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THE MICROBIOME OF THE HABITAT-FORMING BROWN ALGA *FUCUS VESICULOSUS* (PHAEOPHYCEAE) HAS SIMILAR CROSS-ATLANTIC STRUCTURE AND DRIVERS[1]

RUNNING TITLE: *Fucus* bacteria

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ABSTRACT

Latitudinal diversity gradients have provided many insights into species differentiation and community processes. In the well-studied intertidal zone, however, little is known about latitudinal diversity in microbiomes associated with habitat-forming hosts. We investigated microbiomes of *Fucus vesiculosus* because of deep understanding of this model system and its latitudinally large, cross-Atlantic range. Given multiple effects of photoperiod, we predicted that cross-Atlantic microbiomes of the *Fucus* microbiome would be similar at similar latitudes and correlate with environmental factors. We found that community structure and individual amplicon sequencing variants (ASVs) showed distinctive latitudinal distributions, but alpha diversity did not. Latitudinal differentiation was mostly driven by ASVs that were more abundant in cold temperate to subarctic (e.g., *Granulosicoccus*_t3260, *BurkholderiaCaballeroniaParaburkholderia*_t8371) or warm temperate (*Pleurocapsa*_t10392) latitudes. Their latitudinal distributions correlated with different humidity, tidal heights, and air/sea temperatures, but rarely with irradiance or photoperiod. Many ASVs in potentially symbiotic genera displayed novel phylogenetic biodiversity with differential distributions among tissues and regions, including closely related ASVs with differing north-south distributions that correlate with *Fucus* phylogeography. An apparent southern range contraction of *F. vesiculosus* in the NW Atlantic on the North Carolina coast mimics that recently observed in the NE Atlantic. We suggest cross-Atlantic microbial structure of *F. vesiculosus* is related to a combination of past (glacial-cycle) and contemporary environmental drivers.

Keywords: Atlantic phylogeography, fucoid algae, *Granulosicoccus,* macroalgal holobiont, parallel microbiome evolution, *Pleurocapsa*, *Sulfitobacter*

Abbreviations: ASV, amplicon sequence variant; PNA, peptide nucleic acid;

INTRODUCTION

Latitudinal biodiversity gradients provide a robust framework to consider past, present, and future distributions of organisms because they reflect past and present environmental drivers, thereby allowing examination of historical, ecological, evolutionary, and genetic bases of gradients in richness of biodiversity components (Hillebrand 2004, Fuhrman et al. 2008, Amend et al. 2013, Sul et al. 2013, Kinlock et al. 2018, Ibarbalz et al. 2019, Lawrence et al. 2020). In this context, shorelines across latitudes provide gradients of environmental factors (e.g., photoperiod, irradiance, air/sea temperature) that have attracted biologists to the intertidal zone and its biota since the late 1800s (Hillebrand 2004, Bertness et al. 2014, Hurd et al. 2014). Physiological and ecological models developed from such studies contributed greatly to general ecological theory and to understanding of the effects of abiotic and biotic factors on the diversity of marine algae and invertebrates (Hillebrand 2004, Bertness et al. 2014, Hurd et al. 2014). Only recently have comparable studies of intertidal microbiomes begun to appear. There is a particular need to study microbiomes of major habitat-formers due to climate change over their current ranges that might affect the health of the holobiont. Here we investigate microbiomes of the major habitat-forming brown alga *Fucus vesiculosus* over its broad range on both sides of the North Atlantic. The well-known biology of this model organism enables our analysis using the power of cross-Atlantic replication to study latitudinal effects on microbial communities.

Many brown macroalgae are foundation species in marine communities (Assis et al. 2020). They and others (including mussels, oysters [Bertness et al. 2014]) supply essential ecological services to other organisms, providing habitats to other organisms and buffering physical and biological stresses that affect the productivity, diversity, and resilience of coastal ecosystems (Hurd et al. 2014, Wernberg et al. 2018). Intertidal habitat-formers might buffer local

environmental conditions (Brawley and Johnson 1991, Mota et al. 2015, Monteiro et al. 2019) thereby dampening expected latitudinal diversity (Jurgens & Gaylord 2018) of host microbiomes. Furthermore, foundation species contribute significantly to nitrogen (Pfister & Altabet 2019 Pfister et al. 2019), iodine (Gonzales et al. 2017), and carbon biogeochemical cycles (Thomas et al. 2012, Reed et al. 2015, Pfister et al. 2019, Sichert et al. 2020).

Macroalgae depend upon bacteria for normal morphology (*Fucus spiralis* [Fries 1977], *Ectocarpus* sp. [Tapia et al. 2016], *Ulva* spp. [Fries 1975, Provasoli and Pintner 1980, Singh et al. 2011, Ghaderiardakani et al. 2017]), even becoming unicells when axenic (Matsuo et al. 2005). "The composition and structure of macroalgal biomes vary according to host taxonomy (Longford et al. 2007, Lachnit et al. 2009, Lachnit et al. 2011, Roth-Schulze et al. 2018, Weigel and Pfister 2019), host morphology (Lemay et al. 2020), environmental gradients (Weigel and Pfister 2019, Dittami et al. 2020, Quigley et al. 2020), and host tissues (Quigley et al. 2018, Quigley et al. 2020), but differs from the water column or adjacent substratum (Lachnit et al. 2009, Quigley et al. 2020)."

