

LETTER

Giant kelp microbiome altered in the presence of epiphytes

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Scientific Significance Statement

Characterizing patterns in the microbial communities that live in close association with macroalgae is critical to understanding the role of microbes in shaping fundamental aspects of marine ecosystems. Here, we provide the first evidence to indicate that the giant kelp microbiome shifts in the presence of encrusting invertebrates on the surface of kelp laminae (epiphytes). We observed these shifts across two kelp beds in the Santa Barbara Channel over a 4-month period, indicating a broad ecological impact of invertebrate settlement on giant kelp microbiomes. This study highlights the need to better understand the mechanisms driving changes in macroalgal microbiomes to predict the impact of epiphytic communities on algal health and development.

Abstract

Characterizing patterns in the microbial communities associated with canopy-forming kelps is critical to understanding the mechanisms shaping macroalgal microbiomes. Using 16S rRNA gene sequencing, we characterized bacterial and archaeal communities associated with giant kelp in the Santa Barbara Channel. Our results indicate that kelp-associated microbial communities are altered in the presence of epiphytic bryozoans and reflect changes previously observed in other studies of stressed macroalgae. This observation is indicative of patterns of microbiome disruption (dysbiosis) and opportunistic pathogenesis that may have implications for the health and productivity of foundational species of macroalgae. Our study highlights new patterns in host–microbiome associations and reveals the importance of considering host source and condition when investigating the dynamics of macroalgal microbiomes.

Kelp forests provide critical resources such as food and shelter for numerous coastal marine species (Foster and Schiel 1985). These canopy-forming macroalgae contribute substantially to

coastal biogeochemistry and marine capture of atmospheric carbon dioxide through high rates of primary production (see review by Reed et al. 2009 and citations therein). Recent

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Data Availability Statement: Data and metadata are available in the Sequence Read Archive data repository through NCBI (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA612503>). All of the code for this work is publicly available in the GitHub repository (https://github.com/ajamesk10/KELP_SBC_16S_2018).

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research indicates that host-associated microbiomes shape the health and productivity of diverse marine organisms (Peixoto et al. 2017; Singh and Reddy 2016; Wilkins et al. 2019). Yet, despite the ecological and economic importance of kelp forests to coastal ecosystems, we are just beginning to investigate the diversity and function of macroalgal microbiomes. Uncovering patterns in the microbial communities associated with these foundational species will help to elucidate how the microbiome mediates the response of macroalgae to the many stressors facing coastal marine ecosystems.

Previous work has established the ability of giant kelp, *Macrocystis pyrifera*, to develop microbial communities that are distinct from the surrounding water column (Michelou et al. 2013). These communities are enriched in bacteria from the *Planctomycetes* and *Verrucomicrobia* clades which include members that often secrete antimicrobial secondary metabolites, and thus likely minimize biofouling on kelp laminae (e.g. Jeske et al. 2013; Vollmer et al. 2017). In a recent study examining the effects of rising temperatures and ocean acidification on the microbiome of the brown alga, *Ecklonia radiata*, laminae exposed to future ocean conditions exhibited reduced relative abundances (RA) of such microbial taxa with potential antibiofouling abilities (Qiu et al. 2019). These laminae displayed substantial blistering that likely depressed host photosynthetic activity relative to healthy laminae by reducing the surface area available for photosynthesis. This study suggests that host stress, induced prior to or following blistering of the laminae, may have consequences for the kelp microbiome and overall host health.

Similar to blistering in *E. radiata*, settlement of encrusting bryozoans on kelp laminae can negatively impact the kelps

that serve as their substrate, reducing light availability and decreasing the area available for photosynthesis (Cancino et al. 1987). In addition, settlement of bryozoans can weigh down kelp laminae, increasing the laminae's susceptibility to degradation and breakage in the presence of physical perturbations (Lambert et al. 1992). Despite the critical role of giant kelp forests in coastal ecosystems, and the potential for encrusting bryozoans to induce host stress, there have been no characterizations of how macroalgal microbiomes respond to the presence of epiphytic bryozoans prior to our work.

