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REPORT

Algae dictate multiple stressor effects on coral microbiomes

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Abstract Most studies of stressors focus on the response of traditionally classified organisms via effects on growth, mortality or physiology; however, most species have microbial associates that may mediate the response of a host to the stressor. Additionally, species rarely experience one stressor alone, but instead are influenced by multiple, potentially interacting stressors. We evaluated how coral microbiomes responded to two biotic stressors: the vermetid gastropod, *Ceraesignum maximum*, and algal turfs, both of which have previously been shown to decrease coral growth, survival and photophysiology. We collected coral mucus from massive *Porites* colonies in the presence versus absence of both algae and vermetids and sequenced the 16S rRNA gene to characterize the coral surface microbial communities. The presence of algae increased the alpha diversity of the coral microbial community, likely by increasing the relative abundance of rare members of the community. Algae also reduced beta diversity, which we hypothesized was due to algae homogenizing the physical environment. In contrast, vermetids had only small effects on microbial communities, even though vermetids have deleterious effects on coral growth. We previously hypothesized that vermetids would exacerbate algal effects on microbes, but we failed to detect an interaction between vermetids and algae on the coral's microbiome, except for one family, Fusobacteriaceae, which was most abundant in the presence of both stressors. We suggest that algae can affect corals through their effects on microbes, whereas vermetids primarily affect the host directly; these complementary effects may limit the extent to which stressors can interact.

Keywords Vermetid · Algal turf · Microbiome · Coral–algal interactions · Stressors

Introduction

Multiple stressors generally negatively affect individuals and populations in ecological systems. These stressors can be abiotic (e.g., extreme temperatures) as well as biotic (e.g., disease or predation). Although most studies focus on the effects of single stressors, stressors rarely occur in isolation. More frequently, species experience multiple stressors concurrently (Lenihan 1999; Boone et al. 2007; Buck et al. 2011). When stressors combine, their effects can be additive (in which the effects of the stressors are the sum of their effects alone), antagonistic or synergistic (in which effects are smaller or larger, respectively, than the effects predicted under the assumption of additivity; Crain et al. 2008; Darling and Côté 2008). For example, pesticides and predators sometimes combine synergistically, increasing the mortality of tadpoles more than expected based on their individual effects (Relyea and Mills 2001).

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Effects of stressors on individuals are often measured by differences in physiology, growth or survival. However, nearly all organisms are closely associated with microbial communities (Wahl et al. 2012), which also respond to environmental stressors. Studies of microbial responses to stressors may shed insight into effects observed in the host organism, or may provide early indicators of future effects on the host. As a result, studies of holobionts (the microbial communities plus host tissues, Mindell 1992; Rohwer et al. 2001) are beginning to explore how stressors affect host-associated microbial communities.

Host-associated microbial communities respond to changes in the environment and the state of the host. For example, increases in temperature can lead to changes in the diversity (i.e., the number of unique groups present) of the microbiome of sessile species (Vega Thurber et al. 2009), and changes in diet and nutrient regimes can lead to compositional shifts in the human gut microbiome (Turnbaugh et al. 2009). Often, however, microbiome studies have only looked at single stressors and we do not understand how combinations of stressors can influence microbial communities, especially in critical ecosystems that experience a multitude of stressors like coral reefs.

Coral reefs are highly diverse ecosystems that experience local and global stressors, including increased temperature, nutrient pollution and disease. These stressors affect the health, survival and growth of corals, and many effects are likely mediated through effects on the coral's microbial communities. Corals are holobionts that are composed of host tissue and a diverse microbial community (Rohwer et al. 2001). Here, we focused on the bacterial members of the microbial communities. Coral bacterial communities can be found in coral tissues (Sweet et al. 2010; Davy et al. 2012), in their gastrovascular canal (Sweet et al. 2010; Davy et al. 2012), and on their surface mucus layer (Rohwer et al. 2001; Sweet et al. 2010; Davy et al. 2012). Although not well described, these bacterial (hereafter microbial) communities are associated with nutrient cycling (Davy et al. 2012) and defense (Davy et al. 2012; Reshef et al. 2006; Peixoto et al. 2017). Indeed, the surface mucus layer of corals is considered their first line of defense, as it is the first area of a coral that comes in contact with the environment (Shnit-Orland and Kushmaro 2009).

Single stressors, like increased temperature, increased DOC and reduced pH, often cause changes in coral surface microbial communities (e.g., by increasing alpha diversity, increasing beta diversity and altering the taxonomic composition: Vega Thurber et al. 2009; McDevitt-Irwin et al. 2017). Although most studies have focused on effects of single stressors, recent studies have examined how abiotic stressors (increased temperatures and ocean acidification) in combination with biotic stressors (increased nutrients and predation) can change coral microbial communities (Webster et al. 2016; Shaver et al. 2017). However, few studies explore the potential effects of multiple *biotic* stressors on coral microbial communities.

Macroalgae (including turf algae, hereafter referred to as algae) decrease coral growth (Jompa and McCook 2003), increase coral bleaching (Rasher and Hay 2010), and decrease coral survival (Kuffner et al. 2006). Furthermore, when algae are in close proximity to coral, they can alter the coral's microbiome (Barott et al. 2011, 2012). Algal presence increases microbial alpha diversity (Vega Thurber et al. 2012) and drives compositional changes, including increased relative abundance of heterotrophic bacterial groups and groups that are implicated in causing coral diseases (Nugues et al. 2004; Vega Thurber et al. 2012; Sweet et al. 2013). Algae also can lead to increased beta diversity, i.e., increased variation in microbial composition among corals (Zaneveld et al. 2016).

