



Contact- and Water-Mediated Effects of Macroalgae on the Physiology and Microbiome of Three Indo-Pacific Coral Species

Jenny Fong^{1*†}, Lindsey K. Deignan^{2†}, Andrew G. Bauman¹, Peter D. Steinberg^{2,3,4}, Diane McDougald^{2,5} and Peter A. Todd¹

OPEN ACCESS

Edited by:

Linda Wegley Kelly,
San Diego State University,
United States

Reviewed by:

Ty N. F. Roach,
University of Hawai'i at Mānoa,
United States
Maggy Nugues,
Université de Sciences Lettres
de Paris, France
Andrew A. Shantz,
The Pennsylvania State University
(PSU), United States

*Correspondence:

Jenny Fong
jenny.jenny@u.nus.edu

† These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Coral Reef Research,
a section of the journal
Frontiers in Marine Science

Received: 19 September 2019

Accepted: 26 December 2019

Published: 22 January 2020

Citation:

Fong J, Deignan LK, Bauman AG,
Steinberg PD, McDougald D and
Todd PA (2020) Contact-
and Water-Mediated Effects
of Macroalgae on the Physiology
and Microbiome of Three Indo-Pacific
Coral Species. *Front. Mar. Sci.* 6:831.
doi: 10.3389/fmars.2019.00831

¹ Experimental Marine Ecology Laboratory, National University of Singapore, Singapore, Singapore, ² Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore, ³ Centre for Marine Science and Innovation, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia, ⁴ Sydney Institute of Marine Science, Mosman, NSW, Australia, ⁵ The itthree Institute, University of Technology Sydney, Sydney, NSW, Australia

Competitive interactions between corals and macroalgae play an important role in determining benthic community structure on coral reefs. While it is known that macroalgae may negatively affect corals, the relative influence of contact- versus water-mediated macroalgal interactions on corals – such as via an influence on coral-associated microbiomes – is less well understood. Further, the impacts of macroalgae on corals that have persisted in a heavily urbanized reef system have not been explored previously. We examined the effects of the macroalgae *Lobophora* sp. and *Hypnea pannosa* on the physiology and microbiome of three Indo-Pacific coral species (*Merulina ampliata*, *Montipora stellata*, and *Pocillopora acuta*) collected from two reefs in Singapore (Pulau Satumu and Kusu Island), and compared how these effects varied between direct contact and water-mediated interactions. Direct contact by *Lobophora* sp. caused visible tissue bleaching and reduced maximum quantum yield (F_v/F_m) in all three coral species, while direct contact by *H. pannosa* only led to slight, but significant, suppression of F_v/F_m . No detrimental effects on coral physiology were observed when corals were in close proximity to the macroalgae or when in direct contact with algal mimics. However, both direct contact and water-mediated interactions with *Lobophora* sp. and *H. pannosa* altered the prokaryotic community structures in *M. stellata*. For *M. ampliata* and *P. acuta*, the changes in their microbiomes in response to algal treatments were more strongly influenced by the source reefs from which the coral colonies were collected. In particular, coral colonies collected from Kusu Island had proportionately more initial abundances of potentially pathogenic bacteria in their microbiomes than those collected from Pulau Satumu; nevertheless, coral fragments from Kusu Island had the same physiological responses to macroalgal interactions as corals from Pulau Satumu. Overall, our results reveal that, for the species tested, the

coral microbiomes were sensitive to both direct contact and water-mediated interactions with macroalgae, while coral physiology was only compromised when in direct contact. Further, the presence of high levels of potentially pathogenic bacteria in some of the coral samples did not lead to the corals being more susceptible to impacts from macroalgae.

Keywords: competitive interactions, urbanized reef, Singapore, corals, macroalgae, coral microbiome

INTRODUCTION

Competition plays a critical role in shaping the ecology and evolution of species, as well as determining the structure and function of ecological communities (Yodzis, 1978; Hooper et al., 2005). On coral reefs, intense competition for space occurs among sessile benthic organisms, particularly between corals and macroalgae (Miller, 1998; McCook et al., 2001). In recent decades, anthropogenic local stressors such as overfishing and eutrophication, as well as global ocean warming from climate change have resulted in dramatic declines in coral cover and increasing prevalence of macroalgae on degraded reefs (Bellwood et al., 2004; Hughes et al., 2010, 2018). As a consequence, there is a potential for reefs to shift from coral- to macroalgal-dominated systems (Hughes et al., 2010). Macroalgae directly and indirectly impact corals via a suite of physical, chemical, and microbial processes (McCook et al., 2001; Rasher et al., 2011; Barott and Rohwer, 2012). Hence, high macroalgal abundance can reinforce a coral-depauperate state by reducing coral health, growth, reproduction, and recruitment (Tanner, 1995; Box and Mumby, 2007; Evensen et al., 2019; reviewed in McCook et al., 2001 and Birrell et al., 2008). While it is known that macroalgae can negatively affect corals, the relative influence of direct contact and water-mediated interactions with macroalgae are less understood – especially how these interactions may impact coral-associated microbiomes.

Direct macroalgal contact can harm corals through shading, abrasion, and/or production of harmful allelochemicals that cause coral tissue bleaching and/or alter the coral microbiome (River and Edmunds, 2001; Rasher et al., 2011; Morrow et al., 2012). Indirect, water-mediated interactions with macroalgae can also have deleterious effects; however, to what extent this occurs remains unclear. For example, macroalgae can transmit pathogenic bacteria to corals, triggering coral diseases (Nugues et al., 2004). Macroalgae can also exude allelochemicals and compounds (e.g., dissolved organic carbon) into surrounding waters, which has been reported to promote the growth of coral-associated microbes and lead to hypoxic stress in corals (Haas et al., 2011, 2013; Barott and Rohwer, 2012; Jorissen et al., 2016; Roach et al., 2017). Furthermore, advection of macroalgal chemicals and microbes by water flow may indirectly affect the coral microbiome and trigger coral mortality (Barott and Rohwer, 2012). High variability in the competitive abilities among coral and macroalgal species precludes generalizations of the processes involved and the outcomes of these interactions (McCook et al., 2001; Jompa and McCook, 2003).

Corals harbor a range of microorganisms including bacteria, protists, archaea, fungi, and viruses (the “microbiome”) that are critical for maintaining coral health (Rosenberg et al., 2007;

Bourne et al., 2016). For example, some coral-associated bacteria assist corals in assimilation of nutrients and prevention of pathogenic infections through antibiotic production (Lesser et al., 2004; Ritchie, 2006; Lema et al., 2012). Coral microbiomes also play important roles in the ecological interactions between corals and other organisms, including macroalgae. Macroalgae can disturb coral microbial communities by transmitting macroalgal-associated microbes and compounds (Smith et al., 2006; Morrow et al., 2012; Thurber et al., 2012), which may reduce the abundance of beneficial bacteria, promote the growth of rare microbial taxa, or vector new, pathogenic microbes (Nugues et al., 2004; Morrow et al., 2011). Such changes in the microbial communities can be detrimental to corals by increasing their susceptibility to pathogens, bleaching, or other environmental stressors (Rosenberg et al., 2007).

Coral reefs in Singapore persist in a heavily disturbed, urbanized reef environment characterized by high sedimentation and nutrient levels (Browne et al., 2015; Heery et al., 2018). As a result, corals in Singapore may be in a reduced health state and therefore more susceptible to stressors including macroalgal competition (Barott and Rohwer, 2012; Barott et al., 2012). Yet, the persistence of corals in Singapore, especially on the reef flats where macroalgae are abundant and herbivory is low (Guest et al., 2016; Bauman et al., 2017; Low et al., 2019), suggests that the corals are potentially adapted to macroalgal competition. In this study, we examined the direct and indirect effects of macroalgae on corals collected from Singapore and their microbial communities. Specifically, we compared how the effect of macroalgae on corals differed between direct contact and indirect, water-mediated interactions between a brown alga *Lobophora* sp. from a genus previously shown to be allelopathic (Morrow et al., 2011; Evensen et al., 2019; Fong et al., 2019), and a putatively non-allelopathic red alga *Hypnea pannosa*, on three common coral species in Singapore (*Merulina ampliata*, *Montipora stellata*, and *Pocillopora acuta*).

