Aquatic Botany

A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	red algae; seagrass; microbial ecology; grazers; experiment; community; Zostera marina; Smithora naiadum
Corresponding Author:	Mary O'Connor University of British Columbia BC Canada
First Author:	Mary O'Connor
Order of Authors:	Mary O'Connor
	Gwendolyn Griffiths
	Rhea Sanders-Smith
	Margot Hessing-Lewis
	Katharine M Davis
	Coreen Forbes
	Angeleen Olson
	Carolyn Prentice
	Laura Wegener Parfrey
Abstract:	In aquatic foundation species, composition and abundance of associated epibionts can change substantially over small spatial distances. Such spatial variation can reflect top-down control by consumers, bottom-up control by abiotic factors or facilitation, or a combination of processes. We used visual and molecular surveys to describe spatial patterns in the abundance and distribution of the epiphytic red macroalga Smithora naiadum in a meadow of the seagrass Zostera marina on the Central Coast of British Columbia. We detected Smithora using 18S ribosomal RNA molecular marker throughout the seagrass meadow at both interior and edge sites, even in the absence of macroscopic Smithora. We used a reciprocal transplant experiment to test two hypotheses: that patterns in Smithora abundance reflect local environmental conditions, or alternately, that patterns reflect spatial variation in the host plant attributes, microbiota and grazers. Zostera shoots hosted more Smithora at meadow edges relative to meadow interior sites, and shoots with Smithora were associated with distinct invertebrate grazer and bacterial communities relative to shoots 5 m in from the meadow edge without Smithora. Macroscopic Smithora grew on shoots experimentally transplanted from the interior to the meadow edge and shoots hosting Smithora that were transplanted to the interior did not lose Smithora. Our survey and experimental results suggest that presence of macroscopic Smithora blades on Zostera shoots changes the Zostera microbiota. Altogether, we conclude that environmental variation, not host plant attributes or dispersal limitation, affects Smithora colonization on Zostera, and once established, Smithora alters the microbiota on Zostera.
Suggested Reviewers:	Anna K James ajames@ucsb.edu has published research on kelp microbiota / epibiota interactions
	Heike Lotze Heike.Lotze@Dal.Ca expertise in seagrass in canada
	Kelly Ugarelli kugarelli@ufl.edu published on seagrass holobiont

	Carol Thornbur thornber@uri.edu expertise in seagrass-algae interactions
Opposed Reviewers:	

Cover Letter

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Zoology
Faculty of Science
4200 - 6270 University Boulevard
Vancouver, BC V6T 1Z4

Phone 604 822 2131
Fax 604 822 2416
www.zoology.ubc.ca

October 25 2020

Dear Editor:

Please consider our article entitled "A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota" for publication as a Research Paper in Aquatic Botany.

We present results of field surveys and a reciprocal transplant experiment that tested hypotheses about drivers of patterns in the abundance and distribution of the red algal epiphyte *Smithora naiadum* on the meadow-forming seagrass *Zostera marina.* We report molecular evidence that *Smithora* DNA was found throughout the meadow even on Zostera shoots with no visible *Smithora*, yet macroscopic *Smithora* was only observed in some locations, mostly at meadow edges. Our experiment showed that *Smithora* could persist on shoots transplanted to *Smithora*-free zones, and grew rapidly on Zostera shoots that were transplanted to *Smithora*-rich zones. We rejected hypotheses that grazers, shoot attributes, dispersal limitation or shoot microbiota explained differences patterns of *Smithora* across an eelgrass meadow, and are left with a hypothesis that fine-scale spatial variation in abiotic factors is the leading cause. Overall, our study contributes new information to our limited understanding of the ecology of an obligate seagrass epiphyte (*Smithora*) on a major habitat-forming species in coastal waters throughout the northern hemisphere.

We note that a previous version of this manuscript ("An experimental test reveals spatial controls on the seagrass (*Zostera marina*) and epiphyte *Smithora naiadum*" by Griffiths et al) was reviewed by this journal in 2018. The decision was reject with possibility to resubmit. We have entirely rewritten this manuscript, reanalyzed the data, and added new data, and revised the interpretation, so it is reasonable to consider this a new submission at this time. In our revision, we incorporated changes based on the many thoughtful and constructive comments offered by two reviewers, and ultimately our revisions went well beyond what they suggested such that a point-by-point response is no longer relevant (though if requested we could produce one). Here, we present a substantially improved manuscript.

Sincerely, on behalf of co-authors,

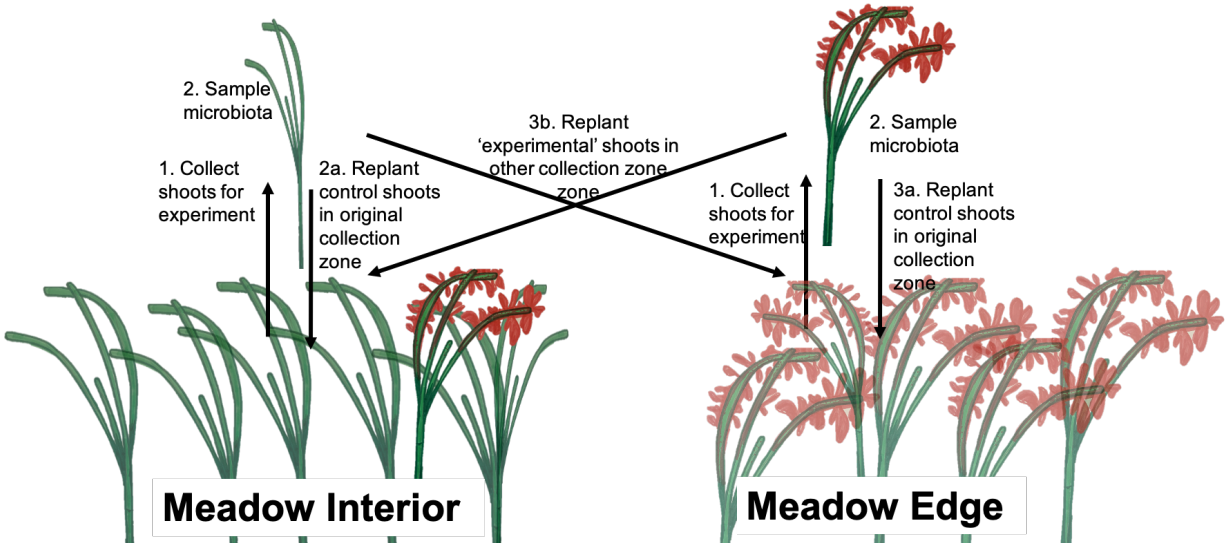
Mary O'Connor

Mary O'Connor
Associate Professor, University of British Columbia
oconnor@zoology.ubc.ca

Highlights

- **Aquatic plants form vast habitats that host rich biodiversity; epiphytic algae play important roles structuring the diversity supported by dominant plants in these ecosystems.**
- **We used a reciprocal transplant experiment to investigate potential drivers of variation in abundance of the algal epiphyte *Smithora naiadum* on the seagrass *Zostera marina*.**
- **We found evidence of *Smithora* DNA on *Zostera* shoots throughout the meadow, but visible abundance of *Smithora* predominantly at meadow edges.**
- **Environmental variation, even over a few meters, explained variation in *Smithora* abundance while *Zostera*-associated microbiota, epifaunal grazers and shoot attributes did not.**
- **Presence of macroscopic *Smithora* appears to influence *Zostera* leaf microbiota, suggesting a possible microbe-mediated feedback between seagrass and algal symbionts.**

Graphical abstract



A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota

Mary I. O'Connor, Gwendolyn Griffiths, Rhea Sanders-Smith, Margot Hessing-Lewis, Katharine M. Davis, Coreen Forbes, Angeleen M. Olson, Carolyn Prentice, Laura Wegener Parfrey

Abstract:

In aquatic foundation species, composition and abundance of associated epibionts can change substantially over small spatial distances. Such spatial variation can reflect top-down control by consumers, bottom-up control by abiotic factors or facilitation, or a combination of processes. We used visual and molecular surveys to describe spatial patterns in the abundance and distribution of the epiphytic red macroalga *Smithora naiadum* in a meadow of the seagrass *Zostera marina* on the Central Coast of British Columbia. We detected *Smithora* using 18S ribosomal RNA molecular marker throughout the seagrass meadow at both interior and edge sites, even in the absence of macroscopic *Smithora*. We used a reciprocal transplant experiment to test two hypotheses: that patterns in *Smithora* abundance reflect local environmental conditions, or alternately, that patterns reflect spatial variation in the host plant attributes, microbiota and grazers. *Zostera* shoots hosted more *Smithora* at meadow edges relative to meadow interior sites, and shoots with *Smithora* were associated with distinct invertebrate grazer and bacterial communities relative to shoots 5 m in from the meadow edge without *Smithora*. Macroscopic *Smithora* grew on shoots experimentally transplanted from the interior to the meadow edge and shoots hosting *Smithora* that were transplanted to the interior did not lose *Smithora.* Our survey and experimental results suggest that presence of macroscopic *Smithora*

blades on *Zostera* shoots changes the *Zostera* microbiota. Altogether, we conclude that environmental variation, not host plant attributes or dispersal limitation, affects *Smithora* colonization on *Zostera*, and once established, *Smithora* alters the microbiota on *Zostera*.

A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota

Mary I. O'Connor[1,2], Gwendolyn Griffiths[1], Rhea Sanders-Smith[3,4], Margot Hessing-Lewis[3], Katharine M. Davis[1,4], Coreen Forbes[1,2], Angeleen M. Olson[3], Carolyn Prentice[3], Laura Wegener Parfrey[1,2,3,4]

1) Biodiversity Research Centre, University of British Columbia, Vancouver, BC V6T 1Z4

2) Department of Zoology, 6270 University Boulevard, University of British Columbia, Vancouver, BC V6T 1Z4

3) Hakai Institute, PO Box 309, Heriot Bay, BC V0P 1H0, Canada

4) Department of Botany, 6270 University Boulevard, University of British Columbia, Vancouver, BC V6T 1Z4

Manuscript in preparation for a Research Paper in Aquatic Botany

Corresponding Author:

Mary I. O'Connor, oconnor@zoology.ubc.ca

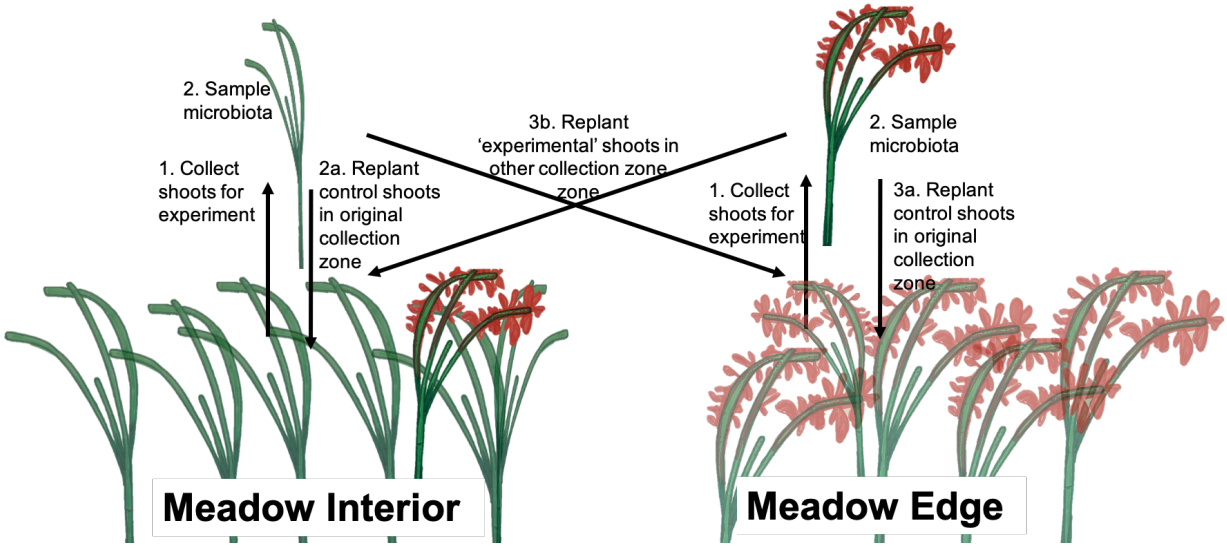
Keywords: red algae, seagrass, microbial ecology, grazers, experiment, community, *Zostera marina*, *Smithora naiadum*

[**ABSTRACT, < 250 words**] In aquatic foundation species, composition and abundance of associated epibionts can change substantially over small spatial distances. Such spatial variation can reflect top-down control by consumers, bottom-up control by abiotic factors or facilitation, or a combination of processes. We used visual and molecular surveys to describe spatial patterns in the abundance and distribution of the epiphytic red macroalga *Smithora naiadum* in a meadow of the seagrass *Zostera marina* on the Central Coast of British Columbia. We detected *Smithora* using 18S ribosomal RNA molecular marker throughout the seagrass meadow at both interior and edge sites, even in the absence of macroscopic *Smithora*. We used a reciprocal transplant experiment to test two hypotheses: that patterns in *Smithora* abundance reflect local environmental conditions, or alternately, that patterns reflect spatial variation in the host plant attributes, microbiota and grazers. *Zostera* shoots hosted more *Smithora* at meadow edges relative to meadow interior sites, and shoots with *Smithora* were associated with distinct invertebrate grazer and bacterial communities relative to shoots 5 m in from the meadow edge without *Smithora*. Macroscopic *Smithora* grew on shoots experimentally transplanted from the interior to the meadow edge and shoots hosting *Smithora* that were transplanted to the interior did not lose *Smithora.* Our survey and experimental results suggest that presence of macroscopic *Smithora* blades on *Zostera* shoots changes the *Zostera* microbiota. Altogether, we conclude that environmental variation, not host plant attributes or dispersal limitation, affects *Smithora* colonization on *Zostera*, and once established, *Smithora* alters the microbiota on *Zostera*.

