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The microbiota of intertidal macroalgae *Fucus distichus* is site-specific and resistant to change following transplant

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Summary

It is unclear how host-associated microbial communities will be affected by future environmental change. Characterizing how microbiota differ across sites with varying environmental conditions and assessing the stability of the microbiota in response to abiotic variation are critical steps towards predicting outcomes of environmental change. Intertidal organisms are valuable study systems because they experience extreme variation in environmental conditions on tractable timescales such as tide cycles and across small spatial gradients in the intertidal zone. Here we show a widespread intertidal macroalgae, *Fucus distichus*, hosts site-specific microbiota over small (meters to kilometres) spatial scales. We demonstrate stability of site-specific microbial associations by manipulating the host environment and microbial species pool with common garden and reciprocal transplant experiments. We hypothesized that *F. distichus* microbiota would readily shift to reflect the contemporary environment due to selective filtering by abiotic conditions and/or colonization by microbes from the new environment or nearby hosts. Instead, *F. distichus* microbiota was stable for days after transplantation in both the laboratory and field. Our findings expand the current understanding of microbiota dynamics on an intertidal foundation species. These results may also point to adaptations for withstanding short-term environmental variation, in hosts and/or microbes, facilitating stable host–microbial associations.

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Introduction

There is widespread evidence of host species specificity in the microbial communities associated with diverse marine eukaryotes, suggesting that host organisms have selective mechanisms to maintain associations with microbes different from those in the surrounding environment (e.g. seawater, sediment, other organisms) (Adair and Douglas, 2017; Cleary *et al*., 2019). These microbes play a role in modulating growth, settlement, nutrition and defence in marine hosts (Egan and Gardiner, 2016; Morris *et al*., 2016; Apprill, 2017; Ugarelli *et al*., 2017; Woznica and King, 2018) but microbial functions important to host biology or biogeochemical processes are often performed by diverse and functionally redundant microbial taxa (Burke *et al*., 2011; Roth-Schulze *et al*., 2016; Louca *et al*., 2017). Within a host species, there can be seasonal turnover in the taxonomic composition of associated microbes (the microbiota) (Bengtsson *et al*., 2010; Lachnit *et al*., 2011; Serebryakova *et al*., 2018) and variation across geographic and/or environmental gradients (Pantos *et al*., 2015; Pfister *et al*., 2019; Weigel and Pfister, 2019; Schellenberg and Clarke, 2020). This taxonomic variation in time and space suggests the environment plays a strong role in the composition of host-associated microbial communities (Adair and Douglas, 2017; Louca *et al*., 2018). In keeping with a strong role of the environment and its influence on a shared microbial source pool, the strength of microbiota host specificity is often reduced for host species in sympatry (Lemay *et al*., 2018; Cleary *et al*., 2019). Yet, other factors are also known to influence the microbiota, including host genetics (Griffiths *et al*., 2019; Díez-Vives *et al*., 2020), phenotypic traits (Carrier and Reitzel, 2018; Lemay *et al*., 2020), organismal behaviours (Pratte *et al*., 2018), as well as biotic interactions such as

microbial cooperation or competition (Coyte *et al*., 2015). Identifying the contribution of extrinsic (e.g. abiotic conditions and source pool of potential microbial colonizers) versus intrinsic (e.g. host traits and biotic interactions) factors to the diversity and structure of the microbiota within and among host species and in changing environments is a major priority in microbial ecology (Trevathan-Tackett *et al*., 2019; Wilkins *et al*., 2019).

Alterations in the microbiota have been linked to disease (Zozaya-Valdes *et al*., 2015; Kumar *et al*., 2016; Zozaya-Valdés *et al*., 2017; Qiu *et al*., 2019) and stress responses (Marzinelli *et al*., 2015; Minich *et al*., 2018) of many marine hosts and could potentially disrupt biogeochemical cycles (Moulton *et al*., 2016; Säwström *et al*., 2016; Pfister *et al*., 2019) and marine food webs (Campbell *et al*., 2014). Changes in the microbiota can also positively impact the host through protective effects (Longford *et al*., 2019; Rosado *et al*., 2019) or facilitation of adaptation (Dittami *et al*., 2016; Lynch and Hsiao, 2019; Voolstra and Ziegler, 2020). Hosts populations may further benefit from microbiota turnover via functional redundancy where microbial functions essential to the host are maintained through associations with diverse microbial taxa from the same functional guild (Burke *et al*., 2011; Louca *et al*., 2016). As it is unclear how changing ocean conditions will impact marine hosts or their microbiota, characterizing how marine microbiota differ across sites with varying environmental conditions and assessing the stability of the microbiota in response to alterations in the environment is a critical step towards understanding and predicting outcomes of change on host-associated microbial communities.

Intertidal macroalgae are informative host systems for studying natural variation in microbiota because these organisms experience marked variation in abiotic conditions on short timescales (i.e. tide cycles) and short distances (i.e. high versus low intertidal zone) in addition to longer seasonal timescales and larger geographic distances. Intertidal macroalgae are also phenotypically diverse and display trait plasticity across intertidal gradients (Johannesson *et al*., 2012; Mueller *et al*., 2015) which could selectively influence the microbiota (Balakirev *et al*., 2012). Furthermore, functional redundancy is increasingly studied in the microbiota of marine macroalgae (Burke *et al*., 2011; Roth-Schulze *et al*., 2016, 2018), yet there is limited existing research on how microbiota vary across the mosaic of habitats and changing environmental conditions experienced by members of the same macroalgal host species.

Here we focus on *Fucus distichus*, an ecologically important species of macroalgae in the northeastern Pacific. It is often the dominant primary producer in the high to mid-intertidal zone of rocky shores and forms dense canopies that serve as habitat for diverse organisms. *Fucus distichus* thrives across a wide range of intertidal conditions and displays phenotypic variation corresponding to environmental conditions (Schonbeck and Norton, 1978; Blanchette, 1997; Dethier and Williams, 2009). Early attempts to propagate other *Fucus* species in the absence of microbes were unsuccessful or showed irregular growth, suggesting microbes are essential for host development and survival (Fries, 1984, 1993). The microbiota of other *Fucus* species show temporal and spatial variation (Stratil *et al*., 2013; Saha *et al*., 2014, 2020; Quigley *et al*., 2020) and regulation via surface metabolites and defence compounds produced by the host (Saha *et al*., 2011; Saha and Wahl, 2013; Saha and Weinberger, 2019). This existing research indicates associations with microbes are important to the ecology of fucoid hosts, but little is known about microbial communities on *F. distichus* throughout the intertidal zone.

