Received: 1 December 2021 | Revised: 21 February 2022 | Accepted: 30 March 2022

DOI: 10.1111/mec.16462

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Salinity and host drive *Ulva*-associated bacterial communities across the Atlantic–Baltic Sea gradient

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Funding information
Portugese nation fund from Foundation for Science and Technology, Grant/Award Number: UIDB/04326/2020; Fonds Wetenschappelijk Onderzoek, Grant/Award Number: 3F020119; Formas national research program for food, Grant/Award Number: 2020-03119; European Marine Biological Resource Centre Belgium, Grant/Award Number: FWO project I001621N

Handling Editor: Henrik Krehenwinkel

Abstract

The green seaweed *Ulva* is a model system to study seaweed–bacteria interactions, but the impact of environmental drivers on the dynamics of these interactions is little understood. In this study, we investigated the stability and variability of the seaweed-associated bacteria across the Atlantic–Baltic Sea salinity gradient. We characterized the bacterial communities of 15 *Ulva sensu lato* species along 2,000 km of coastline in a total of 481 samples. Our results demonstrate that the *Ulva*-associated bacterial composition was strongly structured by both salinity and host species (together explaining between 34% and 91% of the variation in the abundance of the different bacterial genera). The largest shift in the bacterial consortia coincided with the horohalinicum (5–8 PSU, known as the transition zone from freshwater to marine conditions). Low-salinity communities especially contained high relative abundances of *Luteolibacter*, *Cyanobium*, *Pirellula*, *Lacihabitans* and an uncultured Spirosomaceae, whereas high-salinity communities were predominantly enriched in *Litorimonas*, *Leucothrix*, *Sulfurovum*, *Algibacter* and *Dokdonia*. We identified a small taxonomic core community (consisting of *Paracoccus*, *Sulfitobacter* and an uncultured Rhodobacteraceae), which together contributed to 14% of the reads per sample, on average. Additional core taxa followed a gradient model, as more core taxa were shared between neighbouring salinity ranges than between ranges at opposite ends of the Atlantic–Baltic Sea gradient. Our results contradict earlier statements that *Ulva*-associated bacterial communities are taxonomically highly variable across individuals and largely stochastically defined. Characteristic bacterial communities associated with distinct salinity regions may therefore facilitate the host's adaptation across the environmental gradient.

KEYWORDS
bacterial communities, Baltic Sea, microbiome, salinity gradient, *Ulva*

1 | INTRODUCTION

Bacteria are of vital importance to marine multicellular organisms and often play a crucial role throughout their host's life (Bordenstein & Theis, 2015; McFall-Ngai et al., 2013). Seaweeds—important primary producers in coastal ecosystems worldwide—likewise depend on their associated microbiota for optimal functioning, including nutrient exchange, defence mechanisms and reproduction (Egan et al., 2013; Weinberger et al., 2007). The algal host and its associated microbiome are often referred to as a holobiont: a single ecological unit (Egan et al., 2013). The members of these ecological units are connected through complex interactions on multiple levels (Pita et al., 2018). The dynamics of the seaweed holobiont, however, are little understood—especially with regard to environmental drivers (Egan et al., 2013; van der Loos et al., 2019).

The green seaweed *Ulva* is a model to study algae–bacteria interactions (Califano et al., 2020; Kessler et al., 2018; Wichard et al., 2015). *Ulva* relies on specific bacterial partners to obtain its typical morphology (e.g., a blade that is two cells thick or a tube that is one cell thick). In the absence of these specific bacteria, *Ulva* merely grows as a loose aggregation of cells without rhizoids or proper cell wall development. In addition to morphogenesis, bacteria are known to promote *Ulva* growth (Gemin et al., 2019), induce settlement of zoospores (Joint et al., 2000; Patel et al., 2003) and affect the biochemical composition of the seaweed (Polikovsky et al., 2020).

As with other seaweeds, the entire spectrum of interactions between *Ulva*, its associated microbiome and the environment remains largely unknown. Studies so far have only addressed variation in *Ulva*-associated bacterial diversity across small and larger geographical scales (see, e.g., Burke et al., 2011; Roth-Schulze et al., 2018; Tujula et al., 2010), but not across environmental gradients. In the absence of an explicit environmental gradient, neutral or stochastic processes are more likely to drive microbial community structure, thus causing high among-individual variation (Adair & Douglas, 2017). In the presence of an environmental gradient, deterministic mechanisms (i.e., environmental selection) could govern variation in microbial composition (Adair & Douglas, 2017; Martiny et al., 2006). Indeed, previous studies of *Ulva*-associated bacteria with samples taken from one or a few locations have highlighted high levels of intra-individual variation (Burke et al., 2011; Roth-Schulze et al., 2018). Other studies, however, found distinct differences among sampling habitats and *Ulva* host species (Comba González et al., 2021; van der Loos et al., 2021).

Closely related to questions on the *variability* of the *Ulva* microbiome across environmental gradients, is the question on its *stability* (the "core" microbiome). Identifying stable key microbes is important to define "healthy" microbial communities and—especially with regard to spatial and temporal distribution—gain insight into ecological functions (Risely, 2020). Bonthond et al. (2020), for example, identified various prokaryotic and eukaryotic core taxa associated with the red alga *Gracilaria vermiculophylla* on a global scale in both native and introduced populations. This implies that *Gracilaria's* core taxa either have a worldwide distribution, or have been co-introduced with their host during the invasion process. The bacterial communities of the introduced Mediterranean *Caulerpa taxifolia* likewise showed high similarity to the communities of the native populations in eastern Australia (Arnaud-Haond et al., 2017; Meusnier et al., 2001). Core microbes may even facilitate successful introductions (Bonthond et al., 2021). Bacteria probably play an important role in acclimatization and adaptation of *Ulva* to environmental changes, as has been demonstrated in the filamentous brown alga *Ectocarpus*, which depends on bacterial communities for acclimatization to salinity changes (Dittami et al., 2016). Incorporating an environmental gradient can, therefore, provide information on the stochastic vs. deterministic mechanisms controlling the variability and stability of microbial composition in general.

A study on the global, environmental distribution of bacterial diversity marked salinity as the most important driver of bacterial community composition, surpassing the effects of temperature and pH (Lozupone & Knight, 2007). Salinity gradients are often studied in estuaries, but estuarine environments are dynamic and the constant mixing of water bodies causes unstable gradients. The Baltic Sea is the world's largest inland brackish sea and one of the most widely studied coastal areas. This area represents a relatively young (8,000 years), semi-enclosed postglacial sea that stands out by a successive transition from fully marine conditions of the North Sea (Northeast Atlantic) towards a near freshwater state in its innermost parts (Reusch et al., 2018). The lack of tides, as well as the freshwater influx on one side of the gradient combined with limited exchange with North Sea water, allow for stable salinity regions over a large geographical distance. In addition, water retention time in the brackish central Baltic is high (between 3 and 30 years), especially compared to the more dynamic estuaries formed at river mouths (Herlemann et al., 2011). This makes the Baltic Sea an excellent area to study salinity gradients.

The steepest salinity change in the Baltic Sea can be observed at the Danish Straits (Johannesson et al., 2020), and species diversity and distribution are strongly defined by the prevailing salinity regime (Ojaveer et al., 2010). Marine species diversity generally decreases with decreasing salinity, while simultaneously freshwater species increase in number and abundance (Ojaveer et al., 2010). Consequently, only few marine species successfully establish along this entire environmental gradient (Johannesson et al., 2020). Although salinity does not affect bacterial species richness in seawater- and sediment-associated communities in the Baltic, it is a strong driving force behind bacterial community structure and composition (Herlemann et al., 2011; Klier et al., 2018). Work on bacterial communities in the Baltic region has been limited to bacterioplankton, bacteriobenthos and bacteria as components of animal diets (Herlemann et al., 2011; Klier et al., 2018; Skrodenytė-Arbačiauskienė et al., 2021), while host-associated bacteria have rarely been investigated across the entire salinity gradient. The question therefore remains how host-associated bacterial communities are influenced by a gradual environmental transition, and whether the host itself or the prevailing salinity conditions have a larger effect on the associated microbiomes.

This study aims to (i) characterize the dynamics of seaweed-associated bacterial communities as a function of both host and a stable salinity gradient, and (ii) assess whether we can define a taxonomic core community across the Atlantic–Baltic Sea gradient. We sampled 481 *Ulva sensu lato* individuals along 2,000 km of coastline,

spanning the full 3.5–36 PSU salinity gradient in the Baltic Sea and adjacent areas. To examine to what extent the ecological dynamics of the *Ulva*-associated bacterial communities are driven by ecological factors and host species, we generated full-length 16S rRNA gene amplicon sequences with Oxford Nanopore Technologies. Previous studies on *Ulva*-associated bacteria indicated that between-site effects were more important than between-species effects, probably due to the high morphological similarity and close phylogenetic relatedness between *Ulva* species (van der Loos et al., 2021). We therefore hypothesize that *Ulva*-associated bacterial community composition in the Baltic Sea is primarily established under the influence of the prevailing salinity gradient and secondarily affected by host species.

2 | MATERIALS AND METHODS

2.1 | Study area, field collection and sample preparation

Samples of *Ulva sensu lato* individuals ($n = 481$, including *Ulva*, *Blidingia* and *Kornmannia*) used in the present study were collected along the full salinity gradient present in the Baltic Sea and adjacent areas such as the Kattegat, Skagerrak and the eastern North Sea (Figure 1). *Ulva* species are commonly found in the Baltic Sea and on the Northeast Atlantic coast, and are known for their high tolerance towards fluctuations in salinity (Rybak, 2018). Under high nutrient conditions, some species are known to cause nuisance blooms (Smetacek & Zingone, 2013). *Ulva* species are difficult to identify based on their simple morphological characteristics due to the high plasticity within species and high morphological similarities among species. Over 10 species of *Ulva* have previously been identified based on genetic markers in the Baltic area (Steinhagen et al., 2019). Many of these species occur in sympatry and can be found in a wide variety of habitats (Leskinen et al., 2004; Steinhagen et al., 2019). *Ulva* has an isomorphic diplohaplontic life cycle. Morphologically, the gametophytic and sporophytic phases cannot be reliably distinguished (Wichard, 2015). The life stage of the individuals sampled in this study was therefore not checked.

In total, 146 sampling sites, of which 63 in Denmark, 53 in Sweden, 25 in Norway and five in Germany, were visited during summer 2020 (June–September; see also Table S1). The salinity ranged from 3.5 to 36 PSU and is presented in the figures in this study either on a continuous scale (0–36) or in salinity zones defined according to the Venice classification system (0.5–5 = oligohaline, 5–8 = horohalinicum, 8–18 = mesohaline, 18–30 = polyhaline, and 30–36 = euhaline) (Alves et al., 2009; Bleich et al., 2011; Hu et al., 2016). In addition, both water temperature (°C) and oxygen levels (mg L^{-1}) were measured at each site (Figures S1 and S2).

A variety of habitats, such as rock pools, harbours, estuaries, fjords, drain channels as well as exposed and sheltered coastal areas,

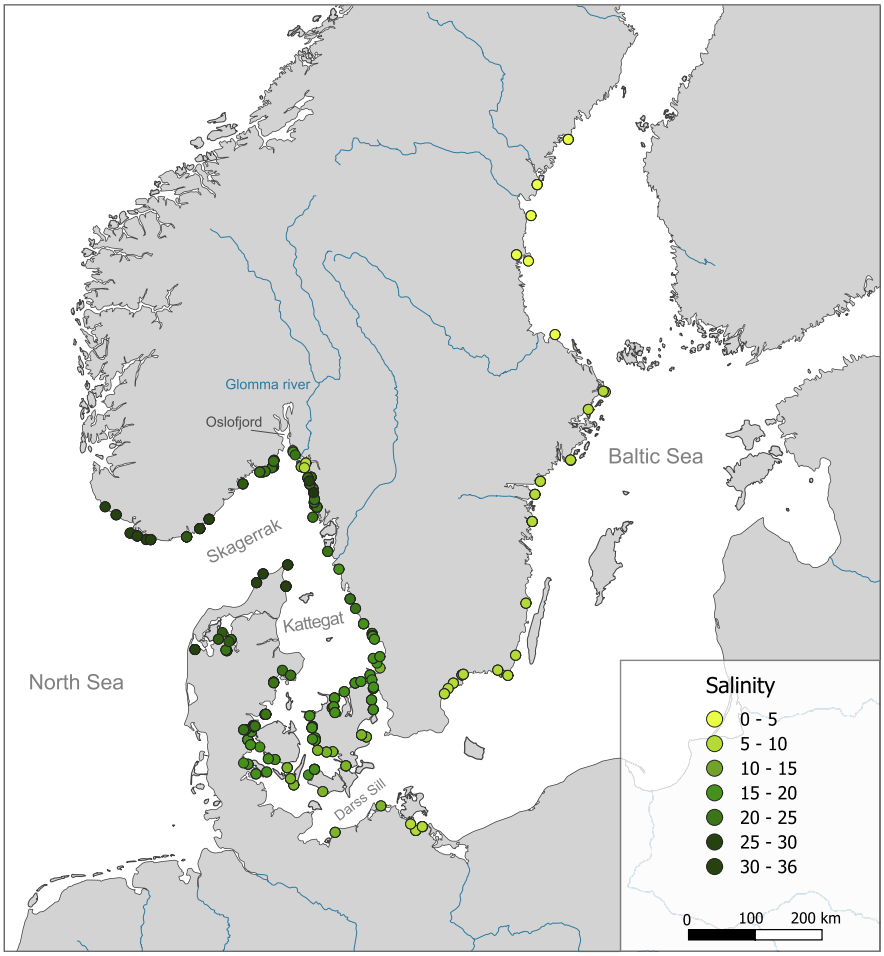


FIGURE 1 Geographical distribution of all 146 sampling sites in the Baltic Sea and adjacent areas (eastern North Sea, Skagerrak and Kattegat) where samples were collected. The colour of the sites corresponds to the measured salinity. Major rivers are shown in blue.

were visited. The different substrates (organic and inorganic) of the attached thalli were recorded. Sampling was performed in the supra- and midlittoral zones using waders, which allowed for sampling to a depth of ~1.5 m below mean sea level. Additional samplings of the mid- and infralittoral zones of chosen sites were conducted via snorkelling. At each site, representative specimens of each morphotype and all observed populations were collected from the supralittoral to the sublittoral (in horizontal transects of ~50 m depending on site accessibility), including drifting and epiphytic green algae. All sampling work in the respective countries was conducted by a single person to ensure repeatability among sites. Sterilized disposable gloves and sterilized equipment were used throughout the sampling procedure to minimize contamination. After rinsing each individual with ~30–50 ml sterile water to remove dirt, a cotton swab sample for microbiome analyses was generated by rubbing for 30 s on the tissue.

