



**HAL**  
open science

## The *Saccharina latissima* microbiome: Effects of region, season, and physiology

Bertille Burgunter-Delamare, Sylvie Rousvoal, Erwan Legeay, Gwenn Tanguy, Stein Fredriksen, Catherine Boyen, Simon M Dittami

### ► To cite this version:

Bertille Burgunter-Delamare, Sylvie Rousvoal, Erwan Legeay, Gwenn Tanguy, Stein Fredriksen, et al.. The *Saccharina latissima* microbiome: Effects of region, season, and physiology. *Frontiers in Microbiology*, 2023, 13, 10.3389/fmicb.2022.1050939 . hal-03951625

**HAL Id: hal-03951625**

**<https://hal.science/hal-03951625>**

Submitted on 23 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 The *Saccharina latissima* microbiome: effects of 2 region, season, and physiology

3 Bertille BURGUNTER-DELAMARE <sup>1</sup> \* [id](#), Sylvie ROUSVOAL <sup>1</sup>, Erwan LEGEAY <sup>2</sup>, Gwenn TANGUY <sup>2</sup>,  
4 Stein FREDRIKSEN <sup>3</sup> [id](#), Catherine BOYEN <sup>1,2</sup> and Simon M. DITTAMI <sup>1</sup> \* [id](#)

5 <sup>1</sup> CNRS, Sorbonne Université, Integrative Biology of Marine Models (LBI2M), Station Biologique  
6 de Roscoff, 29680 Roscoff, France

7 <sup>2</sup> CNRS, Sorbonne Université, FR2424 Station Biologique de Roscoff, 29680 Roscoff, France

8 <sup>3</sup> University of Oslo, Department of Biosciences, PO Box 1066, Blindern, N-0316 Oslo, Norway

9 \*Correspondence: [bertille.burgunter-delamare@sb-roscoff.fr](mailto:bertille.burgunter-delamare@sb-roscoff.fr), [simon.dittami@sb-roscoff.fr](mailto:simon.dittami@sb-roscoff.fr)

## 10 ABSTRACT

11 **Introduction:** *Saccharina latissima* is a canopy-forming species of brown algae and, as such, is  
12 considered an ecosystem engineer. Several populations of this alga are exploited worldwide, and  
13 a decrease in the abundance of *S. latissima* at its southern distributional range limits has been  
14 observed. Despite its economic and ecological interest, only a few data are available on the  
15 composition of microbiota associated with *S. latissima* and its role in algal physiology.

16 **Methods:** We studied the whole bacterial community composition associated with *S. latissima*  
17 samples from three locations (Brittany, Helgoland, and Skagerrak) by 16S metabarcoding analyses  
18 at different scales: algal blade part, regions, season (at one site), and algal physiologic state.

19 **Results and Discussion:** We have shown that the difference in bacterial composition is driven by  
20 factors of decreasing importance: (i) the algal tissues (apex/meristem), (ii) the geographical area,  
21 (iii) the seasons (at the Roscoff site), and (iv) the algal host's condition (healthy vs. symptoms).  
22 Overall, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidia* dominated the general  
23 bacterial communities. Almost all individuals hosted bacteria of the genus *Granulosicoccus*,  
24 accounting for 12% of the total sequences, and eight additional core genera were identified. Our

25 results also highlight a microbial signature characteristic for algae in poor health independent of  
26 the disease symptoms. Thus, our study provides a comprehensive overview of the *S. latissima*  
27 microbiome, forming a basis for understanding holobiont functioning.

28 *Keywords: holobiont; brown macroalgae; natural population; microbiota; metabarcoding*

Accepted Manuscript

## 29 I. INTRODUCTION

30 Brown macroalgae, particularly kelps (Laminariales), play essential ecosystem engineering  
31 roles in coastal temperate marine environments. Depending on the genus, they are distributed  
32 across the western or eastern temperate North Pacific, the Arctic, and North Atlantic Oceans  
33 (Bolton, 2010; Araújo et al., 2016). Kelps contribute to primary productivity and are habitat  
34 formers providing food and shelter to the local biodiversity (Schiel and Foster, 2006; Schiel and  
35 Lilley, 2007). In addition, species of kelps are important in many industries to produce alginates  
36 (Peteiro, 2018), human food, medicine (Smit, 2004), or food for abalone aquaculture (McHugh,  
37 2003; Roussel et al., 2019).

38 *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders is one of the  
39 dominant kelp-forming species of brown macroalgae in Europe. This perennial species (2-5 years)  
40 is widely distributed in the North Atlantic, North Pacific, and the Arctic (Neiva et al., 2018). Its  
41 tissue growth starts from the meristematic region at the base of the blade, with the older tissue  
42 being at the apex part. These older parts can undergo erosion due to senescence and host a higher  
43 bacterial diversity, as shown in previous research on other *Laminariales*, notably *Laminaria*  
44 *digitata* (Corre and Prieur, 1990), *Laminaria hyperborea* (Bengtsson et al., 2010), *Laminaria*  
45 *longicuris* (Laycock, 1974), *Laminaria pallida* (Mazure and Field, 1980), and *Laminaria setchellii*  
46 (Lemay et al., 2021).

47 In recent years, a decrease in the abundance of *S. latissima* at its southern range limits has  
48 been observed (Araújo et al., 2016; Smale, 2020). The exact processes driving this decline are not  
49 fully understood, but it is likely that changes in peak temperature associated with changes in the  
50 microbiota might be at least partially linked to this process, as is the case with corals (Bourne et  
51 al., 2008; Bosch and Miller, 2016; Peixoto et al., 2017).