Highlights

- **Aquatic plants form vast habitats that host rich biodiversity; epiphytic algae play important roles structuring the diversity supported by dominant plants in these ecosystems.**
- **We used a reciprocal transplant experiment to investigate potential drivers of variation in abundance of the algal epiphyte *Smithora naiadum* on the seagrass *Zostera marina*.**
- **We found evidence of *Smithora* DNA on *Zostera* shoots throughout the meadow, but visible abundance of *Smithora* predominantly at meadow edges.**
- **Environmental variation, even over a few meters, explained variation in *Smithora* abundance while *Zostera*-associated microbiota, epifaunal grazers and shoot attributes did not.**
- **Presence of macroscopic *Smithora* appears to influence *Zostera* leaf microbiota, suggesting a possible microbe-mediated feedback between seagrass and algal symbionts.**

Graphical abstract



1. Introduction

Like many aquatic macrophyte foundation species, seagrasses form meadows that host vast biodiversity of algae, invertebrates and fish (Boström et al., 2006b, 2006a; Duffy, 2006; Duffy et al., 2015; Whippo et al., 2018). Central to seagrass ecosystems, seagrass-epiphyte interactions can be competitive for nutrients and light (Hauxwell et al., 1998; McGlathery, 2001) or a symbiosis mediated by nutrient exchange (Harlin, 1973a). Shifts between positive and negative interactions with ecosystem wide consequences can depend on environmental perturbations such as nutrient pollution or trophic cascades resulting from overfishing (Heck et al., 2000; McGlathery, 2001). Epiphytic algae on seagrass also play an important role in the food webs and ecosystems that seagrass support. In temperate zones algae are the base of the green food web associated with seagrass habitats (Duffy et al., 2015; Fry, 1984; Reynolds et al., 2014). Understanding causes of variation in epiphyte abundance, and seagrass-epiphyte interactions, is an important component of understanding energy flow and community structure of a seagrass meadow (Heck et al., 2000; McGlathery, 2001). One biological factor increasingly recognized to play an important role in host - epibiont interactions are microbial assemblages (Barott and Rohwer, 2012; Bengtsson et al., 2017; Chen and Parfrey, 2018; James et al., 2020). On coral reefs, coral-associated microbiota can shift when algae are growing adjacent, leading to a microbial assemblage that can damage coral and facilitate expansion of turf algae (Barott and Rohwer, 2012). In kelps epiphytic organisms block light/alter photosynthetic capacity of host and increase drag/susceptibility to breaking of host tissue. The microbiota of epiphytes can also shift host microbial communities towards assemblages dominated by opportunistic pathogens or taxa specialized in degrading aging host tissue (James et al., 2020). Even in healthy macroalgal assemblages, transmission of microbes between host species occurs (Chen & Parfrey 2018;

Lemay et al 2018) and this may be especially relevant given the spatial proximity of host-epibiont associations.

In coastal habitats of the northeast Pacific Ocean, the meadow-forming seagrass *Zostera marina* (hereafter, *Zostera*) provides food and habitat for a diverse assemblage of invertebrate and fish species (Duffy et al., 2015; Huang et al., 2015; Stark et al., In Press; Whippo et al., 2018), and consequently is considered an ecologically and economically important foundation species (Department of Fisheries and Oceans (DFO) Canada, 2009). One of the most striking macroalgae present on seagrass (*Zostera marina*) on the Pacific Coast from Kodiak Island, Alaska, to Baja, Mexico, is the bright red algae *Smithora naiadum* (herein *Smithora*), also known as seagrass laver, which can grow in abundance on *Zostera* and create brilliant color contrasts in subtidal meadows (Figure 1A). *Smithora* has a complex life cycle with a microscopic form, a small cushion-like form, and a more conspicuous three-dimensional bladed form. The bladed form is only found on seagrasses (Harlin, 1973a; Hollenberg, 1958; Willcocks, 1982), and the cushion form has been detected a few times on other substrates (Harlin, 1973b). *Smithora* gains nutrients from the sediments via *Zostera*'s metabolism and transfer without any internal interaction (Harlin, 1973a), and this nutrient supply could benefit *Smithora* in coastal waters with low water column nutrients. Many seagrass meadows on the west coast of Canada currently experience relatively low levels of nutrient pollution or other conditions known to stimulate algal blooms detrimental to seagrass in other parts of the world (McGlathery, 2001; Nagel et al., 2020).

The *Zostera* – *Smithora* symbiosis is a special example of a host-epiphyte relationship in temperate seagrass systems because *Smithora* is only found on *Zostera marina* and surfgrass, *Phyllospadix spp*. This interaction has served as a model for pioneering work to identify

exchange of nutrients and photosynthate among plants and epiphytes (Harlin 1973). It is also ecologically important: *Smithora* contains much greater essential fatty acid concentrations than other algae (Galloway et al., 2012) and is known to provide a preferred food source to some fish (Horn et al., 1982). The role of microbiota in this interaction remains completely unexplored, despite increasing understanding of the importance of microbiota in host-epiphyte interactions and *Zostera* ecology. Though there is some evidence that *Zostera* leaves influence microbiota relative to seawater microbial assemblages (Sanders-Smith et al., in review), most taxa observed on *Zostera* are common marine bacterial groups (Adamczyk et al., n.d.; Crump et al., 2018; Sanders-Smith et al., in review; Trevizan Segovia et al., Accepted). Seagrass-associated taxa are associated with functions such as nitrogen fixation and sulfide oxidation (Crump et al., 2018), and the *Zostera* microbiome is thought to play a role in seagrass productivity and trophic transfer to higher trophic levels (Ugarelli et al., 2017). It is possible that microbiota on *Zostera* facilitate the colonization or growth of its symbiont *Smithora,* which is only found on *Zostera* and the surfgrass *Phyllospadix.* It is also possible that *Smithora* has a specific microbiota associated with it that could play a role in the *Zostera-Smithora* interaction. Marine epibiota can influence host microbiota (James et al., 2020), and algae can influence their microbial environment (Chen and Parfrey, 2018). Thus, the microbiota on the surface of *Zostera* leaves could be either a cause or consequence of variation in *Smithora* abundance in seagrass meadows.

The red blades of *Smithora* provide a striking visual contrast to the bright green leaves of seagrass, and the patchy patterns of *Smithora* abundance over scales of meters or across sites can be difficult to ignore when observing seagrass across a seascape. Possible causes include the algae's response to dispersal limitation (Willcocks, 1982), fine scale variation in the physical environment (Hollenberg, 1958; Willcocks, 1982), or biotic attributes of the host plant (including

epibiota and grazers) that limit its facilitation of *Smithora* growth. If dispersal limitation explains *Smithora* abundance patterns within a large meadow, *Smithora* would be most abundant at meadow edges close to the rocky intertidal where it can be found on *Phyllospadix* (Willcocks, 1982) or near other *Smithora* on *Zostera* shoots. *Smithora* can reproduce asexually to increase density on a shoot or onto adjacent shoots (Hollenberg, 1958; Willcocks, 1982), but the sexual part of the complex lifecycle and dispersal potential during this microscopic phase is not understood (Hawkes, 1988). Once colonized, variation in local grazer communities, light or nutrient supply via the water column or *Zostera* tissue could affect the growth and persistence of *Smithora* such that zones of the meadow with more grazers, lower light or less resource supply would be expected to have less *Smithora* standing stock. Alternatively, *Zostera* may vary spatially in its morphology, defense chemistry or nutritional status in ways that limit *Smithora* colonization or growth. Understanding whether *Smithora* presence and abundance is due to the physical environment, the host (seagrass) environment, or both, is an important component to understanding the main drivers of eelgrass meadow structure and ecological functioning in healthy ecosystems.

We used visual surveys and environmental DNA sampling to quantify variation in *Smithora* abundance on *Zostera* shoots throughout a highly productive, large eelgrass meadow relatively free of proximate human impacts on the Central Coast of British Columbia (Figure 1). We then used a reciprocal transplant experiment to test hypotheses about possible causes of variation in *Smithora* abundance. We tested the hypothesis that *Smithora* abundance reflects environmental (biotic and abiotic) growing conditions associated with the spatial location: in zones with higher grazer densities we expected *Smithora* biomass to be limited by grazing, and in deeper areas or denser seagrass, we expected *Smithora* growth and biomass to be light limited.

We therefore predicted that *Smithora* abundance would decline when shoots with *Smithora* were transplanted to the no-*Smithora* zone of the meadow, and would increase when *Smithora* shoots were transplanted to the high-*Smithora* zone. If these predictions were supported, we would infer that *Smithora* is most directly affected by environment conditions that favor growth and minimize grazing. Alternatively, *Smithora* presence and abundance may reflect attributes of the *Zostera* biotic environment, such as host plant size, density, chemistry or microbiota, and differences in shoot attributes between meadow edge and interior. In this case, we would predict that *Smithora* abundance on transplanted shoots would persist regardless of their location. Finally, we tested the hypothesis that seagrass leaf surface microbiota is determined by the environment by characterizing bacterial composition on the transplanted shoots before and after the experiment. An additional hypothesis for why *Smithora* abundance varies among meadow zones is dispersal limitation of *Smithora.* Our experiment does not provide a strong test of this hypothesis, but our survey results suggest that dispersal limitation is not the primary cause of the observed patterns. Altogether, *Smithora* appears able to reach and colonize shoots throughout the meadow, reaching highest abundance when environmental conditions (not shoot or epibiota attributes) are most suitable for *Smithora* growth, and potentially influencing the epibiotic microbiota of *Zostera* leaves.

2. Materials and Methods

2.1 Quantifying Zostera and Smithora abundance and distribution: visual surveys

We studied the interaction between *S. naiadum* and *Z. marina* on the Central Coast of BC, Canada, from late May-August 2015 in Choked Passage near Calvert Island (Figure 1D, E). Choked Passage is in a high energy area on the northwest side of Calvert Island, predominantly

ocean influenced (Olson et al., 2019). In a large continuous eelgrass meadow, approximately 367,000 m^2, *Zostera marina* is the only seagrass species present. We sampled *Zostera* shoots and epiphytic *Smithora* abundance at eight subtidal sites at least 100 m apart (Figure 1E). Four sites were in the meadow interior (>50 m from the closest edge of the meadow; hereafter, 'interior sites'), and four sites were in eelgrass within 2 m of the edge of the meadow (hereafter, 'edge sites'; Figure 1E). Depths, standardized to mean lower low water (MLLW) were slightly less for the edge sites (IS = 1.52 m, OS = 4.99 m, WF = 2.01 m, AL = 1.82 m) compared to the interior sites (ID = 3.99, IC = 3.99, IT = 4.30, IA = 3.41), though both zones included at least one site deeper than 4 m. At each site, we sampled shoots along a permanent 40-m transect. Using SCUBA, we haphazardly collected one shoot every 10 m along the transect (n = 5 shoots/transect) by covering shoots with a plastic bag and detaching at the rhizome, sealing the shoot and any epiphytes in the plastic bag with seawater. Surveys were repeated three times throughout the 2015 growing season: late May, late June-early July, and late July – early August (Olson et al., 2019). In the lab, we separated seagrass leaves and epiphytes by gently scraping leaves with a microscope slide. Length and width measurements were taken from the longest leaf per shoot. We measured dry weights of *Zostera* shoots and *Smithora* after 48 hours at 60 ºC. We quantified '*Smithora* load' as the dry weight of *Smithora* divided by the dry weight of its eelgrass host shoot.

2.2 Smithora detection by molecular marker

To test whether *Smithora* might be present on *Zostera* leaves or in the water column in microscopic live stages, we sampled shoots and seawater for presence of *Smithora* genes using the 18S amplicon sequencing. We also tested whether microbiota on epibiotic *Smithora* blades was similar to microbiota on *Zostera* leaves. The 18S rRNA gene is a common molecular marker

for eukaryotic biodiversity surveys. Samples for 18S sequencing were taken from Choked Pass meadow from late May-August 2015 in conjunction with surveys described above (Section 2.1). In that survey, *Zostera* shoots were collected specifically for 18S sequencing of their microbial epibiota and were placed individually in sterile Ziploc bags underwater. *Smithora* tissue on *Zostera* shoots was directly swabbed as a control (n= 5) in late May 2015. Seawater was sampled within the meadow ~ 1 meter above the *Zostera* canopy by filling a 500ml sterile Nalgene bottle underwater and closing in situ. Samples for molecular analysis from *Zostera* shoots and *Smithora* tissue were processed on the boat within two hours of collection using sterile technique. For seagrass shoots, we selected a 10cm length of the second oldest leaf close to the sheath, avoiding large epiphytes, and rinsed for 10 seconds with filtered seawater (0.22µm) to remove unattached organisms. We swabbed this region with Puritan® sterile swabs for 10 seconds. Single blades of *Smithora* were swabbed by the same method. Swabs were placed in sterile cryovials and kept cool and dark on the boat, then transferred to -80°C within 6 hours of sampling until DNA extraction. Water samples were filtered in the lab within 6 hours of collection through 0.22µm Durapore membrane filters using a peristaltic pump and the membrane was then placed in a sterile Whirl-pak bag using sterile forceps. Filters were stored in -80°C until DNA extraction (Section 2.6).