Furthermore, to enable DNA barcoding of the host, clean and epiphyte-free tissue samples (~1 cm^2) of the respective individuals were collected. All samples were stored in a portable freezer (−20°C) until transfer to −80°C in the laboratory.

2.2 | Molecular identification of the algae host

Genomic DNA was extracted from lyophilized host tissue using the Invisorb Spin Plant Mini Kit (Stratec) following the manufacturer's protocol and stored at −80°C. The *tufA* gene was used for species identification of the host. PCR (polymerase chain reaction) amplicons were successfully generated for 461 samples following Steinhagen et al. (2019). The PCR products were first assessed by gel electrophoresis and subsequently purified using the QIAquick PCR Purification Kit (Qiagen). Sanger sequencing of the purified amplicons was performed by Eurofins Genomics. Forward and reverse sequence reads were assembled in SEQUENCHER (version 4.1.4, Gene Codes Corporation). Using the BLAST function in GenBank, first identifications via the specimens' *tufA* sequences were made. To better resolve species identities, a set of peer-reviewed and annotated reference sequences downloaded from GenBank were used in subsequent phylogenetic analyses. Host species were identified according to the latest taxonomic revisions by Hughey et al. (2021). A multiple sequence alignment was constructed using MAFFT (Katoh et al., 2002). An optimal substitution model (GTR+G+I) was determined using MRMODELTEST software version 2.2 (Nylander, 2004). Subsequently, a maximum-likelihood analysis was performed using RAXML (version 8; Stamatakis, 2014) with 1,000 bootstrap iterations. All sequences are publicly available in GenBank (see Table S1 for accession numbers).

2.3 | Molecular characterization of the microbial communities

Bacterial communities were characterized with Oxford Nanopore sequencing following van der Loos et al. (2021). In short, total microbial DNA was extracted with the Qiagen DNeasy mini kit following the manufacturer's protocol, with the addition of a bead beating step before lysis using zirconium oxide beads (RETCH Mixer mill MM400; 5 min at 30 Hz). The full-length 16S rRNA gene was amplified using the primers 27F_BCtail-FW (TTTCTGTTGGTGCTGATATTGC_AGAGTTTGATCMTGGCTCAG) and 1492R_BCtail-RV (ACTTGCCTGTCGCTCTATCTTC_CGGTTACCTTGTTACGACTT), each containing a 5′ extension allowing for subsequent barcoding by PCR. 16S rDNA PCRs were performed using the Phire Tissue direct PCR Master Mix (Thermo Fisher) and amplicons for each sample were barcoded using the Oxford Nanopore "PCR Barcoding Expansion Pack 1-96 (EXP-PBC096)". A total of 481 *Ulva*-associated samples were processed in nine PCRs and the final libraries were prepared with the ligation-based sequencing kit SQK-LSK109 according to the manufacturer's protocol (Oxford Nanopore Technologies). The libraries were subsequently sequenced in six separate sequencing runs on a MinION (with R10.3 flow cells, Oxford Nanopore Technologies) for 72 hr each. Six negative extraction samples were included in this study, as well as nine negative PCR controls, and four positive controls (ATCC microbial standard MSA-1002). In addition, two randomly chosen samples (DK043 from Denmark and NO118 from Norway) were included in all PCRs and divided over the six sequencing runs to verify comparability across PCRs and sequencing runs.

The resulting raw FAST5 reads were basecalled and demultiplexed with GUPPY (version 5.0.7, sup model, Oxford Nanopore Technologies). Data quality and length were first visually inspected with NANOPLOT (De Coster et al., 2018). Subsequently, high-quality reads were obtained using chimaera removal with YACRD (Marijon et al., 2020), and by filtering the data set on quality (Q-score >8) and length (1,000–2,000 bp) with NANOFILT (De Coster et al., 2018). The resulting 23,955,915 high-quality reads were used to assign taxonomy at the genus level with KRAKEN2 in combination with the SILVA 16S database (138.1 release) (Lu & Salzberg, 2020; Quast et al., 2013). In the presented results and figures, we use the nomenclature as implemented in the SILVA database. The sequences are archived at SRA (BioProject PRJNA781821).

After taxonomic assignment, all chloroplast reads (3% of the high-quality reads) were removed from the data set. In addition, rare taxa were discarded (optimal settings based on the positive controls retained operational taxonomic units [OTUs] found more than 70 times in at least 20% of the samples) to protect against OTUs with small mean and trivially large coefficients of variation. Finally, DESEQ2 was used to account for sequencing depth with a variance stabilizing transformation (Love et al., 2014).

2.4 | Statistical analyses

To assess genus-level differences in bacterial composition, Bray–Curtis dissimilarities were calculated and visualized with an NMDS ordination (Bray & Curtis, 1957). Smooth surface lines were fitted to the ordination with the ORDISURF function (VEGAN package) based on the correlation with salinity. The effect of salinity, host species, temperature, oxygen

levels and habitat (substrate from which the host was collected, being either rock, sand, concrete, epiphytic/epizoic, metal, plastic, wood/rubber/rope or drift samples) on community composition was tested using the ENVFIT function of the VEGAN package with 9,999 permutations (model included all factors, with $p < .05$ considered significant) (Oksanen et al., 2020). Multivariate comparisons with 9,999 permutations were made with pairwise ADONIS (Martinez Arbizu, 2020) among all salinity zones and among all host species. A Mantel test was subsequently used to evaluate the correlation between the bacterial community dissimilarity matrix (at the genus level) and the phylogenetic host species distance matrix. Alpha diversity was calculated as the observed genus richness, as well as by using the Shannon Index, Simpson Index and Chao1 Index (Jost, 2007; Willis, 2019). Differences in alpha diversity with salinity were assessed using a generalized linear mixed model based on a negative binomial family ($p < .05$ considered significant). The model included salinity, host species and habitat, as well as the interaction between salinity and host. All categorical variables (host and habitat) were included as random effects.

Significantly differential abundant bacterial genera ($p < .01$, Benjamini–Hochberg corrected) were identified with DESEQ2 (model included salinity, host species and habitat, as well as the interaction between salinity and host) (Love et al., 2014). The amount of explained variation in abundance was quantified using the LME4 (Bates et al., 2015) and VARIANCEPARTITION (Hoffman & Schadt, 2016) packages with a generalized linear mixed model fitted to a negative binomial family (model included salinity, host species and habitat, as well as the interaction between salinity and host, and all categorical variables were included as random effects).

There are many different ways to define and calculate the core microbiome of a given data set (Risely, 2020; Shade & Handelsman, 2012). Both core composition and size differ with relative abundance and prevalence (the number of samples in which the taxa were encountered) threshold settings, and as such defining a "core" microbiome remains relatively arbitrary. Here, the variation in core size (number of core taxa) was calculated for a range of different relative abundances (0.1%–100%) and prevalences (50%–90%) using the MICROBIOME R package (Lahti & Shetty, 2017).

All statistical tests were performed in R (R Core Team, 2020) and data were visualized using the GGPLOT2 (Wickham, 2016), METACODER (Foster et al., 2017) and PHYLOSEQ (McMurdie & Holmes, 2013) packages.

3 | RESULTS

3.1 | Taxonomic identification of host species

A total of 461 *Ulva sensu lato* samples were processed for species discrimination and identification based on *tufA* sequence data. The full data set was subject to phylogenetic analyses to allow for robust identification of host species (see Table S1). The phylogenetic analyses separated the investigated specimens into 15 well-delimited taxonomic entities, including members of the genera *Blidingia*, *Kornmannia* and *Ulva*. Eight entities of the genus *Ulva* could be resolved based on peer-reviewed reference sequences provided by GenBank. Five entities (represented by a total of 25 samples) could not be resolved to species level due to the absence of any similar GenBank entries.

More specifically, the taxa were identified as *Blidingia minima* (Nägli ex Kütz.) Kylin; see also Steinhagen et al. (2021) ($n = 25$ samples), *Kornmannia leptoderma* (Kjellmann) Bliding ($n = 14$), *Ulva australis* Areschoug ($n = 2$), *Ulva compressa* Linnaeus ($n = 48$), *Ulva fenestrata* Postels & Ruprecht ($n = 36$), *Ulva intestinalis* Linnaeus ($n = 116$), *Ulva lacinulata* (Kützing) Wittrock ($n = 32$), *Ulva linza* Linnaeus ($n = 128$), *Ulva prolifera* O.F. Müller ($n = 7$), *Ulva torta* (Mertens) Trevisan ($n = 28$), and unidentified *Ulva* sp. 1 ($n = 1$), *Ulva* sp. 2 ($n = 15$), *Ulva* sp. 3 ($n = 2$), *Ulva* sp. 4 ($n = 4$) and *Ulva* sp. 5 ($n = 3$).

Distinct distribution patterns across the salinity gradient were recorded for the host species. Corroborating previous studies focusing on different taxa, most of the green algal species investigated were absent east of the Danish Straits. *Ulva intestinalis* and *U. linza* showed the widest distribution and were present across the whole salinity gradient (present from 3.5 to >30 PSU). For details on the species distribution see Table S2.

3.2 | Bacterial alpha diversity associated with *Ulva sensu lato*

After filtering out rare taxa (using optimal settings based on the positive controls), we identified 96 bacterial genera, belonging to 28 families and 24 orders, associated with *Ulva*, *Blidingia* and *Kornmannia*. Highly abundant orders across all *Ulva sensu lato* species included the Rhodobacterales, Sphingomonadales, Rhizobiales and Flavobacteriales. Alpha diversity did not change with salinity when calculated as either observed richness ($p = .09$, $z = 1.71$; negative binomial model), or a Shannon Index ($p = .55$, $z = 0.59$; negative binomial model), Simpson Index ($p = .89$, $z = 0.14$; negative binomial model) or Chao1 Index ($p = .27$, $z = 1.11$; negative binomial model).

3.3 | Effect of environment and host species on bacterial community

Bacterial community composition differed significantly with salinity ($p < .001$, $R^2 = .48$) and host species ($p < .0001$, $R^2 = .34$). While pairwise comparisons showed that the oligohaline (0–5 PSU) and horohalinicum (5–8 PSU) shared similar bacterial communities ($p = .816$, $F = 1.77$; pairwise Adonis test), pairwise contrasts among all other salinity zones showed significant differences in bacterial communities (with $p < .001$ for all comparisons; pairwise Adonis test, Table S3). Pairwise comparisons among all host species indicated that, amongst others, *U. linza* and *U. intestinalis* were associated with different bacterial communities ($p = .01$, $F = 25.97$; pairwise Adonis test), as well as *U. compressa* vs. *U. fenestrata* ($p = .01$, $F = 6.09$; pairwise Adonis test) and *U. compressa* vs. *U. lacinulata* ($p = .02$, $F = 5.03$;

pairwise Adonis test). On the contrary, similar bacterial communities were shared between *U. compressa* vs. *U. torta* ($p = .24$, $F = 3.66$; pairwise Adonis test), and *U. prolifera* vs. *U. torta* ($p = 1.00$, $F = 2.23$; pairwise Adonis test). See Table S4 for full statistics.

NMDS plots likewise showed a clear ordination influenced by the salinity gradient as well as host species (Figure 2). This salinity effect was not only observed along the larger Atlantic–Baltic Sea gradient, but also on local scales (e.g., caused by freshwater river input). Sample sites located south in the Oslofjord (Norway, Skagerrak Strait) near the mouth of the Glomma river, for example, have a lower salinity compared to the predominantly higher surrounding salinity levels (Figure 1). Bacterial community composition in these sites was generally more similar to samples collected in distant, low-salinity sites in the Baltic Sea than to samples collected at neighbouring sites in the Skagerrak (Figure S3).

Both habitat ($p < .0001$, $R^2 = .09$) and temperature ($p < .001$, $R^2 = .05$) were found to be significant as well, but with very low explanatory values. Several outliers in the NMDS plot, however, can be explained by habitat. For example, the bacterial communities of two *U. intestinalis* samples collected in high-salinity rock pools located 2 and 10 m away from the main waterbody were more similar to lower salinity communities (Figure 2). The salinity of such rock pools is expected to vary considerably with rainfall and evaporation. Samples collected from green tides (mass accumulation events, $n = 8$), belonging to *U. compressa*, *U. lacinulata* and *U. intestinalis*, were distinctly different from the general host species patterns (Figure 2). Oxygen levels did not have a significant effect on bacterial community composition ($p = .69$, $R^2 \approx 0$).