One of the mechanisms underlying effects of algae on coral involves algal-derived photosynthate (dissolved organic carbon, DOC), which fuels microbial growth and leads to hypoxic conditions on coral surfaces via increased microbial respiration, which results in coral death (Kline et al. 2006; Smith et al. 2006; Barott et al. 2011). Filamentous algal turfs are especially notorious for their high production of DOC and resulting effects on microbial communities (Nelson et al. 2013). This hypothesized mechanism will be most important when water flow is low and diffusive boundary layers (regions of molecular transport) are thick enough to create conditions that would allow for the retention of DOC or low O_2 (Wangpraseurt et al. 2012; Brown and Carpenter 2013; Haas et al. 2013a, b; Jorissen et al. 2016).

Vermetid gastropods, especially the largest species, *Ceraesignum maximum*, also have deleterious effects on corals, decreasing their growth (Shima et al. 2010, 2013), survival (Shima et al. 2010), and photosynthetic yield (Shima et al. 2015). Vermetids are sessile gastropods that feed by casting a mucus net that covers the nearby benthos and collects particles from the water column. The putative mechanism underlying the effects of vermetids on corals likely involves their mucus net, although the exact mechanism is unknown. The effects of vermetids on microbes have not previously been explored.

The nets of vermetids reduce water flow and increase the boundary layers around corals (Brown and Osenberg 2018), which is comparable to some of the effects that underlie algal effects on coral (Brown and Carpenter 2013, 2015). Furthermore, the effects of vermetids on water flow are more pronounced when algae are present (Brown and Osenberg 2018). As a result, we expected that: (1) vermetids and algae would cause similar changes in coral microbial communities; and (2) the combined

presence of vermetids and algae would lead to synergistic changes in coral microbiomes. Specifically, we quantified how algae and vermetids affected coral microbial communities via changes in alpha diversity (rarefied and Chao1 richness measures and Shannon Diversity), beta diversity (i.e., within-group variance) and composition (e.g., via increases in heterotrophic groups, including potential pathogens like *Vibrio*).

Methods

System

Samples were collected from the shallow back reef environment of the north shore of Mo'orea, French Polynesia (S17° 28.466′ W149° 47.313′) on July 31, 2014, during the austral winter. *Ceraesignum maximum* often occur on reefs created by massive *Porites* corals, where the vermetids can reach high abundances (up to 30 snails m^{-2}, Shima et al. 2010, but see Brown et al. 2016). Massive *Porites* is a species complex of visually indistinguishable mounding corals that are generally 1–2 m in height. The taxonomy of the massive *Porites* species complex is ambiguous (Forsman et al. 2009, 2015), but has been previously assigned to *Porites lobata*, *Porites lutea* and *Porites austrialiensis*. However, recent work suggests that *Porites* colonized by vermetids are associated with one clade (Brown 2018). Vermetids can only settle to areas that have been previously disturbed, as they cannot settle to living coral (Phillips et al. 2014). Thus, vermetids are frequently located on reefs that are a mosaic of living coral and other benthic substrates, including algal turfs. Algal turf is a filamentous functional group composed of a multi-species assemblage (Steneck and Dethier 1994). Algal turf is frequently in contact with massive *Porites* corals in the back reef of Mo'orea (Brown and Carpenter 2015). As a result, vermetid nets often cover interactions between massive *Porites* and algal turf.

Sampling

We sampled coral mucus from 10 massive *Porites* bommies (small reefs), all of which also had the vermetid, *C. maximum*, as well as algal turf. Previous work has shown that effects of algae on the coral microbiome dissipate at distances more than 5 cm away from the coral–algal interface (Barott et al. 2011; but see Pratte et al. 2017). Other studies have indicated that vermetid nets do not extend more than 20 cm from the vermetid's aperture (Allen-Jacobson 2018). On each reef, we therefore sampled coral mucus from four locations that were close to and far from algae and/or vermetids. We did this by placing two 5 × 5 cm quadrats around the interface between living *Porites* and the algal turf. One quadrat was placed near a *C. maximum* (i.e., +Vermetid treatments), and the other was placed in a location in which the nearest vermetid was ≥ 20 cm away (i.e., −Vermetid treatment). A 10-mL needleless syringe was then used to collect coral mucus from locations within each quadrat: (1) within 2 cm of the coral–algal interface and (2) 5 cm away from the interface (Fig. 1). Thus, our design yielded four treatments (−Algae, −Vermetid; +Algae, −Vermetid; −Algae, +Vermetid; and +Algae, +Vermetid: Fig. 1), which we analyzed as a crossed design (algal presence/absence crossed with vermetid presence/absence). Because each treatment was obtained from each coral colony, we were able to control for variation among coral colonies (e.g., due to genetic differences) to better isolate the effects of algae and vermetids, i.e., coral colony was treated as a blocking term or random effect in subsequent analyses.

In addition to the coral mucus samples, we also collected water samples (n = 8) and sediment samples (n = 4) from the general area of the reefs we sampled for coral microbiomes. Water was collected with a 10-mL syringe in midwater at haphazard locations along the reef. Sediments were collected haphazardly in Whirlpaks©.

Samples were transported on ice to the laboratory. Mucus was allowed to settle to the bottom of the syringe and then ejected into a 1.5-mL microcentrifuge tube and spun down to a pellet in a centrifuge at 10,000 *g* (©Eppendorf 5418 R), for 5 min, and the supernatant discarded. For the sediment samples, approximately 0.5 g of sediment was inserted into 1.5-mL microcentrifuge tubes. Water samples were transferred directly to 1.5-mL centrifuge tubes. Coral mucus pellets, water and sediment samples were frozen in a − 80 °C freezer and later transported on dry ice to the University of Georgia, where samples were immediately placed in a − 20 °C freezer until extracted.