For epibiotic bacterial sampling in the first survey and in the experiment, we sampled microbiota from a standard location on each shoot - an area halfway up leaf 3 that was typically naturally free of macroscopic *Smithora*, even when macroscopic *Smithora* was present elsewhere on the shoot. This shoot surface was rinsed with filtered seawater for 10 seconds, and then a Puritan® sterile swab was used to swab the area for ten seconds, avoiding any *Smithora* basal thallus cushions. The swab was stored in an individual sterile cryovial (VWR) and placed on ice

for transport back to the lab. The swabs were transferred to -80˚C for storage within 8 hours.

225 *2.3 Quantifying abiotic conditions*

Water column properties were measured throughout the meadow in 2015 as part of an ongoing environmental monitoring program by the Hakai Institute. Because the environmental observations were taken for long-term monitoring, the sites and variables sampled are not exactly aligned with our experimental location and time but can be used to provide a description of the
230 overall eelgrass site and to compare water quality inside vs outside the meadow. We used observations at *Sandspit* (between OS and IS in Figure 1D) for March through August, and in June at three other stations: *Interior* (between IT and ID), *Lower* (west of IC and IA), *Wolf* (east of WF just outside the meadow). The Sandspit, Interior and Lower stations are within the eelgrass meadow, and the Wolf station is outside the meadow on the sand flat.

235 An RBR Conductivity-Temperature-Depth (CTD) Profiler and a YSI Pro plus handheld meter were used to sample the water column at multiple depths (0 m and 5 m) to measure salinity and temperature. Niskin bottle water samples were taken at 0 and 5 m depth to assess chlorophyll-a biomass. Chlorophyll-a samples were analyzed using a Turner Trilogy fluorometer using the JGOFS protocol (Holm-Hansen and Riemann, 1978).

240 The rate of water flow determines the hydrodynamic forces that plants, algae and invertebrates experience, as well as nutrient delivery rates. Differences in flow rates can substantially affect the structure and function of shallow marine communities. To quantify the potential confounding effect of differences in flow rates at our two experimental treatment sites (Figure 1E, Section 2.5), we estimated relative water flow rates as part of another study in

summer for each of two years after our experiment. At each experimental location (edge and interior), pre-weighed Plaster of Paris chalk blocks were attached to PVC posts using zap straps, n=1 block per post in 2016 and n=3 blocks per post (averaged) in 2017. The chalk blocks sat roughly mid-seagrass canopy height (approx. 75 cm above the sediment) and were deployed for 4 days in 2016 and 1 day in 2017, respectively. Blocks were dried in a drying oven upon retrieval from the field and the difference between pre and post weights (g) was divided by the duration of exposure in the field to determine mass lost per hour (g lost/hr). This method produces a relative comparison of water flow rates at the two sites.

2.4 Testing for correlations between Smithora and biotic factors

We identified one area within the meadow (WF, Figure 1E) to focus our efforts on additional surveys and a transplant experiment to test our hypotheses about the distribution of *Smithora* in the meadow. We worked at the WF site because it was an area of continuous meadow that included both edge and interior zones separated by about 5 m with little change in depth or substrate. To test for correlations between *Smithora* abundance and distribution and some of the biotic factors associated with the *Zostera* host plant, we compared eelgrass and their associates at the meadow edge and interior while minimizing potential effects of depth and other larger-scale variation in the meadow. In this second survey, we estimated *Zostera* density, biomass and leaf surface area per unit meadow area ($0.0625 m^2$). We estimated *Smithora* load per *Zostera* aboveground dry weight and cm^2 LAI (max shoot width x max length x number of leaves for each shoot in the plot) and epifaunal invertebrates per *Zostera* above ground weight prior to the transplant experiment.

We collected above-ground biomass and all associated epiphytes and epifauna within 0.0625m^2 quadrats in early July over a two-day period (July 4th and 5th) (n = 6 quadrats in each zone, total 12 quadrats). We collected shoots by gently placing them in a long mesh bag with fine enough mesh to retain invertebrates > 0.5 mm long. After shoots were covered in mesh, we broke eelgrass off at the sediment-water interface and tied the bag to prevent escape by invertebrates. This method is effective at capturing epifaunal invertebrates that may be important grazers (0.5 mm – 4 cm long) on eelgrass shoots, but less effective in capturing larger mobile invertebrates such as adult crabs (Duffy et al., 2015; Reynolds et al., 2014; Whippo et al., 2018). In the lab we separated *Zostera* shoots, epiphytes including *Smithora,* and invertebrates. We counted shoots and dried and weighed algae and seagrass. Following standard processing protocol (Reynolds et al., 2014), all invertebrates were removed from shoots and preserved with 95% ethanol. Invertebrates > 0.5 mm were visually classified to lowest taxonomic level possible (Appendix 1) using a stereo microscope. We counted total invertebrate abundance, and also counted groups of grazers, defined as taxa known to associate with *Zostera* and graze epiphytic algae (Duffy et al., 2015; Whippo et al., 2018). We further divided grazers into gastropods and crustacean groups.

2.5 Reciprocal transplant experiment

We conducted a reciprocal transplant experiment to test the hypothesis that *Smithora* load on eelgrass shoots reflects a shoot's environmental context (interior or edge). The alternative is that *Smithora* abundance reflects the shoot attributes, and therefore would persist after transplant. At the WF site (Figure 1E), we identified two zones 5 meters apart and connected by continuous eelgrass habitat; one in the inner zone of the meadow and one at the edge zone for our transplant

experiment. Depth (between 2 and 3 m MLLW) and substrate (sandy) were similar at the two locations. We collected 12 experimental shoots from the edge zone and 12 from the interior zone (N = 24 shoots total). We exposed shoots to one of two treatments: transplant (edge → interior, interior → edge) and control (edge → edge, interior → interior) (n = 6 shoots per treatment). Transplanted shoots were collected and moved from the edge zone to the interior, and vice versa. Control shoots were collected and replaced in their original location to control for the effect of handling and removing *Zostera* shoots on *Smithora* load and epibiotic community.

We collected shoots with a minimum of 6 rhizome nodes on SCUBA on July 9th. Underwater, shoots were placed in a plastic bag with seawater. At the lab, shoots were swabbed for bacterial community analysis. Initial dry weights, *Smithora* load and invertebrate assemblage were not assessed because these would have been destructive for the experimental shoots. When not being processed, shoots remained submerged in 4-6°C iced seawater. Each shoot was uniquely labeled with flagging tape and returned to the field on July 10th by attaching them by the rhizome with plastic zip-ties to one of two submersible PVC platforms at each treatment site (6 shoots per platform) inserted into the sandy substrate so that shoots floated upright among ambient shoots. Shoots were randomly assigned to each platform so that treatments were not confounded with platform.

To explore potential direct or indirect associations between *Smithora* and the *Zostera* leaf surface bacterial microbiota, we test the hypothesis that *Zostera*-associated surface microbiota reflect their environment and shift after transplanting to a new location. To quantify diversity and composition of each shoot's external microbiota, bacterial samples were taken from each experimental shoot before (n = 11; one sample failed to sequence so n=11 samples were analyzed) and after the transplant (n=12). At the end of the experiment, we sampled two

unmanipulated shoots collected at the time of transplant retrieval from each site, one of which failed sequencing, leaving n=2 ambient shoots at WF – edge and n=1 WF – interior in the analysis.

We left experimental shoots in the field for one month. One month is sufficient time for eelgrass leaves to grow, and for *Smithora* to grow on *Zostera* leaves. We chose an experimental period long enough to allow colonization and community assembly of microbial and invertebrate assemblages, but short enough in duration to minimize risk of losing experimental shoots in the high energy environment. On August 10th, we collected treatment and control shoots, as well as one additional, unmanipulated shoot next to each transplant platform (4 unmanipulated shoots in total) to serve as a reference to compare transplant manipulation shoots to unmanipulated (ambient) shoots in each habitat zone. We discarded experimental shoots that were broken and from which segments were lost during the month in the field and did not process them further for epiphytes or microbes. For the remaining, intact shoots, in the lab we separated epiphytes and shoots. We measured shoot and epiphyte dry weights. We swabbed shoots at the end of the experiment to assess the shoot-associated microbial assemblages.

2.6 Microbiota processing

DNA extraction: 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. Durapore filters containing seawater samples were cut in half under sterile conditions; half of the filter was used for extraction and half archived at -80°C.

Eukaryotic microbial analysis (survey, section 2.2): The V4 region of 18S rRNA gene was targeted for amplification using primers E572F: 5′-CYGCGGTAATTCCAGCTC-3′ and E1009R: 5′-AYGGTATCTRATCRTCTTYG-3′ (Comeau et al., 2011). PCR, library construction, and Illumina MiSeq amplicon sequencing were carried out at the Integrated Microbiome Resource facility at the Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University (Halifax, Canada) according to standard protocols (Comeau et al., 2017).

For 18S data analysis, demultiplexed reads were obtained from the sequencing centre. Sequences were then trimmed to a uniform length of 250 bp using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and processed into operational taxonomic units (OTUs) using the MED method (Eren et al., 2015) as implemented in the Oligotyping microbial analysis software package (Eren et al., 2013). We set the minimum substantive abundance parameter (-M) at 250 reads and used default settings for all other parameters. Taxonomy was then assigned to the representative sequence for each MED node by consensus of the top three hits using UCLUST V1.2.22q (Robert C. Edgar, 2010) and the SILVA 128 (Quast et al., 2013) database clustered at 99% similarity. Chimeras, sequences belonging to *Zostera*, sequences unassigned at the domain level, and prokaryotic sequences were filtered out. We discarded samples with fewer than 1000 total reads after filtering, resulting in n=3 for *Smithora*, n=37 for *Zostera*, and n=3 for seawater. We collated sequence abundances assigned to *Smithora* at the genus-level and used a threshold of >5 reads per sample for calling *Smithora* present. Sequence data and MiMARKs compliant metadata are deposited at the European Bioinformatics Institute, accession number (XXXXXXXX).

Microbiota analyses: 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 12 bp Golay barcode to facilitate sample pooling. Each PCR contained 10 µl of 5-Prime Master Mix, 1µl of each primer (final concentration = 0.2 µM each), 0.5 µl of peptide nucleic acid (PNA) chloroplast blocking primer ((Lundberg et al., 2013); 0.2 µM final concentration, purchased from PNA Bio Inc., Thousand Oaks CA), 2 µl 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 (25 ng) 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 300 bp) was carried out at the Integrated Microbiome Resource facility in the Centre for Genomics and Evolutionary Bioinformatics at Dalhousie University (Halifax, Canada) according to published protocols (Comeau et al. 2017).

For 16S rRNA data analysis, raw sequencing reads were demultiplexed using split libraries within the Quantitative Insights into Microbial Ecology (QIIME v.1.9) analysis pipeline (J G Caporaso et al., 2010), and then trimmed to 250 base pairs using FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). MED uses Shannon entropy to separate out meaningful patterns of nucleotide diversity from sequencing noise and partition the data into MED nodes, which in practice are analogous to ≥99% OTUs. The minimum number of reads

was 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 (R. C. 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). We removed OTUs annotated as chloroplasts, mitochondria, or unassigned at the domain level. OTUs that occurred in only a single sample or had fewer than 500 reads across the data set were also removed. To minimize the impact of barcode switching, OTUs with fewer than two reads for given sample were removed. Finally, samples with fewer than 100 total reads were removed from the analysis. The final bacterial dataset from the experiment consisted of 744214 sequences clustered into 311 OTUs from 26 samples out of an initial 28 samples (one sample from the initial timepoint and one ambient sample from the final timepoint had fewer than 1000 sequences after filtering), 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 (J. G. Caporaso et al., 2010) 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).

2.7 Statistical analyses

In the meadow-wide survey, we quantified spatial patterns in the presence and abundance of *Smithora* using a mixed-effects model. Observed ratios of *Smithora* dry biomass / *Zostera* dry weight ('*Smithora* load') were zero-inflated and were not normally distributed. Zeroes in these

405 data could represent very low (but unobserved) *Smithora* biomass, or in fact an absence of *Smithora* on *Zostera*. We used a gamma hurdle model to analyze the data in two stages: first, we used a negative binomial mixed effects model to analyze the frequency of zero vs non-zero values across zones (edge sites vs interior sites) and observation times (May, July, August), considering site as a random effect. Next, we used a gamma mixed effects model to analyze the 410 non-zero *Smithora* load values. We used the lme4 package in R (R core team 2019).

For the second set of comprehensive surveys at the experimental site, we used analysis of variance (ANOVA) to test for variation in *Zostera* attributes (shoot density and biomass) and epifaunal grazer density between the edge and interior zones at the Wolf Site. We tested for covariation in *Zostera* attributes and invertebrate density using linear models and ANOVA 415 comparisons. To test hypotheses about drivers of *Smithora* load on *Zostera*, we used ANOVA with zone, sample time and *Zostera* above ground dry mass and density as predictors. We tested for correlations between *Zostera* dry mass and shoot density with regression.

We used a non-metric multidimensional scaling (NMDS) approach on untransformed invertebrate abundances to visualize invertebrate communities based on Bray-Curtis 420 dissimilarity. Invertebrate community data were analyzed in the Vegan 2.5-6 package in R (Oksanen et al., 2019). PERMANOVA was used to test the effect of zone (edge vs. interior) on invertebrate community composition, and a one-way ANOVA was used to test the effect of zone on total abundance.

