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RESEARCH ARTICLE

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A metabarcoding analysis of the wrackbed microbiome indicates a phylogeographic break along the North Sea–Baltic Sea transition zone

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Abstract

Sandy beaches are biogeochemical hotspots that bridge marine and terrestrial ecosystems via the transfer of organic matter, such as seaweed (termed wrack). A keystone of this unique ecosystem is the microbial community, which helps to degrade wrack and re-mineralize nutrients. However, little is known about this community. Here, we characterize the wrackbed microbiome as well as the microbiome of a primary consumer, the seaweed fly *Coelopa frigida*, and examine how they change along one of the most studied ecological gradients in the world, the transition from the marine North Sea to the brackish Baltic Sea. We found that polysaccharide degraders dominated both microbiomes, but there were still consistent differences between wrackbed and fly samples. Furthermore, we observed a shift in both microbial communities and functionality between the North and Baltic Sea driven by changes in the frequency of different groups of known polysaccharide degraders. We hypothesize that microbes were selected for their abilities to degrade different polysaccharides corresponding to a shift in polysaccharide content in the different seaweed communities. Our results reveal the complexities of both the wrackbed microbial community, with different groups specialized to different roles, and the cascading trophic consequences of shifts in the near shore algal community.

INTRODUCTION

Sandy beaches comprise 31% of the world's ice free coastline (Luijendijk et al., 2018) and represent some of the most ecologically and economically valuable landforms (Barbier et al., 2011). Beaches bridge marine and terrestrial ecosystems and provide critical ecosystem functions to both, such as recycling nutrients (Hyndes et al., 2022; Koop et al., 1982b; Rodil et al., 2019) and supporting key habitats, for example, bird nesting sites (Schlacher et al., 2017).

Unlike other ecosystems, most sandy beaches have little to no primary production (Colombini & Chelazzi, 2003; McLachlan & Brown, 2006; Speybroeck et al., 2008). Instead, the basis of the sandy beach ecosystem is often formed by deposited organic matter,

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such as algae and carrion (Colombini & Chelazzi, 2003; Hyndes et al., 2022). These deposits, called wrackbeds, are primarily decomposed by physical processing, invertebrate consumption, and bacteria (Colombini & Chelazzi, 2003; Hyndes et al., 2022; Jêdrzejczak, 2002; Lastra et al., 2014; Rodil et al., 2019). Wrackbeds are biogeochemical hotspots with extremely high metabolic activity (Rodil et al., 2019) partly due to bacteria; after algae are deposited on the beach, bacterial densities increase by up to four orders of magnitude (Cullen et al., 1987; Koop et al., 1982a; Urban-Malinga & Burska, 2009). Along with detritivores, these bacteria mineralize nutrients, which are then exported back to the sea (Dugan et al., 2011; Koop et al., 1982b; Rodil et al., 2019; van Erk et al., 2020).

The microbial biomass of these wrackbeds also serves as the basis for secondary production, providing food for macro and meiofauna, such as dipteran larvae, nematodes, and amphipods (Cullen et al., 1987; Griffin et al., 2018; Porri et al., 2011; Singh et al., 2021; Urban-Malinga & Burska, 2009). In turn, secondary consumers, such as spiders and beetles, and scavengers such as birds and mammals then prey on the macro and meiofauna (Hyndes et al., 2022). As such, wrackbed-decomposing microbiota form the basis of the beach ecosystem. However, we understand little about these communities: if they have a core microbiome (here defined as operational taxonomical units [OTUs] present in at least 95% of samples with a prevalence >0.5%, but see Neu et al., 2021), the extent of functional redundancy and how communities vary over space and time. We also know little about the ecological consequences of variation in the wrackbed microbiome (e.g., the bacterial species composition), and its effects on associated species. Changes in the wrackbed microbiome likely affect the composition of the eukaryotic consumer community by exerting different selective pressures on individual species, similar to the interaction between soil microbiomes and plant communities (Trivedi et al., 2020).

Seaweed flies are one such consumer group and common inhabitants of wrackbeds, including *Coelopa frigida* in Northern Europe. Eggs of this species are laid on the seaweed and emerging larvae feed primarily on the microorganisms in the wrackbed (Cullen et al., 1987). The flies experience high mortality during the early larval phase (Butlin & Day, 1984; Cullen et al., 1987), with mortality and growth rates differing based on the seaweed composition within the wrackbed (Cullen et al., 1987; Edward, 2008). As bacterial assemblages likely shift with seaweed composition, this suggests that the wrackbed microbiome could exert significant selective pressure on *C. frigida* (Edward, 2008; Edward & Gilburn, 2013), pointing towards a potential importance of the wrackbed microbiome for higher trophic levels. However, whether or not the wrackbed microbiome is a driver of selection in this species remains unknown.

Here, we examine the community structure and function of the wrackbed microbiome along one of the most studied ecological gradients in the world, the transition from the marine North Sea to the brackish Baltic Sea. Multiple abiotic factors vary along this transition zone, including salinity, temperature, and alkalinity (Møller Nielsen et al., 2016; Snoeijs-Leijonmalm et al., 2017). This is accompanied by shifts in algal, seagrass, and seawater microbial communities (Herlemann et al., 2011, 2016; Schubert et al., 2011; Takolander et al., 2017). For example, kelp and green algae dominate wrackbeds in the North Sea but are mostly replaced by fucoids (brown algae) and red algae in the Baltic. First, we sampled wrackbeds from five sites spanning the environmental transition zone and investigated how the species and functional composition of the bacterial communities changed over this gradient. Second, to understand how changes in the wrackbed microbiome composition impact the food chain, we sequenced the microbiome of seaweed fly larvae from the same sites. We used these data to investigate two key questions: (1) How does the species composition and functionality of the wrackbed microbiome vary over the transition zone, and how is this linked with environmental factors? (2) Can we detect effects of changing microbiome community composition on the microbiome of seaweed fly larvae?

EXPERIMENTAL PROCEDURES

Sample collection

Samples were collected in July and August 2016 from five sites along the Scandinavian Coastline. Three sites are located in the North Sea (including the Kattegat and Skagerak): Skeie (58°41′50.4″ N, 5°32′27.0″ E) and Justøya (58°13′08.2″ N, 8°23′12.1″ E) in Norway and Magnarp (56°17′51.0″ N, 12°47′18.4″ E) in Sweden. The remaining two sites Smygehuk (55°20′17.3″ N, 13°21′48.7″ E), and Ystad (55°25′27.9″ N, 13°46′23.1″ E) in Sweden are located within the Baltic Sea (Figure 1; Snoeijs-Leijonmalm & Andrén, 2017).