52 Indeed, macroalgal functioning needs to be seen as the result of the interactions between  
53 the algal hosts and their associated microbiota, constituting a singular entity termed the algal  
54 holobiont (Egan et al., 2013). It has been shown that macroalgal health, fitness, pathogen  
55 resistance (Wiese et al., 2009), acclimation to a changing environment (Dittami et al., 2016), and  
56 metabolism (Burgunter-Delamare et al., 2020) are regulated and supported by bacterial partners

57 (Goecke et al., 2010). Considering the biofilm composition and deciphering the interactions within  
58 the holobiont is thus essential to fully understand the biology of algae. Previous studies were  
59 carried out on microbiota of different kelp species like *L. digitata* (Ihua et al., 2020), *L. hyperborea*  
60 (Bengtsson et al., 2010), *L. religiosa* (Vairappan et al., 2001) and *L. setchellii* (Lemay et al., 2021),  
61 but little is known about the *S. latissima* microbiota. Notably, Staufenberg et al. (2008) analyzed  
62 the bacterial composition of *Saccharina* from two locations and seasons (Baltic and North Sea;  
63 January and April 2006) using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene  
64 clone libraries. Later, Tourneroche et al. (2020) used 16S metabarcoding and FISH to decipher the  
65 bacterial microbiota of young tissues of *S. latissima* sampled in Scotland on one date.

66 In the present study, we compared the microbiota composition of young *S. latissima*  
67 samples from several locations in the Atlantic Ocean (Roscoff, Helgoland, and Skagerrak) by 16S  
68 metabarcoding analyses to test if the microbiota is specific to the area of origin, season, and algal  
69 blade part (apex/meristem). Lastly, we compared the microbiota composition of healthy thalli  
70 with those of thalli exhibiting one or several symptoms (holes, bleaching, twisted blades). This  
71 enabled us to identify a microbial signature characteristic of algae in poor health, regardless of  
72 the precise symptoms.

## 73 II. MATERIAL & METHODS

### 74 1. Biological material & Environmental Variables

75 *S. latissima* were sampled at different sites and dates (**Table 1**). Briefly, samples were  
76 taken from three regions (Roscoff, Helgoland, and Skagerrak) at low tide (or diving when  
77 necessary at the Helgoland and Skagerrak sites). Please note, however, that the tidal differences  
78 at Roscoff are up to 9m, whereas they reach 3m at Helgoland and only 30cm in the Skagerrak on  
79 the coast of Norway. Among young individuals (<1m length), five healthy algae and five with  
80 physical symptoms (holes, bleaching, twisted blades) were selected for each sampling session.  
81 We focused on a general “symptoms” category rather than on a specific disease because it was  
82 impossible to find enough individuals with the same symptoms throughout the sampling sessions  
83 and sites. The algal material was immediately placed in sterile plastic bags and rapidly (<3h)  
84 transported to the laboratory in a cooling box at ca. 4°C.

85 Two parts of the blades were sampled: the basal meristem and the tip (**Figure 1**). A disc  
86 with  $\varnothing 2\text{cm}$  was punched out for each part of the blade and placed in a 15 ml Falcon tube  
87 containing 5ml of clean silica gel (2-6mm; VWR), following the protocol developed in our  
88 laboratory (Burgunter-Delamare et al., 2022). Tubes were stored at room temperature for up to  
89 15 days before DNA extraction.

90 For the samples from Roscoff, corresponding environmental variables (temperature,  
91 salinity and ammonium, nitrites, nitrates, and phosphate concentrations) were obtained from the  
92 Service d'Observation en Milieu Littoral (SOMLIT) database (<https://www.somlit.fr/mysomlit/>;  
93 Astan point; approximately 3.6km North-East of the sampling point). They are available in **Figure**  
94 **S1**. For the other sites, no corresponding environmental data was available.

## 95 2. DNA extraction

96 DNA extraction was carried out with the silica-gel stored samples, according to the  
97 protocol described by Bernard et al. (2017). Briefly, samples were freeze-dried, and  $\frac{1}{2}$  of a disk  
98 was ground using a Qiagen TissueLyser II bead beater (3 sessions, 45sec, 30Hz, 3mm stainless  
99 steel beads). Nucleic acids were then extracted using a 2% CTAB extraction buffer (100 mM Tris-  
100 HCl [pH 7.5], 1.5 M NaCl, 2% CTAB, 50 mM EDTA [pH 8], 50 mM DTT; shaker 250 rpm at room  
101 temperature). Supernatants were purified with one volume of chloroform/isoamyl alcohol (24:1)  
102 followed by 15min centrifugation at 10 000 rpm (16°C). The upper phase was transferred to a new  
103 tube, and ethanol (0.3 vol) was added drop by drop until polysaccharide precipitation was visible,  
104 followed by a second chloroform/isoamyl alcohol extraction and recovery of the aqueous phase.  
105 The pre-purified DNA was purified using Nucleospin plant II columns (Macherey-Nagel, Germany)  
106 according to the manufacturer's instructions. Finally, DNA was eluted in 50 $\mu\text{l}$  of elution buffer  
107 (Macherey-Nagel). Blank extractions were also performed. These extracts were used to identify  
108 potential contaminations introduced during the extraction and downstream processing of the  
109 samples.

## 110 3. 16S Metabarcoding

111 The bacterial community composition associated with algal cultures was determined by  
112 16S metabarcoding. A mock community comprising a mix of DNA from 26 cultivated bacterial

113 strains (Thomas et al., 2019) and negative control were run and treated in parallel to the DNA  
114 extracts. For all of these samples, the V3 and V4 regions of the 16S rDNA gene were amplified  
115 using the NOCHL primers including Illumina adapters (Thomas et al., 2019), to avoid plastid DNA  
116 amplification. Then a standard Illumina protocol for metabarcoding (Illumina, 2013) was run using  
117 the Q5® High-Fidelity PCR Kit (New England BioLabs, MA, USA), the AMPure XP for PCR  
118 Purification Kit (Beckman Coulter, Brea, CA, USA), and the Nextera XT DNA Library Preparation Kit  
119 (Illumina, San Diego, CA, USA). Libraries were quantified with a Quantifluor® ds DNA System  
120 (Promega, WI, USA), and mean fragment size was determined using a LabChip® GX Touch™  
121 (Perkin Elmer, MA, USA). An equimolar pool of all samples was generated at a concentration of 4  
122 nM, diluted to 3 pM, spiked with 10% PhiX (Illumina), and sequenced on an Illumina MiSeq  
123 sequencer at the Genomer platform (Station Biologique de Roscoff) using a MiSeq v3 kit  
124 (2x300bp, paired-end). Raw Illumina reads were deposited at the European Nucleotide Archive  
125 under project accession number PRJEB47035.