3.4 | Differentially abundant bacteria

The largest shift in bacterial community composition was observed passing the horohalinicum (salinity 5–8 PSU; Figure 3a). This shift in community composition was attributed mostly to large differences in abundance, rather than presence/absence patterns. Lower salinity communities were enriched in Cyanobiaceae ($p < .0001$), Rubritaleaceae ($p = .0002$), Sphingomonadaceae ($p = .0002$) and Spirosomaceae ($p < .0001$) (contrasts between 0–5 PSU and 30–36 PSU, all *p*-values Benjamini–ochberg corrected; Figure 3a). High-salinity communities were characterized by high relative abundances of amongst others Alteromonadaceae ($p < .0001$), Granulosicoccaceae ($p = .001$), Hyphomonadaceae ($p < .0001$), Sulfurovaceae ($p < .0001$) and Thiotrichaceae ($p < .0001$) (contrasts between 0–5 PSU and 30–36 PSU, all *p*-values Benjamini–Hochberg corrected; Figure 3a). These differences become more pronounced when comparing oligohaline communities with increasingly higher salinity communities (i.e., the differences between the euhaline and oligohaline form a starker contrast than the differences between the mesohaline and oligohaline).

A total of 70 bacterial genera were differentially abundant with changing salinity levels (with $p < .01$, Benjamini–Hochberg corrected; see Table S5 for an overview of all $\log_2$fold change and *p*-values). Low-salinity communities especially contained high relative abundances of *Luteolibacter* (Rubritaleaceae), *Cyanobium* (Cyanobiaceae), *Pirellula* (Pirellulaceae), *Lacihabitans* (Spirosomaceae) and an uncultured Spirosomaceae (Figure 3b). High-salinity communities were predominantly enriched in *Litorimonas* (Hyphomonadaceae), *Leucothrix* (Thiotrichaceae), *Sulfurovum* (Sulfurovaceae), *Algibacter* and *Dokdonia* (both Flavobacteriaceae) (Figure 3b; Figure S4).

As *U. intestinalis* and *U. linza* co-occurred over the entire salinity gradient from the North Sea to the Baltic Sea (Table S2), they provided a good opportunity to assess differences in host species. Both *Ulva* species contained high relative abundances of *Luteolibacter* and *Lacihabitans* in low-salinity sites, but low-salinity communities of *U. intestinalis* were further characterized by *Pirellula*, *Rhizobium* and an uncultured Spirosomaceae, whereas *U. linza* communities mainly contained *Cyanobium*, *Flavobacterium* and *Pseudorhodobacter* (Figure 3c,d). Likewise in high-salinity environments, both host species had high abundances of *Algibacter*, but *U. intestinalis* had significantly more *Litorimonas*, *Sulfurovum*, *Rubritalea* and an uncultured Flavobacteriaceae with increasing salinity. *U. linza*, on the other hand, typically contained more *Leucothrix*, *Glaciecola*, *Dokdonia* and *Alteromonas* in high-salinity environments (Figure 3c,d).

When comparing the bacterial communities of *Ulva* species (Ulvaceae) with the more distantly related *Kornmannia leptoderma* (Kornmanniaceae), *Ulva* harboured significantly higher abundances of *Algitalea*, *Marinagarivorans* and *Algibacter* compared to *K. leptoderma*, whereas the latter typically contained more *Cellulophaga*, *Sulfurovum* and *Altererythrobacter* ($p < .01$, Benjamini–Hochberg corrected). Compared to *Blidingia minima* (Kornmanniaceae), *Ulva* was enriched in *Rubritalea*, *Algitalea* and *Roseitalea*, while *Phormidesmis*, *Roseibacillus* and *Jannaschia* were associated with *Blidingia* ($p < .01$, Benjamini–Hochberg corrected).

Despite host species having a clear effect on the associated bacteria, the correlation between host phylogeny and bacterial community composition was very weak (Mantel test, $p = .004$, $r = .03$).

3.5 | Variance partitioning

Salinity, host species and habitat together explained 34%–91% of the variation in the abundance of the bacterial genera (Figure 4; Figure S5). In concordance with the differential abundance analyses (based on $\log_2$fold change), the variation was best explained for *Lacihabitans* (91% of the variation explained), *Leucothrix* (86%), *Algitalea* (84%), *Dokdonia* (84%), *Luteolibacter* (83%) and *Algibacter* (81%). For most genera, the interaction between salinity and host species explained the highest proportion, followed by the single effects of salinity and host species (Figure 4). Salinity explained much of the variation for *Litorimonas* (39%) and *Cyanobium* (29%), whereas host species explained a high proportion of the variation in *Mesorhizobium* (49%, especially abundant in *Ulva linza*), *Roseitalea* (47%, less abundant in *Blidingia* and *Kornmannia*), *Fuerstia* (44%, especially abundant in *U. compressa*, *U. fenestrata* and *U. lacinulata*), *Ensifer* (43%, enriched in *Kornmannia*),

Marinagarivorans (40%, enriched in *Kornmannia*) and *Jannaschia* (31%, enriched in *Blidingia*).

Habitat explained little of the variation in most genera, except for *Roseivivax* (61%) and *Olleya* (21%) (Figure 4). Additional DESEQ2 analyses indicated *Roseivivax* was especially abundant on algae collected from sandy habitats and *Olleya* on algae growing on metal. Although relatively few samples in green tide events were collected ($n = 8$), patterns could be distinguished. For example, the green tide

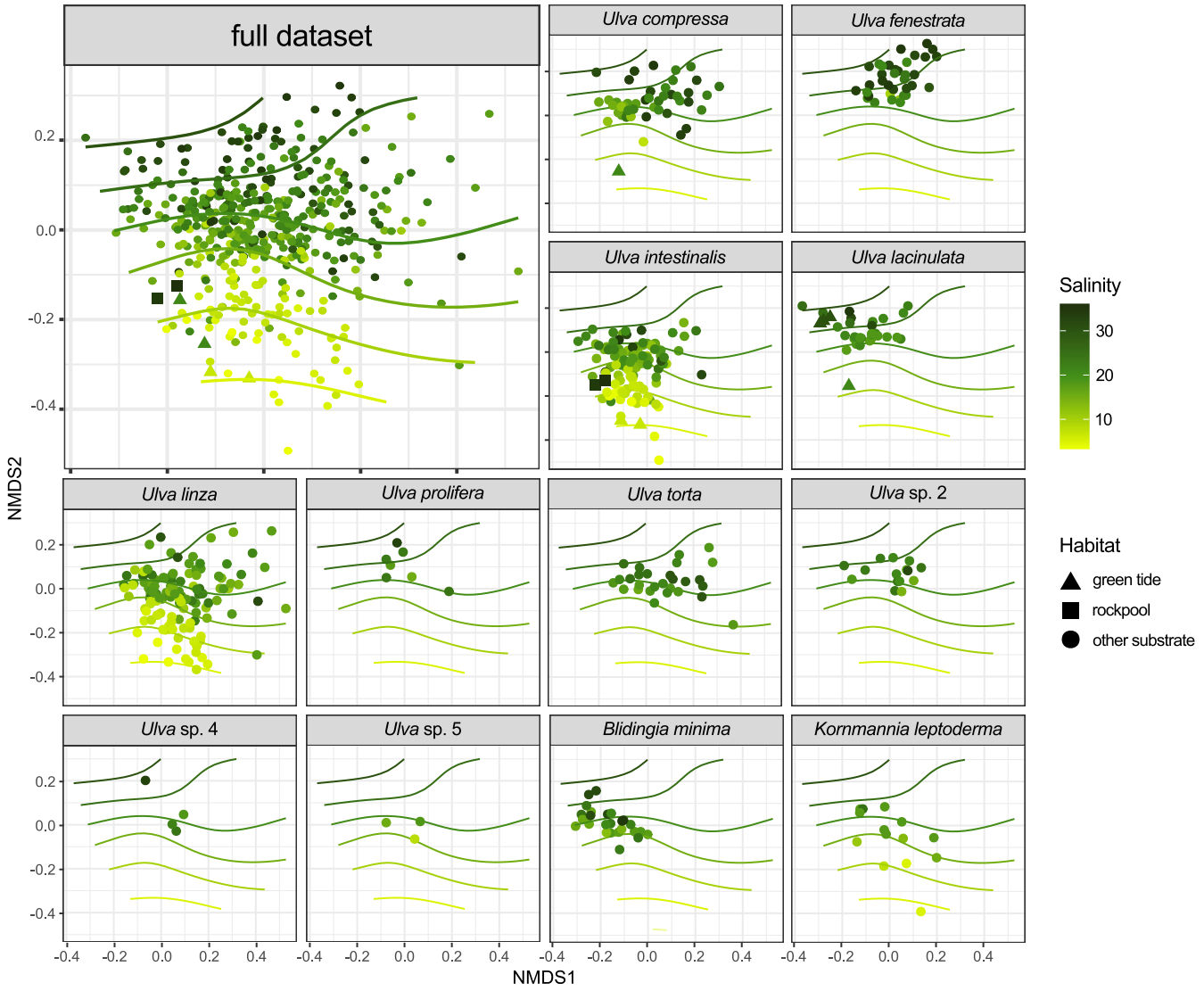
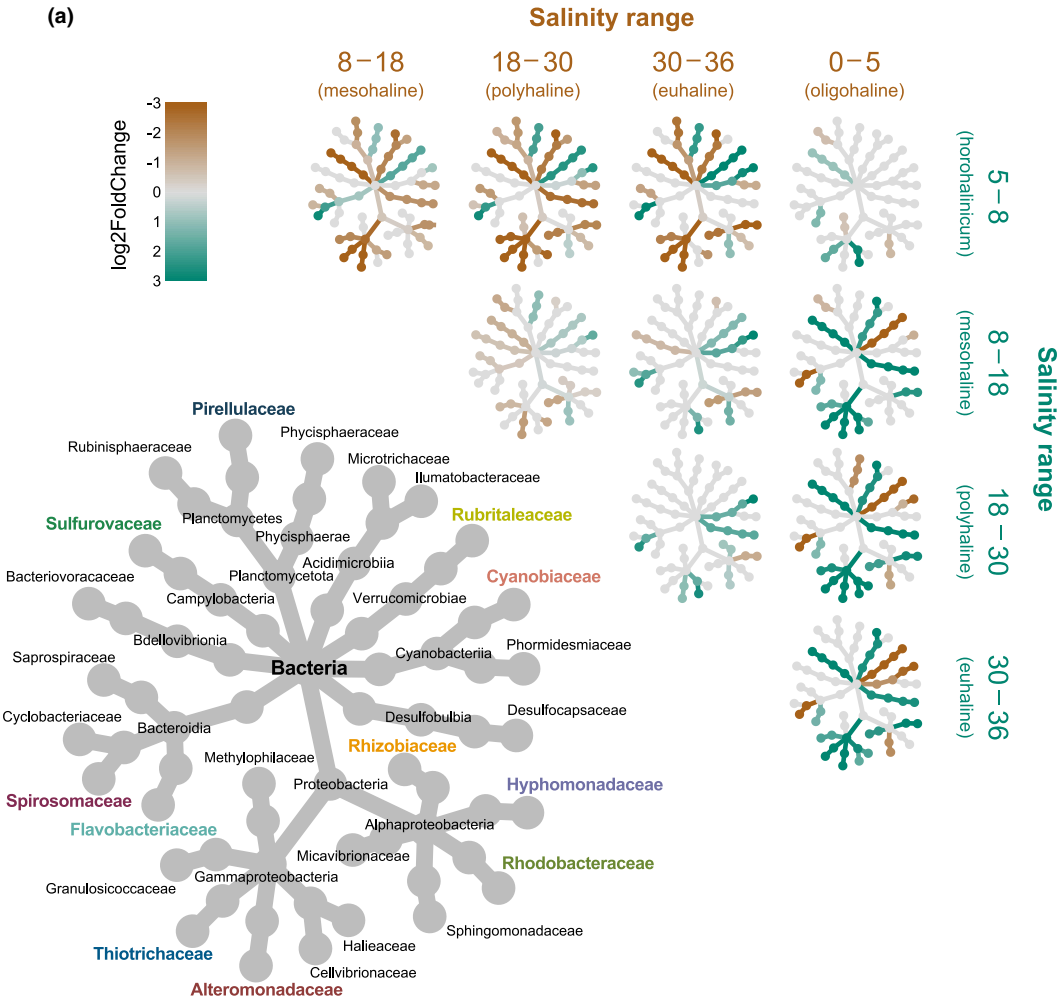
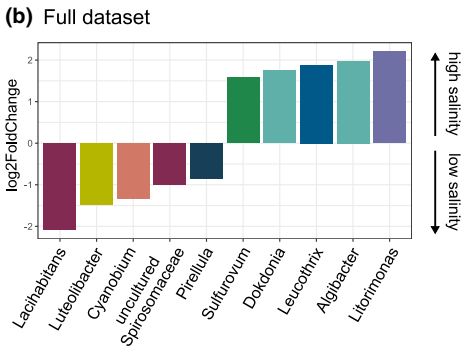
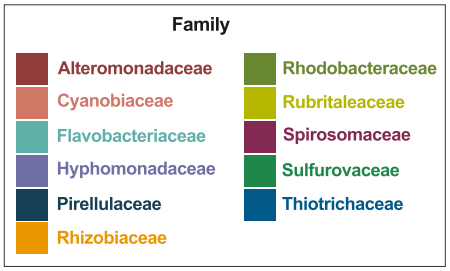


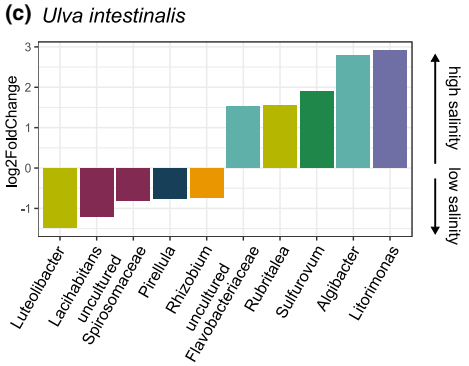
FIGURE 2 NMDS plots (stress =0.01, $k = 4$) of *Ulva sensu lato* associated bacterial community composition (based on Bray–Curtis dissimilarities and genus-level identifications). The first panel shows the full data set ($n = 481$ samples). The remaining panels are split by host species (*Ulva compressa*, *U. fenestrata*, *U. intestinalis*, *U. lacinulata*, *U. linza*, *U. prolifera*, *U. torta*, *Ulva* sp. 2, *Ulva* sp. 4, *Ulva* sp. 5, *Blidingia minima* and *Kornmannia leptoderma*). Note that separate plots for *U. australis*, *Ulva* sp. 1, and *Ulva* sp. 3 are not shown due to the few data points collected for these species. Colours represent salinity and symbols represent the habitat of the host species. The contour lines (smooth surface lines) are fitted to the ordination based on the correlation with salinity.