We collected both wrackbed and larval samples from each site. The wrackbed at each site was mostly continuous and we sampled only from above the high tide line. For replicate samples, we collected three handfuls of seaweed from widely spaced parts of the wrackbed (>1 m apart) where *C. frigida* larval density was high (more than 50 larvae in approximately one handful of wrackbed). We removed as many larvae as possible from this seaweed and then placed the remaining matter in a 50 mL tube filled with 99%

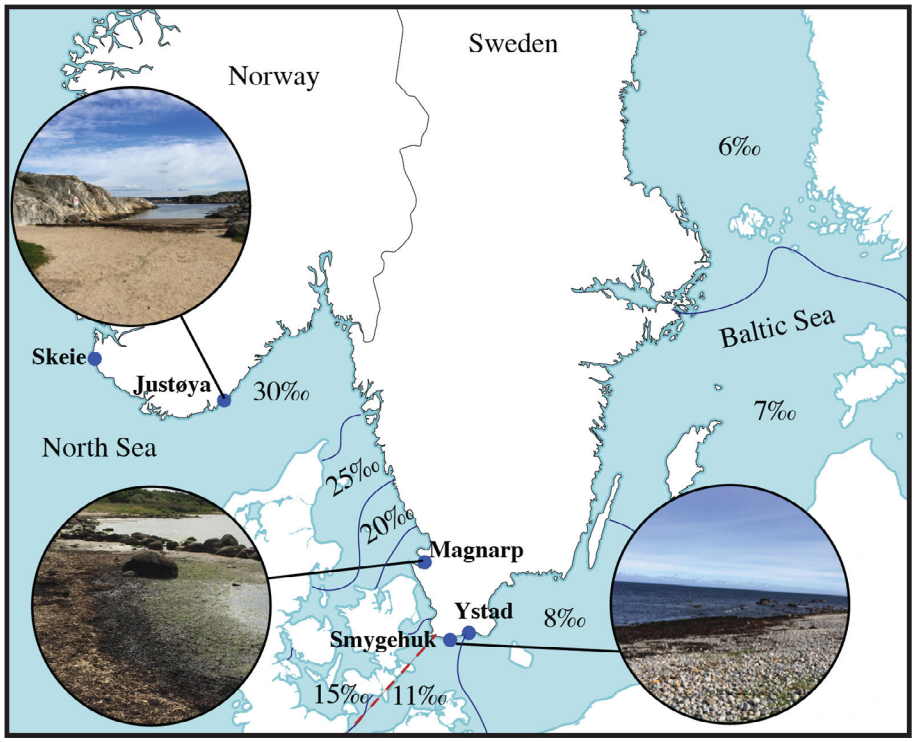


FIGURE 1 Map of sampling sites with representative photographs of wrackbeds from some sites and the salinity gradient from the North Sea into the Baltic Sea. The red dashed line indicates the split between Baltic and North Sea sites.

ethanol. All other handfuls were placed in two to three ventilated plastic containers. After collection, we chose 15–17 random larvae per wrackbed from all our collections (i.e., per site), which were placed in groups of 5 in 1.5 mL Eppendorf tubes filled with 99% ethanol. All wrackbeds were highly degraded preventing the identification of different species of seaweed. As a proxy for wrackbed composition, fresh seaweed samples were also collected from the tideline (with the exception of Justøya). We blindly took 1–3 kg of fresh seaweed from each site to assess the species present and their relative abundances. All samples were transported back to Tjärnö Marine Laboratory in Sweden, where they were stored at −20°C until processing.

DNA extraction and library preparation

We separately extracted DNA from wrackbed samples and individual larvae. All remaining larvae were removed from the wrackbed samples, the wrackbed material was spun down for 10 min at 3220 rcf, and excess ethanol was poured off. The samples were flash frozen using liquid nitrogen and subsequently ground with a mortar and pestle. Two technical replicates of 0.25 g of each wrackbed sample (six extractions per site) were extracted using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA) following the manufacturer's instructions. Individual larvae were removed from the ethanol and allowed to dry before being extracted in the same manner. We extracted 10–17 larvae per site.

To examine the relationship between the microbiome and the genetic structure in *C. frigida*, we genotyped all larvae for the Cf-*Inv*(*1*) inversion which has extensive phenotypic effects (see Mérot et al., 2020 for a recent summary). In *C. frigida*, frequencies of the Cf-*Inv*(*1*) inversion vary, depending on a variety of abiotic and biotic factors, including the seaweed composition of the wrackbed (Butlin & Day, 1989; Day et al., 1983; Mérot et al., 2018). Thus, we hypothesized that there may be a relationship between larval microbiome and Cf-*Inv*(*1*) genotype. Larvae were genotyped for the Cf-*Inv*(*1*) inversion using a diagnostic single nucleotide polymorphism (SNP) by PCR and then enzyme digest as described in the study by Mérot et al. (2018).

To examine the wrackbed microbiome, we used amplicon sequencing targeting the V3–V4 loops of the bacterial and archaeal 16S genes with the 341F and 805R primers. Primer sequences can be found in Supplementary text S1. We followed the protocol from the Andersson lab (Hugerth et al., 2014) (https://github.com/EnvGen/LabProtocols/blob/master/Amplicon_dual_index_prep_EnvGen.rst) to generate individually-barcoded libraries for each of our samples (four wrackbed samples (including one technical replicate) and 15–17 larvae per site). Samples were then pooled and sequenced on one flowcell of MiSeq v3 (paired-end 300 bp reads) at the National Genomics Infrastructure in Stockholm, Sweden.

Data processing

After de-multiplexing, primer and adaptor removal, as well as trimming, were done using cutadapt enabling wildcards in reads and specifying that the first five bases of each read should be trimmed (Martin, 2011). We assembled these quality-filtered reads into error-corrected amplicon sequence variants (ASVs) (Callahan et al., 2017), using DADA2 v1.18.0 (Callahan et al., 2016), largely following the DADA2 pipeline tutorial. In brief, read quality of primer-trimmed forward and reverse reads was visualized and after manual inspections of the profiles we chose a truncation parameter of 270 bp for the forward reads and 200 bp for the reverse reads—ensuring an overlap of 45 base pairs. During quality filtering, we allowed for two (forward) and five (reverse) expected errors after trimming. This setting resulted in a median loss of 40% of the reads (interquartile range 37%–45.5%). De-replication, de-noising, and merging of the paired reads were performed using default parameters, choosing the 'pseudo' option for de-noising. After merging, sequences with a length of greater than 431 bp or shorter than 399 bp were discarded. This excluded 30% of the ASVs accounting for 2.7% of the (remaining) reads. We checked for chimeric sequences using the 'consensus' method from the removeBimeraDenovo function and all sequences identified as likely chimeras were discarded (30% of ASVs, 1% of the reads). Assembled ASVs were assigned a taxonomy using the Ribosomal Database Project (RDP) naïve classifier method (Wang et al., 2007) implemented in the assignTaxonomy function in DADA2—using the SILVA ribosomal RNA gene database v138.1 (Quast et al., 2012) as reference. Taxonomic assignment at any rank was only maintained if the taxon was assigned a probability of $>= 80\%$ (default setting) by the RDP classifier. Reads that were not classified at Kingdom level or were classified as one of Eukaryota, Chloroplast or Mitochondria were discarded (2% of ASVs, 0.7% of the reads). As an additional quality-filtering step, we aligned all sequences using the AlignSeqs function from the DECIPHER R package (Wright, 2016) and calculated the sum of the distance of each sequence to all other sequences. Visual inspection of the distribution of distance-sums revealed a group of sequences that was almost twice as different from all other sequences and the vast majority of these sequences had no taxonomic annotation at phylum level. This makes it likely that these ASVs represent non-biological sequences (e.g., undetected chimeras) or undiscovered lineages and therefore we excluded them (2% of ASVs and 0.07% of the reads).