#### 126 4. Analyses

127 Sequence analysis was performed using the DADA2 1.14.0 package (Callahan et al., 2016)  
128 on R 3.6.2 following the protocol established by Benjamin Callahan  
129 (<https://benjjneb.github.io/dada2/tutorial.html>). Sequences were filtered, allowing for a  
130 maximum of 2 expected errors and reducing the read length to 291 bp for forward reads and 265  
131 bp for reverse reads. An amplicon sequence variant (ASV) table was constructed, and chimeras  
132 were removed. The taxonomy of the remaining ASVs was assigned using the Silva\_SEED 138  
133 database. The resulting abundance table and taxonomic classification were analyzed using  
134 Phyloseq 1.30.0 (McMurdie and Holmes, 2013). ASVs that were more abundant in the blank  
135 samples than in the algal samples, organellar and eukaryote reads, rare ASVs (<0.01% of total  
136 reads), and samples with less than 7688 remaining reads were removed. Non-Metric  
137 Multidimensional Scaling analyses (NMDS) were carried out using the Bray-Curtis dissimilarities  
138 and the vegan R package version 2.6-2. We tested for factor effects on community dissimilarity  
139 using permutational analysis of variance (PERMANOVA; Anderson, 2001) using the adonis2  
140 function (vegan R package). The most important factor separating the samples in the NMDS was  
141 then further explored. The Shannon H diversity index was calculated using Past version 4.02

142 (Hammer et al., 2001). The normality of the input data was verified with a Shapiro-Wilk test. If  
143 they followed a normal distribution, the variance homogeneity was tested by a Levene test,  
144 followed by a t-test. If the data were not normal, a Kruskal Wallis test or a Mann-Whitney test  
145 was performed. Statistical analysis of differential abundance was performed at the genus and  
146 phylum level using ANCOM-BC version 1.4.0 (Lin and Peddada, 2020) with default parameters.  
147 Binomial tests followed by a Benjamini and Hochberg (BH) correction (Benjamini and Hochberg,  
148 1995) were carried out to determine the overrepresented genera among the ASVs identified by  
149 ANCOM-BC. Then, the factor in question was eliminated from the dataset if possible (grouping of  
150 apex and meristem sample, focus on specific region), and the analyses were repeated to  
151 determine the next factor. Even if there is no consistent definition of the core and threshold  
152 values can range from 50% to 100% (Shade and Handelsman, 2012; Risely, 2020), we choose to  
153 determine the bacterial core at the genus level and define it as genera present in 90% of replicates  
154 for each algal part, season, and location. This is a rather stringent definition excluding, for  
155 instance, bacteria that are associated with the algal hosts only at particular ages or seasons.

### 156 III. RESULTS

#### 157 1. General taxonomy

158 16S metabarcoding analyses were carried out for all control and algal samples. A total of  
159 4,028,372 raw sequences were generated and, after filtering, assembled into 1,658,746 merged  
160 contigs. The taxonomic assignation of mock samples was consistent with the mock composition,  
161 and a total of 18,028 ASVs were identified in the dataset. The sequences obtained corresponded  
162 predominantly to *Alphaproteobacteria* (34,1% of total reads), followed by *Gammaproteobacteria*  
163 (29,5% of total reads) and *Bacteroidota* (26% of total reads).

#### 164 2. Comparison of apex and meristem samples

165 Global NMDS analysis of all samples demonstrated a clear separation between the apex  
166 and meristem samples (PERMANOVA  $p=0.001$ ; **Figure 2A**). We also observed a significant  
167 interaction between the algal part and the region factors (PERMANOVA  $p=0.001$ ), as several apex  
168 samples from Helgoland group with meristem samples, while one meristem sample from Roscoff  
169 grouped with the apex sample. Overall, alpha diversity, as calculated using the Shannon H index



170 (**Figure 2B**), was higher in apex samples than in meristem samples ( $p$ -value  $< 0.0001$ ). Several  
171 phyla were found to differ significantly in relative abundance between the apex and meristem  
172 samples. The *Actinobacteriota*, *Firmicutes*, and unclassified *Proteobacteria* ( $p < 0.0001$ ) were  
173 found in higher relative abundance in the meristem samples. The *Alphaproteobacteria*  
174 ( $p = 0.00016$ ) class, and the *Bacteroidota* ( $p = 0.0037$ ), and *Planctomycetota* ( $p = 0.004$ ) phyla were  
175 relatively more abundant in the apex samples (**Figure 2C**). ANCOM-BC analyses revealed a total  
176 of 122 ASVs to differ significantly (adjusted  $p$ -value  $< 0.05$ ) in relative abundance between the  
177 apex and meristem samples (28 ASVs were more abundant in apex and 94 in meristem samples;  
178 **Table S1**). The taxonomic groups overrepresented (adjusted  $p$ -value  $< 0.05$ ; BH correction) among  
179 these significant ASVs are shown in **Table 2**: one genus was significantly overrepresented in the  
180 apex samples (*Ki89A\_clade*, 39%) and seven in the meristem samples (four of them belong to the  
181 *Gammaproteobacteria*; **Table 2**). The bacterial core in the apex and meristem samples comprises  
182 the four genera *Granulosicoccus*, *Litorimonas*, *Hellea*, and *Blastopirellula*, accounting for 32% of  
183 the total reads for all samples. Five additional genera were systematically present in the apical  
184 part: *Algitalea*, *Arenicella*, *Portibacter*, *Tenacibaculum*, and *Bdellovibrio* and accounted for 15%  
185 of the total reads.