To test our hypothesis that source location and experimental transplant treatment affected 425 *Smithora* load, we used a two-way ANOVA with an interaction term to test for effects of transplant treatment (experimental vs control) and shoot source zone (edge vs interior) on *Smithora* load at the end of the experiment.

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 NMDS plots using the phyloseq (McMurdie and Holmes, 2013) and Vegan packages in R. PERMANOVA (Permutational Analysis of Variance) tests implemented in PRIMER E (Clarke and Gorley, 2006) and Vegan were used to compare the effect of location, experimental treatment, *Smithora* presence and host species on bacterial community composition. We also used PERMDISP implemented in PRIMER E to test for significant differences in bacterial community dispersion. Indval analysis from the indispecies package in R (De Caceres et al., 2020) was used to identify bacterial ASVs that were significantly associated with sites at the edge versus interior of the seagrass bed as well as with *Smithora* or *Zostera* hosts.

We used a model selection approach to determine drivers of meadow-wide *Smithora* load, and to identify patterns in experimental-site *Zostera* biomass, invertebrate load, and *Smithora* load. For each linear model analysis, we competed multiple possible and relevant models. We ranked models using Akaike's Information Criterion (AIC) values using the MuMin package in R (Barton, 2019) adjusted for small sample sizes (AIC_C) and model weights based on log-likelihood and parameter parsimony. We used coefficients from the best model to produce estimates of variables of interest (e.g., *Smithora* load).

3. Results

3.1 *Abiotic conditions*

Salinity at the *Sandspit* monitoring station in the eelgrass meadow interior ranged from 27.2 PSU at the surface (0 m) in May 2015 to 31.1 PSU at 5 m depth in July 2015. Differences between surface and 5 m depth were minimal, and salinity over the meadow did not differ from salinity outside the meadow (Table 1). Inside the meadow, water temperatures ranged from 9.2 ºC inside the meadow in March to 14.9 ºC in July. Overall, we do not have evidence of differences in environmental regimes between sampling sites in the meadow.

Comparison of mass remaining in flow blocks after deployment at sites near the experimental interior and edge location indicate slightly higher mass loss rates at the meadow edge (Wolf site: 1.13 g lost / hour vs IC: 0.97 g lost/ hour in 2016, and 2.62 g lost / hour vs 2.33 g lost / hour in 2017). This suggests that in 2015 when we did our experiment, the meadow edge may have experienced slightly higher flow rates, though we note that our interior experiment site was 5 m into the meadow from the edge, while the IC site is 20 m in from the edge, and likely experiences a greater reduction in flow than our interior experimental site. In the absence of better data from our exact experimental sites, we proceed on the assumptions that these differences in flow rates are negligible.

3.2 Smithora abundance and distribution within the meadow

Throughout the meadow, we observed macroscopic *Smithora* more often and in greater abundance at meadow edge sites relative to interior sites (Figure 1B-C). Based on the best model with a negative binomial error distribution (Table 2A), we took the inverse of the coefficients estimated on a logit scale to find that the probability of observing *Smithora* on *Zostera* increased by 0.86 between successive sample times in the summer of 2015 and by a negligible 0.00024 in

edge relative to interior sites (main effects). There was an interaction term of 0.92 for the interaction between zone and time, reflecting the much greater frequency of *Smithora* later in the summer at edge sites. This interaction term was not included in the second-best model, which cannot be rejected ($\delta AIC_C < 2$; Table 2). There was no support for site depth as a predictor of macroscopic *Smithora* presence (Table 2).

When macroscopic *Smithora* was observed (Table 2), modeled *Smithora* load was 0.46 g *Smithora* / g *Zostera* at edge sites and 0.24 g *Smithora* / g *Zostera* at interior sites, and ranged at edge sites from 0.18 g at OS to 1.17 g at IS. Interior sites had an estimated 1.43 g *Smithora*/g *Zostera* less than shoots at edge sites. *Smithora* load declined with depth based on support for models with depth as a predictor (Table 2B). In contrast to the increase over time observed for *Smithora* presence, sampling date was not a strong predictor of *Smithora* load (Table 2B). *Smithora* load estimated in terms of *Zostera* surface area (LAI) instead of above ground biomass exhibited a similar pattern (Figure A2), reflecting the strong correlation between *Zostera* surface area and biomass (Figure A2).

18S amplicon surveys of *Zostera* shoots in the Choked Pass meadow in summer 2015 revealed a high prevalence of *Smithora* sequences (Table 3). Only one of these sites corresponded with interior sites from our non-molecular shoot survey (Figure 1E). At this site, IT, our visual methods detected no macroscopic *Smithora* but we did detect *Smithora* 18S sequences in 100% of shoot swabs collected concurrently (May 2015). We did not detect *Smithora* by molecular method in any of the seawater samples (Table 3).

3.3 Correlations between Smithora and biotic factors

The survey of *Zostera* and its epibiota (grazers, *Smithora* and microbiota) at the

experimental site before the experiment revealed differences in both *Zostera* attributes and microbiota between edge and interior zones. In this survey, zones were separated by about 5 m at a constant depth, yet edge samples had more epifaunal invertebrates, *Smithora* and *Zostera* than interior samples. *Zostera* and *Smithora* abundance differed among edge and interior zones, consistent with trends observed in the larger meadow survey reported in Section 3.2 (model selection results given in Table 4). In July, the edge zone had higher *Zostera* shoot density (N/m^2) (Figure 2A). In both June and July, *Zostera* biomass (Figure 2B) and *Smithora* load (Figure 2C) were higher at the edge zone. We do not have eelgrass morphometric data (e.g., LAI) for this survey. In a second analysis of this pre-experiment survey, we found little support for *Zostera* covariates (shoot density, dry weight) as predictors of *Smithora* load (Table 5). Three models were among the best, and these were the models with a) zone (interior/edge), time and *Zostera* dry weight, b) zone, and c) zone, and time. An analysis of model sets with only a *Zostera* weight term (no density term) revealed the same patterns. Though *Zostera* density was included in one of these models, the effect size was low: -0.01 g *Smithora* / g *Zostera* per increasing g *Zostera* dry weight.

Patterns in invertebrate abundance on *Zostera* were consistent with patterns of *Smithora*, such that there were more invertebrates / g *Zostera* in the meadow edge zone compared to the interior (Figure 2D). In July, we observed 10,637 individual invertebrates on *Zostera* shoots, and of these, 8690 were taxa considered to be grazers. Invertebrate load – number of invertebrates / g *Zostera* -- was 3-4 x higher at the meadow edge relative to the interior in July, at the start of the experiment (Figure 2D). Although the pattern between zones was significant for both invertebrates and *Smithora*, there was no significant correlation between *Smithora* load and grazer load (Figure A3), and consequently little support for a negative effect of total grazers on

Smithora abundance. Invertebrate species composition differed between the two zones (Figure 2E). Differences were driven by high abundances of amphipods and copepods in most meadow edge plots, while some gastropods were more abundant in the interior (*Alia carinata*, *Margarites papillus*). Overall, though, gastropods were equally abundant in both zones (Figure A1). Two grazer taxa - *Idotea resecata* and *Lacuna sp.* - were abundant in both zones. Grazer abundance, a subset of total invertebrates, followed similar trends as total invertebrates (one-way ANOVA $F = 13.38$, $df = 1, 10$, $P < 0.05$), as did crustaceans (a further subset of the total, and mostly grazers) (one-way ANOVA $F = 14.42$, $df = 1,10$, $P < 0.05$, Figure A1). In contrast, gastropod grazers were equally abundant at the meadow edge and interior (one-way ANOVA $F = 0.001$, $df = 1, 10$, $P < 0.97$) (Figure A1).

3.4 Reciprocal transplant experiment

At the end of the reciprocal transplant experiment, shoots at the meadow edge (edge → edge, interior → edge) had high *Smithora* load regardless of source location (Figure 3). Shoots transplanted to the edge (interior → edge shoots) gained *Smithora* biomass comparable to the edge control (edge → edge) shoots (Figure 3; Table 6; two-way ANOVA with interaction term: $F = 12.33$, $df = 3, 11$, $P < 0.001$). Shoots transplanted from the edge to the interior zone (edge → interior) retained *Smithora*, while we observed no *Smithora* on interior shoots that stayed in the interior (interior → interior) (Figure 3B).

Over the course of the experiment, 9 shoots were broken or lost. *Smithora* abundance tended to be greater toward leaf tips, and not evenly distributed along the length of the *Zostera* leaf, thus we could not estimate total *Smithora* abundance for the broken shoots. This leaf loss

decreased biomass and microbial community sample size from the initial N = 24 shoots. Shoots that were transplanted experienced leaf loss at a greater rate than control (uprooted and relocated at the same site) shoots. Three of the initial six replicates for each of the transplant treatments (interior → edge and edge → interior) lost leaves (final sample sizes are n = 3 per transplant treatment), while the control shoots remained intact in 4 replicates in the interior, and 5 at the edge site. In total, 15 of the original 24 shoots were recovered, swabbed and measured. Of those 15 swabs, 12 yielded usable microbial data.

Microbial communities differed on shoots from the meadow edge and interior before the experiment (Figure 4A; one-way PERMANOVA, df = 1, 9; $F = 7.51$, $p < 0.01$). Additionally, microbial communities on *Zostera* shoots were significantly different than those on *Smithora* blade tissue (PERMANOVA, df = 1, 17; $F = 3.68$, $p < 0.001$). At the end of the experiment, the origin of the shoot no longer explained variation in the final shoot microbial assemblage. Instead, our analysis that combined our experimental data with the broader survey data showed that *Smithora* presence can also be a significant predictor of microbial composition on *Zostera* (Figure 4B; Table 7). We also found a significant effect of sampling time for microbial communities on ambient controls, suggesting natural temporal turnover in the *Zostera* associated microbiota (Supplementary Table). When considering only the samples taken at the end of the experiment, we noted many bacterial ASVs significantly associated with *Smithora* blade tissue were also found on *Zostera* shoots hosting *Smithora* epiphytes, independent of where the shoot originated; few *Smithora* indicator ASVs were found on *Zostera* controls from the bed interior (Figure 5).

4. Discussion

Understanding the causes and consequences of variation and community structure in host-epibiota systems is an essential and sometimes overlooked component of understanding the processes that maintain stable and healthy ecosystems (Barott and Rohwer, 2012; Thornber et al., 2016). We found that a conspicuous algal epiphyte (*Smithora*) on a seagrass foundation species (*Zostera*) varied substantially in abundance over relatively small spatial scales within a coastal seagrass meadow. Variation in *Smithora* abundance was most likely explained by local variation abiotic conditions and in turn appeared to drive variation in *Zostera* microbial epibiota. *Zostera* microbial epibiota have been shown to differ somewhat from the surrounding environment indicating some host selectivity (Adamczyk et al., n.d.; Bengtsson et al., 2017; Crump et al., 2018; Trevizan Segovia et al., Accepted). We found that fine scale environmental variation can have large effects on seagrass epiphyte biomass, and our results further suggest that this variation in epiphyte biomass could be affecting the *Zostera* leaf-associated microbiota. We could not assess whether the direct or indirect effects of *Smithora* on *Zostera* were positive or negative, and other studies have shown that both epiphyte biomass and that algae-induced shifts in host microbiota can have either positive, negative or neutral effects on seagrass hosts (Brodersen et al., 2015; Duarte, 1995; Harlin, 1975; Ruesink, 2016). The consequences of *Smithora* blooms for *Zostera* health in this region require further investigation.

Our finding that *Smithora* was present on *Zostera* shoots even when it was not observable to the naked eye was somewhat surprising, and we rejected dispersal limitation as a plausible explanation of variation in *Smithora* abundance. *Smithora* was most likely to be noticeable and abundant at meadow edge and at shallower sites later in the summer, forming billowing sheets of algae that covered the eelgrass blades (Figure 1A). *Smithora* load was low or absent from meadow-interior shoots in our surveys. Such patchy distribution has been observed for *Smithora*

before and is potentially consistent with possible dispersal limitation as a driver of variation (Willcocks, 1982). However, in contrast to the visible spatial and temporal patterns, we detected microscopic *Smithora* in molecular surveys of *Zostera* leaves much more broadly – in May, and at all meadow zones including interior and deeper zones. This is the first comparison of macroscopic and microscopic distribution of *Smithora* patterns, and suggest that *Smithora* may often occur in microscopic forms (e.g., spores) on *Zostera* in the meadow interior but does not grow into blades even in mid-summer when growth conditions are good. More quantitative methods (such as qPCR) are needed to confirm abundance of spores.