Extractions

All samples were extracted using methods outlined in Boström et al. (2004) with minor modifications. We included a bead-beating step, in which we initially added 0.04 g glass beads (Omega Biotech), and then after adding lysozyme (concentration: 1 mg mL^{-1}), vortexed the samples for 10 min at full speed using a vortex adapter (©MoBio). At the end of the extraction, pellets were eluted in 25 µL of Omega Elution buffer. To remove PCR inhibitors, we added equal volumes of SPRI magnetic beads in PEG solution. PEG coats the beads and "grabs" DNA (Rudi et al. 1997). Following two wash steps in 200 µL of 80% ETOH, 25 µL of Omega Elution buffer was added to the beads to suspend DNA.

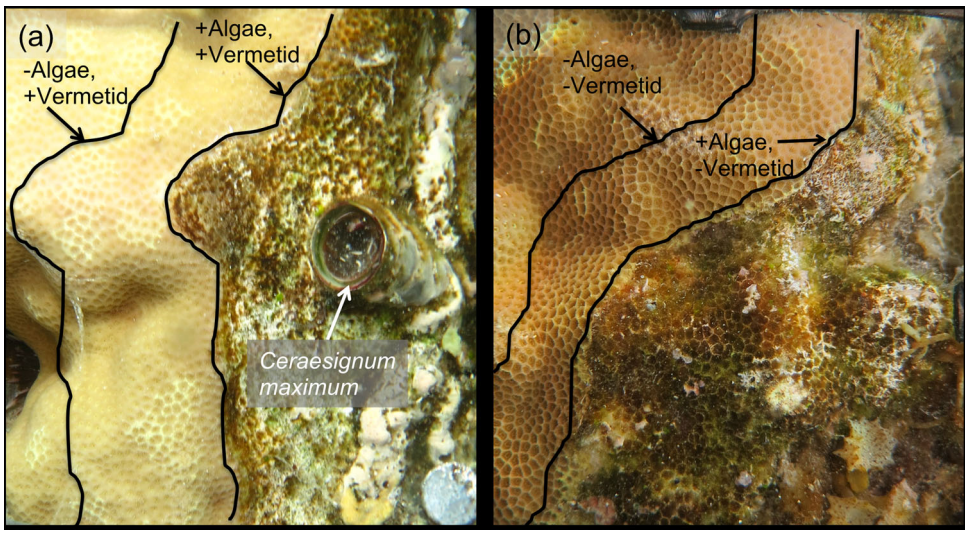

Fig. 1 Images of sampling areas where coral–algal interactions were in the **a** presence (+Vermetid) and **b** absence (−Vermetid) of vermetids. Lines show sampling transects for the +Algae and −Algae samples in the presence and absence of vermetids. Previous studies suggest the effect of algae is minimal at distances ≥ 5 cm away from the interface; therefore, we consider the samples taken at 5 cm to be "−Algae" (Barott et al. 2011). The four sample types were each taken from 10 different *Porites* coral colonies

Sequencing

Extracted DNA was sent to a commercial laboratory for sequencing (www.mrdnalab.com, Shallowater, TX, USA) on an Illumina MiSeq platform, paired-end, 250 base pairs. The V4 region of the 16S rRNA gene was sequenced using the 515F/806R primer pair with the barcode on the forward primer. The company performed PCR on the samples using a 30-cycle PCR with HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min, 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. Samples were purified using calibrated AMPure XP beads. The PCR products then were used to prepare the DNA library using the Illumina TruSeq DNA library preparation protocol. Raw forward and reverse reads were returned to UGA for analysis.

Bioinformatics and analysis

We assembled the sequences using the QIIME pipeline (version 1.9.1, Caporaso et al. 2010, 2011). We used SeqPrep to demultiplex and assemble the forward and reverse reads. Chimeras (incorrectly merged sequences) were identified using usearch61 (Edgar 2010) and the Greengenes database (Feb 2011; DeSantis et al. 2006) and subsequently removed. OTUs (operational taxonomic units) were assembled using open reference frame OTU picking, which matches sequences to the Greengenes database at 97% sequence similarity (McDonald et al. 2013; Werner et al. 2012) and clusters the remaining OTUs de novo (using uclust: Caporaso et al. 2010; Edgar 2010). Following OTU classification, chloroplast sequences were removed, and data were compiled into a biom table that was imported into R (version 3.3.2, R Core Team, 2016) where mitochondrial OTUs were removed. OTUs were filtered to only include bacteria using phyloseq. We used SourceTracker (v 0.9.5) in QIIME to identify which OTUs from water or sediment may have contributed to those in coral surface samples (i.e., are shared) using the default conditions in QIIME.

Microbial data were analyzed using the phyloseq and vegan packages (Oksanen et al. 2017). We rarefied sequences to an even sampling depth to obtain the number of unique OTUs given a comparable sampling effort to compute alpha diversity. We used the phyloseq package to calculate three measures of alpha diversity: (1) Chao1 richness (which considers missed rare members), (2) Shannon diversity (H': a combined measure of richness and evenness) and (3) richness. These analyses were performed with and without the water and sediment samples. Treatment effects on rarefied richness, Chao1 richness and Shannon diversity were determined using a linear model with vermetid presence/absence crossed with algal presence/absence, and reef as a random effect. Water and sediment were excluded from this analysis, but are included in a graphical presentation: see ESM Figure 1.

For compositional and beta diversity comparisons, we summarized sequences (not rarefied) to relative abundance of OTUs within a sample and then summarized the data at the family level. We examined differences in beta diversity (pairwise dissimilarity among samples within a treatment) and evaluated variation across treatments using multivariate homogeneity of group dispersions (betadisper the equivalent of PERMADISP, in the vegan package in R, Oksanen et al. 2017) based on Bray–Curtis dissimilarity matrices. Group dispersions quantify distances from each sample to its group's centroid.