MATERIALS AND METHODS

Corals and Macroalgae Collection

This study was conducted over 8 weeks at St John’s Island National Marine Laboratory (SJINML), Singapore from November 2017 to January 2018. Two weeks prior to the experiment, four colonies each of *M. ampliata*, *M. stellata*, and *P. acuta* were collected from the reef flats at two fringing reefs in the Southern Islands of Singapore (Kusu Island: 1°13’32”N, 103°51’35”E; Pulau Satumu: 1°09’39”N, 103°44’26”E). For *P. acuta* and *M. ampliata*, two colonies were each collected

from Pulau Satumu and Kusu Island, while all four colonies of *M. stellata* were collected from Pulau Satumu due to lack of suitable colonies at Kusu Island. Samples of two locally abundant macroalgae, *Lobophora* sp. and *H. pannosa*, were also collected from Kusu Island on the same day. We chose *M. ampliata*, *M. stellata*, and *P. acuta* as study species because they are abundant on Singapore's reefs (Huang et al., 2009) and within genera that vary in susceptibility to macroalgal competition (Bonaldo and Hay, 2014). We selected the foliose brown alga *Lobophora* sp. because this genus is known to be strong competitors against corals (Jompa and McCook, 2002; Nugues and Bak, 2006), while previously studies have shown the corticated mat-forming red alga *H. pannosa* have negligible impacts on corals (Jompa and McCook, 2003). DNA barcode analysis revealed that the *Lobophora* sp. studied here is an undescribed species, with its closest sister species being an undescribed *Lobophora* species from Oman (see Fong et al., 2019).

Coral and macroalgal samples were brought back to SJINML. Seven 4 to 5 cm fragments were obtained from each coral colony and epoxied to small cement tiles (6 × 6 cm). Samples of each macroalgal species were separated into twenty-four equal sized clumps of ~7 g wet weight. On each cement tile, two 1 cm stainless-steel nails were embedded on the opposite ends so that polyethylene string could be secured over each nail head to hold the macroalgae in place (following Rasher and Hay, 2010). Both corals and algae were allowed to acclimate for 2 weeks in separate holding tanks in the outdoor aquariums before the start of the experiment.

Experimental Design

To investigate the effects of direct macroalgal contact and indirect water-mediated interactions on coral physiology and microbiome, coral fragments from *M. ampliata*, *M. stellata*, and *P. acuta* were assigned to one of the following seven treatments for 8 weeks: (1) direct contact with *Lobophora* sp., (2) direct contact with *H. pannosa*, (3) in close proximity with *Lobophora* sp. (coral and macroalgae were on separate cement tiles ~5 cm apart but not in direct contact), (4) in close proximity with *H. pannosa*, (5) direct contact with mimic *Lobophora* sp., (6) direct contact with mimic *H. pannosa*, and (7) without any algal treatment (control). The inert algal mimics were constructed by tying together several semicircles of brown foam paper (mimic *Lobophora* sp.) and by grouping several lengths of wool yarn (mimic *H. pannosa*). These mimics served to control for the physical effects caused by macroalgal abrasion as well as any shading effects. Each treatment was replicated four times.

Replicates were placed in individual 3 l plastic tanks, which received independent flow through filtered seawater and were aerated. Tanks were distributed randomly among three large water baths to reduce water temperature fluctuations. To avoid any positional effects, the arrangement of the plastic tanks was changed randomly every week. Minimal variations in water temperature and light levels were recorded among the water baths throughout the experiment (mean ± SE: 28.10 ± 0.08°C, 57.14 ± 8.68 μmol s⁻¹ m⁻²; HOBO data loggers), and the values

were comparable to *in situ* conditions (Browne et al., 2015). Tanks were cleaned every 3 to 4 days.

Assessment of Coral Physiology

The physiological state of the coral fragments was monitored using image analysis and PAM fluorometry. Previous research indicates that the effects of macroalgae on corals are often localized to areas of physical contact only (Rasher et al., 2011). Therefore, for each coral fragment that was in direct contact with live and mimic algae, we assessed the side of the fragment that was exposed to the algal treatment and also its opposite side away from the exposure. Photographs of coral fragments were taken weekly to quantify the percentage of tissue bleaching using ImageJ photoanalysis software (Schneider et al., 2012). In addition, we examined maximum quantum yield (F_v/F_m) of coral fragments using pulse amplitude modulated fluorometer (Diving-PAM Underwater Fluorometer) every 2 weeks. F_v/F_m is a measure of the quantum efficiency of photosystem II centers in dark-adapted organisms, and many studies have found F_v/F_m a reliable proxy for coral health (Ralph et al., 1999; Fitt et al., 2001). For each side of each coral fragment, three F_v/F_m values were taken and averaged. All PAM measurements were taken between 0400 and 0600 h to ensure adequate dark acclimation in coral samples.

To determine the effects of algal treatments on the percent coral tissue bleaching and F_v/F_m, linear mixed-effect (LME) models were performed using the *lme* function from the *nlme* package (Pinheiro et al., 2019) in R v3.6.1 (R Core Team, 2019). Parent colony was added as a random effect to account for the nested data structures, and only data from the final time point (i.e., week 8) were fitted to the models. Models were weighted to allow variance to differ by treatments and corals to ensure homogeneity of variance. Significance tests were based on likelihood ratio tests and changes in Akaike's Information Criterion (AIC). *Post hoc* analyses were conducted using the *emmeans* package with Tukey's adjusted *p*-values. To compare the physiological responses between coral fragments sides (i.e., in direct contact with macroalgae and the opposite sides), Wilcoxon signed-rank tests were performed. Assumptions of normality and homoscedasticity were validated by visual inspection of the residual plots for all models (Supplementary Figure 1).

Assessment of Coral Microbiome

To characterize the natural microbiome of the three coral species, a 5 cm fragment was sampled from each individual coral colony at the time the colonies were collected in the field. An additional 5 cm fragment from each colony was sampled 2 weeks after acclimation in the aquarium. At the end of the experiment (and after the physiological testing described above), all coral fragments from the seven treatments were collected for microbial sampling. All samples were immediately frozen in a dry shipper or -80°C freezer. Coral tissue was then removed from the entire fragment using compressed air and subsequently stored at -80°C. DNA was extracted using the Qiagen DNeasy PowerBiofilm Kit and stored at -20°C. PCR was run with 10 μl HotStarTaq Plus Master Mix, 1 μl each of 10 μM forward and reverse primers, 5 μl water, 1 μl 100% DMSO, and 2 μl template

DNA ($5 \text{ ng } \mu\text{l}^{-1}$). The 515F and 806R primers were used to amplify the V4 region of the 16S rRNA gene (Caporaso et al., 2011). Triplicate PCR reactions were run using the following conditions: an initial denaturation at 95°C for 5 min, followed by 37 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, then a final extension of 10 min at 72°C . For *M. stellata* samples, no DMSO was used during PCR, and the reaction only ran for 35 cycles. All samples were pooled and cleaned with Agencourt AMPure XP beads and quantified using a Qubit 2.0 fluorometer. All PCR products were quality checked on an Agilent 2200 TapeStation before the samples were delivered to the sequencing facility at the Singapore Centre for Environmental Life Sciences Engineering (Nanyang Technological University, Singapore) for library preparation and amplicon sequencing on an Illumina MiSeq platform.

Amplicon sequence data were processed in Mothur v. 1.39.5 (Schloss et al., 2009) using the corresponding MiSeq SOP analysis pipeline (Kozich et al., 2013). In brief, paired sequences were combined and trimmed, then aligned to the Silva v4 reference database. Sequences that matched within two base pairs were pre-clustered, and chimeric sequences were removed using VSEARCH. Any non-bacterial or non-archaeal sequence reads (i.e., chloroplast, mitochondria, or unknown Eukaryota) were filtered out. Individual treatment replicates were rarefied to the lowest number of sequence reads by coral species to account for variation in sampling depth. Mean relative abundance of the representative taxa for each sample group was calculated. Rarefied data were used to calculate alpha diversity (richness, chao, Shannon evenness, and Inverse Simpson Diversity) and beta diversity. Alpha diversity measures were calculated using Mothur.