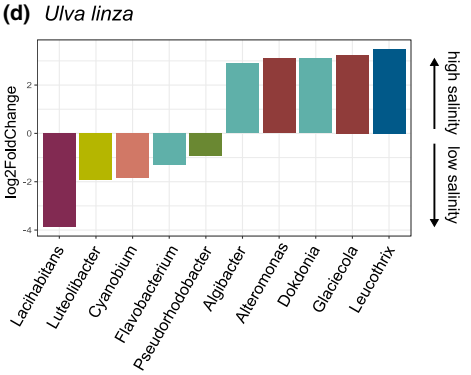
FIGURE 3 Overview of the significantly differentially abundant bacterial families and genera associated with *Ulva sensu lato* across Atlantic–Baltic salinity ranges. (a) Pairwise comparisons of phylogenetic heat trees depicting the 28 bacterial families associated with *Ulva*, *Blidingia* and *Kornmannia*. The larger, grey tree on the lower left functions as a taxonomic key for the smaller unlabelled trees. The smaller trees provide contrasts between five salinity zones: 0–5 PSU (oligohaline), 5–8 PSU (horohalinicum), 8–18 PSU (mesohaline), 18–30 PSU (polyhaline) and 30–36 PSU (euhaline). The colour (brown to green) of the nodes and edges corresponds to the $\log_2$fold change (only significant differences are coloured, $p < .05$, Benjamini–Hochberg corrected). Taxa coloured brown are enriched in the salinity zones in columns, whereas taxa coloured green are enriched in salinity zones in rows. For example, Rubritaleaceae, Spirosomaceae and Cyanobiaceae are enriched in the oligohaline (brown) compared to most of the higher salinity zones (green). (b–d) Bar graphs of the top 10 differentially abundant genera between high and low salinity, based on (b) the full data set when controlled for host species, (c) *Ulva intestinalis* samples only and (d) *Ulva linza* samples only. The $\log_2$fold change is expressed on the *y*-axis and genus on the *x*-axis. Colours of the bars correspond to family level (similar colours as used in the phylogenetic heat tree).


(a)
Salinity range
8−18 (mesohaline)
18−30 (polyhaline)
30−36 (euhaline)
0−5 (oligohaline)
log2FoldChange
-3
-2
-1
0
1
2
3
5−8 (horohalinicum)
8−18 (mesohaline)
18−30 (polyhaline)
30−36 (euhaline)
Salinity range
Pirellulaceae
Phycisphaeraceae
Rubinisphaeraceae
Microtrichaceae
Ilumatobacteraceae
Sulfurovaceae
Planctomycetes
Rubritaleaceae
Phycisphaerae
Acidimicrobiia
Bacteriovoracaceae
Planctomycetota
Cyanobiaceae
Campylobacteria
Verrucomicrobiae
Bdellovibrionia
Phormidesmiaceae
Saprospiraceae
Cyanobacteriia
Bacteria
Cyclobacteriaceae
Desulfobulbia
Desulfocapsaceae
Bacteroidia
Rhizobiaceae
Methylophilaceae
Proteobacteria
Hyphomonadaceae
Spirosomaceae
Flavobacteriaceae
Alphaproteobacteria
Micavibrionaceae
Rhodobacteraceae
Gammaproteobacteria
Granulosicoccaceae
Sphingomonadaceae
Thiotrichaceae
Halieaceae
Cellvibrionaceae
Alteromonadaceae


(b) Full dataset
log2FoldChange
high salinity
low salinity
Lacihabitans
Luteolibacter
Cyanobium
uncultured
Spirosomaceae
Pirellula
Sulfurovum
Dokdonia
Leucothrix
Algibacter
Litorimonas


Family
Alteromonadaceae
Cyanobiaceae
Flavobacteriaceae
Hyphomonadaceae
Pirellulaceae
Rhizobiaceae
Rhodobacteraceae
Rubritaleaceae
Spirosomaceae
Sulfurovaceae
Thiotrichaceae


(c) Ulva intestinalis
log2FoldChange
high salinity
low salinity
Luteolibacter
Lacihabitans
uncultured
Spirosomaceae
Pirellula
Rhizobium
uncultured
Flavobacteriaceae
Rubritalea
Sulfurovum
Algibacter
Litorimonas


(d) Ulva linza
log2FoldChange
high salinity
low salinity
Lacihabitans
Luteolibacter
Cyanobium
Flavobacterium
Pseudorhodobacter
Algibacter
Alteromonas
Dokdonia
Glaciecola
Leucothrix

in Gryt on the Baltic coast of Sweden (salinity = 7.0 PSU), consisted of *Ulva intestinalis* (n = 2 samples). These Gryt green tide algal microbiomes were mainly characterized by the abundant presence of *Rhodopirellula* and *Rubripirellula* (both Planctomycetota) compared to the bacterial communities of non-green tide *U. intestinalis* specimens collected at the same site or neighbouring sites (n = 3 samples) (Figure S6). The green tide in Frederikshavn in Denmark (salinity = 30.0 PSU) was caused by *Ulva lacinulata*. Compared to *U. lacinulata* specimens growing attached in the same harbour (n = 2), the green tide communities (n = 3) were enriched in *Thiothrix*, *Limibaculum*, *Pseudophaeobacter*, *Octadecabacter* and *Sulfitobacter* (all Proteobacteria) (Figure S6).

3.6 | Bacterial core

The number of core taxa and members of the core bacterial community varied tremendously depending on the threshold settings of relative abundance and prevalence (percentage of samples in which the taxon occurs) (Figure 5). When setting the limits to ≥0.1%

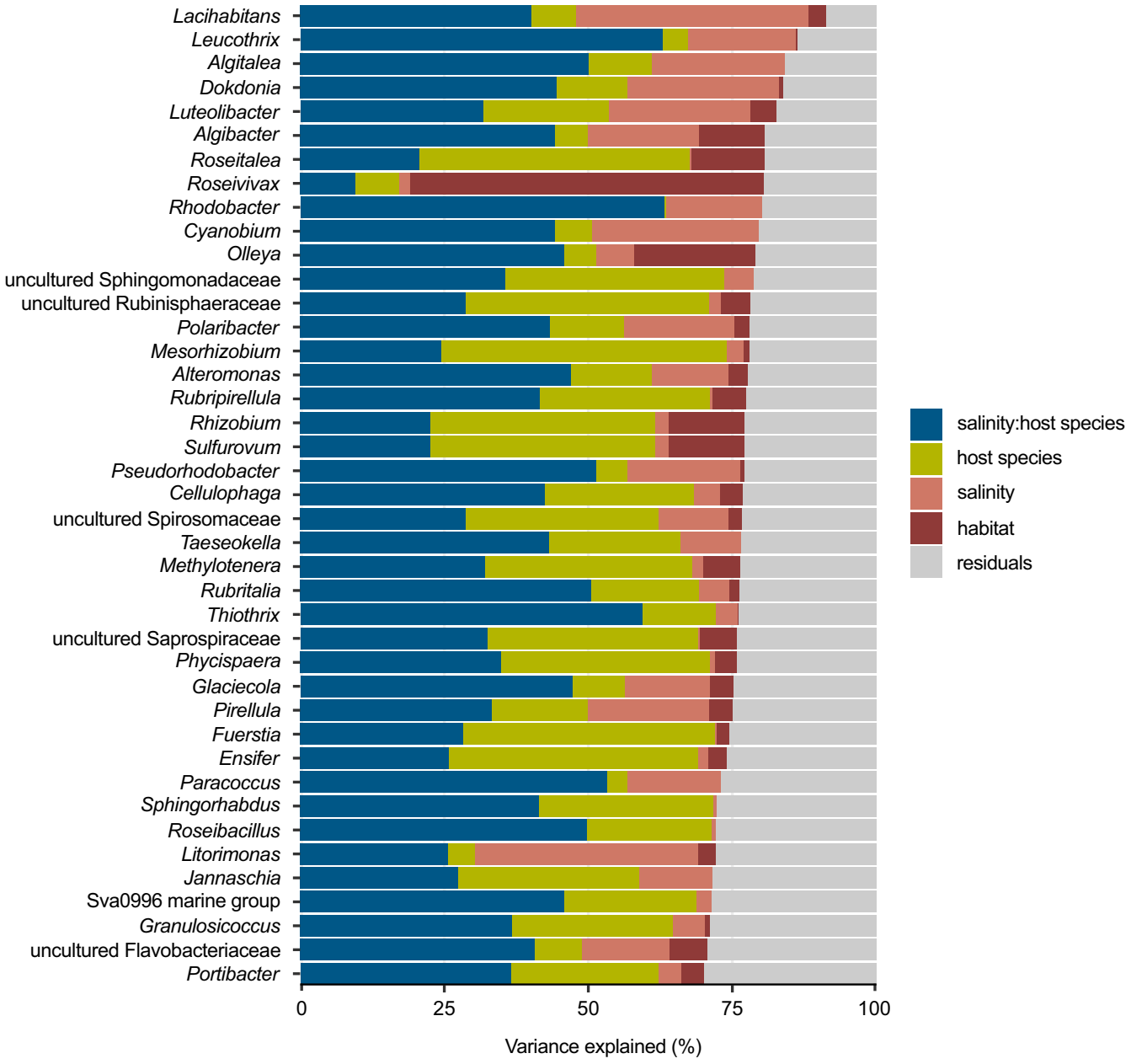


FIGURE 4 Variance partitioning, showing the amount of variance in abundance of *Ulva sensu lato* associated bacterial genera explained (%) by the interaction between salinity and host species (salinity:host species), host species, salinity and habitat. This is based on a generalized linear mixed model (negative binomial family). Only genera for which >70% of the variation was explained are shown. For a graph containing all genera, see Figure S5

abundance and ≥50% prevalence, >60 genera were considered part of the core of *Ulva sensu lato* along the entire salinity gradient. However, with strict thresholds of ≥1% relative abundance and ≥90% prevalence, only two genera were defined as core taxa: an uncultured Rhodobacteraceae and *Sulfitobacter*. When the prevalence threshold was lowered to ≥80%, *Paracoccus* became part of the core bacterial community as well, and when the prevalence was set to ≥70%, an uncultured Rhizobiaceae, *Yoonia-Loktanella* and an uncultured Saprospiraceae became additional members.

Across the salinity gradient, a shift in core community composition occurred. Five taxa were considered core across all species in the oligohaline region (0–5 PSU) and four taxa in the horohalinicum (5–8 PSU) with ≥75% prevalence and ≥1% relative abundance (Figure 6a). In addition to the three taxa considered core across the entire salinity gradient (*Sulfitobacter*, *Paracoccus* and an uncultured Rhodobacteraceae), these low salinity ranges also shared *Luteolibacter* as a core genus. The mesohaline samples (8–18 PSU) contained five core taxa, the polyhaline samples (18–30 PSU) six core taxa and the euhaline samples (30–36 PSU) five core taxa. These higher salinity regions all shared an uncultured Rhizobiaceae in their core. In addition, the mesohaline and polyhaline core both included *Yoonia-Loktonella*, and the polyhaline and euhaline shared an uncultured Saprospiraceae (Figure 6a).

Core membership not only shifted with salinity. The different host species were also associated with distinct core consortia (Figure 6b). *Ulva intestinalis* and *U. linza* shared the same geographical range, but the *U. intestinalis* core was larger (seven taxa) and included amongst others *Yoonia-Loktanella*, an uncultured Sphingomonadaceae, *Erythrobacter* and *Roseovarius*, while the *U. linza* core was smaller (four taxa) and included only an uncultured Saprospiraceae in addition to the three main core members. *Ulva fenestrata*, a more typical marine species, was the only host with *Granulosicoccus* and *Blastopirellula* in its core. *Blidingia minima* shared a large proportion of its core with *U. intestinalis*, but additionally included *Jannaschia* and *Altererythrobacter*. *Kornmannia leptoderma* in particular had high relative abundances of the core taxon *Altererythrobacter* as well.

