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KELP-ASSOCIATED MICROBIOTA ARE STRUCTURED BY HOST ANATOMY[1]

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Seaweed-associated microbiota are essential for the health and resilience of nearshore ecosystems, marine biogeochemical cycling, and host health. Yet much remains unknown about the ecology of seaweed–microbe symbioses. In this study, we quantified fine-scale patterns of microbial community structure across distinct anatomical regions of the kelp *Laminaria setchellii*. These anatomical regions represent a gradient of tissue ages: perennial holdfasts can be several years old, whereas stipe epicortex and blades are younger annual structures. Within blades, new growth occurs at the base, while the blade tips may be several months old and undergoing senescence. We hypothesized that microbial communities will differ across anatomical regions (holdfast, stipe, blade base, and blade tip), such that younger tissues will harbor fewer microbes that are more consistent across replicate individuals. Our data support this hypothesis, with the composition of bacterial (16S rRNA gene) and microeukaryote (18S rRNA gene) communities showing significant differences across the four anatomical regions, with the surfaces of older tissues (holdfast and blade tips) harboring significantly greater microbial richness compared to the younger tissues of the meristematic region. Additional samples collected from the surfaces of new *L. setchellii* recruits (<1y old) also showed differences in microbial community structure across anatomical regions, which demonstrates that these microbial differences are established early. We also observed this pattern in two additional algal species, suggesting that microbial community structure across host anatomy may be a common feature of the seaweed microbiome.

Key index words: **bacteria; holobiont; *Laminaria setchellii*; marine microbes; protists**

***Abbreviations*: ASV, amplicon sequence variant; BT, blade tip; DGGE, denaturing gradient gel electrophoresis; HF, holdfast; MS, meristem; PCoA, principal coordinates analysis; PERMANOVA, permutational analysis of variance; ST, stipe**

Kelps are a morphologically diverse clade of large canopy-forming marine macroalgae (order: Laminariales), which have long been appreciated for their significant cultural, commercial, and ecological importance (Krumhansl et al. 2016). Kelps form dense assemblages along rocky coastlines, providing critical habitat for large communities of mammals, fish, invertebrates, and other algae (Dayton 1985, Steneck et al. 2002), as well as a significant source of energy and nutrients for marine (Duarte and Cebrian 1996, Dethier et al. 2014) and terrestrial food webs (Polis and Hurd 1996, Orr et al. 2005, Lastra et al. 2008).

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Kelp-associated microbial symbionts are an essential component of these nearshore ecosystems. Microbial symbionts support the health of their hosts by providing nutrients and defending them against pathogens (reviewed by Singh and Reddy 2016), and also facilitate fundamental ecosystem services by mediating the transfer of kelp-derived carbon to higher trophic levels. However, recent studies have shown that the ecological association between kelp and their microbial symbionts can be disrupted by increased seawater temperatures (Minich et al. 2018, Qiu et al. 2019) and that microbial communities shift when hosts experience stress (Marzinelli et al. 2015). Thus, research into the ecology of kelp–microbe associations is critical for understanding factors that affect the health and resilience of kelp forest ecosystems (Qiu et al. 2019).

Laminaria is a species-rich genus of kelp with a broad distribution across coastal regions of the Pacific and Atlantic oceans (Lane et al. 2006, Bolton 2010). The anatomy of *Laminaria* is a composite of tissues of different ages and functions. Holdfasts are perennial structures that persist for several years. Stipes are also perennial structures, but the epicortex (surface tissue) goes through an annual exfoliation each spring to accommodate increased stipe girth (Klinger and Dewreede 1988). Blades are annual structures that erode during the winter and grow anew each spring and continue to grow through summer (Nielsen et al. 2014). New blade tissue grows from a meristematic region at the base of each blade, adjacent to the top of the stipe. Therefore, tips of the blade may be several months old and undergoing erosion due to senescence, while the tissue at the meristematic regions is newly formed.

This gradient of differently aged tissues on the same individual has made *Laminaria* species (and their relatives in the family Laminariaceae) an important model system for investigating fine-scale microbial structure across a single host. Previous research using bacterial cultures and microscopy found an increase in the richness of bacteria at the blade tip compared to the meristem and mid-blade in *L. digitata* (Corre and Prieur 1990), *L. pallida* (Mazure and Field 1980), and *L. longicruris* (Laycock 1974). Subsequent genetic research using denaturing gradient gel electrophoresis (DGGE) found that bacterial communities on *Saccharina latissima* (previously *L. saccharina;* Lane et al. 2006) differ along the length of the thallus, with bacteria showing more specific associations in the meristematic and stipe regions compared to the blade tip (Staufenberger et al. 2008). Similarly, Bengtsson et al. (2012) used 16S rRNA amplicon sequencing to show that there are differences in bacterial community composition between the meristematic and older regions of the blade.

In this study, we expand on previous analyses of kelp-associated microbiota, using amplicon sequencing of both bacterial (16S rRNA gene) and microeukaryote (18S rRNA gene) surface communities from all anatomical regions of *Laminaria setchellii* (holdfast, stipe, meristem, and blade tip) to quantify taxonomic differences across regions of different tissue age. We augmented our study by sampling new recruits of *L. setchellii*, which were in their first season of growth and thus have greater homogeneity of tissue ages compared to adult samples, as well as two additional sympatric brown-algal species in order to test the generality of these patterns.

MATERIALS AND METHODS

We focused our microbial survey on *Laminaria setchellii*, a northeast Pacific species that is accessible on rocky shores at low tide. We collected microbial communities from four anatomical regions (holdfast, stipe, blade base = meristem, and blade tip; Fig. 1) of mature *L. setchellii* individuals ($n = 29$) between June 5 and 9, 2016, from a low intertidal bench on North Beach, Calvert Island British Columbia (51.666, −128.135). Each anatomical region was first rinsed with sterile seawater to remove transient microbes and then sampled using a Puritan® sterile swab stored in an individual sterile cryovial (VWR). Eight additional *L. setchellii* individuals of similar size were collected to estimate the average age of thalli in this population. For each of these specimens, the stipes were sectioned and the concentric annual growth rings were counted following the methods described by Klinger and DeWreede (1988). These samples were collected from the same population and of the same size as the individuals used for microbial analyses. This analysis found that the individuals in this study were all 2–3 years old (Table S1, Fig. S1 in the Supporting Information), making these specimens much younger than the maximum age (17 y) previously observed in a population from Vancouver Island, BC (Klinger and Dewreede 1988).

