chemical environment, potentially involving shifts in organic

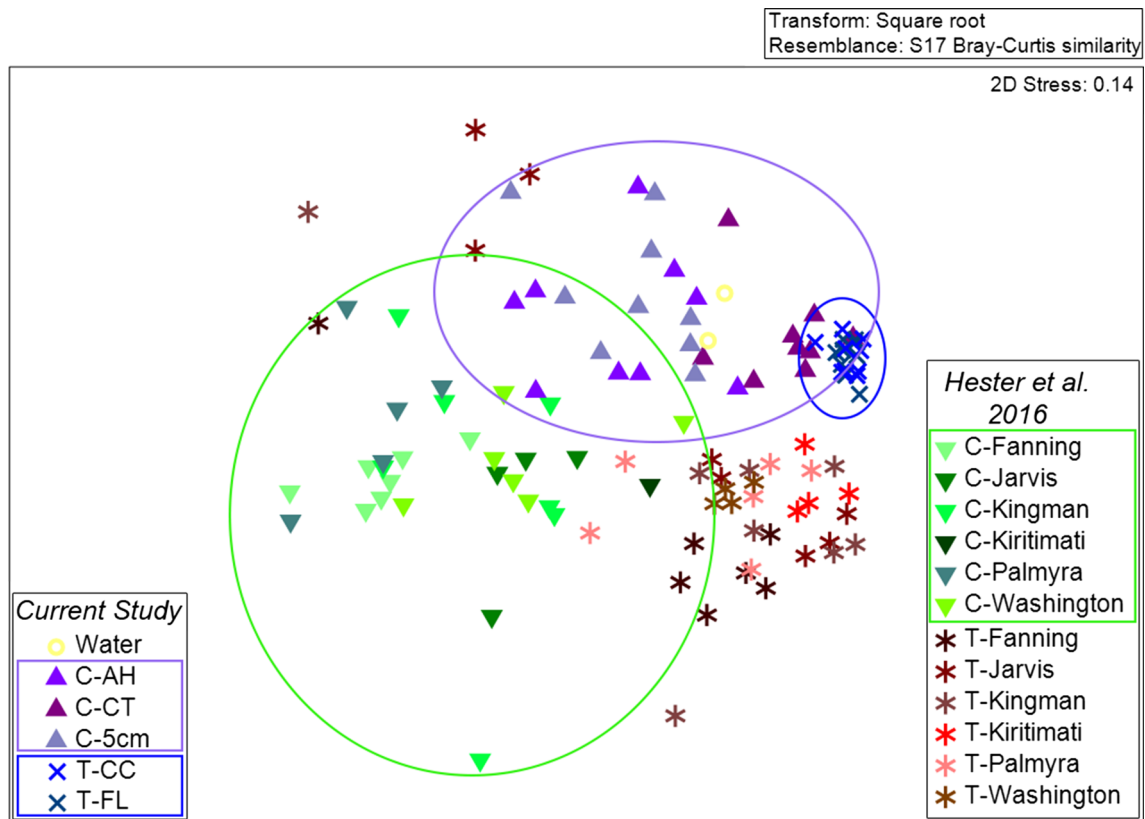


Fig. 5 Microbiome community relatedness across years (2010, 2016) and sample type based on non-metric multi-dimensional (NMDS) analysis of microbiomes associated with *Porites* sp. coral (C) and algal turf (T) from the current study (triangle and cross symbols, respectively; March 2016 samples) and previously published data

from Hester et al. (2016) (inverted triangle and asterisk symbols, respectively; October 2010 samples). NMDS is based on Bray–Curtis dissimilarities. *C-AH* apparently healthy coral, *C-5cm* coral 5 cm from turf, *C-CT* coral contacting turf, *T-CC* turf contacting coral, *T-FL* free-living turf

substrate availability. The exact functional contributions of these taxa to the holobiont remain to be determined, but may vary among species and in response to host health state and environmental conditions.

The genus *Dermaococcus*, a member of the widespread gram-positive Actinobacteria phylum, was also enriched in coral portions 5 cm from turf contact. *Dermaococcus* has been detected previously in stony corals (Yang et al. 2013) and was one of several actinobacterial genera recently isolated from thermally stressed corals in the Arabian Gulf (Mahmoud and Kalendar 2016). In our study, proportional abundances of this genus differed significantly between coral 5 cm from turf contact versus coral in contact with turf, as well as turf samples (DESeq2; ESM Table S1), with *Dermaococcus* detected in nearly all (eight of nine) samples of coral 5 cm from turf, but only in a minority of samples of coral in contact with turf (two of nine) and turf samples (one of nine, of both turf types; Fig. 4). Furthermore, *Dermaococcus* was not detected in any of the apparently healthy coral samples, although DESeq2 did not identify this genus as differing significantly between coral 5 cm from turf contact versus apparently healthy coral samples

(ESM Table S1), likely because a zero sum (i.e., *Dermaococcus* was not detected) across all healthy coral replicates prevented accurate estimation of fold changes between sample types (ESM Fig. S1).