Most macroalgal microbiome studies are conducted at scales smaller than geographic gradients (Brodie et al. 2016, Califano et al. 2020, Quigley et al. 2020). However, Marzinelli *et al.* (2015) discovered less continental-scale variation in the microbial communities of the kelp *Ecklonia radiata* than that between healthy and climatically stressed, diseased individuals. Local comparisons of the microbiomes of *Ulva australis and U. ohnoi* in Australia and between Spanish and Australian *U. australis* found that taxonomic composition was too variable to describe a common core of operational taxonomic units (OTUs), yet metagenomic studies identified functional redundancy (Roth-Schulze et al. 2018). These studies mirror the lottery

hypothesis for community assembly of coral reef fishes (Sale 1979) to explain functionally similar but taxonomically different microbial assemblages (Burke et al. 2011, Ghaderiardakani et al. 2017). However, for latitudinally varying bacterial communities, the core bacteria essential to host function and structural integrity might change at retreating edge distributions if hosts have altered genetic variability that coincides with range contractions (Neiva et al. 2015, Jueterbock et al. 2018, Casado-Amezúa et al. 2019, Qiu et al. 2019, Coleman et al. 2020).

This study investigates whether the microbiome of intertidal *Fucus vesiculosus* varies with latitude similarly on both sides of the North Atlantic, as hypothesized if driven by latitudinally replicated environmental factors. We compared host bacterial communities along the entire Atlantic range from Greenland to North Carolina (USA) in the western Atlantic and from Norway to Spain in the eastern Atlantic. Cross-Atlantic replication allowed tests of these hypotheses: 1) alpha diversity of *F. vesiculosus* microbiomes will increase from northern to southern sites, 2) environmental parameters predict contemporary community structure of the *F. vesiculosus* microbiome over latitude, and 3) a stable core of bacterial taxa is associated with *F. vesiculosus* across its range in the North Atlantic, in spite of expansions and contractions in the host metapopulations over recent glacial cycles and contemporary climate change (Muhlin and Brawley 2009, Cánovas et al. 2011, Coyer et al. 2011, Nicastro et al. 2013, Assis et al. 2014, Neiva et al. 2016). Lastly, in exploring latitudinal differences in bacterial communities, we examined the phylogenetic relationships within specific bacterial genera, including some known algal symbionts, across the latitudinal gradients.

METHODS

Site descriptions and sample collection We collected *F. vesiculosus* individuals from 16 sites covering its latitudinal distribution in the western (7 sites) and eastern (9 sites) Atlantic Ocean during the summers of 2015 and 2016 (Fig. 1, Suppl. Table 1). Most sites were sampled in both years, but some only in one year for logistical considerations or local-scale comparisons (Suppl. Table 1). To test the latitudinal hypothesis, we divided sites into three regions: North (~1700 km, 70°N - 55°N), Central (~1700 km, 55°N - 40°N) and South (~700 km, 40°N - 34°N), consistent with summer sea surface temperatures (Fig. 1). Host canopies typically occupy rocky mid-intertidal shores on both Atlantic coasts. The population at Cádiz (Spain) grew on emergent patches of hard substratum in a sandy, well-flushed bay. Populations in Lewes (DE, USA; Suppl. Fig. 1) and Beaufort (NC, USA; Suppl Fig. 1) were growing on a variety of natural (oysters, mussels, marsh grass rhizomes) or artificially placed substrates (concrete, rocks).

We sampled yearly on two days (typically 3-20 days apart in July, Suppl. Table 1) along two fixed 30-m transects. Using random numbers, we selected three individuals/transect from which we removed several receptacles (reproductive organs), vegetative tips, and the holdfast using sterile techniques. Each tissue was immediately rinsed in sterile seawater in 50-mL Falcon tubes and stored on ice until flash-freezing in liquid nitrogen within 3 h of collection or stored on dry ice until transfer to -80 °C. Uummannaq samples were desiccated with silica gel (Quigley et al. 2018). Simultaneously, we collected environmental samples by (1) collecting seawater (1 l) from the shore, prefiltering through a 1.0- or 5.0-µm filter, and retaining the sample on a 0.2-µm filter, and (2) scraping ~4 cm^2 of the substratum at a random position/transect. Samples were

stored at -80 °C as before. Sampled macroalgae were conserved as herbarium specimens in the University of Maine Herbarium (MAINE).

Sample and bioinformatic processing DNA was extracted from 25 mg lyophilized samples, pulverized using a Geno/Grinder (SPEX SamplePrep, Metuchen, NJ; 2 min, 1600 strokes/min, 2.4-mm zirconium beads). DNAs were extracted with the Qiagen DNeasy Plant protocol (Germantown, MD). The bacterial 16S rRNA gene V4 hypervariable region was amplified in the presence of peptide nucleic acid clamps (PNAs, 25μM) that blocked the amplification of host 18S and plastid genes. The amplification primers contained Illumina-specific sequences for binding to the flow cell and sequencing primers fused to 16S GT-515F (GT-GTGYCAGCMGCCGCGGTAA) and CC-806RB (CCGGACTACNVGGGTWTCTAAT). The forward primer contained an in-line barcode, and the reverse primer contained an index captured by a short indexing read. Cycling conditions were 94°C for an initial denaturation of 3 min, 30 cycles of 94°C for 45 s, 78°C for 10 s, 50°C for 1 min and 72°C for 90 s and a final extension at 72°C for 10 min. The products were cleaned, quantified, and pooled [15]. We used the V4 region because of its extensive testing and adoption by the Earth Microbiome Project [54]. Amplicon pools (up to 96 amplicon libraries) were sequenced on an Illumina MiSeq (manufacturer's protocol, v.3 sequencing kit). Paired-end reads were demultiplexed by index using on-instrument software and by barcode using a custom python script [14]. Paired-end reads were merged, trimmed of primer sequences, and quality filtered (Eren et al., 2013).

Samples with low amplification were excluded from the analyses (see Suppl. Table 2 for final sample and library sizes). The final datasets served as input to Minimum Entropy Decomposition (MED) analysis [55]. MED identified 1779 amplicon sequence variants (1,779 ASVs; Suppl.

