**An experimental test reveals spatial controls on the seagrass (*Zostera marina*) and epiphyte *Smithora naiadum***

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[**ABSTRACT, < 250 words**] In aquatic benthic communities defined by foundation species, composition and abundance of associated species can change substantially over small spatial distances. Such spatial turnover can reflect top-down control by consumers, bottom up control by abiotic factors or facilitation, or a combination of the two types of process. We used a reciprocal transplant experiment to test causes of striking spatial patterns patterns in the abundance and distribution of the red macrophyte *Smithora naiadum* in meadows of the seagrass *Z. marina* on the Central Coast of British Columbia. We hypothesized that *S. naiadum* presence and abundance reflects grazing by invertebrate epifaunal grazers, facilitation by microbiota on individual eelgrass shoots, or abiotic conditions such as hydrodynamic conditions. When placed in a high *Smithora* environment, *Smithora*-free eelgrass blades were colonized, while existing *Smithora* was not lost when colonized shoots were moved to a *Smithora* free environment. Illumina sequencing of bacterial DNA isolated from surface swabs of seagrass transplants revealed significantly different bacterial communities on shoots with and without *Smithora*. Further, we found that shoots with *Smithora* had higher invertebrate grazer abundance and distinct grazer and bacterial communities. We conclude that once colonized, *S. naiadum* can persist on eelgrass shoots in interior meadow areas without the epiphyte, and that variation in epibiotic communities of microbes and grazers was better explained by the presence of *S. naiadum* than by location in the meadow.

**Highlights**

* **Aquatic plants form vast habitats that host rich biodiversity. Within these habitats, there is spatial variation in the abundance and composition of hosted species.**
* **We used a reciprocal transplant to investigate potential drivers of variation in abundance of an abundant algal epiphyte *Smithora naiadum* on *Zostera marina***.
* **We observed effects of spatial location on *Smithora* colonization but not persistence, suggesting that spatial factors affecting colonization are most important in this system. We also observed significant correlation between *Smithora* abundance and grazer and bacterial communities on the seagrass blades.**
* **Correlated changes in abundance of microbial, invertebrate and algal epibiont groups suggest general spatial processes operating within the meadow, or indirect effects of one epibiotic taxon (*S. naiadium*) facilitating others (grazers and microbes).**

**Graphical Abstract (anyone?)**

*Smithora* influences invertebrate and bacterial community composition



Does not vary between edge and interior

Much higher abundance at edge vs. interior of meadow, but *Smithora* can survive in meadow interior.

1. **Introduction**

Like many aquatic macrophyte foundation species, seagrasses form meadows that host vast biodiversity of algae, invertebrates and fish (Bostrom et al. 2006, Duffy 2006). Variation within meadows in shoot-level epibiotic species composition contributes to high biodiversity at the meadow-scale, yet the causes of this variation within meadows are not well understood (Johnson et al. 2005; Lavery and Vanderklifft 2002; Prado et al., 2007; Saunders et al., 2003). Variation in epibiotic composition and diversity has been attributed to a range of biotic and abiotic factors, including grazing pressure (Amundrud et al. 2015; Huang et al. 2015; Duffy et al. 2015; Reynolds et al. 2015; Montfrans et al. 1984), environmental conditions (flow, nutrients, temperature, light) (Milchakova 2000; Alcoverro, Duarte, and Romero 1997; Kendrick and Burt 1997; Cebrian et al. 1999; Reyes and Sansón 1997; Johnson et al. 2005), or even shoot specific microbial community (Harder, 2008; Silva et al. 2013; Holmström et al., 2002b, Meja et al. 2016).

One of the main food sources for the diverse seagrass-associated animal communities is not the seagrass itself, but epiphytic algae living on seagrass blades (Valentine and Heck, Edgar and Shaw 1995, Taylor 1998). Epiphytic algae play an important role in the seagrass ecosystem by supporting animal productivity, but they also can have detrimental impacts on seagrass plants by reducing the level of light and nutrients that seagrass plants receive (Mcroy and Goering 1974; Sand-Jensen 1977; Penhale 1977; Harlin 1975; Coleman and Burkholder 1994; Lin et al. 1996; Morgan and Kitting 1984, Fry 1984). Thus, like other foundation species, seagrass and the biodiversity it hosts exist in a complex network of positive and negative interactions (Connolly 1994; Boström and Bonsdorff 1997; Sheridan 1997; Webster, Rowden, and Attrill 1998; Heck and Orth 1980; Heck Jr and Orth 1980; Attrill, Strong, and Rowden 2000; Tolan, Holt, and Onuf 1997; Harlin 1975; Fong, Lee, and Wu 2000), whose outcomes vary over space with variation in environmental and biotic conditions.