We conclude that macroscopic *Smithora* abundance likely reflects environmental conditions favorable to growth – possibly light or nutrient supply in the water column. Variation in epiphyte abundance on *Zostera* and other seagrasses over fine scales, within and among meadows, has been reported often in surveys and correlative studies have pointed to abiotic variation as a driver of the patterns (Johnson et al., 2005; Leliaert and Vanreusel, 2001), though we are aware of no experimental transplants to test this hypothesized explanation. Though little *Smithora* growth was observed in the meadow interior naturally, our reciprocal transplant experiment indicated that summertime conditions in the meadow interior were not inhospitable to macroscopic *Smithora* survival. Final (August) *Smithora* standing stock on shoots transplanted to the interior was similar to what we observed at the edge in July, implying no net gain or loss of *Smithora* on experimental shoots in the meadow interior over the experimental period. Additionally, when shoots free of visible *Smithora* were transplanted to the meadow edge, by the end of the experiment their *Smithora* load was indistinguishable between transplanted shoots and edge controls, and higher than observed *Smithora* load in July. We infer that *Smithora* spores were already present on interior shoots when they were transplanted to the edge, based on the rapid

growth of *Smithora* after transplant and our prior survey in which nearly every interior shoot we sampled had *Smithora* DNA. The close proximity, similar depth and landward orientation of our experimental sites controlled for major differences in abiotic conditions such as light supply to the top of the meadow or wave exposure from ocean waves. Light supply may differ on the edge because of the increased penetration of light and reflection off the sand onto shoots at the edge; perhaps also combined with moderately higher flow rates and exposure to seawater coming from outside the meadow, nutrient supply may have also been higher. It is also possible that fish grazing could have varied spatially between meadow edge and interior.

We rejected the hypotheses that variation in *Smithora* abundance is due to grazing that reduces *Smithora* biomass. We considered grazers as part of the environment (in our 'environmental hypothesis') rather than an attribute of the *Zostera* shoots themselves. Observed grazer abundance patterns between the two zones in our surveys are not consistent with grazer control of *Smithora* biomass. Grazers, in general, were much more abundant when *Smithora* was abundant. Epibiotic invertebrates in eelgrass meadows with *Smithora* have been observed to take on the color of *Smithora*, suggesting they consume it (O'Connor and Galloway personal observation, Olson and Prentice personal observation), and perhaps might be more abundant in areas of high *Smithora* growth. This indicates that *Smithora* may provide habitat and food to some invertebrate taxa. When grazers consume algae in sufficient quantities to limit algal biomass and minimize any competitive effects of epiphytes on hosts, grazing benefit seagrass by releasing it from competition with epiphytic for light and nutrients (the "mutualistic grazer hypothesis", Duffy et al., 2013; Reynolds et al., 2014). Our results lead us to reject this hypothesis in this case, but our conclusion remains provisional because a full experimental test (grazer manipulation) would be necessary to make a stronger inference. Reciprocal transplant

experiments have revealed the potential for invertebrate grazing to reduce epiphyte algal abundance for diatoms (Reynolds et al., 2014).

An alternate hypothesis for spatial variation in epiphyte abundance is that the host plant selects for epiphytes through inhibition (e.g., defensive compounds) or direct or indirect facilitation, and these host plant attributes vary spatially (Honkanen and Jormalainen, 2005). Though we did observe differences in shoot attributes among zones, our transplant experiment results lead us to reject the hypothesis that these shoot attributes explain spatial and temporal variation in *Smithora* abundance. The differences in shoot attributes we observed include their propensity to break, shoot density, and different epibiotic microbiota. The fact that shoots transplanted to a different site were more likely to break than shoots that were replanted at their source site suggests that there may have been subtle differences in biomechanical or morphological properties that allowed shoots to be more robust in their local hydrological regimes. Shoot density differed among meadow edge and interior zones but total biomass did not (Figure 2), suggesting shoots were smaller in the meadow interior in June but not July. The combined density and biomass results from June to July suggest that the shoots in the different zones might have been on different growth trajectories during the experiment, such that interior shoots were becoming larger and less dense than edge shoots. Still, these biological differences did not explain *Smithora* abundance patterns in our surveys.

We observed that shoots with and without *Smithora* hosted different microbial communities in the field (Figure 4, Table 7), such that the presence of *Smithora* was correlated with a distinct seagrass-associated microbial community. This result was unexpected, because *Z. marina* tissue free of macroscopic *Smithora* was sampled from all shoots. We suggest that differences in bacterial assemblages between edge and interior shoots reflect the presence of *Smithora* itself

rather than differences in shoot defenses. Macroalgae alters nearby microbial communities in the water column and in biofilms and this effect is attributed to seaweed exudates that either promote (e.g. polysaccharides) or deter (e.g. defense compounds) particular microbes (Chen and Parfrey, 2018; Lam et al., 2008). The distinct microbial communities on *Zostera* shoots harboring *Smithora* may reflect particular bacteria being attracted to sugars exuded by *Smithora* and others deterred by antimicrobials released from *Smithora*. Our indicator species analysis supports the conclusion that *Smithora* drives changes in the microbes because *Smithora*-associated microbes became abundant on interior → edge transplants which gained *Smithora* biomass (Figure 3). Though we cannot completely rule out microbial community shifts preceding *Smithora* development on transplanted shoots, we find this scenario more likely than microbial community variation driven strictly by variation in the abiotic conditions in the edge versus interior because our results contrast with a previous study reporting no difference in the leaf microbiome between the edge and interior in a meadow without spatial variation in macroalgal epiphytes (Ettinger et al., 2017). Similarly, Ugarelli and colleagues found no difference in the microbiome of *Thalassia testudinum* across small spatial scales (Ugarelli et al., 2018). Variation in the seagrass microbiome has been attributed to variation in environmental conditions at broad spatial scales (Fahimipour et al., 2017) and when environmental conditions strongly contrast (Mejia et al., 2016; Bengtsson et al, 2017), and we find that microbial communities on *Zostera* differ by sampling month (Table 7). We conclude that microbial community composition on *Zostera* is dynamic over time and likely influenced by the presence of epiphytic macroalgae.

We note several caveats to our inferences. We did not detect *Smithora* DNA in three water samples taken from above the meadow where we find a high prevalence of *Smithora* reads on *Zostera* shoots. This suggests that microscopic *Smithora* may not be circulating in high

abundance in the water column, though our three samples are far too few to constitute a systematic sampling effort. Alternatively, we may have detected free environmental DNA shed from macroscopic of *Smithora*. If this is the case, the lack of detection in the water column would point to a longer residence time of DNA in leaf biofilms. Furthermore, we cannot infer a true absence from a failure to detect *Smithora* by PCR in our sample; for example the presence of PCR inhibitors is one possible cause of PCR failure (Jin et al., 1997). Additional work targeting this question is required to make a more robust conclusion about the prevalence of *Smithora* in the water column on *Zostera* shoots and in microscopic forms.

Foundation species host biodiversity and interaction webs in all kinds of habitats, and for this reason are a central focus of conservation and management strategies. Foundation macrophytes provide habitat for epibiota and in turn support intricate interaction webs such as food webs (e.g., bromeliads) and competitive networks (Amundrud et al., 2015; Duffy, 2006; Heck Jr. et al., 2008; Heck Jr. and Valentine, 2006; Thornber et al., 2016). In rainforests, entire food webs are based in epiphytic bromeliads; similarly in nearshore marine systems, kelps and seagrasses host abundant algal, microbial and animal epibiota on and within their fronds and leaves. Though it is widely acknowledged that such interdependencies can be hallmarks of healthy, stable ecosystems, we still understand relatively little about how host plants and their epibiota interact and what role epiphytes play in the fuller ecosystem.

In conclusion, we found that variation in environmental conditions over just a few meters drives conspicuous change in seagrass epibiotic community. Through an experimental transplant, we are able to attribute this variation to abiotic conditions and we reject grazing and other biotic causes. We show for the first time that *Smithora* DNA is present throughout the meadow, but only grows to high biomass in some areas, mostly at meadow edges. In the presence of *Smithora*,

Zostera associated microbiota was distinct compared to shoots without *Smithora* across all meadow zones. This suggests a possible role for *Smithora*-associated microbiota in the *Zostera* – *Smithora* obligate symbiosis in which the epiphyte is known to take up nutrients and carbon from the host. Overall, our results provide a novel example of the interconnections between aquatic macrophytes and microbiota in interaction networks that can have ecosystem-scale consequences.

Acknowledgments

We thank the Hakai Institute Calvert marine station field teams and staff for their support. This work was supported by the Hakai Institute (Microbes to Macrophytes grant to LWP, MIO). Further support provided by NSERC Discovery Grants to LWP and MIO.

References

Adamczyk, E., O'Connor, M.I., Parfrey, L.W., n.d. Seagrass (Zostera marina) transplant experiment uncovers microbial selectivity and core microbiome.

Amundrud, S.L., Srivastava, D.S., O'Connor, M.I., 2015. Indirect effects of predators control herbivore richness and abundance in a benthic eelgrass (*Zostera marina*) mesograzer community. J Anim Ecol 84, 1092–1102. https://doi.org/10.1111/1365-2656.12350

Barott, K.L., Rohwer, F.L., 2012. Unseen players shape benthic competition on coral reefs. Trends in Microbiology 20, 621–628. https://doi.org/10.1016/j.tim.2012.08.004

Barton, K., 2019. MuMIn: Multi-Model Inference.

Bengtsson, M.M., Bühler, A., Brauer, A., Dahlke, S., Schubert, H., Blindow, I., 2017. Eelgrass Leaf Surface Microbiomes Are Locally Variable and Highly Correlated with Epibiotic Eukaryotes. Front. Microbiol. 8, 1312. https://doi.org/10.3389/fmicb.2017.01312

Boström, C., Jackson, E.L., Simenstad, C.A., 2006a. Seagrass landscapes and their effects on associated fauna: A review. Estuarine, Coastal and Shelf Science 68, 383–403. https://doi.org/10.1016/j.ecss.2006.01.026

Boström, C., O'Brien, K., Roos, C., Ekebom, J., 2006b. Environmental variables explaining structural and functional diversity of seagrass macrofauna in an archipelago landscape. Journal of Experimental Marine Biology and Ecology 335, 52–73. https://doi.org/10.1016/j.jembe.2006.02.015

Brodersen, K.E., Lichtenberg, M., Paz, L.-C., Kühl, M., 2015. Epiphyte-cover on seagrass (Zostera marina L.) leaves impedes plant performance and radial O2 loss from the below-ground tissue. Front. Mar. Sci. 2. https://doi.org/10.3389/fmars.2015.00058

Caporaso, J. G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010.

PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267.

Caporaso, J G, Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303

Chen, M.Y., Parfrey, L.W., 2018. Incubation with macroalgae induces large shifts in water column microbiota, but minor changes to the epibiota of co-occurring macroalgae. Mol Ecol 27, 1966–1979. https://doi.org/10.1111/mec.14548

Clarke, K.R., Gorley, R.N., 2006. PRIMER V6: User Manual/Tutorial. PRIMER-E, Plymouth.

Comeau, A.M., Douglas, G.M., Langille, M.G.I., 2017. Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. mSystems 2, 1–11. https://doi.org/10.1128/msystems.00127-16

Comeau, A.M., Li, W.K.W., Tremblay, J.É., Carmack, E.C., Lovejoy, C., 2011. Arctic ocean microbial community structure before and after the 2007 record sea ice minimum. PLoS ONE 6. https://doi.org/10.1371/journal.pone.0027492

Crump, B.C., Wojahn, J.M., Tomas, F., Mueller, R.S., 2018. Metatranscriptomics and Amplicon Sequencing Reveal Mutualisms in Seagrass Microbiomes. Front. Microbiol. 9, 388. https://doi.org/10.3389/fmicb.2018.00388

De Caceres, M., Jansen, F., Dell, N., 2020. Package “indicspecies.”

Department of Fisheries and Oceans (DFO) Canada, 2009. Does eelgrass (Zostera marina) meet

the criteria as an ecologically significant species? Canadian Science Advisory Secretariat Science Advisory Report (No. 2009/018). Department of Fisheries and Oceans, Ottawa, Ontario.

DeSantis, T.Z., Hugenholtz, P., Larson, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied Environmental Microbiology 72, 5069–5072.

Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41, 87–112.

Duffy, J., 2006. Biodiversity and the functioning of seagrass ecosystems. Mar. Ecol. Prog. Ser. 311, 233–250. https://doi.org/10.3354/meps311233

Duffy, J.E., Hughes, A.R., Moksnes, P.-O., 2013. Ecology of Seagrass Communities, in: Bertness, M.D., Bruno, J.F., Silliman, B.D., Stachowicz, J.J. (Eds.), Marine Community Ecology and Conservation. Sinauer Associates Incorporated, pp. 271–297.

Duffy, J.E., Reynolds, P.L., Boström, C., Coyer, J.A., Cusson, M., Donadi, S., Douglass, J.G., Eklöf, J.S., Engelen, A.H., Eriksson, B.K., Fredriksen, S., Gamfeldt, L., Gustafsson, C., Hoarau, G., Hori, M., Hovel, K., Iken, K., Lefcheck, J.S., Moksnes, P.-O., Nakaoka, M., O'Connor, M.I., Olsen, J.L., Richardson, J.P., Ruesink, J.L., Sotka, E.E., Thormar, J., Whalen, M.A., Stachowicz, J.J., 2015. Biodiversity mediates top-down control in eelgrass ecosystems: a global comparative-experimental approach. Ecology Letters 18, 696–705. https://doi.org/10.1111/ele.12448

Edgar, Robert C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Eren, A.M., Maignien, L., Sul, W.J., Murphy, L.G., Grim, S.L., Morrison, H.G., Sogin, M.L., 2013. Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene data. Methods in Ecology and Evolution 4, 1111–1119. https://doi.org/10.1111/2041-210X.12114

Eren, A.M., Morrison, H.G., Lescault, P.J., Reveillaud, J., Vineis, J.H., Sogin, M.L., 2015. Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME Journal 9, 968–979. https://doi.org/10.1038/ismej.2014.195

Ettinger, C.L., Voerman, S.E., Lang, J.M., Stachowicz, J.J., Eisen, J.A., 2017. Microbial communities in sediment from *Zostera marina* patches, but not the *Z. marina* leaf or root microbiomes, vary in relation to distance from patch edge. PeerJ 5, e3246. https://doi.org/10.7717/peerj.3246

Fry, B., 1984. 13C/12C ratios and the trophic importance of algae in Florida Syringodium filiforme seagrass meadows. Marine Biology 79, 11–19.