Table 3) within summer *Fucus* and environmental samples as defined in our custom ASV database (github.com/kacf24/FucusLatitude). Taxonomy was assigned to these ASVs using VSEARCH (Rognes et al., 2016)* and our custom V4 database, derived from the SILVA reference taxonomy v.132 (Quast et al., 2013).

We subsampled without replacement (rarefied) to an even sampling depth of 4,918 high-quality, merged sequences/sample. We refer to ASVs by concatenating their assigned ASV number to their genus-level taxonomy (e.g., ASV 3260 is *Granulosioccus*_t3260). ASVs found in our previous study of *Fucus spiralis, F. vesiculosus*, and *F. distichus* bacterial communities [15] include the letter 't' (transplant study) whereas novel ASVs include the letter 's' (summer trans-Atlantic). Raw sequence fastq files are contained in NCBI Sequence Read Archive, accession number PRJNA658993.

Alpha and beta diversity To investigate presence of any significant patterns in ASV alpha diversity, a Monte Carlo simulation calculated 5 metrics (ASV richness, Shannon-Wiener Diversity Index, Simpson's Diversity Index, Inverse Simpson Index, and Pielou's Evenness Index) on 1000 independent data rarefactions. The results were analyzed by tissue and site.

We analyzed beta diversity (i.e., community differentiation among sites) through a permutation multivariate analysis of variation (PERMANOVA) based on a Bray-Curtis (community structure) distance matrix calculated from the square root-transformed ASV table, using 9999 permutations (Anderson 2001). We considered the factors region (3 levels), tissue (3 levels), and year (2 levels) as fixed, while site (16 levels), day (2 levels, nested within year) and transect (2 levels, nested within site) were random. We used a total of 845 sequence datasets, from 116 environmental controls (seawater and substrates) and 270 individuals. PERMDISP tested homogeneity of group dispersions. Pairwise-testing of significant factors and interactions

was also performed with PERMANOVA. When fewer than 200 permutations were possible, Monte Carlo simulation-based *p*-values were used. Pairwise *p*-values were corrected for multiple comparisons (Benjamini and Hochberg 1995). ASVs driving differentiation were identified by calculating the contribution of each ASV to the dissimilarity between groups (SIMPER; Clarke 1993).

Environmental parameters Because of our broad latitudinal range and the vast literature on the effects of different environmental conditions on macroalgae (reviewed in Hurd et al. 2014), we tested correlations between the abundance of some ASVs and environmental parameters. We obtained meteorological, oceanographic, and astronomical information covering the two weeks (Langenheder and Ragnarsson 2007) before each sampling date to account for lag time in environmental conditions affecting bacterial abundances. Hourly meteorological data from DarkSky (Rudis 2017), a global weather-aggregating service, ensured standardization between data sources and included air temperature, dew point, humidity, wind speed, and wind bearing. We used modeled tidal data on a 5-min timescale from worldtides.info, because nearby tidal gauges were absent at many sites, but checked the data against tidal gauges where possible. Tidal data were transformed to proportional tidal height based on the highest and lowest tide from May 1, 2015 - May 05, 2017, because a relatively short period (i.e., 2 weeks) would have missed longer-frequency harmonics. Oceanographic data (photosynthetically active radiation [PAR] and sea surface temperature [SST]) from the NASA MODIS-AQUA satellite (NASA Goddard Space Flight Center, Ocean Ecology Laboratory, Ocean Biology Processing Group, 2014) were daily means on a 4-km^2 grid with large patches of no data (cloud interference); hence, we used the nearest valid grid cell to each site to create a daily time series of the previous two weeks. Photoperiod represents the difference in time between sunrise and sunset each day

(Sefick, 2016). For each variable, we used the minimum, maximum, and median values as possible explanatory variables for the abundance of some ASVs in bidirectional (i.e., variables added and removed from model) stepwise regression limited to 5 explanatory variables (Lumley 2020). We tested only the seven ASVs that had the highest variation in SIMPER analysis, because they would be expected to show the strongest latitudinal differentiation. Values of the two most significant variables for each ASV were plotted against ASV abundance and produced a trendline fit using a general additive model (GAM).

Tests for correlations between community structure and the environment used distance-based redundancy analysis (dbra; Oksanen et al. 2019). This was undertaken stepwise in both directions to select variables that minimized the AIC (Akaike information criterion) of the model (ordistep; Oksanen et al. 2019). Because this method leads to large, complex models that may have statistical, but not biological significance, only the first 3 terms were considered significant (Babyak 2004).

Phylogenetic trees To determine how closely our ASVs were related to previously described strains, we downloaded reference sequences (Suppl. Table 4) belonging to *Blastopirellula, BurkholderiaCaballeroniaParaburkholderia* complex*, Granulosicoccus, Maribacter, Octadecabacter, Pleurocapsa, Roseobacter,* and *Sulfitobacter* from a custom-curated database (VAMPS2.mbl.edu; Huse et al. 2014) and trimmed them to the V4 region. We also obtained V4 ASV sequences from Pacific brown algae *Nereocystis* and *Macrocystis* (Weigel and Pfister 2019). For each genus, the trimmed sequences were aligned using EMBL-EBI's Clustal Omega (Madeira et al. 2019). The resulting alignment served as input to RAXmlHPC using the GTR substitution model under the Gamma model of rate heterogeneity with bootstrap values calculated using 1000 replicates (Stamatakis 2014). We annotated resulting trees using the

Interactive Tree of Life (itol.embl.de; Letunic and Bork 2019). The distribution of each ASV across tissues was visualized by 1) calculating the average abundance of each ASV on each sampling for every tissue, 2) summing all the values for each tissue, and 3) calculating the proportional abundance of each tissue. Similarly, we found the distribution of each ASV across sample regions (North, Central, South) by calculating 1) the mean abundance of each ASV in each region, and 2) the proportional abundance of each region. When available, sample provenance was noted on the phylogenetic tree.