Top-down control by grazers and bottom-up facilitation by microbes or seagrass hosts may interact to influence the spatial patterns of seagrass associated epiphytes (Meja et al. 2016; Ettinger et al. 2017, Lavery and Vanderklift 2002; Milchakova 2000; Alcoverro, Duarte, and Romero 1997; Kendrick and Burt 1997; Cebrian et al. 1999; Reyes and Sansón 1997; Johnson et al. 2005; Schanz et al. 2002). Bacterial communities that form microbial films on the surface of eelgrassblades may facilitate colonization of seagrasses by epifaunal organisms (Sieburth and Thomas 1973). Unique bacterial groups are often associated with a specific blade surface chemistry (Bagwell et al. 2002; Crump and Koch 2008; Hamisi et al. 2009; Weidner et al. 2000; Duarte, Holmer, and Marba 2005), and the growth of unique bacterial communities can discourage the attachment of spores on marine macrophytes (Bell, Lang, and Mitchell 1974; Mejia et al. 2016). Evidence is mixed on how much within-meadow variation in microbial films - for example, between a meadow edge and interior – could affect colonization of epifaunal algae (Meja et al. 2016, Ettinger et al.2017). There is stronger evidence that spatial variation in grazer abundance both between and within meadows can shift spatial patterns algal abundances through grazing (Amundrud, Srivastava, and O’Connor 2015; Boström and Mattila 1999; Tanner 2005). Both grazer abundances and bacterial communities can be highly variable across spatial gradients. To investigate their relative top-down and bottom-up effects on *Smithora* abundance it follows that their effects should be investigated co-currently and at a small spatial scale.

In coastal habitats of the northeast Pacific Ocean, the meadow-forming eelgrass *Zostera marina* provides food and habitat for hundreds of invertebrate and fish species, and one of the most conspicuous epibiotic residents is the epiphytic red alga *Smithora naiadum*. *S. naiadum* is notable not only for its bright red color in a green landscape, but also for its the spatial variation in its abundance and distribution meadows (Harlin 1975). There is limited understanding about what could be causing this variation in *S. naiadum*, but because of its high quality as a food source (Galloway et al., 2012), potentially severe biomechanical consequences and limitation of light and nutrients to the *Z. marina* blades, understanding why it varies so much over relatively short distances is essential to understanding the relative importance of top down and bottom up influences in *Z. marina* ecosystems (Kitting, Fry, and Morgan 1984).. It is possible that epibiotic microbial films or hyperlocal abiotic conditions such as hydrodynamics could affect *S. naiadum’s* success in colonizing as a microscopic spore, after which it forms tough basal cushions and then grows into lobed blades (Hansen 1986, Harlin 1973b, Hawkes 1988).

Reciprocal transplant experiments have been used to test for the importance of local drivers in determine species abundance in the field. So far, transplant experiments have revealed the potential for invertebrate grazing to reduce epiphyte algal abundance (Reynolds et al. 2017). But it remains unclear whether bottom up processes (including facilitation by microbial assemblages) can also drive changes in epiphyte abundance. If a blade-level community exhibited bottom up control of epiphytes, high epiphyte abundance would be expected to correlate with specific environmental (e.g., nutrient) or microbial communities (Reynolds et al. 2017). However, few studies have investigated the potential for bottom up processes to influence algal abundance and no studies have done this using a reciprocal transplant.

Here, we investigated the relative importance of bottom-up and top-down drivers of spatial variation in abundance of a dominant epiphyte, the red algae *S. naiadum,* on the eelgrass *Z. marina*. We first quantified the abundance and distribution of *S. naiadum* on *Z. marina* at our study site. We then performed a reciprocal transplant experiment of seagrass shoots between zones of high and low *S. naiadum* abundance within a single, large *Z. marina* meadow. Our experimental design allowed us to test whether epiphyte abundance was determined by the environment surrounding the seagrass shoot, or by the host plant and its associates. We tested the hypothesis that *S. naiadum* abundance on eelgrass is determined by biotic characteristics of the host plant (shoot biomass, morphology), its epibiotic microbial assemblage, or the host’s environment (location). Our experiment also allowed us to compare the microbial communities of *Z. marina* before and after colonization by *S. naiadum* to determine whether there were identifiable community shifts in microbial community that could be correlated with a decline in shoot health, a metric of the outcome of interactions between seagrass and its dominant epiphyte.