Here, we present a comparative study of the microbial communities found on giant kelp (*M. pyrifera*) laminae with and without the encrusting bryozoan, *Membranipora serilamella*. Our results from two kelp beds located along the Santa Barbara Channel reveal a significant difference in the microbial communities found on laminae lacking visual signs of bryozoans, compared to those where greater than 20% of the laminae surface area was encrusted by bryozoans. This work provides new insights into how epiphytes can disrupt the microbiome (dysbiosis), and the ecological processes shaping the microbiome of canopy-forming kelps.

Methods

We sampled seawater and the epibiotic microbial communities of kelp laminae (the “kelp microbiome”) at two kelp beds in the Santa Barbara Channel: Arroyo Quemado (AQ; 34°28.127'N, 120°07.285'W) and Mohawk Reef (MR; 34°23.660'N, 119°43.800'W), from May to August, 2018 (Fig. 1). These kelp forests range in size from approximately 300 m (MR) to 1500 m (AQ) in length, and 120–300 m in



Fig. 1. Map indicating sampling locations at AQ (purple) and MR (yellow) kelp beds in the Santa Barbara Channel. Sampling was conducted at AQ in June and July, 2018, and at MR during May and August, 2018. Insert highlights the visual differences in laminae lacking bryozoans (healthy), compared with those containing $\geq 20\%$ bryozoan coverage (bryozoan) at a single site within the MR kelp bed during August 2018.

width. Mean water depths range from 5 to 6 m along the inshore edge of the forests and 9 m (MR) to 10.7 m (AQ) at the offshore edge. In addition to giant kelp, both reefs are characterized by a diversity of understory algae, invertebrates, mobile grazers and predators, and contain numerous fish species, as is common of many kelp forest communities (Foster and Schiel 1985).

Sampling

MR was sampled during May and August (2018), and AQ during June and July (2018). For each sampling period, we used stand-up-paddle boards to sample microbial communities on kelp laminae from the surface canopy. At each site, we sampled two to three laminae per frond located approximately 2 m from the frond tip. Sampled laminae were separated into two categories based on the presence or absence of encrusting bryozoan communities: laminae lacking visual signs of bryozoans are hereafter referred to as *healthy laminae*, while those visually identified as having $\geq 20\%$ bryozoan coverage are referred to as *bryozoan laminae* (see Fig. 1 insert). Where possible, we sampled three laminae from both a healthy frond and a bryozoan frond at a single reef site, in order to minimize the effects of reef site on the comparison (see Fig. 1 insert). In total, we sampled 63 kelp laminae: 4 from MR in May (all healthy as there were no signs of bryozoans at this time), 29 from AQ in June, 18 from AQ in July, and 12 from MR in August.

The kelp laminae were placed on a hard-plastic surface for sampling. To dislodge microbial communities from the laminae surfaces, we used a device called a supersucker (Lim et al. 2014). The supersucker functions as a closed-circuit syringe, dislodging microbial communities from the kelp laminae by pumping double 0.2- μm filter (polyethersulfone filter; Supor-200, Pall) sterilized artificial seawater (0.4 mol L⁻¹ NaCl, 0.02 mol L⁻¹ Na₂SO₄, 0.01 mol L⁻¹ KCl, 0.05 mol L⁻¹ MgCl₂, plus 0.01 mol L⁻¹ CaCl₂) at the laminae. Once dislodged, the microbial communities in artificial seawater were filtered onto a 0.2- μm polyethersulfone filter cartridge (Sterivex-GP, Millipore). For samples collected at AQ in July, microbial communities were filtered onto a 0.2- μm polyethersulfone filter (Supor-200, Pall). Because we did not have as many supersucker devices as laminae or fronds sampled, the supersuckers were rinsed with roughly 50 mL of artificial seawater and completely emptied between uses.