4 | DISCUSSION

Seaweeds and associated bacteria show interdependent and complex dynamics. Here, we tested the stability of *Ulva*-associated bacterial communities across a stable salinity gradient in the Atlantic–Baltic Sea. In addition, we made use of the rich diversity of *Ulva* species in the study area, with some species covering the entire salinity gradient, to characterize species-specific responses vs. environmentally driven variation.

4.1 | Salinity-driven seaweed–bacterial interactions

The Baltic Sea is characterized by its strong and stable salinity gradient. Salinity has been identified as the most important structuring factor on seawater and sediment microbial consortia

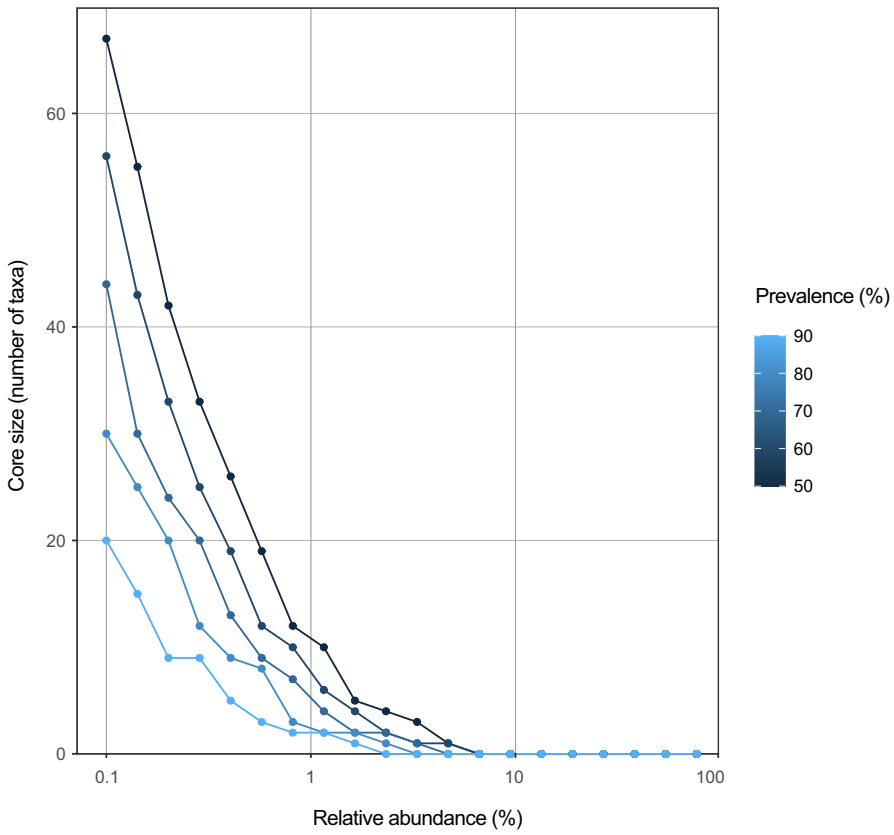


FIGURE 5 Bacterial core size (number of genera) of *Ulva sensu lato* across the entire Baltic salinity gradient with different relative abundance (based on read counts) and prevalence (based on the number of samples in which the taxa was encountered) thresholds.

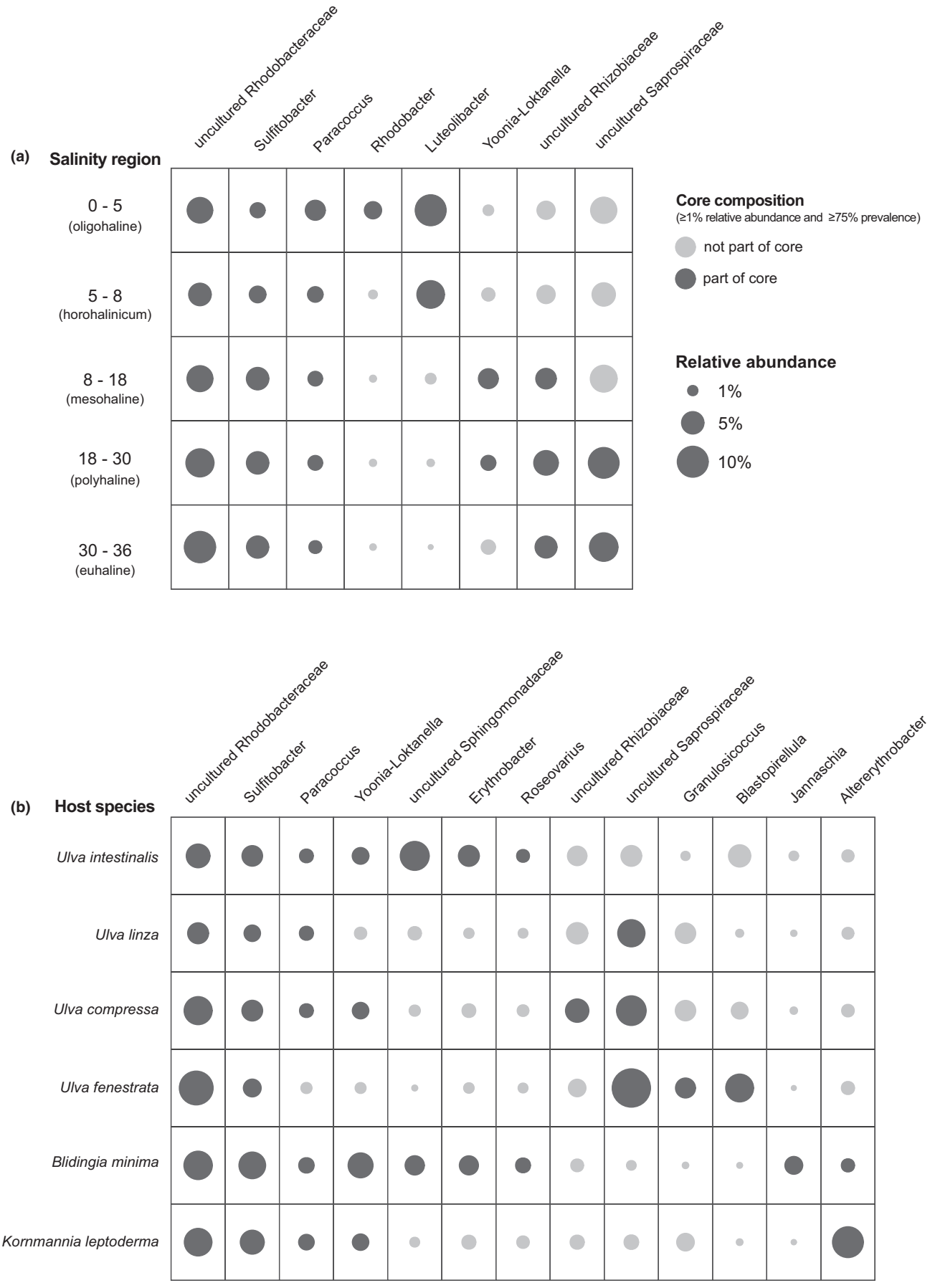


FIGURE 6 Mean relative abundance of bacterial core taxa of *Ulva sensu lato*, split by (a) salinity and (b) host species. The circle size corresponds to relative abundance, and the dark grey shade indicates whether the taxon is part of the core community (based on >1% relative abundance and >75% prevalence).

(Dupont et al., 2014; Herlemann et al., 2011). This agrees with our study, which showed that *Ulva*-associated bacterial composition is strongly structured primarily by salinity and secondarily by host species.

The largest shift in the bacterial consortia of *Ulva sensu lato* was observed passing the horohalinicum (5–8 PSU) from low salinity to higher salinity. The brackish to full marine transition has also been termed the "critical salinity region", as this is the salinity range where many chemical, physical and biological processes abruptly change (Telesh & Khlebovich, 2010). For example, the ion Ca/Cl ratio is stable down to 7 PSU, but below this salinity level the ratio drastically changes. Similar nonlinear dynamics are observed for, among others, silicon concentration, suspended matter concentration and the stability of phosphorus compounds. In the Baltic Sea, the horohalinicum coincides with the Darss Sill (situated east of the Danish Straits; Figure 1). This probably explains why most of the *Ulva* species' distribution is limited to the North Sea and Danish Straits. The distribution of bacteria associated with *Ulva sensu lato* is clearly affected as well and this study provides the first report of a host-associated bacterial community changing drastically in the horohalinicum.

The effect of salinity on seaweed bacteria has rarely been investigated in laboratory experiments. Two exceptions include the long-term mesocosm studies conducted on the red seaweed *Gracilaria vermiculophylla* collected along the North Sea coast of Germany (as *Agarophyton vermiculophyllum*, Saha et al., 2020), and the brown seaweed *Fucus vesiculosus* collected in the Kiel fjord in the western Baltic (Stratil et al., 2014). *Gracilaria vermiculophylla* is non-native in Europe and has also been introduced in the Baltic Sea where it occurs across a wide salinity range. Both the bacterial communities of *Gracilaria* and *Fucus* were strongly impacted by salinity. Despite the occurrence of some shared bacterial taxa in *Ulva* and *Gracilaria* microbiomes, for example *Dokdonia* in higher salinity communities, the bacterial microbiomes of respective seaweeds are very different. Comparisons between *Ulva*- and *Fucus*-associated bacteria likewise result in very few shared taxa. Although, in our study, salinity overall had a greater effect than host species, possibly the evolutionary distances between the genera *Ulva*, *Gracilaria* and *Fucus* are large enough to overrule salinity (Lachnit et al., 2009).

Our results may suggest that some of the typical low-salinity bacteria in *Ulva* microbiomes (e.g., *Lacihabitans*, *Luteolibacter* and *Cyanobium*) could facilitate acclimatization of the host to low salinity in the Baltic. The effect of specific bacterial taxa on host tolerance to lower salinities has so far only been tested in the brown algal genus *Ectocarpus* (Dittami et al., 2016). In *Ectocarpus*, two bacterial OTUs—*Haliea* and an uncultured Sphingomonadales—were linked to increased host performance when the algae were first cultivated in seawater medium and subsequently in freshwater medium (Dittami et al., 2016). Axenic (bacteria-free) cultures of an *Ectocarpus* strain originally isolated from a freshwater environment did not survive in freshwater medium, nor did they survive the change from seawater to freshwater medium. Acclimatization to freshwater medium was only possible if the axenic strain was inoculated with medium containing bacteria of the nonaxenic cultures (Dittami et al., 2016). To be able to experimentally test whether characteristic low-salinity consortia in *Ulva* bacterial communities likewise stimulate host acclimatization to freshwater, isolation and cultivation of the associated bacteria and subsequent experimental work with axenic *Ulva* are required.

4.2 | Disentangling the effects of spatial distance and salinity

In essence, all environmental gradients in the Baltic are spatial gradients in a northeast–southwest direction. The samples in this study were collected on a 2,000-km transect. The shortest route across water from the most inland sampling site (Skepssmalen, Sweden) to the most outer sampling site (Egersund, Norway) was over 1,670 km. The spatial effect is statistically hard to separate from the environmental salinity gradient. However, 13 samples from five different sampling sites near the mouth of the Glomma river in the Oslofjord (Skagerrak; Figure 1) provide a good test case. The Glomma is Norway's longest and most voluminous river. Sampling at the sites close to the Glomma river mouth took place in early July, immediately after its discharge flow peak in May–June (Frigstad et al., 2020). Measured salinity at these sites was 5.1–13.6 PSU, which corresponds to prevailing central and northern Baltic salinity ranges, whilst the surrounding sites in the Skagerrak are characterized by salinity levels >20 PSU. As bacterial community composition at the sites influenced by the Glomma discharge was in general more similar to central–northern Baltic microbiomes >1,000 km away, than to the Skagerrak sites only 20–50 km further south or west, salinity seems to overrule spatial distance (Figure S3). The effect of host species is visible here as well. Regarding *Kornmannia leptoderma*, for example, the samples at the mouth of the Glomma river represent the lowest salinity levels in which the species was found in this study, but also the most northern records in our data set. The samples were found at least 300 km more to the north than other *K. leptoderma* samples collected in the Danish Straits (salinity ~25 PSU), but are more similar to samples collected in the Baltic Proper (salinity ~7 PSU) that are geographically even further away. For seaweeds to recruit similar bacterial communities in specific environmental conditions despite large spatial distances requires the bacteria to be widely dispersed in the environment. This agrees with the Baas-Becking hypothesis: "everything is everywhere but the environment selects" (Martiny et al., 2006).

4.3 | The Baltic Sea and its multiple environmental gradients

*Ulva sensu lato*harbours distinct bacterial communities across the different salinity regions along the Atlantic–Baltic Sea transect. However, the Baltic is not only characterized by a pronounced salinity gradient. Although less dramatic than the salinity gradient, the Baltic Sea also accommodates both a horizontal oxygen

gradient (with the Kattegat and North Sea area being in general oxygen-rich and the remainder of the Baltic Sea oxygen-poor) and a vertical oxygen gradient (oxygen depletion at >50 m depth) (Villnäs & Norkko, 2011). In addition, the Baltic experiences strong seasonal dynamics and is subjected to anthropogenic pressure (e.g., eutrophication, pollution, fisheries, shipping) (Ojaveer et al., 2010). Whilst both seawater surface temperature and oxygen levels were measured in this study and explained limited variance of the bacterial community composition, the sample distribution was not designed to capture the full dynamics associated with these environmental factors.