We also sampled microbial communities from the rocky substrate adjacent to *Laminaria setchellii* individuals using swabs as described above ($n = 20$). Seawater communities were sampled using sterile 500 mL plastic containers ($n = 25$), which were filtered using a Cole-Parmer MasterFlex L/S peristaltic pump with a 0.22 µm Durapore® membrane filter (Merck KGaA, Darmstadt Germany). Sampling microbial communities from the environment provides important information about the source community of ambient bacteria and microeukaryotes available to colonize seaweed surfaces. Data from the rocky substrate also provide a reference for how nearby abiotic surfaces are being colonized in the absence of host-driven biological filters.

We extracted DNA from swabs and water filters using the using MoBio PowerSoil®-htp 96 well DNA extraction kit. Amplicon sequencing was used to identify bacterial (V4 region of the 16S rRNA gene) and microeukaryote (V4-V5 region of the 18S rRNA gene) communities from each sample following the library preparation methods described by (Lemay et al. 2018). Raw MiSeq reads were clustered into amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). The resulting bacterial and microeukaryote ASVs were annotated to sequences in the SILVA 128 ribosomal RNA database (Quast et al. 2013, Yilmaz et al. 2014) and aligned using RAxML (Stamatakis 2014). We removed 18S ASVs that annotated as the macroalgal host, as well as all 16S

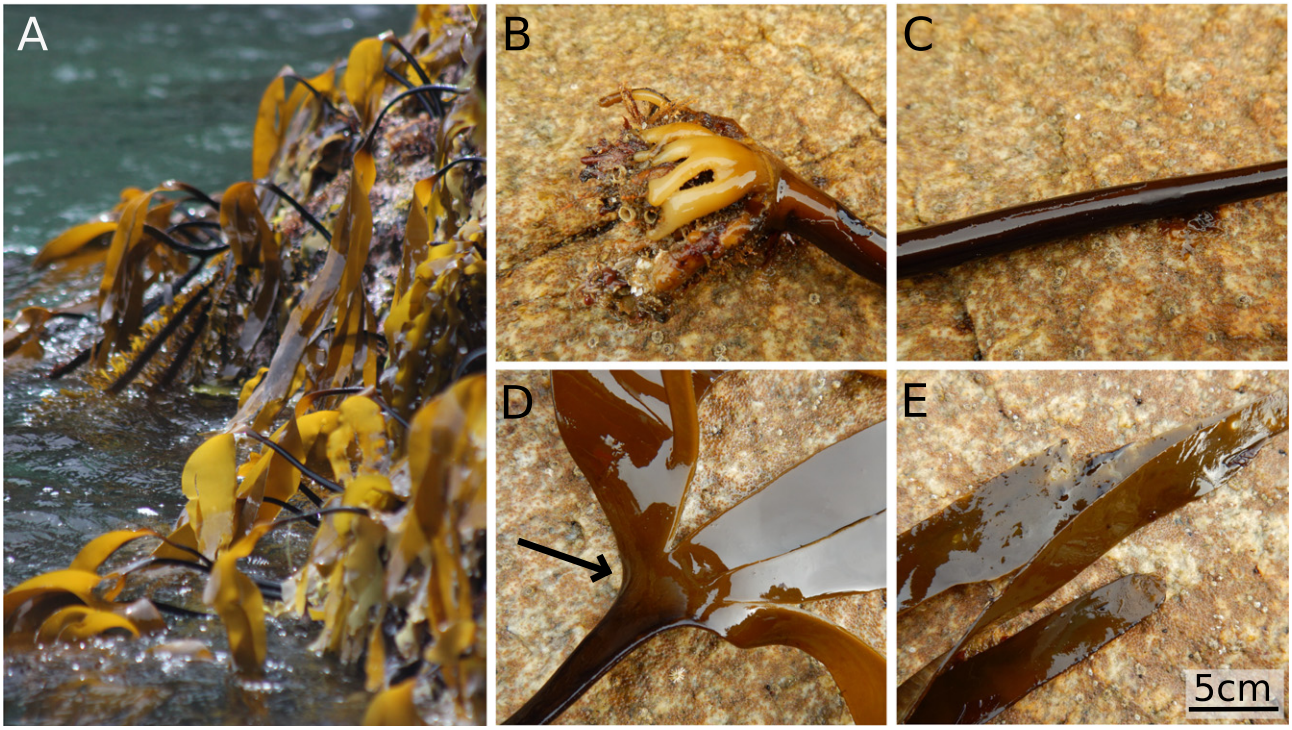

FIG. 1. Microbial communities on the surface of *Laminaria setchellii* (A) were sampled from four discrete anatomical regions: holdfast (B); stipe (C); meristem (D); and blade tips (E). Scale bar is approximate and refers to parts B-D. Photos by B. Clarkston and M. Lemay.

ASVs annotated as chloroplast and mitochondria. Additional ASVs were removed if they occurred in only a single sample, had fewer than 100 sequences, or had unassigned taxonomy. Bacterial data were rarefied to 3,000 reads per sample before statistical analyses, and microeukaryote data were rarefied to 1,000 reads per sample before statistical analyses. These rarefaction thresholds were selected to balance the need for statistical comparison across equal read depths while retaining the majority of samples. At this level of rarefaction, all 16S rRNA data were retained; however, six 18S rRNA samples fell below this threshold and were excluded from further statistical analysis.

Taxonomic richness was estimated for each sample using the Chao1 index (Chao 1984) and was computed separately for the 16S and 18S rRNA data. We tested for statistical differences in taxonomic richness among anatomical regions of *Laminaria setchellii* (blade tip, meristem, holdfast, and stipe) using a mixed model with the individual coded as a random factor, and this was carried out using the lmerTest package (Kuznetsova et al. 2016) in R v.3.2.3 (R Development Core Team 2015). We carried out a similar statistical test to include the samples from seawater and rocky substrate; this test was carried out with the source of the data (rocky substrate, seawater, blade tip, meristem, holdfast, and stipe) coded as a fixed factor and did not include a random factor since it was not possible to apply the factor "individual" to the environmental samples. Pairwise contrasts were computed using the lsmeans package (Lenth 2016) with the Tukey correction.

We compared microbial community structure among anatomical regions using a permutation analysis of variance (PERMANOVA) on Bray–Curtis dissimilarity as implemented in PRIMER v. 6 (Clarke and Gorley 2006) with 9,999 permutations and individual coded as a random factor. As with the test for taxonomic richness, this statistical analysis was repeated without the random factor (individual) so that the environmental samples could be included in the model.

