Weigel Brooke (Orcid ID: 0000-0002-6271-9083)

Oxygen metabolism shapes microbial settlement on photosynthetic kelp blades compared to artificial kelp substrates

Running title: Oxygen metabolism shapes the kelp microbiome

Brooke L. Weigel[1#], Catherine A. Pfister[1,2]

[1]Committee on Evolutionary Biology, University of Chicago, Chicago, IL, USA

[2]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA

[#]**Corresponding Author:** Brooke L. Weigel, brookeweigel@uchicago.edu

Address: Culver Hall 402, University of Chicago, 1025 E. 57th Street, Chicago, IL 60637

Phone number: 608-333-5975

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Originality-Significance Statement: Marine macroalgae host dense microbial biofilms on their photosynthetic blade surfaces. Here, we used an *in situ* experimental approach to demonstrate differential settlement of marine microbial taxa onto newly produced kelp tissues and artificial kelp substrates from the same pool of seawater microbes. Moreover, we found a functional enrichment of obligately aerobic microbial taxa on living kelp blades, while nonliving kelp substrates were dominated by facultative anaerobes, suggesting that photosynthetic production of O_2 may be an important factor structuring the composition of the macroalgal microbiome.

Summary

We examined factors shaping community assembly of the bull kelp (*Nereocystis luetkeana*) microbiome by comparing biofilm formation on photosynthetic kelp blade tissues and artificial kelp substrates ("agar substrates") deployed into a kelp forest. New kelp blade tissues were colonized by markedly distinct microbial taxa relative to agar substrates during the same time interval, even when agar substrates were infused with *N. luetkeana* blades, suggesting that microbial settlement onto kelp surfaces is more than just attraction to a polysaccharide-rich surface. Further, common seawater taxa such as *Colwellia sp*. and *Psychromonas sp*. became abundant on agar substrates but avoided new kelp blade tissues, indicating that host-specific factors may deter certain surface-associated marine microbial taxa. Over two-thirds of taxa in the kelp microbiome were associated with strictly aerobic metabolisms; thus, photosynthetic production of O_2 may favor aerobic microbial metabolisms. While living kelp blades primarily recruited aerobic microbes, including the obligate aerobe *Granulosicoccus sp.*, microbes that colonized agar substrates were predominantly facultative anaerobes. We also found that infusion of kelp tissues into agar substrates altered microbial community composition and lowered taxonomic diversity relative to control agar substrates, suggesting that non-living components of the kelp blade also impact microbial community assembly.

Introduction

The importance of microbial communities to the health and functioning of multicellular organisms is becoming increasingly recognized across the tree of life, from the human gut to the plant rhizosphere. Association with eukaryotic hosts plays a large role in structuring microbial communities globally; host-associated microbiomes are generally less speciose than free-living

microbial communities (Thompson *et al.*, 2017). In the ocean, host-associated microbiomes can impact marine ecosystem functioning by altering the way that hosts reproduce and develop, metabolize nutrients, or respond to environmental stressors (Wilkins *et al.,* 2019). Marine host-associated microbiomes may be useful for microbial-based management and conservation, yet knowledge about the basic rules of microbiome assembly and persistence is still lacking for most marine organisms (Wilkins *et al.*, 2019). Marine macroalgae host dense microbial biofilms on their photosynthetic surfaces, with roughly 10^6-10^8 microbial cells per cm^2 of algal tissue (Mazure and Field, 1980; Stratil *et al.*, 2013). Microorganisms that live in biofilms on the macroalgal surface may greatly affect the biology of their host by influencing development, disease susceptibility, biofouling, or nutrient acquisition (Goecke *et al.*, 2010; Egan *et al.*, 2013). Despite strong differentiation of macroalgal microbiomes from free-living microbial communities in the surrounding seawater (Bengtsson *et al.*, 2010; Mancuso *et al.*, 2016; Weigel and Pfister, 2019), little is known about the factors that structure microbial biofilm assembly on macroalgal hosts compared to the surrounding environment.

In the ocean, bacteria can rapidly colonize available substrates, forming dense biofilms with more than 10^5 cells/cm^2 on artificial surfaces in just 20 hours (Fischer *et al.*, 2014). Algal tissues may be colonized partially by microbes from the seawater, as algal hosts share 32% (Weigel and Pfister, 2019) to 86% (Lemay *et al.*, 2018) of their microbial taxa with the surrounding seawater. It is possible that motile microbes from the seawater are especially likely to settle onto kelp surfaces, as microbial motility and chemotaxis genes were enriched in the kelp blade surface biofilm relative to the seawater microbial community (Minich *et al.*, 2018). Common properties of the macroalgal surface may attract generalist microbial taxa that can colonize many algal host species (Florez *et al.*, 2017). For example, new blade tissues on nine

sympatric macroalgal species shared a large number (36%) of overlapping microbial taxa (Lemay *et al.*, 2018). Given the proclivity of seawater microbes to adhere to surfaces and rapidly generate biofilms (Fischer *et al.*, 2014), it is possible that the macroalgae are "just a surface" for microbial settlement from the seawater. However, microbial settlement onto macroalgal surfaces is likely shaped by a variety of host-associated factors.

The macroalgal surface environment may select for a subset of potential colonizing microbes from the seawater using certain biological, morphological, or chemical cues. Macroalgal blade surfaces are rich in organic nutrients and carbohydrates, provision microbes with a source of dissolved organic matter (Reed *et al.*, 2015, Weigel and Pfister, 2020), and produce oxygen, which may provide a preferred habitat for certain microbes. In addition, surface-associated metabolites from macroalgal hosts may shape microbial membership by attracting or deterring certain microbial groups (Lachnit *et al.*, 2013, Saha and Weinberger, 2019). For example, Lachnit *et al.* (2010, 2013) discovered that certain algal-produced compounds had a "profouling effect" on the artificial algal surfaces, while others such as fucoxanthin deterred bacterial colonization in a nondiscriminatory manner. Macroalgae shape not only organic matter availability but also local oxygen concentrations (Irwin and Davenport, 2002; Noisette and Hurd, 2018; Pfister *et al.*, 2019), which can be a critical determinant of microbial community structure due to differing microbial respiratory metabolisms and defenses against reactive oxygen species (Morris and Schmidt, 2013). Finally, morphology alone can be a determinant of microbial community structure. For example, a recent study using artificial seaweed cut to mimic different thallus morphologies found that morphology and structural complexity shaped surface microbial communities in the absence of biological or chemical

interactions (Lemay et al. *in review*). Despite all of these factors, we are still just beginning to understand the processes shaping microbial community assembly on macroalgal surfaces.

Macroalgae known as kelp (order Laminariales) play a vital role in coastal ecosystems, creating kelp forest habitat and fixing tremendous amounts of carbon, yet we know little about microbial community assembly on kelps. Photosynthetic blade tissues of the canopy-forming kelp *Nereocystis luetkeana* host an abundant and spatially structured microbiome of 10^7-10^8 cells/cm^2 (Ramirez-Puebla *et al.*, 2020). Despite high cell density, the biofilm community is dominated by a few highly abundant bacterial taxa that displayed repeatable spatial structure over multiple months (Ramirez-Puebla *et al.*, 2020) and persisted over large geographical distances (Weigel and Pfister, 2019). As an annual species, *N. luetkeana* rapidly grows from a microscopic gametophyte to a massive sporophyte during the spring and summer, becoming one of the largest primary producers in the ocean by mid-summer. The photosynthetic blades of *N. luetkeana* elongate rapidly and produce 1 to 5 cm of new blade tissue per day (Maxell and Miller, 1996; Weigel and Pfister, 2019), constantly providing new tissues for microbial colonization. By comparing microbial biofilm formation on new kelp blade tissues with artificial kelp substrates, this study examined the processes shaping recruitment of microbiota from the seawater onto living and non-living kelp substrates.

Here, we deployed artificial kelp substrates consisting of agar gel plates with and without infusion of crushed *N. luetkeana* blades into a kelp forest in Washington, USA (Fig. 1). Artificial kelp substrates were deployed at the north-facing Main Beach site on Tatoosh Island, Washington, USA (48.39°N, 124.74°W), where *N. luetkeana* forests are abundant and have persisted over decades (Fig. 1A, Pfister *et al.*, 2018). Agar is a polysaccharide extracted from red algal cell walls, composed of agarose (a polymer of galactose and galactopyranose) and

agaropectin, and thus is an excellent mimic for the macroalgal surface. Given that motile marine bacteria exhibit chemotaxis towards phytoplankton cells and their extracellular compounds (Seymour *et al.*, 2010), we hypothesized that infusing crushed kelp blades into the "kelp agar" treatment will alter microbial recruitment. After 4 days of deployment in the kelp forest, we sampled control and kelp agar artificial substrates, nearby *N. luetkeana* blade tissues, and surrounding seawater. Detailed experimental procedures, including 16S rRNA gene sequencing and analysis, can be found in Appendix 1: Experimental Procedures. We characterized microbial communities on control agar, kelp agar, *N. luetkeana* blade base, *N. luetkeana* blade tip and seawater samples with 16S rRNA gene sequencing to answer the following questions: 1) Do artificial kelp substrates and new *N. luetkeana* blade tissues develop similar microbial communities after 4 days in a kelp forest? 2) Does infusion of agar with crushed *N. luetkeana* blades ("kelp agar") alter microbial settlement or increase microbiome similarity between artificial substrates and real *N. luetkeana* blades? 3) Do any of the common microbial taxa associated with *N. luetkeana* settle onto artificial kelp substrates?

Results and Discussion

Artificial kelp substrates recruit a distinct microbiome compared to real kelp blades

We found that artificial kelp substrates deployed in a *N. luetkeana* forest for 4 days recruited a distinct microbial biofilm compared to microbial taxa associated with *N. luetkeana* blade surfaces and the surrounding seawater (Fig. 2A). Microbial community structure varied significantly with substrate type between control agar, kelp agar, *N. luetkeana* blade base, *N. luetkeana* blade tip and seawater samples (PERMANOVA, df = 4, total df = 24, $F = 13.87$, $R^2 = 0.74$, $P = 0.001$; Fig. 2A; Table 1), and these differences were not due to unequal dispersion of

variance among sample types (PERMDISP, df = 4, total df = 24, F = 0.95, P = 0.46). Further, after samples were rarefied to the lowest sample read count, the differences between sample types were still significant (PERMANOVA, df = 4, total df = 24, F = 11.29, R^2 = 0.69, P = 0.001; Appendix 2: Table S2), and there were still no differences in dispersion (PERMDISP, df = 4, total df = 24, F = 0.38, P = 0.82). After 3.7 days in the *N. luetkeana* kelp forest, both agar substrates had significantly different microbial communities from those living on blades of *N. luetkeana* (PERMANOVA pairwise comparisons, $p < 0.01$; Table 1; Fig. 2A). There were also significant differences in ASV richness among sample types (ANOVA, df = 4, total df = 24, F = 123, $P < 0.001$), where seawater samples had the highest ASV richness, the base of the kelp blade had the lowest ASV richness, and both kelp agar and control agar had similar ASV richness to the blade tip samples (Fig. 2B). Microbial community composition on *N. luetkeana* blades was significantly different from that of the agar substrates (Fig. 3A). Across all *N. luetkeana* samples, the most abundant taxon was *Granulosicoccus sp.*, a *Gammaproteobacteria* from the family *Granulosicoccaceae*, with a mean relative abundance of 38.7% (± 6.25, std. error; Fig 3A). While 25 ASVs were detected on *N. luetkeana* blades, 3 *Granulosicoccus* ASVs comprised an average of 38.1% (± 6.34% std. error) of the *N. luetkeana* blade microbiome, while the others had mean abundances of less than 1% of the community (Appendix 2: Fig. S1). In contrast, *Granulosicoccus* were hardly detected on agar substrates (Fig. 3A, Fig S1B), making up negligible proportions of the total microbial community (0.34% ± 0.50). Despite extremely low abundances, 11 *Granulosicoccus sp.* ASVs were detected on control agar substrates, many of which differed from the ASVs that recruited to kelp blades (Appendix 2: Fig. S1A). The microbiome of *N. luetkeana* blades was also enriched in *Alphaproteobacteria* from the *Hyphomonadaceae* family, *Bacteroidetes* from the *Flavobacteriaceae* and *Saprospiraceae*

families, *Verrucomicrobia* from the *Rubritaleaceae*, and *Planctomycetes* from the *Planctomycetaceae* compared to agar substrates (Fig. 3A).

Examining the distribution of the 50 most abundant ASVs across sample types revealed that the abundant microbial symbionts from *N. luetkeana* blade tip communities colonized at a lower abundance onto artificial agar surfaces, while many of the ASVs abundant on agar substrates were not found on *N. luetkeana* blades (Fig. 4). In contrast to the kelp blade surface, agar substrate biofilm communities were comprised mainly of *Gammaproteobacteria* from the families *Colwelliaceae* (mostly *Colwellia sp.*), *Pseudoalteromonadaceae* and *Psychromonadaceae*, *Alphaproteobacteria* from the *Rhodobacteraceae* family, as well as *Arcobacter sp.* from the *Epsilonproteobacteria* family *Campylobacteraceae* (Fig. 3A). A few of the most abundant ASVs found in seawater were highly abundant on agar substrates (Fig. 4), including ASVs classified as *Colwellia sp.*, *Pseudoalteromonas sp.*, and *Psychromonas sp.*, demonstrating settlement of these taxa from the seawater onto agar substrates. Both agar substrates and seawater samples are enriched in these three families, while they are nearly absent or found in low abundance on *N. luetkeana* blades (Fig. 3A). These results are well-matched with those of Lachnit *et al.* (2013), where artificial hydrogel surfaces hosted significantly different microbial communities from those of *Fucus vesiculosus* after 3 days in the field, with abundant *Epsilonproteobacteria* and *Gammaproteobacteria* on artificial substrates. The same families of *Gammaproteobacteria* reported here, including *Colwelliaceae* and *Pseudoalteromonadaceae*, were enriched in seawater amended with alginate and agarose in the Arctic Ocean (Jain *et al.*, 2020), suggesting that polysaccharides such as agarose may select for common microbial taxa in the seawater, even in different ocean basins.

While short-term artificial surfaces might not resemble the microbiome of a mature macroalgal host given the differences in biofilm age or succession, examining microbial settlement on artificial surfaces compared to new algal tissues can reveal how the macroalgal microbiome is structured differently than inert substrates exposed to the same field conditions and pool of potential colonizing microbes. Sampling the extremely rapid new blade growth of the annual kelp *N. luetkeana* (~2 to 4 days old) revealed that kelp blade biofilms recruited a distinct community from those that developed on agar substrates (3.7 days old) during the same time interval, even when agar substrates were infused with *N. luetkeana* blades (Table 1; Fig. 2A). We found that *Granulosicoccus sp.* made up an average of 59% (± 24%) of the microbiome of *N. luetkeana* blade base samples but were nearly absent from agar substrates (Fig. 3A, Appendix 2: Fig. S1B), indicating selective recruitment of this taxon to new kelp tissues rather than nearby artificial kelp substrates. *Granulosicoccus sp*. reaches high densities on the tip of *N. luetkeana* blades, with up to $9x10^6$ cells/cm^2, but only sparse cells are visible on new tissue at the base of the blade (Ramirez-Puebla *et al.*, 2020). While we cannot differentiate between colonization of these taxa and performance differences post-settlement (cell growth, differential survival), the presence of *Granulosicoccus sp.* on new kelp blade tissues suggests possible colonization from the seawater or from older, mature portions of the *N. luetkeana* blade.

As demonstrated previously (Weigel and Pfister 2019, Ramirez-Puebla *et al.*, 2020), *N. luetkeana* blade base and tip microbial biofilms had significantly different microbial community structure (Table 1; Fig. 2A). Newer tissues from the base of the kelp blade had a lower number of bacterial sequences, lower overall diversity, and many of the abundant ASVs on the tips of the blade were absent from the base of the blade (Fig. 4). Further, the diversity of the most abundant bacterium on kelp blades, *Granulosicoccus sp.*, increased from new to mature kelp tissues; while

one *Granulosicoccus* ASV was most abundant on new kelp tissues at the base of the blade, 3 *Granulosicoccus* ASVs reached high abundances on mature blade tip tissues (Appendix 2: Fig. S1A). We found similar microbiome composition when sequencing DNA from whole kelp blade tissue extracts and surface swabs (Fig. 2A, Fig. 3A), suggesting that surface swabs are a useful proxy for whole tissue extracts, while minimizing host chloroplast contamination. However, we caution that the surface swabs more closely resembled the microbial communities from whole tissue extracts of the kelp blade tip (Fig. 2A), while the kelp blade base swabs had much higher ASV richness than the kelp blade base whole tissue extracts (Fig. 2B). While our low replication of kelp surface swabs (n = 2 per base and tip) prohibits strong inference, we suspect that whole tissue extracts from the base of the blade more accurately the reflect low microbial abundance environment of new kelp tissues (Ramirez-Puebla *et al.*, 2020), while the swabs gathered a higher number of microbial taxa by covering a larger surface area. Finally, we note that this study has limitations including a low sample size (5-6 replicates per treatment) and geographic restriction to one field site, when we know that the *N. luetkeana* blade microbiome displays geographic variation across the Salish Sea in Washington (Weigel and Pfister 2019). A recent study found that plastic seagrass mimics deployed in a *Zostera marina* bed for 6 days developed different microbiomes than real seagrass blades, despite showing similar biogeographic trends to real seagrass microbial communities (Adamczyk et al., in review), suggesting that geographic factors may affect both host-associated and nonliving substrates.

Oxygen metabolisms shape microbial composition on kelp blades vs. agar substrates

We found that aerobic and facultatively anaerobic metabolisms were not distributed evenly across kelp blades; rather, living kelp blades recruited ASVs belonging to families with

primarily aerobic metabolisms, while ASVs that colonized agar substrates were predominantly facultative anaerobes (Fig. 3B, chi-squared = 57.10, df = 1, P = < 0.001). Grouping the 23 most abundant microbial families by metabolic oxygen requirements revealed that 67% of kelp blade-associated bacterial ASVs belonged to families with strictly aerobic metabolisms, 17% of ASVs were aerobic or facultatively anaerobic, 5% were facultatively anaerobic and only 0.002% were microaerobic or strictly anaerobic (Fig. 3B). In contrast, only 6% of ASVs associated with agar substrates belonged to strictly aerobic families, while 51% were facultative anaerobes, 27% were aerobic or facultatively anerobic, 9% were microaerobic, and 3% were strictly anaerobic (Fig. 3B). This trend is largely driven by the high relative abundance of *Granulosicoccus sp.* on kelp blades, which are known to be obligately aerobic (Baek *et al.*, 2014), and *Colwellia sp*. (family Colwelliaceae) on agar substrates, which are facultative anaerobes (Bowman, 2014). We note that these results are based on assignments of oxygen metabolisms to bacterial families (Rosenberg *et al.*, 2014), which may not always be consistent at the family level as some microbes are metabolically diverse, thus they should be taken as putative oxygen metabolisms until these findings are confirmed with physical measurements and culture-based assays.

Kelp forests produce oxygenated seawater through photosynthesis during the daytime (Pfister *et al.*, 2019), and kelp blades produce highly oxygenated microenvironments at the diffusive boundary surface layer (0.1 to 1 mm from the kelp blade) relative to the surrounding seawater (Irwin and Davenport, 2002; Noisette and Hurd, 2018). Further, there is evidence that motile bacterial cells are capable of chemotactic behavior to orient themselves towards optimum oxygen concentrations (Thar and Fenchel, 2001). Kelp forests also create strong diel cycles in oxygen concentrations (Pfister *et al.*, 2019), producing highly oxygenated conditions during the day but relatively oxygen-depleted conditions at night through respiration (Noisette and Hurd,

2018). We found that ~67% of microbial ASVs from highly oxygenated kelp blade surface biofilms were associated with strictly aerobic metabolisms, suggesting that photosynthetic production of O_2 may be an important factor structuring the composition of the macroalgal microbiome. In contrast to kelp blade-associated microbes, colonists of agar substrates had a much greater proportion of facultatively anaerobic (51%), microaerobic (9%), and obligately anaerobic (5%) metabolisms (Fig. 3B). The strictly anaerobic purple sulfur bacterium *Thiorhodospira sp*. (Bryantseva *et al.*, 1999) from the family *Ectothiorhodospiraceae* was abundant on agar substrates but absent was from kelp blade surfaces (Fig. 3B). In aquatic microbial biofilms, oxygen concentrations decay over time as oxygen is consumed through aerobic respiration, creating low-O_2 microoxic and anoxic zones within the biofilm (Rubol *et al.*, 2018). It is possible that agar substrates, without a photosynthetic host, developed oxygen-depleted or anoxic microsites that supported anaerobic microbial metabolisms. Finally, we note that the morphological disparity between real *N. luetkeana* blades and artificial kelp substrates, with the latter lacking the undulating movements that kelp blades experience in fluid environments (Koehl *et al.*, 2008), may have contributed further to the differences in microbial biofilm development and oxygen metabolisms.

Infusion of kelp tissues altered microbial community assembly on agar substrates

We found that infusing dried and crushed *N. luetkeana* blades into agar substrates significantly altered microbial biofilm communities (PERMANOVA pairwise comparison, P = 0.017; Table 1). This difference in community structure is visible on the NMDS plot, where each agar type formed a distinct cluster of samples (Fig. 2A). We also found that kelp agar substrates had significantly lower ASV richness than the control agar substrates (Fig. 2B), providing

evidence that some component of the *N. luetkeana* blade likely inhibits the settlement or growth of some bacterial taxa. While the kelp blade metabolite composition was not characterized here, dried *N. luetkeana* blades likely contributed additional carbohydrates, nutrients and cell wall components to the kelp agar treatment. Despite infusion of dried *N. luetkeana* tissues into the kelp agar treatment, microbial communities on the kelp agar did not become more similar to the kelp blade surface (Table 1). To test whether common kelp-associated microbes were more abundant on the kelp agar compared to the control agar, we used differential abundance testing with DESeq2 (Appendix 1: Experimental Procedures). We detected 14 ASVs that were significantly enriched on kelp agar and 17 ASVs that were depleted on kelp agar compared to control agar substrates (Benjamini–Hochberg adjusted p-values < 0.01; Appendix 2: Fig. S2). However, none of the ASVs that were significantly enriched on the kelp agar, including *Psychromonas sp*., *Arcobacter sp.*, *Colwellia sp*., and *Alteromonas sp*. (Appendix 2: Fig. S2) were common on real kelp blades; rather, these taxa were more abundant in the surrounding seawater samples (Fig. 3A).

In addition to the oxygen environment and the chemical composition of the kelp surface, compounds actively exuded by macroalgae can attract or deter settlement or growth of certain microbes (see review in Goecke *et al.*, 2010). Kelps in particular release large quantities of dissolved organic carbon (DOC) into the surrounding seawater, and *N. luetkeana* blades release ~16% of the carbon that they fix as DOC (Weigel and Pfister, 2020). DOC exuded by primary producers can contribute to microbial growth in surface biofilms (Espeland *et al.*, 2001), and it likely provides an important carbon resource to heterotrophic microbes such as *Granulosicoccus sp*. that reside in the photosynthetic blade biofilm. In contrast to provisioning microbes with carbon, organic compounds such as fucoxanthin can deter bacterial colonization (Lachnit *et al.*,

2013). Surface-associated metabolites from the red algae *Agarophyton vermiculophyllum* reduced the settlement of pathogens and recruited beneficial bacterial strains, while microbe-microbe interactions provided additional protection against invading pathogens (Saha and Weinberger, 2019), possibly through the production of antibiotics (Florez *et al.*, 2017). We found that common seawater microbes settled and became abundant on agar substrates, while at the same time, new tissues at the base of the kelp blade largely avoided colonization by the same seawater microbes (Fig. 4). Thus, it is possible that chemical defenses or physical cues by *N. luetkeana* or its associated microbiome limited colonization of these common seawater microbial taxa. Future experiments using artificial macroalgal substrates with different combinations of factors such surface metabolites, dissolved organic carbon or oxygen will continue to elucidate the factors affecting microbial biofilm formation on macroalgae.

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Figure legends

Figure 1. A. Artificial kelp substrates were deployed at the north-facing Main Beach site on Tatoosh Island, WA, USA in close proximity to a *N. luetkeana* forest. The substrates consisted of B. control agar plates and C. kelp agar plates, infused with dried *N. luetkeana* blades. Agar substrates were deployed for 4 days by suspending them from a rope D. connected to a surface buoy and an anchor, so that they remained underwater at all times.

Figure 2. A. Microbial community structure of each sample type visualized with a non-metric multidimensional scaling (NMDS) plot. Each point represents one microbial community sample, and points clustered more closely together have more similar microbial communities. Color and ellipses around data points help to visually group samples by treatment. B. Mean amplicon sequence variant (ASV) richness in each sample type. Different letters indicate significant differences in ASV richness among sample types (ANOVA pairwise comparisons, $P < 0.05$), but swabs of base and tip tissues were not included due to low replication ($n = 2$) per sample type.

Figure 3. Barplots depicting A. the relative abundances of bacterial families across substrate types, and B. the relative abundances of bacterial families across substrate types, colored by the oxygen metabolism of each bacterial family. Each bar represents one microbial community sample. For *N. luetkeana* blade base and tip samples, two swabbed samples demonstrated compositional similarity between whole tissue and swabbed microbial communities. In the legend of A, families are grouped by phylum, or class for *Proteobacteria*, to add taxonomic information. For B, families are colored by oxygen metabolisms, as indicated in the legend.

Figure 4. Heatmap showing the $\log_{10}$ transformed relative abundance of the 50 most common amplicon sequence variants (rows) across all samples (columns), grouped by sample type. ASVs are labelled by bacterial family on the y-axis, colored by phylum (legend at the bottom of the graph). Within the heatmap, each cell indicates the log-transformed relative abundance of ASVs, where lighter green indicates greater relative abundance, dark blue indicates extremely low abundance, and black indicates absence.

Tables

Table 1. PERMANOVA pairwise comparisons of microbial community structure among substrate types (control agar, kelp agar, *N. luetkeana* blade base and tip, seawater), based on a Bray Curtis distance matrix of microbial community similarity among samples. Swabs of base and tip tissues were not included due to low replication (n = 2) per sample type.

PERMANOVA Pairwise Tests	**df**	**Sum Squares**	***F* statistic**	***p*-value**
Control agar vs. Kelp agar	1	0.80	8.38	0.017
Control agar vs. *N. luetkeana* blade base	1	2.23	20.6	0.017
Kelp agar vs. *N. luetkeana* blade base	1	2.15	16.8	0.008
Control agar vs. *N. luetkeana* blade tip	1	1.98	14.8	0.002
Kelp agar vs. *N. luetkeana* blade tip	1	1.96	12.8	0.002
Control agar vs. Seawater	1	1.25	20.5	0.015
Kelp agar vs. Seawater	1	0.93	10.3	0.017
N. luetkeana blade base vs. tip	1	2.03	12.8	0.001
N. luetkeana blade base vs. Seawater	1	1.68	15.7	0.013
N. luetkeana blade tip vs. Seawater	1	1.30	9.28	0.017

Figures

Figure 1

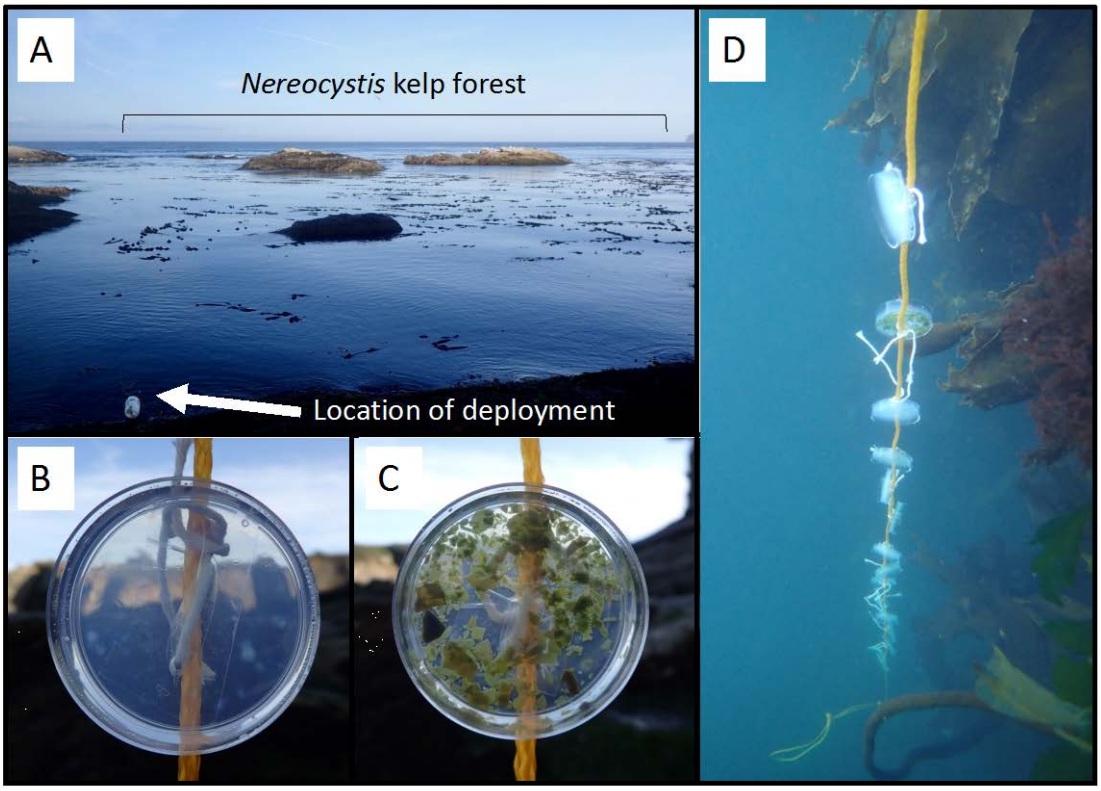

Figure 2

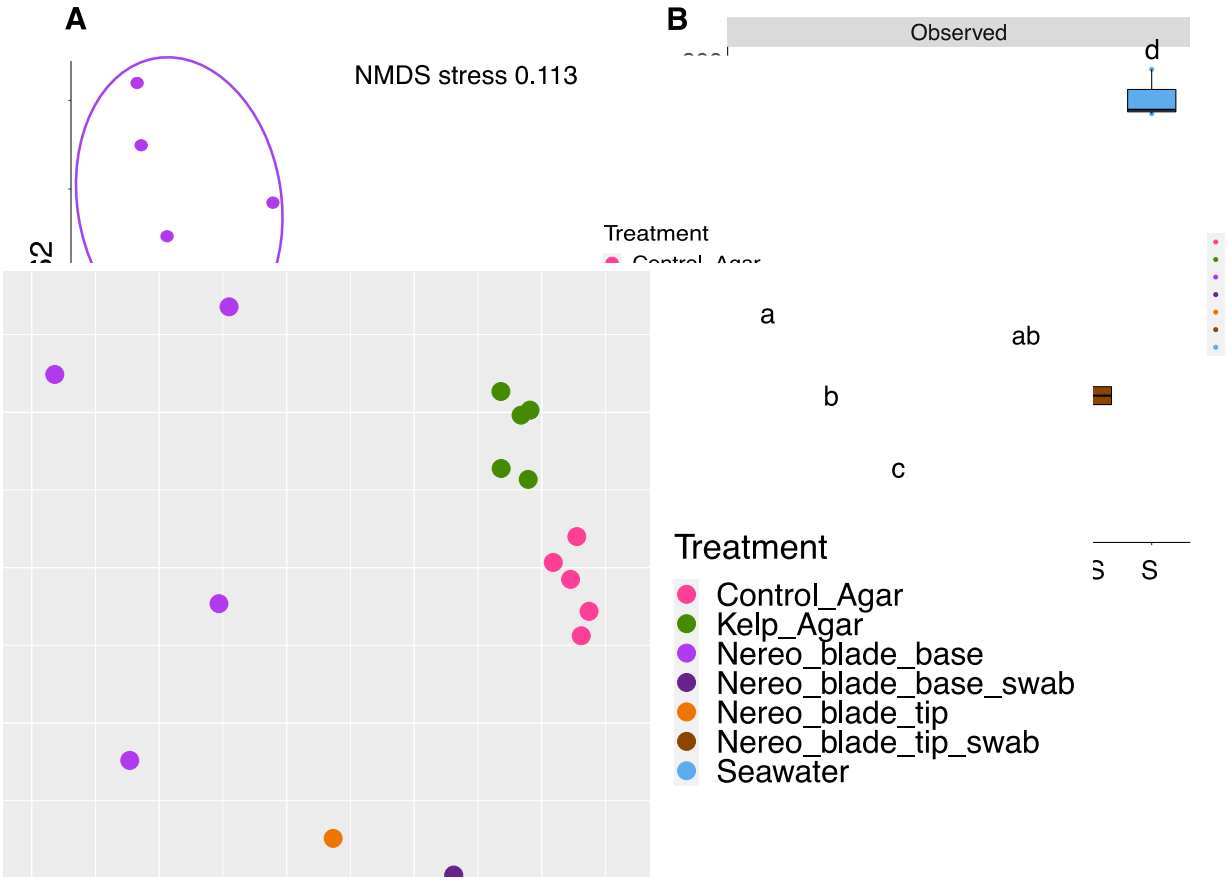

Figure 3

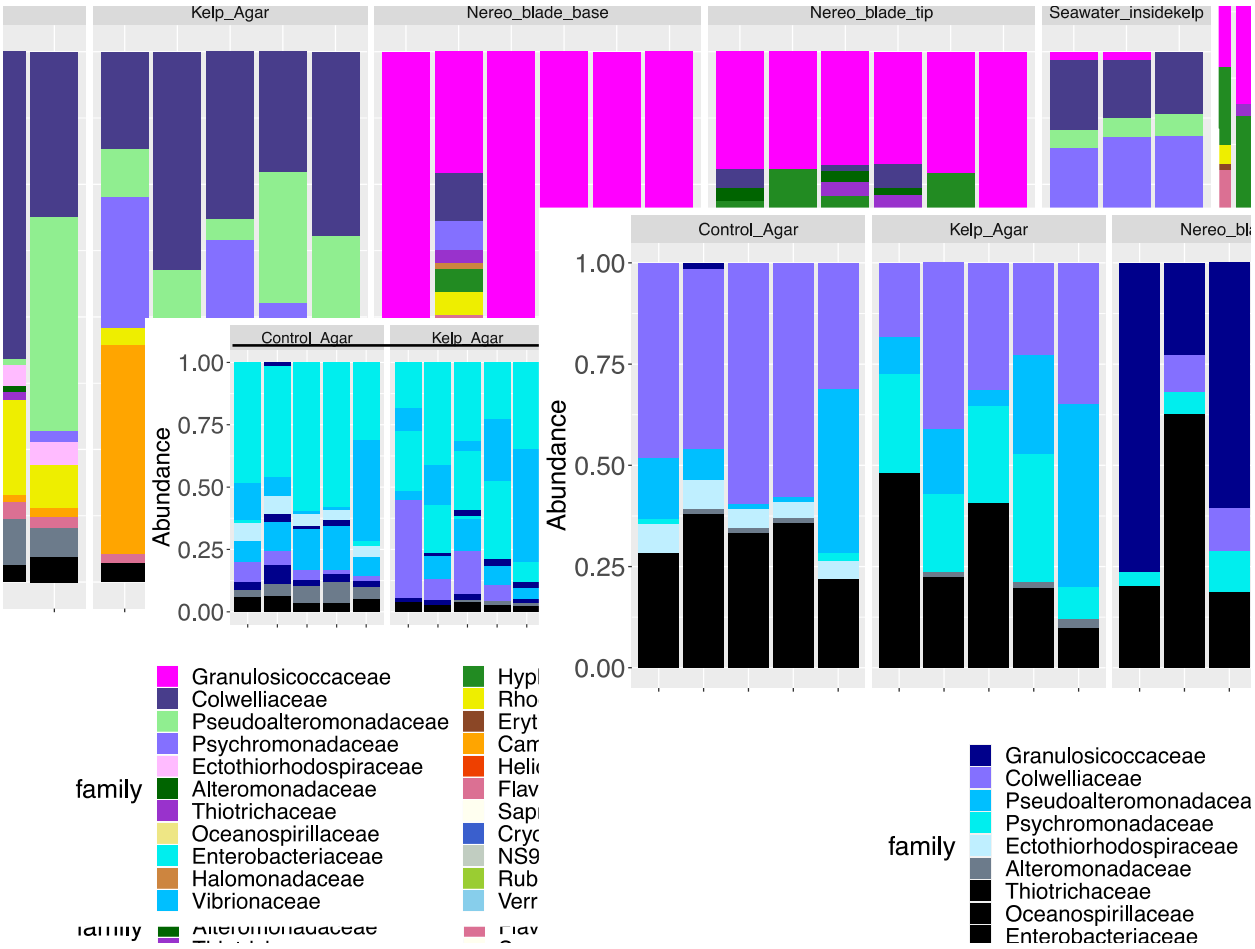

Figure 4