Community description We tested the hypothesis that *F. vesiculosus* has a stable core of symbionts through core community analysis. We pooled all replicates of a tissue sampled on the same day at each site to identify core ASVs, using non-rarified data to minimize the effects of ASV abundance (McMurdie and Holmes 2014). Core ASVs were present in all or all but one sample of a given tissue on a sampling day (i.e., 5/6 or 4/5 replicates), but if 4 or fewer replicates were available, the ASV had to be present in all. Bar charts showing the proportional abundance of the ten most abundant contributors at each taxonomic level display a mean (± SE) of ASV reads for a given taxonomic level from all replicates of a given sample location and tissue.

RESULTS

General description of sequence numbers The MED analysis returned 130,465,846 high-quality reads and an average number of reads across the 1305 samples of 99,974 (± 57,884), ranging from 5868 - 707,042 reads (rarefied to 4918 reads). MED identified 1779 ASVs (amplicon sequence variants; Suppl. Table 3) that had ≥ 50,000 assigned reads across all sequenced samples.

General description of community Proteobacteria dominated bacterial communities collected from each site in all tissues, but within the phylum, two orders (Alphaproteobacteria_or_fa and Rhodobacteraceae) dominated holdfast communities whereas receptacles and vegetative tissue hosted primarily Burkholderiaceae and Thiohalorhabdaceae (Suppl. Fig. 6). The abundance of Rhodobacteraceae decreased with decreasing latitude. Other abundant phyla included Bacteroidetes and Verrucomicrobia (Suppl. Fig. 6). The Verrucomicrobia were especially prominent in vegetative tips collected from Oban and Torriera. The relative abundance of Cyanobacteria in bacterial communities of receptacles and vegetative tissue generally increased with decreasing latitude, a relationship primarily driven by presence/absence of Xenococcaceae (Suppl. Fig. 6).

Latitude None of the five alpha diversity metrics showed a distribution consistent with a latitudinal gradient (Suppl. Fig. 2). In contrast, community structure of *F. vesiculosus* microbiomes showed significant differentiation among North, Central, and South regions when tested at each taxonomic level (all $p < 0.03$, 6-14% total variation; Suppl. Table 5). However, only at the ASV level was there a pairwise difference ($p = 0.0001$) between the North and Central regions (all others, $p > 0.4$). Microbiomes of all tissues differed between North and South (Fig. 2). Pairwise-testing revealed that the structure of Central microbiomes did not significantly differ from North in vegetative and receptacle tissue (Suppl. Table 6a); however, some significant differences may reflect heterogeneity of variances between latitudes (Ppermdisp < 0.05; Suppl. Table 6a). Differentiation between regions was driven by only a few ASVs, including *Granulosicoccus*_t3260, *Alphaproteobacteria_or_fa_ge*_t3536, *BurkholderiaCaballeroniaParaburkholderia*_t8371 and *Pleurocapsa* _t10392 (SIMPER analysis; Table 1).

In addition to regional differentiation, community structure of the *F. vesiculosus* microbiome significantly differed ($p < 0.05$) across all tested factors and most interactions (Suppl. Table 6a) where tissue and site accounted for most variation (22% and 14% respectively). Holdfast communities were not similar to vegetative or receptacle communities (7-29% mean similarity, Suppl. Table 6d), which were not significantly different in 2/3 of comparisons (~44% mean similarity, Suppl. Table 6d). Heterogeneity of variances rarely explained tissue differentiation (Suppl. Table 6). Principal coordinate ordination (Fig. 2) shows latitudinal variation in the microbiome and strong differentiation between receptacles/vegetative tissues and holdfasts. *Fucus vesiculosus* microbiomes were strikingly different from environmental microbiomes collected from adjacent seawater or substrata (Suppl. Fig. 3).

Environmental model Many ASVs contributed to the overall variation across the grouping gradient (North-Central-South), but the seven most significant ASVs in the SIMPER analysis had different distributions that correlated with different environmental variables (Fig. 3, Suppl Fig. 4a-e). Generally, trends were similar across both North Atlantic coasts at similar latitudes despite some ASV distributions exhibiting a multimodal distribution on one shore and a linear one on the other (e.g., Suppl. Fig. 4a). Some ASVs correlated with a single parameter. For example, the relative abundance of *BCP*_t8371 across all tissues tended to decrease with increasing air temperature (Suppl. Fig 4a). However, some environmental correlations were tissue-specific despite similar latitudinal distributions in individual tissues, such as *Pleurocapsa*_t10392's negative association with lower air (receptacle) and sea surface (vegetative) temperatures (Fig. 4). Likewise, *Alphaproterobacteria_or_fa_ge*_t3536 was highly abundant on holdfasts of sites with mean air and sea temperatures warmer than 20 °C (Suppl. Fig. 4b). Sometimes, an interaction between parameters supported more complex latitudinal