We visualized the relative abundances of OTUs at the family level using non-multidimensional scaling of Bray–

Curtis dissimilarity matrices, in which each sample was represented as a point in the nMDS space. We conducted two visualizations: one with and one without the water and sediment samples. The data from the crossed design (Algae, Vermetid, Algae × Vermetid) were analyzed for differences in composition with PERMANOVA using the adonis function in the vegan package in R (Oksanen et al. 2017), in which we treated reefs as a random effect.

For each of the families that significantly contributed to differences observed in the nMDS plot (significance based on 999 permutations), we compared their relative abundances using the crossed design (Algae × Vermetid) blocked by reef. Lastly, because the genus *Vibrio* includes coral pathogens (Vezzulli et al. 2010; Peixoto et al. 2017; Kemp et al. 2018), we also analyzed the effects of treatment on the relative abundance of OTUs that were assigned to the genus *Vibrio*.

For all of the linear models, data were analyzed using a linear mixed effects model from the lme4 (Bates et al. 2015) and lmerTest packages (Kuznetsova et al. 2016) with Satterthwaite approximation for degrees of freedom and reef treated as a random effect.

Results

After quality filtering, we had a total of 4,777,381 sequences across all samples. The average number of sequences per sample was 78,317 ± 44,176 (± sd; $n = 52$ samples). After rarefaction (without water and sediment samples), each sample contained 11,629 sequences. Rarefied OTU richness (alpha diversity) increased approximately twofold in the presence of algae ($F_{1,30} = 33.70$, $p < 0.001$) but did not change significantly in the presence of vermetids ($F_{1,30} = 2.18$, $p = 0.15$); the interaction was not significant ($F_{1,30} = 0.08$, $p = 0.77$, Fig. 2a) suggesting the absence of a synergistic or antagonistic effect between algae and vermetids. The Shannon diversity index, which takes into account evenness as well as richness, showed similar results (Fig. 2c, Shannon: algae: $F_{1,30} = 42.4$, $p < 0.001$; vermetids: $F_{1,30} = 1.7$, $p = 0.2$; interaction: $F_{1,30} = 0.09$, $p = 0.76$). Similarly, Chao1 richness, which estimates total richness by considering the number of rare species, also showed an effect of algae (Fig. 2b, Chao1: algae: $F_{1,30} = 38.15$, $p < 0.001$), a weak (but not significant) effect of vermetids (vermetids: $F_{1,30} = 3.0$, $p = 0.09$), and no interaction ($F_{1,30} = 0.06$, $p = 0.81$). Sediment and water samples had lower rarefied richness, Chao1 richness and Shannon diversity (ESM Figure 1).

Beta diversity (compositional variation among corals within the same treatment assessed using pairwise dissimilarity) was high (i.e., close to 1) for all coral treatments (ESM Figure 2), but lower when algae were present (Fig. 3, Algae: $F_{1,40} = 13.25$, $p = 0.0008$, Vermetid: $F_{1,40} = 0.67$, $p = 0.42$, Algae × Vermetid: $F_{1,40} = 1.12$, $p = 0.30$; see also ESM Figure 2). Although this result indicates that treatments varied in multivariate dispersion, we continued to analyze the data with a PERMANOVA because the balanced design should be robust to departures from multivariate homogeneity of variances (Anderson and Walsh 2013).

Sediment and water samples had markedly different microbial communities than did the coral samples (ESM Figures 1, 3, 4), which indicates that the samples we took from coral surfaces reflected the microbiomes of corals, and not sediment or water. Additionally, the coral samples and combined water and sediment samples shared less than 50% of their OTUs (average ± se contribution of water: 29 ± 0.6%, mean contribution of sediment: 17 ± 0.5%), suggesting that the mucus assemblages are distinct and not simply the result of incidental colonization by microbes associated with the water and sediment. We next focus on the core analysis (Algae x Vermetid) of the coral microbiomes.

Microbial composition on corals changed due to algae (Table 1; Fig. 4), but not vermetids (Table 1); again, there

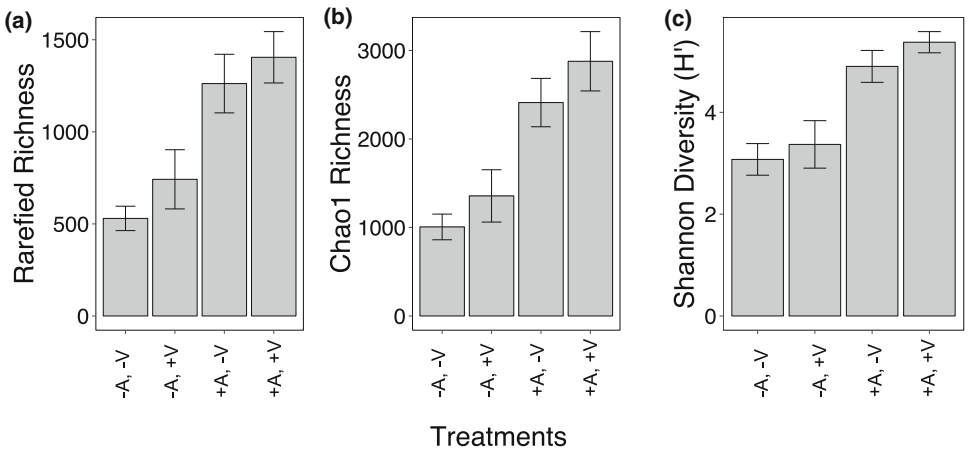

Fig. 2 **a** Rarefied OTU richness, **b** Chao1 richness, **c** Shannon diversity (H′) for treatment combinations that cross the presence/absence of algae with the presence/absence of vermetids. Each bar gives the mean ± SE; $n = 10$. For each panel, algae had a significant effect on alpha diversity measures, whereas the effects of vermetids and the interaction between algae and vermetids were not significant