The enrichment of specific microbial members (e.g., Pseudomonadaceae, *Dermaococcus*) in coral microbiomes 5 cm from turf relative to healthy coral samples suggests a potential impact on microbiome composition near, but beyond, the point of turf contact. Prior studies have demonstrated spatial variation in coral microbiome composition (Rohwer et al. 2002; Daniels et al. 2011), and that direct algal contact is necessary for substantial restructuring of the *Porites* microbiota (Morrow et al. 2013). Our analyses indicate that direct contact is necessary to cause substantial changes in the coral microbiome in response to turf, but also that contact may induce subtle, non-random shifts in microbial structure that occur a few centimeters from the contact site. The observed shifts in structure away from the contact point may elicit relatively minor effects on microbiome function, as apparently healthy coral samples and those 5 cm from turf are similar at the community level. This prediction requires testing, particularly as the

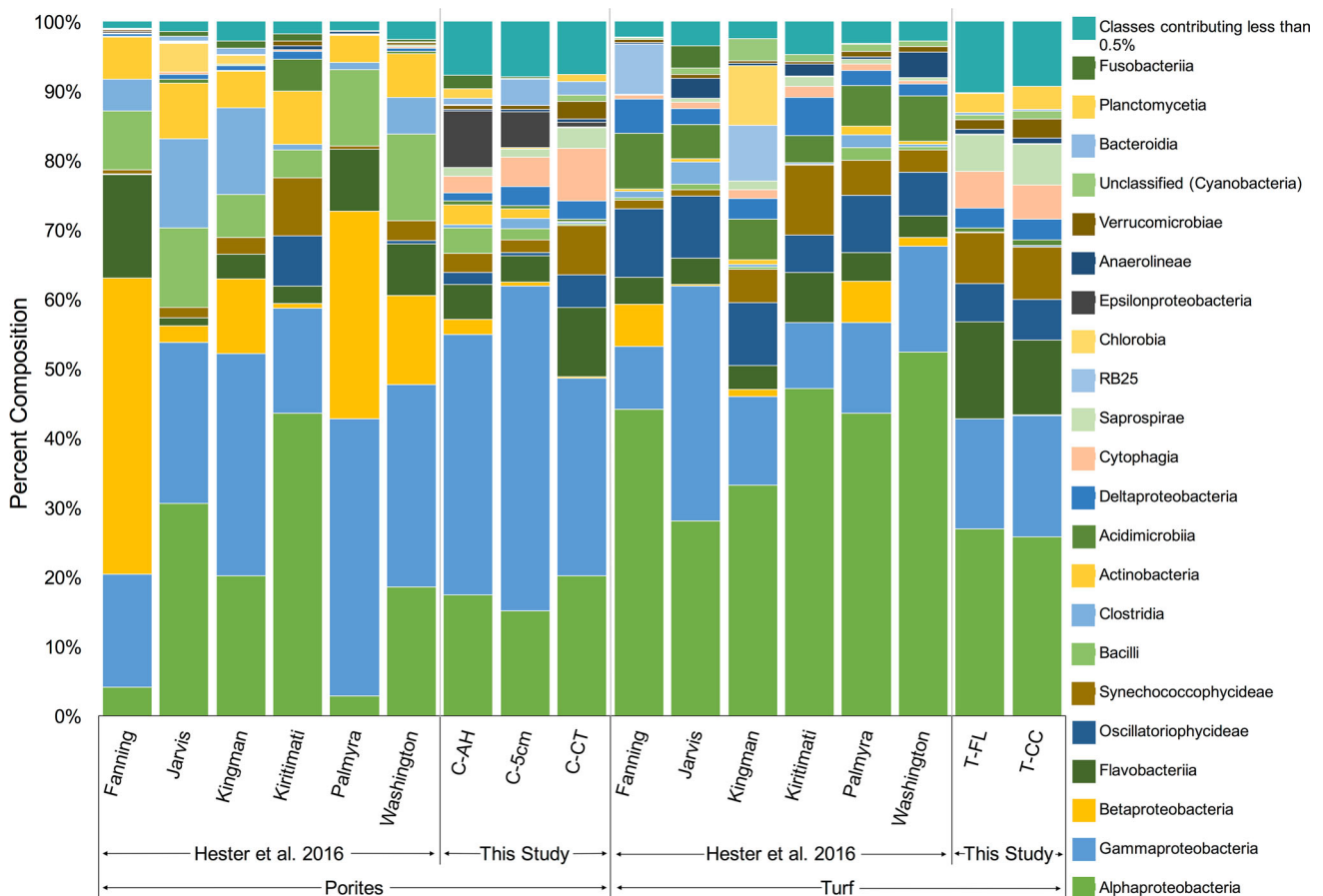


Fig. 6 Community composition (class) across years (2010, 2016) and sample type. Data reflect microbiomes associated with *Porites* sp. coral and algal turf collected in March 2016 for the current study (C-AH apparently healthy coral, C-5cm coral 5 cm from turf, C-CT coral

contacting turf, T-CC turf contacting coral, and T-FL free-living turf) and in October 2010 from six locations in the Line Islands (Hester et al. 2016). Replicates for each sample type were averaged

ecological function of the vast majority of coral-associated microbes remains uncharacterized.