348 distributions, such as the high relative abundance of *Granulosicoccus*_t3260 in sites with
349 temperatures between 10 – 15 °C and with tides closest to lowest low tide on both receptacles
350 and vegetative tips (Fig. 3). All these ASVs were highly correlated with one or more of a small
351 subset of environmental parameters (air temperature, humidity, SST, tidal height), and not with
352 the one (photoperiod) caused directly by latitude, with the possible exception of PAR influencing
353 abundance of *Alphaproterobacteria_or_fa_ge*_t3536 associated with vegetative tissue (Suppl.
354 Fig. 4b). Overall, these ASVs correlated with specific stresses such as desiccation, inferred by
355 wind speed and tidal height (*Blastopirellula*_t628, Suppl. Fig. 4c), or heat stress
356 (*Sulfitobacter*_7351, Suppl. Fig. 4e).
357 At the community level, environmental variables were not strongly correlated with
358 microbial structure. SST explained the most variation when all tissues were combined (R^2 =
359 0.06; Suppl. Table 7) or analyzed separately (Holdfast R^2 = 0.08, Receptacle R^2 = 0.10,
360 Vegetative R^2 = 0.15). Holdfast and receptacle microbial structures were associated with
361 humidity, but microbiomes of vegetative tissue were associated with tidal height. The model of
362 combined tissues most closely resembled that of vegetative tissue. Overall, the microbiome
363 structure of vegetative tissue had the highest correlation with the environment (R^2 = 0.23) while
364 holdfasts had the lowest (R^2 = 0.09).
365 *Granulosicoccus phylogenetics* Our study identified 86 ASVs assigned to the genus
366 *Granulosicoccus* that present novel phylogenetic diversity (Fig. 5). Most ASVs from the South
367 region were different from reference sequences. The highly abundant *Granulosicoccus*_t3260 is
368 identical to the V4 region of a bacterial sequence cloned from the surface of *F. vesiculosus*
369 (Lachnit et al., 2011), and many North-Central ASVs were similar/identical to reported
370 sequences from brown algae (Fig. 5). Several reference coral/sponge isolates formed a clade that

371 excluded related *Fucus* associates (Clade 1; Fig. 5). There was no clear phylogenetic pattern in
372 the tissue distribution of *Granulosicoccus* ASVs, even though most *Granulosicoccus* ASVs were
373 strictly either holdfast or thallus specialists. Forty-two ASVs were found predominantly in either
374 the North (22), Central (7), or South (13) latitudinal distribution range. Closely related ASVs had
375 strongly contrasting tissue and/or regional distributions (Fig. 5). The sister holdfast-associated
376 ASVs *Granulosicoccus*_t3363 and *Granulosicoccus*_t3364 differed by a single nucleotide, but
377 the former had a strong South and the latter a North-Central distribution (Fig. 5). In contrast,
378 ASVs *Granulosicoccus*_t3376 and *Granulosicoccus*_t3369 differed in both tissue and regional
379 distribution with *Granulosicoccus*_t3369 restricted to southern holdfasts and t3376 to central
380 receptacles (Fig. 5).
381 *Pleurocapsa phylogenetics* Overall, most *Pleurocapsa* ASVs were associated with
382 South latitudes (Fig. 6). As noted earlier, the relative abundance of *Pleurocapsa*_t10392 is
383 negatively associated with lower temperatures. However, *Pleurocapsa*_t10392, which has a
384 predominantly southern distribution is identical to the V4 region from an OTU reported from the
385 surface of *F. vesiculosus* collected from the Baltic Sea (Lachnit et al. 2013). ASVs from this
386 study that share a node with an ASV from the kelp microbiome (Weigel and Pfister 2019) exhibit
387 varied distributions: t10412 and 310c944c6, central; s10343 and 3920c18b, primarily southern;
388 and t10409 and cb129acc, northern and central (Fig. 6).
389 *Sulfitobacter phylogenetics* We identified 13 ASVs in *Sulfitobacter*, four of them
390 largely restricted to holdfast tissue (Fig. 7). As with the *Granulosicoccus* ASVs, the phylogenetic
391 distance between ASVs did not predict tissue distribution, and related ASVs, e.g.,
392 *Sulfitobacter*_t14484 and *Sulfitobacter*_s16545, exhibited different latitudinal distributions

393 (North-Central and Southern, respectively). Similarly, ASVs *Sulfitobacter*_t14490 and
394 *Sulfitobacter*_s17161 showed both different distributions and different tissue specificities.
395 *Phylogenetics of other genera of interest for their abundance/function* Generally,
396 many ASVs are tissue or region specific within individual genera (Suppl. Fig. 5); however, the
397 analysis of the *Burkholderia/Caballeronia/Paraburkholderia* ASVs (Suppl. Fig. 5a) showed that
398 their distributions and tissue locations were nearly identical. Each latitudinal region (North,
399 Central, and South) had one or more ASVs specific to the holdfast and one or more specific to
400 the thallus. *Blastopirellula* ASVs included pairs that were nearly identical but showed very
401 different geographic distributions and tissue affinities (Suppl. Fig. 5b). For example,
402 *Blastopirellula*_s17026 was more prevalent in Northern holdfast communities while
403 *Blastopirellula*_s17027 exclusively occurred in communities of Central receptacles. Similarly,
404 *Blastopirellula*_s18367, was also exclusive to Central receptacle communities, but was nearly
405 identical to *Blastopirellula*_s18368 that principally occurred only in Southern holdfast
406 communities. We identified 26 *Octadecabacter* ASVs, nearly half of them largely from North
407 and Central holdfast samples (Suppl. Fig. 5c). Weigel and Pfister (2019) reported many
408 *Octadecabacter* ASVs from north Pacific kelps *Macrocystis* and *Nereocystis,* some nearly
409 identical to the ASVs from our *Fucus* samples (70c73119 and *Octadecabacter*_t7491, 8f932656
410 and *Octadecabacter*_t1742, 202fc719 and *Octadecabacter*_t12129; bootstrap support > 50). Our
411 phylogenetic analysis of *Roseobacter* ASVs (Suppl. Fig. 5d) contained a clade that included
412 reference isolate KT461667, *Roseobacter*_t12218 and *Roseobacter*_t12219. Other well-
413 supported clades contained only ASVs from this study: *Roseobacter*_s16949 and
414 *Roseobacter*_t12539, with South-only and mixed North-Central distributions, respectively; and
415 *Roseobacter*_s18276, *Roseobacter*_t15161, *Roseobacter*_s18281, and *Roseobacter*_t12394.

*Roseobacter*_t12394 is of interest as it was almost absent from Central samples. Finally, recovered *Maribacter* ASVs (Suppl. Fig. 5e) were identical or nearly identical to sequences associated with red (*Maribacter*_s14677) and green algae (*Maribacter*_t10716, *Maribacter*_t5410, *Maribacter*_t5408, and *Maribacter*_t5327).