The Baltic temperature gradient covaries to some extent with salinity. In our study, lower temperatures were measured on the North Sea coast of Norway, and higher temperatures in the Baltic Proper (Figure S1). As sampling was only carried out in summer, yearly seasonal fluctuations were not represented. In addition, sampling was not restricted to a specific time of the day, hence small variations in the measured temperature between sites may have been caused by daily fluctuations. In spring, sea surface temperatures usually increase earlier in the year in the south and west areas of the Baltic Sea compared to the north and east areas (Mück & Heubel, 2018). Bacterial community composition of seawater therefore not only changes with salinity as a primary factor, but secondarily also with seasons (Andersson et al., 2010; Herlemann et al., 2016). In fact, these bacterioplankton community shifts display repeated patterns between years (Lindh et al., 2015). It is likely that across the entire Baltic, *Ulva*-associated bacterial communities also experience seasonal dynamics, as has been shown before in local populations in the Kiel fjord (Lachnit et al., 2011), as well as in the Caribbean (Comba González et al., 2021).

Nutrients were not measured during this study but may drive some of the unexplained variation. The Atlantic–Baltic Sea salinity gradient is caused by freshwater input from subarctic rivers on one side of the gradient and limited water exchange with the marine water body of the North Sea on the opposite side of the gradient (Seidel et al., 2017). Freshwater river discharge not only affects salinity, but simultaneously increases nutrient influx, including nitrate, phosphate and dissolved organic carbon (Frigstad et al., 2020; Korth et al., 2012). Southern, high-salinity areas in the Baltic are characterized by high levels of autochthonous DOM (dissolved organic matter derived from, for example, phytoplankton primary production), whereas northern, low-salinity areas are richer in allochthonous DOM of terrestrial origin discharged by rivers (Rowe et al., 2018). This increased nutrient load is a major stimulator of bacterioplankton growth and pelagic productivity (Stepanauskas et al., 2002), and pelagic bacterial growth efficiency is highest in the low-salinity regions (Rowe et al., 2018). The community composition of bacteria living in association with *Ulva* may be impacted by prevailing nutrient conditions as well, but not necessarily following the same patterns as bacterioplankton, as the *Ulva* host probably provides its microbial partners (and other associates) with carbon and nutrients (Hudson et al., 2019).

4.4 | Green tides: *Ulva* on the drift

Green tides are mass accumulations of unattached green seaweeds and are often caused by *Ulva* spp. They have profound negative effects on the environment, including reduced biodiversity, and smothering of the sea bed and its inhabitants (Wan et al., 2017; Ye et al., 2011). Decomposition of the *Ulva* biomass results in anoxic conditions and the release of gaseous sulphur compounds. In the Baltic Sea, where oxygen levels have already deteriorated over the past decades due to eutrophication, these anoxic conditions in particular pose a problem (Reusch et al., 2018). In the formation of green tides too, eutrophication plays a major role (Smetacek & Zingone, 2013). The microbial communities of green tide-forming ulvoid species have rarely been sequenced, but mass growth events of seaweeds are likely to induce a change in both the seaweed and environmental microbiome. Qu et al. (2020), for example, demonstrated that sulphate-reducing bacteria and heterotrophic bacteria increased in abundance in sediment directly under an *Ulva prolifera* bloom in the Yellow Sea, especially towards the end of the bloom. In surface seawater samples, the abundance of heterotrophic diazotrophic bacteria increased likewise during *U. prolifera* blooms (Zhang et al., 2015). Diazotrophic bacteria are involved in N_2-fixation. Hence, altered microbial communities during green tide events may affect the sulphur and nitrogen cycles (Aires et al., 2019).

In the current study, green tides were encountered at Skive in Denmark (caused by *Ulva compressa* and *U. lacinulata*), Frederikshavn in the north of Denmark (caused by *U. lacinulata*) and Gryt in Sweden (caused by monostromatic *U. intestinalis*). Several of these samples were visible as distinct outliers in the NMDS plot (Figure 2). In Frederikshavn, particularly high relative abundances of *Thiothrix* and *Sulfitobacter* were observed in green tide samples compared to attached thalli growing in the same harbour. These are sulphur-oxidizing bacteria (SOB) and *Sulfitobacter* is additionally known to promote *Ulva* growth (Grueneberg et al., 2016; Krishnani et al., 2010). Growth-promoting bacteria produce metabolites and chemical compounds such as thallusin that induce cell division and thallus differentiation, including rhizoid formation and the proper development of cell walls, in *Ulva* (Alsufyani et al., 2020). In turn, *Ulva* can attract growth-promoting bacteria through the release of the chemoattractant dimethylsulfoniopropionate (DMSP). In some cases, SOB form visible mats on the sediment or on degrading organic material, such as seaweed tissue (Fenchel et al., 2012). Depending on the green tide phase, autotrophic as well as mixotrophic SOB could take advantage of degrading *Ulva* tissue for carbon and sulphur sources.

In Gryt, green tide samples were enriched in *Rhodopirellula* and *Rubripirellula* compared to non-green tide *U. intestinalis* thalli in neighbouring sites. Both bacterial genera belong to the Planctomycetota. These bacteria are adapted to life in marine biofilms, as they have a holdfast that accommodates surface colonization, they can reproduce by budding and can quickly adapt to environmental changes (Kallscheuer et al., 2020). In addition, they have large genomes that often encode a large number of sulfatase

genes. The genome of *Rhodopirellula baltica*, for example, contains 110 sulfatases (Wegner et al., 2013). Sulfatases enable the degradation of sulphated polysaccharides such as ulvan in the *Ulva* cell wall. Planctomycetota are known to be abundant on algal surfaces and their high abundance in green tide samples may simply be caused by the large accumulation of biomass (Bondoso et al., 2017; Wiegand et al., 2021).

Drifting seaweeds are not always necessarily green tides. All foliose *U. compressa* samples in this study were collected as unattached specimens, most without the occurrence of mass accumulations. Interestingly, all tubular shaped *U. compressa* were found growing attached to substrates, such as rock and concrete. The bacterial communities of foliose vs. tubular *U. compressa* were distinctly different. However, all foliose samples were collected at low salinity (<20 PSU) and the tube-shaped samples at high salinity (>20 PSU). It is therefore not possible with the current data set to resolve whether differences in the *U. compressa* bacterial communities fundamentally differ with morphology or salinity.

4.5 | *Ulva* core bacterial communities along an environmental gradient

Although the *Ulva*-associated bacterial communities varied with salinity and host species, a small, stable consortium can be identified as well. The term "core microbiome" was initially used to describe a set of microbes or genes shared by the majority of host specimens in a given habitat (Turnbaugh et al., 2007). This is often referred to as a "common core", but the core concept has since been used in a broader context. Core microbiome members are, for example, hypothesized to play a key role in ecosystem functioning or may significantly affect host fitness and resilience to disturbance (Shade & Handelsman, 2012). Such "functional cores" are based on commonly occurring functional genes rather than taxonomic units (Risely, 2020). Whether based on taxonomy or function, the nature of a core can vary from being "substantial" (the majority of individuals/samples share a large proportion of the microbial consortia), to "minimal" (all individuals/samples only share a few core members) or even "nonexistent" (no taxa or genes in common across the majority of individuals/samples) (Hamady & Knight, 2009). In addition, there are "gradient" core models (in which individuals close to each other on a gradient share more microbial components than individuals at opposite ends of the gradient) and "subpopulation" core models (distinct subpopulations of host species each have their own, unique core) (Hamady & Knight, 2009).

Following the different core models described in Hamady and Knight (2009), we can define *Ulva*-associated bacterial communities across the Baltic as having a "minimal" taxonomic core (only three taxa are shared across the entire gradient), with in addition a "gradient" core (more taxa are shared between neighbouring salinity ranges than between ranges at opposite ends of the Atlantic–Baltic Sea gradient). Depending on the chosen prevalence and relative abundance settings, the minimal core consisted of *Sulfitobacter*, *Paracoccus* and an uncultured Rhodobacteraceae. Together they made up on average 14% of the reads per sample. *Sulfitobacter* and *Paracoccus* are known growth-promoting and morphogenesis-inducing bacteria of *Ulva* (Ghaderiardakani et al., 2017, 2019), and are thus unsurprising core members. Possible beneficial interactions with the uncultured Rhodobacteraceae are unknown, but several of the reads assigned to this family did have a strong match to sequences in the NCBI database extracted from an *Ulva prolifera*–seawater interface (GenBank accession no. JF769698.1).

Some gradient core taxa were clearly defined by differences in relative abundance. *Luteolibacter*, for example, was highly abundant at low salinity (7%–9% relative abundance) and scarcely present at higher salinity (<1% relative abundance), which was also demonstrated by the differential abundance analyses. Other gradient core taxa were defined predominantly by prevalence. An uncultured Saprospiraceae, for example, was quite abundant across the entire salinity gradient (5%–9% relative abundance), but was only part of the bacterial core in higher salinities due to low prevalence at lower salinity levels. These varying prevalence levels might be due to differences among host species, as the uncultured Saprospiraceae was a highly abundant core member of *U. linza*, *U. compressa* and *U. fenestrata*, but did not have a high prevalence and abundance in *U. intestinalis*, *Blidingia minima* and *Kornmannia leptoderma*.

At host species level, the core consortia were slightly larger, varying from four to nine core members. Interestingly, the core community of *U. intestinalis* was more similar to the core of *B. minima* than to *U. linza*, despite *U. intestinalis* and *U. linza* sharing a similar geographical range. The same pattern was observed in the NMDS plot, in which *U. intestinalis* and *B. minima* samples were clustered to the left of the plot, while the *U. linza* cluster was located to the right of the plot. The Mantel test showed that overall bacterial community composition did not differ with host phylogeny, indicating that differences in bacterial communities between host species were caused by intrinsic factors (e.g., biochemical composition and defence mechanism).

As the definition of a core community is flexible, the decision on which taxa should be considered core members and whether a core community exists at all remains arbitrary. Some studies define core taxa purely based on prevalence (Aires et al., 2015), others use both relative abundance and prevalence (Ainsworth et al., 2015), and others use models (Bonthond et al., 2020; Shade & Stopnisek, 2019). The threshold settings used vary tremendously as well, and rarely have biological justifications, so the resulting core depends on the authors (Risely, 2020). In *Ulva*, the taxonomic variability has often been deemed too large to contain a core consortium. A study on *U. australis*, for example, demonstrated only six bacterial species were consistently present in all samples ($n = 6$), and while these did make up on average 15.6% relative abundance per sample, a core was considered nonexistent (Burke et al., 2011). These results are relatively similar to our data set, with three genera contributing up to 14% of the reads per sample. The larger the data set and the wider the geographical scale investigated, the less likely it becomes to define a large core

microbiome (Turnbaugh et al., 2007). Roth-Schulze et al. (2018) investigated the bacterial communities of three *Ulva* species in Spain and Australia, but found only one common OTU representing only 0.33% of the total number of sequences. By contrast, >70% of the functional genes were shared across the microbiomes of all three *Ulva* species independent of biogeography, and the remaining 30% could possibly be linked to environmental adaptation. The large biogeographical scales in the aforementioned study, however, were not associated with obvious environmental gradients, and bacterial communities may therefore seem to be influenced mostly by stochastic processes. The results from our study, on the other hand, indicate a large deterministic effect of the environment. Future studies investigating the functional repertoire of bacterial communities across the Atlantic–Baltic gradient could show whether the *Ulva*-associated functional core likewise follows a gradient model, and if such functional patterns could be linked to the taxonomic core.

5 | CONCLUSIONS

Salinity and host play a major role in *Ulva*-associated bacterial community structure. Pronounced differences between low- and high-salinity communities manifest themselves through defined patterns in differential abundance rather than presence/absence patterns of certain bacteria. Deviations from the predominant pattern at a distinct salinity can often be ascribed to microhabitats (e.g., high-salinity rock pools, green tides, river mouths) that differ from the prevailing conditions on surrounding sites. We identified a small taxonomic core consortium with in addition a few gradient core members that change across the salinity gradient. Future studies with experimental work could focus on causal relationships between bacteria and host tolerance towards fluctuating salinity, as well as functional analyses across the entire Atlantic–Baltic salinity gradient.

ACKNOWLEDGEMENTS

The research leading to the results presented in this publication was carried out with infrastructure funded by the FWO PhD Fellowship fundamental research (3F020119), the EMBRC Belgium—FWO project I001621N, the Formas national research programme for food (grant no. 2020-03119), and Portuguese national funds from FCT—Foundation for Science and Technology through project UIDB/04326/2020 and contract CEECINST/00114/2018. We would like to thank Samanta Hoffmann for her assistance during field work.

AUTHOR CONTRIBUTIONS

S.S., G.B.T and H.P. designed the study. S.S. and F.W collected the sample. L.M.L., S.D. and S.S. carried out the molecular work. L.M.L. performed bioinformatics and statistical analyses. L.M.L and S.S. wrote the original draft. O.D.C, A.W., G.B.T., H.P., F.W. and A.H.E. edited the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Raw sequence reads are deposited in the SRA (BioProject PRJNA781821). Related metadata are also stored in SRA (BioProject PRJNA781821) and can be found in Table S1. The *tufA* sequences generated in this study to identify host species are deposited in GenBank and accession numbers can be found in Table S1.