To examine the patterns of coral-associated prokaryotic communities, non-metric multidimensional scaling (nMDS) plots were created using Bray-Curtis distance matrices of square root transformed data with the *Vegan* package in R v3.4.3. Permutational multivariate analysis of variance (PERMANOVA) and permutational multivariate analysis of dispersion (PERMDISP) were performed using the PERMANOVA+ add-on in PRIMER 7 – both with the controls and with only algal treatments for each coral-algal pairing. PERMANOVA was also performed to compare the response of the prokaryotic community structure between colonies collected from Pulau Satumu and Kusu Island for *M. ampliata* and *P. acuta* interactions with *Lobophora* sp. and *H. pannosa*. To determine the specific OTUs that were contributing to the differences detected by the PERMANOVA, multivariate generalized linear models (GLMs) with negative binomial distribution were performed using the *mvabund* package (Wang et al., 2012) with residual plots inspected to ensure fit. Each dataset was subsampled to 500 OTUs for the GLM analysis.

RESULTS

Macroalgal Effects on Coral Physiology

Interactions with macroalgae had significant effects on coral tissue and F_v/F_m (Table 1). Direct contact with *Lobophora* sp. caused $12.26 \pm 2.11\%$ (mean \pm SE) of the coral tissue

TABLE 1 | Summary of LME models on coral maximum quantum yield (F_v/F_m) and percentage of coral tissue bleaching after 8 weeks of interactions with macroalgae.

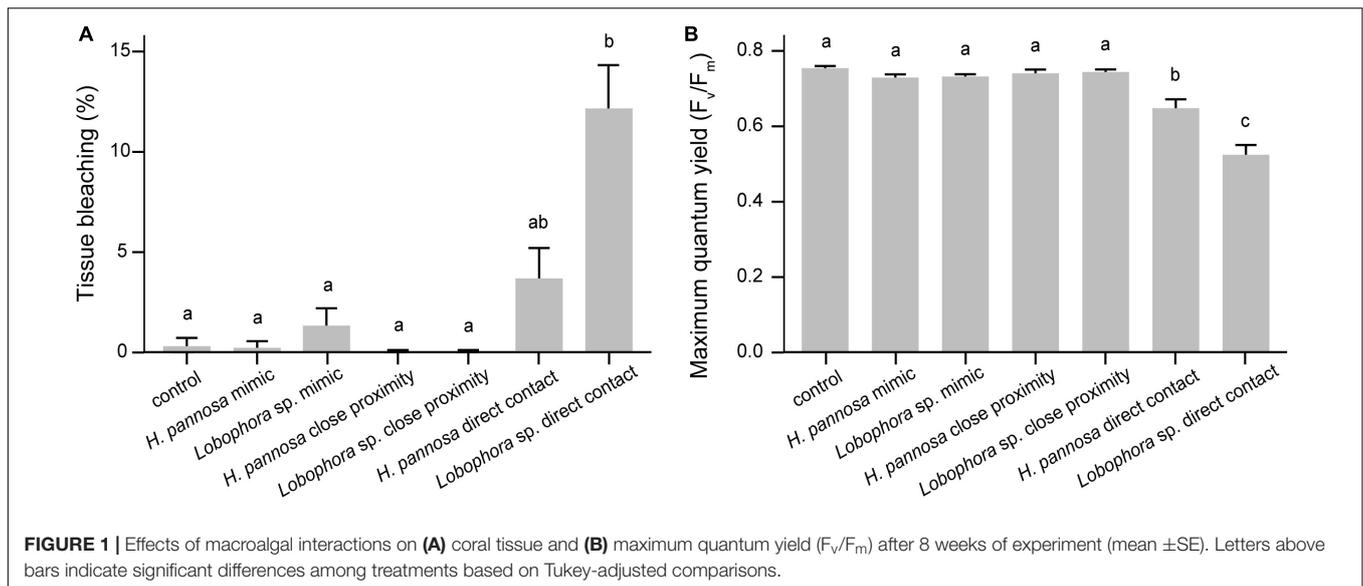
Variables	df	ΔAIC	LR test	<i>P</i>
F_v/F_m				
Treatment	6	−25.09	51.67	<0.001
Coral	2	6.11	2.75	0.253
Treatment : Coral	12	4.98	19.02	0.088
Tissue bleaching				
Treatment	6	−14.14	26.14	<0.001
Coral	2	3.24	0.76	0.685
Treatment : Coral	12	8.13	15.87	0.197

Bold values denote statistical significance when $\alpha = 0.05$.

area to bleach, while direct *H. pannosa* contact did not cause significant tissue bleaching compared to control corals (Figure 1). Direct macroalgal contact also caused significant suppression of F_v/F_m , from 0.760 ± 0.005 in control corals to 0.529 ± 0.023 in *Lobophora* sp. treatment and 0.653 ± 0.021 in *H. pannosa* treatment (Figure 1). Macroalgal impacts were limited to the areas of direct contact (Wilcoxon signed-rank test; $p < 0.001$; Supplementary Figures 2, 3) and became evident after 4 weeks (Supplementary Figure 4). No detrimental effects on coral physiology were observed when corals were in close proximity (i.e., ~ 5 cm apart) to *Lobophora* sp. and *H. pannosa* or when in direct contact with algal mimics (Figure 1).

Macroalgal Effects on Coral Microbiome

Amplicon sequence data were subsampled by species to 115,813 sequences for *M. stellata*, 55,399 sequences for *P. acuta*, and 35,566 sequences for *M. ampliata*. The dominant bacterial phylum in all coral species was *Proteobacteria*, particularly *Alphaproteobacteria* and *Gammaproteobacteria* (Supplementary Figures 5–7). In general, macroalgal treatments led to higher richness and diversity in the microbiome of all coral species compared to samples collected from the field at the start of the experiment and tank-acclimated controls (Supplementary Tables 1–3). For *M. stellata*, there was a significant shift in the prokaryotic community structure between the field control samples and the control treatments by the end of the experiment (Pairwise PERMANOVA: $t = 2.202$, $p = 0.007$; Supplementary Figure 8). Due to the temporal shift in microbiomes observed throughout the experiment, all subsequent analyses to assess the specific response of the coral microbiome to algal treatment focused on comparisons to the control fragments that were sampled at the same time as those that received algal treatments. For *M. ampliata* and *P. acuta*, the temporal shift was less apparent due to the confounding effect of source reef; however, for consistency all coral species were analyzed in the same manner with treatments being compared to experimental controls and not directly to field controls. For each coral species, there were also significant differences in the prokaryotic community structure across all treatments (PERMANOVA: *M. stellata* Pseudo- $F = 2.544$, $p = 0.001$; *P. acuta* Pseudo- $F = 1.549$, $p = 0.001$; *M. ampliata* Pseudo- $F = 1.577$, $p = 0.001$).



For *M. stellata*, the coral microbiomes differed significantly among the four macroalgal treatments (i.e., direct algal contact, in close proximity with algae, direct contact with algal mimic, without any algal treatment) for both *Lobophora* sp. and *H. pannosa* treatments when examined for each algal species separately (Table 2). Particularly in *Lobophora* sp. treatments, the nMDS demonstrates how prokaryotic community structures of corals that were in contact or close proximity with the algae were more closely clustered together and distinct from controls and algal mimic treatments (Figure 2A). In contrast, in the *H. pannosa* treatments, the treatments involving direct contact, either with the algae or algal mimic, were clustered similarly (Figure 2B). For *P. acuta* and *M. ampliata*, PERMANOVA analysis revealed no significant differences in prokaryotic community structure among treatment groups for each algal treatment; however, there were significant differences in the response of the coral microbiome to both *Lobophora* sp. and *H. pannosa* based on the source reef from which the colony was collected (Table 2). The nMDS plots demonstrate marked separation in the microbiomes of corals collected from Kusu Island and Pulau Satumu (Figure 3).