We characterized the microbiota on *F. distichus* from multiple habitats within a small geographic area (<5 km). We hypothesized that environmental variation, and the corresponding host morphological variation across *F. distichus* habitats, would be accompanied by compositional differences in the microbiota because of the selective filters exerted by differing abiotic conditions. We used common garden and reciprocal transplant experiments to test the stability of associations between *F. distichus* and the microbiota. Field-based transplant studies are a crucial yet underutilized tool to understand host–microbiota associations in ecologically realistic conditions (Greyson-Gaito *et al*., 2020). We hypothesized that the microbiota of transplanted *F. distichus* would rapidly shift to reflect the contemporary environment of the host because environmental conditions pose a strong selective filter and also alter the microbial source pools available to colonize the host. Characterizing the interactions between foundation species and their microbiota, particularly in highly variable intertidal habitats, is a necessary step towards understanding how host–microbiota associations and their ecosystem functions will respond to environmental fluctuations associated with changing oceans (Smale and Wernberg, 2013; Harris *et al*., 2018; Trevathan-Tackett *et al*., 2019; Wilkins *et al*., 2019).

Results

Fucus distichus *microbiota composition is correlated with site and host morphology*

We examined the microbiota composition on the surface of 52 *F. distichus* individuals from five sites on Calvert Island, British Columbia, Canada, by comparing *in situ* unmanipulated, initial samples from our two independent experiments conducted in the same week (Fig. 1). These

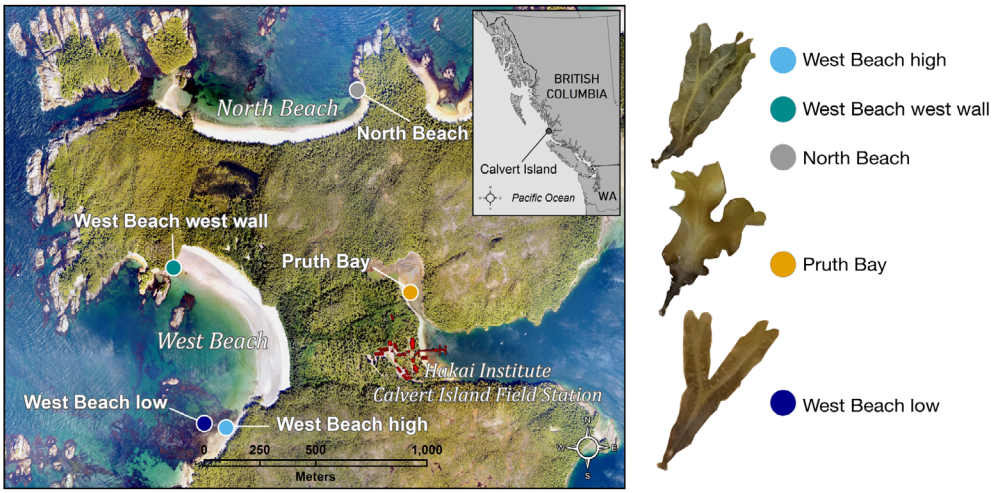

Fig 1. Map of sampling sites on Calvert Island, BC (coloured circles) and corresponding *F. distichus* morphotypes. High intertidal sites with morphotype A are North Beach (grey), West Beach west wall (teal), West Beach high (light blue). The high intertidal site with morphotype B is Pruth Bay (yellow). The low intertidal site with morphotype C is West Beach low (dark blue). [Color figure can be viewed at wileyonlinelibrary.com]

samples included a high intertidal, wave-exposed host morphotype A at three sites: West Beach (WB) high (n = 7), WB west wall (n = 8), North Beach (NB) (n = 7); a high intertidal, wave-protected morphotype B at one site: Pruth Bay (PB) (n = 17); and a low intertidal, wave-exposed morphotype C at one site: WB low (n = 13) (Fig. 1). Each site is characterized by a distinct physical habitat (Fig. S1). Morphotype and site explained significant variation in microbiota composition [permutational analysis of variance (PERMANOVA): morphotype pseudo-$F_{2,47}$ = 18.591, p-value <0.001; morphotype:site pseudo-$F_{2,47}$ = 4.552, p-value <0.001]. We examined the effect of site alone by testing for a site effect within the one morphotype that was sampled at multiple locations separated by hundreds of meters to kilometres (Fig. 1). The high intertidal, wave-exposed morphotype A (sampled at WB high, WB west wall and NB) also showed significant variation in microbiota composition among sites [PERMANOVA: pseudo-$F_{2,19}$ = 4.086, p-value <0.001]. We sampled underlying rock substrate for a subset of sampling sites (PB and WB low) and found that *F. distichus* microbiota (PB, n = 23; WB low, n = 17) were significantly different from biofilm communities on rocks sampled at those sites (PB, n = 15; WB low, n = 17) [PERMANOVA: pseudo-$F_{1,70}$ = 18.605, p-value <0.001] (Fig. 4A). Rock biofilm communities also showed significant site specificity [PERMANOVA: pseudo-$F_{1,34}$ = 15.059, p-value <0.001] (Fig. 4A).

Differences in F. distichus *microbiota among sites and morphologies are maintained in a common environment*

To determine if a shared microbial source pool and consistent environmental conditions would result in *F. distichus* individuals converging to a common microbiota, regardless of site of origin, we performed a common garden experiment. From the individuals sampled *in situ*, we selected a subset of eight individuals from each of the five sites, transported them to the lab and attached them to sterilized rocks (see Experimental procedures; Fig. S1F). At the start of the experiment (sample day 1), before transplanting them into a common flow-through seawater tank, we confirmed that *F. distichus* microbiota from each site of origin were statistically different from *F. distichus* microbiota from every other site, in agreement with the above. There was no significant effect of experimental manipulation and transportation to the lab [PERMANOVA: pseudo-$F_{4,27}$ = 6.958, p-value <0.001] (Fig. 2). The most abundant ASVs associated with *F.*

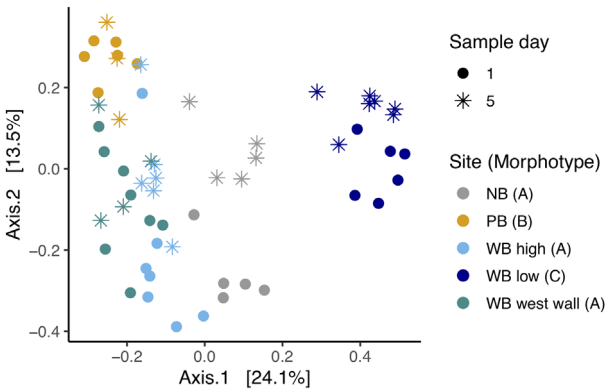

Fig 2. *Fucus distichus* microbiota in the common garden remains differentiated by site over time. PCoA of Bray–Curtis dissimilarities for *F. distichus* microbiota in the common garden from initial sampling on day 1 (circles) and final sampling on day 5 (stars). Points are coloured by site of origin. [Color figure can be viewed at wileyonlinelibrary.com]

distichus from each site were predominantly from the same families: Flavobacteriaceae, Rhodobacteraceae, Rubritaleaceae, Saprospiraceae and Thiohalorhabdaceae; although the relative abundances of dominant genera within these families varied by site (Fig. 3A).

After 5 days in a common environment, differences in microbiota composition between sites remained significant [PERMANOVA: pseudo-$F_{4,20}$ = 5.787, p-value <0.001] (Fig. 2). Strikingly, we did not observe convergence to a similar or shared microbiota composition over time (Figs 2 and 3A). We used pairwise comparisons of microbial community composition on *F. distichus* individuals from the same site versus between individuals from different sites to determine if more ASVs were shared between sites after 5 days in the common garden compared with original microbiota compositions at the time of collection from the field. If all *F. distichus* individuals were colonized by the same microbial taxa in the common garden, or if microbes were transmitted between hosts from different sites, we would expect to see significant increases in community similarity between samples from different sites over time. Pairwise comparisons of Jaccard beta-diversity, a metric based on presence/absence of shared ASVs, showed more shared ASVs for individuals from within the same site than comparisons between hosts from different sites; these results were similar at the start and end of the experiment (Fig. 3B). When comparing Jaccard metrics from the initial day in the common garden to day 5, paired t-tests showed no significant change in beta-diversity within or between sites, with the exception of comparisons between PB and other sites and comparisons within WB low and WB west wall. The samples from WB low became significantly more dissimilar to each other from day 1 to day 5 in the common garden (Fig. 3B; Table S1).

Indicator species analysis was used to identify ASVs that had colonized all individuals in the common garden, regardless of the site of origin, by the end of the experiment (day 5). Indicator species analysis assesses the specificity and fidelity of ASVs to specific sites or groups of sites via permutation tests (see Experimental

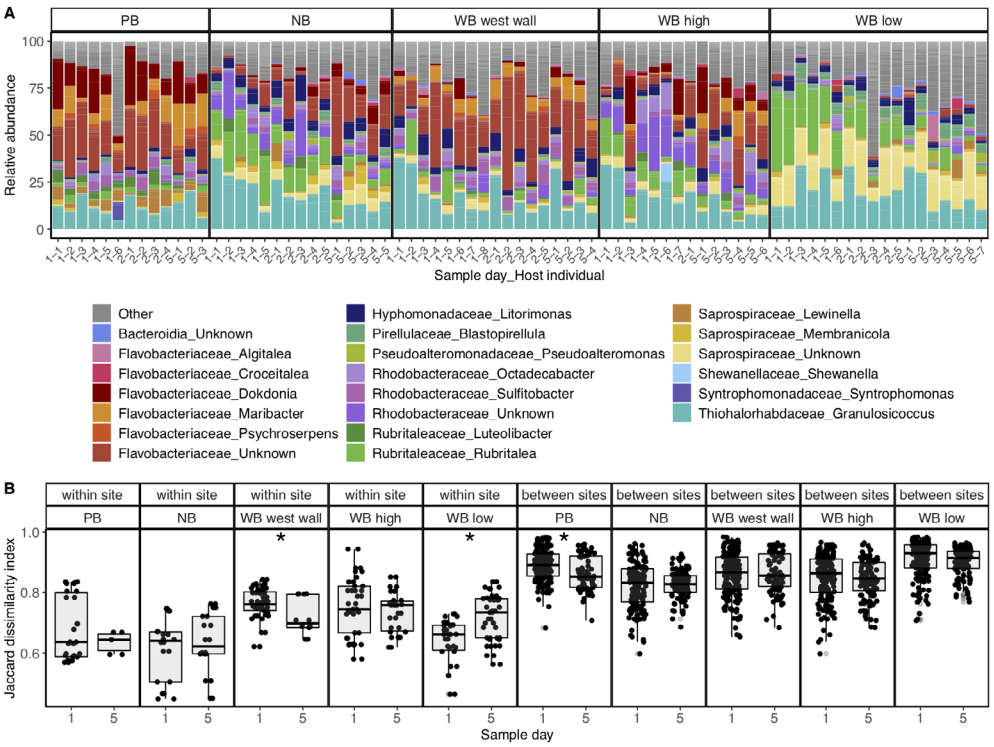

Fig 3. Broad taxonomic groups are common to all *Fucus distichus,* but compositional differences based on the presence/absence of specific ASVs (as measured by the Jaccard dissimilarity index) is greater between sites than within sites at both the start and end of the common garden experiment.

(A) Stacked bar plots of the most abundant ASVs on *F. distichus*, grouped by family and genus. Each column represents an individual *F. distichus* sample; samples are ordered by day of sampling and grouped by origin site. The first number of *x*-axis labels is sampling day, the second number is a unique *F. distichus* individual.

(B) Jaccard dissimilarity values for pairwise comparisons of *F. distichus* microbiota samples from within the same site or between different origin sites at the initial sampling (1) and fifth, final day in the common garden (5). We represent between-site comparisons for each site independently, plotting the dissimilarity values between a focal site and all the others. Asterisks indicate a significant difference in Jaccard dissimilarity between initial and final time points for a given site based on paired t-tests. [Color figure can be viewed at wileyonlinelibrary.com]

procedures) (De Cáceres and Legendre, 2009). We found few ASVs that indiscriminately colonized *F. distichus* regardless of the site of origin (Table S2), these included members of Bdellovibrionaceae. Most indicator ASVs were significantly associated with specific sites of origin, even at the end of the experiment (Table S2). These indicator ASVs belong to clades typically associated with *Fucus* species and other marine macroalgae, including Saprospiraceae, *Granulosicoccus* and Piruellaceae (Table S2) (Bondoso *et al*., 2017; Parrot *et al*., 2019; Weigel and Pfister, 2019). The site specificity of indicator taxa further highlights the strong distinction between *F. distichus* microbiota from different sites and the persistence of those distinct microbial communities throughout the experiment.

Differences between sites in F. distichus *microbiota and rock biofilm communities are maintained following transplantation to novel environments*

To determine if the stability observed in the common garden experiment translates to natural conditions, we performed *in situ* transplantation between the two sites with the most divergent environmental conditions: high intertidal protected bay (PB) and low intertidal wave-exposed site (WB low). Among the individuals initially sampled *in situ*, we selected a subset of 16 individuals (and eight rocks) from each of the two sites to transplant. Some samples of specific individuals and timepoints during the experiment were lost in the field to natural processes such as wave action and grazing. Others were excluded due to poor sequencing quality or sample contamination (for details see Experimental procedures), and ultimately, we analysed 162 high-quality samples (103 *F. distichus* and 59 rock samples taken over the 5-day experiment). We observed resistance to rapid change in the biofilm community of *F. distichus* individuals (Fig. 4B) and rock substrate (Fig. 4C) following transplant to a new environment. Over the course of the experiment, both *F. distichus* and rock biofilm microbial communities remained more similar in composition to controls in the origin site than to *F. distichus* individuals (or rock substrate) in the environment to which they were transplanted (i.e. transplanted microbiota stayed clustered with microbiota of the origin site rather than the destination site) (Fig. 4B and C). For transplants from PB to WB low, we found no significant difference in microbial community composition over time (3 days) on *F. distichus* (n = 13) or rocks (n = 9) [PERMANOVA PB->WB low rock transplants by sample day: pseudo-$F_{1,7}$ = 0.913, p-value = 0.543, brown shapes in Fig. 4C; PB->WB low *F. distichus* transplants by sample day: pseudo-$F_{1,11}$ = 1.391, p-value = 0.164, brown shapes in Fig. 4B].

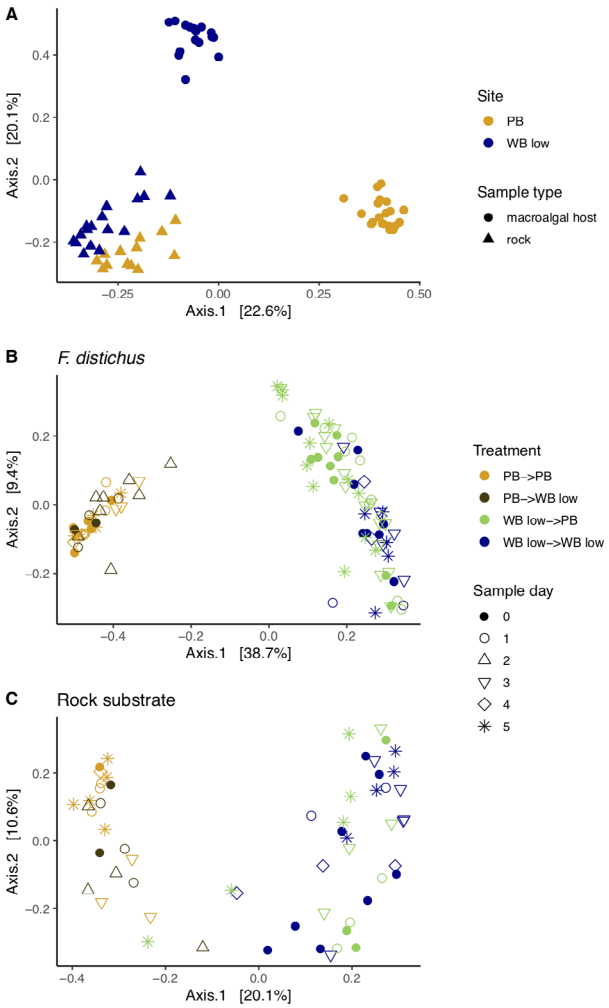

Fig 4. Microbiota on *Fucus distichus* and rock substrate from wave-protected and wave-exposed habitats are distinct and experimental transplants remain more similar to the origin site than to the destination site.
(A) PCoA plot based on Bray–Curtis dissimilarity of microbiota on unmanipulated *F. distichus* and underlying rock substrate from wave-exposed West Beach low (WB low) and protected Pruth Bay (PB). PCoA plots of reciprocally transplanted (WB low->PB and PB->WB low) and unmanipulated controls at each site (WB low->WB low and PB->PB) over time (initial sample = day 0 to final sample = day 5) for (B) *F. distichus* and (C) rock substrate. [Color figure can be viewed at wileyonlinelibrary.com]

In contrast, for transplants from WB low to PB, we found a significant difference in microbial community composition over time (6 days) on *F. distichus* (n = 47) and rock (n = 15) [PERMANOVA WB low->PB rock transplants by sample day: $F_{1,13}$ = 2.415, p-value = 0.016, green shapes in Fig. 4C; WB low->PB *F. distichus* transplants by sample day: $F_{1,45}$ = 5.102, p-value <0.001, green shapes in Fig. 4B]. We also compared the pseudo-F statistic of PERMANOVA models that compare *F. distichus* microbiota composition to origin and destination site controls across

sampling days with regression; we found no significant relationship between pseudo-*F* statistic and day of sampling at either site (Fig. S3). This supports our finding that transplanted hosts did not become progressively, and significantly, more similar (i.e. less differentiated and smaller pseudo-*F*-value) to *F. distichus* hosts native to the destination site over time.

Indicator species analysis found numerous ASVs significantly associated with *F. distichus* from both WB low and PB (Table S3). The majority of ASVs significantly associated with an origin site (indicator group) did not rapidly colonize *F. distichus* transplanted to that indicator site. Instead, most ASVs identified as indicators from the origin site remained associated with transplants originating from that site when transplanted to the new environment (Fig. 4). Visual inspection revealed few indicator ASVs from the destination site that consistently colonized individuals transplanted there; for example, Rhodobacteraceae ASV29 and ASV37 and Saprospiraceae ASV332 characteristic of WB low colonized transplants from PB; Flavobacteriaceae ASV4, Rubritaleaceae ASV25 and Saprospiraceae ASV177 characteristic of PB notably colonized transplants from WB low (Fig. 5; Table S3).

Discussion

We investigated variation in the surface microbiota of *F. distichus* with three distinct morphotypes from five sites that are separated by small spatial distances on Calvert Island, BC, Canada (Fig. 1). Morphological variation in *F. distichus* is correlated with prevailing environmental conditions such as wave exposure and vertical position in the intertidal (Coyer *et al*., 2009; Pearson *et al*., 2010; Wahl *et al*., 2011). Much like macroalgal morphology, microbial communities are also shaped by environmental conditions at local (Weigel and Erwin, 2016; Pfister *et al*., 2019; Quigley *et al*., 2020) and regional spatial scales (Campbell *et al*., 2015; Marzinelli *et al*., 2018; Weigel and Pfister, 2019). We predicted that abiotic factors and correlated host characteristics across sites influence the source pool of microbes colonizing *F. distichus* such that microbiota composition would be differentiated in accordance with host morphology. As expected, morphotype A individuals from three high-intertidal, wave-exposed sites hosted microbiota that were more similar to each other than to morphotype B or C (Fig. 2). The clear differences we observed between *F. distichus* microbiota from WB low (morphotype A) and WB high (morphotype C), sites that are separated by only tens of

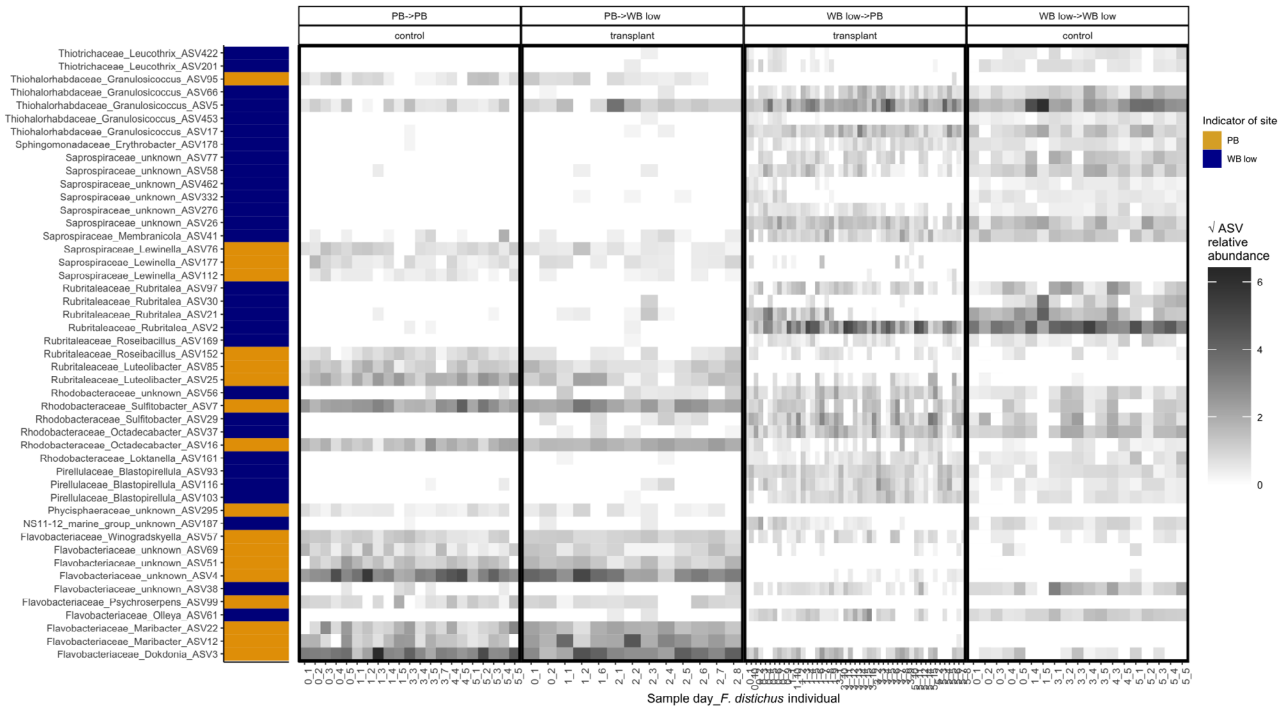

Fig 5. Indicator ASVs for a given site do not readily colonize *Fucus distichus* transplanted to that site. ASVs (with family and genus-level taxonomic assignments) significantly associated with Pruth Bay (PB, yellow label) or West Beach low intertidal (WB low, dark blue label) with an Indval statistic ≥0.9 (SI Table S3 for all indicator ASVs). Each column represents an individual *F. distichus* sample. Samples are grouped by treatment (transplant or unmanipulated control), origin location and sampling day following transplant (initial = 0 to final = 5). The higher relative abundance an ASV is in a sample, the darker the grey rectangle. Relative abundance of ASVs per sample was square-root transformed to better visualize right-skewed abundance distributions. [Color figure can be viewed at wileyonlinelibrary.com]

meters, emphasizes the role selective filtering by local environments (e.g. host morphology and/or tidal height) plays in establishing microbiota differences on *F. distichus* compared with other mechanisms such as dispersal. Unexpectedly, we also found strong and significant differences in microbiota composition by site within a single morphotype, morphotype A, for sites separated by hundreds of meters to kilometres (Figs 1 and 2). The site-specific microbiota on *F. distichus* with the same morphology is surprising as macroalgal hosts at these sites are likely experiencing similar wave action, seawater immersion time and UV exposure due to their position at roughly the same tide height. It is possible that more subtle differences in local abiotic conditions, such as levels of desiccation, UV exposure and temperature extremes, known to vary within an *F. distichus* population and throughout a tidal cycle (Joux *et al*., 1999; Wright *et al*., 2004; Rothrock and Garcia-Pichel, 2005; Alster *et al*., 2018), could affect the success of specific microbes in colonizing a host individual. Host factors, such as morphological complexity (Lemay *et al*., 2020), exudate composition (Bengtsson *et al*., 2011; Saha *et al*., 2011; Parrot *et al*., 2019) and tissue chemistry (Dethier and Williams, 2009; Weigel and Pfister, 2020) that vary across space (Van Alstyne, 1988; Saha and Wahl, 2013) and season (Rickert *et al*., 2016) in *Fucus* species can also promote or inhibit the growth of particular microbes (Stratil *et al*., 2013; Saha *et al*., 2014) and drive the significant variation in microbiota within *F. distichus* we report. We expect that the varied microbial taxa contributing to intraspecific differences in the microbiota are filling similar ecological roles on *F. distichus*; functional consistency and taxonomic variability are commonly observed in the microbiota of macroalgae (Burke *et al*., 2011; Louca *et al*., 2016, 2018; Roth-Schulze *et al*., 2016, 2018) and in other systems (Louca *et al*., 2016, 2018). Whether intraspecific differences in microbiota composition would be maintained after abrupt environmental change is not well known.

To test the stability of site-specific differences in *F. distichus* microbiota composition in the face of changing environmental conditions we used experimental transplants. We hypothesized that transplantation to a common garden would homogenize site-specific microbiota differences due to rapid community turnover driven by selective filtering imposed by the common abiotic environment (Hernandez-Agreda *et al*., 2017; Lemay *et al*., 2018; Marzinelli *et al*., 2018; Weigel and Pfister, 2019), recruitment from a shared microbial source pool, or transmission between hosts. We can reject this hypothesis, at least for short time scales (hours to days), as we found that microbial communities on *F. distichus* originating from different sites did not converge to a shared composition in our common garden environment. Site-specific microbiota differences remained significant after 5 days; showing that resident *F. distichus* microbiota are not quickly replaced or outcompeted in new environmental conditions. The constructed environment of our common garden had a continuous flow of natural seawater but did not fully mimic the variation typical of natural intertidal habitats and microbial source pools. Thus, we used reciprocal transplants in the field to investigate the stability of the *F. distichus* microbiota in ecologically realistic conditions (Greyson-Gaito *et al*., 2020). Again, we found that existing differences by site of origin were resistant to change over the 6-day experiment (Figs 3 and 4) and significant differences in microbiota by site of origin persisted. Furthermore, we found little evidence that bacterial taxa significantly associated with the new (destination) site colonized transplants (Fig. 5). This implies the microbiota of *F. distichus* does not rapidly turnover in response to altered environmental conditions or microbial source pools at both the community level and with respect to individual bacterial taxa. Surprisingly, we observed this pattern of site specificity and resistance to immediate colonization by microbes in a new environment in both *F. distichus* and the underlying rock substrate. Again, these results point to selective filtering by local abiotic conditions and priority effects (Fukami, 2015) or the dynamics of biofilm turnover as factors that shape the structure and stability of marine surface-associated microbial communities whether they are on living hosts or inert substrates such a rock.