Galloway, A.W.E., Britton-Simmons, K.H., Duggins, D.O., Gabrielson, P.W., Brett, M.T., 2012. Fatty Acid Signatures Differentiate Marine Macrophytes at Ordinal and Family Ranks. Journal of Phycology 48, 956–965. https://doi.org/10.1111/j.1529-8817.2012.01173.x

Harlin, M.M., 1975. Epiphyte—host relations in seagrass communities. Aquatic Botany 1, 125–131.

Harlin, M.M., 1973a. Transfer of products between epiphytic marine algae and host plants. Journal of Phycology 9, 243–248.

Harlin, M.M., 1973b. Obligate algal epiphyte S. naiadum naiadum grows on a synthetic substrate. Journal of Phycology 9, 230–232.

Hauxwell, J., McClelland, J., Behr, P.J., Valiela, I., 1998. Relative Importance of Grazing and Nutrient Controls of Macroalgal Biomass in Three Temperate Shallow Estuaries. Estuaries 21, 347. https://doi.org/10.2307/1352481

Hawkes, M.W., 1988. Evidence of sexual reproduction in S. naiadum naiadum (Erythropeltidales, Rhodophyta) and its evolutionary significance. British Phycological Journal 23, 327–336.

Heck Jr., K.L., Carruthers, T.J.B., Duarte, C.M., Hughes, A.R., Kendrick, G., Orth, R.J., Williams, S.W., 2008. Trophic Transfers from Seagrass Meadows Subsidize Diverse Marine and Terrestrial Consumers. Ecosystems 11, 1198–1210. https://doi.org/10.1007/s10021-008-9155-y

Heck Jr., K.L., Valentine, J.F., 2006. Plant–herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology and Ecology 330, 420–436.

Heck, K.L., Pennock, J.R., Valentine, J.F., Coen, L.D., Sklenar, S.A., 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: An experimental assessment. Limnol. Oceanogr. 45, 1041–1057. https://doi.org/10.4319/lo.2000.45.5.1041

Hollenberg, G.J., 1958. Smithora, an interesting new algal genus in the Erythropeltidaceae. Pacific Naturalist 1, 3–12.

Honkanen, T., Jormalainen, V., 2005. Genotypic variation in tolerance and resistance to fouling in the brown alga Fucus vesiculosus. Oecologia 144, 196–205.

Horn, M.H., Murray, S.N., Edwards, T.W., 1982. Dietary selectivity in the field and food preferences in the laboratory for two herbivorous fishes (Cebidichthys violaceus and

Xiphister mucosus) from a temperate intertidal zone. Marine Biology 67, 237–246. https://doi.org/10.1007/BF00397664

Huang, A.C., Essak, M., O'Connor, M.I., 2015. Top-down control by great blue herons *Ardea herodias* regulates seagrass-associated epifauna. Oikos 124, 1492–1501. https://doi.org/10.1111/oik.01988

James, A.K., English, C.J., Nidzieko, N.J., Carlson, C.A., Wilbanks, E.G., 2020. Giant kelp microbiome altered in the presence of epiphytes. Limnol Oceanogr 5, 354–362. https://doi.org/10.1002/lol2.10157

Jin, H., Kim, J., Sohn, C., DeWreede, R., Choi, T., Towers, G., Hudson, J., Hong, Y., 1997. Inhibition of Taq DNA polymerase by seaweed extracts from British Columbia, Canada and Korea. Journal of Applied Phycology 9, 383–388.

Johnson, M.P., Edwards, M., Bunker, F., Maggs, C.A., 2005. Algal epiphytes of Zostera marina: Variation in assemblage structure from individual leaves to regional scale. Aquatic Botany 82, 12–26. https://doi.org/10.1016/j.aquabot.2005.02.003

Lam, C., Stang, A., Harder, T., 2008. Planktonic bacteria and fungi are selectively eliminated by exposure to marine macroalgae in close proximity: Antibacterial and antifungal waterborne macroalgal metabolites. FEMS Microbiology Ecology 63, 283–291. https://doi.org/10.1111/j.1574-6941.2007.00426.x

Leliaert, F., Vanreusel, W., 2001. Epiphytes on the seagrasses of Zanzibar Island (Tanzania), floristic and ecological aspects. BELGIAN JOURNAL OF BOTANY 18.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D., Dangl, J.L., 2013. Practical innovations for high-throughput amplicon sequencing. Nature Methods 10, 999–1002. https://doi.org/10.1038/nmeth.2634

McGlathery, K., 2001. Macroalgal blooms contribute to the decline in seagrasses in nutrient-enriched coastal waters. Journal of Phycology 37, 453–456.

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible INteractive Analysis and Grapics of Microbiome Census Data. PLoS ONE 8, E61217.

Nagel, E.J., Murphy, G., Fast, J., Bittick, S.J., Adamczyk, E., O'Connor, M.I., Wong, M.C., Lotze, H.K., 2020. Application of a coastal human impact metric and nitrogen loading model to 10 eelgarss (Zostera marina) meadows in British Columbia, Canadian Technical Report of Fisheries and Aquatic Sciences ####. Fisheries and Oceans Canada, Bedford Institute of Oceanography.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. The Vegan Package.

Olson, A.M., Hessing-Lewis, M., Haggarty, D., Juanes, F., 2019. Nearshore seascape connectivity enhances seagrass meadow nursery function. Ecol Appl 29. https://doi.org/10.1002/eap.1897

Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS ONE 5, e9490.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41, 590–596. https://doi.org/10.1093/nar/gks1219

Reynolds, P.L., Richardson, J.P., Duffy, J.E., 2014. Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. Limnol. Oceanogr. 59,

1053–1064. https://doi.org/10.4319/lo.2014.59.3.1053

Ruesink, J.L., 2016. Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk. Journal of Experimental Marine Biology and Ecology 481, 1–8. https://doi.org/10.1016/j.jembe.2016.03.022

Sanders-Smith, R., Trevizan Segovia, B., Forbes, C., Hessing-Lewis, M., Morien, E., Lemay, M., O'Connor, M.I., Wegener Parfrey, L., in review. Host-specificity and core taxa of seagrass leaf microbiome identified across tissue age and geographical regions. Frontiers in Ecology and Evolution.

Stark, K.A., Thompson, P.L., Yakimishyn, J., Lee, L., Adamczyk, E., Hessing-Lewis, M., O'Connor, M.I., In Press. Beyond a single patch: local and regional processes explain diversity patterns in a seagrass epifaunal community. Marine Ecology Progress Series.

Thornber, C., Jones, E., Thomsen, M., 2016. Epibiont-Marine Macrophyte Assemblages, in: Ólafsson, E. (Ed.), Marine Macrophytes as Foundation Species. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742, pp. 43–65. https://doi.org/10.4324/9781315370781-4

Trevizan Segovia, B., Sanders, R.D., Adamczyk, E., Forbes, C., Hessing-Lewis, M., O'Connor, M.I., Parfrey, L.W., Accepted. Microeukaryotic Communities Associated With the Seagrass Zostera marina Are Spatially Structured. Journal of Environmental Microbiology.

Ugarelli, K., Chakrabarti, S, Laas, P., Stingl, U., 2017. The seagrass holobiont and its microbiome. Microorganisms 5, 81.

Ugarelli, K., Laas, P., Stingl, U., 2018. The Microbial Communities of Leaves and Roots Associated with Turtle Grass (Thalassia testudinum) and Manatee Grass (Syringodium

filliforme) are Distinct from Seawater and Sediment Communities, but Are Similar between Species and Sampling Sites. Microorganisms 7, 4. https://doi.org/10.3390/microorganisms7010004

Whippo, R., Knight, N.S., Prentice, C., Cristiani, J., Siegle, M.R., O'Connor, M.I., 2018. Epifaunal diversity patterns within and among seagrass meadows suggest metacommunity structure 9, 1–34.

Willcocks, P.A., 1982. Colonization and distribution of the red algal epiphytes Melobesia mediocris and Smithora naiadum on the seagrass Phylospadix torreyi. Aquatic Botany 12, 365–373.

Tables and Figures

Table 1. Abiotic attributes of the Choked Pass eelgrass meadow in 2015. Sites are shown in Figure 1B.

Site	Zone	Month	Depth (m)	Salinity (PSU)	Temperature (C)	[Chlorophyll a] (ug/L)
Wolf (WF)	Edge	5	0	28.3	9.8	-
Wolf (WF)	Edge	6	0	26.95	10.2	-
Wolf (WF)	Edge	6	5	-	-	-
Wolf (WF)	Edge	7	0	31.40	13.3	-
Wolf (WF)	Edge	7	5	31.70	12.6	-
Sandspit (IS / OS)	Interior	5	0	27.20	10.25	1.79
Sandspit (IS / OS)	Interior	5	5	28.30	10.13	1.66
Sandspit (IS / OS)	Interior	6	0	27.27	12.6	2.64
Sandspit (IS / OS)	Interior	6	5	27.30	12.5	1.34
Sandspit (IS / OS)	Interior	7	0	30.70	13.02	4.54
Sandspit (IS / OS)	Interior	7	5	31.10	11.22	1.60
Sandspit (IS / OS)	Interior	8	0	30.41	14.96	2.68
Sandspit (IS / OS)	Interior	8	5	30.98	13.58	3.25
Interior (IT)	Interior	6	0	-	-	-
Lower (SP)	Interior	6	0	-	-	-

915

Table 2. Results of statistical analysis on *Smithora* load on *Zostera* shoots in the summer of 2015. We used a negative binomial mixed effects model to analyze the frequency of zero vs non-zero values across zones (Zn: edge sites vs interior sites), depths and observation times (Tm: May, July, August). Coefficients given here are not inverted or exponentiated.

A. Models for *Smithora* presence vs absence											
Md	**Int**	**Zn**	**Tm**	**Zn*Tm**	**D**	**Tm*D**	**df**	**logLike**	**AIC_c**	**Delta**	**w**
A	-0.68	+	1.78	+			5	-35.99	82.5	0.00	0.70
B	-2.44	+	3.04				4	-38.02	84.4	1.86	0.28
D	-4.43		3.06				3	-41.54	89.3	6.77	0.02
E	3.51	+			-0.55		4	-61.18	130.7	48.20	0.00
C	1.94	+					3	-63.05	132.2	49.79	0.00
F	3.93				-.91		3	-63.68	133.6	51.05	0.00
B. Models for *Smithora* abundance, when present											
Md	**Int**	**Zn**	**Tm**	**Zn*Tm**	**D**	**Tm*D**	**df**	**logLike**	**AIC_c**	**Delta**	**w**
E	0.59	+			-0.53		5	12.00	-13.1	0.00	0.68
F	0.23	+	0.16	+	-0.53		7	12.40	-9.2	3.99	0.09
G	-0.02	+	0.27		-0.42	-0.05	7	12.40	-9.1	4.01	0.09
H	0.04		0.32		-0.52	-0.07	6	10.94	-9.7	4.49	0.07
C	-0.77	+					4	8.03	-7.5	5.64	0.04
B	-1.17	+	0.18				5	8.61	-6.4	6.79	0.02
A	-1.24	+	0.21	+			6	8.70	-4.2	8.97	0.01
D	-1.86		0.18				4	5.51	-2.5	10.68	0.00

920

Table 3. Prevalence *Smithora naiadum* on *Zostera marina* shoots in the summer of 2015 based on 18S amplicon sequencing. Prevalence is based on presence (> 5 reads per sample) in shoot or seawater samples taken from sites in Choked Pass meadow in May, June, July, August. Site names as in Figure 1B.

Sample type	Site	Month	Samples (n)	*S. naiadum* present (n)	Prevalence
Z. marina	IT	May	8	8	100
Z. marina	between OS and IS	May	5	5	100
Z. marina	between OS and IS	July	5	3	60
Z. marina	SP	June	4	4	100
Z. marina	SP	July	6	5	83.3
Z. marina	SP	August	3	3	100
Z. marina	WF	July	6	6	100
seawater	SP	June	3	0	0

930 **Table 4. Model comparison for *Zostera* density, biomass, *Smithora* load and invertebrate abundance observed in survey at experimental sites**.