The stringent quality filtering described above resulted in an ASV table with 13,125 unique ASVs across all samples. As a final step, we clustered the sequences to operational taxonomical units (OTUs) at 99% identity using vsearch v2.17.0 (Rognes et al., 2016) because our goal was to compare the community composition across sites and not to study any specific strains. The clustering resulted in 7775 unique OTUs. OTUs present in only a single sample or with fewer than five reads across all samples were further excluded (40% of the ASVs and 0.8% of the sequences), resulting in a final OTU table with 4655 OTUs. To account for differences in sequencing depth, we standardized our OTU table using the cumulative sum standardization method as suggested by Paulson et al. (Paulson et al., 2013). This method performed well in a comparative investigation of standardization methods (Weiss et al., 2017).

We included six pairs of technical replicates of wrackbed samples to test that our sequencing and data processing pipeline was reproducible. Each technical replicate was a separate DNA extraction from the original ground wrack sample that was processed as an individual sample. To test our pipeline, we visualized all wrackbed samples in an NMDS plot with Bray–Curtis dissimilarity using the package 'phyloseq' (McMurdie & Holmes, 2013). All technical replicates were close to their partner with the exception of a single sample from Skeie that appeared to have been mislabeled as it grouped with samples from the Smygehuk population (Figure S1). To avoid pseudoreplication, we removed one technical replicate from each pair (the one with fewer summed ASV counts) and the anomalous Skeie sample.

Statistical analysis

To examine diversity in our samples, we calculated the effective number of species of order $q = 1$. The effective number of species represents the number of species in a hypothetical community that has the same entropy (Shannon index, for $q = 1$) as the community at hand but completely even abundance. Similar to the Shannon index, it weights species by their relative abundance, but unlike the Shannon index it is a true diversity metric (see Jost, 2006 for details). We tested for differences in the effective number of species using a GLM model with a negative binomial distribution implemented with the MASS package v7.3 (Venables & Ripley, 2002) in R. Our model included sample type, salinity, and their interaction. *P* values were obtained by using the anova function on our model object.

We built a phylogenetic tree of all OTU sequences for use in downstream analyses. We used the DECIPHER R package v2.26 (Wright, 2016) to create a multiple-alignment of all of our sequences. We then staggered our alignments using the StaggerAlignment function and built an approximate-maximum-likelihood tree using FastTree (v2.1.11), then used the phangorn R package v2.11.1 (Schliep, 2010) to construct a

neighbour joining tree. Using this as a starting point, we then fitted a maximum likelihood tree assuming the GTR + G + I mutation model (generalized time-reversible with gamma rate variation).

This tree was used with the PhILR R package v1.24 (Silverman et al., 2017) to perform a Phylogenetic Isometric Log-Ratio Transformation on our data. This is a compositionally aware approach that controls for false positives by testing for the changes in log ratios between microbial abundances (called balances) that are constructed using evolutionary history (i.e., the phylogenetic tree). In other words, the phylogenetic tree is used as a sequential binary partition as an Isometric Log-Ratio transformation is applied to the data. This technique fully accounts for the correlation structure of the data as well as the compositional nature of the data (Gloor et al., 2017). After the PhILR transformation, we performed ordinations with Euclidian distance using the phyloseq package v1.42 (McMurdie & Holmes, 2013) which indicated a difference between Baltic and North Sea samples. We identified balances that separated Baltic and North Sea samples using a sparse logistic regression from the glmnet package v.4.1 (Friedman et al., 2010) implemented in R. This analysis is a penalized logistic regression, which imposes a penalty on the variables. The coefficients of less contributive variables are shrunken (minimized) by a lambda penalty. An increase in lambda means that the coefficients are shrunken to a greater extent. The lambda penalty for this regression was estimated using our data and the cv.glmnet function. We extracted the PhiLR Euclidian distance using the vegdist function implemented in the vegan package v2.6 (Dixon, 2003). We tested whether data were homogenously dispersed among groups using a PERMDISP analysis implemented in the vegan package in R (Oksanen et al., 2008).

Additionally, we used the ANCOM framework (Mandal et al., 2015) to test for differential abundance of different taxonomic groups between our factors of interest. This methodology had the lowest false discovery rate in a recent comparison study, especially with smaller sample sizes (Weiss et al., 2017). We ran ANCOM v2.0.2 on phylum abundance, as well as order abundance in two clades of interest (Bacteroidetes and Proteobacteria). Prior to analysis, all clades that made up <1% of the total count were removed. We used the default settings and our model included type of sample (wrack or larvae), location (Baltic vs. North Sea) and their interaction. We also ran a second analysis looking at the effect of sample, salinity (as a continuous variable), and their interaction.

To examine the functional structure of our data we estimated the potential functional roles of OTUs using the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database v1.2.4 and following the method of Louca, Parfrey, & Doebeli, (2016) and Louca et al. (2016). We were able to assign at least one function to 1136 of our 4655 OTUs (24%). Overall, 71 functional groupings were associated with at least one OTU, but we removed all groupings associated with fewer than three OTUs (14 groups) and groups that had a similarity of 1 (Jaccard similarity index) with one other group (9 groups) leaving 48 groups. The count given for each functional group for a given sample corresponds to the sum of the number of occurrences of each OTU within that group. We used these functional data to perform an ordination in phyloseq using Bray–Curtis distances. The Bray–Curtis ordination indicated that there were potentially significant differences in dispersion between groups. To test this, we used the vegan package in R (Oksanen et al., 2008), to perform a PERMDISP analysis. As dispersion was not homogenous between groups a PERMANOVA was not possible so we examined the relative abundance of the different functions, further removing groups that had a Jaccard similarity index of >0.75 with another group.

RESULTS

Variation in wrackbed microbiome composition over the environmental transition zone

Sampled microbiomes were highly site-specific and variable across 15 wrackbed and 74 larval samples from five sites along the North Sea to the Baltic Sea transition zone. Seaweed composition at these sites was also highly variable (Table S1). We identified 4655 OTUs, most of which were present in only a subset of samples (Figure S2); mean prevalence was 0.141 ± 0.002 across the 89 samples, and there were no OTUs present in all samples. Each sample contained between 52 and 1691 OTUs (median—610, interquartile range—651; full sample information can be found in Table S2). The distribution of OTUs across sites was non-random, only 13.9% of OTUs (646) were found in at least one sample at all five sites, whereas 28% (1304) were only found at a single site. Some OTUs were sample-type specific; 10.1% of OTUs (472) were unique to wrackbed samples and 1.4% (66) were unique to larval samples. Wrack samples grouped strongly by site (Figures S3 and S4), but larval samples overlapped somewhat. Rarefaction curves calculated from non-normalized data showed that most samples were asymptotic (Figure S5) indicating that further sequencing effort would not greatly affect the results. After calculating the effective number of species, we tested differences in the effective number of species using a GLM approach. Sample type ($df = 1$, deviance = 27.28, $P = 1.76$ E-07), salinity (deviance = 33.60, $P = 6.79$ E-09), and their interaction ($df = 1$, deviance = 11.34, $P = 7.6$ E-04) were all significant. In general, wracksamples had higher

diversity and diversity increased in larvae as salinity decreased but remained similar in wrack samples (Figure 2).