We used the supersuckers to sample three conserved locations along each lamina (directly adjacent to the nematocyst, in the middle of the lamina, and at the end of the lamina) to ensure potential differences in communities along the laminae did not interfere with our comparison between healthy and bryozoan laminae. These three samples were all filtered onto a single filter, such that each filter represents the integrated microbial communities across each of the sampled laminae. Sterivex filters were capped with autoclaved luer-lock plugs and placed onto ice for transport back to the University

of California, Santa Barbara; flat filters used at AQ in July were held in sterile cryogenic vials. Filters were loaded with 1 mL of sucrose lysis buffer (40 mM ethylene diamine tetra acetic acid, 50 mM Tris-hydrochloric acid (HCl), 750 mM sucrose, 400 mM NaCl, pH adjusted to 8.0) and placed at -40°C once back at the laboratory.

To assess differences in the microbial communities found on laminae from those in the surrounding seawater, we collected 1.2 L of seawater into acid-washed (10% HCl) polycarbonate biotainers (ThermoFisher Scientific) at locations within and near the kelp beds. This water was transported back to the laboratory at UC Santa Barbara and filtered onto 0.2- μm polyethersulfone filters (Supor-200, Pall) and placed into sterile cryogenic vials. Filters were then frozen at -40°C .

DNA extraction and 16S rRNA gene sequencing

Samples were lysed in sucrose lysis buffer with 1% wt/vol sodium dodecyl sulfate and 0.2 mg mL⁻¹ proteinase-K at 55°C for 2 h. Genomic DNA was extracted and purified using a modified spin-column filtration method (Macherey-Nagel) described previously (Kelly et al. 2014).

Gene amplicons of the V4 region of the bacterial 16S rRNA gene were generated using 515F (GTGYCAGCMGCCGCGGTAA) and 806R-B (GGACTACNVGGGTWTCTAAT) primers as described by Wear et al. (2018). Briefly, the V4 region was amplified in duplicate 25 μL reactions via polymerase chain reaction (PCR) reactions consisting of 12.5 μL Kapa Robust Hotstart ReadyMix, 1 μL each of the respective forward and reverse primers (10 μM), 1 μL (10 ng μL^{-1}) bovine albumin serum, 6.5 μL PCR water (5'), and 3 μL genomic DNA, cycled for 3 min at 95°C ; 30 cycles of 30 s at 95°C , 30 s at 57°C , and 1 min at 72°C ; and 10 min at 72°C . One negative control of PCR-grade water was added to each plate and exhibited less than 1–4% of total mean sequence read counts per sample. Duplicate reactions were pooled together and then cleaned and normalized using SequalPrep plates (Invitrogen). Normalized amplicons were pooled at equal volumes, concentrated using Amicon Ultra 0.5 mL 30k centrifugal filters (Millipore), gel extracted to remove nontarget bands (Qiagen Qiaquick), and sequenced at University of California, Davis DNA Technologies Core on an Illumina MiSeq using PE250. Data and metadata are available in the Sequence Read Archive data repository through NCBI (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA612503>). All of the code for this work is publicly available in the GitHub repository (https://github.com/ajamesk10/KELP_SBC_16S_2018; James et al. 2020).

16S rRNA gene sequence analysis

Raw sequence reads were processed using the DADA2 algorithm (Callahan et al. 2016; v. 1.10.0) which detects and removes low quality sequences and merges paired-end reads to generate amplicon sequence variants (ASVs). In contrast to similarity-based clustering techniques, ASV analysis provides finer sequence resolution and enables cross-study comparisons (Callahan et al. 2017). Sequence quality control was performed through the identification and removal of chimeric sequences, sequences containing unknown (ambiguous) bases, and

singleton reads. Forward reads were then trimmed to 200 bp and reverse to 160 bp based on per-base pair sequence quality graphs. The resulting sequences were classified with the SILVA taxonomy (v.132; silva_nr_v132_train_set.fa; Glöckner et al. 2017) and

ASVs identified as mitochondria, chloroplasts, and eukaryotes were removed. Quality filtering sequence reads resulted in 2.8×10^6 sequence reads from the 80 samples, which clustered into 3841 ASVs. Sequence read counts ranged from 5510 to 7.0×10^4 per sample and showed no clear bias by environment or blade condition so were not rarefied (Supporting Information Fig. S1; Willis 2019; McMurdie and Holmes 2014).