Biofilm dynamics may be a particularly important determinant of the timescale of turnover in host-associated microbial communities. Rapid microbiota turnover, on the order of hours to days, has been demonstrated for highly disturbed or previously uncolonized host surfaces and substrates in marine systems including corals (Ziegler *et al*., 2017, 2019), macroalgae (Rao *et al*., 2006; Longford *et al*., 2019), seagrass (Wang *et al*., 2021), artificial macroalgal substrates (Lemay *et al*., 2020; Weigel and Pfister, 2020) and marine particles (Datta *et al*., 2016). Long-term observations of transplanted marine hosts with mature and unmanipulated biofilms see more gradual microbiota turnover, if any. In these studies, transplanted microbiota often become more similar to the new, destination environment on the timescale of months or years (Ziegler *et al*., 2017, 2019). In some cases, however, long-term microbiota change is attributed to seasonal turnover (Weigel and Erwin, 2017) or effects of transplantation (Campbell *et al*., 2015; Casey *et al*., 2015; Uren Webster *et al*., 2020) while signatures of origin-site specificity are maintained. In this study, where we do not find evidence of rapid microbiota turnover, we propose that new colonization of mature biofilm communities could be constrained by slow microbial growth and recruitment rates (Kirchman, 2016) as well as competition and

antagonism among microbes. We surmise that transplantation to a new environment did not represent a disturbance to the biofilm community severe enough to facilitate rapid colonization by new microbial taxa. There is evidence for antagonism among microbes also defending *Fucus* species against new microbial colonization; for example, by antibiotic-producing Rhodobacteraceae (Dogs *et al*., 2017). We observed high prevalence and relative abundance of Rhodobacteraceae and other microbes in the *F. distichus* microbiota that have been shown to produce antibiotics (Wiese *et al*., 2009; Singh *et al*., 2015; Chakraborty *et al*., 2017), suggesting active competitive strategies may be a stabilizing force against taxonomic turnover in new environments (Hibbing *et al*., 2010). More experimental studies on microbially mediated mechanisms of microbiota assembly are needed to understand the importance of biotic interactions in the structure and stability of the macroalgal microbiota.

We predict that natural microbial community turnover occurring on longer timescales of weeks to months might eventually lead to shifts in microbiota composition on experimentally transplanted *F. distichus*. In support of this prediction, we observed a trend towards increasing similarity to the destination environment in the transplants that were sampled for a longer duration (WB low to PB = 6 days) compared with the reciprocal transplants of PB to WB low which were sampled over 3 days (Fig. 4B).

There is an emerging pattern across marine systems of greater resistance to turnover in microbiota originating from more variable environments following experimental manipulation. Subtidal sponges show greater turnover following transplant than sponges from the more variable intertidal environment (Weigel and Erwin, 2017); corals adapted to cooler, more constant thermal regimes show greater turnover following transplant than corals adapted to hotter and more variable thermal regimes (Ziegler *et al*., 2017, 2019); and offshore, surface bacterioplankton communities show greater turnover in response to environmental change than bacterioplankton from more dynamic nearshore waters (Wang *et al*., 2020). Our findings of general stability in the microbiota of host species adapted to highly dynamic intertidal environments are congruent with this pattern. Within our study, *F. distichus* individuals that underwent the greatest amount of microbiota change following transplant to a new environment were from the WB low site (Figs 3B and 4B; Fig. S3). *Fucus distichus* from the WB low intertidal site experience overall more stable conditions compared with individuals at higher intertidal sites. These individuals and their associated microbes are exposed to the air for less time during a given low tide event and also experience fewer low tide cycles overall, resulting in decreased exposure to desiccation and temperature extremes (Wright *et al*., 2004; Wahl *et al*., 2011). Thus, exposure to environmental variation, even within a single host species, may influence the sensitivity of associated microbial communities to environmental change. Additional experiments run for longer durations and specifically designed to test if adaptations to more variable environments decrease sensitivity to environmental change are needed to better understand the timescales and drivers of microbiota turnover on macroalgae.

In conclusion, we have shown that *F. distichus* hosts a surface microbiota that differs significantly over small spatial scales (meters to kilometres), probably in response to varied environmental conditions (e.g. wave action, tidal exposure, salinity) and/or host factors (e.g. physiology, tissue chemistry). Following transplantation to a new, natural environment or controlled common garden we found the microbiota of this intertidal host is stable over a short period of time (5 days). Our findings imply that the native and site-specific microbiota is resistant to invasion by microbes from distinct, conspecific hosts or novel source pools. Our findings expand the current understanding of microbial community assembly in highly variable environments and show surprising stability of the microbiota in response to environmental change over a few days to a week. Future work on macroalgae and other systems are needed to test the generality of our findings and the hypothesis that variable environments harbour microbial communities that are more resilient in the face of changing environmental conditions.

Experimental procedures

Site and Fucus distichus *morphotype descriptions*

We sampled *Fucus distichus* from five sites within the Hakai Lúxvbálís Conservancy at the Hakai Institute's Calvert Island Ecological Observatory on Calvert Island, BC, Canada in June 2018 (Fig. 1). The high intertidal, wave-exposed site at West Beach (WB high) is characterized by *F. distichus* with short blades that twist slightly (morphotype A). This high intertidal morph grows attached to boulders, approximately 1 m above mean lower low water (MLLW) (Fig. S1C). The low intertidal, wave-exposed site at West Beach (WB low) is characterized by *F. distichus* with deeply forked receptacles, long blades and a flat growth habit (morphotype C). WB low *F. distichus* grows attached to large boulders approximately 1 m below MLLW (Fig. S1E). The WB low site has the highest macroalgal diversity of all sites in this study (Lemay *et al*., 2018). WB high and WB low sites are in the same boulder field on WB, with a clear vertical zonation pattern between *F. distichus* morphs (personal observation). The high intertidal morph is separated from the low intertidal morph by a zone of boulders and cobble where little to

no *F. distichus* grows (Fig. S1C and S1E). A high intertidal, wave-exposed site was sampled at the opposite end of West Beach (WB west wall). The WB west wall *F. distichus* has short, twisting blades (morphotype A) and grows on boulders and vertical rock walls 0–1 m above MLLW (Fig. S1B). The North Beach (NB) *F. distichus* is from a high intertidal, wave-exposed site on the north end of Calvert Island (Fig. 1). This is a short-bladed, twisting morph (morphotype A) growing on rock walls approximately 1 m above MLLW (Fig. S1A). The Pruth Bay (PB) *F. distichus* occurs in a shallow, protected bay with a freshwater stream at the head of the bay. *Fucus distichus* at this site has wide blades with undulate margins and upright growth habit (morphotype B) and is found attached to cobble in the high intertidal, 0–1 m above MLLW (Fig. S1D). Similar morphological differentiation between habitats is well documented within other species of *Fucus* (Anderson and Scott, 1998; Scott *et al*., 2001; Kucera and Saunders, 2008).

Common garden experiment

For the common garden experiment, we combined *F. distichus* from the five sites described above in a controlled environment (Fig. S1F) to see if individual microbiota converged to a common composition when the microbial source pool and environmental conditions were homogenized. *Fucus distichus* ($n = 10$) were collected from each site by selecting individuals that could be easily dislodged without damaging the holdfast (e.g. *F. distichus* growing attached to small barnacles). All individuals were roughly the same size; less than 10 cm long at the longest blade. Collected individuals were brought back to the lab, rinsed with 0.22 μm filtered sterile seawater and sampled by rubbing the first 2–3 cm from the apical tip (the site of new growth) with a Puritan® sterile swab for 10 s to characterize the initial microbial community (sample day 1). Swabs were deposited in individual 2 ml cryovials (VWR) and stored at −80°C until DNA extraction.

A random subsample of individuals ($n = 8$ per site) were attached to small, 10% bleach sterilized rocks (~7 cm in diameter) using Splash Zone Epoxy (Z-Spar, New Jersey, USA) and allowed to air dry until the epoxy hardened (~1 h). Samples from each site were marked with a unique identifier on the rock substrate using enamel paint. Each experimental unit (sterile rock with attached *F. distichus* individual) was randomized in a continuous flow seawater table to account for variability in experimental conditions (Fig. S1F). Seawater was pumped directly from Pruth Lagoon at the Calvert Island Ecological Observatory dock (51°39′17.2″N,128°07′45.1″W). The seawater table was completely drained for 3–4 h every day to mimic tidal exposure. Light was provided by natural light through one window and four 75 W fluorescent bulbs. Light averaged 499 ± 20.2 μmol m^{-2} s^{-1} at the light source, 9.24 ± 1.65 μmol m^{-2} s^{-1} at the top of the seawater table and 7.80 ± 1.38 μmol m^{-2} s^{-1} below the seawater surface. *Fucus distichus* individuals in the flow-through table were sampled daily for 5 consecutive days by rinsing a blade with 0.22 μm filtered sterile seawater for 10 s to remove transient environmental microbes and then rubbed at the first 2–3 cm from the apical tip (the site of new growth) with a Puritan sterile swab for 10 s. The sterilized rock substrate was swabbed in a 2–3 cm^2 patch as above to see how biofilm communities developed on an abiotic surface in the common garden. Swabs were deposited in individual 2 ml cryovials (VWR) and stored at −80°C until DNA extraction. Experimental *F. distichus* individuals were routinely inspected visually for tissue necrosis or other evidence of negative effects of the experimental conditions but none were observed. On average, individuals in the common garden increased in length of the longest blade by 0.80 ± 0.52 mm over 5 days (0.16 mm day^{-1}).

Reciprocal transplant experiment

We tested if strong differences in environmental conditions and associated biotic interactions shape *F. distichus* microbiota composition with a reciprocal transplant experiment. We asked if transplanting *F. distichus* hosts from one habitat to a new habitat would lead to the acquisition of a different microbiota, more similar to the *F. distichus* microbiota of the new habitat, either by transmission from the new abiotic environment (e.g. seawater or rocks) or nearby *F. distichus* native to the new habitat. We compared compositional stability (or change) of the *F. distichus* transplants to the biofilm communities on the rock substrate where *F. distichus* individuals were attached. Rock substrate represents an abiotic surface that also has an environmentally acquired microbial community but lacks biologically driven selective filtering. As a result, we hypothesized that transplanted rock substrate would be more readily influenced by the abiotic conditions and microbial source pool of the new environment than *F. distichus* transplants.

Two sites on Calvert Island, BC with marked differences in physical and ecological characteristics and distinct *F. distichus* morphotypes were selected for the reciprocal transplant experiment: Pruth Bay (PB) and West Beach low intertidal zone (WB low) (Fig. 1). These sites are the same as included the common garden experiment, but different individuals were selected for the transplant experiment. PB and WB low are separated by approximately 15 km of coastline; due to the geography

and prevailing ocean currents, these sites are expected to have limited seawater exchange in the short term (hours to days). At each site, juvenile *F. distichus* individuals (non-reproductive; length of the longest blade from holdfast 5–8 cm) growing attached to rocks 30 cm or smaller in diameter, were selected for transplanting (n = 16 *F. distichus* individuals per site; n = 8 rocks per site). Selected *F. distichus* individuals were sampled by rinsing the length of a thallus blade with 0.22 μm filtered sterile seawater for 10 s to remove transient environmental microbes and then rubbed along the first 2–3 cm from the apical tip with a Puritan sterile swab for 10 s. Swabs were deposited in individual 2 ml cryovials (VWR), placed in coolers on ice and, upon return to the lab (4 h or less after collection), stored at −80°C until DNA extraction. The rock substrate to which these individuals were attached was gently scraped with a sterile blade to remove other macroalgal species and sessile invertebrates and then rinsed and sampled by swabbing a 2–3 cm^2 patch as detailed above. Transplanted rocks were marked with unique identifiers using enamel paint for identification in the field. Additional rocks with attached juvenile *F. distichus* individuals were selected, marked and swabbed, but not transplanted (n = 16 individuals per site); these served as origin site controls. Transplants (*F. distichus* attached to rocks) were moved to the non-native, destination site in 5-gal buckets and swabbed again at the time of transplant (day 0). Transplants and unmanipulated *F. distichus* comparisons were swabbed every 24 h within 2 h of the lowest low tide for 5 consecutive days. Corresponding rock substrate swabs were also collected at each sampling.

DNA extraction and 16S amplicon sequencing

DNA was extracted from swabs and filters using the MoBio PowerSoil®-htp 96 well DNA extraction kit (Carlsbad, CA) following the manufacturer's recommended protocol. Extracted DNA was sent to Integrated Microbiome Resource (IMR), Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University for PCR amplification and library construction. Primers targeted the V4–V5 region of the 16S rRNA gene for bacteria and archaea, 515f: 5′-GTGYCAGCMGCCGCGGTAA-3′ and 926r: 5′-CCGYCAATTYMTTTRAGTTT-3′ (Comeau *et al*., 2011). Amplicon library preparation and sequencing with Illumina MiSeq using paired-end (2 × 300 bp) v3 chemistry was performed at the IMR at Dalhousie University, Halifax, Nova Scotia, Canada according to the published protocols (Comeau *et al*., 2017). Quality filtering, trimming, dereplication, chimera removal, inference of true amplicon sequence variants (ASVs) and taxonomic assignment against the SILVA database (v.1.3.2) were done with DADA2 (Callahan *et al*., 2016).