2. Materials and Methods

2.1 Quantifying epibiotic abundance and distribution at meadow edge and interior.

*Zostera marina* is a meadow forming eelgrass common along coastlines in the northern hemisphere (Phillips, Macmillan, and Bridges 1983), where in many locations, the red alga *Smithora naiadum* is one of the more common macroalgal epiphytes on eelgrass. We studied the interaction between *S. naiadum* and *Z. marina* on the Central Coast of British Columbia, Canada, in June-August 2015 in Choked Pass, Calvert Island (Figure 1). In a large continuous eelgrass meadow approximately 367,000 square meters in area, *S. naiadum* is prevalent on *Z. marina* blades along the edges of the meadow, but not in the meadow interior. The site is strongly ocean influenced, with salinities between 29 and 31 ppt and temperatures between 6 and 10°C in summertime. Within the meadow, depths vary (max 10m), but a consistent depth of 5m was held across our experimental transplant locations.

Within the Choked Pass eelgrass meadow, we quantified spatial variation in *S. naiadum* abundance on eelgrass. We surveyed *S. naiadum* on *Z. marina* along 8 40-m transects, four in the meadow interior (>200m from the closest edge) and four at the meadow edge (2m from bordering sand habitats) throughout the primary growing season, May to August (Olson 2017; Fig. 1). All transects were located in permanently subtidal areas, and were separated by at least 100 m. Survey data consisted of randomly selected shoots along each transect. Using SCUBA, we collected one shoot every 10m (n = 5 shoots per site visit) by covering shoots with a Ziplock bag and detaching at the rhizome. From each shoot, we measured *Z. marina* shoot dry weight and *S. naiadum* dry weight (see lab processing methods in section 2.4), and grouped data from these 5 shoots for each transect.

In a second survey to estimate spatial patterns in epifaunal invertebrates, we quantified grazer abundance and diversity on *Z. marina* shoots at the meadow edge (WF) and interior (IA) (Figure 2). *Z. marina* shoots were collected from 0.25m x 0.25m quadrats (n = 6) from these two locations in mid June and July 2015. Following standard processing protocol (Duffy et a 2015), all invertebrates were removed from shoots and preserved with 95% ethanol. Invertebrates > 500 um in diameter were visually classified to the lowest possible taxonomic group (Appendix 1), usually family but sometimes to species, using a stereo microscope, and invertebrates known to associate with *Z. marina* and graze epiphytic algae were enumerated (Whippo et al in revision, Duffy et al 2015).

2.3 Reciprocal transplant experiment

To test whether *S. naiadum* abundance on an eelgrass shoot reflects the shoot’s location or the shoot’s biological characteristics (e.g., defenses, microbiota, size, etc), we conducted a reciprocal transplant experiment. We identified two adjacent source locations (transplant edge and interior locations) within the Choked Pass meadow at the WF site. These zones differed in *S. naiadum* abundance on shoots, from 0.37 + 0.39 *S. naiadum* / *Z. marina* (g/g dry wt) in the high *S. naiadum* zone at WF edge to 0.02 + 0.06 *S. naiadum* / *Z. marina* (g/g dry wt) in the low *S. naiadum* zone at WF interior. Depth and substrate (sandy) were consistent, and the two sites were 10 meters apart and connected by continuous eelgrass habitat.