Reported RAs of sequences represent the average of samples pooled by environment (water vs. kelp) or laminae condition (healthy vs. bryozoan). We evaluated overall differences in microbial communities in seawater and on kelp laminae, as well as on healthy vs. bryozoan laminae using one-way permutational multivariate ANOVA (PERMANOVA) with the “adonis” function in the R package “vegan” (Oksanen et al. 2013). To account for the effects of sampling event (a factor with four levels including MR in May and August, and AQ in June and July) on differences in laminae communities, we used the “strata” argument. This analysis indicated that the main effect of bryozoans was significant and robust to the decision to include sampling event in the model. Linear mixed-effects (LME) models were then used to assess if there were significant differences between the mean RAs of individual bacterial families by laminae condition, with the random effect of sampling event accounted for using the “lmer” function in the R package “lme4” (Bates et al. 2015). Statistical analyses were performed on arcsine square root transformed RAs of ASVs and family-level taxa. Arrows indicating the correlation between key family-level taxa and nonmetric multidimensional scaling ordinations were calculated using the “envfit” function in the R package “vegan” (Fig. 2; Oksanen et al. 2013).

Significant differences in the mean RAs of individual ASVs by laminae condition were evaluated using “DESeq2” in the R package “DESeq2” (Love et al. 2014). Following the recommendations in the DESeq vignette, we applied a zero-inflated negative binomial distribution to our raw ASV counts using the “sfType = ‘poscounts’” in the DESeq call. “Poscounts” estimates a modified geometric mean for ASVs with multiple zero counts across samples. Due to limited replication by location and month, we were unable to account for “sampling event” variance in our DESeq2 analysis. However, our results provide insight to overall changes in the RAs of ASVs by laminae condition. Differentially abundant ASVs are those exhibiting $\geq$ two-fold increase ($\log_2$ fold-change ≥ 1.5) in either healthy or bryozoan laminae communities and significant false discovery rate (FDR)-adjusted p values (≤ 0.02).

Results and discussion

The free-living microbial communities found in seawater within and surrounding the kelps beds differed significantly from the epibiotic microbial communities found on kelp laminae (p value = 0.001, $R^2 = 0.23$; PERMANOVA; Supporting Information Fig. S2), consistent with previous reports (Michelou et al. 2013; Vollmers et al. 2017; Weigel and Pfister 2019). Seawater communities were characterized by significantly higher mean