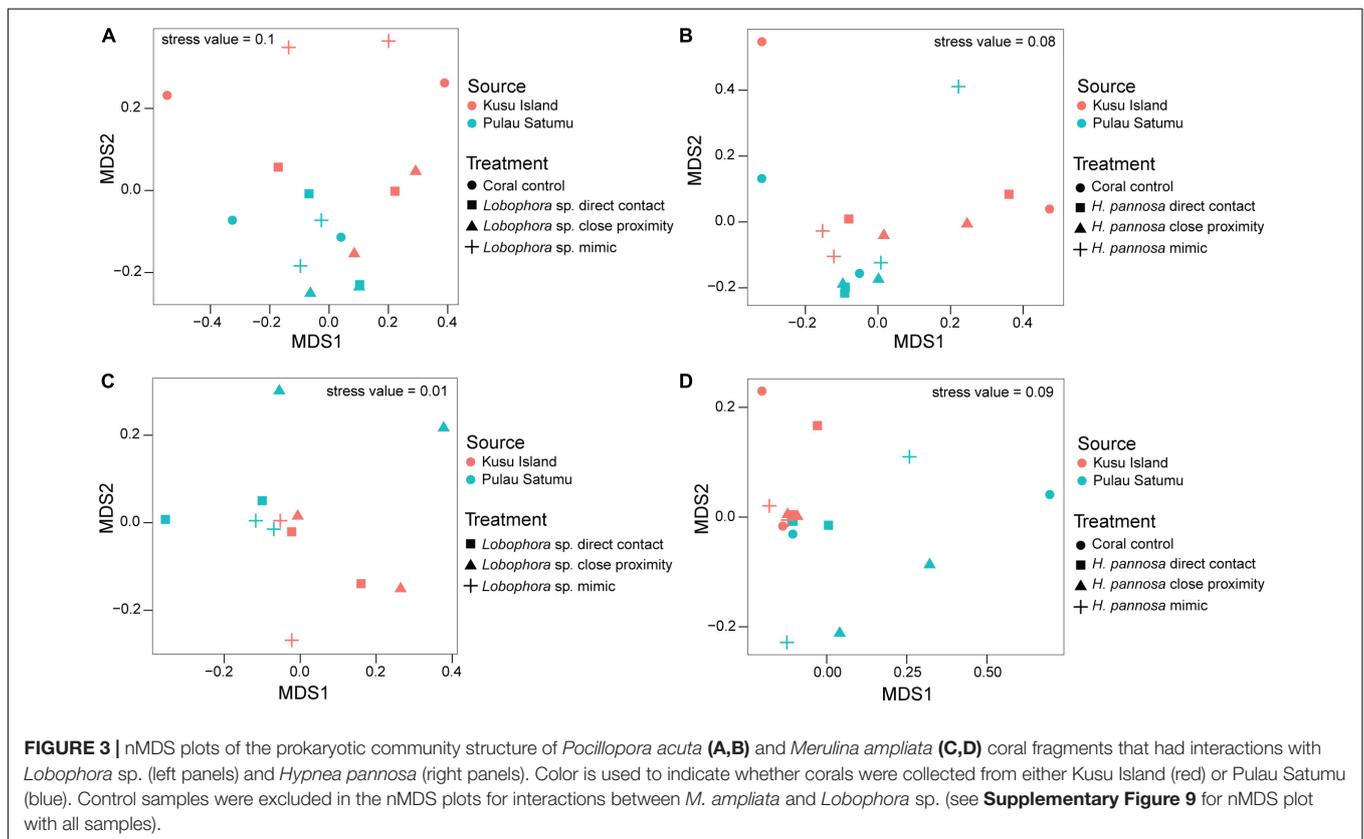
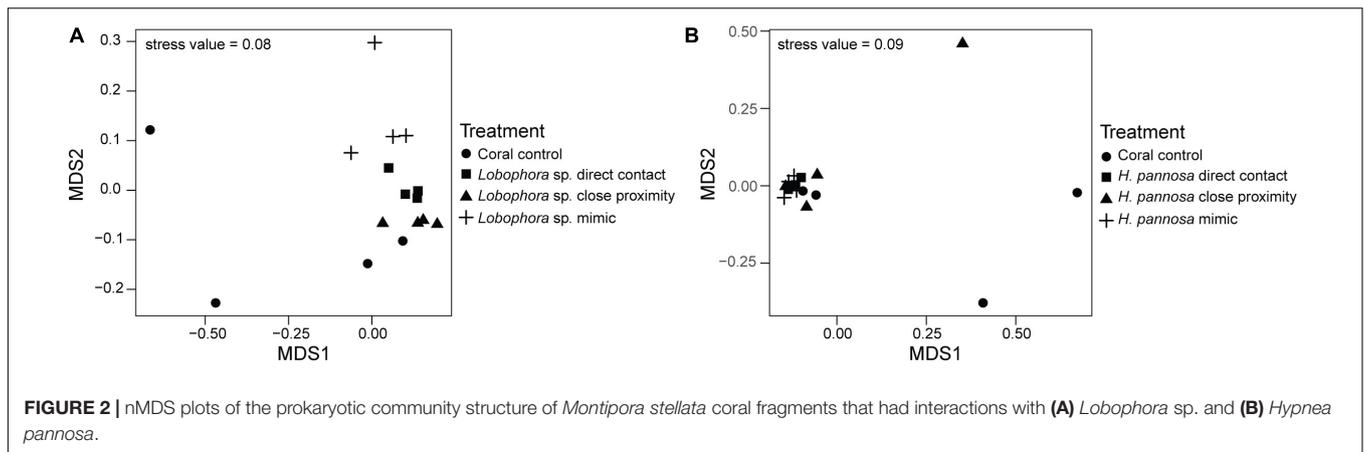
Generalized linear models identified significant differences among treatment groups and source reef consistent with the PERMANOVA results (Table 2). For *M. stellata*, 18.2% of the OTUs were significantly different in *Lobophora* sp. treatments while 8.2% of the OTUs were significantly different in *H. pannosa* treatments (Supplementary Data). For *P. acuta* and *M. ampliata*, GLM revealed that significant differences in the coral microbiomes were driven by the colony source reef (Table 2). In the *P. acuta* treatments, 19.6% of the OTUs were identified as contributing to the significant differences between the colonies from either Kusu Island or Pulau Satumu in response to *Lobophora* sp. treatments and 18.0% for *H. pannosa* treatments (Supplementary Data). For *M. ampliata*, the difference was attributed to 8.8% of the OTUs in the *Lobophora* sp. treatments and 13.0% for *H. pannosa* (Supplementary Data). However,

when accounting for only the highly abundant OTUs (>1%), the number of significant OTUs between treatment groups ranged from 3 to 11 OTUs (*M. stellata*: Table 3; *P. acuta* and *M. ampliata*: Table 4). Notably in the *M. stellata* treatments with *Lobophora* sp., the largest difference between control and algal treatments was due to a high abundance of the coral-associated *Endozoicomonas* sp. in the control samples (0.175 ± 0.247), compared to a lower relative abundance (0.031 ± 0.021) across all of the

TABLE 2 | PERMANOVA, PERMDISP, and GLMs results for the coral microbiome.

	PERMANOVA		PERMDISP		GLM
	Pseudo-F	P	F	P	P
1. Treatment					
a. <i>M. stellata</i>					
<i>Lobophora</i> sp.	1.68	<0.001	5.52	0.018	0.064
<i>H. pannosa</i>	1.28	0.015	9.03	0.092	0.019
b. <i>P. acuta</i>					
<i>Lobophora</i> sp.	0.97	0.557	5.53	0.219	0.013
<i>H. pannosa</i>	1.02	0.400	1.82	0.159	0.335
c. <i>M. ampliata</i>					
<i>Lobophora</i> sp.	1.05	0.246	3.71	0.183	0.115
<i>H. pannosa</i>	0.94	0.706	2.24	0.313	0.309
2. Source					
a. <i>P. acuta</i>					
<i>Lobophora</i> sp.	1.75	0.003	6.29	0.011	0.036
<i>H. pannosa</i>	1.72	0.016	1.80	0.023	0.284
b. <i>M. ampliata</i>					
<i>Lobophora</i> sp.	1.37	0.014	1.48	0.069	0.369
<i>H. pannosa</i>	1.67	0.006	9.10	0.023	0.025

The first set shows the differences in the coral microbiome by algal treatment (i.e., direct algal contact, close proximity to alga, algal mimic, control) for each coral and algal species. The second set shows the differences in the coral microbiome by the source reef of the coral colony for each coral and algal species. Bold values denote statistical significance when $\alpha = 0.05$.



algal treatment groups. The single most abundant, significant OTU was a *Desulfovibrio* sp. found in the *P. acuta* treatments originating from Kusu Island. *Pocillopora acuta* was also the only coral species to have *Archaea*, specifically *Thaumarchaeota* of the Order *Nitrosopumilus*, accounting for a large proportion of the difference between groups.