### 186 3. Comparison of regions

187 For the following analyses, reads from the apex and meristem samples of the same alga  
188 were pooled as individuals to remove the apex/meristem effect. On the NMDS plot, the samples  
189 are now grouped according to their region of origin (PERMANOVA  $p = 0.001$ ; **Figure 3A**). The alpha  
190 diversity did not differ significantly between the regions (**Figure 3B**). However, at the phylum  
191 level, the *Firmicutes* and unclassified *Proteobacteria* were underrepresented in the Norwegian  
192 samples compared to Roscoff (ANCOM-BC,  $p = 0.013$  and  $p < 0.0001$ ) and Helgoland (ANCOM-BC,  
193  $p = 0.004$  and  $p < 0.0001$ ; **Figure 3C**). *Bacteroidota* and *Alphaproteobacteria* exhibited significantly  
194 higher relative abundance in Roscoff than in Helgoland (ANCOM-BC,  $p = 0.003$  for both phyla). At  
195 the ASV level, 234 ASVs were represented in higher proportions in the Roscoff samples, 243 in  
196 the Helgoland samples, and 18 in the samples from Skagerrak (**Table S1**). The taxonomic affiliation  
197 of significantly over-expressed ASVs (adjusted  $p$ -value  $< 0.05$ ; BH correction) is shown in **Table 3**.  
198 Twelve genera were significantly overrepresented in Helgoland samples (including

199 *Alphaproteobacteria* 28,4% of ASVs and *Bacteroidota* 13,5% of ASVs), four genera in the Norwegian  
200 samples (including *Rhizobiaceae\_NA*; 50% of ASVs), and eleven genera in Roscoff samples (nine  
201 of them belong to the *Proteobacteria*; **Table 3**).

#### 202 4. Seasonality

203 Only Roscoff samples (apex and meristem reads of the same alga pooled as individuals)  
204 were used to assess the impact of season on the microbiome because these were the only  
205 samples with four sampling points from different seasons available. The NMDS analysis shows a  
206 separation between different seasons' samples. The spring and summer samples formed separate  
207 clusters, whereas the autumn and winter samples overlapped in an intermediate position  
208 between the two spring and summer samples (PERMANOVA  $p=0.001$ ; **Figure 4A**), even if the  
209 alpha diversity (**Figure 4B**) did not differ significantly between the seasons. *Actinobacteria* were  
210 exclusively found in summer. *Firmicutes* (ANCOM-BC,  $p=0.032$ ) were more abundant in autumn  
211 samples than in spring samples (ANCOM-BC,  $p=0.032$ ). *Alphaproteobacteria* were significantly  
212 more abundant in autumn than summer (ANCOM-BC,  $p=0.0017$ ) and winter (ANCOM-BC,  
213 ANCOM-BC  $p=0.014$ ). *Gammaproteobacteria* were significantly more abundant in spring than in  
214 autumn (ANCOM-BC,  $p=0.032$ ) and winter ( $p=0.022$ ; **Figure 4C**). ANCOM-BC analyses revealed  
215 422 ASVs with higher relative abundance in one or several seasons. 126 ASVs were most abundant  
216 in winter samples, 85 ASVs in spring samples, 95 ASVs in summer samples, and 115 ASVs in  
217 autumn samples (**Table S1**). The taxa significantly overexpressed among these ASVs (adjusted  $p$ -  
218  $value < 0.05$ ; BH correction) are shown in **Table 4**. Most ASVs with higher relative abundance  
219 belonged to the *Alphaproteobacteria* in the spring (*Litorimonas*, *Robiginitomaculum* and *Yoonia-*  
220 *Loktanella*; 27.1% of ASVs), and autumn samples (*Algitalia*, *Octadecabacter*, *Tateyamaria*,  
221 *Litorimonas* and *Yoonia-Loktanella*; 37.4% of ASVs). In winter the over-represented ASVs belong  
222 to the *Gammaproteobacteria* (three genera; 28.6% of ASVs), *Alphaproteobacteria*  
223 (*Octadecabacter*, *Litoreibacter* and *Yoonia-Loktanella*; 17.5% of ASVs), and *Bacteroidota* (one  
224 genus; 8% of ASVs). In autumn, the *Cytophagales\_NA* and *Algitalia* genera were also over-  
225 represented (*Bacteroidota*, representing 14.8% of ASVs). And in summer, eight genera were over-  
226 represented, and 33% of ASVs belonged to the *Gammaproteobacteria*. Also, the  
227 Sva0996\_marine\_group (*Actinobacteria*; 12%) was overrepresented only in these samples.

## 228 5. Comparison Healthy / Symptoms

229 Both healthy samples and samples with symptoms were found only in Roscoff and  
230 Helgoland, and the symptoms were diverse: holes, twisted blade, bubbling in the blade and  
231 bleaching (**Figure 5**). The NMDS shows no separation between the healthy individuals and those  
232 with symptoms (PERMANOVA  $p>0.05$ ; **Figure 6A**), and the Shannon H index indicated no alpha  
233 diversity differences ( $p>0.05$ ; **Figure 6B**). No phyla significantly and systematically differed  
234 between healthy algae and algae with symptoms (**Figure 6C**). This observation also remains true  
235 when we distinguish samples between the different types of symptoms (**Figure 6D**), and the  
236 samples are still separated depending on the region. However, ANCOM-BC analyses revealed 9  
237 ASVs that were characteristic in either of the groups: four ASVs were more abundant in samples  
238 with symptoms (*Alteromonadaceae\_NA*, *Octadecabacter* sp., *Tenacibaculum* sp., *Yoonia-*  
239 *Loktanella* sp.) and five that were more abundant in the healthy ones (*Escherichia/Shigella* sp.,  
240 *Granulosicoccus* sp., *K189A\_clade*, *Rhodobacteraceae\_NA*, *Zobellia* sp.; **Table S1**).

## 241 IV. DISCUSSION

242 Knowledge of the bacterial communities within holobionts is key to understanding the  
243 dynamics of these systems. Here we studied the diversity and composition of bacterial  
244 communities of *S. latissima* by 16S metabarcoding analysis. The impacts of several factors on  
245 bacterial communities were examined: algal blade part, origin of the host, season, and host  
246 condition.