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REFERENCES

Adair, K. L., & Douglas, A. E. (2017). Making a microbiome: the many determinants of host-associated microbial community composition. *Current Opinion in Microbiology*, *35*, 23–29. https://doi.org/10.1016/j.mib.2016.11.002

Ainsworth, T. D.,Krause, L.,Bridge, T.,Torda, G.,Raina, J. B.,Zakrzewski, M., & Leggat, W. (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME Journal*, *9*(10), 2261–2274. https://doi.org/10.1038/ismej.2015.39

Aires, T.,Moalic, Y.,Serrao, E. A., & Arnaud-Haond, S. (2015). Hologenome theory supported by cooccurrence networks of species-specific bacterial communities in siphonous algae (*Caulerpa*). *FEMS Microbiology Ecology*, *91*(7), 1–14. https://doi.org/10.1093/femsec/fiv067

Aires, T.,Muyzer, G.,Serrão, E. A., & Engelen, A. H. (2019). Seaweed loads cause stronger bacterial community shifts in coastal lagoon sediments than nutrient loads. *Frontiers in Microbiology*, *9*, 3283. https://doi.org/10.3389/fmicb.2018.03283

Alsufyani, T.,Califano, G.,Deicke, M.,Grueneberg, J.,Weiss, A.,Engelen, A. H., & Wichard, T. (2020). Macroalgal-bacterial interactions: Identification and role of thallusin in morphogenesis of the seaweed *Ulva* (Chlorophyta). *Journal of Experimental Botany*, *71*(11), 3340–3349. https://doi.org/10.1093/jxb/eraa066

Alves, A. S.,Adão, H.,Patrício, J.,Neto, J. M.,Costa, M. J., & Marques, J. C. (2009). Spatial distribution of subtidal meiobenthos along estuarine gradients in two southern European estuaries (Portugal). *Journal of the Marine Biological Association of the United Kingdom*, *89*(8), 1529–1540. https://doi.org/10.1017/S0025315409000691

Andersson, A. F.,Riemann, L., & Bertilsson, S. (2010). Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. *ISME Journal*, *4*(2), 171–181. https://doi.org/10.1038/ismej.2009.108

Arnaud-Haond, S.,Aires, T.,Candeias, R.,Teixeira, S. J. L.,Duarte, C. M.,Valero, M., & Serrão, E. A. (2017). Entangled fates of holobiont genomes during invasion: nested bacterial and host diversities in *Caulerpa taxifolia*. *Molecular Ecology*, *26*(8), 2379–2391. https://doi.org/10.1111/mec.14030

Bates, D.,Mächler, M.,Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), https://doi.org/10.18637/jss.v067.i01

Bleich, S.,Powilleit, M.,Seifert, T., & Graf, G. (2011). B-Diversity as a measure of species turnover along the salinity gradient in the Baltic Sea, and its consistency with the Venice System. *Marine Ecology Progress Series*, *436*, 101–118. https://doi.org/10.3354/meps09219

Bondoso, J.,Godoy-Vitorino, F.,Balagué, V.,Gasol, J. M.,Harder, J., & Lage, O. M. (2017). Epiphytic *Planctomycetes* communities associated with three main groups of macroalgae. *FEMS Microbiology Ecology*, *93*(3), 1–9. https://doi.org/10.1093/femsec/fiw255

Bonthond, G.,Bayer, T.,Krueger-Hadfield, S. A.,Barboza, F. R.,Nakaoka, M.,Valero, M.,Wang, G.,Künzel, S., & Weinberger, F. (2020). How do microbiota associated with an invasive seaweed vary across scales? *Molecular Ecology*, *29*(11), 2094–2108. https://doi.org/10.1111/mec.15470

Bonthond, G.,Bayer, T.,Krueger-Hadfield, S. A.,Stärck, N.,Wang, G.,Nakaoka, M., & Weinberger, F. (2021). The role of host promiscuity in the invasion process of a seaweed holobiont. *ISME Journal*, *15*(6), 1668–1679. https://doi.org/10.1038/s41396-020-00878-7

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology*, *13*(8), 1–23. https://doi.org/10.1371/journal.pbio.1002226

Bray, J., & Curtis, J. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographies*, *27*, 325–349. https://doi.org/10.2307/1942268

Burke, C.,Thomas, T.,Lewis, M.,Steinberg, P., & Kjelleberg, S. (2011). Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *ISME Journal*, *5*(4), 590–600. https://doi.org/10.1038/ismej.2010.164

Califano, G.,Kwantes, M.,Abreu, M. H.,Costa, R., & Wichard, T. (2020). Cultivating the macroalgal holobiont : Effects of integrated multi-trophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). *Frontiers in Marine Science*, *7*(52), https://doi.org/10.3389/fmars.2020.00052

Comba González, N. B.,Niño Corredor, A. N.,López Kleine, L., & Montoya Castaño, D. (2021). Temporal changes of the epiphytic bacteria community from the marine macroalga *Ulva lactuca* (Santa Marta, Colombian-Caribbean). *Current Microbiology*, *78*(2), 534–543. https://doi.org/10.1007/s00284-020-02302-x

De Coster, W.,D'Hert, S.,Schultz, D. T.,Cruts, M., & Van Broeckhoven, C. (2018). NanoPack: Visualizing and processing long-read sequencing data. *Bioinformatics*, *34*(15), 2666–2669. https://doi.org/10.1093/bioinformatics/bty149

Dittami, S. M.,Duboscq-Bidot, L.,Perennou, M.,Gobet, A.,Corre, E.,Boyen, C., & Tonon, T. (2016). Host-microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *ISME Journal*, *10*(1), 51–63. https://doi.org/10.1038/ismej.2015.104

Dupont, C. L.,Larsson, J.,Yooseph, S.,Ininbergs, K.,Goll, J.,Asplund-Samuelsson, J., & Bergman, B. (2014). Functional tradeoffs underpin salinity-driven divergence in microbial community composition. *PLoS One*, *9*(2), https://doi.org/10.1371/journal.pone.0089549

Egan, S.,Harder, T.,Burke, C.,Steinberg, P.,Kjelleberg, S., & Thomas, T. (2013). The seaweed holobiont: Understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews*, *37*(3), 462–476. https://doi.org/10.1111/1574-6976.12011

Fenchel, T.,King, G. M., & Blackburn, T. H. (2012). Chapter 7. Aquatic Sediments. In *Bacterial Biogeochemistry* (Third edit, pp. 121–142). Elsevier. https://doi.org/10.1016/B978-0-12-415836-8.00007-4

Foster, Z. S. L.,Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology*, *13*(2), 1–15. https://doi.org/10.1371/journal.pcbi.1005404

Frigstad, H.,Kaste, Ø.,Deininger, A.,Kvalsund, K.,Christensen, G.,Bellerby, R. G. J., & King, A. L. (2020). Influence of riverine input on Norwegian coastal systems. *Frontiers in Marine Science*, *77*, 332. https://doi.org/10.3389/fmars.2020.00332

Gemin, M.,Peña-Rodríguez, A.,Quiroz-Guzmán, E.,Magallón-Servín, P.,Barajas-Sandoval, D., & Elizondo-González, R. (2019). Growth-promoting bacteria for the green seaweed *Ulva clathrata*. *Aquaculture Research*, *50*(12), 3741–3748. https://doi.org/10.1111/are.14336

Ghaderiardakani, F.,Califano, G.,Mohr, J.,Abreu, M.,Coates, J., & Wichard, T. (2019). Analysis of algal growth- and morphogenesis- promoting factors in an integrated multi-trophic aquaculture system for farming *Ulva* spp. *Aquaculture Environment Interactions*, *11*(August), 375–391. https://doi.org/10.3354/aei00319

Ghaderiardakani, F.,Coates, J. C., & Wichard, T. (2017). Bacteria-induced morphogenesis of *Ulva intestinalis* and Ulva mutabilis (Chlorophyta): A contribution to the lottery theory. *FEMS Microbiology Ecology*, *93*(8), 1–12. https://doi.org/10.1093/femsec/fix094

Grueneberg, J.,Engelen, A. H.,Costa, R., & Wichard, T. (2016). Macroalgal morphogenesis induced by waterborne compounds and bacteria in coastal seawater. *PLoS One*, *11*(1), 1–22. https://doi.org/10.1371/journal.pone.0146307

Hamady, M., & Knight, R. (2009). Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. *Genome Research*, *19*(7), 1141–1152. https://doi.org/10.1101/gr.085464.108

Herlemann, D. P. R.,Labrenz, M.,Jürgens, K.,Bertilsson, S.,Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal*, *5*(10), 1571–1579. https://doi.org/10.1038/ismej.2011.41

Herlemann, D. P. R.,Lundin, D.,Andersson, A. F.,Labrenz, M., & Jürgens, K. (2016). Phylogenetic signals of salinity and season in bacterial community composition across the salinity gradient of the Baltic Sea. *Frontiers in Microbiology*, *7*, 1883. https://doi.org/10.3389/fmicb.2016.01883

Hoffman, G. E., & Schadt, E. E. (2016). variancePartition: Interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics*, *17*(1), 17–22. https://doi.org/10.1186/s12859-016-1323-z

Hu, Y. O. O.,Karlson, B.,Charvet, S., & Andersson, A. F. (2016). Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the Baltic Sea. *Frontiers in Microbiology*, *7*, 679. https://doi.org/10.3389/fmicb.2016.00679

Hudson, J.,Kumar, V., & Egan, S. (2019). Comparative genome analysis provides novel insight into the interaction of *Aquimarina* sp. AD1, BL5 and AD10 with their macroalgal host. *Marine Genomics*, *46*(January), 8–15. https://doi.org/10.1016/j.margen.2019.02.005

Hughey, J. R.,Gabrielson, P. W.,Maggs, C. A., & Mineur, F. (2021). Genomic analysis of the lectotype specimens of European *Ulva rigida* and *Ulva lacinulata* (Ulvaceae, Chlorophyta) reveals the ongoing misapplication of names. *European Journal of Phycology*, 1–11. https://doi.org/10.1080/09670262.2021.1914862

Johannesson, K.,Le Moan, A.,Perini, S., & André, C. (2020). A Darwinian laboratory of multiple contact zones. *Trends in Ecology and Evolution*, *35*(11), 1021–1036. https://doi.org/10.1016/j.tree.2020.07.015

Joint, I.,Callow, M. E.,Callow, J. A., & Clarke, K. R. (2000). The attachment of *Enteromorpha* zoospores to a bacterial biofilm assemblage. *Biofouling*, *16*(2–4), 151–158. https://doi.org/10.1080/08927010009378440

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. *Ecological Society of America*, *88*(10), 2427–2439. https://doi.org/10.1890/06-1736.1

Kallscheuer, N.,Jogler, M.,Wiegand, S.,Peeters, S. H.,Heuer, A.,Boedeker, C., & Jogler, C. (2020). Three novel *Rubripirellula*species isolated from plastic particles submerged in the Baltic Sea and the estuary of the river Warnow in northern Germany. *Antonie Van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *113*(12), 1767–1778. https://doi.org/10.1007/s10482-019-01368-3

Katoh, K.,Misawa, K.,Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier

transform. *Nucleic Acids Research*, *30*(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Kessler, R. W.,Weiss, A.,Kuegler, S.,Hermes, C., & Wichard, T. (2018). Macroalgal–bacterial interactions: Role of dimethylsulfoniopropionate in microbial gardening by *Ulva* (Chlorophyta). *Molecular Ecology*, *27*(8), 1808–1819. https://doi.org/10.1111/mec.14472

Klier, J.,Dellwig, O.,Leipe, T.,Jürgens, K., & Herlemann, D. P. R. (2018). Benthic bacterial community composition in the oligohaline-marine transition of surface sediments in the Baltic Sea based on rRNA analysis. *Frontiers in Microbiology*, *9*(FEB), 1–12. https://doi.org/10.3389/fmicb.2018.00236

Korth, F.,Deutsch, B.,Liskow, I., & Voss, M. (2012). Uptake of dissolved organic nitrogen by size-fractionated plankton along a salinity gradient from the North Sea to the Baltic Sea. *Biogeochemistry*, *111*(1–3), 347–360. https://doi.org/10.1007/s10533-011-9656-1

Krishnani, K. K.,Kathiravan, V.,Natarajan, M.,Kailasam, M., & Pillai, S. M. (2010). Diversity of sulfur-oxidizing bacteria in greenwater system of coastal aquaculture. *Applied Biochemistry and Biotechnology*, *162*(5), 1225–1237. https://doi.org/10.1007/s12010-009-8886-3

Lachnit, T.,Blümel, M.,Imhoff, J. F., & Wahl, M. (2009). Specific epibacterial communities on macroalgae: Phylogeny matters more than habitat. *Aquatic Biology*, *5*(2), 181–186. https://doi.org/10.3354/ab00149

Lachnit, T.,Meske, D.,Wahl, M.,Harder, T., & Schmitz, R. (2011). Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environmental Microbiology*, *13*(3), 655–665. https://doi.org/10.1111/j.1462-2920.2010.02371.x

Lahti, L., & Shetty, S. (2017). Tools for microbiome analysis in R. *Microbiome Package Version*, *1*(15), 1. Retrieved from http://microbiome.github.com/microbiome

Leskinen, E.,Alström-Rapaport, C., & Pamilo, P. (2004). Phylogeographical structure, distribution and genetic variation of the green algae *Ulva intestinalis* and *U. compressa* (Chlorophyta) in the Baltic Sea area. *Molecular Ecology*, *13*(8), 2257–2265. https://doi.org/10.1111/j.1365-294X.2004.02219.x