From each zone, we collected twelve shoots and exposed them to one of two treatments (n = 6 shoots per treatment): transplant and control. Transplanted shoots were collected and moved: transplant edge shoots moved to transplant interior, and vice versa. To control for the effect of uprooting on *Smithora* abundance and bacterial community, control shoots were collected and replaced in their original location. Collection, initial sampling, and replanting procedures consisted of the following steps: a) shoots with a minimum of 6 rhizome nodes were collected on SCUBA on July 9th, placed in a Ziplock bag underwater, and subsequently swabbed for bacterial community analysis (when not being processed they remained submerged in 4-6°C seawater); b) each shoot was uniquely labeled with flagging tape so that it could be re-sampled at the end of the experiment; c) shoots were placed in field on July 10th by attaching them by the rhizome with zip-ties to PVC submersible platforms attached to the sandy substrate so that shoots floating upright. On August 10th, all 24 treatment and control shoots were collected, processed and photographed in the lab. We also collected 1 ambient shoot next to each transplant platform to compare transplant manipulation shoots to unmanipulated shoots. We removed mesograzers from the shoots upon collection. Several shoots were lost or torn during the experimental period and sufficient biomass could not be recovered. This decreased the sample size from the initial N=24 (see Table 1 for all sample sizes used in analyses).

2.4 Sampling shoot and environment attributes before and after the experiment

We sampled experimental independent and response variables at the beginning and end of the experiment (Table 1). For all shoots collected for the transplant experiment (N = 12 treatment shoots + 2\*2 ambient control shoots) and transect surveys (N = 120), we measured the following shoot characteristics: leaf length, leaf width, biomass (dry weight, after 48 hours at 60°C), and microbiota. For shoots collected as part of environmental surveys, we also counted the number of blades per shoot. Shoots were brought to the lab, where epiphytes were gently scraped off with a microscope slide and grouped taxonomically. *Z. marina* shoots and associated *S. naiadum* epiphytes were then dried at 60°C for 48hrs to obtain dry weights.

To quantify the diversity and composition of each shoot’s external microbiota, bacterial samples were taken before and after the transplant, as well as from ambient shoots collected at the time of transplant retrieval. We sampled microbiota from a standard location on each shoot - an area halfway up the third youngest? leaf that was free of *S. naiadum*. This area was rinsed with filtered sterilized seawater for 10 seconds, and then a Puritan® sterile swab was used to swab the area for ten seconds, avoiding any *S. naiadum* basal thallus cushions. The swab was stored in an individual sterile cryovial (VWR) and placed on ice for transport back to the lab, and were transferred to -80˚C for storage within 8 hours.

DNA extraction and protocols followed standard procedures. DNA was extracted from swabs and water filters using the MoBio PowerSoil®-htp 96 well DNA extraction kit (Carlsbad, CA) following the manufacturer’s recommended protocol. The V4 region of 16S rRNA in Bacteria and Archaea was targeted for amplification using redesigned versions of the primers 515f/806r (Caporaso et al. 2012): 515f: 5’–GTGYCAGCMGCCGCGGTAA–3’, 806r: 5’–GGACTACNVGGGTWTCTAAT–3’. Forward primers were tagged with a 12bp Golay barcode to facilitate sample pooling. Each PCR contained 10µl of 5-Prime Master Mix, 1µl of each primer (final concentration = 0.2µM each), 0.5µl of peptide nucleic acid (PNA) chloroplast blocking primer (Lundberg et al. 2013; 0.2µM final concentration, purchased from PNA Bio Inc., Thousand Oaks CA), 2µl of DNA, and PCR grade water to a final volume of 25µl. PCR was carried out with an initial denaturation step at 94˚C for 3 minutes, followed by 25 cycles of denaturation at 94˚C for 45 seconds, PNA clamping at 75˚C for 60 seconds, primer annealing at 50˚C for 60 seconds, and extension at 72˚C for 90 seconds, with a final extension step of 72˚C for 10 minutes. PCR products were quantified using Quant-IT Pico Green® ds DNA Assay Kit (Life Technologies). Equal amounts (25ng) of each sample were pooled and then purified using the MoBio UltaClean® PCR clean-up kit. Pooled library quantitation and paired-end Illumina MiSeq sequencing (2 x 300bp) was carried out at the Integrated Microbiome Resource facility in the Centre for Genomics and Evolutionary Bioinformatics at Dalhousie University (Halifax, Canada).