### 247 The blade part is the primary driver of samples separation

248 Distinct bacterial communities were demonstrated to be associated with different parts  
249 of *S. latissima* by 16S metabarcoding, and this is the primary factor of separation regardless of  
250 region, season, and physiology. Staufenberger et al. (2008) found the same dynamics when  
251 working on several *S. latissima* tissue from the Baltic Sea and the North Sea, sampled in winter  
252 and spring.

253 *S. latissima*'s type of growth can explain this difference in bacterial communities. *S.*  
254 *latissima* is a short-lived but perennial species, and growth occurs mainly in the meristem region.

255 From there, the proliferating cells form the thallus. Young algae only have short blades and no  
256 access to the surrounding sediment as they only stand upright in the water column. As the thallus  
257 grows, it becomes heavier, and the apex finally bends and touches the ground (Kain, 1979; Lüning,  
258 1991). Water currents move the old blade, resulting in access to nearby substrates and a broader  
259 environment. Mechanical stress also occurs in this part, which becomes vulnerable to bacterial  
260 decomposition, offering new ecological niches for different bacteria (Bengtsson and Øvreås,  
261 2010). Therefore, the younger meristem tissues are typically less colonized by bacteria and exhibit  
262 lower bacterial diversity, as previously found (Staufenberger et al., 2008; Goecke et al., 2010; Ihua  
263 et al., 2020; Lemay et al., 2021). Furthermore, the synthesis or release of compounds that either  
264 have an antimicrobial effect or act as nutrients for the bacteria may vary between the different  
265 parts of the blade. This was described for phenolic substances in the kelp *L. hyperborea* and likely  
266 contributed to differences in the microbial composition (Bengtsson et al., 2012).

267 We found a higher proportion of *Planctomycetes* at the apex and *Actinobacteriota* almost  
268 exclusively in the meristem. Both phyla are found in brown macroalgae (Hollants et al., 2013) and  
269 on *S. latissima* [apex: (Staufenberger et al., 2008); meristem: (Tourneroché et al., 2020)].  
270 *Planctomycetes* contain many sulfatase genes (Wegner et al., 2013), which help degrade sulfated  
271 polysaccharides. They may also be involved in degrading polysaccharides from the extracellular  
272 matrix of microbial biofilms (Parrot et al., 2019), which may explain their higher relative  
273 abundance in older tissues that exhibit first signs of degradation. *Actinobacteriota*, on the other  
274 hand, is a diverse phylum that has successfully colonized a wide range of habitats (Ul-Hassan and  
275 Wellington, 2009), but we currently do not know what features make them successful colonizers  
276 of the *S. latissima* meristem.

277 The bacterial core also reveals the shift from a low to a higher diversity as the blade ages,  
278 with four genera found in both algal parts and five additional genera found in >90% of apex  
279 samples. Those taxa were also found on the meristem part of *L. digitata* (Ihua et al., 2020) and on  
280 the blade of *L. setchellii* (Lemay et al., 2021), *Taonia atomaria* (Paix et al., 2021), and *U. lactuca*  
281 (Comba González et al., 2021). Our results are furthermore consistent with the core microbiota  
282 found in *S. latissima* and *L. hyperborea* in the United Kingdom (King et al., 2022). *Granulosicoccus*  
283 sp. (*Gammaproteobacteria*) was one of the most abundant genera (12% of total reads) and

284 included several ASVs overexpressed in the meristem samples. This genus was also found  
285 abundantly on the youngest parts of the sister species *S. japonica* (Balakirev et al., 2012; Zhang  
286 et al., 2020) and other kelps like *L. setchellii* (Lemay et al., 2021), *L. hyperborea* (Bengtsson et al.,  
287 2012), *Macrocystis pyrifera*, and *Nereocystis luetkeana* (Weigel and Pfister, 2019; Ramírez-Puebla  
288 et al., 2022), reinforcing the idea of a strong association between *Granulosicoccus* and the kelp  
289 tissue. This genus might help its host by providing vitamins (vitamin B12, for example) and  
290 reduced nitrogen (Kang et al., 2018; Capistrant-Fossa et al., 2021; Weigel et al., 2022). In the same  
291 vein, the genus *Algिताlea* (*Flavobacteriaceae*; 3% of total reads) was one of the “apex” bacterial  
292 core genera. This genus belonged to the pioneer bacterial communities found on the apical parts  
293 of *T. atomaria* (Paix et al., 2020). Also, the *Flavobacterium* lineage has been recognized as  
294 necessary in the decomposition processes of organic matter during algae blooms (Riemann et al.,  
295 2000; Pinhassi et al., 2004) and thus may participate in the decay process occurring at the algal  
296 apices.

#### 297 [Regional specificities: tides, seawater, and genetic background](#)

298         Aside from the apex/meristem duality, the region of origin also was a decisive separation  
299 factor. Lachnit et al., 2009 have already shown this region-dependent separation by comparing  
300 global epibacterial communities of algae from the North and Baltic Seas. In their study, only *S.*  
301 *latissima* showed regional differences within conspecific algae (contrary to the two other studied  
302 *Phaeophyceae*). At a larger geographical scale, results on *Ulva* sp. and *Agarophyton vermiculosum*  
303 (Roth-Schulze et al., 2018; Bonthond et al., 2020) suggested that the seaweed microbiota  
304 composition, diversity, and functions strongly depend on the local scale, but also shows that  
305 processes are acting at larger scales to shape this microbial community, and they need to be  
306 identified.

307         In our study, the regional differences were more pronounced than the seasonal  
308 differences obtained for one sampling site, suggesting that region and not just variability between  
309 sampling dates was the driving factor. The regional differences might be due to several abiotic  
310 factors. For instance, the tidal ranges for the three regions decrease going north: 10 meters in  
311 Roscoff, 3 meters in Helgoland, and less than 1 meter in Norway. In the same vein, winter  
312 seawater temperatures are lower in the north, which might favor psychrophilic strains over

313 mesophilic communities, as found in a culture-based study on the surface bacteria of *L. longicruris*  
314 (Laycock, 1974). Also, increasing time exposure to rain, wind, or sunlight (UV), waves, currents,  
315 hydrostatic pressure, pH, and salinity can lead to cellular stress and, by extension, senescence.  
316 This changing environment can offer new ecological niches for different bacteria. Lastly, bacterial  
317 communities can be altered by nutrient supply, interspecies competition, and viral infection  
318 (Fuhrman et al., 2015; Stal and Cretoiu, 2016).