DISCUSSION

Competitive interactions between corals and macroalgae are becoming increasingly pervasive on coral reefs

(Hughes et al., 2010; Brown et al., 2018), yet, there remains considerable uncertainties regarding the extent to which macroalgae can impact corals, particularly within heavily disturbed reef systems. While direct macroalgal contact has largely been shown to harm corals, there is limited understanding regarding the water-mediated effects of macroalgae on corals. In this study, we found direct macroalgal contact caused tissue bleaching and reduced F_v/F_m of all three coral species, particularly when in direct contact with *Lobophora* sp., while macroalgae that were only in close proximity but not touching corals had no effects on coral physiology. Both contact- and water-mediated interactions with *Lobophora* sp. and *H. pannosa*

TABLE 3 | Highly abundant (>0.01 mean relative abundance of total community \pm SD) OTUs identified as contributing significantly to the differences between treatment groups for *Montipora stellata* interactions with *Lobophora* sp. and *Hypnea pannosa*.

OTU	Mean relative abundance				Phylum	Lowest taxonomic classification
	Control	Direct contact	Close proximity	Algal mimic		
<i>Lobophora</i> sp.						
3	0.006 \pm 0.006	0.078 \pm 0.067	0.037 \pm 0.033	0.080 \pm 0.062	Proteobacteria	Order Rhodobacteraceae
4	0.175 \pm 0.247	0.040 \pm 0.024	0.011 \pm 0.007	0.040 \pm 0.017	Proteobacteria	Endozoicomonas sp.
6	0.000 \pm 0.000	0.001 \pm 0.001	0.002 \pm 0.030	0.010 \pm 0.015	Proteobacteria	Order Erythrobacteraceae
14	0.007 \pm 0.003	0.026 \pm 0.023	0.008 \pm 0.003	0.023 \pm 0.009	Proteobacteria	Order Rhodobacteraceae
17	0.000 \pm 0.000	0.004 \pm 0.005	0.001 \pm 0.001	0.076 \pm 0.079	Bacteroidetes	Tenacibaculum sp.
22	0.008 \pm 0.007	0.012 \pm 0.015	0.001 \pm 0.001	0.034 \pm 0.029	Proteobacteria	Thalassotalea sp.
30	0.003 \pm 0.002	0.000 \pm 0.000	0.033 \pm 0.034	0.002 \pm 0.002	Unclassified	Bacteria
47	0.000 \pm 0.000	0.000 \pm 0.000	0.000 \pm 0.000	0.021 \pm 0.022	Proteobacteria	Class Gammaproteobacteria
48	0.028 \pm 0.032	0.002 \pm 0.003	0.002 \pm 0.002	0.000 \pm 0.000	Bacteroidetes	Phylum Bacteroidetes
60	0.001 \pm 0.001	0.004 \pm 0.003	0.001 \pm 0.001	0.019 \pm 0.012	Proteobacteria	Order Alteromonadaceae
<i>H. pannosa</i>						
21	0.003 \pm 0.001	0.006 \pm 0.002	0.024 \pm 0.020	0.005 \pm 0.003	Proteobacteria	Vibrio sp.
45	0.000 \pm 0.000	0.008 \pm 0.010	0.020 \pm 0.022	0.000 \pm 0.000	Proteobacteria	Order Alteromonadaceae
76	0.000 \pm 0.000	0.005 \pm 0.006	0.012 \pm 0.024	0.000 \pm 0.000	Proteobacteria	Thalassolituus sp.

Each OTU is listed to its lowest taxonomic classification.

altered the coral-associated microbiomes in *M. stellata*, while the effects on the microbiomes of *M. ampliata* and *P. acuta* were more strongly associated with the source reefs from which they were collected rather than algal treatments. Overall, our results reveal that, for the species tested, the coral microbiomes were sensitive to both direct contact and water-mediated interactions with macroalgae, while coral physiology was compromised only when in direct contact with macroalgae.

Coral fragments of *M. ampliata*, *M. stellata*, and *P. acuta* that were in direct contact with *Lobophora* sp. had visible tissue bleaching and approximately 30% reduction in F_v/F_m , whereas coral fragments that were in direct contact with *H. pannosa* exhibited a \sim 14% reduction. These results are consistent with patterns reported in previous studies showing *Lobophora* spp. were competitively superior to corals (Rasher and Hay, 2010; Morrow et al., 2012) whereas *H. pannosa* only caused minor damage to corals (Jompa and McCook, 2003). The damage caused by *Lobophora* sp. and *H. pannosa* was only observed in the area of direct contact, providing further evidence of the limited spatial impact of macroalgae on coral health (Rasher et al., 2011; Clements et al., 2018). This is in contrast with previous findings that show macroalgal waterborne compounds (e.g., dissolved organic compounds) could cause coral mortality (Kline et al., 2006; Smith et al., 2006). For instance, Smith et al. (2006) reported that *Pocillopora verrucosa* fragments placed next to the green alga *Dictyosphaeria cavernosa*, but not in direct contact, suffered 100% mortality within 2 days. Additionally in our experiment, coral tissue bleaching was only observed after 4 weeks (**Supplementary Figure 4**), compared to previous studies which have reported more immediate macroalgal impacts within the first few days to a week (e.g., Smith et al., 2006; Andras et al., 2012), or slightly longer period (e.g., 20 days in Rasher et al., 2011). These differences could be due to species-specific variability in coral susceptibility or macroalgal potency among the species tested

(McCook et al., 2001). Alternatively, lag effects of macroalgae on coral physiology observed in this study could be related to the highly urbanized nature of Singapore's reef environment; for instance corals from Singapore may be adapted to algal competition, but additional work is required to clarify this link.

Interactions with macroalgae also caused substantial changes to the coral microbiomes. Macroalgal treatments generally led to higher richness and diversity in the microbiomes of all coral species (**Supplementary Tables 1–3**), which are consistent with previous research suggesting coral stress can result in higher microbiome beta diversity (McDevitt-Irwin et al., 2017; Zaneveld et al., 2017). For *M. stellata*, the extent of changes to the microbiome differed between the *Lobophora* sp. and *H. pannosa* treatments. Coral microbiomes that were in contact or close proximity to *Lobophora* sp. were significantly different from the control and algal mimic treatments, indicating that *Lobophora* sp. was able to impact the coral microbiomes through indirect interactions. The genus *Lobophora* is known to contain allelopathic chemicals that can harm corals (Vieira et al., 2016; Evensen et al., 2019), including the species in this study (Fong et al., 2019). Crude extracts from *Lobophora* have also been found to display strong antimicrobial activities (Morrow et al., 2012). Some studies have shown that macroalgae can indirectly impact coral microbiomes that are several centimeters away via water-mediated macroalgal compounds and microbes (Barott and Rohwer, 2012; Morrow et al., 2013; Pratte et al., 2018). For example, Morrow et al. (2013) reported that *Halimeda opuntia* and *Dictyota menstrualis* altered bacterial communities associated with *Montastraea faveolata* that were up to 5 cm away from the direct macroalgal contact zone. Similarly, Pratte et al. (2018) found significant shifts in the microbial communities of *Porites* sp. 5 cm from turf contact, although there were more pronounced shifts in direct contact zone. While the allelopathic effects of *Lobophora* sp. has been established (Fong et al., 2019), it

TABLE 4 | Highly abundant (>0.01 mean relative abundance of total community \pm SD) OTUs identified as contributing significantly to the differences between Pulau Hantu and Kusu Island for each coral and algal species interaction.