Our data agree broadly with those of prior studies. Our Shannon–Weiner diversity values for apparently healthy *Porites* and algal turf microbiomes are similar to those reported in Barott et al. (2011) and Hester et al. (2016). Also consistent with our findings, Vega Thurber et al. (2012) reported that coral in contact with algae had higher microbiome richness (Chao1) compared to healthy corals. Similar to our results, Hester et al. (2016) reported that beta diversity (measured by distance to sample type centroid) was higher in *Porites* corals than in algal turf. Here, we also found that contact with algae decreased beta diversity of coral microbiomes, consistent with these microbiomes becoming more similar to those of turf following contact (Fig. 3). This result agrees with that of Zaneveld et al. (2016), who also observed a decrease in within-group beta diversity for corals in contact with algae in the genera *Halimeda* and *Amphiroa*. However, Zaneveld et al. (2016) also showed an increase in coral microbiome beta diversity

upon contact with the brown alga *Dictyota*, demonstrating that effects of algal contact on coral microbiomes vary with algal type.

The mechanisms by which algal contact reshapes coral microbiomes may include release of algal DOC that stimulates growth of specific bacteria, allelopathy from compounds produced by the algae, the creation of zones of hypoxia, and abrasion (Kline et al. 2006; Smith et al. 2006; Rasher et al. 2011; Morrow et al. 2011; Vega Thurber et al. 2012). Here, the large change in microbial composition in coral portions in immediate contact with algae and the relatively minor effect only 5 cm away suggest that critical aspects of algae–microbiome–coral interactions were restricted to millimeter- to centimeter-sized spatial scales at the point of contact between algal turf and coral. This is consistent with the notion of contact-mediated transfer of allelopathic metabolites from algal surfaces (Rasher et al. 2011; Andras et al. 2012; Longo and Hay 2017) or with release of water-soluble metabolites (DOC) that affect coral microbiomes at only very small scales because

compounds are rapidly transported away (Jorissen et al. 2016) or because algal-associated microbes consume almost all DOC at the point of release (Haas et al. 2013), limiting the spatial scale over which DOC may influence the coral microbiome. Thick algal turf dominated our sampling site in 2016 (82% cover), but we did not detect evidence that this high turf cover affected the coral's microbiome at a reef scale, as might be expected given microbiome differences between coral- versus algae-dominated reefs (Barott and Rohwer 2012). Although further work is needed to test hypotheses about how diverse algae affect coral, this study confirms the potential for microbial transfer from algal turf to corals upon contact.

It remains uncertain how other stressors such as increased water temperature affect how coral microbiomes respond in situ to algal contact. Comparisons to existing data offer preliminary insight. The *Porites* sp. and algal turf microbiomes described by Hester et al. (2016), collected from the Line Islands in 2010 at a time of reduced water temperatures following a previous period of thermal stress only broadly resembled the 2016 coral and turf microbiomes analyzed here, with the 2010 and 2016 datasets of the same sample type clearly separated based on bacterial class composition (Figs. 5, 6). This separation is likely affected by a range of between-study differences, including differences in sequencing method, collection site, year and month of collection, and level of thermal stress. Nonetheless, we also observed similarities between studies. Notably, the proportional representation of Alphaproteobacteria was comparable between years (15.4 and 17.7% in 2010 and 2016, respectively) and similar to levels reported previously for stressed or diseased corals (Sunagawa et al. 2009; Cardenas et al. 2012). Both 2010 and 2016 datasets also exhibited low relative abundances (average of 0.48% for all coral samples) of Endozoicimonaceae, a bacterial family that is often observed at higher abundances in healthy corals (Klaus et al. 2005; Vega Thurber et al. 2012; Bayer et al. 2013; Meyer et al. 2014; Neave et al. 2017). However, many bacterial groups commonly observed in coral microbiomes exhibit substantial variation among host individuals, species, and studies. Furthermore, *Porites* microbiome composition prior to the 2010 and 2016 thermal anomalies is unknown, preventing strong conclusions regarding the effect of environmental stress on microbiome structure in the Line Islands. Nonetheless, these results suggest that despite methodological differences between studies (see Methods), as well as likely biological differences between 2010 and 2016, both studies may have captured coral microbiomes in a state of coral stress. Despite these potential signatures of stress and a relatively high turf cover in both years, coral microbiomes remained clearly distinguishable from algal microbiomes. *Porites* and their associated microbiomes, though potentially under

stress in 2010 and in 2016, appear somewhat resistant to the dual threats of high temperature and high algal cover, at least at non-contact portions of the coral colony. At the point of algal contact, however, *Porites* experiences microbiome restructuring. It remains unclear how such contact-dependent restructuring, as well as minor shifts in microbiome composition away from the contact site, may affect overall dynamics and coral stability during coral–algae phase shifts.

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