Core communities No ASV was a core member for all *F. vesiculosus* tissues over all sampling locations (Suppl. Table 8). The closest candidate was *Granulosicoccus*_t3260, the only ASV present in all vegetative tips and receptacles, but it was not prevalent in holdfast samples from Oban or Beaufort. Two ASVs (*Ilumatobacter*_t6062 and *Granulosicoccus*_t3356) represented core community members for holdfasts over all sampling locations.

Changing geographic distribution of Fucus vesiculosus The population at the southernmost limit of *F. vesiculosus* on the western Atlantic (Beaufort, NC, USA) became increasingly sparse (June 2015-July 2016; Suppl. Table 9. Heavy rains, flooding, and high tides associated with tropical storms and a hurricane after July 2016 (Armstrong 2016, Kunkel et al., 2020) led us to survey our sites in November 2016. No *F. vesiculosus* were found along our transects, but three individuals (1 male, 2 females) were attached to marsh grass and oysters near our study site adjacent to Pivers Island. These individuals bore receptacles with mature gametes. We failed to find any other *F. vesiculosus* within the greater Morehead City-Beaufort-Topsail region, and subsequent surveys through December 2020 also failed to find *F. vesiculosus* (Suppl. Fig. 1).

DISCUSSION

439 The scale of replication and geographic range in our study allowed a comprehensive
440 analysis of the hypothesis (Burke et al. 2011, Ghaderiardakani et al. 2017, Roth-Schulze et al.
441 2018) that stable taxonomic core is not associated with macroalgal hosts. Our study did find
442 three tissue-specific core ASVs (*Ilumatobacter*_t6062, *Granulosicoccus*_t3356,
443 *Granulosicoccus*_t3260) that may be critical symbionts of *F. vesiculosus*. In contrast, many
444 ASVs were localized to specific latitudes despite sometimes differing by only a single
445 nucleotide. We were rarely able to match these to a described strain; however, many were
446 identical or nearly so to the corresponding V4 marker of known algal associates, suggesting a
447 putative role in host fitness. This suggests that the bacteria represented by these differentially
448 localized ASVs might exhibit differences across latitudinal conditions in environmental tolerance
449 and metabolic function, a hypothesis that could be examined by isolation/cultivation, genomic
450 sequencing, and experimentation.
451 We found numerous members of the *Burkholderia/Caballeronia/Paraburkholderia*
452 (BCP) complex in North and Central latitudes; this complex includes bacteria that produce the
453 morphogen indoleacetic acid, synthesize catalase, fix nitrogen, and provide resistance to many
454 antibiotics while producing potent antifungal and antibacterial compounds (Panhwar et al. 2015,
455 Vandamme and Eberl 2018, Dias et al. 2019). Higher temperatures, especially higher air
456 temperatures, correlate with low relative abundances of *BCP* in the Southern region of the North
457 Atlantic Ocean.
458 Cyanobacteria and Alphaproteobacteria represented by ASVs identified here as abundant
459 in the Southern region (e.g. *Pleurocapsa*_t10392, Alphaproteobacteria*_or_fa_ge*_t3536, and
460 multiple *Octodecabacter*), might contribute to host survival in hot and nutrient-limited summer
461 conditions through the fixation of nitrogen and production of vitamin B_{12} (Rippka et al. 2015,

462 Dogs et al. 2017). *Octadecabacter* was also reported from the north Pacific kelps *Macrocystis*
463 and *Nereocystis* (Weigel and Pfister 2019), suggesting that the bacteria represented by these
464 ASVs may have similar effects on all these brown algae.
465 We identified many ASVs belonging to *Granulosicoccus*, which has many CAZymes
466 (including alginate lyase, DMSP demethylase, and other enzymes involved in sulfur cycling) and
467 affects nitrogen cycling via its nitrate reductase, nitrite reductase, and urease (Kang et al. 2018).
468 *Granulosicoccus* spp. are associated with a variety of macroalgae (Weigel and Pfister 2019,
469 Califano et al. 2020, Quigley et al. 2020). In our environmental modeling, the relatively high
470 abundance of *Granulosiccocus*_t3260 correlated with cooler minimum sea surface temperatures
471 on both receptacles and vegetative tissue, especially above 45^{o} N, although it is a core ASV over
472 all latitudes. Among the three tissue-specific core ASVs, the association of
473 *Granulosicoccus*_t3260 across vegetative tips/receptacles and nearly all sites' holdfasts, as well
474 as the core association with holdfasts of *Granulosicoccus*_t3356, illustrate their comparative
475 value in elucidating the basis for tissue-specificity when isolates become available for genomic
476 analyses.
477 Canopy protection of fronds/bacteria from environmental stress may account for the
478 absence of a latitudinal richness gradient in the *F. vesiculosus* microbiome in the North Atlantic
479 Ocean. Our analysis showed that bacteria of holdfasts (covered by canopy) exhibited the lowest
480 correlation with environmental factors over the trans-Atlantic replication (vegetative > receptacle
481 > holdfast), suggesting environmental buffering by the overlying canopy. However, this may
482 also reflect biochemical and structural differences among tissues.
483 We discovered a strong north-south latitudinal gradient in structure of the microbiome of
484 *F. vesiculosus* over its biogeographic range in which structure was similar across shores of the

485 western and eastern North Atlantic Ocean at comparable latitude. Environmental conditions
486 during our collection times in summer 2015 and 2016 were sufficient to explain the gradient
487 based on correlative modeling. However, there are two additional influences to consider. The
488 most important is probably the phylogenetic history of the host *F. vesiculosus* during its
489 expansions and contractions during glacial cycles in the North Atlantic Ocean (Neiva et al.
490 2016). *Fucus vesiculosus* is the most widely distributed species and ecologically adaptable
491 within the Atlantic-speciated genus, and its range has fluctuated over glacial cycles (Coyer et al.
492 2011, Nicastro et al. 2013, Assis et al. 2014). Using occurrence records of the recent past where
493 changes in distribution of *F. vesiculosus* are recorded historically, Assis et al. (2014) derived a
494 model constrained by sea and air temperatures of the hottest summer month, availability of hard
495 substratum for attachment, and humidity of the wettest summer month. Notably, these factors
496 affecting the host, other than substrate, are consistent with those we have modeled for 2015 and
497 2016 summer distributions of different ASVs. Thus, we hypothesize *F. vesiculosus* and its
498 microbiome appear to have the same environmental drivers. Overall, the correlations between
499 environmental variables and community structure and the relative abundance of select ASVs
500 were weak, indicating complex interactions control these communities.
501 *Fucus vesiculosus* diversified in the NE Atlantic Ocean into two different genetic groups
502 (North and South) (Cánovas et al. 2011, Coyer et al. 2011, Assis et al. 2014; Neiva et al. 2016)
503 that both extend into NW Portugal. The southern group is genetically adapted to be stress
504 tolerant (Ladah et al. 2003, Saada et al. 2016), but this local adaptation has not prevented it from
505 suffering a recent southern range edge contraction (Nicastro et al. 2013), stranding small
506 populations (e.g., Cádiz). Our phylogenetic analyses of bacteria on *F. vesiculosus* show closely
507 related ASVs with different distributional abundances, particularly North versus South, that

508 correlate with different levels of environmental factors, but also suggest their possible
509 diversification on hosts that diversified into the different northern versus southern groups in the
510 NE Atlantic. The bacteria have likely adapted to different environmental factors experienced by
511 the host (Ladah et al. 2003, Saada et al. 2016), potentially expanding and contracting their ranges
512 with the host. A third genetically distinct group likely existed in the NW Atlantic before the last
513 glacial maximum (LGM; Coyer et al. 2011, Assis et al. 2014, Neiva et al. 2016). Existing
514 microsatellite genotype data (Muhlin and Brawley 2009) include most of our present sampling
515 locations. In that study, populations of *F. vesiculosus* were differentiated into three groups: South
516 (Lewes and Beaufort), Central (Connecticut, near Newport and Woods Hole), and North
517 locations (Maine, Nova Scotia). Thus, host differentiation mirrors the North South differentiation
518 of ASVs on the host in the NW Atlantic. It seems likely that this is related to both past (pre/post
519 LGM) environments and contemporary latitudinal gradient of environmental stress. Hosts such
520 as *F. vesiculosus* have fine-scaled phylogenetic signals because they lack any widely dispersive
521 stages such as larvae. The strong ASV associations within particular genera (e.g.,
522 *Granulosicoccus*) on such macroalgae offer further opportunity to understand the effect of
523 glacial cycles on holobiont community organization in the northern hemisphere.
524 Another explanation for the latitudinal differentiation of microbial community structure
525 on both sides of the Atlantic Ocean could be habitat-specific differences in North versus South
526 parts of the Atlantic host metapopulation. Below Montauk Point (NY) in the NW and below
527 Brittany (France) in the NE, hard substratum is interrupted by sandy shores. In its southern
528 distributions, *F. vesiculosus* is restricted to sheltered bays, mouths of estuaries, and coastal
529 lagoons (Ladah et al. 2003). The varying biological context (mussel-rich marshes, oyster-rich
530 bays) may influence the microbiomes of *F. vesiculosus* to create an apparent "South" grouping

by latitude. Although ecological habitat differences could contribute to our bacterial community differentiation across latitudes, the microbiome of *F. vesiculosus* (and other brown macroalgae (Weigel and Pfister 2019, Quigley et al. 2020) are well differentiated from environmental samples, and a consistent group of bacterial genera (e.g., *Granulosicoccus, Octadectabacter, Maribacter, Ilumatobacter, Roseobacter*) are associated with macroalgae that have long persisted and moved in time and space in the North Atlantic Ocean. Moreover, adjacent but very different habitats of estuarine (Lima) and open-coast (Viana do Castelo) populations did not noticeably differ in their associated bacterial communities.

It is possible that bacterial ecotypes on *F. vesiculosus* in southern parts of the biogeographic range that might aid its stress tolerance are being lost at the current speed of effect of anthropogenic climate change. The contraction of *F. vesiculosus* in the NE Atlantic Ocean is described (Nicastro et al. 2013), and we do not predict recovery of the Beaufort NC population. Similar retractions have occurred for *Mytilus edulis* and *Semibalanus balanoides* (Jones et al. 2010, Jones et al. 2012). *Fucus vesiculosus* was once abundant in Beaufort, North Carolina (1910s; Hoyt 1917) and still common there as late as 2005 despite its low genetic diversity (Muhlin et al., 2009). Higher temperatures and the lengthening tropical storm season on the North Carolina coast appear to have had catastrophic population effects in 2016. Furthermore, *F. vesiculosus* is less likely to recover easily, because both male and female individuals are required for recruitment. By understanding the membership, dynamics, and potential environmental drivers of the stressful summer months, we can better predict how the bacterial communities of *F. vesiculosus*, and ultimately the host itself, will fare in the North Atlantic under shifts of temperature, wind, and precipitation**.**

We found striking similarities in microbial community structure and distributions of individual ASVs at similar latitudes with the power of our cross-Atlantic sampling over the host's biogeographic range. Contemporary environmental conditions are sufficient to explain the regionality of ASV distributions, but North-South differentiation of closely related ASVs within multiple genera also suggests possible effects of the host's phylogeographic history in the North Atlantic over recent glacial cycles. This hypothesis could be tested with comparative studies of other intertidal species with broad cross-Atlantic distributions. The comparative importance to the holobiont of ecotypes having different tissue or regional specificities can be evaluated following their isolation, culture, and functional analysis.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest with this work.