Lindh, M. V.,Sjöstedt, J.,Andersson, A. F.,Baltar, F.,Hugerth, L. W.,Lundin, D., & Pinhassi, J. (2015). Disentangling seasonal bacterioplankton population dynamics by high-frequency sampling. *Environmental Microbiology*, *17*(7), 2459–2476. https://doi.org/10.1111/1462-2920.12720

Love, M. I.,Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 1–21. https://doi.org/10.1186/s13059-014-0550-8

Lozupone, C. A., & Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(27), 11436–11440. https://doi.org/10.1073/pnas.0611525104

Lu, J., & Salzberg, S. L. (2020). Ultrafast and accurate 16S rRNA microbial community analysis using Kraken2. *Microbiome*, *8*(1), 1–11. https://doi.org/10.1186/s40168-020-00900-2

Marijon, P.,Chikhi, R., & Varré, J. S. (2020). Yacrd and fpa: Upstream tools for long-read genome assembly. *Bioinformatics*, *36*(12), 3894–3896. https://doi.org/10.1093/bioinformatics/btaa262

Martinez Arbizu, P. (2020). *pairwiseAdonis: Pairwise multilevel comparison using adonis*. R package version 0.4. Retrieved from https://github.com/pmartinezarbizu/pairwiseAdonis

Martiny, J. B. H.,Bohannan, B. J. M.,Brown, J. H.,Colwell, R. K.,Fuhrman, J. A.,Green, J. L., & Staley, J. T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112. https://doi.org/10.1038/nrmicro1341

McFall-Ngai, M.,Hadfield, M. G.,Bosch, T. C. G.,Carey, H. V.,Domazet-Lošo, T.,Douglas, A. E., & Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(9), 3229–3236. https://doi.org/10.1073/pnas.1218525110

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), https://doi.org/10.1371/journal.pone.0061217

Meusnier, I.,Olsen, J. L.,Stam, W. T.,Destombe, C., & Valero, M. (2001). Phylogenetic analyses of *Caulerpa taxifolia* (Chlorophyta) and of its associated bacterial microflora provide clues to the origin of the Mediterranean introduction. *Molecular Ecology*, *10*(4), 931–946. https://doi.org/10.1046/j.1365-294X.2001.01245.x

Mück, I., & Heubel, K. U. (2018). Ecological variation along the salinity gradient in the Baltic Sea area and its consequences for reproduction in the common goby. *Current Zoology*, *64*(2), 259–270. https://doi.org/10.1093/cz/zoy006

Nylander, J. A. A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre Uppsala University, 2(October), 1–2. Retrieved from https://github.com/nylander/MrModeltest2

Ojaveer, H.,Jaanus, A.,Mackenzie, B. R.,Martin, G.,Olenin, S.,Radziejewska, T., & Zaiko, A. (2010). Status of biodiversity in the Baltic sea. *PLoS One*, *5*(9), 1–19. https://doi.org/10.1371/journal.pone.0012467

Oksanen, J.,Blanchet, F. G.,Friendly, M.,Kindt, R.,Legendre, P.,McGlinn, D., & Wagner, H. (2020). *vegan: Community Ecology Package*. R package version 2.5-7.

Patel, P.,Callow, M. E.,Joint, I., & Callow, J. A. (2003). Specificity in the settlement–modifying response of bacterial biofilms towards zoospores of the marine alga *Enteromorpha*. *Environmental Microbiology*, *5*(5), 338–349. https://doi.org/10.1046/j.1462-2920.2003.00407.x

Pita, L.,Rix, L.,Slaby, B. M.,Franke, A., & Hentschel, U. (2018). The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome*, *6*(1), 46. https://doi.org/10.1186/s40168-018-0428-1

Polikovsky, M.,Califano, G.,Dunger, N.,Wichard, T., & Golberg, A. (2020). Engineering bacteria-seaweed symbioses for modulating the photosynthate content of *Ulva* (Chlorophyta): Significant for the feedstock of bioethanol production. *Algal Research*, *49*, 101945. https://doi.org/10.1016/j.algal.2020.101945

Qu, T.,Zhao, X.,Hao, Y.,Zhong, Y.,Guan, C.,Hou, C., & Wang, Y. (2020). Ecological effects of *Ulva prolifera* green tide on bacterial community structure in Qingdao offshore environment. *Chemosphere*, *244*, 125477. https://doi.org/10.1016/j.chemosphere.2019.125477

Quast, C.,Pruesse, E.,Yilmaz, P.,Gerken, J.,Schweer, T.,Yarza, P., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), 590–596. https://doi.org/10.1093/nar/gks1219

R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Reusch, T. B. H.,Dierking, J.,Andersson, H. C.,Bonsdorff, E.,Carstensen, J.,Casini, M., & Zandersen, M. (2018). The Baltic Sea as a time machine for the future coastal ocean. *Science Advances*, *4*(5), https://doi.org/10.1126/sciadv.aar8195

Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *Journal of Animal Ecology*, *89*(7), 1549–1558. https://doi.org/10.1111/1365-2656.13229

Roth-Schulze, A. J.,Pintado, J.,Zozaya-Valdés, E.,Cremades, J.,Ruiz, P.,Kjelleberg, S., & Thomas, T. (2018). Functional biogeography and host specificity of bacterial communities associated with the marine green alga *Ulva* spp. *Molecular Ecology*, *27*(8), 1952–1965. https://doi.org/10.1111/mec.14529

Rowe, O. F.,Dinasquet, J.,Paczkowska, J.,Figueroa, D.,Riemann, L., & Andersson, A. (2018). Major differences in dissolved organic matter characteristics and bacterial processing over an extensive brackish water gradient, the Baltic Sea. *Marine Chemistry*, *202*(May), 27–36.https://doi.org/10.1016/j.marchem.2018.01.010

Rybak, A. S. (2018). Species of *Ulva* (Ulvophyceae, Chlorophyta) as indicators of salinity. *Ecological Indicators*, *85*(February), 253–261.https://doi.org/10.1016/j.ecolind.2017.10.061

Saha, M.,Ferguson, R. M. W.,Dove, S.,Künzel, S.,Meichssner, R.,Neulinger, S. C., & Weinberger, F. (2020). Salinity and time can alter epibacterial communities of an invasive seaweed. *Frontiers in Microbiology*, *10*, 2870. https://doi.org/10.3389/fmicb.2019.02870

Seidel, M.,Manecki, M.,Herlemann, D. P. R.,Deutsch, B.,Schulz-Bull, D.,Jürgens, K., & Dittmar, T. (2017). Composition and transformation of dissolved organic matter in the Baltic Sea. *Frontiers in Earth Science*, *5*, 31. https://doi.org/10.3389/feart.2017.00031

Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: The hunt for a core microbiome. *Environmental Microbiology*, *14*(1), 4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

Shade, A., & Stopnisek, N. (2019). Abundance-occupancy distributions to prioritize plant core microbiome membership. *Current Opinion in Microbiology*, *49*, 50–58. https://doi.org/10.1016/j.mib.2019.09.008

Skrodenytė-Arbačiauskienė, V.,Virbickas, T.,Lukša, J.,Servienė, E.,Blažytė-Čereškienė, L., & Kesminas, V. (2021). Gut microbiome of wild Baltic salmon (*Salmo salar* L.) Parr. *Microbial Ecology*, 0123456789, 1–5. https://doi.org/10.1007/s00248-021-01910-9

Smetacek, V., & Zingone, A. (2013). Green and golden seaweed tides on the rise. *Nature*, *504*(7478), 84–88. https://doi.org/10.1038/nature12860

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Steinhagen, S.,Düsedau, L., & Weinberger, F. (2021). DNA barcoding of the German green supralittoral zone indicates the distribution and phenotypic plasticity of *Blidingia* species and reveals *Blidingia cornuta* sp. nov. *Taxon*, *70*(2), 1–17. https://doi.org/10.1002/tax.12445

Steinhagen, S.,Karez, R., & Weinberger, F. (2019). Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas. *European Journal of Phycology*, *54*(3), 466–483. https://doi.org/10.1080/09670262.2019.1597925

Stepanauskas, R.,JØrgensen, N. O. G.,Eigaard, O. R.,Žvikas, A.,Tranvik, L. J., & Leonardson, L. (2002). Summer inputs of riverine nutrients to the baltic sea: Bioavailability and Eutrophication relevance. *Ecological Monographies*, *72*(4), 579–597.

Stratil, S. B.,Neulinger, S. C.,Knecht, H.,Friedrichs, A. K., & Wahl, M. (2014). Salinity affects compositional traits of epibacterial communities on the brown macroalga *Fucus vesiculosus*. *FEMS Microbiology Ecology*, *88*(2), 272–279. https://doi.org/10.1111/1574-6941.12292

Telesh, I. V., & Khlebovich, V. V. (2010). Principal processes within the estuarine salinity gradient: A review. *Marine Pollution Bulletin*, *61*(4–6), 149–155. https://doi.org/10.1016/j.marpolbul.2010.02.008

Tujula, N. A.,Crocetti, G. R.,Burke, C.,Thomas, T.,Holmström, C., & Kjelleberg, S. (2010). Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. *ISME Journal*, *4*(2), 301–311. https://doi.org/10.1038/ismej.2009.107

Turnbaugh, P. J.,Ley, R. E.,Hamady, M.,Fraser-Liggett, C. M.,Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*(7164), 804–810. https://doi.org/10.1038/nature06244

van der Loos, L. M.,D'hondt, S.,Willems, A., & De Clerck, O. (2021). Characterizing algal microbiomes using long-read nanopore sequencing. *Algal Research*, *59*(August), 102456. https://doi.org/10.1016/j.algal.2021.102456

van der Loos, L. M.,Eriksson, B. K., & Falcão Salles, J. (2019). The macroalgal holobiont in a changing sea. *Trends in Microbiology*, *27*(7), 635–650. https://doi.org/10.1016/j.tim.2019.03.002

Villnäs, A., & Norkko, A. (2011). Benthic diversity gradients and shifting baselines: Implications for assessing environmental status. *Ecological Applications*, *21*(6), 2172–2186. https://doi.org/10.1890/10-1473.1

Wan, A. H. L.,Wilkes, R. J.,Heesch, S.,Bermejo, R.,Johnson, M. P., & Morrison, L. (2017). Assessment and characterisation of Ireland's green tides (Ulva species). *PLoS One*, *12*(1), https://doi.org/10.1371/journal.pone.0169049

Wegner, C. E.,Richter-Heitmann, T.,Klindworth, A.,Klockow, C.,Richter, M.,Achstetter, T., & Harder, J. (2013). Expression of sulfatases in Rhodopirellula baltica and the diversity of sulfatases in the genus Rhodopirellula. *Marine Genomics*, *9*, 51–61. https://doi.org/10.1016/j.margen.2012.12.001

Weinberger, F.,Beltran, J.,Correa, J. A.,Lion, U.,Pohnert, G.,Kumar, N., & Potin, P. (2007). Spore release in *Acrochaetium* sp. (Rhodophyta) is bacterially controlled. *Journal of Phycology*, *43*(2), 235–241. https://doi.org/10.1111/j.1529-8817.2007.00329.x

Wichard, T. (2015). Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Frontiers in Plant Science*, *6*(MAR), 1–19. https://doi.org/10.3389/fpls.2015.00086

Wichard, T.,Charrier, B.,Mineur, F.,Bothwell, J. H.,De Clerck, O., & Coates, J. C. (2015). The green seaweed Ulva: A model system to study morphogenesis. *Frontiers in Plant Science*, *6*(FEB), 1–8. https://doi.org/10.3389/fpls.2015.00072

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. Retrieved from https://ggplot2.tidyverse.org

Wiegand, S.,Rast, P.,Kallscheuer, N.,Jogler, M.,Heuer, A.,Boedeker, C., & Jogler, C. (2021). Analysis of Bacterial Communities on North Sea Macroalgae and Characterization of the Isolated Planctomycetes Adhaeretor mobilis gen. nov., sp. nov., Roseimaritima multifibrata sp. nov., Rosistilla ulvae sp. nov. and Rubripirellula lacrimiformis sp. nov. *Microorganisms*, *9*(7), 1–34. https://doi.org/10.3390/microorganisms9071494

Willis, A. D. (2019). Rarefaction, alpha diversity, and statistics. *Frontiers in Microbiology*, *10*, 1–5https://doi.org/10.3389/fmicb.2019.02407

Ye, N.,Zhang, X.,Mao, Y.,Liang, C.,Xu, D.,Zou, J.,Zhuang, Z., & Wang, Q. (2011). "Green tides" are overwhelming the coastline of our blue planet: Taking the world's largest example. *Ecological Research*, *26*(3), 477–485. https://doi.org/10.1007/s11284-011-0821-8

Zhang, X.,Song, Y.,Liu, D.,Keesing, J. K., & Gong, J. (2015). Macroalgal blooms favor heterotrophic diazotrophic bacteria in nitrogen-rich and phosphorus-limited coastal surface waters in the Yellow Sea. *Estuarine, Coastal and Shelf Science*, *163*, 75–81. https://doi.org/10.1016/j.ecss.2014.12.015

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How to cite this article: van der Loos, L. M., D'hondt, S.,Engelen, A. H.,Pavia, H.,Toth, G. B.,Willems, A.,Weinberger, F.,De Clerck, O., & Steinhagen, S. (2023). Salinity and host drive *Ulva*-associated bacterial communities across the Atlantic–Baltic Sea gradient. *Molecular Ecology*, *32*, 6260–6277. https://doi.org/10.1111/mec.16462