Raw sequencing reads were demultiplexed using split libraries within the Quantitative Insights into Microbial Ecology (QIIME v.1.9) analysis pipeline (Caporaso et al. 2010b), and then trimmed to 250 base pairs using FastX Toolkit (<http://hannonlab.cshl.edu/fastx_toolkit/>). Reads were then clustered into “species” level operational taxonomic units (OTUs) using Minimum Entropy Decomposition (MEDs; Eren et al. 2015), with the minimum number of reads per MED node set to 500 (-M parameter). All other parameters were run with default settings; the maximum variation allowed per node (-V) was automatically set at three nucleotides. Taxonomy was assigned to MED-nodes (hereafter referred to as operational taxonomic units; OTUs) using uclust (Edgar 2010) as implemented in the Assign Taxonomy function of QIIME v.1.9 retrained on the GreenGenes (gg\_13\_8) database (DeSantis et al. 2006). OTUs annotated as chloroplasts, mitochondria, or unassigned, and OTUs with fewer than 500 reads across the data set were removed. Additional OTUs were removed if they occurred in only a single sample. Samples with fewer than 1000 reads were removed from the analysis. The final dataset for analysis consisted of 744214 sequences clustered into 311 OTUs from 27 samples, with a range of 7177 – 45672 sequences per sample. The dataset was rarefied to 7100 sequences per sample for diversity analyses. Representative sequences for these OTUs were aligned with PyNAST v.1.2.2 (Caporaso et al. 2010a) using the GreenGenes 13\_8 alignment as a template, and a tree was constructed using FastTree (Price et al. 2010) as implemented in QIIME v.1.9. Sequence data and MiMARKs compliant metadata are deposited at the European Bioinformatics Institute, accession number (XXXXXXXX).

**2.5 Statistical analyses**

To identify significant variation in S. naiadum abundance and distribution between the meadow edge and interior, we fit linear models and used two-way ANOVA to test for an effect of site and interior or edge on *S. naiadum* abundance.

To test our hypothesis that source location and transplant treatment affected *S. naiadum*, we fit linear models and used ANOVA to compare *Z. marina* and *S. naiadum* biomass and density among locations and treatments (R. 325 statistical software). Independent (predictor) variables were four treatment levels (edge/transplant, edge/control, interior/transplant, interior/control). Independent one-way anovas were performed on the following response variables: smithora biomass (final), …?.

To quantify differences among the edge and interior experimental sites so that we might identify potential environmental or shoot-level drivers of patterns in *S. naiadum* abundance, wshoot and environmental attributes among experimental locations at the beginning and end of the experiment. We used two-way anova with location and sample time as independent variables (Table 1) to analyze patterns in the dependent variables of epifaunal abundance and diversity. ies *. naiadum*y composition

To compare bacterial community composition among treatments, we constructed a dissimilarity matrix in QIIME on rarefied data (7100 sequences/sample) using Bray-Curtis dissimilarity, which takes relative abundance into account. Beta-diversity patterns were visualized with non-metric Multi Dimensional Scaling (NMDS) plots created in PRIMER E v. 6 (Clarke & Gorley 2006). PERMANOVA (Permutational Analysis of Variance) tests implemented in PRIMER E were used to compare the effect of *S. naiadum* presence, time, and transplant on bacterial community. PERMDISP implemented in PRIMER E was used to test for significant differences in bacterial community dispersion. The Chao1 index (Chao 1984) was used to estimate the richness of bacterial taxa for each sample. Chao1 richness was compared using t-tests.

**3. Results**

3.1 Spatial patterns in epibiota between meadow edge and interior

Across eight sites at the landward side of the Choked Pass eelgrass meadow (Figure 1), *S. naiadum* presence and abundance on eelgrass shoots varied strongly from site to site, and there was a significant difference in *S. naiadum* abundance on *Z. marina* between meadow edge and interior sites (Figure 1C, two-way ANOVA: site type (interior vs edge): F = 63.46, df = 1, p = < 0.001; site: F = 8.06, df = 6, p = < 0.001, residuals: df = 108).

Additional, plot-scale sampling at the WF edge and interior sites, where the experiment was conducted, revealed similar patterns of high *S. naiadum* abundance at the meadow edge and less in the interior in June 2015 (Figure 2). *Z. marina* shoot density was higher at the edge vs the interior (one-way ANOVA: F = 15.29, df = 1, 10, p = 0.003; Appendix A1), and so was *Z. marina* and *S. naiadum* biomass per *Z. marina* shoot (one-way anova: F = 6.57, df = 1, 10, p = 0.028) (Figure 2A and B). Grazers were more abundant on *Z. marina* shoots at the edge of the meadow compared to the interior (df=1,t=7.995 p=<0.0001*,* Figure 2C). Grazer density on *Z. marina* increased over the course of the experiment, between June and July (df=1,t=9.908, p=<0.0001) but the trend of higher invertebrate abundances at the edge remained the same regardless of month although the effect of edge was stronger in July (df=1, t=5.515, p=<0.0001). Epifaunal invertebrate community composition also varied over time (PERMANOVA F=4.3221, df=1, p=0.065) and with *S. naiadum* abundance (PERMANOVA F = 4.7201, df = 1, p=0.048) (Appendix A2 and A3).