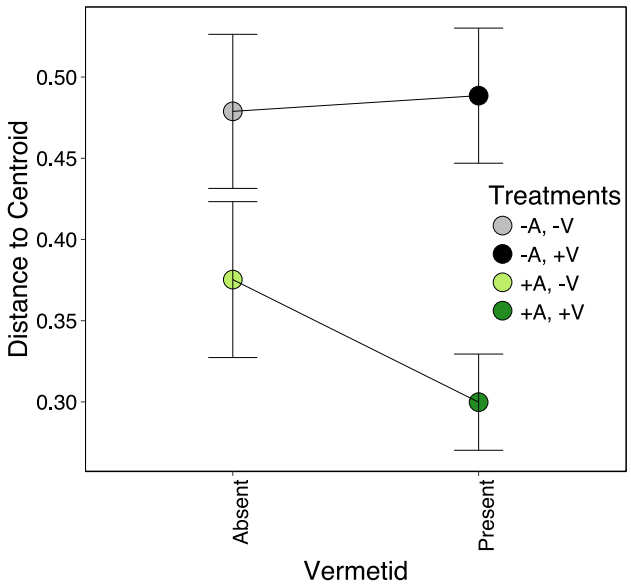

Fig. 3 Beta diversity (mean ± SE) measured by dispersion within a treatment (i.e., distance to centroid). Values are based on Bray–Curtis distance matrices of data summarized by family. Green circles (light and dark) indicate the presence of algae (+A), and gray/black indicate the absence of algae (−A). Algae significantly reduced dispersion ($p < 0.001$), but there was no significant effect of vermetids nor a significant interaction between the effects of algae and vermetids

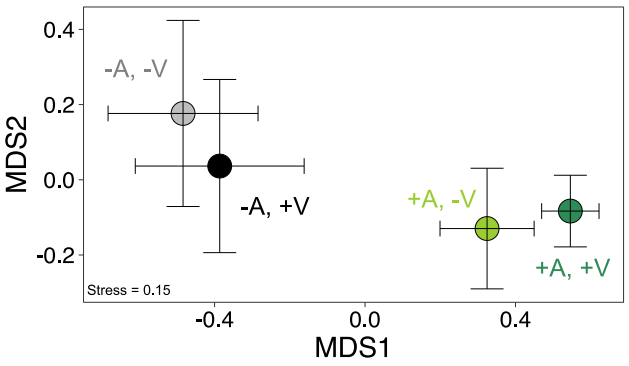

Fig. 4 Non-multidimensional scaling plot, where each dot represents the mean (± SE) of each treatment's microbial community. Points that are further away indicate communities are more different from each other than are points that are close together. Data are based on Bray–Curtis dissimilarity matrices of sample data summarized at the family level. Stress value indicates the fit is acceptable (Legendre and Legendre 1998). Dark green dots indicate both algae (A) and vermetids (V) are present (+A, + V), light green indicates algae are present but vermetids are absent (+A, −V), black indicates vermetids are present but algae are absent (−A, + V), and gray indicates neither algae nor vermetids are present (−A, −V)

was no significant interaction between algae and vermetids (Table 1). We detected 254 families in the coral mucus samples, of which 44 were common (i.e., had relative abundances > 3% in at least one sample, Fig. 5). Endozoicimonaceae, Phyllobacteriaceae, Comamonadaceae, Verrucomicrobiaceae, and Rhodobacteraceae made up 25–80% of sequences in a sample. Treatments with algae had a greater number of rare families (rare defined as groups whose relative abundance was < 3% in the sample). There were several families that separated the treatments in nMDS space, and likely led to the significant effect of algae on community composition (Fig. 6). For example, Endozoicimonaceae, a family that includes potential coral symbionts, were 3 × more abundant in the absence of algae (Fig. 6a, $p < 0.001$), but showed no effect of vermetids, nor an interaction between algal and vermetid presence. In contrast, Planctomycetaceae (Fig. 6f) were 2× more abundant on corals in the presence of algae. These microbes are common in marine habitats although their function is not well described. Other families (i.e., Flavobacteriaceae, Bdellovibrionaceae, Piscirickettsiaceae, Clostridiaceae, Legionellaceae and Rhodobacteraceae) are heterotrophic and potentially pathogenic and also were more abundant in the presence of algae (Fig. 6b–f). Two of these families, Legionellaceae and Rhodobacteraceae, also showed a slight increase in the presence of vermetids (Fig. 6g, h). Fusobacteriaceae, which includes pathogenic taxa as well as members that require lower oxygen concentrations (Olsen 2014; Staley and Whitman 2010), increased the most when both algae and vermetids were present (Fig. 6i). The relative abundance of *Vibrio* did not differ among treatments (Fig. 7, Algae: $F_{1,27} = 0.95$, $p = 0.34$; Vermetid: $F_{1,27} = 0.002$, $p = 0.96$; Algae × Vermetid: $F_{1,27} = 1.25$, $p = 0.27$).