OTU	Mean relative abundance		Phylum	Lowest taxonomic classification
	Pulau Satumu	Kusu Island		
<i>P. acuta-Lobophora</i> sp.				
1	0.005 \pm 0.007	0.241 \pm 0.273	Proteobacteria	<i>Desulfovibrio</i> sp.
8	0.060 \pm 0.037	0.002 \pm 0.002	Unclassified	Bacteria
12	0.025 \pm 0.026	0.004 \pm 0.004	Thaumarchaeota	Order Nitrosopumilus
15	0.000 \pm 0.001	0.037 \pm 0.050	Chlorobi	<i>Prosthecochloris</i> sp.
27	0.012 \pm 0.008	0.003 \pm 0.004	Proteobacteria	Class Gammaproteobacteria
<i>P. acuta-H. pannosa</i>				
8	0.023 \pm 0.018	0.004 \pm 0.003	Unclassified	Bacteria
24	0.016 \pm 0.009	0.003 \pm 0.003	Thaumarchaeota	Order Nitrosopumilus
27	0.011 \pm 0.006	0.003 \pm 0.003	Proteobacteria	Class Gammaproteobacteria
31	0.020 \pm 0.023	0.001 \pm 0.001	Bacteroidetes	Family Flavobacteriales
<i>M. ampliata-Lobophora</i> sp.				
4	0.043 \pm 0.048	0.000 \pm 0.000	Bacteroidetes	Order Flavobacteriaceae
5	0.00 \pm 0.000	0.046 \pm 0.069	Proteobacteria	Class Gammaproteobacteria
8	0.000 \pm 0.000	0.030 \pm 0.048	Bacteroidetes	Order Flavobacteriaceae
11	0.000 \pm 0.001	0.021 \pm 0.024	Proteobacteria	Class Gammaproteobacteria
12	0.036 \pm 0.073	0.002 \pm 0.002	Proteobacteria	<i>Arenicella</i> sp.
14	0.005 \pm 0.007	0.028 \pm 0.033	Proteobacteria	<i>Ruegeria</i> sp.
17	0.001 \pm 0.002	0.017 \pm 0.025	Proteobacteria	Class Alphaproteobacteria
<i>M. ampliata-H. pannosa</i>				
4	0.074 \pm 0.085	0.000 \pm 0.000	Bacteroidetes	Order Flavobacteriaceae
5	0.001 \pm 0.002	0.077 \pm 0.088	Proteobacteria	Class Gammaproteobacteria
6	0.031 \pm 0.038	0.000 \pm 0.000	Proteobacteria	<i>Ralstonia</i> sp.
8	0.00 \pm 0.000	0.040 \pm 0.047	Bacteroidetes	Order Flavobacteriaceae
11	0.003 \pm 0.006	0.023 \pm 0.022	Proteobacteria	Class Gammaproteobacteria
12	0.047 \pm 0.076	0.002 \pm 0.002	Proteobacteria	<i>Arenicella</i> sp.
14	0.002 \pm 0.002	0.023 \pm 0.021	Proteobacteria	<i>Ruegeria</i> sp.
17	0.001 \pm 0.001	0.021 \pm 0.026	Proteobacteria	Class Alphaproteobacteria
25	0.023 \pm 0.039	0.000 \pm 0.000	Bacteroidetes	Order Flavobacteriaceae
27	0.000 \pm 0.000	0.015 \pm 0.026	Proteobacteria	Class Gammaproteobacteria
36	0.011 \pm 0.019	0.000 \pm 0.000	Proteobacteria	<i>Salmonella</i> sp.

Each OTU is listed to its lowest taxonomic classification. Note that OTU identification numbers correspond to different taxa for each coral species.

remains unclear what role the algal microbiome may have played in the strong response of the coral microbiomes to the presence of *Lobophora* sp., particularly given that the *M. stellata* colonies and *Lobophora* sp. were collected from two different reefs. Future studies should examine the effects of site-specific algal microbial communities on coral microbiomes. In the *H. pannosa* treatments, *M. stellata* microbiomes were more similarly affected by the direct contact of live and mimic algae than by the presence of algae nearby, suggesting that the changes in the coral microbiomes were more strongly driven by the effects of physical contact. In contrast to the leathery surface of *Lobophora* sp., the corticated thallus of *H. pannosa* might inflict surface abrasions on corals triggering shifts in the coral microbiomes (Mydlarz et al., 2006). Nevertheless, the presence of *H. pannosa* on coral reefs appears to be less harmful to the coral holobiont than the presence of a strongly allelopathic alga as the impact is restricted to direct interactions.

While *Lobophora* sp. and *H. pannosa* induced shifts in the microbial communities of *M. ampliata* and *P. acuta*, the

source reefs from where the coral colonies were collected (Pulau Satumu and Kusu Island) had a greater effect. The differences in the coral microbiomes between the two reefs were first identified in the field samples collected from Kusu Island, which had proportionately higher abundance of potentially human-associated pathogens in their microbiomes than those collected from Pulau Satumu. For example, the most abundant taxon in the two *P. acuta* colonies collected from Kusu Island was *Salmonella* sp. and, in one of the *M. ampliata* colonies from Kusu Island, the most abundant taxon was *Streptococcus* sp. Closer proximity to human influences, i.e., the main island of Singapore (~4 km for Kusu Island versus ~13 km for Pulau Satumu) might explain why the microbial communities on the corals collected from Kusu Island were dominated by bacterial genera associated with human pathogens (Kaczmarek et al., 2005; Dinsdale et al., 2008). Differences in environmental conditions among reefs, such as light, nutrients, and water currents, can also have a strong influence on the specificity of coral microbiome (Klaus et al., 2007; Apprill and Rappé, 2011; Pantos et al., 2015).

Ng et al. (2019) reported that water motion was significantly greater at Kusu Island than Pulau Satumu. Our results were also consistent with patterns reported by Wainwright et al. (2019), who found that the bacterial communities associated with *P. acuta* collected from Kusu Island were markedly different from those collected Pulau Satumu. Nevertheless, despite higher levels of potentially pathogenic bacteria, *P. acuta* and *M. ampliata* from Kusu Island had the same physiological response to environmental perturbation (interaction with algae) as corals from Pulau Satumu. This is in contrast with the patterns observed in the Line Islands of the Central Pacific, where reefs closer to human influences, which presumably have higher prevalence of pathogens and coral disease, were poorer competitors against algae compared to corals from more pristine habitats (Dinsdale et al., 2008; Barott and Rohwer, 2012; Barott et al., 2012).

Only direct contact with macroalgae initiated a physiological response in the corals, while we found evidence of water-mediated effects of *Lobophora* sp. and *H. pannosa* on the microbiome. Coral microbiomes have been shown to be highly dynamic and capable of responding to environmental perturbations, particularly those induced by the presence of macroalgae, and their diffusible compounds (i.e., chemicals and dissolved organic carbon) and microbes (Ainsworth et al., 2010; Bourne et al., 2016). It has been proposed that the coral microbiome may be composed of a small conserved core while the majority of microbes in the microbiome change routinely in response to environmental conditions with little negative effect on the functioning of the coral holobiont (Hernandez-Agreda et al., 2018). While our ability to determine a core microbiome for *P. acuta* and *M. ampliata* is limited by the size of the current study, the shared proportion of prokaryotic symbionts for each species within each reef was small, with 31 OTUs shared among the four *P. acuta* control colonies and 27 OTUs shared among the four *M. ampliata* control colonies (Supplementary Table 4). Given the variability that exists in their natural microbiomes, it is not surprising to see variability in the response of those microbiomes to the same algal treatments. However, some caution is required when interpreting our results because coral microbiomes were likely different between *ex situ* and *in situ* samples, as evidenced in *M. stellata* fragments, and this might influence the effects of macroalgae on coral microbial communities. Further investigations would be required to (1) compare the impacts of macroalgae on coral microbiomes under different environmental settings, and (2) determine whether a core or resident microbiome is maintained for each of the coral species examined here and the specific symbiotic benefits imparted to the coral from that microbiome.

Overall, this study provides important insights into the impacts of direct contact and water-mediated interactions with macroalgae on corals and their microbiomes. Importantly, our results demonstrate that contact- and water-mediated

interactions with *Lobophora* sp. and *H. pannosa* altered coral-associated microbiomes in corals, but only direct macroalgal contact resulted in reduced coral health. Notably, the presence of high levels of bacterial genera associated with human pathogens in some of the coral samples did not lead to the corals being more susceptible to macroalgal competition.

DATA AVAILABILITY STATEMENT

The raw sequence data were cataloged in the NCBI Sequence Read Archive under BioProject accession number PRJNA548204.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study, manuscript revision, read, and approved the submitted version. JF carried out the experiment and conducted the coral physiology assessment. LD conducted the coral microbiome work. JF and LD performed the statistical analysis and wrote the first draft of the manuscript.

FUNDING

This study was supported by the National Research Foundation, Prime Minister's Office, Singapore under the Marine Science Research and Development Programme (MSRDP-P03), Wildlife Reserves Singapore Conservation Fund, and the Ministry of Education, Singapore under its Research Centre of Excellence Program to the Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University.