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Figure Legends

Figure 1. Locations (black circles) where *Fucus vesiculosu*s holdfast, vegetative and receptacle tissues were sampled across the North Atlantic Ocean. Background colors represent daily mean sea surface temperature conditions for summer from 2002-2020 (NASA Goddard Space Flight Center, 2014) rounded to the nearest 5 °C. The white dashed lines divide the regions into North (Bodø, Oban, Uummannaq), Central (Halifax, Lima, Minehead, Newport, Schoodic, Sidmouth, Torreira, Viana, Woods Hole), and South (Beaufort, Cádiz, Lewes, Tagus).

Figure 2. Principal coordinate analysis (PCO) of samples of bacterial ASVs by A) tissue, B) latitudinal region, C) and side of Atlantic. D) Pairwise comparisons (n = 359,976) of Bray-Curtis similarity and geographic distance among samples.

Figure 3. The distribution of *Granulosicoccus*_t3260 against its most significant explanatory environmental variables and latitude. Red indicates samples from the Eastern Atlantic Ocean, black indicates samples from the Western Atlantic Ocean, and blue represents both sides of the Atlantic Ocean. Trendlines were fit using general additive models (GAM). Note, many points are often overlapping.

Figure 4. Proportional distribution of the top 10 most abundant genera by tissue. BCP = *Burkholderia/Caballeronia/Paraburkholderia* and unk_Alpha = *Alphaproteobacteria_or_fa_ge.*

Figure 5. Phylogenetic relationships among *Granulosicoccus* ASVs identified in this study and in Weigel and Pfister (2019). Bootstrap confidence is indicated by branch width. Symbols denote the host from which the isolate or sequence was obtained. Pie charts show the relative abundance of each ASV in different tissue types. Bar graphs show their relative abundance in northern, central, and southern latitudes. *Granulosicoccus* ASVs 0855314e, 38910e1c, 3c4a4240, 68b6abf1, 6add9d51, 794283f9, a5ff1b4d, a985d165, aad1cf6d, bf89d33d, e0091b3b, ef0e252f, and f7a6d1a9 were identified in a marker-gene survey of the brown algae *Nereocystis leutkeana* and *Macrocystis pyrifera* (Weigel and Pfister 2019).

Figure 6. *Pleurocapsa* ASVs with grey shading indicate associations with South latitudes. Green shading indicates ASV t10392, which contributes significantly in the SIMPER analysis. Brown shading indicates an identical V4 sequence to a *Pleurocapsa* (ref GU451368) on Baltic *F. vesiculosus* in late summer (Lachnit et al. 2013). ASVs from this study that appear closely related to those from the microbiome of Pacific kelps (Weigel and Pfister 2019) are indicated with arrows. Annotations as in Fig. 5.

Figure 7. Phylogenetic relationships among *Sulfitobacter* ASVs and reference sequences. ASV pairs *Sulfitobacter*_t14484 and Sulfitobacter_s16545 (gray shading) and *Sulfitobacter*_t14490 and *Sulfitobacter*_s17161 (lavender shading) show very different latitudinal distributions and

tissue specificities. Annotations as in Fig. 5. *Sulfitobacter* ASVs 03fd1834, 4a106f4d, 74a460ef, bc4017df, 2a953fbd, 4e375c7f, 69d70be8, 75e5baac, 86b1f362, a8169c7c, b2014b64, dc63c55d, e1a3c928, eb9bd91a, f5a360f6, faf69120, 7a97f982, 2df75eb6, 46ca9bec, 60abedd5, 661abd89, 7695e9a0 (alga); 02c5e080, 159b98f6, 1e58dead, 2dc38804, 37b57d09, 41499ea2, 78d549cb, 7dc652da, 81373e26, 854c78a9, 9a895521, de259408 (seawater) were identified in a marker-gene survey of the brown algae *Nereocystis leutkeana* and *Macrocystis pyrifera* (Weigel and Pfister 2019).

Tables

Table 1. Top 5 ASVs Contributing to Differentiation Among North, Central, and South Regions

North (N) vs Central (C)				
ASV	**Mean N Abundance**	**Mean C Abundance**	**Variation Contribution (%)**	**Cumulative %**
*Granulosicoccus*_t3260	22.57	23.62	2.37	2.37
*BCP*_t8371	17.52	11.74	2.14	4.51
*Alphaproteobacteria_or_fa_ge*_t3536	12.18	12.08	1.84	6.35
*Blastopirellula*_t628	5.9	8.12	0.8	7.15
*Litorimonas*_t5725	4.99	4.66	0.64	7.79
Central (C) vs South (S)				
ASV	**Mean C Abundance**	**Mean S Abundance**	**Variation Contribution (%)**	**Cumulative %**
*Granulosicoccus*_t3260	23.62	11.26	2.2	2.2
*Alphaproteobacteria_or_fa_ge*_t3536	12.08	15.14	1.86	4.06
*BCP*_t8371	11.74	2.84	1.41	5.47
*Pleurocapsa*_t10392	1.3	10.51	1.12	6.59
*Blastopirellula*_t628	8.12	4.47	0.81	7.41
South (S) vs North (N)				
ASV	**Mean S Abundance**	**Mean N Abundance**	**Variation Contribution (%)**	**Cumulative %**
*Granulosicoccus*_t3260	11.26	22.57	2.06	2.06
*Alphaproteobacteria_or_fa_ge*_t3536	15.14	12.18	1.93	3.99
*BCP*_t8371	2.84	17.52	1.87	5.86
*Pleurocapsa*_t10392	10.51	2.28	1.12	6.98
*Sulfitobacter*_t7351	6.68	2.41	0.67	7.65