Because we used the Greengenes database to assign taxonomy, which uses the family Endozoicimonaceae instead of Hahellaceae (Neave et al. 2016), we checked the sequences of the most abundant OTUs against multiple databases (using SINA, Pruesse et al. 2012) and found they

Table 1 PERMANOVA results based on the Bray–Curtis dissimilarity index

	df	Sum of squares	Mean squares	*F* ratio	R^2	*P*
Algae	**1**	**1.91**	**1.91**	**9.16**	**0.20**	**0.01**
Vermetids	1	0.13	0.13	0.63	0.01	0.62
Algae × vermetids	1	0.06	0.05	0.26	0.01	0.96
Residuals	36	7.49	0.21		0.78	
Total	39	9.58			1	

Terms in bold indicate significant differences in communities due to treatment

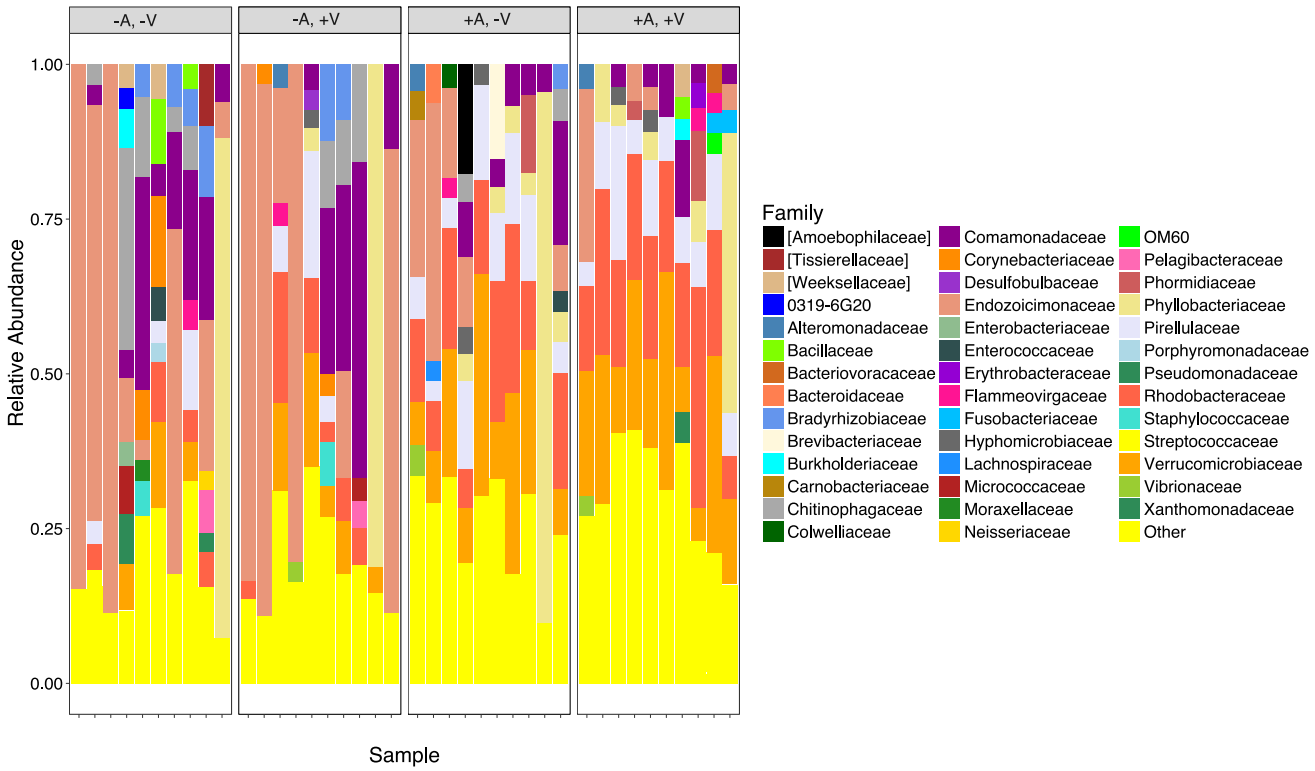

Fig. 5 Relative abundance of OTUs belonging to different microbial families in each of the treatments that varied in the presence/absence of vermetids (+V or −V) and algae (+A or −A). Bars represent a separate coral sample. Each color represents a different family. "Other" indicates the combined relative abundance of OTUs from families whose relative abundance was < 3% in a sample

match genus *Endozoicomonas* at 97% similarity in the Silva database.

Discussion

Frequently, multiple stressor studies have focused on the growth, survival and physiology of an organism. Recent work suggests that many of these effects might be mediated through changes in the organism's microbiome (Vega Thurber et al. 2012; Shaver et al. 2017), although such effects have not been investigated for most organisms in their natural environment. Here, we examined whether we could detect effects of two stressors (algal turf and vermetids) on the microbial communities of corals. The observed effects were primarily due to the effects of algae; there was little indication of an effect of vermetids. Nor was there much evidence to support that hypothesis of a synergism or antagonism between the effects of vermetids and algae.

Because vermetids have demonstrable effects on coral growth (Shima et al. 2010, 2013), we hypothesized that they these effects were mediated through effects on the coral microbiome. However, vermetids caused only a slight (and non-significant) increase in Chao1 richness, and no clear effect on rarefied richness or Shannon diversity (Fig. 2) or taxonomic composition (Fig. 5). Even if vermetids had no strong effects on microbes, their nets decrease water flow, trap conditions near coral surfaces (Brown and Osenberg 2018), and contain bioactive compounds (Klöppel et al. 2013). We therefore hypothesized that vermetids should intensify deleterious effect of algae on corals, as mediated through their microbiomes. This would manifest as a synergism (e.g., exacerbation of an effect) between algae and vermetids. We observed a synergism for only one microbial family, Fusobacteriaceae. Fusobacteriaceae include pathogenic species and are associated with low-oxygen environments (Olsen 2014; Staley and Whitman 2010). Thus, it appears that algae and vermetids combine to create environmental conditions conducive to the growth of Fusobacteriaceae (i.e., low flow, low oxygen, Brown and Osenberg 2018; Haas et al. 2013a), but otherwise do not have synergistic effects on the coral microbiome.