Phylum composition was similar among wrackbed sites but no significant core microbiome (here defined as OTUs with a prevalence >0.5% in at least 95% of samples) was found. We were able to assign 4625 of our OTUs (99.4%) to 30 phyla. The most abundant phylum was Proteobacteria followed by Bacteroidetes. Our ANCOM model indicated that there were clear and consistent differences between wrack and larvae in the relative abundance of different phyla (Figure 3, Table 1). Spirochaetota, Campylobacterota, and Bacteroidota were more abundant in wrack samples while Actinobacteria and Patescibacteria were more abundant in larval samples (Table 1A). However, using our criteria, there was no strong core wrack nor larval microbiome. Two OTUs, were found in >95% of larval samples but had prevalence <0.5% in at least one fourth of the samples. In wrack, only 28 OTUs were found in >95% of the samples and none comprised >0.5% of the counts in even half of the samples. ANCOM also revealed differences between Baltic and North Sea sites in the relative abundance of different phyla (Table 1A). Spirochaeta was more prevalent in the Baltic while Proteobacteria was more prevalent in the North Sea samples. Additionally, Actinobacteriota had a significant interaction between sample type and site type (Figure S6A): There was little difference between sample type at Baltic sites but a higher prevalence in larvae at North Sea sites. A model replacing location (Baltic vs. North Sea) with salinity had identical interaction effects but slightly different main effects (Table S3).

As Bacteroidetes and Proteobacteria were the dominant phyla (Figure 3), we examined lower classification levels as well. An examination of the order distribution within Bacteroidetes revealed that the Flavobacteriales order was more prevalent in larval and in North Sea samples (Table 1B) while the Bacteroidales order was more dominant in the Baltic samples (Figure S7). Flavobacteriales also showed an interaction between sample type and site type with no difference in the Baltic but higher prevalence in larvae in North Sea samples (Table 1B, Figure S6B). Finally, orders of Proteobacteria also showed significant differences using the ANCOM framework. Sphingomonadales and Legionellales were more prevalent in larvae while Xanthomonadales was more prevalent in the Baltic (Table 1C, Figure S8). Sphingomonadales and Enterobacterales had significant interactions (Table 1C). Sphingomonadales was more prevalent in Baltic wrack samples but showed a much weaker effect of sample type in North Sea sites (Figure S6C). Enterobacterales was more prevalent in larvae in North Sea samples but showed an opposite but weak effect in Baltic samples (Figure S6D). Full data prevalence and counts for all OTUs can be found in Table S4.

Effects of changing microbiome community composition on seaweed fly larvae

Ordination of samples after the PhILR transformation revealed effects of site and sample type on community composition. Although all sites showed overlap between wrack and larval samples, wrack samples grouped more strongly showing significantly lower dispersion (Figure 4A, Table S5A). We observed an effect of salinity with sites decreasing in salinity along the first axis representing 23.3% of the variation. When the two first axes were viewed together, we observed a split between Baltic and North Sea samples (Figure 4C). Due to significantly different dispersions for all of these groups (Table S5A–C), we did not perform any statistical tests.

The functional community profile indicated differences between sample types as well as a distinction between Baltic and North Sea sites. FAPROTAX identified chemoheterotrophy and fermentation as the most abundant categories (Figure S9). These results are in line with the known role of bacteria in the wrackbed but since FAPROTAX is a computational approximation of functionality, these results should be taken as a working hypothesis to be tested with metagenomic data. Unlike the phylum composition, there were no consistent functional differences between wrack and larval microbiomes. However, ordination based on functional categories revealed that larvae and wrack overlapped in functional space and that microbiomes of North Sea larvae were more variable (Figure 4B). A PERMDISP analysis confirmed this difference as significant preventing further analysis with a PERMANOVA (Table S5D). There were also major changes in variance depending on the site (Figure 4D) with samples from the two Baltic sites (Smygehuk and Ystad) tightly clustering together. A PERMDISP analysis confirmed that both site and location (e.g., Baltic vs. North Sea) were significant predictors of dispersion (Table S5E,F).

We further investigated the differences between the Baltic and North Sea sites by identifying balances that distinguish the two groups. Balances are log-ratios of the geometric mean abundances of the two separate groups of taxa that descend from a node. Using a sparse logistic regression, we identified eight significant balances (Figure 5) at different levels of taxonomy. The deepest node was the one separating Alphaproteobacteria from the other Proteobacteria (n16). The other seven significant nodes were at lower taxonomic levels (Figure 5B).

There was no pattern connecting microbiome composition and genotype at Cf-*Inv*(*1*) in the *C. frigida* larvae. We were able to genotype 57 of the 74 larval samples and found an over-abundance of heterozygotes (38/57) consistent with previous studies (Day