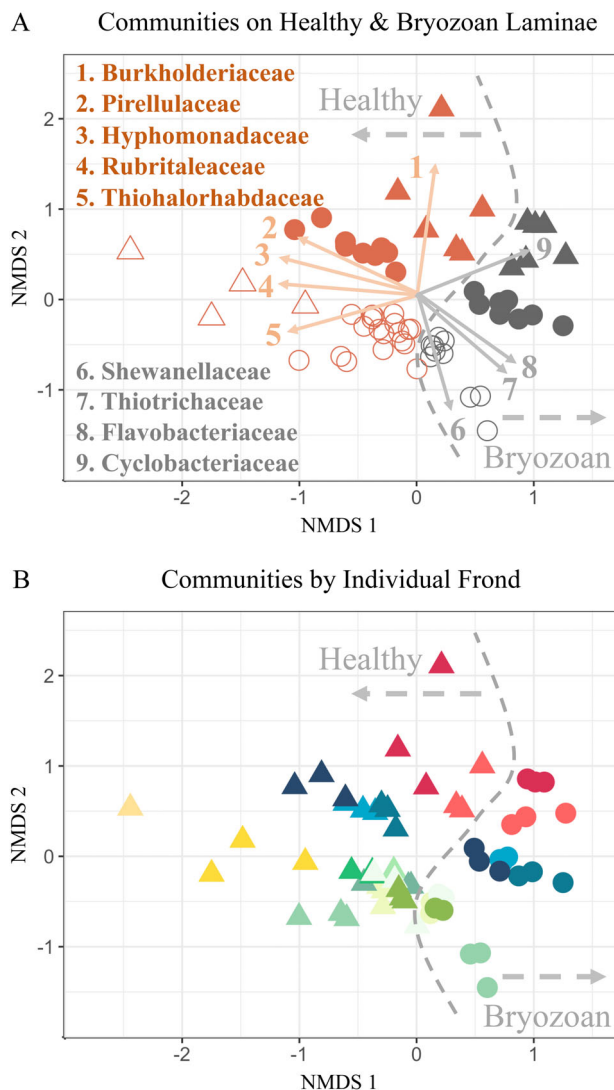


Fig. 2. Nonmetric multidimensional scaling ordination plot of the arcsine square root transformed relative abundances (RAs) of ASVs observed as a function of bryozoan coverage (A) and frond (B). (A) Shapes indicate sampling at MR in May (open triangles) and August (filled triangles), and AQ in June (open circles) and July (filled circles). Colors indicate healthy (orange) and bryozoan (gray) laminae. Arrows represent ASVs aggregated to family level and fitted to the ordination. Family levels shown are those exhibiting mean RAs $\geq 3\%$ and significant differences between healthy and bryozoan laminae (FDR-adjusted p values ≤ 0.01). Arrows point in the direction of increasing RAs. (B) Shapes indicate healthy (triangles) and bryozoan (circles) laminae while colors indicate sampling location with MR in May/August in yellow/red and AQ in June/July in green/blue. Hues within each color are indicative of individual fronds. For the majority of fronds, there exist three points of the same hue—these represent the communities from three laminae on a single frond. Dashed line demarcates healthy and bryozoan covered laminae.

RAs of *Alphaproteobacteria* and *Oxyphotobacteria* (Fig. 3, FDR-adjusted p values ≤ 0.001 , LME), which were dominated by SAR11 and Rhodobacteraceae clades (33% and 45% of *Alphaproteobacteria*) and *Synechococcus* CC9902 (95% of *Oxyphotobacteria*; Supporting Information Fig. S3). Kelp laminae exhibited significantly higher mean abundances of *Gammaproteobacteria* and *Planctomycetacia* (Fig. 3, FDR-adjusted p values < 0.001 , LME). *Bacteroidia* and *Verrucomicrobia* showed similar RAs in free-living and laminae communities, comprising 30% and 4% of free-living, and 38% and 9% of laminae communities, respectively (Fig. 3). These trends broadly agree with published studies of coastal free-living (Lemay et al. 2018; Rappé et al. 2000; Wear et al. 2015) and giant kelp associated microbial communities (Michelou et al. 2013; Vollmers et al. 2017; Weigel and Pfister 2019).

In addition to differences between free-living and kelp associated microbial communities, we found significant differences in overall microbial community composition on healthy laminae compared with bryozoan laminae (p value = 0.001, $R^2 = 0.10$; PERMANOVA; Fig. 2A). Of the most relatively abundant taxa (RAs $\geq 3\%$), *Verrucomicrobiae* and *Planctomycetacia* exhibited greater mean RAs on healthy laminae and included higher abundances of Rubritaleaceae and Pirellulaceae (Figs. 2A, 4, and Supporting Information Fig. S3, FDR-adjusted p values ≤ 0.01 , LME). These differences were driven in part by significant enrichment of seven ASVs ($\geq 0.5\%$) belonging to four genera within Rubritaleaceae including *Roseibacillus* and *Persicirhabdus* (Table 1 and Supporting Information Table S1, FDR-adjusted p values < 0.02 , DESeq2). In addition, four ASVs belonging to the genus *Blastopirellula* comprised nearly 6% of healthy communities and contributed to the enrichment of *Planctomycetacia* on healthy laminae (Table 1 and

Supporting Information Table S1, FDR-adjusted p values < 0.02 , DESeq2).

Blastopirellula has been identified on various macroalgal species (see review by Lage and Bondoso 2014), while *Roseibacillus* and Pirellulaceae were shown to exhibit higher RAs on healthy vs. blistered laminae in a recent study of the effects of low pH and elevated temperature on the microbiome of the brown alga, *E. radiata* (Qiu et al. 2019). In that study, kelps exposed to ocean acidification conditions were significantly more likely to develop a putative disease that manifested in blistering of the laminae. These blisters minimized photosynthetic area and reduced photosynthetic efficiency, mimicking the potential effects of bryozoan settlement on giant kelp in our study. Moreover, a recent metagenomics study of the giant kelp microbiome identified secondary metabolite gene clusters with the potential to produce antimicrobial compounds in multiple taxa of *Planctomycetacia* and *Verrucomicrobia*, including *Persicirhabdus* and Rubritaleaceae (Vollmers et al. 2017). Along with the patterns we observed, these studies indicate a potentially key role of *Planctomycetacia* and *Verrucomicrobia* in maintaining healthy microbiomes through biofouling defense via the production of antimicrobial metabolites on the kelp surface (reviewed by Lage and Bondoso 2014).