ACKNOWLEDGMENTS

We thank Zhiyuan Chen and Jing Jie Teh for their assistance with the experiment. We acknowledge the St. John's Island National Marine Laboratory, a National Research Infrastructure under the National Research Foundation Singapore, for providing the facility necessary for conducting the research. All research was carried out with permission of the Singapore National Parks Board (Permit no. NP/RP17-046).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00831/full#supplementary-material>

REFERENCES

- Ainsworth, T. D., Thurber, R. V., and Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends Ecol. Evol.* 25, 233–240. doi: 10.1016/j.tree.2009.11.001
- Andras, T. D., Alexander, T. S., Gahlana, A., Parry, R. M., Fernandez, F. M., Kubanek, J., et al. (2012). Seaweed allelopathy against coral: surface distribution of a seaweed secondary metabolite by imaging mass spectrometry. *J. Chem. Ecol.* 38, 1203–1214. doi: 10.1007/s10886-012-0204-9

- Apprill, A., and Rappé, M. S. (2011). Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii, USA. *Aquat. Microb. Ecol.* 62, 251–266. doi: 10.3354/ame01471
- Barott, K. L., and Rohwer, F. L. (2012). Unseen players shape benthic competition on coral reefs. *Trends Microbiol.* 20, 621–628. doi: 10.1016/j.tim.2012.08.004
- Barott, K. L., Williams, G. J., Vermeij, M. J. A., Harris, J., Smith, J. E., Rohwer, F. L., et al. (2012). Natural history of coral–algae competition across a gradient of human activity in the Line Islands. *Mar. Ecol. Prog. Ser.* 460, 1–12. doi: 10.3354/meps09874
- Bauman, A. G., Hoey, A. S., Dunshea, G., Feary, D. A., Low, J., and Todd, P. A. (2017). Macroalgal browsing on a heavily degraded, urbanized equatorial reef system. *Sci. Rep.* 7:8352. doi: 10.1038/s41598-017-08873-3
- Bellwood, D. R., Hughes, T. P., Folke, C., and Nyström, M. (2004). Confronting the coral reef crisis. *Nature* 429, 827–833. doi: 10.1038/nature02691
- Birrell, C. L., McCook, L. J., Willis, B. L., and Diaz-Pulido, G. A. (2008). Effects of benthic algae on the replenishment of corals and the implications for the resilience of coral reefs. *Oceanogr. Mar. Biol.* 46, 25–63. doi: 10.1201/9781420065756.ch2
- Bonaldo, R. M., and Hay, M. E. (2014). Seaweed–coral interactions: variance in seaweed allelopathy, coral susceptibility, and potential effects on coral resilience. *PLoS One* 9:e85786. doi: 10.1371/journal.pone.0085786
- Bourne, D. G., Morrow, K. M., and Webster, N. S. (2016). Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annu. Rev. Microbiol.* 70, 317–340. doi: 10.1146/annurev-micro-102215-095440
- Box, S. J., and Mumby, P. J. (2007). Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. *Mar. Ecol. Prog. Ser.* 342, 139–149. doi: 10.3354/meps342139
- Brown, K. T., Bender-Champ, D., Kubicek, A., van der Zande, R., Achlatis, M., Hoegh-Guldberg, O., et al. (2018). The dynamics of coral–algal interactions in space and time on the southern great barrier reef. *Front. Mar. Sci.* 5:181. doi: 10.3389/fmars.2018.00181
- Browne, N. K., Tay, J. K. L., Low, J., Larson, O., and Todd, P. A. (2015). Fluctuations in coral health of four common inshore reef corals in response to seasonal and anthropogenic changes in water quality. *Mar. Environ. Res.* 105, 39–52. doi: 10.1016/j.marenvres.2015.02.002
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4516–4522. doi: 10.1073/pnas.1000080107
- Clements, C. S., Rasher, D. B., Hoey, A. S., Bonito, V. E., and Hay, M. E. (2018). Spatial and temporal limits of coral–macroalgal competition: the negative impacts of macroalgal density, proximity, and history of contact. *Mar. Ecol. Prog. Ser.* 586, 11–20. doi: 10.3354/meps12410
- Dinsdale, E. A., Pantos, O., Smriga, S., Edwards, R. A., Angly, F., Wegley, L., et al. (2008). Microbial ecology of four coral atolls in the Northern Line Islands. *PLoS One* 3:e1584. doi: 10.1371/journal.pone.0001584
- Evensen, N. R., Doropoulos, C., Morrow, K. M., Motti, C. A., and Mumby, P. J. (2019). Inhibition of coral settlement at multiple spatial scales by a pervasive algal competitor. *Mar. Ecol. Prog. Ser.* 612, 29–42. doi: 10.3354/meps12879
- Fitt, W. K., Brown, B. E., Warner, M. E., and Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* 20, 51–65. doi: 10.1007/s003380100146
- Fong, J., Lim, Z. W., Bauman, A. G., Valiyaveetil, S., Liao, L. M., Yip, Z. T., et al. (2019). Allelopathic effects of macroalgae on *Pocillopora acuta* coral larvae. *Mar. Environ. Res.* 151:104745. doi: 10.1016/j.marenvres.2019.06.007
- Guest, J. R., Tun, K., Low, J., Vergés, A., Marzinelli, E. M., Campbell, A. H., et al. (2016). 27 years of benthic and coral community dynamics on turbid, highly urbanised reefs off Singapore. *Sci. Rep.* 6:36260. doi: 10.1038/srep36260
- Haas, A. F., Nelson, C. E., Rohwer, F., Wegley-Kelly, L., Quistad, S. D., Carlson, C. A., et al. (2013). Influence of coral and algal exudates on microbially mediated reef metabolism. *PeerJ* 1:e108. doi: 10.7717/peerj.108
- Haas, A. F., Nelson, C. E., Wegley Kelly, L., Carlson, C. A., Rohwer, F., Leichter, J. J., et al. (2011). Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. *PLoS One* 6:e27973. doi: 10.1371/journal.pone.0027973
- Heery, E. C., Hoeksema, B. W., Browne, N. K., Reimer, J. D., Ang, P. O., Huang, D., et al. (2018). Urban coral reefs: degradation and resilience of hard coral assemblages in coastal cities of East and Southeast Asia. *Mar. Pollut. Bull.* 135, 654–681. doi: 10.1016/j.marpolbul.2018.07.041
- Hernandez-Agreda, A., Leggat, W., Bongaerts, P., Herrera, C., and Ainsworth, T. D. (2018). Rethinking the coral microbiome: simplicity exists within a diverse microbial biosphere. *mBio* 9, e812–e818. doi: 10.1128/mBio.00812-18
- Hooper, D. U., Chapin, F. S. III, Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., et al. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3–35. doi: 10.1890/04-0922
- Huang, D., Tun, K. P. P., Chou, K. M., and Todd, P. A. (2009). An inventory of zooxanthellate scleractinian corals in Singapore, including 33 new records. *Raffles Bull. Zool.* 22, 69–80.
- Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., et al. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* 259, 80–83. doi: 10.1126/science.aan8048
- Hughes, T. P., Graham, N. A. J., Jackson, J. B. C., Mumby, P. J., and Steneck, R. S. (2010). Rising to the challenge of sustaining coral reef resilience. *Trends Ecol. Evol.* 25, 633–642. doi: 10.1016/j.tree.2010.07.011
- Jompa, J., and McCook, L. J. (2002). Effects of competition and herbivory on interactions between a hard coral and a brown alga. *J. Exp. Mar. Biol. Ecol.* 271, 25–39. doi: 10.1016/S0022-0981(02)00040-0
- Jompa, J., and McCook, L. J. (2003). Coral–algal competition: macroalgae with different properties have different effects on corals. *Mar. Ecol. Prog. Ser.* 258, 87–95. doi: 10.3354/meps258087
- Jorissen, H., Skinner, C., Osinga, R., De Beer, D., and Nugues, M. M. (2016). Evidence for water-mediated mechanisms in coral–algal interactions. *Proc. R. Soc. B* 283:20161137. doi: 10.1098/rspb.2016.1137
- Kaczmarek, L. T., Draud, M. A., and Williams, E. H. (2005). Is there a relationship between proximity to sewage effluent and the prevalence of coral disease? *Caribb. J. Sci.* 41, 124–137.
- Klaus, J. S., Janse, I., Heikoop, J. M., Sanford, R. A., and Fouke, B. W. (2007). Coral microbial communities, zooxanthellae and mucus along gradients of seawater depth and coastal pollution. *Environ. Microbiol.* 9, 1291–1305. doi: 10.1111/j.1462-2920.2007.01249.x
- Kline, D. I., Kuntz, N. M., Breitbart, M., Knowlton, N., and Rohwer, F. (2006). Role of elevated organic carbon levels and microbial activity in coral mortality. *Mar. Ecol. Prog. Ser.* 314, 119–125. doi: 10.3354/meps314119
- Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* 79, 5112–5120. doi: 10.1128/AEM.01043-13
- Lema, K. A., Willis, B. L., and Bourne, D. G. (2012). Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Appl. Environ. Microbiol.* 78, 3136–3144. doi: 10.1128/AEM.07800-11
- Lesser, M. P., Mazel, C. H., Gorbunov, M. Y., and Falkowski, P. G. (2004). Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science* 305, 997–1000. doi: 10.1126/science.1099128
- Low, J. K. Y., Fong, J., Todd, P. A., Chou, L. M., and Bauman, A. G. (2019). Seasonal variation of *Sargassum ilicifolium* (Phaeophyceae) growth on equatorial coral reefs. *J. Phycol.* 55, 289–296. doi: 10.1111/jpy.12818
- McCook, L. J., Jompa, J., and Diaz-Pulido, G. (2001). Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* 19, 400–417. doi: 10.1007/s003380000129
- McDevitt-Irwin, J. M., Baum, J. K., Garren, M., and Vega Thurber, R. L. (2017). Responses of coral-associated bacterial communities to local and global stressors. *Front. Mar. Sci.* 4:262. doi: 10.3389/fmars.2017.00262
- Miller, M. W. (1998). Coral/seaweed competition and the control of reef community structure within and between latitudes. *Oceanogr. Mar. Biol.* 36, 65–96.
- Morrow, K. M., Liles, M. R., Paul, V. J., Moss, A. G., and Chadwick, N. E. (2013). Bacterial shifts associated with coral–macroalgal competition in the Caribbean Sea. *Mar. Ecol. Prog. Ser.* 488, 103–117. doi: 10.3354/meps10394
- Morrow, K. M., Paul, V. J., Liles, M. R., and Chadwick, N. E. (2011). Allelochemicals produced by Caribbean macroalgae and Cyanobacteria have species-specific effects on reef coral microorganisms. *Coral Reefs* 30, 309–320. doi: 10.1007/s00338-011-0747-1