319 However, the regional differences might also be due, in part, to the algal genotype. Using  
320 single nucleotide polymorphisms (SNPs) and microsatellites, Guzinski et al. (2016, 2020)  
321 determined that *S. latissima* individuals from Brittany, Helgoland, and Norway are genetically  
322 distinct, and this might lead to the attraction of different bacterial species (Griffiths et al., 2019).  
323 Furthermore, *S. latissima* displays a unique lipidomic signature depending on its geographic origin  
324 (Monteiro et al., 2020), as the content of chemical elements (C, H, N, S), fatty acids, and lipids  
325 varies depending on the region. These molecules are common components of membranes  
326 (Harwood, 2004) and might influence the attractiveness of the algal surface for several bacterial  
327 strains. Lastly, variability, as described by Zhu et al. (2021), was not monitored in this study but  
328 could be included in future sampling schemes to elucidate the relationships between algal  
329 morphology and microbiome or vice versa.

### 330 [Shifts in bacterial communities depending on the season](#)

331 The third factor we examined was seasonality. We also provided the available abiotic data  
332 from a nearby monitoring site to discuss possible environmental drivers of the seasonality, but  
333 please note that our dataset comprising only four time points is insufficient to establish  
334 statistically meaningful correlations. Laycock (1974) observed that as the seasonal temperatures  
335 decreased, the bacterial communities of *L. longicruris* shifted from mesophilic to psychrophilic  
336 strains. When working with *L. hyperborea*, Bengtsson et al. (2010) hypothesized that the seasonal  
337 succession in the bacterial communities might be explained by abiotic factors like seawater  
338 temperature and biotic factors such as seasonal changes in the kelp substrate. Indeed, seawater  
339 temperature alone does not seem to have been the most important factor in our data, as the  
340 seawater was coldest in winter and spring (<12°C), but the samples from autumn and winter were  
341 more similar in their bacterial communities. Other physicochemical parameters might also play a

342 role. Nitrogen is an important element for organisms, and microorganisms can take up nitrogen  
343 in different forms such as nitrate, nitrite, ammonium, urea, organic nitrogen, and in some cases,  
344 dinitrogen gas (N<sub>2</sub>), depending on the organism (Zehr and Ward, 2002). Nitrate, nitrite, and  
345 ammonium concentrations follow seasonal variations, with nitrates being lower in summer and  
346 higher in winter and nitrites at their highest in autumn. Phosphorus is another essential nutrient  
347 for primary production in the euphotic zone. Most of the phosphorus is present in the oxidized  
348 form as free phosphate or bound to organic matter. The phosphate concentration was lowest in  
349 springtime. Several ASVs overrepresented in spring belong to the *Roseobacter* clade, and Atlantic  
350 strains of this genus are known to possess abundant high-affinity phosphorus uptake systems,  
351 constituting likely adaptations to low environmental phosphate concentrations (Newton et al.,  
352 2010).

353 Lastly, seasonal variations may also be due to seasonal changes in the alga's chemical  
354 composition. For instance, Schiener et al. (2015) demonstrated that in *S. latissima*, polyphenol  
355 levels are higher between May and July and then decrease, reaching their lowest in March. This  
356 could be interpreted as a defense against bacterial colonization as the seawater temperature  
357 rises, polyphenols being known for their wide range of antimicrobial properties (Zhang et al.,  
358 2006; Daglia, 2012). Also, carbohydrate content (laminarin and mannitol) is higher in summer  
359 (Schiener et al., 2015), and these are both substrates easy to degrade by the bacteria (Alderkamp  
360 et al., 2007; Jeske et al., 2013; Groisillier et al., 2015). Similarly, algal iodine content is generally  
361 lower in summer (Nitschke et al., 2018), and the algae's production of toxic iodine compounds  
362 may control the surface biofilm and repulse microbial pathogens (Rodeheaver et al., 1982; Gobet  
363 et al., 2017). Regardless of the mechanisms, please note that seasonal changes may vary from  
364 one site to another, and this has also been previously shown to impact the seasonal changes in  
365 the microbial communities associated with *Macrocystis pyrifera* (Florez et al., 2019). Therefore,  
366 any conclusions drawn here about seasonality are valid only for the Roscoff site.

367 [Is there a microbial signature characteristic for algae in poor health?](#)

368 Some bacteria affect the alga in a deleterious manner by decomposing cell material, like  
369 alginate and laminarin (Laycock, 1974; Dimitrieva and Dimitriev, 1997; Sawabe et al., 1998b;  
370 Ivanova et al., 2003) or by causing diseases like *Alteromonas* species (Vairappan et al., 2001; Peng



371 and Li, 2013) and species of *Pseudoalteromonas* (Sawabe et al., 1998a). Ihua et al. (2019) have  
372 shown that the microbial communities (phyla level) associated with intact *Ascophyllum* differ  
373 from rotting algae, suggesting that the decay process might shape the associated bacterial  
374 community. Similarly, the microbial communities of *Ecklonia* are strongly associated with the algal  
375 condition (stressed or not) more than with other variables (Marzinelli et al., 2015). Moreover, the  
376 core bacterial community characteristic of healthy algae may be lost when hosts are subjected to  
377 stress, and the microbiota of stressed individuals of *Ecklonia* were more similar to each other at  
378 a given location than those on healthy hosts (Marzinelli et al., 2015); which is contrary to the so-  
379 called Anna Karenina principle (Zaneveld et al., 2017; Ma, 2020), stating that all “healthy”  
380 microbiomes are alike and each “symptom” microbiome is “sick” in its own way. Lastly, in the red  
381 alga *Delisea pulchra*, certain bacteria have been shown to mitigate bleaching disease, likely by  
382 preventing dysbiosis (Li et al., 2022). If such effects are also common in *S. latissima*, and if they  
383 frequently involve the same bacteria, such bacteria could be statistically overrepresented in algae  
384 without symptoms.