We tested for core bacteria and eukaryotes that were consistently present on *Laminaria setchellii* and enriched compared to the environment to identify potentially important host–microbe associations. To be considered part of the core microbiome for an anatomical region, an ASV had to be present in >95% of the samples from that region. We removed ASVs from this analysis if they were also present in >95% of samples from substrate and seawater as these represent ubiquitous ASVs. We tested each anatomic region separately and then compared the core across regions and with the environment to determine whether there are stable core taxa always present on *L. setchellii* or specifically associated with different regions. We hypothesized that the meristem is most likely to harbor fewer specific taxa because it is new tissue and more highly chemically defended. There is no consistent definition of the core (Hernandez-Agreda et al. 2017, Risely 2020), and threshold values for prevalence range from 50% to 100%. While the best evidence for a stable and biologically important core relationship would require the microbial taxa to be present on 100% of hosts, we used this slightly relaxed threshold that allows for rare taxa to be missed on some replicates. This high threshold is appropriate here because all samples are from one location.

We augmented our study with samples collected from the surface of new recruits of *Laminaria setchellii* ($n = 5$) that were growing at the same location and tide height as the adult individuals. These individuals were in their first year of growth, which means that differences in the age of their tissues are much lower in comparison to adult samples. Bacterial communities from recruits were collected at the same four anatomical regions and processed as described above. These individuals were less than half the size of the adults, so in addition to the anatomical regions being more similar in

tissue age, the anatomical regions are also physically closer to each other than on adults.

In addition to our data from *Laminaria setchellii*, we opportunistically collected a small number of bacterial samples from two other brown-algal species (*Alaria marginata* and *Fucus distichus*; $n = 4$ individuals of each species). *Alaria marginata* is a kelp (order: Laminariales) with annual growth, which means that unlike *L. setchellii*, all anatomical regions of *A. marginata* grow anew each year. *Fucus distichus* (order: Fucales) is not a kelp, but does have perennial growth and can survive for several years (Ang 1991). Unlike the kelp species sampled in this study, new blade growth in *F. distichus* occurs from an apical meristem at the blade tips rather than at the base of the blades. Bacterial communities from these samples were quantified from three anatomical regions: holdfast, stipe, and blades. Data processing and statistical analyses were carried out as described above.

RESULTS

Amplicon sequencing the V4 region of the 16S rRNA gene resulted in 1,947 ASVs with a mean coverage of 44,432 quality filtered reads/sample. Sequencing the V4-V5 region of the 18S rRNA gene resulted in 391 ASVs with a mean coverage of 30,870 quality filtered reads/sample. The composition of microbial surface communities (both 16S and 18S rRNA gene data) was significantly different among anatomical regions of *Laminaria setchellii* (PERMANOVA of Bray–Curtis dissimilarity: bacteria pseudo-$F_{3,84} = 22.6$, $P = 0.0001$, Table S2 in the Supporting Information; microeukaryotes pseudo-$F_{3,64} = 8.2$, $P = 0.0001$, Table S3 in the Supporting Information). Pairwise comparisons revealed that all anatomical regions were significantly different from each other and also differed from microbial communities in the environment (Fig. 2). The richness of microbial communities (Chao1 index) was also significantly different among anatomical regions (ANOVA of Chao1 Index; bacteria $F_{3,84} = 92.4$, $P < 0.0001$, Table S4 in the Supporting Information; microeukaryotes $F_{3,77} = 22.4$, $P < 0.0001$, Table S5 in the Supporting Information) and was correlated with the age of the tissues (Fig. 2); new growth regions (meristem and stipe) have significantly lower ASV richness compared to the older regions (holdfast and blade tips).

The youngest tissue surfaces (blade meristems) were overwhelmingly dominated by sequences annotated to the genus *Granulosicoccus* (family Thiohalorhabdales) within the Gammaproteobacteria (Fig. 3). *Granulosicoccus* ASVs accounted for 55% of all meristem bacterial sequences averaged across all meristem samples (range: min = 6%, max = 94%). Indeed, the single most abundant bacterial ASV observed on *Laminaria setchellii* samples was from this taxonomic group and is 98% similar to previously published *Granulosicoccus* clones obtained from the meristem of the closely related kelp, *Saccharina japonica*, collected from the Sea of Japan (Balakirev et al. 2012). *Granulosicoccus* was also dominant on meristems of *L. hyperborea* (Bengtsson et al. 2012) and is common on *Nereocystis* and *Macrocystis* (Weigel and Pfister 2019), suggesting a consistent association of *Granulosicoccus* with kelp surface tissues. By contrast, the surfaces of older tissues hosted a significantly greater diversity of bacteria and were not dominated by any single taxa. These tissues showed an increase in ASVs from the Flavobacteria, Acidiomicrobiia, and Verrucomibia, with the oldest tissues of the holdfast having greater taxonomic similarity with rocky substrate than with other kelp tissues (Figs. 2 and 3).

The most abundant eukaryote (18S rRNA gene) sequences were overwhelmingly from diatoms (family: Bacillariophyceae). These were most abundant on stipe tissues, accounting for an average of 65% of eukaryote reads per replicate (Fig. 3). Sequences annotated to the Bacillariophyceae were also highly abundant on meristem tissues (44% of reads/sample), and while they had relatively lower abundance on the surfaces of the holdfast (23%) and blade tip (34%), they still occurred at much greater abundance on these surfaces than in samples from the environment (Fig. 3).

We found that each anatomical region of the host supported a small core group of bacterial ASVs that were present on 95% of replicate individuals (Fig. 4). Two ASVs (annotated as *Vibrio* sp. and *Algitalea* sp.) were present in the core group of all four tissue types and were also at high prevalence in the rocky substrate and seawater (>95% of replicates); these two ASVs are therefore abundant everywhere and were not retained in our list of core *Laminaria setchellii* ASVs. The oldest tissue (blade tip and holdfast) supported the largest core group of bacteria with 18 and 15 ASVs, respectively. In contrast, the youngest tissue (meristem) supported a much smaller core group of 4 ASVs, all from the Alpha- and Gammaproteobacteria (Genus: *Glaciecola* sp., *Teredinibacter* sp., *Granulosiciccus* sp., and sp.). Stipe tissue had the lowest number of core ASVs (2); both of these ASVs were from Gammaproteobacteria (*Granulosiciccus* sp. and *Leucothrix* sp.) and were also members of the core group of other tissues (Fig. 4). A fasta formatted list of all core bacterial ASV sequences is included in Appendix S1 in the Supporting Information. In contrast to bacteria, microeukaryote communities had much greater variability among replicate samples and as a result they lacked a common core community on each type of *L. setchellii* tissue.