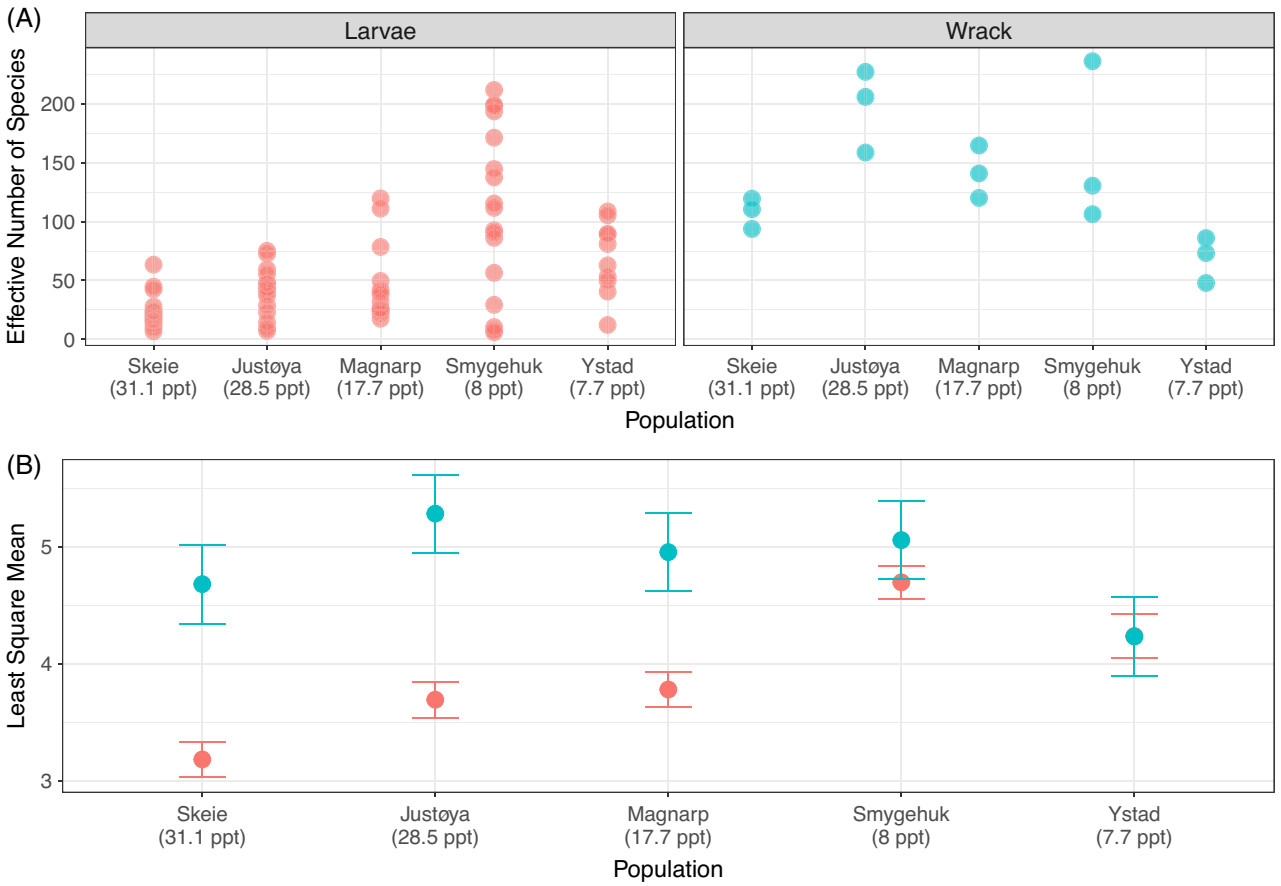


FIGURE 2 (A) Effective number of species ($e^{shannon\ index}$). Samples are coloured by type, red-larvae, blue-wrack and labelled by site (with salinity in ppt listed underneath). (B) Least square means from the GLM model. Colours indicate sample type (red-larvae, blue-wrack) and error bars indicate standard errors.

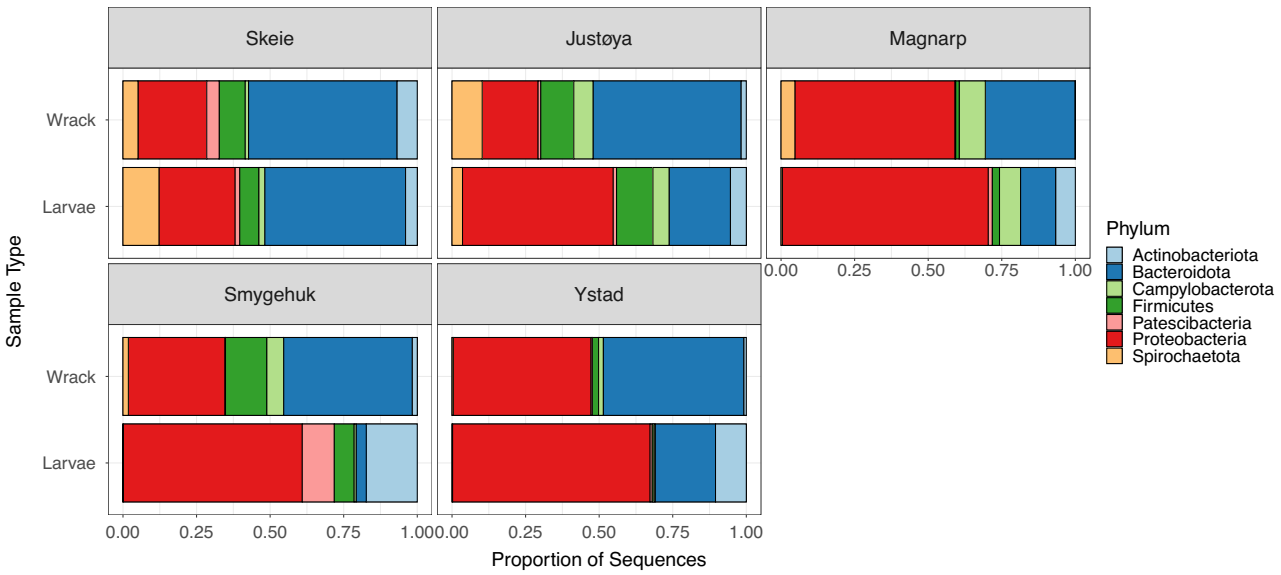


FIGURE 3 Taxonomic composition by site and sample type. All unassigned OTUs (30/4655) have been removed. All phyla that made up less than 1% of total counts have been removed.

et al., 1983). In particular, larvae from Ystad could not be genotyped, potentially due to low DNA yields as they were particularly small. Ordination after PhiLR transformation revealed no pattern based on genotype (Figure S10) although genotype sampling was not consistent between sites (Table S2).

TABLE 1 ANCOM results for (A) all phyla, (B) Bacteroidetes orders and (C) Proteobacteria orders.

	Larvae versus Wrack			Baltic versus North Sea			Interaction		
(A) Phylum	**LFC**	***P***	***Q***	**LFC**	***P***	***Q***	**LFC**	***P***	***Q***
Proteobacteria	−0.175769	0.65608231	0.8856118	**−1.3969683**	**0.00068688**	**0.00412126**	0.24067393	0.65353107	1
Firmicutes	0.40061867	0.4428059	0.8856118	0.87320853	0.03516892	0.15768083	−0.0041154	0.99517239	1
Actinobacteriota	−2.6461582	**0.00030805**	**0.00154023**	−1.0171575	0.03153617	0.15768083	**2.92317302**	**0.00103596**	**0.0072517**
Patescibacteria	−1.5328518	**0.00624497**	**0.02497989**	−0.7716209	0.15148102	0.45444305	2.04732896	0.02427819	0.14566917
Spirochaetota	2.55732282	**0.00018431**	**0.00110588**	**3.08695533**	**9.78E-09**	**6.85E-08**	−2.0386223	0.03485103	0.17425515
Campylobacterota	1.61264252	**0.00644868**	**0.02497989**	0.54512274	0.33988246	0.67976493	−1.2520546	0.10896374	0.43585494
Bacteroidota	1.40836336	**0.00011502**	**0.00080516**	−0.0407972	0.92244121	0.92244121	−0.0742704	0.86737719	1
	Larvae versus Wrack			**Baltic versus North Sea**			**Interaction**		
(B) Order	**LFC**	***P***	***Q***	**LFC**	***P***	***Q***	**LFC**	***P***	***Q***
Chitinophagales	−0.0584426	0.8894356	1	0.04991507	0.885034	1	0.21016726	0.69666911	1
Bacteroidales	0.38959403	0.54718866	1	**2.29507042**	**6.98E-08**	**2.10E-07**	−0.1386243	0.86626403	1
Flavobacteriales	**−1.3938755**	**5.99E-05**	**0.00023946**	**−1.7186334**	**3.82E-08**	**1.53E-07**	**1.54244896**	**0.00120486**	**0.00481945**
Cytophagales	−0.124063	0.81991295	1	−0.0848266	0.83748334	1	−0.2726623	0.66737863	1
	Larvae versus Wrack			**Baltic versus North Sea**			**Interaction**		
(C) Order	**LFC**	***P***	***Q***	**LFC**	***P***	***QI***	**LFC**	***P***	***Q***
Rhodobacterales	−0.0559295	0.90931338	1	0.70940462	0.10094179	0.50470897	−1.0269745	0.07264548	0.43587287
Sphingomonadales	**−2.1363058**	**2.13E-05**	**0.0001488**	−0.7926528	0.12562776	0.50470897	**2.34640595**	**0.00069339**	**0.00554715**
Legionellales	**−3.7000703**	**5.22E-08**	**4.18E-07**	−0.627196	0.32730114	0.65460227	0.90689876	0.27336446	1
Burkholderiales	−0.1916605	0.76635847	1	0.02718924	0.94681769	0.94681769	−0.3312108	0.68757456	1
Enterobacterales	1.27376942	0.09470251	0.47351254	1.09306373	0.03200234	0.19201404	**−2.4086507**	**0.00601759**	**0.04212312**
Pseudomonadales	0.08305229	0.88017578	1	−0.7235645	0.1101916	0.50470897	−0.458107	0.52685692	1
Xanthomonadales	0.11898911	0.85435393	1	**2.53968271**	**5.02E-07**	**4.01E-06**	0.30311633	0.71521542	1
Rhizobiales	−0.9025545	0.04820461	0.28922764	1.03896133	0.00817026	0.0571918	−0.0423965	0.93698624	1

Note: Bold indicates significant *Q* values ($Q < 0.1$).
Abbreviations: LFC, log fold change; P, *p*-value; Q, FDR controlled Q value.

DISCUSSION

Understanding the structure and function of complex microbial communities, and how they vary across space and time, is a major goal of microbial ecology. Here, we report on the taxonomic and functional composition of the wrackbed microbiome as well as the microbiome of one common wrackbed bacterivore across the North Sea–Baltic Sea environmental transition zone. To our knowledge, this is the first report on the microbiome of deposited beach wrack at the OTU level. We observed strong overlap between sites following PhILR transformation but were unable to identify a core microbiome. We also observed a separation between Baltic and North Sea sites in both taxonomic composition and functional variance, consistent with the environmental gradient in that zone, although sampling of additional sites will be necessary to confirm this association and experimental work will be needed to establish causation. Below we discuss these patterns and their potential causes and consequences.

Sites differed at the OTU level but overlapped somewhat in PhILR ordination space. We were not able to find a strong core microbiome for the wrackbed; none of the 24 OTUs found in all wrack samples were highly prevalent (prevalence >0.5%) in even half of the samples. This result may be partly explained by the idea of 'functional redundancy', that is, the idea that different metabolic functions can be performed by a wide range of taxa (Burke et al., 2011; Louca et al., 2018). This hypothesis is supported by the fact that the functional composition of the wrack samples overlapped substantially (Figure 4D), while the samples were completely separated in the Bray–Curtis NMDS (Figure S1) based on the species composition. In line with other studies, we suggest that colonization of the wrackbed environment is likely a neutral process occurring via random dispersal (Hubbell, 2006) with certain

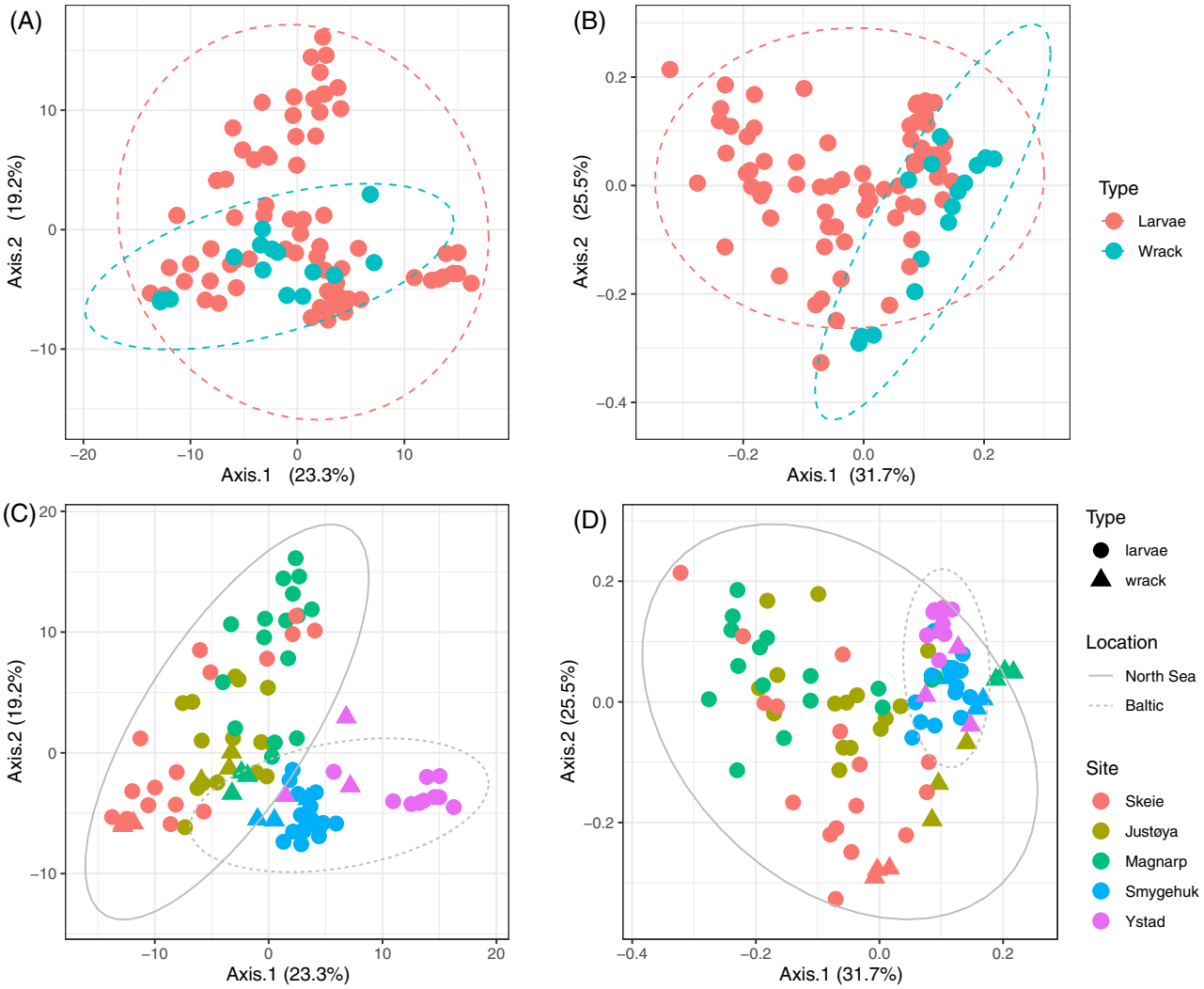


FIGURE 4 Baltic samples are compositionally and functionally separate. Ordination of samples after PhILR transformation (A, C) and ordination based on functional categories (B, D). Samples are coloured by type (A, B) or by site (C, D). Sample type is indicated by shape in (C) and (D). Dashed and solid lines indicate 95% ellipses.

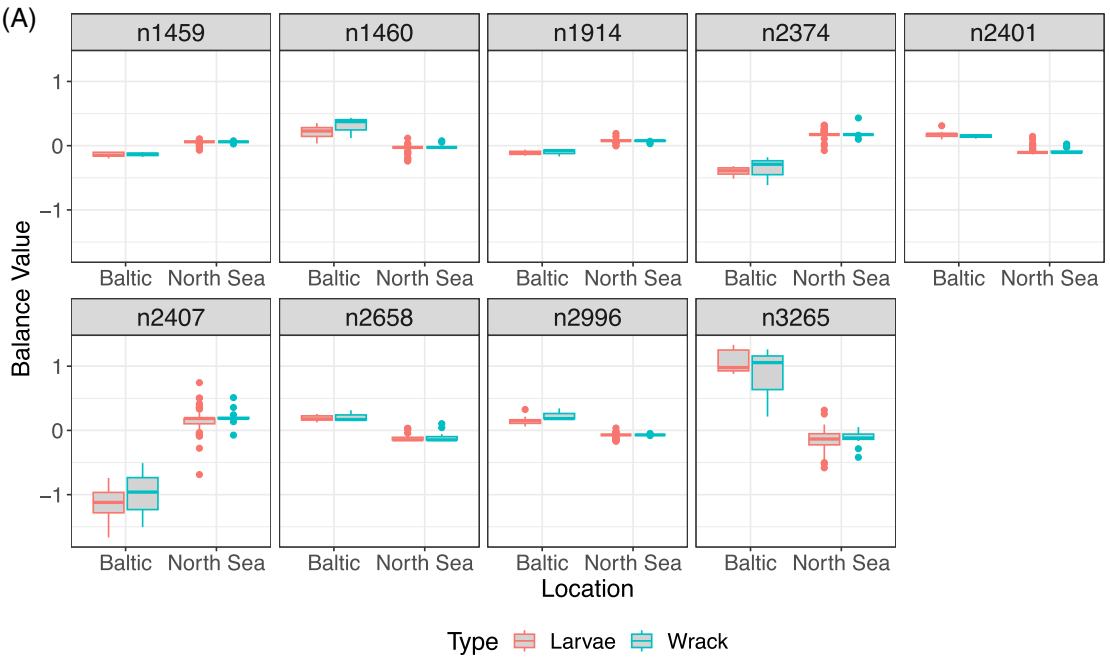
OTUs becoming abundant due to their functional properties (Burke et al., 2011; Louca et al., 2018). However, we note that we are not able to test this directly with our data. We were not able to determine the origin of these microbes (i.e., the path of colonization), although it is likely that a large number of them originated from the macroalgal microbiomes. A recent study on kelp detritus on the seafloor found that the microbiome shifted greatly as the kelp degraded (Brunet et al., 2021), which may obscure the signal of origin.

The similarity between the North Sea wrack sites in PHiLR ordination space is surprising as microbial communities are often highly dynamic (Louca, Jacques, et al., 2016; Tully et al., 2018). We suggest that the functional space of the wrackbed may be narrower than in other environments and that this may impose taxonomic constraints. Some functions are more redundant than others, for example, photoautotrophy is a more redundant function than sulfate respiration (Louca, Parfrey, & Doebeli, 2016), and the wrackbed environment likely requires a large number of specific functions. For example, macroalgae contain secondary metabolites, such as phlorotannins, which are polyphenolic compounds unique to brown seaweed (Glombitza & Kno, 1992; Hierholtzer et al., 2013). These phlorotannins are often used as chemical defences and are known inhibitors of anaerobic digestion systems (Chen et al., 2008). Macroalgae also contain complex polysaccharides, the degradation of which requires highly specialized enzymes (Chauhan & Saxena, 2016; Sichert et al., 2020).

Polysaccharides are major structural components in macroalgae and can comprise up to 50% of macroalgal biomass (Mabeau & Kloareg, 1987). Our results show that chemoheterotrophy was the major functional category in all samples (Figure S9) and we found a compositional abundance of polysaccharide degraders. Note that polysaccharide degradation is not a functional category in FAPROTAX. Members of the phylum Bacteroidetes are the primary polysaccharide degraders in marine environments (Arnosti et al., 2021; Fernández-Gomez et al., 2013), although Gammaproteobacteria (Sarmento et al., 2016), Planctomycetales (Reintjes et al., 2017), and Verrucomicrobia (Sichert et al., 2020) are also known polysaccharide degraders. Bacteroidetes was the second most abundant phylum and Proteobacteria was the most abundant phylum (Figure 3) with 59%–96% of Proteobacteria in wrackbeds being Gammaproteobacteria. Bacteroidetes comprised 28%–65% of the wrackbed microbiome compared to <10%–30% in many ocean water samples (Alonso-Sáez & Gasol, 2007; Okamoto et al., 2022; Sunagawa et al., 2015), 10%–20% in sand (Okamoto et al., 2022), and 8%–25% in interstitial tidal communities (Okamoto et al., 2022). However, Bacteroidetes are more common in macroalgal epiphytic bacterial communities (Florez et al., 2017) and are highly abundant on particulate organic matter (POM) (Fernández-Gomez et al., 2013). Overall, our functional and compositional data suggest that these polysaccharide degraders may be a dominant component of the wrackbed microbiome (Figure 3).

Given the abundance of Bacteroidetes and Gammaproteobacteria, we hypothesized that the specific polysaccharide composition of the nearshore macroalgal community, and so of the wrackbed, may be a major force shaping the microbial community. While all macroalgae contain polysaccharides, different groups of macroalgae contain different polysaccharides and the concentration can range from 4% to 76% of the dry weight (Kraan, 2012). For example, in brown algae, alginate can represent up to 60% of the total cell wall polysaccharides (Mabeau & Kloareg, 1987). In red algae, the most common polysaccharides are agarose and carrageenan (Popper et al., 2011) while porphyran is limited to the red alga *Porphyra* (Kraan, 2012). Green algae contain sulphated polysaccharides such as ulvan, which is a cell wall polysaccharide present in species of *Ulva* (Kidgell et al., 2019). Different carbohydrate-active enzymes (CAZymes) are needed to catabolize these compounds (Lombard et al., 2014). A unique feature of Bacteroidetes genomes is that CAZymes are organized into polysaccharide utilization loci (PULs) that encode co-regulated enzyme and protein complexes for degradation of specific polysaccharides (Grondin et al., 2017). Different species of Bacteroidetes contain different PULs specific to categories of polysaccharides (Grondin et al., 2017). Closely related species of *Bacteroides* have been shown to be highly specialized on specific polysaccharide bonds, even to the point of neglecting the simple sugars these polysaccharides are built from (Martens et al., 2011). There is a strong shift from brown and green to red algae between the North Sea and the Baltic sites that likely corresponds to a shift in wrackbed polysaccharide composition from ulvan, fucoidans, and alginate to agarose and carrageenan. This is accompanied by shifts in taxonomic composition of Bacteroidetes. Four of the nine significant balances identified by our PhiLR analysis are within Bacteroidetes (n2401, n2658, n4401, and n2374; Figure 5) and an additional two are in Gammaproteobacteria (n3265, n996). Furthermore, an examination of the class distribution within Bacteroidetes showed that the Flavobacteriales order was more prevalent in North Sea sites while the Bacteroidales order was more dominant in the Baltic sites (Figure S7). Within Gammaproteobacteria, Xanthomondales was more prevalent in the Baltic Sea (Figure S8). We note that we are only inferring function from 16S data, not directly analysing functional data. Thus, a metagenomic analysis looking at the frequency of different PULs and other polysaccharide degrading enzymes in different wrackbeds will be necessary to test this link formally.