In addition to higher RAs of *Verrucomicrobia* and *Planctomycetacia*, healthy laminae were enriched with Thiohalorhabdaceae, Burkholderiaceae, and Hyphomonadaceae (Figs. 2A, 4, and Supporting Information Fig. S3, FDR-adjusted

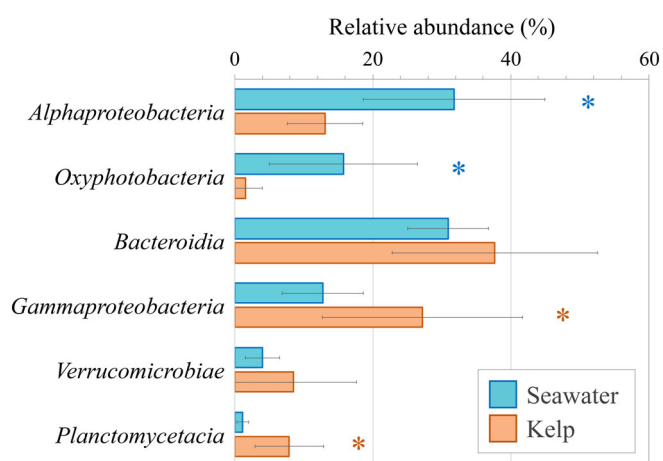


Fig. 3. The average relative abundance (RA) of class-level clades for microbial communities sampled in seawater (blue) and on kelp laminae (orange); considering only groups that exhibited $\geq 5\%$ RA in seawater or kelp samples. Asterisks indicate significantly higher mean transformed RA in seawater (blue) or on kelp laminae (orange). Error bars represent means $\pm$ SD.

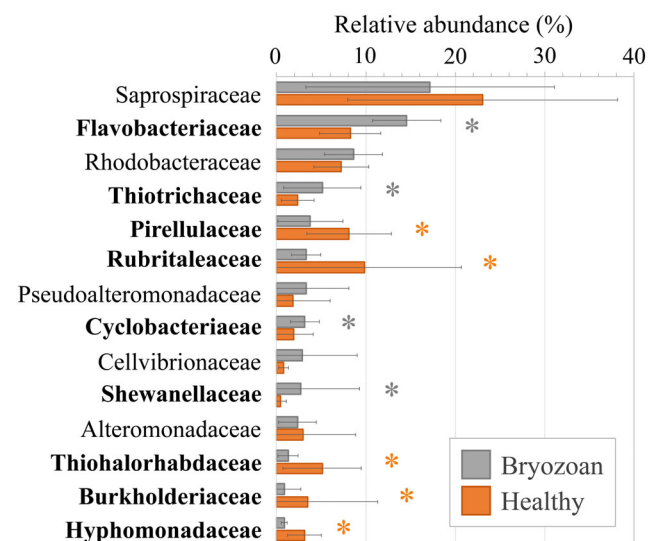


Fig. 4. The average relative abundance (RA) of family-level clades for microbial communities sampled on bryozoan laminae (gray) and healthy laminae (orange); considering only groups that comprised $\geq 3\%$ RA in bryozoan or healthy laminae samples. Asterisks indicate significantly higher mean transformed RA on either bryozoan laminae (gray) or on healthy kelp laminae (orange). These families are highlighted in bold. Error bars represent means $\pm$ SD.

Table 1. Mean relative abundance (RA) of top differentially abundant ASVs (RA $\geq 0.5\%$ on either healthy or bryozoan laminae) summed to genera and exhibiting increased RA on healthy (H) or bryozoan (B) laminae (FDR-adjusted p values ≤ 0.02 , log2 fold change ≥ 1.5 , DESeq2).

Class	Family	Genus	RA (%)		# of ASVs	> RA on H or B
			Healthy	Bryozoan		
<i>Verrucomicrobiae</i>	Rubritaceae	Unknown Rubritaceae	0.66	0.04	1	H
<i>Verrucomicrobiae</i>	Rubritaceae	<i>Persicirhabdus</i>	1.83	0.14	3	H
<i>Verrucomicrobiae</i>	Rubritaceae	<i>Roseibacillus</i>	1.27	0.03	2	H
<i>Verrucomicrobiae</i>	Rubritaceae	<i>Rubritalea</i>	1.44	0.05	1	H
<i>Planctomycetacia</i>	<i>Pirellulaceae</i>	<i>Blastopirellula</i>	5.99	0.74	4	H
<i>Gammaproteobacteria</i>	Alteromonadaceae	<i>Alteromonas</i>	0.73	0.02	1	H
<i>Gammaproteobacteria</i>	Arenicellaceae	<i>Arenicella</i> —H	0.67	0.00	1	H
<i>Gammaproteobacteria</i>	<i>Thiohalorhabdaceae</i>	<i>Granulosicoccus</i>	3.72	0.67	3	H
<i>Bacteroidia</i>	Cryomorphaceae	Unknown Cryomorphaceae	0.80	0.01	1	H
<i>Bacteroidia</i>	Saprospiraceae	<i>Lewinella</i>	1.35	0.00	1	H
<i>Bacteroidia</i>	Saprospiraceae	Unknown Saprospiraceae	1.10	0.06	2	H
<i>Alphaproteobacteria</i>	Hyphomonadaceae	<i>Litorimonas</i>	1.40	0.20	2	H
<i>Alphaproteobacteria</i>	Rhodobacteraceae	<i>Sulfitobacter</i>	1.01	0.02	1	H
<i>Gammaproteobacteria</i>	<i>Shewanellaceae</i>	<i>Shewanella</i>	0.15	2.47	1	B
<i>Gammaproteobacteria</i>	Arenicellaceae	<i>Arenicella</i>	0.21	0.67	1	B
<i>Bacteroidia</i>	Cryomorphaceae	Unknown Cryomorphaceae	0.04	0.55	1	B

Note: Bolded values represent ASVs summed to genera and comprising $\geq 2.5\%$ of the communities on healthy or bryozoan laminae.

p values ≤ 0.01 , linear mixed-effects (LME)). These family level shifts were driven in part by enhancement of ASVs belonging to the genera *Litorimonas* and *Granulosicoccus* (Table 1 and Supporting Information Table S1, FDR-adjusted p values < 0.02 , DESeq2). These genera have been identified in numerous macroalgal-associated communities (Lemay et al. 2018; Miranda et al. 2013), and *Granulosicoccus* was recently shown to comprise the majority of giant kelp microbiomes sampled off the coast of Washington (Weigel and Pfister 2019). These studies did not document changes in these genera relative to host condition; however, our results, in combination with the prevalence of these taxa across geographic location and algal species, highlight the potential for members of the *Litorimonas* and *Granulosicoccus* clades to play a key role in healthy macroalgal microbiomes, with reductions in these genera, along with *Verrucomicrobia* and *Planctomycetacia*, potentially serving as indicators of host stress.

In contrast to healthy laminae, bryozoan laminae were characterized by higher mean RAs of Flavobacteriaceae, Thiotrichaceae, Cyclobacteriaceae, and Shewanellaceae (Figs. 2A, 4, and Supporting Information Fig. S3, FDR-adjusted p values ≤ 0.01 , LME). Concomitant with the decreases in *Verrucomicrobia* and *Planctomycetacia* on blistered *E. radiata* laminae mentioned above, Qiu et al. (2019) observed substantial reductions in the RAs of Flavobacteriaceae on blistered as compared to healthy laminae. Furthermore, increases in the RA of

Flavobacteriaceae in epibiotic microbial communities found on diseased laminae of the red alga, *Delisea pulchra* (Kumar et al. 2016; Zozaya-Valdés et al. 2017), indicate a potential for this lineage to play a critical role in algal dysbiosis across algal divisions.