Sampling bacterial communities from *Laminaria setchellii* recruits provided an interesting basis for comparison with adult samples. We found significant differences in the richness of bacterial communities across the anatomical regions of new recruits (ANOVA of Chao1: $F_{3,11} = 15.4$, $P = 0.0003$; Table S6 in the Supporting Information), in which the holdfasts of these recruits had greater ASV richness compared to all other anatomical regions (Fig. 5). There was also a significant difference in

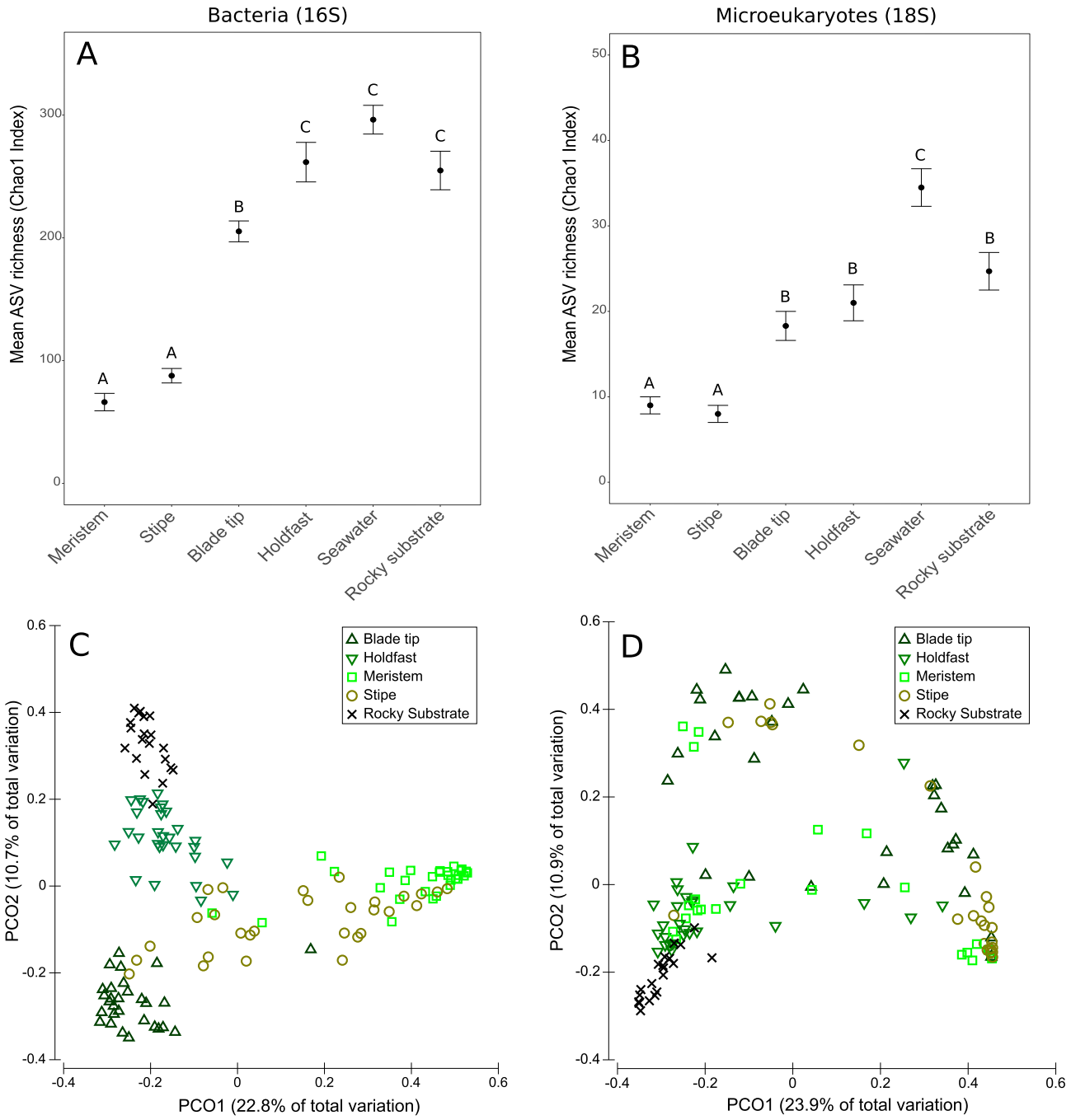

FIG. 2. Microbial ASV richness increases with the age of host tissue (A, B) and community composition (based on Bray–Curtis dissimilarity) differs among anatomical regions (C, D) of individual *Laminaria setchellii*. Anatomical locations include blade tip (BT), holdfast (HF), meristem (MS), and stipe (ST). Seawater samples are not included on PCOA (parts C, D) because they are so distinct that they distort the figure. Note that parts A and B use a different scale on the *y*-axis. [Color figure can be viewed at wileyonlinelibrary.com]

bacterial community structure among the meristem, holdfast, and blade tip of the recruits (PERMANOVA of Bray–Curtis dissimilarity: pseudo-$F_{3,11} = 2.6$, $P = 0.0001$, Fig. 5, Table S7 in the Supporting Information), suggesting that a relatively short time-frame, on the order of days or weeks in the case of these recruits, is sufficient to promote differences in bacterial communities along the length of the blade surface. Further, we found that the diversity of these communities was similar to those observed on adults (Fig. S2 in the Supporting Information).

From our small sample of sympatric brown-algal species, we found that communities on the surface