385 In our study, the changes in bacterial communities between healthy and diseased  
386 individuals are visible only at the ASV level, and we found 9 ASVs that were differentially  
387 expressed between the healthy (5 ASVs) and diseased samples (4 ASVs). ASVs characteristic for  
388 the latter belong to the genus *Tenacibaculum* and the *Alteromonadales*, known for their alginate  
389 lyase activities (Thomas et al., 2021), and to the *Roseobacter* clade, known for their production of  
390 quorum-sensing molecules, a phenomenon involved in virulence and pathogenicity (Buchan et  
391 al., 2005; Wagner-Döbler and Biebl, 2006; Brinkhoff et al., 2008). One of the healthy specific ASVs  
392 is a *Granulosicoccus* sp., emphasizing the importance of this genus in the algal microbiota. The  
393 fact that several ASVs were found to differ indicates that, regardless of the type of disease, an  
394 alga that is not well will undergo characteristic changes in the microbiome. Moreover, these ASVs  
395 signatures are probably stable because they are derived from different places, times of the year,  
396 and symptoms, as shown for *Ecklonia* (Marzinelli et al., 2015). Although this would require  
397 additional developments, these signatures might also be helpful as bioindicators for kelp health.

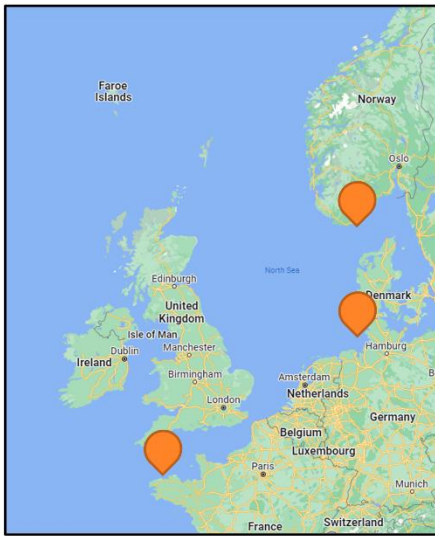


## 398 V. CONCLUSION

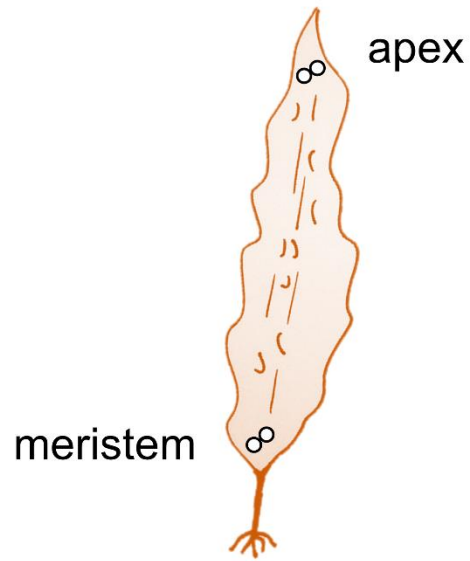
399 In conclusion, our study provides an extensive overview of the *S. latissima* microbiome  
400 and highlights several factors driving its variability. In particular, the observation that the blade  
401 part had a more profound impact on the microbial composition than region or season underlines  
402 the extent to which algal hosts select their associated microbiota. Our discovery of microbial  
403 signatures characteristic for diseased *S. latissima* individuals that persist in our dataset  
404 independently of the disease symptoms further supports this hypothesis. Given the variety of  
405 symptoms observed in our samples, it is unlikely that the same bacteria could be the causative  
406 agents in all cases. Rather, the different types of disease likely cause similar changes in the host,  
407 which would lead to similar microbial changes. Understanding these signatures will be of interest  
408 for fundamental research on the different algal diseases, and may lead to the development of  
409 molecular markers of host health to survey natural populations or aquacultures.