Compared to wrack samples, larval samples showed higher variation within site and lower site-specific signatures. Both the Bray–Curtis ordination



(B)

Node ID	Balance	Numerator	Denominator	Taxonomic level
n1459	Order Gastranaerophilales/Order Gastranaerophilales	no information	no information	Class
n1460	Order Gastranaerophilales/Order Gastranaerophilales	no information	no information	Class
n1914	Class_Alphaproteobacteria/Phylum_Proteobacteria	Alphaproteobacteria	all other Proteobacteria	Class
n2374	Family_Paludibacteraceae/Genus_Paludibacter	all other Paludibacteracae	Paludibacter	Genus
n2401	Genus_Bacteroides/Genus_Bacteroides	no information	no information	Species
n4401	Genus_Prevotella_7/Family_Prevotellaceae	no information	*Prevotella, Prevotella_7, Prevotella_9*	Genus
n2658	Genus_Flavobacterium/Genus_Flavobacterium	no information	no information	Species
n996	Order_Eneterobacterales/Class_Gammaproteobacteria	Enterobacterales	all other Gammapretobacteria	Order
n3265	Family_Pseudomonadaceae/Family_Pseudomonadaceae	all other genera	*Pseudomonas*	Genus

FIGURE 5 Balances separating Baltic and North Sea sites. Balances represent the log-ratio of the geometric mean abundance of the two groups of taxa that descend from the given node. Internal nodes are numbered from base of the tree to the tips. (A) Boxplot showing the distribution of these balances for Baltic and North Sea wrack samples (blue) and larval samples (red). The number above corresponds to the node. (B) Full data on each node with information on taxonomic groups that make up the numerator and denominator (when available) and the taxonomic level of the split.

(Figure S3) and the PhILR approach (Figure 4A) showed that wrack samples tended to separate by site, but most larval samples did not. Only larvae from the Baltic sites (Ystad and Smygehuk) seemed to group by site. The functional analysis and PERMDISP also showed that larvae were more functionally variable than wrack samples, although seemingly with the exception of larvae from Ystad and Smygehuk (Figure 4D, Table S5A). Furthermore, no single ASV was present in every larval sample and although genotype sampling was highly uneven, none of the observed variance could be explained by genotype at the Cf-*Inv* (*1*) inversion (Figure S10). Some of this variation may be explained by experimental design: Wrackbeds are highly heterogeneous and contain many microhabitats, and the sampled larvae may have come from any number of these microhabitats whereas wrack samples were homogenized before sequencing, destroying the microhabitat structure. Another potential source of variation is larval age. Larvae were taken directly from the field sites and were a variety of ages. Studies in honeybees (Vojvodic et al., 2013), leafworms and bollworms (Mason et al., 2020), and silkworms (Chen et al., 2018) shows that there are strong shifts in larval microbiomes across instars.

Despite the high variation in the larval samples, we observed consistent wrack-larval differences in the prevalence of Actinobacteria, Patescibacteria, Spirochaetota, Campylobacterota, and Bacteroidota (Figure 3, Table 1). Our observed data include a combination of the bacteria that provide nutrition for larvae, that is, bacteria that the larvae have ingested, along with their own resident microbiome. As larval microbiomes do not directly match wrack microbiomes, it is clear that there is some level of selection in regards to either (1) which bacteria the larvae are eating and/or (2) which bacteria are colonizing and are becoming established in the gut. As polysaccharides are degraded, digestive enzymes as well as sugar oligomers and monomers can be released (Allison, 2005; Arnosti et al., 2021; Teeling et al., 2012). These can then be used by a wide variety of organisms including cheater or scavenger bacteria that cannot digest polysaccharides themselves. For example, in terrestrial ecosystems, detritivores

show strong preferences for microbe digested substrates (Frainer et al., 2016). The relative rates by which *C. frigida* larvae consume bacteria (and if they preferentially consume certain bacteria) versus metabolic byproducts of these bacteria is still unknown. However, it is possible that they preferentially take up simple sugars and other easily used nutrients. More detailed studies of wrackbed microbial ecology and the economics of polysaccharide degradation are clearly needed.

The observed Baltic–North Sea split coincides with numerous other physical and biological changes occurring over the same spatial gradient (Snoeijs-Leijonmalm et al., 2017). Perhaps the most powerful physical driver of biological systems that varies across this gradient is salinity, which ranges from 8 to 10 psu in the southern Baltic Sea up to >30 psu in the North Sea and can vary seasonally (Møller Nielsen et al., 2016). Our data show that salinity can explain a large proportion of the major axis of variation in our samples (Figure 4), but this association may not be causal. This and other biological gradients can have powerful effects on the marine life of the region (Pearson et al., 2000), resulting in observed genetic breaks in many species (Johannesson et al., 2020), a pattern which holds true in our data. However, we note that we only sampled five sites along the gradient and this pattern might change with more intensive sampling. Despite this caveat, the observed pattern is consistent with dependence of the wrackbed environment on the seaweeds that grow nearby. Still it is remarkable that these differences are sustained through multiple trophic linkages and spatial subsidy events as the seaweed is washed ashore and degrades.

CONCLUSION

Wrackbeds are biogeochemical hotspots where a combination of microbes and grazers degrade stranded seaweed and provide the base of a complex food web. Polysaccharides make up the major component of algal carbon and our results indicate that the wrackbed microbiome is specialized for polysaccharide degradation. Furthermore, the microbiome composition potentially alters based on the polysaccharides present. This change of microbiome composition co-occurs with a strong change over a natural marine environmental transition zone (the entrance of the Baltic Sea), which may be directly influenced by the changes in abiotic factors like salinity, or indirectly through the changing seaweed community, which is controlled by those abiotic factors. This shift carries up through trophic levels to the microbiome of seaweed fly larvae although larvae were more variable than the wrackbed itself. However, no connection between genotype at the Cf-*Inv*(*1*) inversion and larval microbiome was found, indicating that the wrackbed microbiome may not be a driver of selection on this inversion. The microbial food web of the wrackbed is potentially very complex, but studies of wrackbeds are currently in their infancy and the diverse roles of the various bacterial groups remain a black box at present.

AUTHOR CONTRIBUTIONS

Emma Berdan: Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Fabian Roger:** Data curation (equal); formal analysis (equal); methodology (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Maren Wellenreuther:** Conceptualization (equal); funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Alexandra Kinnby:** Writing – original draft (supporting); writing – review and editing (equal). **Gunnar Cervin:** Writing – review and editing (equal). **Ricardo Pereyra:** Methodology (supporting); resources (supporting); writing – review and editing (equal). **Mats Topel:** Conceptualization (equal); writing – review and editing (equal). **Kerstin Johannesson:** Conceptualization (equal); funding acquisition (supporting); project administration (equal); supervision (lead); writing – review and editing (equal). **Roger Butlin:** Conceptualization (equal); formal analysis (supporting); funding acquisition (supporting); methodology (equal); writing – review and editing (lead). **Carl Andre:** Funding acquisition (lead); methodology (supporting); project administration (lead); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

We declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Trimmed reads with adaptors removed are available on the NCBI SRA (BioProject PRJNA881340). Analysis scripts are available at the Harvard Dataverse (https://doi.org/10.7910/DVN/FUIOG9).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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