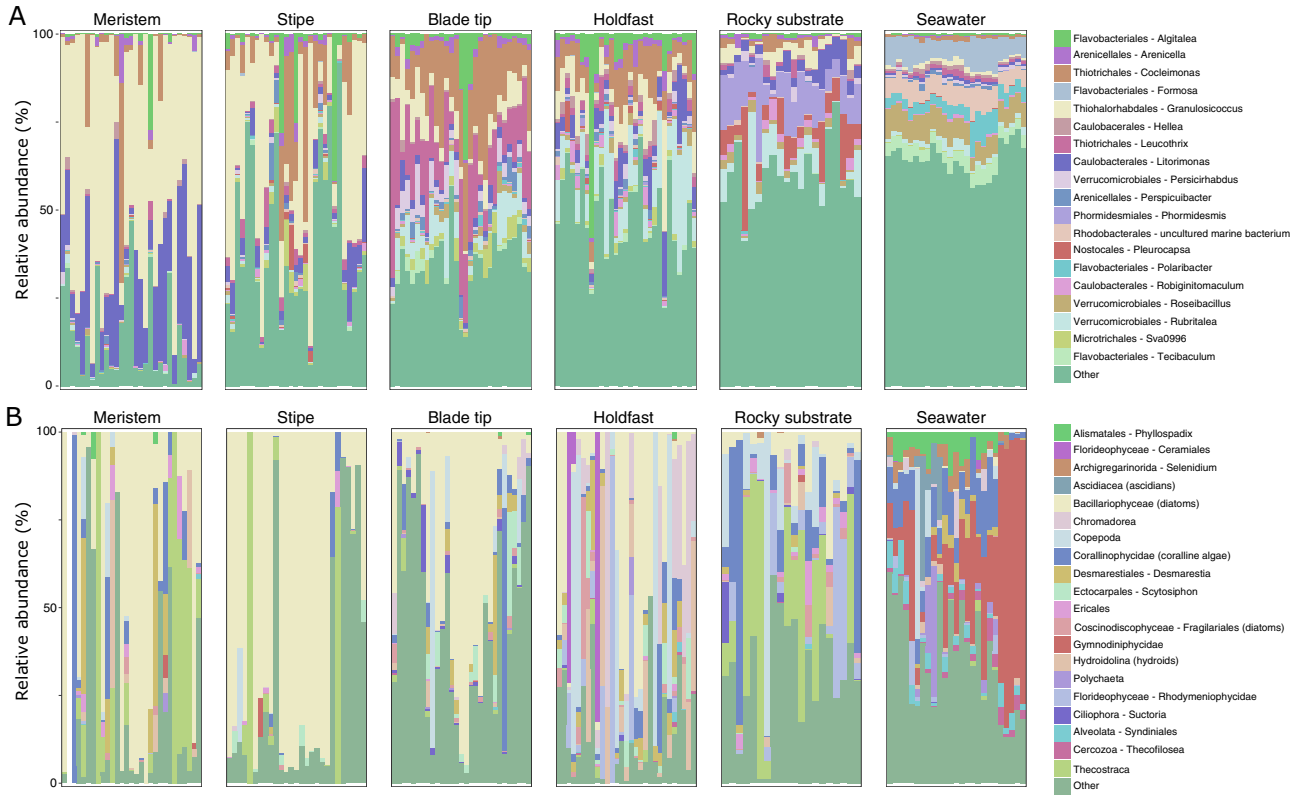

FIG. 3. The taxonomic composition of (A) bacterial and (B) microeukaryote communities become more complex on older algal tissues and in environmental samples. Each column represents a replicate individual, and anatomical regions are presented from left to right in order of increasing tissue age. The 20 most abundant taxonomic groups are presented with the remaining taxa combined as "other."

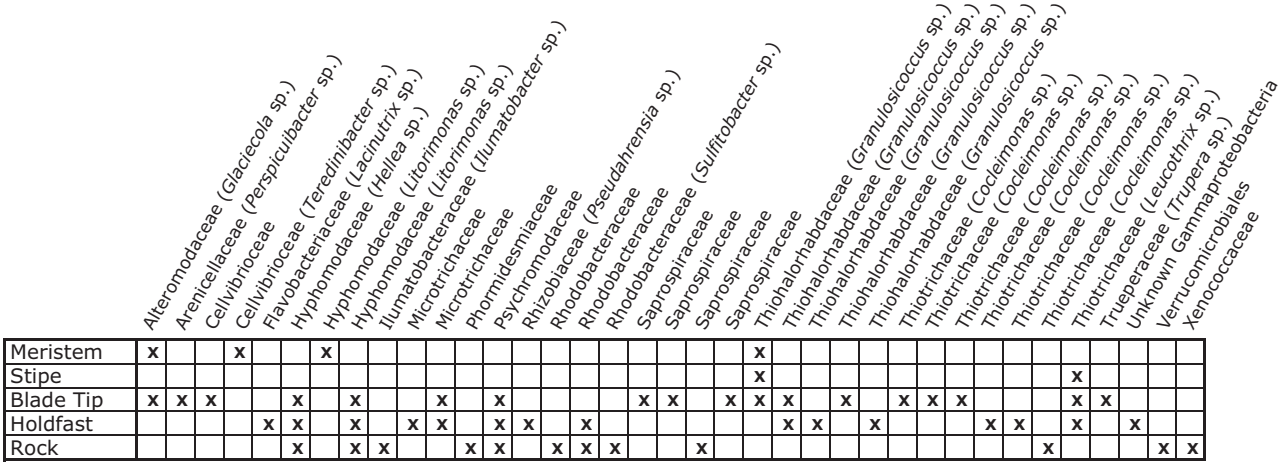

FIG. 4. The core group of bacterial ASVs expands and becomes more taxonomically diverse with tissue age. This figure displays the identity of core bacterial ASVs present on the surface of each anatomical region of *Laminaria setchellii*. For this analysis, inclusion in the core microbiome required that the ASV was observed in >95% of replicate samples for each anatomical region. Annotations are presented to the lowest available taxonomic resolution, and the core ASVs on rock, those present on >95% of samples, group present on rocky substrate is included for comparison. Anatomical regions in the figure are listed in order from youngest to oldest. The nucleotide sequences of all core ASVs are included in Appendix S1.

of *Alaria marginata* ($n = 4$), an annual kelp species, were significantly different across all three anatomical regions that were tested (blade, holdfast, and stipe; PERMANOVA of Bray–Curtis: pseudo-$F_{2,6} = 3.5$, $P = 0.0005$; Fig. 6, Table S8 in the Supporting Information). Holdfast tissue supported significantly greater ASV richness than the stipe tissue (Fig. 6, Table S9 in the Supporting Information).

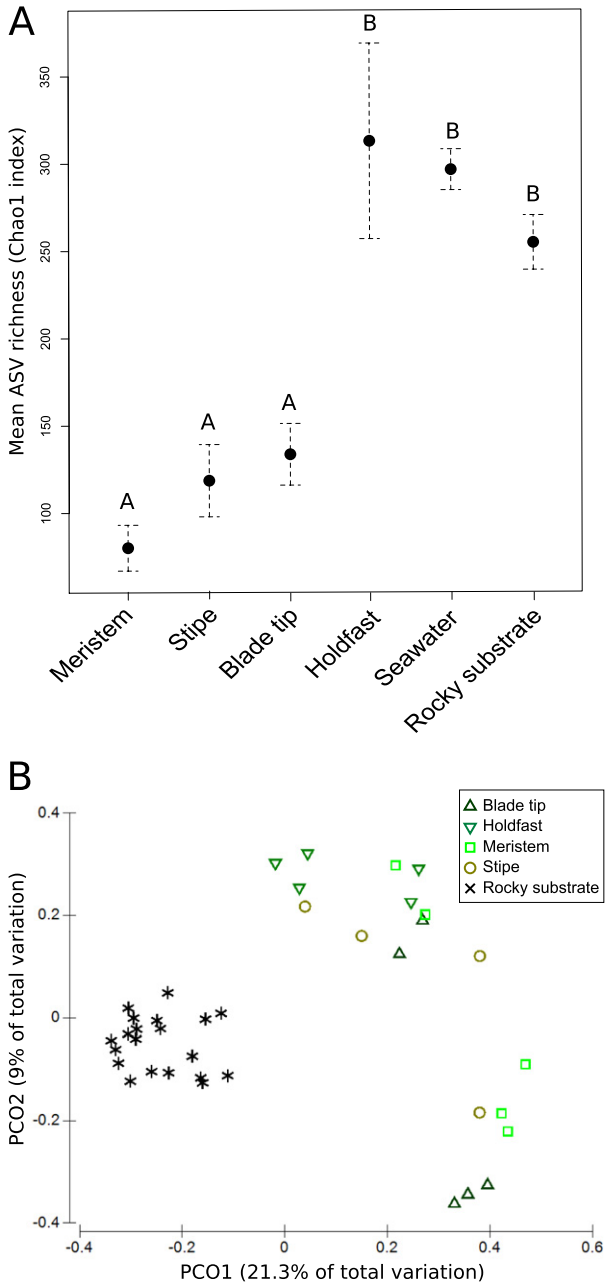

FIG. 5. (A) Richness (mean of Chao1 + SE) and (B) PCoA of Bray–Curtis dissimilarity of bacteria from the surfaces of *Laminaria setchellii* recruits ($n = 5$). Anatomical locations include blade tip (BT), holdfast (HF), meristem (MS), and stipe (ST). [Color figure can be viewed at wileyonlinelibrary.com]

Similarly, samples from *Fucus disticus* ($n = 4$), a brown alga from the order Fucales (i.e., not a kelp), also had significant patterns of community structure across host tissues (PERMANOVA of Bray–Curtis: pseudo-$F_{2,5} = 4.0$, $P = 0.0003$) with communities on blades differing from those on holdfast and stipes (Fig. 6, Table S8). The richness of the bacterial communities on *F. distichus* also varied across host anatomy, with the holdfast having greater richness than blade tissue (Fig. 6, Table S9).

DISCUSSION

In this study, we show that the surfaces of each anatomical region of the intertidal kelp, *Laminaria setchellii*, support statistically distinct communities of bacteria and microeukaryote symbionts. These results suggest that selection for kelp-associated microbial communities occurs at a very fine spatial scale over the surface of the host (on the order of centimeters) and that the mechanism is conserved among replicates of the same species. Indeed, we found that differences in microbiological communities among anatomical regions of the same individual were greater than the differences within any anatomical region across replicate individuals. It is also notable that the observed differences in microbial community structure across host anatomy are maintained despite their constant contact with microbes in the surrounding seawater and adjacent seaweeds.

In previous research, Staufenberger et al. (2008) used DGGE to quantify bacterial richness among anatomical regions of a related species, *Saccharina latissima*, and found comparable patterns of increasing diversity at the blade tip compared to the stipe and meristem. Similarly, in a new and highly relevant study, Ihua et al. (2020) used amplicon sequencing to quantify bacterial communities (V3-V4 region of the 16S rRNA gene) on the surface of *Laminaria digitata* from the Atlantic coast. They sampled communities from the same four anatomical regions as in our study and at the same point in time (summer 2016), providing an excellent basis of comparison with our data. In support of our results, they found the greatest bacterial richness on the holdfast, followed by the blade, while meristem tissue supported the lowest taxonomic diversity of bacteria, and stipe tissues were intermediate. The remarkable consistency of these patterns on different species of *Laminaria* from different continents suggests that microbial community structure across host anatomy is a common feature of the *Laminaria* microbiome.

Our data from *Alaria marginata* and *Fucus distichus* add further breadth to these observations by illustrating that microbial differences across host anatomy are robust across host taxonomic orders and host life-histories (annual and perennial). These results are supported by a recent study that found differences in bacterial communities between holdfasts and blades in three species of *Fucus* (Quigley et al. 2020). Likewise, in their examination of microbial communities from the annual kelp species, *Nereocystis luetkeana*, Weigel and Pfister (2019) found significantly greater richness of bacterial ASVs associated with the older tissue of the blade tip compared to the younger meristematic region. The occurrence of parallel patterns of bacterial community structure across different brown-algal species suggests that differences in microbial communities across host anatomy may be common in

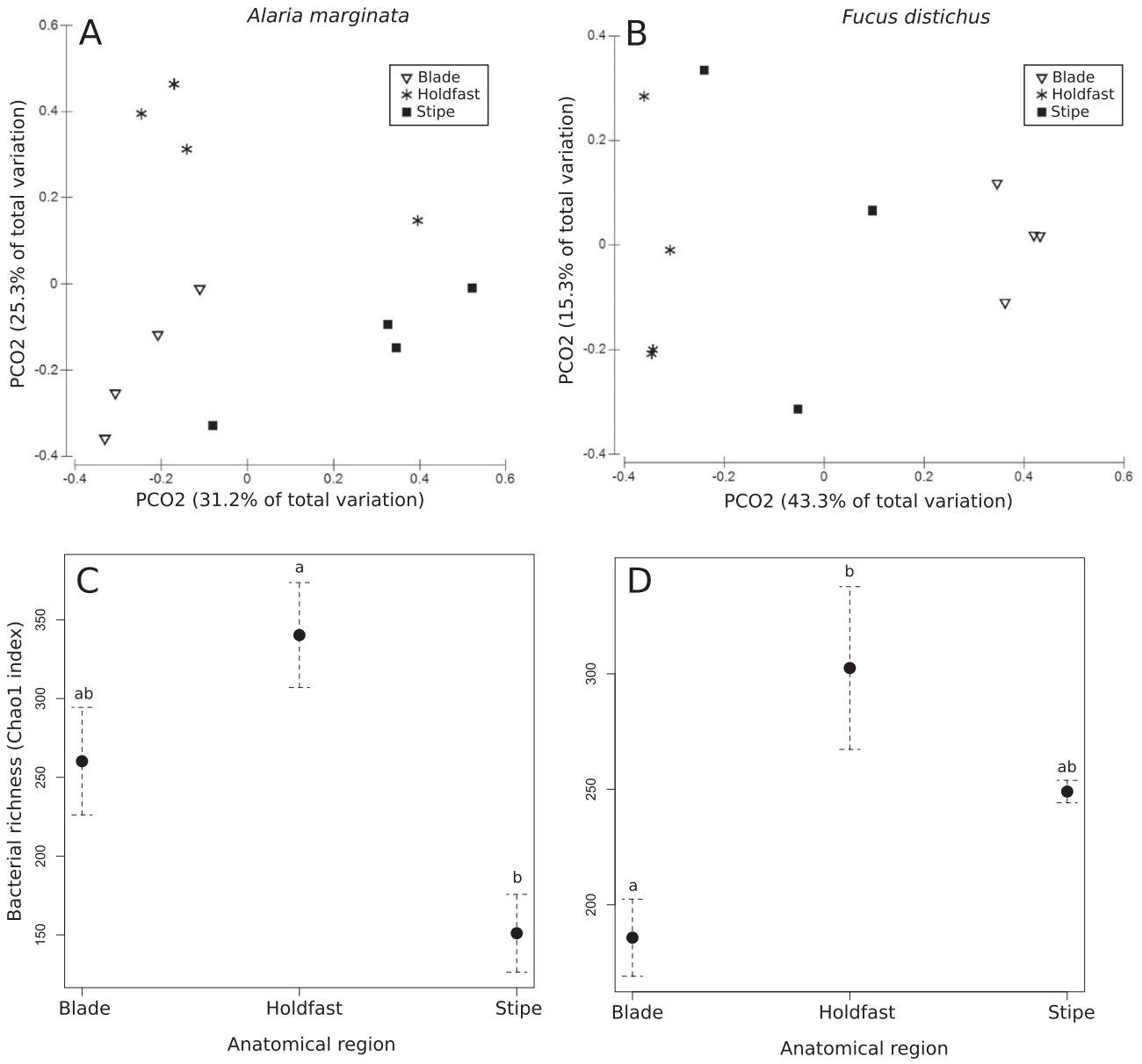

FIG. 6. Bacteria community composition is structured across anatomical regions of (A) *Alaria marginata* and (B) *Fucus distichus*. These PCoAs are based on Bray–Curtis dissimilarity of 16S rRNA ASVs. The richness of these communities (C, D) also differed among anatomical regions.

seaweeds, yet the mechanism driving this pattern is unknown.

We suggest several mechanisms that could drive the patterns observed in our study. First, the structure of microbial communities across anatomical regions of kelp is consistent with the hypothesis that these communities represent discrete successional stages of microbial symbionts on kelp surfaces of different age (Bengtsson et al. 2010, Weigel and Pfister 2019). Our samples from new recruits provide additional insight into this hypothesis. We found that the greatest differences between recruits and adults are in older tissues of the blade tips and holdfasts, whereas microbial communities on the meristems and stipe tissues of recruits are relatively similar to those of adults (Fig. S2). The similarity between meristem tissue of new recruits and adults provides additional support that their microbial communities represent an early successional stage – despite the adults being ~3 y old, the adult meristem tissue is newly formed and comparable in age to recruit meristems. Conversely, greater differences in the holdfast and blade tip suggest that microbiota on these tissues change over time via colonization, succession, and biofilm development. We suggest that future work should investigate the temporal

dynamics of these successional patterns. For example, our results from new recruits suggest that community turnover occurs on the blade surface at the scale of months, yet it is unknown how these changes progress. Finer resolution of sampling across the blade length will likely provide greater insight into succession and colonization patterns. An improved understanding of the ecological interactions between the host and early successional communities is also needed: Do the early colonizers (i.e., *Granulosicoccus*) perform functions that benefit kelp, or are they simply the best at exploiting this newly exposed habitat?

Differences in the biochemistry and function of host tissues could also explain the observed microbial community structure among anatomical regions. For example, in their study of the perennial kelp, *Macrocystis pyrifera*, Arnold and Manley (1985) found that anatomical regions significantly differ in their rates of photosynthesis, with blades having a greater photosynthetic rate than stipes, and holdfasts exhibiting zero photosynthetic capacity. They also found that within blades, the amount of total chlorophyll (chl *a* and *c*) increased from the base of the blade to the tips (Arnold and Manley 1985). Similarly, Starko et al. (2018) found that older, over-wintering, blade tissues of *Laminaria setchellii* have greater abundance of cellulose and fructose-containing polysaccharides, and are stiffer and thicker compared to meristem blade tissue. Tissue-specific differences in the abundance of polysaccharides and kelp-derived carbon may affect the diversity of microbes that colonize these surfaces.

Finally, we suggest that differences in the concentration of host defensive compounds could provide an alternative mechanism for structuring microbial communities across kelp anatomy. Several studies have shown that phlorotannin extracts from brown-algal tissues have anti-microbial properties (Nagayama et al. 2002, Lopes et al. 2012, Ford et al. 2020). Van Alstyne et al. (1999) hypothesized that seaweeds may preferentially allocate chemical defense to tissues with the highest fitness value, including meristem and reproductive tissue. This hypothesis is supported by the observation that the meristem region of kelps and rockweeds has increased concentrations of phlorotannin. Reproductive structures of kelps have also been shown to contain higher concentrations of phenolic compounds compared to vegetative tissues (Steinberg 1984). It is possible that differences in the concentration of phlorotannin in different host tissues could explain the comparatively low diversity of microbes that we observed on meristem tissues. Tissue-specific differences in the chemistry, rate of photosynthesis, and abundance of defensive compounds likely affect the assembly of surface microbiota, but experimental approaches will be needed to disentangle these factors from the confounding effect of tissue age.

Microbial communities from the surface of stipe epicortex of *Laminaria setchellii* show some unexpected patterns of diversity. Tissue from the stipe surface is replaced annually, making it older than the tissue of the meristem, but variable in relative age compared to the blade tips (depending on the time of year). We found that the microbial richness on stipes did not significantly differ from the meristematic region, was significantly lower than the blade tips and holdfasts, and supported the lowest number of core bacteria. We suggest that the stipe tissue that we sampled was at the early stage of its seasonal growth and thus this tissue's surface was newly formed, but there are other important differences between the stipe and blade tips that could explain these patterns. Notably that blade tips are in the process of being lost by erosion and are not likely defended by the host plant in comparison to the stipe tissue. Significant decay on the blade tips could mean that any host-filtering is absent from these tissues, resulting in increased microbial richness compared to other tissues of comparable age. Alternatively, the relatively low photosynthetic activity of stipes compared to the blades means that these tissues produce less carbon, which may result in a lower diversity of microbes on those surfaces compared to areas with higher rates of photosynthesis. Qualitatively, the stipe surface is much cleaner and clear of visible epiphytes compared to other anatomical regions. It may be that the sloughing external tissue of this region reduces the accumulation of epiphytes, including microbial symbionts.

Given the importance of seaweeds as foundation species in nearshore ecosystems, there is a rapidly growing body of research to quantify the microbiota of seaweed hosts (reviewed by: Egan et al. 2013, Hollants et al. 2013, Singh and Reddy 2014). To date, the majority of these studies have focused on the prokaryote component of the seaweed microbiome (using 16S rRNA amplicon sequencing), with very few studies quantifying the microeukaryote communities associated with seaweed surfaces (18S rRNA gene diversity). In the current study, we found that patterns of microeukaryote community richness and composition mirror those observed for bacteria (Fig. 2). However, the observed microeukaryote community is more variable across replicates and treatments, suggesting that these organisms form weaker associations with their hosts compared with bacterial symbionts. This pattern is exemplified by the lack of a common core group of eukaryote ASVs on any of the anatomical regions. The general pattern of weak but significant eukaryote community structure compared with bacteria mirrors our previous results from a red algal species (Lemay et al. 2018).

Extensive research on human skin has shown that the composition of surface communities is not homogeneously distributed across the host (The Human Microbiome Project et al. 2012). Rather, the composition of surface microbial communities is largely determined by body habitat (Costello et al. 2009, Grice et al. 2009), with each anatomic region supporting distinct ecosystems of microbial symbionts. This research

on the human microbiome demonstrates that a meaningful and comprehensive characterization of an organisms microbiota depends on sampling across all body habitats, as these may be strikingly divergent. Yet, this has rarely been applied to non-human systems, which may lead to an underappreciation of the complexity of non-human microbial ecology. We demonstrate that the microbiome of *Laminaria setchellii* shows considerable community structure across host anatomy and suggest that this pattern may be a common feature of the seaweed microbiome. The presence of microbial community structure across anatomical regions of *L. setchellii* has important implications for the design of experiments characterizing kelp-associated microbial communities. In order to promote robust comparisons, it is important that microbial samples are collected from the same tissue or anatomical region when comparing communities across different groups (individuals, populations, species, etc.). If the goal is to fully characterize the surface microbiota, then comprehensive sampling across all anatomical regions is necessary. Understanding the mechanisms driving community turnover across these anatomical regions is an important and exciting area for future research.

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AUTHOR CONTRIBUTIONS

Matthew A. Lemay: Conceptualization (equal); formal analysis (equal); investigation (equal); writing-original draft (equal); writing-review & editing (equal). **Katherine M. Davis**: Conceptualization (equal); investigation (equal); writing-original draft (equal); writing-review & editing (equal). **Patrick T. Martone**: Conceptualization; supervision (equal); writing-original draft (equal); writing-review & editing (equal). **Laura Wegener Parfrey**: Conceptualization (equal); supervision (equal); writing-original draft (equal); writing-review & editing (equal).

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DATA AVAILABILITY STATEMENT

Raw Illumina MiSeq reads and associated MiMARKS compliant metadata have been accessioned at the NCBI Sequence Read Archive (BioProject: PRJNA682229). A list of BioSamples is presented in Table S10 in the Supporting Information.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Cross-section of a mature *Laminaria setchellii* stipe with the growth rings highlighted using black lines. This individual is 3 y old.

Figure S2. Comparison of the bacterial diversity present on the surfaces of adult *Laminaria setchellii* compared to the same regions of new recruits. The top panel recreates Figure 1, with the recruit samples added. The bottom figures highlight each anatomic region individually. The four anatomical regions are: blade tip (BT), holdfast (HF), stipe (ST), and meristem (MS).

Table S1. Age of eight adult *Laminaria setchellii* individuals as inferred using an analysis of their growth rings.

Table S2. Statistical comparisons of bacterial (16S rRNA gene) community composition using PERMANOVA on Bray-Curtis dissimilarity. (A) The analysis was first restricted to samples collected from adult *Laminaria setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor in the model. (B) The analysis was

repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model.

Table S3. Statistical comparisons of eukaryote (18S rRNA gene) community composition using PERMANOVA on Bray-Curtis dissimilarity. (A) The analysis was first restricted to samples collected from adult *Laminaria setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor in the model. (B) The analysis was repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model.

Table S4. Statistical comparisons of bacterial (16S rRNA gene) taxonomic richness (ANOVA of Chao1 Index). (A) The analysis was first restricted to samples collected from adult *Laminaria setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor. (B) The analysis was repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model using the Tukey correction for multiple comparisons.

Table S5. Statistical comparisons of eukaryote (18S rRNA gene) taxonomic richness (ANOVA of Chao1 Index). (A) The analysis was first restricted to samples collected from adult *Laminaria setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor. (B) The analysis was repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model using the Tukey correction for multiple comparisons.

Table S6. Comparison of bacterial (16S rRNA gene data) taxonomic richness (ANOVA of Chao1 Index) among anatomical regions of *Laminaria setchellii* recruits ($n = 5$). (A) The analysis was first restricted to samples collected from recruit *L. setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor. (B) The analysis was repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model using the Tukey correction for multiple comparisons.

Table S7. Comparison of bacterial (16S rRNA gene) community structure (PERMANOVA of Bray-Curtis dissimilarity) among anatomical regions of *Laminaria setchellii* recruits ($n = 5$) using PERMANOVA. (A) The analysis was first restricted to samples collected from recruit *L. setchellii*; the fixed factor 'Anatomy' has 4 levels (Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and each individual was included as a random factor. (B) The analysis was repeated to include environmental samples. The fixed factor 'Sample Type' has 6 levels (Seawater, Rocky substrate, Blade tip [BT], Holdfast [HF], Meristem [MS], and Stipe [ST]) and the random factor was removed. (C) Pairwise comparisons were computed for this model using the Tukey correction for multiple comparisons.

Table S8. Comparison of bacterial community structure (PERMANOVA of Bray-Curtis dissimilarity) among anatomical regions of *Alaria marginata* ($n = 4$) and *Fucus distichus* ($n = 4$) using PERMANOVAs. For each test, anatomical regions was a fixed factor and the individual host was a random factor in this model. No environmental samples were collected for comparison with these samples.

Table S9. Comparison of bacterial richness (ANOVA of Chao1 Index) among anatomical regions of *Alaria marginata* ($n = 4$) and *Fucus distichus* ($n = 4$). For each test, anatomical regions was a fixed factor and the individual host was a random factor in this model.

Table S10. Raw Illumina reads and associated MiMARKS compliant metadata have been accessioned at the NCBI Sequence Read Archive (BioProject: PRJNA682229). The table below contains the list of BioSamples included in this study.

Appendix S1. Nucleotide sequences of all ASVs identified as part of the common core microbiome of *Laminaria setchelli*. The header information includes the ASV number, taxonomic information, and the anatomical region where each core taxa was identified (holdfast = hf; meristem = ms; blade tip = bt; stipe = st).