*Initial microbial assemblages.* Blades with *Smithora* from the meadow edge harbor significantly different microbial communities than blades from the interior without (PERMANOVA p=0.009, pseudo-F=7.3624, df =1, Figure 2D). Microbial community composition shifted from July to August (PERMANOVA for date p=0.001, pseudo-F=4.818, df = 1).

3.3 Reciprocal transplant experiment

At the end of the reciprocal transplant experiment, the meadow edge had high *Smithora* load regardless of source location; shoots transplanted to the edge gained Smithora biomass comparable to the edge control shoots (Figure 3; two-way ANOVA with interaction term: Source (interior vs edge): F = 32.04, df = 1, p = < 0.001; Treatment (control vs unmanipulated: F = 0.28, df = 1, p = 0.61, Source X Treatment: F = 4.67, df = 1, p = 0.05; residuals: df = 11). Shoots transplanted from the edge to the interior location retained *Smithora,* albeit in lower abundance than their source location, while interior shoots that stayed in the interior (controls) were not colonized (Fig 3). Controls (uprooted but locally planted) and ambient shoots did not differ in *Smithora* load at the time of the end of the experiment (two-way anova: Source (interior vs edge): F = 26.34, df = 1, p = < 0.001; Treatment (control vs unmanipulated: F = 1.59, df = 1, p = 0.27, residuals: df = 10).

*Bacterial results on transplanted shoots.* There was a significant effect of initial *Smithora* presence on shoot level bacterial community. Both at the beginning and end of the experiment shoots with *Smithora* had a different community than those without, regardless of whether the shoot with *Smithora* was at the edge or interior (PERMANOVA for *Smithora* presence/absence p=0.027, pseudo-F=2.03,df=1). Bacterial community dispersion was not different (PERMDISP p=0.441). There was no significant difference in blade bacterial assemblages associated with edge vs. interior with respect to the shoot source (PERMANOVA for source location p=0.583, pseudo-F=0.800,df=1), or shoot final location (edge vs. interior) (PERMANOVA for destination p=0.573,pseudo-F=0.94, df=1). While microbial taxonomic composition was different overall, microbial taxonomic richness at the end of the experiment was not significantly different between blades with or without *Smithora* (t-test p=0.59) or between shoots with different transplant locations (t-test p=0.60), or source locations (t-test p = 0.664).

**4. Discussion**

We tested the hypothesis that a host’s associated floral and faunal communities can be controlled by host specific characteristics, by their location, or both.We found that *Smithora* abundance on eelgrass shoots did not change to match *Smithora* on neighboring shoots when transplanted in one direction (from meadow edge to interior), but it did change in the reciprocal direction (interior to edge) (Fig. 3). Thus, we reject the hypothesis that only local factors determine *Smithora* abundance on *Zostera* shoots. Rather, our results indicate that, once settled, *Smithora* can persist on *Z. marina* even in locations where it has not colonized. The directional trends also support interactions between factors, leading to rapid colonization at the meadow edge, and gradual loss of epiphytes in the meadow interior. This suggests that limited *Smithora* abundance in the meadow interior could reflect limited dispersal and different environmental conditions in this area of the meadow.

We observed that shoots with and without *Smithora* had different microbial communities in the field (Fig. 2D), such that the presence of *Smithora* is correlated with a unique seagrass microbial community. This result was unexpected, because we sampled *Smithora*-free areas of eelgrass shoots. This could be due to a change in shoot phenolics following colonization (Harder 2008, Silva et al. 2013, Holstrom et al. 2002). Past studies have found that secondary metabolites produced by seagrasses deter the attachment of fouling organisms; the differences we observed between shoots with and without *Smithora* could be due to an underlying difference in phenolic content between edge and interior plants that allows *Smithora* to only colonize edge shoots (Dworjanyn et al. 1999; Butman 1987; Davis and Targett 1989).However, the distinct microbial communities on shoots without *Smithora* did not prevent *Smithora* from colonizing shoots transplanted once moved to a high *Smithora* area. When control shoots were uprooted and replanted in the interior, this may have lowered shoot health and phenolic content. Despite this, no *Smithora* colonization occurred in the interior, suggesting that it is unlikely that *Smithora* colonization at the edge was due to decreased seagrass metabolite defense alone. The initial differences in bacterial assemblages detected between edge and interior shoots is most likely due to the presence of *Smithora* itself rather than any small scale changes in shoot defenses.