Enrichment of Shewanellaceae on bryozoan blades was driven almost exclusively by the increase in a single ASV identified as *Shewanella* (Table 1 and Supporting Information Table S1). This ASV comprised roughly 2.5% of the total bryozoan laminae community, and was one of three top ASVs enriched on bryozoan laminae (Table 1, Supporting Information Table S1). Culture-dependent studies of microbial communities associated with various species of bryozoan have revealed a consistent presence of *Shewanella* in bryozoan microbiomes (Heindl et al. 2010; Heindl and Thiel 2012; Pukall et al. 2001). Though *Shewanella* has also been observed on various species of macroalgae (Goecke et al. 2013; Kumar et al. 2016), the substantial enrichment of this single ASV on bryozoan laminae and the prevalence of these genera in association with various species of bryozoan suggest that bryozoans may uniquely alter macroalgal microbiomes through the introduction of bryozoan associated microbial genera. The presence of this *Shewanella* ASV on healthy laminae may indicate a capacity for these microbes to metabolize kelp material, or may be indicative of early settlement of bryozoans. Future studies are needed to clarify this discrepancy and to

understand the role of this *Shewanella*—and other potential bryozoan microbes—as they interact with kelp.

Potential mechanisms

The mechanisms driving our observed changes in the kelp microbiome remain unclear. However, for our study, as well as in Qiu et al. (2019), decreases in RAs of *Planctomycetacia* and *Verrucomicrobia* were concomitant with increases in Flavobacteriaceae. Several taxa belonging to Flavobacteriaceae have been implicated as opportunistic marine pathogens associated with temperature-induced bleaching in *E. radiata* (Marzinelli et al. 2015; Qiu et al. 2019) and natural bleaching events of *D. pulchra* (Kumar et al. 2016). One of the genera observed in higher abundances during these natural bleaching events, *Dokdonia*, was also enriched on bryozoan laminae for our study (Supporting Information Table S1, FDR-adjusted *p* value < 0.01, DESeq2). Moreover, flavobacterial isolates that were abundant on diseased blades experimentally induced bleaching of healthy *D. pulchra* (Kumar et al. 2016). The mechanisms for such opportunistic flavobacterial pathogenesis remain, as yet, uncharacterized. Intriguingly, however, several different Flavobacteriaceae cultured from marine macroalgae have demonstrated enzymatic abilities to degrade components of macroalgal cell wall polymers (e.g., alginases, fucoidanases; Miyashita et al. 2010), implying that such organisms might be able to lyse macroalgal cells and cause lesions. Polysaccharide-degrading abilities have also been observed in *Shewanella* (Martin et al. 2015), such that depressed RAs of antimicrobial producing bacteria—*Planctomycetacia* and *Verrucomicrobiae*—on bryozoan laminae may compound the negative impacts of encrusting bryozoans on kelp health by enabling increases in potential opportunistic pathogens such as members of Flavobacteriaceae and *Shewanella*. This result has potentially critical consequences for kelp health, as a rise in potential pathogens concurrent with encrusting bryozoan settlement may further contribute to host stress and degradation.

In addition to revealing novel patterns in kelp-associated microbial communities in the presence of epiphytic bryozoans, our dataset points to an intriguing pattern that may be important for understanding the ecological and physiological mechanisms driving the development of the kelp microbiome. Comparing mean RAs of ASVs found in kelp-associated microbial communities revealed that in the majority of cases, communities on laminae from a single frond were more similar to one another than to communities on laminae from other fronds (Fig. 2B). While the mechanisms for this similarity are yet unclear, it could indicate frond-level similarities driven by factors as diverse as frond position within the kelp bed, frond age, intraspecific host genetic variation, or inoculation of frond microbiomes from the tip of growing fronds (the apical meristem). Exploring the mechanisms driving this pattern is key to developing a comprehensive understanding of how kelp engineer the physical and chemical conditions that may shape their microbiome, and highlights the need to

address host source and condition in future studies of macroalgal microbiomes.

Conflict of interest

The authors declare no conflicts of interest related to the study.

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