For DADA2 processing, the filter and trim step were set to a minimum read length of 150 bp forward and 120 bp reverse. Reads were truncated after a quality score of less than or equal to two. Reads with higher than 8 forward and 10 reverse maxEE 'expected errors' were discarded. Chimera detection was done using the pooled method. Singletons and 16S reads from chloroplast or mitochondria were removed for downstream analyses. We detected signals of contamination in our amplicon sequencing reads from mammalian gut microbiota. All samples that contained more than one ASV from any member of Clostridia or Negativicutes were excluded from downstream analysis, as those taxonomic groups are known to be prevalent in the mammalian gut but not in marine ecosystems. Sequences and metadata are deposited in the European Nucleotide Archive under the project accession PRJEB42717.

Statistical analyses

All statistical analyses were conducted in R (R Core Team, 2019; version 3.6.2). 16S amplicon data were rarefied to 1500 reads per sample. To test for differences among bacterial communities between morphotypes and sample sites, we performed permutational analysis of variance (PERMANOVA) on Bray–Curtis dissimilarities of initial samples from both experiments using the vegan package (Oksanen *et al*., 2019) in R.

To test for differences in microbiota of samples originating from different habitats in the common garden experiment, we used PERMANOVA on Bray–Curtis dissimilarity and performed independent analyses for each sampling day (initial sample before individuals were placed in the common garden, 2 days, and 5 days after the start of the experiment). To test for significant differences in microbiota composition between specific sites of origin or morphotypes at the start and end of the common garden experiment we used pairwise PERMANOVAs with Bray–Curtis dissimilarities using the wrapper function pairwise.adonis for vegan (Martinez Arbizu, 2020) and adjusted p-values with the Benjamini–Hochberg correction for multiple comparisons. Community differences by site and sampling day in the common garden were visualized with principal coordinates analysis (PCoA) using the phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen *et al*., 2019) packages in R.

If microbial community composition in the common garden became homogenized across all *F. distichus* individuals over time, we would expect microbial community dissimilarity between samples from different sites to decrease from the start of the experiment to day 5 and to increase between samples originating from the same site

over time. To test for this, we used the Jaccard dissimilarity metric, based on the presence or absence of bacterial ASVs, for *F. distichus* individuals from each of the five sites. We used paired *t*-tests of Jaccard distance to determine if more ASVs were shared between *F. distichus* individuals originating from the same site or between those originating from different sites at the start (day 1) or end (day 5) of the common garden experiment. We used indval analysis from the indicspecies package (De Cáceres and Legendre, 2009) to identify ASVs that were indicative of each site of origin (WB low, WB high, WB west wall, NB, PB) as well as ASVs that were shared among all *F. distichus* in the common garden at initial sampling and on day 5 of the experiment. The indval analysis assesses the relationship between ASV occurrence or abundance values from a set of samples and the classification of the same samples into groups, which may represent habitat types, sampling points, experimental treatments, etc. The method calculates an IndVal index value based on specificity, or the proportion of samples of a group where the ASV is found, and the fidelity, or the proportion of the number of individuals (abundance) of the ASV that are in the group (Dufrêne and Legendre, 1997). An index value is calculated for every ASV in each group and then ASVs with the highest association value for a particular group are identified using permutation tests to assess the statistical significance of the relationship.

To test for differences among bacterial communities between treatments in the reciprocal transplant experiment, we used PERMANOVA on Bray–Curtis dissimilarity for transplanted samples (transplants) compared with *F. distichus* native to the transplant location or site of origin (controls). To visualize the PERMANOVA results, we conducted PCoA using the phyloseq package. Independent PERMANOVAs were performed on samples from each sampling day (initial sample to 5 days after the start of the experiment) with treatment as the explanatory variable. To determine if microbial communities on transplanted *F. distichus* changed in compositional similarity relative to *F. distichus* controls from the site of origin or destination site over time, we used ANOVA on a linear model of pseudo-*F* statistics from PERMANOVAs regressed against sampling day.

In the reciprocal transplant experiment, we wanted to know if bacterial ASVs specific to one site (PB or WB low) readily colonized *F. distichus* which were transplanted to that site. We used indval analysis to identify specific bacterial ASVs that prevail in a site of origin (PB or WB low) while being absent or having low/irregular abundance elsewhere, suggesting a significant, stable and site-specific association with *F. distichus* at that origin or indicator site. After identifying significant indicator ASVs for each site of origin (PB and WB low controls), we examined the relative abundance of significant indicator ASVs on *F. distichus* individuals from the transplant treatments to determine if indicator ASVs colonized individuals transplanted to the indicator site (increased in relative abundance) or were lost from individuals transplanted away from the indicator site (decreased in relative abundance).

All PERMANOVA analyses were run with 999 permutations. All R code associated with these analyses is available on GitHub: https://github.com/katherine-m-davis/Fucus_transplant.

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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:

Fig. S1. Benthic contour maps and aerial photographs of sampling sites. 0 m is the MLLW line and brown contours are every 1 m; tan contours are every 0.25 m. A) North Beach (NB), morphotype A; B) West Beach west wall (WB west wall), morphotype A; C) West Beach high (WB high, morphotype A; D) Pruth Bay (PB), morphotype B; E) West Beach low (WB low), morphotype C; F) Experimental setup for the common garden. Red arrows in C) and E) indicate zone at WB without *F. distichus* growth, separating morphotypes A and C.

Fig. S2. *F. distichus* and rock biofilm communities are distinct and stable following transplant to a new environment. Stacked bar plots of the most abundant ASVs on A) *F. distichus* and B) rock substrate. Each column represents an individual sample; samples are ordered by day of sampling and grouped by site of origin. The first number of x-axis labels is sampling day, the second number is a unique sample identifier.

Fig. S3. Microbial communities on *F. distichus* transplants do not become more differentiated from unmanipulated controls at the native origin site over time or more similar to controls at destination site over time. ANOVA on linear regressions of pseudo-*F* values over time for WB low - > PB transplants and controls at the destination site (p-value = 0.054) or origin site (p-value = 0.630); PB - > WB low and controls at the destination site (p-value = 0.728) or origin site (p-value = 0.597).

Table S1. Pairwise comparisons of compositional dissimilarity (Jaccard) of *F. distichus* microbiota samples from within the same site and between a focal site and all other sites at the start (day 1) and end (day 5) of the common garden experiment.

Table S2. Indicator analysis for ASVs significantly associated with site(s) of origin in the common garden. Specific indicators were identified for each site at initial sampling (day 1) and final sampling (day 5) in common garden as well as for all possible combinations of sites and time points using the indicspecies package in R (Cáceres and Legendre, 2009). The Indval statistic is comprised of A, specificity, and B, fidelity of an ASV.

Table S3. Indicator analysis for ASVs on *F. distichus* significantly associated with origin sites in the reciprocal transplant experiment. Specific indicators were identified for each site based on initial samples (day 0) and unmanipulated controls at West Beach low or Pruth Bay over the 5 days experiment using the indicspecies package in R (Cáceres and Legendre, 2009). The Indval statistic is comprised of A, specificity, and B, fidelity of an ASV.