***Zostera* Density (N/m^2)**									
Model	**Int**	**Zone**	**Time**	**Zone*Time**	**Df**	**logLike**	**AIC$_c$**	**Delta**	**w**
D	5.57	+	1.63	+	5	-43.11	100.0	0.00	0.42
B	8.09	+			3	-46.44	100.2	0.26	0.37
A	7.09				2	-48.90	102.4	2.48	0.12
C	8.68	+	-0.38		4	-46.34	103.0	3.08	0.09
***Zostera* Dry weight (g)**									
Model	**Int**	**Zone**	**Time**	**Zone*Time**	**Df**	**logLike**	**AIC$_c$**	**Delta**	**w**
C	1.05	+	8.24		4	-59.04	128.4	0.00	0.80
D	-0.72	+	9.39	+	5	-58.75	131.3	2.82	0.19
B	13.79	+			3	-68.41	144.1	15.71	0.00
A	11.15				2	-70.75	146.1	17.69	0.00
***Smithora* load (*Smithora* g / *Zostera* g)**									
Model	**Int**	**Zone**	**Time**	**Zone*Time**	**Df**	**logLike**	**AIC$_c$**	**Delta**	**w**
B	0.32	+			3	11.90	-16.5	0.00	0.44
C	0.47	+	-0.10		4	13.41	-16.5	0.00	0.44
D	0.45	+	-0.09	+	5	13.43	-13.1	3.36	0.08
A	0.22				2	8.07	-11.5	4.96	0.04
Invertebrate grazer load (N/ *Zostera* g)									
Model	**Int**	**Zone**	**Time**	**Zone*Time**	**Df**	**logLike**	**AIC$_c$**	**Delta**	**w**
A	90.13	+	NA	NA	3	-58.59	126.2	0.00	0.93

B	56.70		NA	NA	2	-63.03	131.4	5.21	0.07

Table 5. Model comparison for *Smithora* load in survey at interior and edge zones near experimental site. Model terms include zone (Zn, levels: interior, edge), Sampling time (Tm, levels: June, July), *Zostera* density (Z_D) and *Zostera* dry weight (Z_W).

Md	Int	Zn	Tm	Zn*Tm	Z_D	Z_W	df	logLik	AIC_c	Delta	W
I	0.49	+	0.02			-0.01	5	15.21	-16.7	0.00	0.280
B	0.32	+					3	11.91	-16.5	0.20	0.254
C	0.47	+	-0.10				4	13.41	-16.5	0.20	0.254
H	0.36	+	0.06		0.03	-0.02	6	15.75	-13.9	2.77	0.070
D	0.45	+	-0.09	+			5	13.43	-13.1	3.55	0.047
J	0.47	+	-0.10				5	13.41	-13.1	3.59	0.046
A	0.23						2	8.07	-11.5	5.16	0.021
N	0.08						3	8.88	-10.4	6.24	0.012
F	0.36	+	0.06	+		-0.02	7	15.75	-9.5	7.17	0.008
O	0.27				0.01	-0.00	3	8.31	-9.3	7.38	0.007

Table 6. Results of two-way ANOVA on reciprocal transplant experiment.

940

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
***Smithora* load**				
Intercept	0.42725	0.04958	8.618	3.2e-06 ***
Type: Transplanted	-0.07183	0.08096	-0.887	0.393924
Source: Interior	-0.42725	0.07436	-5.745	0.000129 ***
Type * Source	0.25310	0.11714	2.161	0.053643

Table 7. Permutational multivariate analysis of variance (PERMANOVA) of 16S bacterial community dissimilarity (Bray-Curtis) before and after reciprocal transplant experiment (July and August 2015). Host species is *Zostera* shoot compared to *Smithora* blade communities. Site is meadow edge versus interior. Tests performed using 999 permutations.

PERMANOVA	**n**	**Degrees of freedom**	**pseudo-F value**	**R^2**	**p-value**
Host species	27	1	3.735	0.130	**0.001***
Month/Site = Month Site	27	1 2	6.126 4.325	0.162 0.229	**0.001*** **0.001***
Site before experiment	11	1	7.510	0.455	**0.005 ***
Transplant origin site	16	1	0.836	0.0564	0.607
Transplant destination site	16	1	1.865	0.118	**0.039 ***
Smithora presence after transplant	16	1	2.025	0.126	**0.026 ***

FIGURE 1. A) *Smithora naiadum* (red) growing on *Zostera marina* (green). Observed *Smithora* load (g *Smithora* / g *Zostera* dry weight) when *Smihtora* was present at B) meadow edge sites and C) interior sites (Table 3). Study site at Calvert Island, British Columbia, Canada. B) Choked pass, site of experimental transplants and surveys of *Smithora naiadum* and *Zostera marina.* Eight sites in the meadow were surveyed using 40 m transects (lines) at edge (OS, IS, AL, WF) vs interior (ID, IT, IS, IA) sites, and at some of these sites plus site SP, microbial assemblages were sampled (*). The experiment was conducted near WF (indicted by the black box).

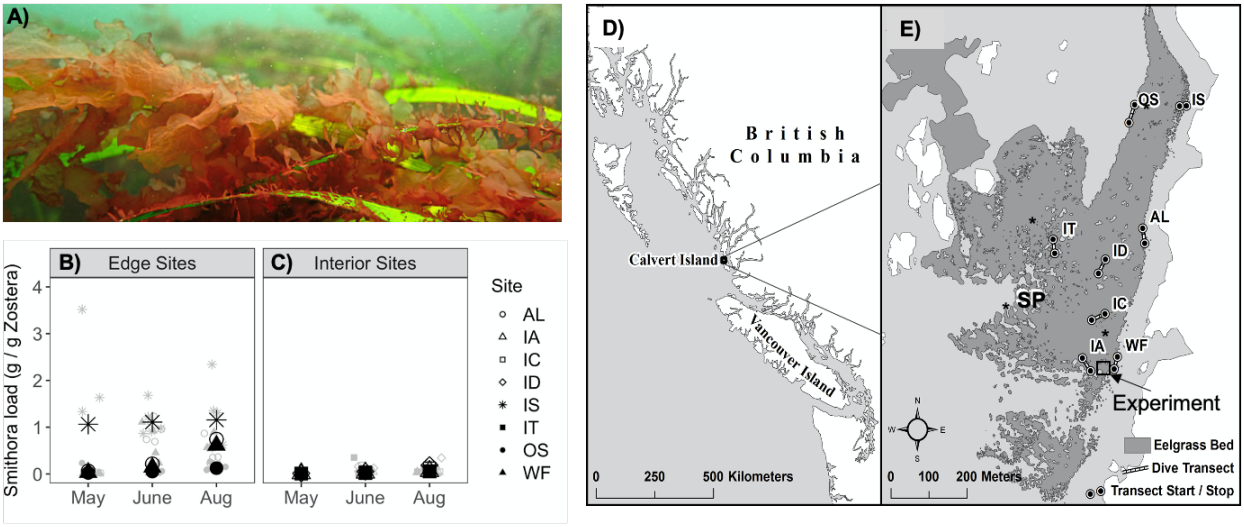


Figure 2 *Zostera marina*, *Smithora naiadum* and eipfuanal invertebrate abundance near the experimental sites prior to the start of the experiment. A) *Zostera* shoot density (N/m^2) varied among zones in July but not June (Two-way ANOVA: $F = 4.163$, $df = 3, 18$, $P < 0.05$), the only significant model term was the interaction between sample time and location ($P < 0.05$), B) *Zostera* dry weight (g) varied significantly among zones and sampling dates (Two-way ANOVA: $F = 18.04$, $df = 2, 19$, $P < 0.001$), C) *Smithora* load (g/ *Zostera* g) dry weight varied significantly between meadow zones (One-way ANOVA: $F = 8.33$, $df = 1, 20$, $P < 0.01$), D) epibiotic invertebrate load (g / *Zostera* g) in July differed significantly between zones (one-way ANOVA: $F = 10.96$, $df = 1,10$, $P < 0.01$), and E) epibiotic invertebrate composition differed among edge and interior zones (PERMANOVA: F.model = 6.37, $df = 1,10$, $P < 0.01$). See Table 4 for model comparisons.

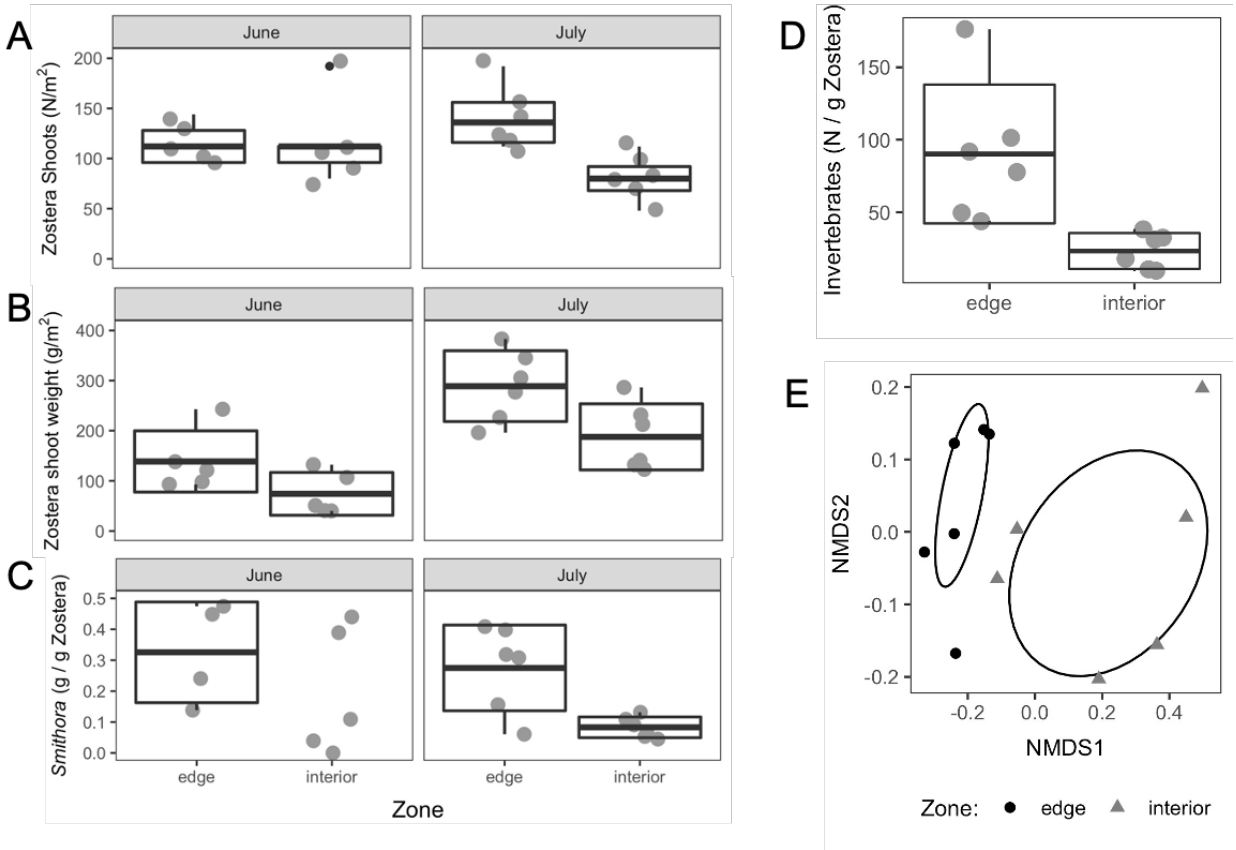


Figure 3. Final *Smithora naiadum* abundance (g / g *Zostera* dry wt) for experimental and control shoots in the reciprocal transplant experiment. Horizontal lines in boxplots indicate the mean and standard deviations.

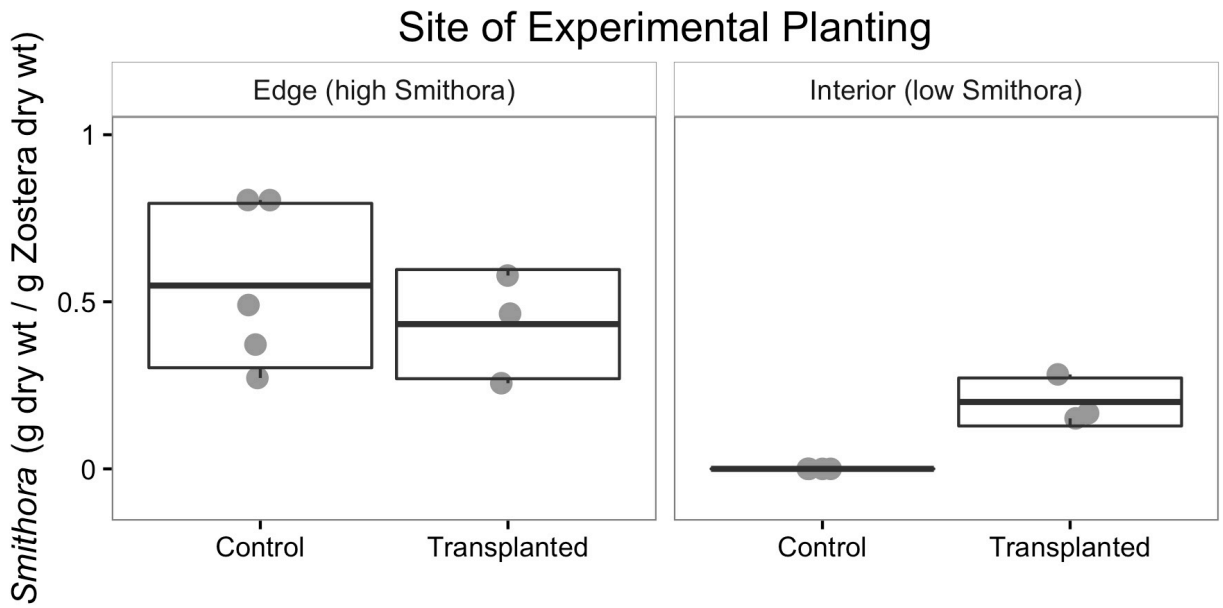


Figure 4. **Location within the bed and presence of *S. naiadum* influence the *Z. marina* microbiome.** Non-parametric multidimensional scaling (NMDS) of bacterial beta diversity (Bray-Curtis) based on relative abundance of OTUs on *Zostera* shoots before and after reciprocal transplant experiment. Each point represents the community on one shoot. **A)** Comparison of microbial communities on shoots from interior site with *S. naiadum* absent (black) to shoots from edge site with *S. naiadum* present (grey) and control shoots in August 2015, one month after transplant. Ambient controls were unmanipulated; interior-interior and edge-edge refer to controls transplanted back to the site of origin; edge-interior and interior-edge are experimental transplants. *S. naiadum* persisted on edge-interior transplants and had established on interior-edge transplants by the end of the experiment.