The limited effects of vermetids and the limited interaction between algae and vermetids seem to conflict with previous results demonstrating deleterious effects of vermetids on corals (e.g., Shima et al. 2010). We suggest several explanations for this apparent discrepancy. First, it is possible that the effects of vermetids on corals are not

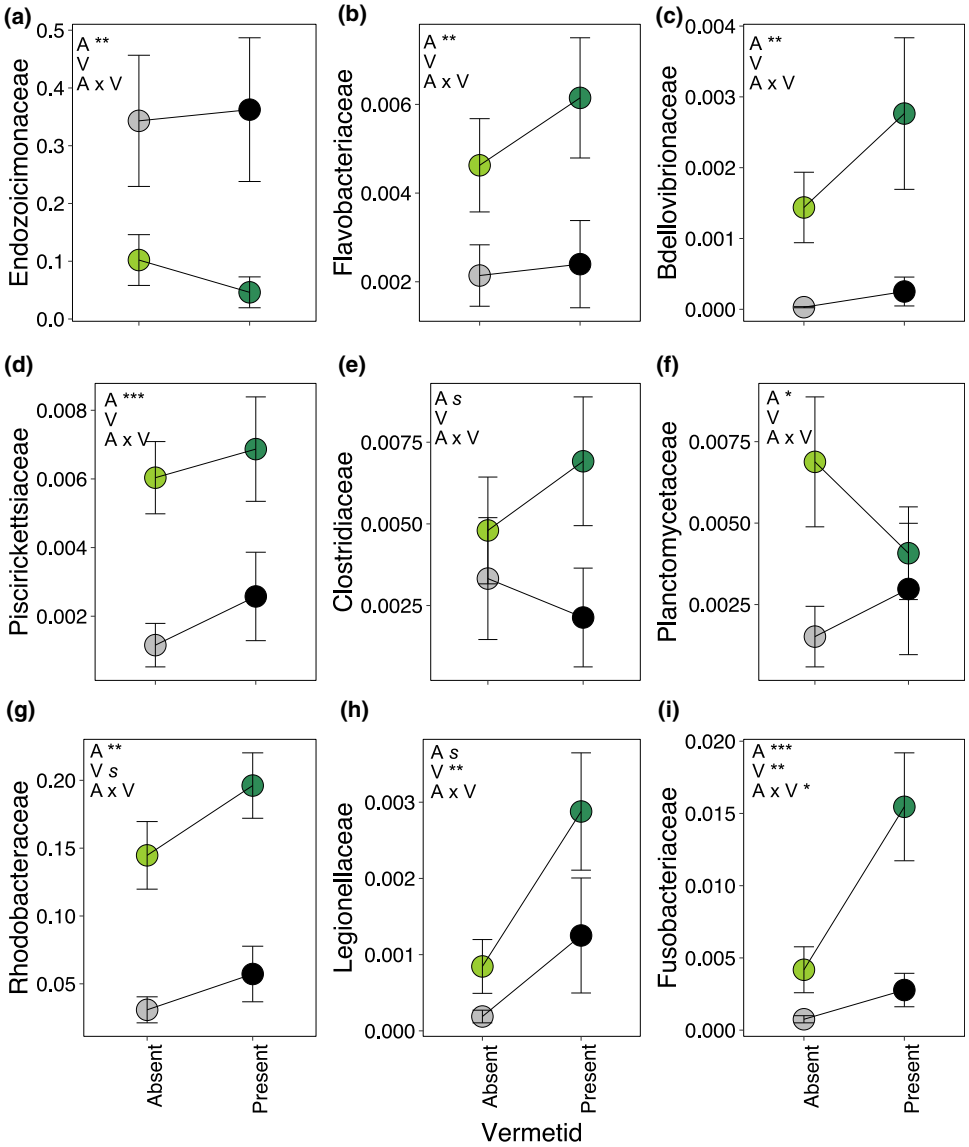

Fig. 6 Mean (± SE) relative abundance for families that significantly contribute to compositional differences in nMDS space (significance determined by permutation test, 1000 permutations, Oksanen et al. 2017). Green (light and dark) indicates the presence of alga, and gray/black indicates the absence of algae. Darker shades indicate the presence of vermetids. Asterisks indicate significant main or interactive effects ($s = 0.05$, *$0.01 < p \leq 0.05$, **$0.001 < p < 0.01$, ***$0 < p < 0.001$)

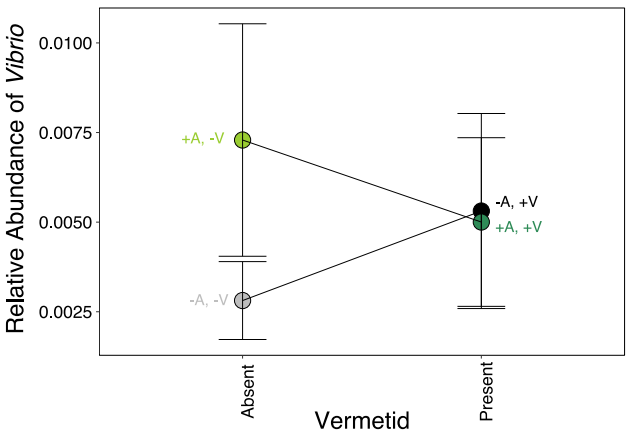

Fig. 7 Relative abundance of OTUs in the *Vibrio* genus (mean ± SE). There was a threefold increase of *Vibrio* due to algae (but only in the absence of vermetids), although neither the main effects nor interaction was significant ($p > 0.05$)