- Morrow, K. M., Ritson-Williams, R., Ross, C., Liles, M. R., and Paul, V. J. (2012). Macroalgal extracts induce bacterial assemblage shifts and sublethal tissue stress in Caribbean corals. *PLoS One* 7:e44859. doi: 10.1371/journal.pone.0044859
- Mydlarz, L. D., Jones, L. E., and Harvell, C. D. (2006). Innate immunity, environmental drivers, and disease ecology of marine and freshwater invertebrates. *Annu. Rev. Ecol. Evol. Syst.* 37, 251–288. doi: 10.1146/annurev.ecolsys.37.091305.110103
- Ng, C. S. L., Lim, J. X., Sam, S. Q., Kikuzawa, Y. P., Toh, T. C., Wee, T. W., et al. (2019). Variability in skeletal bulk densities of common hard corals in Southeast Asia. *Coral Reefs* 38, 1133–1143. doi: 10.1007/s00338-019-01852-2
- Nugues, M. M., and Bak, R. P. (2006). Differential competitive abilities between Caribbean coral species and a brown alga: a year of experiments and a long-term perspective. *Mar. Ecol. Prog. Ser.* 315, 75–86. doi: 10.3354/meps315075
- Nugues, M. M., Smith, G. W., Van Hoodonk, R. J., Seabra, M. I., and Bak, R. P. M. (2004). Algal contact as a trigger for coral disease. *Ecol. Lett.* 7, 919–923. doi: 10.1111/j.1461-0248.2004.00651.x
- Pantos, O., Bongaerts, P., Dennis, P. G., Tyson, G. W., and Hoegh-Guldberg, O. (2015). Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*. *ISME J.* 9, 1916–1927. doi: 10.1038/ismej.2015.3
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team, (2019). *Nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-142*.
- Pratte, Z. A., Longo, G. O., Burns, A. S., Hay, M. E., and Stewart, F. J. (2018). Contact with turf algae alters the coral microbiome: contact versus systemic impacts. *Coral Reefs* 37, 1–3. doi: 10.1007/s00338-017-1615-4
- R Core Team, (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Ralph, P. J., Gademann, R., Larkum, A. W. D., and Schreiber, U. (1999). In situ underwater measurements of photosynthetic activity of coral zooxanthellae and other reef-dwelling dinoflagellate endosymbionts. *Mar. Ecol. Prog. Ser.* 180, 139–147. doi: 10.3354/meps180139
- Rasher, D. B., and Hay, M. E. (2010). Chemically rich seaweeds poison corals when not controlled by herbivores. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9683–9688. doi: 10.1073/pnas.0912095107
- Rasher, D. B., Stout, E. P., Engel, S., Kubanek, J., and Hay, M. E. (2011). Macroalgal terpenes function as allelopathic agents against reef corals. *Proc. Natl. Acad. Sci. U.S.A.* 108, 17725–17731. doi: 10.1073/pnas.1108628108
- Ritchie, K. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar. Ecol. Prog. Ser.* 322, 1–14. doi: 10.3354/meps322001
- River, G. F., and Edmunds, P. J. (2001). Mechanisms of interaction between macroalgae and scleractinians on a coral reef in Jamaica. *J. Exp. Mar. Biol. Ecol.* 261, 159–172. doi: 10.1016/S0022-0981(01)00266-0
- Roach, T. N., Abieri, M. L., George, E. E., Knowles, B., Naliboff, D. S., Smurthwaite, C. A., et al. (2017). Microbial bioenergetics of coral-algal interactions. *PeerJ* 5:e3423. doi: 10.7717/peerj.3423
- Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nat. Rev. Microbiol.* 5, 355–362. doi: 10.1038/nrmicro1635
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. doi: 10.1128/AEM.01541-09
- Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to imageJ: 25 years of image analysis. *Nat. Methods* 9:671. doi: 10.1038/nmeth.2089
- Smith, J. E., Shaw, M., Edwards, R. A., Obura, D., Pantos, O., Sala, E., et al. (2006). Indirect effects of algae on coral: algae-mediated, microbe-induced coral mortality. *Ecol. Lett.* 9, 835–845. doi: 10.1111/j.1461-0248.2006.00937.x
- Tanner, J. E. (1995). Competition between scleractinian corals and macroalgae: an experimental investigation of coral growth, survival and reproduction. *J. Exp. Mar. Biol. Ecol.* 90, 151–168. doi: 10.1016/0022-0981(95)00027-O
- Thurber, R. V., Burkepile, D. R., Correa, A. M. S., Thurber, A. R., Shantz, A. A., Welsh, R., et al. (2012). Macroalgae decrease growth and alter microbial community structure of the reef-building coral, *Porites astreoides*. *PLoS One* 7:e44246. doi: 10.1371/journal.pone.0044246
- Vieira, C., Engelen, A. H., Guentas, L., Aires, T., Houllbreque, F., Gaubert, J., et al. (2016). Species specificity of bacteria associated to the brown seaweeds *Lobophora* (Dictyotales, Phaeophyceae) and their potential for induction of rapid coral bleaching in *Acropora muricata*. *Front. Microbiol.* 7:316. doi: 10.3389/fmicb.2016.00316
- Wainwright, B. J., Afiq-Rosli, L., Zahn, G. L., and Huang, D. (2019). Characterisation of coral-associated bacterial communities in an urbanised marine environment shows strong divergence over small spatial scales. *Coral Reefs* 38, 1097–1106. doi: 10.1007/s00338-019-01837-1
- Wang, Y., Naumann, U., Wright, S. T., and Warton, D. I. (2012). mvabund—an R package for model-based analysis of multivariate abundance data. *Methods Ecol. Evol.* 3, 471–474. doi: 10.1111/j.2041-210X.2012.00190.x
- Yodanis, P. (1978). *Competition for Space and the Structure of Ecological Communities*. Berlin: Springer.
- Zaneveld, J., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat. Microbiol.* 2:17121. doi: 10.1038/nmicrobiol.2017.121

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Fong, Deignan, Bauman, Steinberg, McDougald and Todd. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.