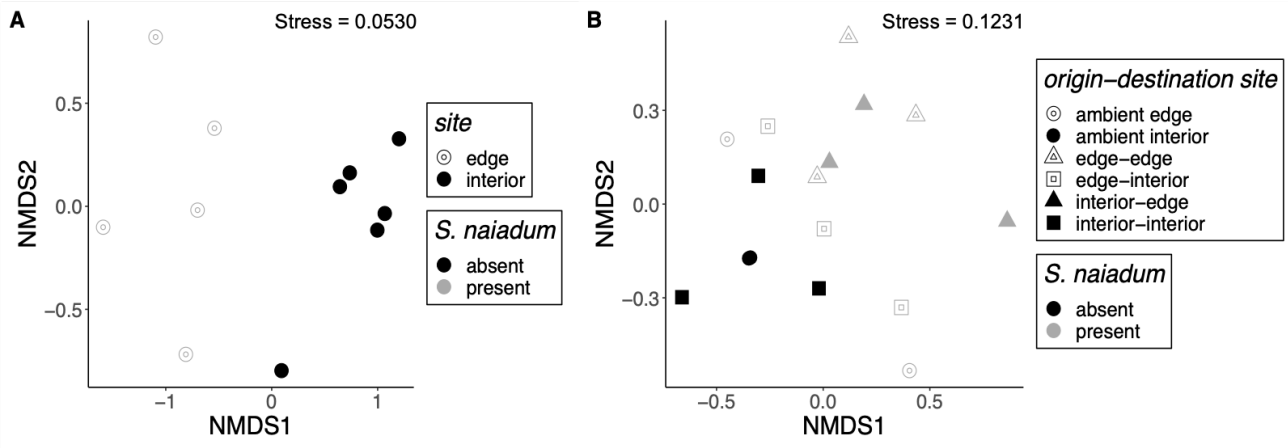
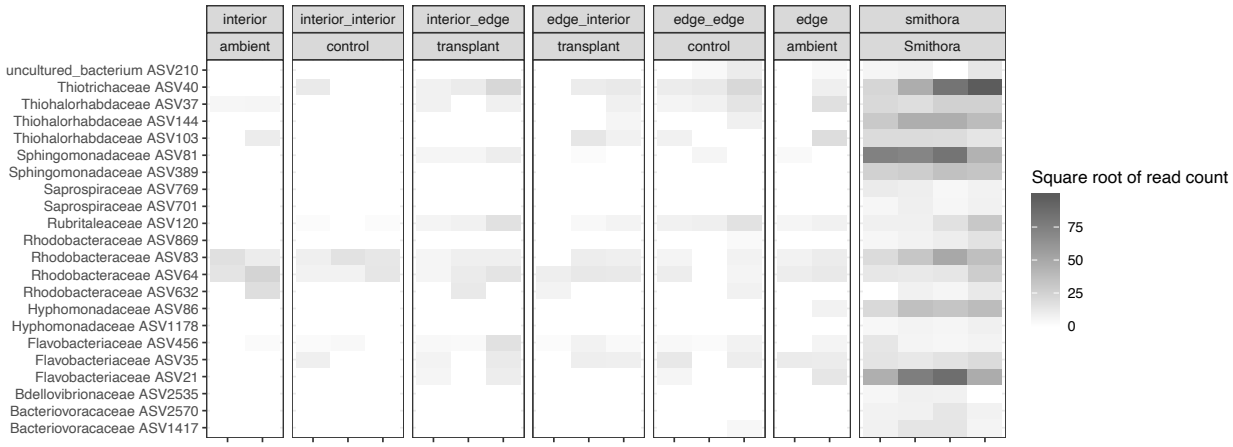


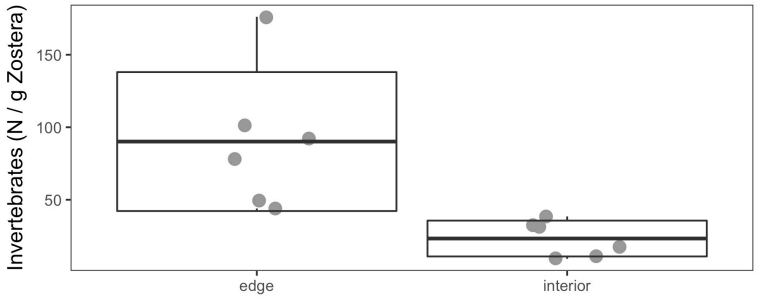
Figure 5. **Bacterial ASVs significantly associated with *Smithora* blade tissue are more abundant on *Zostera* shoots in the meadow edge compared to interior.** The heatmap represents the square root of read counts (to account for fold-change abundance differences) of the most significant indicator amplicon sequence variants (ASVs) in *Zostera* samples taken at the end of the transplant experiment and *Smithora* blade tissue samples. Each column is a *Zostera* shoot sample. Significant indicator taxa were selected as > 0.8 indval statistic for *Smithora* compared to *Zostera* communities.



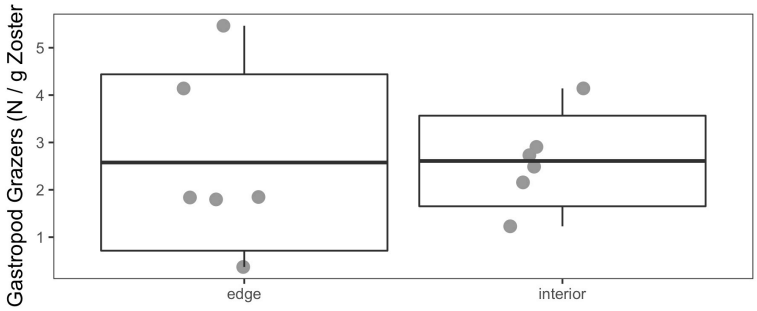
APPENDIX:

Figure A1. Abundance of epifaunal invertebrates on *Zostera marina* from survey before and during the reciprocal transplant experiment.

A



B



C

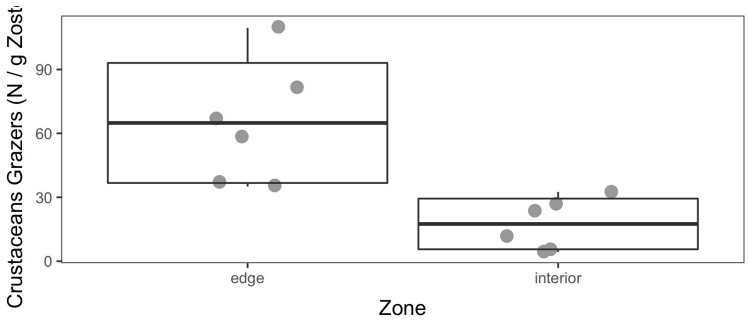


Figure A2. A) *Smithora* load estimated relative to *Zostera* surface area. Surface area is estimated as leaf area index (LAI, cm^2), calculated as 2*(shoot max width*shoot max length)*number of leaves. B) Correlation between *Zostera* surface area and biomass. The correlation between biomass and surface area was estimated with linear regression (F-ratio: 256.3, df = 1, 77, P < 0.001).

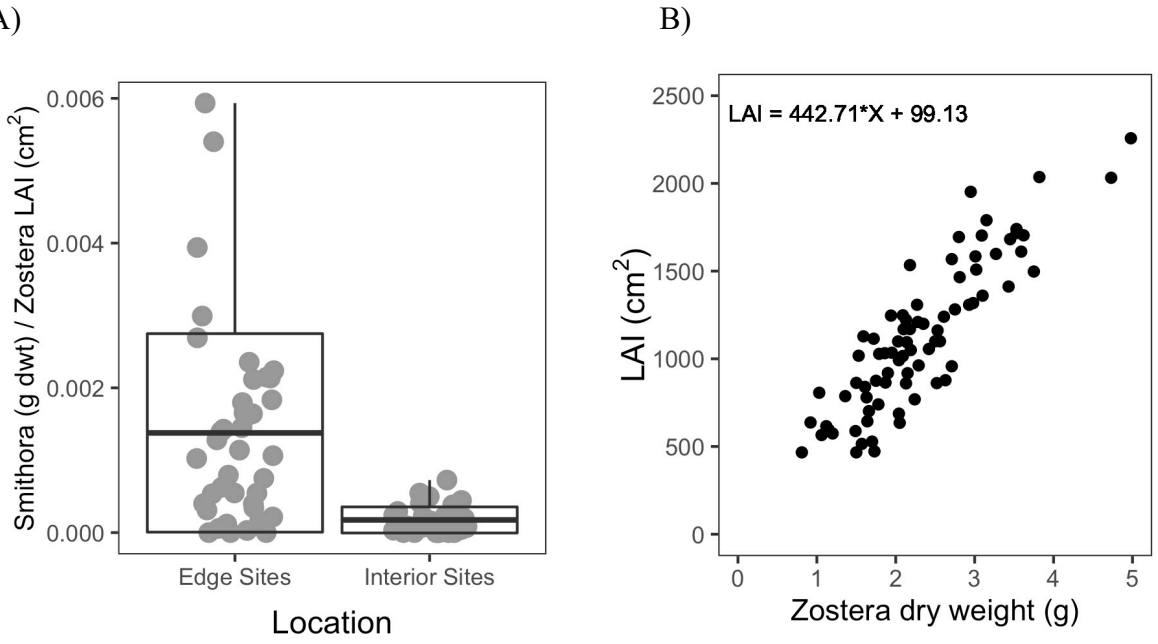
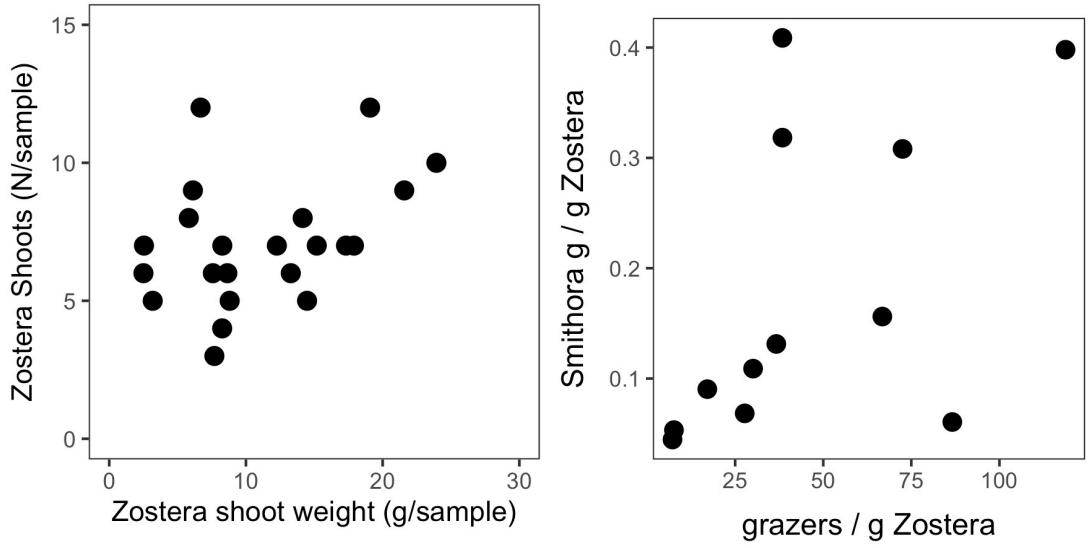


Figure A3. In the pre-experiment survey, A) *Zostera* density and dry weight were not correlated (regression: F = 3.42, df = 1, 20, P > 0.05), and neither are B) *Smithora* load and grazer load (regression: F = 3.88, df = 1, 10, P > 0.05).



1035

A reciprocal transplant experiment sheds new light on a classic marine seagrass-algal symbiosis and suggests influence of epiphytic symbiont on seagrass microbiota

Mary I. O'Connor, Gwendolyn Griffiths, Rhea Sanders-Smith, Margot Hessing-Lewis, Katharine M. Davis, Coreen Forbes, Angeleen M. Olson, Carolyn Prentice, Laura Wegener Parfrey

Author Statement:

Mary I. O'Connor: Validation, Formal Analysis, Resources, Writing – Review and Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Gwendolyn Griffiths**: Conceptualization, Methodology, Formal Analysis, Investigation, Writing – Original Draft, Visualization. **Rhea Sanders-Smith**: Conceptualization, Methodology, Data curation, Investigation, Writing – Review and Editing, Project Administration**; Margot Hessing-Lewis:** Conceptualization, Investigation, Methodology, Writing – Review and Editing, Resources, Project Administration, Funding Acquisition; **Katharine M. Davis:** Data curation, Methodology, Formal Analysis, Writing – Review and Editing, Visualization. **Coreen Forbes:** Methodology, Data curation, Writing – Review and Editing. **Angeleen M. Olson:** Methodology, Investigation, Data curation, Writing – Review and Editing. **Carolyn Prentice:** Methodology, Investigation, Data curation, Writing – Review and Editing. **Laura Wegener Parfrey:** Validation, Resources, Writing – Review and Editing, Supervision, Project Administration, Funding Acquisition.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: