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ARTICLE

Morphological complexity affects the diversity of marine microbiomes

Matthew A. Lemay[1,2] · Melissa Y. Chen[2] · Florent Mazel[2] · Katharine R. Hind[1,2,3] · Samuel Starko[2,3] · Patrick J. Keeling[1,2] · Patrick T. Martone[1,2] · Laura Wegener Parfrey[1,2,4]

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Abstract

Large eukaryotes support diverse communities of microbes on their surface—epibiota—that profoundly influence their biology. Alternate factors known to structure complex patterns of microbial diversity—host evolutionary history and ecology, environmental conditions and stochasticity—do not act independently and it is challenging to disentangle their relative effects. Here, we surveyed the epibiota from 38 sympatric seaweed species that span diverse clades and have convergent morphology, which strongly influences seaweed ecology. Host identity explains most of the variation in epibiont communities and deeper host phylogenetic relationships (e.g., genus level) explain a small but significant portion of epibiont community variation. Strikingly, epibiota community composition is significantly influenced by host morphology and epibiota richness increases with morphological complexity of the seaweed host. This effect is robust after controlling for phylogenetic non-independence and is strongest for crustose seaweeds. We experimentally validated the effect of host morphology by quantifying bacterial community assembly on latex sheets cut to resemble three seaweed morphologies. The patterns match those observed in our field survey. Thus, biodiversity increases with habitat complexity in host-associated microbial communities, mirroring patterns observed in animal communities. We suggest that host morphology and structural complexity are underexplored mechanisms structuring microbial communities.

Introduction

Microbes are fundamental to the biology of multicellular organisms. Host-associated microbes (symbionts) influence the growth, health, and development of their hosts [1–3], leading to the assertion that we cannot understand multicellular organisms without considering their microbiome [2, 4]. This task is complicated by the fact that the microbiome is not a constant, but instead is a collection of microbes that varies tremendously across individuals and populations. We have limited understanding of the extent to which microbiome composition and diversity are determined by the host versus the environment, and which attributes of the host are most important. Host identity explains the largest portion of microbiome variation in many organisms, as comparisons of the microbiota across closely related species of plants and animals have shown [5–7]. We also know that host-associated microbiota are distinct from neighbouring environmental microbiota in nearly all cases. What attributes of the host drive this pattern? Many traits are wrapped up in host identity, such as life history, physiology, diet, and habitat use, and there is

These authors contributed equally: Matthew A. Lemay, Melissa Y. Chen, Florent Mazel

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✉ Matthew A. Lemay
matt.lemay@hakai.org

✉ Laura Wegener Parfrey
lwparfrey@botany.ubc.ca

[1] Hakai Institute, 1002 Wharf Street, Victoria, BC V8W 1T4, Canada

[2] Department of Botany, University of British Columbia, 3529-6270 University Blvd., Vancouver, BC V6T 1Z4, Canada

[3] Department of Biology, University of Victoria, PO BOX 1700 Station CSC, Victoria, BC V8W 2Y2, Canada

[4] Department of Zoology, University of British Columbia, 4200-6270 University Blvd., Vancouver, BC V6T 1Z4, Canada

debate as to whether host phylogenetic relationships or host ecology primarily structure microbiome variation, and how this varies across systems. Insight into the factors that structure the microbiome will help predict responses of the host and their associated microbiota to changing global conditions.

Microbial community structure correlates with host relatedness—a pattern termed phylosymbiosis—in systems ranging from the mammalian gut to termites to the plant rhizosphere [8–10]. Such correlations between the microbiota and host phylogeny are of interest because they may suggest long term associations between host and microbe. However, strict co-evolution is rare, and phylosymbiosis patterns can readily be generated when microbes colonize hosts in response to phylogenetically correlated host traits [10, 11]. Phylogenetic relationships structure the microbiota across a wide range of hosts, but the signal is weaker and less often recovered for surface-associated symbionts [5, 6, 10, 12]. This reduced signal on surface communities likely occurs because surface symbionts are assumed to be acquired from the environment anew each generation, because the rate of interaction with the pool of environmental microbes is much higher, and because the differences between host and non-host surfaces are less pronounced (e.g., similar temperature, pH) in aquatic environments.

Host ecology, the interaction of a host with its environment, also has a strong influence on microbial community structure. Distantly related host species that show evolutionary convergence in key aspects of their biology often support convergent microbial communities [13, 14]. For example, a survey of 29 cichlid fish species found that host trophic niche is significantly correlated with the composition of gut bacterial communities [14], independent of host relatedness and geographical location. Similarly, host diet strongly predicts the structure of gut microbial communities across a diversity of host systems including insects, fish, and mammals [13–15]. In seaweeds, host life-history (annual vs perennial) influences kelp microbiota [12, 16].

In many cases it has been shown that both host ecology and host phylogeny influence microbial community structure, and do not function independently [8, 13, 17]. For example, in termites, microbial community structure is more similar within than between subfamilies (reflecting host phylogeny), but termite diet explains most of the variation in microbial community structure overall and the phylogenetic patterns are nested within each diet type [13]. Diet and host phylogenetic relationships are similarly nested in hosts such as cichlid fishes [14] and mammals [15], resulting in complex patterns of microbial diversity across host species that are challenging to disentangle.

Seaweeds are foundation species that provide essential habitat, nutrients, and carbon to coastal ecosystems [18]. Microbes influence many important aspects of seaweed biology ranging from development to disease resistance and also likely influence carbon and nutrient cycling [19]. ‘Seaweed’ is not a taxonomic term, but rather a descriptive term for a large polyphyletic group of multicellular photosynthetic eukaryotes (algae) that have arisen from single celled ancestors many times within lineages of eukaryotes (Rhodophyta, Chlorophyta, and Ochrophyta: Phaeophyceae) that diverged more than a billion years ago [20]. Across seaweed lineages, species differ in many aspects of their biology including their chemical exudates, life-history strategy, longevity, and life cycle, yet occupy the same habitats and show remarkable convergence of morphologies. Diverse communities of seaweeds from distant algal lineages with convergent morphologies that co-occur in the same location provides a tractable system to disentangle the effects of host phylogeny and ecology in marine surface symbioses.

Morphological characteristics (i.e., body shape) of seaweeds are strongly tied to their ecology and dictate how species interact with their environment (e.g., [21, 22]). For example, seaweeds have evolved several morphological adaptations to resist hydrodynamic forces resulting from waves and tidal currents [23–25]. When struck by waves, upright seaweeds minimize the surface area exposed to water flow, changing shape into streamlined forms that minimize drag coefficients [24, 26, 27], while crustose seaweeds have a low profile, avoiding drag altogether. Crustose seaweeds also resist stresses such as herbivory, sand scouring, and winter storms [28], allowing them to be long-lived with low rates of mortality [29]. In contrast, upright species tend to grow more rapidly at the expense of being shorter-lived and generally more palatable to herbivores [28, 30, 31]. The red algal genus *Mastocarpus* alternates between crustose and upright life history phases, and it has previously been shown that these life-history phases support distinct microbial communities [32].

We predict that ecological differences associated with seaweed morphology will be reflected in their microbiota, such that hosts with convergent morphology will support convergent microbial communities. Moreover, we predict that hosts with complex morphologies will support a greater richness of microbes, consistent with well-established paradigm that structural complexity increases biodiversity in many animal systems. To test this hypothesis, we used a large field-survey to quantify microbial communities (bacteria and microeukaryotes) from 38 sympatric species of intertidal seaweed encompassing three algal phyla and 16 orders. The sympatric occurrence of these evolutionarily diverse—yet morphologically convergent—species allows us to disentangle the effects of habitat, host phylogeny, and host morphology on the seaweed microbiome. We also experimentally deployed artificial seaweeds that differed

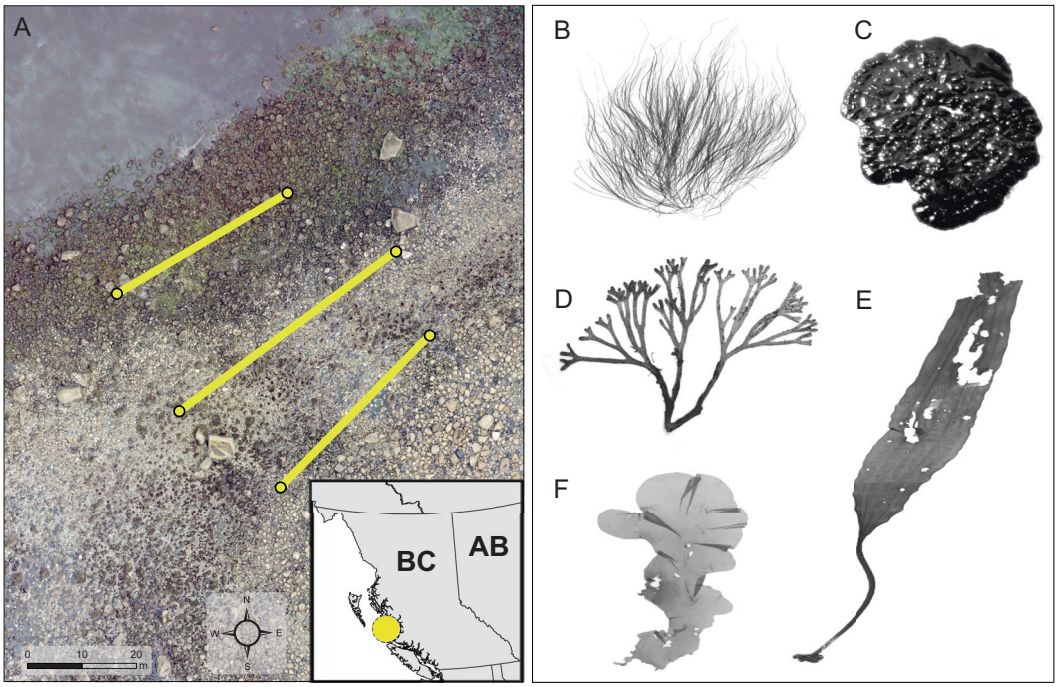

Fig. 1 Field-sampling seaweed microbiota. Sympatric seaweeds ($n = 38$ species) were sampled at a rocky intertidal site on Calvert Island, British Columbia (**a**). These species were grouped into five discrete categories used to explore the effect of seaweed morphology on microbial community structure: (**b**) finely branched, (**c**) crustose, (**d**) coarsely branched, (**e**) large blades, and (**f**) thin blades.

only in morphological complexity to further isolate the effect of host morphology from biotic interactions.

Materials and methods

Microbial sampling

We sampled microbial communities (bacteria and micro-eukaryotes) from 38 sympatric species of intertidal seaweeds ($n = 288$ individuals) at a rocky shoreline on the west coast of British Columbia, Canada (51.651°, −128.145°). These species encompassed a diversity of algal lineages (Table S1). Sampling was carried out along three horizontal transects that differed in tidal elevation (1.3 m, 1.9 m, and 2.5 m elevation above chart datum; Fig. 1). At each low tide event between March 17-23, 2015 we walked the length of each transect and sampled microbial communities from every macroalgal species present; $n \geq 5$ was attempted for each species at each tide height. We first rinsed each host alga with sterile seawater in order to remove non-host associated environmental microbes and then sampled microbial communities using a Puritan® sterile swab. The swab was stored in an individual cryovial (VWR) and placed on ice for transport back to the lab where they were transferred to −80 °C for storage. Swabbing was carried out at the base of the blade, which for many species represents the area of newest blade growth. Sampling consisted of rubbing a consistent surface area (~4 cm^2) for 10 s. For species that lack a blade, the surface area and time where consistent and sampling occurred at analogous regions. Swabbing the area of newest growth was carried out to control for the potential effect of microbial succession on the algal surfaces.

Host species were keyed to the lowest possible taxonomic level and their identity was confirmed using DNA barcoding (Table S1). In addition to the taxonomic identity of each host, we grouped all seaweed species into five discrete morphological categories (Fig. 1; Table S1). These categories were loosely based on those introduced by Littler and Littler [21] and used in subsequent analyses to examine the relative effect of host species and morphology on microbial community structure. Additional microbial samples were collected from rocky substrate at locations with exposed rock along each transect ($n = 29$) and from seawater collected at the water's edge, adjacent to the lowest transect ($n = 27$). Microbial communities were quantified by sequencing the V4 regions of both the 16 S rRNA and 18 S rRNA genes. Illumina MiSeq reads were clustered into taxonomic units using the Minimum Entropy Decomposition method described by Eren et al. [33], with a subset of analyses repeated using data clustered with DADA2 [34] to confirm the robustness of our main conclusions (see Figs. S1, S2, and supplemental text). Detailed methods for microbial sampling, amplicon sequencing, and DNA barcoding are presented in the supplementary material.

Beta diversity

Compositional dissimilarities (i.e., beta-diversity) are usually described with a single measure, either using taxonomic metrics, such as Bray Curtis distance, or using phylogenetic metrics, such as UniFrac [35]. However, relying on a single number to describe community dissimilarities may be too simplistic if different factors (here host phylogeny, morphology, or substrate type) shape compositions in OTUs at different microbial phylogenetic scales [15, 36, 37]. To overcome this limitation, we used the Beta Diversity Through Time (BDTT) analysis as described previously [15, 36], to quantify beta-diversity at different depths of the microbial phylogeny (R scripts available at https://github.com/FloMazel/BDTT). Note that "time" in this context refers to evolutionary time separating microbial taxa. Standard phylogenetic distance metrics such as UniFrac use a single distance value to summarize turnover at all microbial phylogenetic scales (the UniFrac value for a given pair of samples), BDTT slices it further, providing distance values at different scales of the microbial tree (hence we have many beta-diversity values for each pair of samples). For all BDTT analyses we used Bray Curtis dissimilarity after rarefying to 1000 sequences/sample and analyzed the 16 S and 18 S rRNA data separately. In the main text, we only present the statistical results using the initial OTUs (i.e., the tips of the microbial phylogeny) but we graphically present the results for all the slices of the microbial trees in the supplementary material.

Statistical analyses

To test the effects of substrate type, tidal height, host taxonomy and morphology on microbial community composition we used PERMANOVA as implemented in the adonis2 function from the vegan R package [38]. BDTT was applied by running the models described below with different delineation of microbial units (i.e., at different depth of the microbial phylogeny).

To test the effect of substrate type (three levels: seaweed, rocky substrate, seawater) and tidal height, we used the following model: Bray-Curtis ~ Substrate type + Tidal height, with a type III sum of squares, i.e., testing the effect of each factor while taking into account the other ones (using the adonis2 function in vegan, option "by" set to "margin"). As preliminary analysis showed that dispersion was also significant (i.e., different substrates show contrasted dispersion) and the PERMANOVA can be biased by dispersion effect if the sampling is unbalanced, we also ran the same model but with a sub-sampled dataset. We randomly subsampled 25 rocky samples and 25 seaweed samples (seawater contained 25 samples) and re-ran the model. Results were quantitatively similar to the full set of samples so we only present the results from the full set of samples in the main text.

In order to statistically test for an effect of morphology on microbial community structure, we first needed to understand the effects of host relatedness that may confound our conclusions about morphology. Specifically, we tested whether species-level patterns are nested within broader host lineages. To test the effect of seaweed relatedness on their microbiome we used PERMANOVA on five distinct datasets, in the spirit of a nested PERMANOVA. Because host taxonomy presents a nested structure (i.e., species are nested with genera, genera are nested within families, etc.), typical PERMANOVA cannot be used. Instead, we successively tested the effect of host species, genus, family, order and class. To test the effect of species alone, we used Bray Curtis dissimilarities between individuals for those species for which we had more than one individual. To test the effect of host genus alone, we averaged Bray Curtis dissimilarities between species and only include genera with more than one species (i.e., four genera: *Acrosiphonia*, *Laminaria*, *Mastocarpus*, *Pyropia*). To test the effect of host family alone, we averaged Bray Curtis dissimilarities between genera and only include families with more than one genus (i.e., three families: Ceramiaceae, Corallinaceae, Endocladiaceaea). To test the effect of host order alone, we averaged Bray Curtis dissimilarities between families and only include order with more than one family (i.e., four orders: Ceramiales, Gigartinales, Laminariales, Ulvales). To test the effect of host class alone, we averaged Bray Curtis dissimilarities between order and only include classes with more than one order (i.e., three classes: Florideophyceae, Phaeophyceae, Ulvophyceae).

Many species were only found at one or two tidal heights, and microbiota differences across tide heights likely contribute to the apparent variability within species. To test the effect of host species identity and tidal height, we used PERMANOVA on a set of samples only containing host species that occur in all three tidal heights (*Endocladia muricata*, *Fucus distichus*, *Hildenbrandia sp.*, *Mastocarpus alaskensis*, and *Pyropia perforata*). We used the following model Bray Curtis ~ Species Identity + Tidal height with a type III sum of squares (option 'by' in adonis2 function set to "margin").

We tested for the effect of host morphology while taking into account host relatedness using a PERMANOVA test that included both taxonomy and morphology and incorporated either host order or family as a covariate. We used this approach to account for host phylogeny instead of a tree-based method because we include taxa that do not share a recent common ancestor (e.g., brown and red algae). This analysis was carried out at the host genus level to avoid pseudo-replication because, within our data, all species within the same genus also share the same morphological

category. The genus *Mastocarpus* was excluded from this analysis because species in this genus have two morphologies as they alternate between crustose (sporophyte) and coarsely branched (gametophyte) life history phases, which would confound our analysis. We have previously demonstrated that these alternate life history phases of *Mastocarpus spp*. have distinct microbial communities [32] and wanted to avoid biasing our results.

To further disentangle host relatedness and morphology and provide an additional line of evidence, we used phylogenetic comparative analyses on the subset of red algal samples from our survey (phylum Rhodophyta), which is the most diverse clade of seaweeds sampled in this study ($n = 23$ species). We focused only on the red algal host species in this analysis to minimize negative effects of phylogenetic uncertainty that would result from including distantly related host taxa from the green and brown algae. To facilitate these analyses, we first reconstructed a phylogenetic tree using published and newly acquired sequence data, focusing on the two loci that were best represented across the taxa of interest (the mitochondrial gene, COI, and the plastid gene, *rbcL;* See Supplemental Text and Table S2 for detailed phylogenetic methods). This tree was used to construct a phylogenetic distance matrix. For these same red algal species, we assigned a complexity ranking to each morphological category present in the Rhodophyta samples (1 = crust, 2 = thin blade, 3 = coarsely branched, 4 = finely branched) and used this ranking to construct a morphological complexity distance matrix. We then used multiple regression on matrices (MRM) and variance partitioning to tease apart the effects of morphology and phylogeny on a Bray-Curtis dissimilarity matrix of bacterial community composition. Using variance partitioning, we estimated the partial and shared effects of host morphology and phylogeny. These phylogenetic analyses were conducted using the R packages ape [39], nlme [40], phytools [41] and ecodist [42].

To test for an effect of host phylogenetic relatedness on bacterial and microeukaryotic OTU richness, we used Blomberg's K [43] and Pagel's Lambda [44], two widely used indices of phylogenetic signal. We then used a lambda model of evolution [44, 45], which is a tree transformation used to identify correlations between phylogenetic distance and trait dissimilarity, to test whether morphology influences microbial diversity while controlling for phylogeny by using a phylogenetic least squares (PGLS) model. We did this analysis using the four categories of morphology present in our red algal samples.

Artificial seaweed experiment

We constructed artificial seaweed to experimentally test the effect of host morphology on the structure of microbial surface communities in the absence of biological or chemical host-microbe interactions. Artificial seaweeds were constructed from 0.4 mm thick latex sheeting (Radical Rubber olivegrn40, Elastica Engineering) in shapes that emulate the three most distinct morphological groups from our survey: crustose, thin blade, and branched forms. Latex has previously been used to simulate natural seaweeds as it has similar flexibility and performance in flow (i.e., drag coefficient) [24]. These were each attached to separate 7.5 × 7.5 cm laminate tiles with silicon glue; the thin blade and branched forms were glued by the base of the stipe, whereas the crustose forms were glued flat to each tile. Importantly, the two-dimensional surface area for each morphology was consistent at ~44 cm^2: The branched morphology was simply the bladed morphology cut into filaments whereas the crustose morphology was created by rounding off the pointed tip of the bladed morphology before gluing it flat.

To test for differences in the accumulation of bacterial communities among morphologies, we submerged replicate artificial seaweeds at two sites. To create a consistent microenvironment, samples at each site were suspended from a dock ~1 m below the water surface. This location placed them in constant contact with source microbial communities from the seawater and not within or adjacent to any naturally occurring seaweed communities, though each site was within ~50 m of *Fucus* beds. At site 1 (Calvert Island Field Station, August 2016, 51.654° −128.1298°) 9–10 replicates of each morphology were sampled at each time point: 20 min, 1 h, 6 h, 12 h, and 4 days. At site 2 (Port Moody, March 2016; 49.2918° −122.8897°) three replicates of each morphology were sampled destructively at each of five different time points 20 min, 1 h, 3 h, 6 h, 12 h, and 24 h. Repeated sampling was carried out because we were unsure the rate at which biofilms would accumulate, however the statistical analyses were designed to contrast differences in morphology rather than investigate succession changes across sampling intervals (see below).

Microbial communities were sampled using swabs as described above for real seaweed surfaces, and all library preparation, amplicon sequencing, and sequence processing was carried out using the same protocols as described above. Bacterial richness was calculated using the Chao1 index for each artificial seaweed sample, Bray Curtis distance was used to construct dissimilarity matrices among samples after rarefying to 4000 reads/sample. We tested for differences in community composition among morphologies using a PERMANOVA. This model included time points as a fixed factor and was carried out separately at each site (because the spacing of the points was different between sites). We used an ANOVA to test for differences in OTU richness (Chao1 index) with the model constructed as described above.

Results

We quantified microbial communities from 288 individual seaweeds, which encompass a diversity of algal lineages, morphologies, and tidal heights ($n = 38$ species; Fig. 1; Fig. S3, Table S1). We also sampled microbial communities from seawater ($n = 27$) and rocky substrate ($n = 29$), which confirmed that the composition of microbial communities on seaweed is distinct from the environment (16 S: pseudo-F = 15.8, $R^2 = 0.09$, $P = 0.001$; 18 S: pseudo-F = 18.7, $R^2 = 0.1$, $P = 0.001$; Table S3, Fig. S4). In general, samples from rocky substrate have a much greater similarity to samples collected from algal surfaces, compared to seawater samples (Figs. S1, S2), which we hypothesize is due to the common presence of microbial taxa that adhere to or are associated with surfaces (i.e., biofilm-forming) such as Hyphomonadaceae (Alphaproteobacteria) and Saprospiraceae (Bacteroidetes). Vertical zonation on the shoreline (tidal height) also had a significant effect on the composition of microbial communities on these substrates (Table S3, Fig. S5).

Differences in the microbial communities on these substrates (seaweed surfaces, rocky substrate, seawater) extend beyond the OTU level; our BDTT analysis shows that these substrates also differ in the entire clades of microbes that occurred preferentially on one substrate or the other (Fig. S5). Indeed, using the BDTT approach we find that varying the phylogenetic scale of the analysis also does not change the overall patterns in our data for host taxonomy, morphology, or tide height: significant differences observed at the OTU level are also significant at deeper levels (Figs. S3–S6). Given that analyses at different microbial phylogenetic scales were all consistent with the OTU level, we present the standard results at the OTU level in main paper, and include supplemental figures that illustrate this pattern (Figs. S3–S6).

Microbial community structure: disentangling host morphology and phylogeny

To visualize broad relationships between host species and their bacterial symbionts, we constructed an UPGMA dendrogram by calculating the Bray Curtis distance among microbial communities present on each host species, where OTU abundance was averaged across individuals from the same tide height (Fig. 2). Clustering within the dendrogram portrays a complex mixture of taxonomic and ecological patterns. For example, the largest cluster is predominantly composed of morphologically similar red and brown algal crusts and rocky substrate. Yet, within this crustose group, host species from the genus *Mastocarpus* cluster together, illustrating a role for host relatedness shaping microbial assemblages. Similarly, for many species, samples from different tidal heights cluster together, suggesting that host factors select for similar communities despite differences in abiotic factors such as emersion time and salinity stress (e.g., *Fucus*, *Endocladia*, *Mastocarpus*, *Acrosiphonia*, *Hildenbrandia*). However, other taxa (e.g., *Ulva*, *Pyropia*) lack consistency among transects. The lack of consistency among *Ulva* samples echoes previous research, which found the bacterial surface communities were highly variable among *Ulva australis* individuals [46, 47].

To approximate host phylogeny, we used seaweed taxonomy, and ran a series of nested PERMANOVAs to test whether there is greater similarity of individuals within-versus-among species, species within-versus-among genera; genera within-versus-among families, etc. Across the whole dataset we found that sympatric seaweed species support distinct bacterial (PERMANOVA: pseudo-F = 5.1, $R^2 = 0.44$, $P = 0.001$) and eukaryotic (PERMANOVA: pseudo-F = 2.9, $R^2 = 0.31$, $P = 0.001$) surface communities (Fig. S6; Table S4). At deeper levels of host relatedness, there was also a significant signal for host genus and family, but not order, in structuring bacterial communities, and a significant signal for host genus and order, but not family for microeukaryote communities (Fig. 3; Table S4; Fig. S6). This pattern is apparent in the dendrogram: for example, species within *Mastocarpus*, *Laminaria* and *Acrosiphonia* cluster together; *Ralfsia* and *Analipus* cluster together (order Ralfsiales), as do *Corallina* and *Bossiella* (order Corallinales). The observed phylosymbiosis pattern in our data decreases at deeper host taxonomic ranks: species explain much more variation than genera and above (Fig. 3; Fig. S6). This pattern of species explaining much more variability than deeper phylogenetic levels is consistent with results from other systems such as mammalian gut symbionts [15], leaf microbiota [5], and sponge surfaces [6].

In a restricted set of five species that were present at all three tide heights, we found that host species still explained 30% of variation in bacterial community structure (PERMANOVA: pseudo-F = 7.5, $R^2 = 0.31$, $P = 0.001$; Fig. S7, Table S5), and 15% of variation in microeukaryote communities (PERMANOVA: pseudo-F = 3.1, $R^2 = 0.15$, $P = 0.001$). For microeukaryotes these values are comparable to the magnitude of the influence of tide height on microeukaryote communities, which explained 10% of variation (Fig. S7, Table S5).

Having demonstrated that host phylogeny significantly influences microbial community structure, we then tested for an effect of host morphology while including higher level taxonomy as a covariate. After controlling for host taxonomy (family and order) we find a weak but significant effect of host morphology on the composition of bacterial (pseudo-F = 1.1, df=4, $P = 0.03$) and microeukaryote (pseudo-F = 1.1, df=4, $P = 0.048$) communities (Fig. 4; Fig. S8, Table S6). This pattern is driven largely by crustose

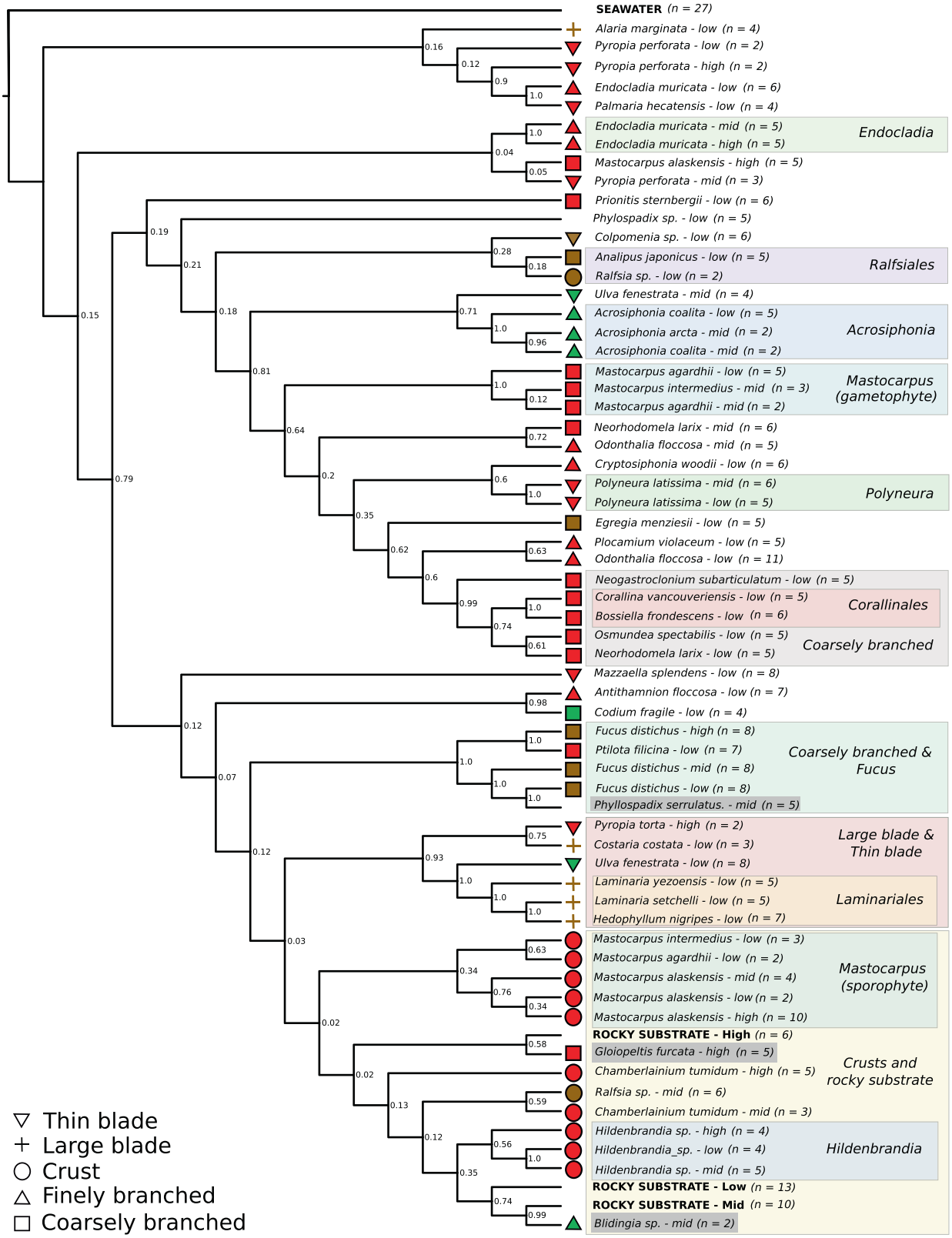

Fig. 2 The structure of the seaweed microbiota is the result of taxonomic and ecological factors. UPGMA phylogram of bacterial diversity (16 S rRNA gene) constructed based on the mean Bray Curtis distance of microbial communities from each species at each intertidal elevation (low, mid, high). Jackknife support values are based on 100 trees. Coloured boxes are used to draw attention to morphological and taxonomic patterns; dark grey shading indicates species that don't conform to the rest of their clade.

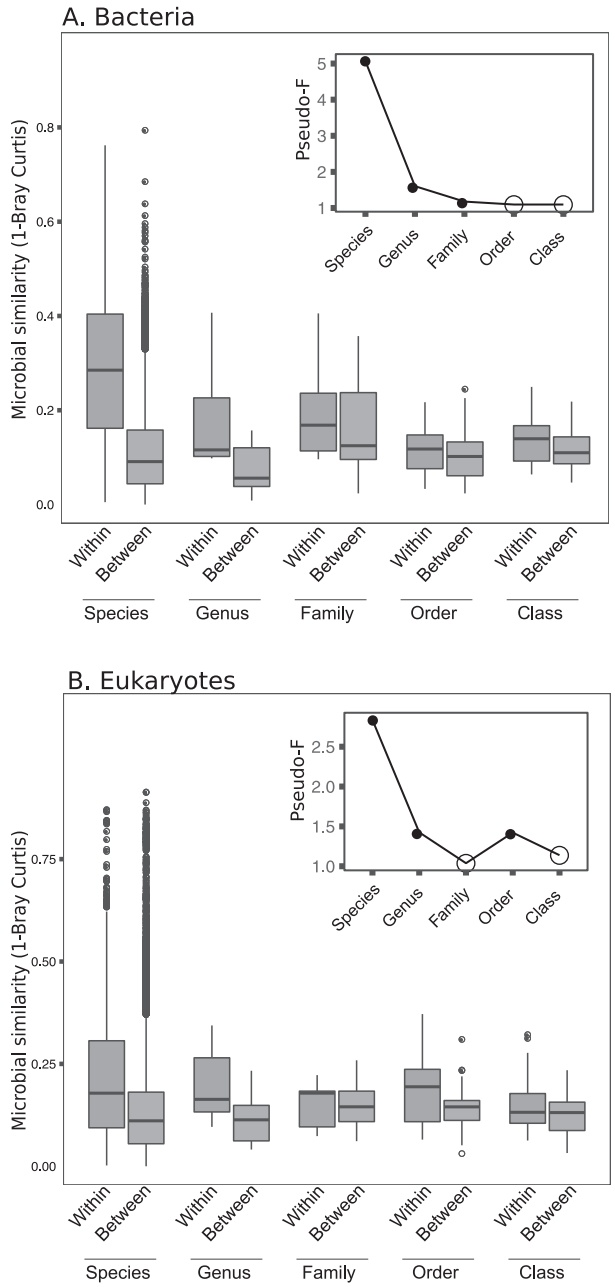

Fig. 3 Taxonomic diversity of seaweeds influences the assembly of their microbial communities. These plots present (**a**) bacterial and (**b**) eukaryote community similarity between and within groups at each taxonomic rank of macroalgal hosts. The inset graphs depict the pseudo-F value derived from PERMANOVA at each taxonomic rank, solid circles indicate $P < 0.05$.

seaweed species: when crustose genera are removed from this analysis we no longer see a significant effect of host morphology (Table S7).

We also used tree-based methods to disentangle the effects of phylogeny and morphology on bacteria associated with the subset of host species in the phylum Rhodophyta, the only monophyletic lineage for which we sampled adequate diversity for this analysis (Fig. S9). MRM analysis revealed that there were significant effects of both phylogeny ($F = 21.964$, $p = 0.02$) and morphological category ($F = 29.299$, $p = 0.002$) on the composition of bacterial communities with host phylogeny explaining ~8% of the variation ($R^2 = 0.08$) in bacterial community and host morphology explaining ~11% of the variation ($R^2 = 0.11$). We found that morphological category is significantly correlated with phylogeny ($K = 1.104$, $p = 0.007$; Lambda = 0.9999, $p = 0.004$), but only 2% of the variation in bacterial community composition is shared between host phylogeny and host morphology. While this analysis is restricted to the Rhodophyta, it provides an additional line of support for the role of host morphology in shaping seaweed microbial communities.

Morphological complexity and microbial richness

We identified significant differences in the richness of bacterial OTUs among morphological categories of seaweed hosts (rarefied data using the Chao1 Index, ANOVA: df = 4, $F = 21.4$, $P < 0.0001$; Fig. 5, Table S8). These differences point to a correlation between host morphological complexity and the diversity of microbes that colonize their surfaces, such that finely branched and coarsely branched species have a greater bacterial richness compared to simple blades and crusts. The richness of microeukaryote communities was low compared with bacteria, but still showed significant differences among morphological categories (ANOVA: df = 4, $F = 3.0$, $P = 0.018$).

To further investigate this pattern, we also conducted phylogenetic tests for an effect of morphology on microbial richness associated with host species in the phylum Rhodophyta (using the red algal phylogeny in Fig. S9). This analysis found that for red algae, there was a significant effect of host phylogeny on bacterial richness (Chao1 Index) using Blomberg's K ($K = 0.912$, $p = 0.01$), but this trend was not significant with Pagel's Lambda (Lambda = 0.821, $p = 0.06$), and there was no significant phylogenetic signal of microeukaryotic richness using either Blomberg's K ($K = 0.760$, $p = 0.07$) or Pagel's Lambda (Lambda = 0.678, $p = 0.06$), however in both cases p values were <0.1. Controlling for host phylogeny, we then found that there were significant differences in both bacterial (PGLS 4 levels: $F = 4.8$, $p = 0.01$) and eukaryotic (PGLS 4 levels: $F = 4.0$, $p = 0.03$) diversity among the four morphological categories present in our red algal samples.

Artificial seaweed experiment

We experimentally tested for the role of host morphology on the accumulation of surface communities by constructing artificial seaweed from 0.4 mm thick latex sheets cut into shapes that approximated three morphological categories

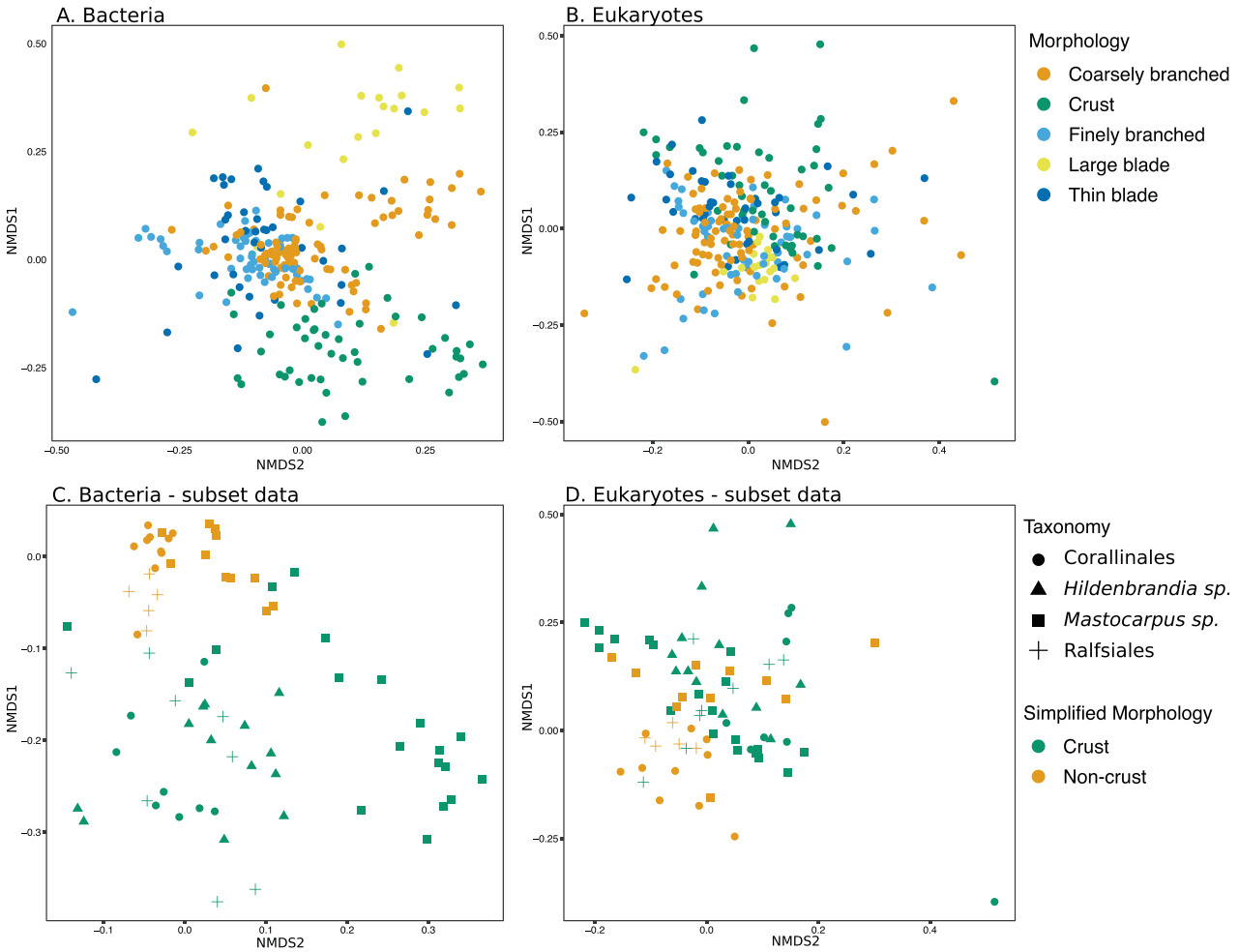

Fig. 4 Morphological diversity of seaweeds influences the composition of their microbial communities. NMDS plots based on Bray Curtis distance showing the relationship between (**a**) bacterial and (**b**) eukaryote communities associated with each macroalgal morphological category (Stress values: bacteria = 0.238, eukaryotes = 0.249). For this figure, all species with the same morphology are pooled and coded with the same colour. In the bottom panels (**c** & **d**) we present a reduced subset of only the crustose taxa (green symbols: *Mastocarpus sp.*, *Hildenbrandia sp.*, *Ralfsia sp.*, *Chamberlainium sp.*) paired with closely related upright species from the same family or genus as the crustose taxa (orange symbols), highlighting the significant effect of host morphology over relatedness at structuring these microbial communities.

(crustose, thin blade, coarsely branched; Fig. 6). We deployed replicates of each morphology at two subtidal locations, sampled them destructively over several days, and quantified bacterial communities across time-points and morphologies. In agreement with results from the field survey, this experiment found significant differences in the richness of bacterial communities among morphologies (ANOVA of Chao1: Site 1, $F = 19.1$, $p < 0.0001$; Site 2 $F = 23.2$, $p < 0.0001$; Table S9, Fig. S10). At both locations, the coarsely branched morphology had significantly greater bacterial OTU richness than both the crustose and thin bladed artificial seaweed (Fig. 6), which is consistent with the results observed on real seaweed surfaces. Richness of bacterial communities increased at each time-point, but there was no significant interaction between "time" and "morphology". Interestingly, differences in bacterial richness among artificial seaweed morphologies became apparent within 20 min after deployment (Fig. S10), and were maintained through all timepoints.

We also identified differences in community structure at each site (PERMANOVA of Bray Curtis distance: Site 1 pseudo-F = 4.9, $p = 0.0001$; Site 2 pseudo-F = 4.5, $p = 0.0001$). Pairwise comparisons revealed significant differences in bacterial community structure between all three artificial seaweed morphologies at each site (Fig. 7, Table S10). The relative abundance of the dominant taxa were different between the two locations that the artificial seaweeds were deployed, and also differed from the surfaces of real seaweed (Fig. 7).

While the two-dimensional surface area for each artificial seaweed morphology was consistent at ~44 cm^2, we note that the branched form has a greater total surface area due to the cuts that create the branching pattern. When swabbing the branched morphology we attempted to control for this

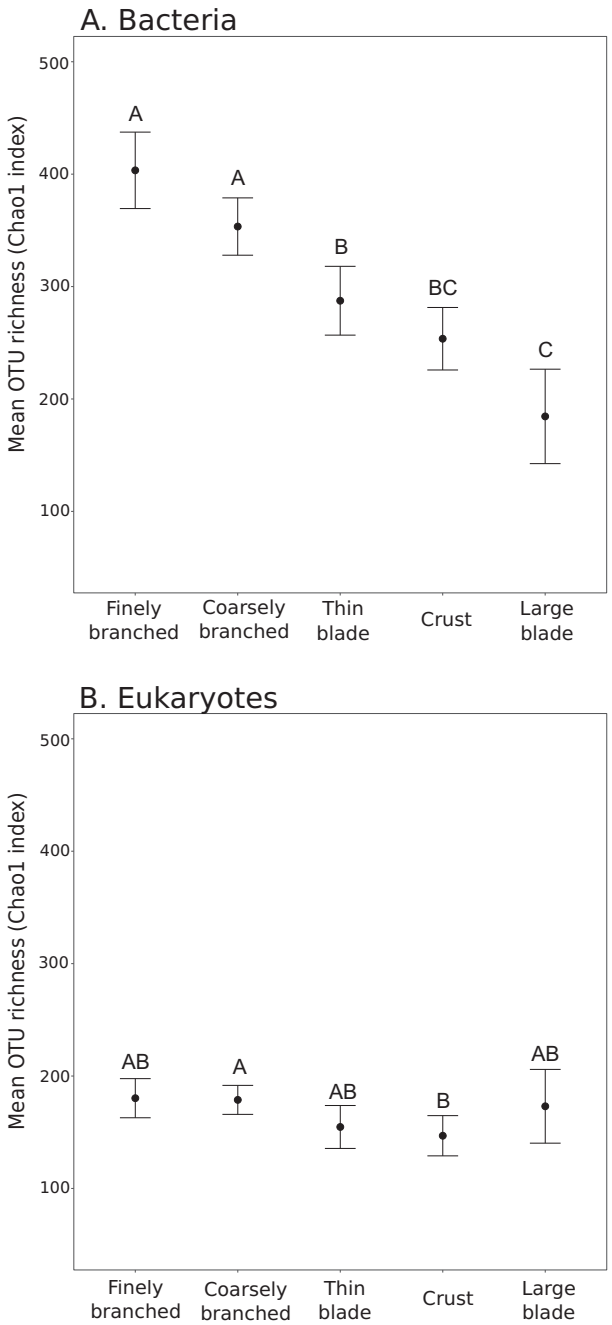

Fig. 5 Morphological diversity of seaweeds influences the richness of their microbial communities. This plot shows the mean OTU richness (±SE) of (**a**) bacterial and (**b**) eukaryote communities present on the surface of each morphological category. Letters above the bars denote significant pairwise differences (Tukey contrasts).

by laying it as flat as possible so that only the two-dimensional surface was swabbed, but it is reasonable to assume that microbes from the cut surface were also swabbed. We believe that this was still is an appropriate comparison because: [1] The crustose and simple blade morphologies had significant differences in their bacterial community structure. In this case the blades were identical in shape, the only difference being that the "crust" was glued flat to the ceramic tile, while the blade was attached by a single anchor point and able to move in the seawater [2]; The purpose of these artificial seaweeds was to emulate real seaweed morphologies, and the same issue of a branched seaweed having greater surface area than an unbranched seaweed of the same two-dimensional size also occurs in real seaweeds. Indeed, we hypothesize that the increased complexity created by these differences in surface area likely contribute to the differences that we observed.

Discussion

Using a multi-species field-survey combined with a manipulative experiment, we found evidence that the morphology of marine hosts affects the composition of their surface microbial communities. These results provide an interesting complement to previous research showing that (macroscopic) animal biodiversity is often greater in more structurally complex habitats (reviewed by [48]). For example, the morphological complexity of aquatic macrophytes correlates with the biodiversity of epiphytic invertebrate communities that they support [49–52], the richness and diversity of fishes is correlated with the structural complexity of their habitats [53, 54], and the diversity of bird species increases in mixed stands with greater variety of foliage types [55]. Our data suggest that this relationship between habitat complexity and biodiversity can be further generalized to include the diversity of microbial symbionts on their hosts.

There are a number of different mechanisms hypothesized to increase biodiversity in more complex habitats (reviewed by [48]), and these are not mutually exclusive. For example, topographic complexity is expected to increase biodiversity due to increased diversity of available niches [54]. The increased surface area in complex habitats might promote increased species diversity as a result of general species area relationships [56]. Greater habitat complexity may also provide increased physical protection against predators [56]. Future work in this system should be aimed at disentangling the relative contributions of these factors in the microbial realm.

Studies on the microbiota of terrestrial plants provide additional evidence that host-associated microbiota are structured in part by the morphology of the host. For example, research using scanning electron microscopy has shown that the presence of morphological features such as trichomes, stomata, cell wall junctions, and leaf vein grooves results in a greater observed abundance of bacteria compared to featureless leaf surfaces [57–59]. It is notable that in terrestrial plants both glandular and non-glandular trichomes increase bacterial richness, suggesting that

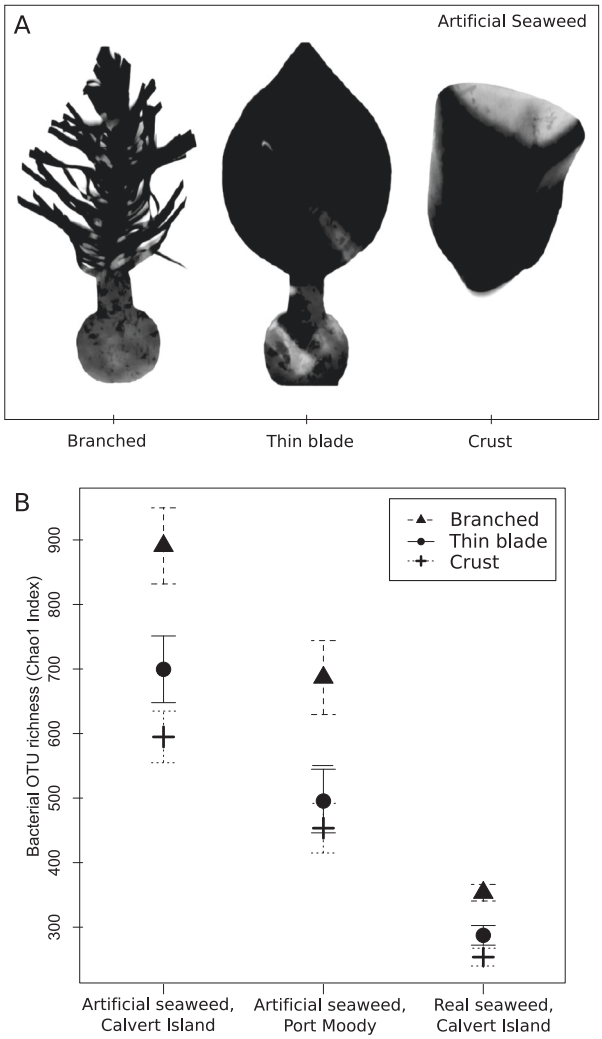

Fig. 6 Microbial communities on artificial seaweed reflect what we observed in the field survey. a The three representative morphologies constructed from latex sheets. **b** Bacterial OTU richness (Chao1 index) was significantly greater on the coarsely branched artificial and real seaweed compared with the blade and crustose morphologies. Bacterial data from the surfaces of real seaweeds are included for comparison.

structure itself rather than metabolites, which are secreted only by glandular trichomes, affect bacterial richness [60].

For marine organisms, the influence of gross morphology on the flow of water across host surfaces may also have consequences for the composition of their microbial surface communities. We know that the direction and speed of water flow across solid surfaces affects microbial settlement and biofilm development, in which biofilm formation is increased when water speeds are high [61, 62] and on surfaces with complex topology [63]. However fast flow may also shear off layers of biofilm [64] and dislodge larger epiphytes [65]. Given the different morphological adaptations that seaweeds use to attenuate the flow of water around their thallus [24, 27], it is conceivable that these differences in water flow will also affect the horizontal transfer of microbes from seawater to algal surfaces. Teasing apart the causal mechanisms for this pattern is challenging because seaweeds with different morphologies interact differently with waves and currents [24, 25, 27]; thus, disentangling the effect of thallus morphology from the effect of thallus behaviour in flow will require further experimentation. We suggest that a closer examination of water dynamics around the seaweed thallus may be an important area for future research examining the relationship between surface complexity and microbial colonization.

Our results provide evidence that some variation in marine microbial communities is associated with host morphology alone, and not from selection for microbes with a particular function from either the host (e.g., defence, homoeostasis) or microbe (e.g., metabolism of exuded polysaccharides or tolerance of host defence). For example, the convergence of microbial communities on unrelated crustose species (Fig. 4) and the clustering of crusts with the similarly shaped rocky substrate (Fig. 2) suggests that structural similarity is driving microbial colonization to some extent. Furthermore, our experiment on artificial seaweed surfaces suggests that biotic interactions and biochemical processes are not necessary to reproduce the patterns from our field survey—structural complexity itself can increase microbial richness and alter microbial community structure. This conclusion echoes recent theoretical work showing that phylosymbiosis patterns can be established via environmental filtering of phylogenetically correlated host traits; direct host control or any sort of co-evolution need not be invoked [10].

Previous research from a broad range of study systems has shown that host ecology and host phylogeny can both influence microbial community structure and can function jointly [8, 13, 17]. For the seaweeds examined in this study, morphology is a novel factor structuring epiphytic microbial communities, but it was not the only significant driver of microbial community structure. We find that host identity, and to a lesser extent host phylogeny (Fig. 3), also contributes to the structure of microbial surface communities. For example, in the dendrogram of microbial community similarity (Fig. 2), within the large clade of crustose species there is also host phylogenetic structure, as species within the same genus cluster together. Furthermore, we find significant difference in microbial communities across tide heights. Taken together, these results suggest a highly complex system in which host taxonomy, host morphological complexity, and the physical location inhabited by a host all contribute to the assemblage of their microbial communities.

Across all of our analyses we find that bacterial communities (16 S rRNA data) show increased community

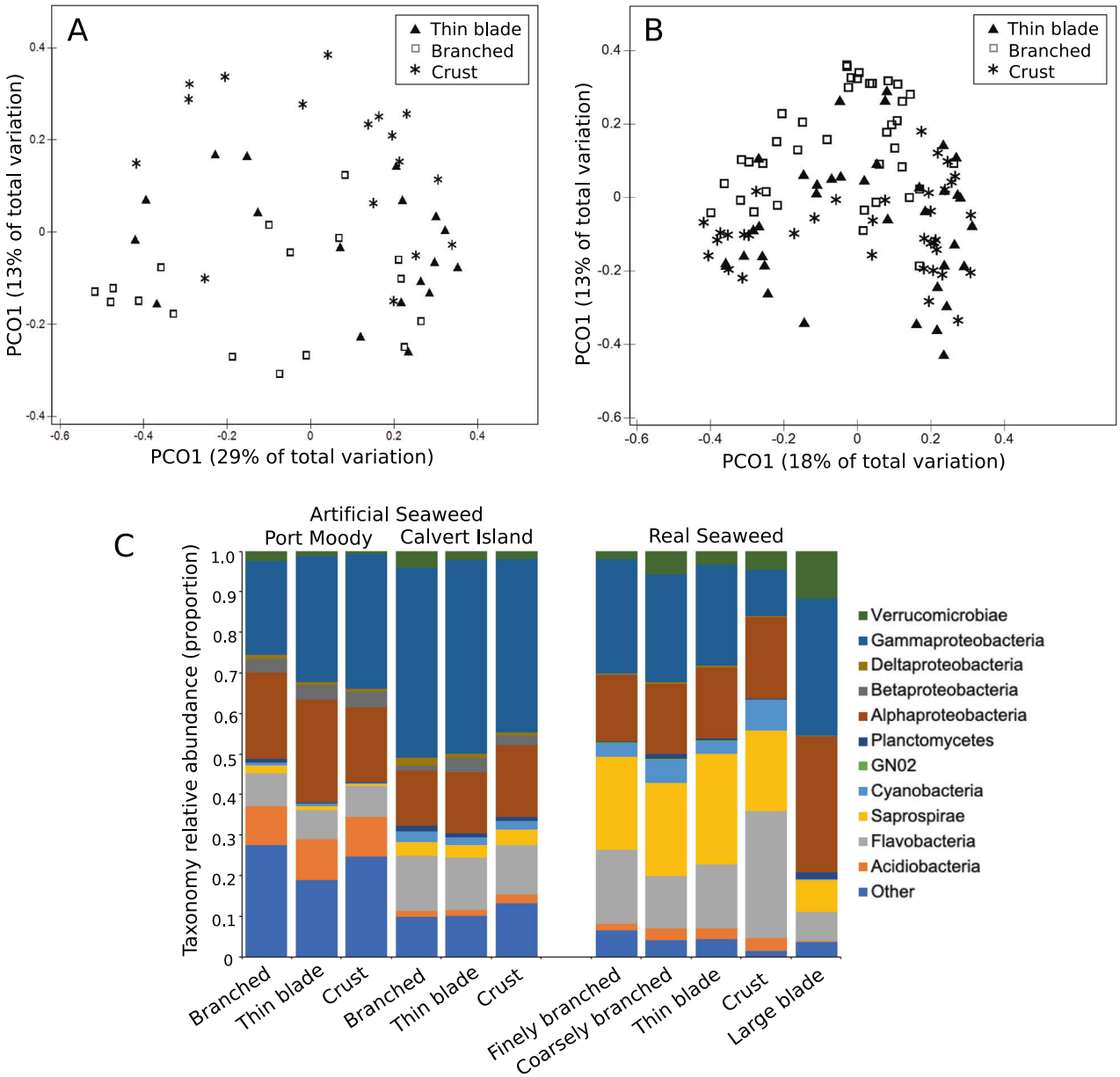

Fig. 7 The morphology of artificial seaweeds influences the composition of their microbial communities. Principal Coordinates plot (PCO) illustrating the differentiation of bacterial communities on artificial seaweed (based on Bray-Curtis Distance) among morphologies at Calvert Island (**a**) and Port Moody (**b**). The bottom panels show the dominant taxa present on the three artificial morphologies at each site (**c**) compared with the dominant bacteria in real macroalgal morphologies (**d**).

structure and less variability compared to micro-eukaryote communities (18 S rRNA data). For example, the NMDS plot of bacterial samples (Fig. 4) shows a separation of samples by morphology, which is highlighted in the strong differentiation between crust and upright morphologies (Fig. 4). In contrast, the eukaryote data shows much more community overlap across morphologies, though differences are still statistically significant. OTU richness is much lower for microeukaryotes compared to bacteria, and microeukaryote richness is much less variable across host types (Fig. 5). These results suggest that bacteria may have closer, more specific associations with host species compared to eukaryotic microbes.

The findings of this study are broadly applicable to a wide range of other marine systems. For example, colonial invertebrates, such as corals, bryozoans, sponges and hydroids all have unique symbiont communities that are still poorly understood [66–69]. As with seaweeds, these taxa possess a wide range of forms that vary in their morphological complexity, and it has been hypothesized that differences in coral morphology may affect the composition of microbial surface communities [70, 71]. Our results

support this hypothesis and suggest that this pattern may be wide-spread across marine species. In particular, the results of our artificial seaweed experiment point to the generality of this pattern—we essentially removed every aspect of the seaweed except for its shape, and still see differences in the surface microbial communities among morphological groups. Discerning the extent to which different aspects of host biology select for their microbiota may have implications for protecting organisms, such as corals, that rely on microbiota to resist bleaching and disease [72].

Conclusion

Large eukaryotes live with microbial symbionts that can profoundly influence their biology. This dependence on symbionts promotes an expectation that symbiont communities are selectively determined, for example by host biology. However, we show that seaweed symbiont communities are also influenced by the physical shape—morphology—of the host, suggesting that host characteristics can impact the microbiome simply by altering the way hosts come into contact with microbes, independent of biotic interactions.

Data availability

Raw amplicon sequence data for both the 16 S and 18 S rRNA genes from our field survey have been deposited at the European Bioinformatics Institute (EBI; www.ebi.ac.uk; Accession number PRJEB25010). Raw 16 S rRNA gene data from artificial seaweeds have also been accession at EBI (Accession number: PRJEB25951). Sanger sequences used to confirm host species identity have been deposited at NCBI and the corresponding accession numbers are listed in Table S1.

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Compliance with ethical standards

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

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