meditated through the microbial community. Alternatively, the effects may be mediated through only a narrow set of microbes (e.g., Fusobacteriaceae) with most members of the microbiome unaffected. Another alternative is that the effect is mediated not through changes in the composition of the microbial community but through shifts in community metabolism, other microbial members (e.g., viral components), or total abundance (e.g., as suggested by Brown and Osenberg 2018). Because mucus nets are a carbohydrate matrix, they also have the potential to fuel microbial growth, which could enhance microbial abundances or metabolism without changing the community composition. It is also possible that our study was not designed appropriately to detect actual effects. For example, if the effects of vermetids persist beyond the expected 20 cm threshold, then our design would not have included any samples that were actually free of vermetid effects.

Despite the limited effects of vermetids, our data demonstrate clear and strong effects of algae on the coral microbiome, above the variation we observed among reefs (ESM Figures 5 and 6). Algae increased alpha diversity (rarefied richness, Chao1 richness and Shannon diversity) of the coral microbial community (Fig. 2). The effects we observed were likely caused by an increase in rare taxa (Fig. 5), which contributed to the distinct separation in multivariate space for microbial communities sampled near versus far from algae (Fig. 5). We hypothesize that the increased number of taxa and relative abundance of other taxa (e.g., Planctomycetaceae and Flavobacteriaceae) in the presence of algae was the result of increased availability of dissolved organic carbon produced by algal turf and retained near the coral surface by the complex topography created by turf (Carpenter and Williams 1993; Wangpraseurt et al. 2012; Jorissen et al. 2016). Algal turfs are among the most prolific producers of DOC, can increase diffusive boundary layer thickness (Carpenter and Williams 1993; Brown and Carpenter 2013), especially when filaments are ungrazed (Carpenter and Williams 1993; Jorissen et al. 2016), and are known to encourage microbial growth (Kline et al. 2006; Haas et al. 2011). Previous studies that examined changes in microbial communities on *Montastrea* and *Porites* corals also have shown increased alpha diversity near algae (Barott et al. 2011; Pratte et al. 2017).

In many ecosystems, increased alpha diversity is hypothesized to increase ecosystem function (Tilman et al. 2014); however, in coral microbial systems, increased diversity is often associated with disruption in the normal functioning of an organism and has often been associated with disease (Mera and Bourne 2017) and stress (McDevitt-Irwin et al. 2017). Thus, it is likely that an increase in microbial diversity reflects an instability in host-associated (e.g., coral) microbial communities, which facilitates the invasion of deleterious microbes.

Increased beta diversity is also associated with the presence of stressors (McDevitt-Irwin et al. 2017; Pratte et al. 2017) and hypothesized to indicate instability in host–microbe relationships (Zaneveld et al. 2016, 2017). In contrast to this expectation, we observed high beta diversity in all treatments and a decrease in beta diversity (at the family level) in the presence of algae (Fig. 4). The high variation we observed among samples within a treatment may be due, in part, to variation among reefs (ESM Figures 5 and 6), due either to genetic differences among corals or variation in the species composition of algal turf. Algal turfs are known to exhibit high variability in their algal composition (Harris et al. 2015), which could contribute to variation in their effects on the coral microbiome. However, we observed lowered beta diversity in the presence of algae turfs, suggesting that differences in algal composition is likely not the main driver of these patterns. Instead, we suggest that the reduced beta diversity attributed to algae may be driven by a homogenization of the physiochemical conditions created at the coral–algal interface due to low flow, reduction in mixing and retention of chemical conditions (Brown and Carpenter 2013; Brown and Osenberg 2018). Indeed, algae and, to a lesser extent, vermetids lead to similar microbial communities that are composed of families that are associated with pathogens and/or can withstand low-oxygen conditions (Fig. 6).

The responses of the microbiome, and especially the shifts in alpha and beta diversity, suggest that insights about coral responses to stressors may be gained through better understanding of the interactions that arise within the microbial community and between the microbes and the coral. For example, in the presence of algae, we observed increases in families that are associated with pathogens (Piscirickettsiaceae, Bdellovibrionaceae, Legionellaceae, Rhodobacteraceae). The increase in Bdellovibrionaceae is especially interesting, as this family contains predatory bacteria that attack other gram-negative bacteria, which may include other potential pathogens or beneficial microbes of coral mucus (Martin 2002). Thus, this group has the potential to actively reduce other microbial groups, including beneficial coral symbionts. Endozoicimonaceae (or Hahellaceae) includes potentially beneficial microbes in the genus *Endozoicomonas* that are associated with pathogen resistance (Morrow et al. 2012; Bourne et al. 2013; Meyer et al. 2014; Peixoto et al. 2017). We observed reduced relative abundance of this family (and the genus *Endozoicomonas*) in the presence of algae, suggesting that the coral's resistance to pathogens may have been compromised. Thus, algae might disrupt beneficial symbioses between corals and their microbial partners, allowing an increase in the pathogenic and opportunistic groups.

Testing for the role of multiple stressors on reefs is an important task, as coral reefs are experiencing a multitude of new stressors that can result in both lethal and sublethal effects on corals (Harborne et al. 2017). Some of the effects of biotic stressors on coral microbes are likely due to direct effects the stressors have on the physical environment (e.g., through oxygen or DOC concentration), but other microbial changes are likely due to shifting interactions among microbes. Future insights might be facilitated if we can better unravel the complex microbial interactions (e.g., predator–prey) arising within the coral microbiome while simultaneously understanding how stressors influence microbial dynamics and the physical environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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