Shoot-level microbial communities appeared to be unaffected by transplant location. Edge shoots that had *Smithora* had similar microbial communities to shoots with *Smithora* in the interior (Fig 4.). Our result is consistent with observations that microbial communities do not vary between the edge and interior of a meadow (Ettinger et al. 2017). All microbial communities on uprooted shoots changed following transplant and this could be due to a change in shoot health following uprooting or a temporal change in community composition. The blade level microbial community of *Zostera* has been shown to vary along a spatial gradient (Meja et al. 2016), however we found that shoot location did not explain microbial compositional shift in our study. We assumed that the small spatial scale of our study removed the possibility of environmental variation causing the blade level microbial differences between edge and interior shoots. Instead, the microbial changes we observed were a reflection of the physiological responses of *Zostera* to algal colonization and experimental manipulation (i.e., uprooting).

In general we found that *Smithora* biomass and *Zostera* density and biomass differed between edge and interior sites. It is unclear why there is consistently more *Smithora* biomass at the edge of the meadow. The adult sexual stage of *Smithora* is unknown and it could be that the diploid sporophyte stage of *Smithora* is a conchocelis phase that grows outside the seagrass meadow in shell fragments (Harlin 1973; Hawkes 1988). This could explain why there is higher *Smithora* biomass at edge areas close to the rocky intertidal and unvegetated sandy habitats with high coverage of shells. This could also explain why we see high abundances of *Smithora* on *Phyllospadix* spp. in the intertidal (Harlin 1973). It could be that the dispersal distance of *Smithora* propagules is quite small and so it settles relatively quickly into the seagrass meadow. We could be observing dispersal limitation of *Smithora* as its spores are released from the intertidal and then trapped at the meadow edge without dispersing farther into the subtidal meadow.

Our final comparison between high and low *Smithora* sites was a grazer community comparison. If grazers were controlling *Smithora* presence or abundance through consumption, we would have observed a negative correlation between *Smithora* abundance and grazer abundance. But we observed the opposite pattern, with higher abundances of invertebrates at the edge where there was also a high abundance of *Smithora*. This could be due to the presence of *Smithora* creating a more structurally complex habitat which in turn provides food and shelter for epifaunal invertebrates (Burkepile et al. 2006). We also observed that invertebrate communities from quadrats with *Smithora* were significantly different from those without in terms of relative abundances of different species (A3). This indicates that *Smithora* could be providing more than substrate on the blade surface. *Smithora* may provide a specialized habitat to a unique assemblage of invertebrates or is an important food source to certain species. These results may also support higher level trophic dynamics, including predation. For instance, higher abundances of recruiting rockfish at the high Smithora meadow have been documented (Olson 2017).

An important consideration of our study is that our comparisons across shoots with and without *Smithora* are also comparing between the edge and interior of a seagrass meadow. Large changes in epifaunal communities can be associated with seagrass meadow edges (Bowden, Rowden, and Attrill 2001; Bell et al. 2001, Tanner 2005, Prado et al. 2007). In separate studies edge effects have been shown to effect algal and invertebrate communities. We observed patterns similar to other studies with a strong correlation in edge effects between invertebrate and algal communities and with the added correlation of bacterial community differences, indicating that edge effects could be acting on multiple community levels.

In summary our research highlights important community forming processes occurring at the edges of seagrass meadows. We noted differences in *Smithora* abundances between the edge and interior of meadows, and we showed that different seagrass microbial communities are correlated with this variation in algal abundance. We also showed that grazer communities vary with these changes in *Smithora* abundances. Contrary to other seagrass systems, however, we find that invertebrate abundance is positively correlated with ephiphyte biomass. It is unclear whether all three trophic assemblages are influenced by the same edge effects or they are interacting together to form the ecological pattern we are noting. Understanding community forming processes in seagrass epifaunal communities at small spatial scales is important for recognizing large scale patterns in seagrass communities.

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