410 FIGURES

A

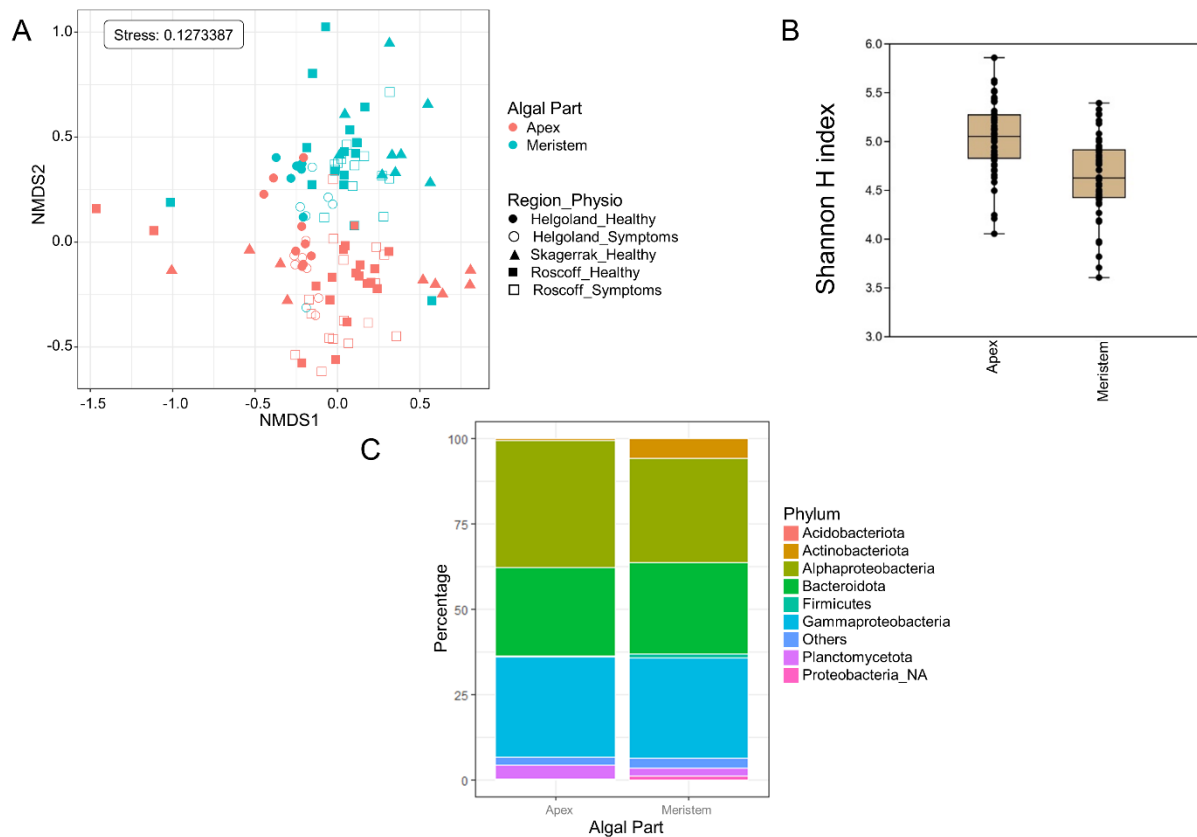


B

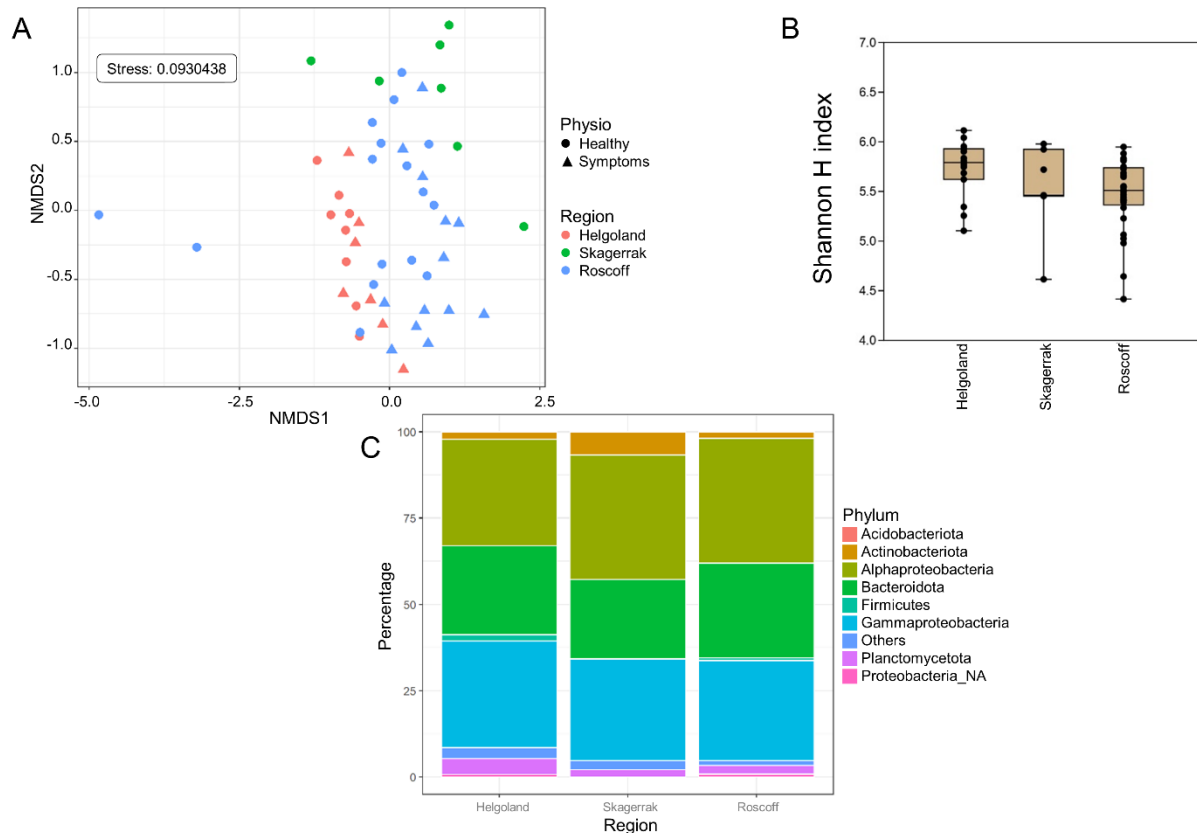


411  
412 **Figure 1 – Sampling.** (A) Map of sampling locations and (B) Sampled parts of the *Saccharina*  
413 *latissima* thalli (<1m). Two discs (Ø2cm) were punched out in immediate proximity for each part  
414 of the blade and preserved in silica gel. Please refer to Table 1 for details on the sampling sites.

Accepted Manuscript



415  
 416 **Figure 2 – Algal blade part analysis.** (A) NMDS analysis of the microbiome composition. Results  
 417 show a clear separation of the apex and meristem samples (PERMANOVA  $p=0.001$ ). (B) Box plot  
 418 of alpha-diversity (Shannon H index) across different sample types calculated at the ASV level.  
 419 Error bars correspond to the range;  $n=111$ ; mean alpha-diversity differs significantly between  
 420 conditions (t-test,  $t=5.47$ ,  $p<0.0001$ ) (C) Comparison of microbiome composition between apex  
 421 and meristem samples at the phylum level. Bars show the relative abundance of 16S rRNA gene  
 422 metabarcoding sequences; all phyla were detected in all sample types, and only their relative  
 423 abundance varied. *Proteobacteria\_NA*: unclassified as *Proteobacteria*.



424

425 **Figure 3 – Region analysis. (A)** NMDS analysis of the microbiome composition. Results show a

426 clear separation of the samples according to their origin (PERMANOVA  $p=0.001$ ). **(B)** Box plot of

427 alpha-diversity (Shannon H index) across different sample types calculated at the ASV level. Error

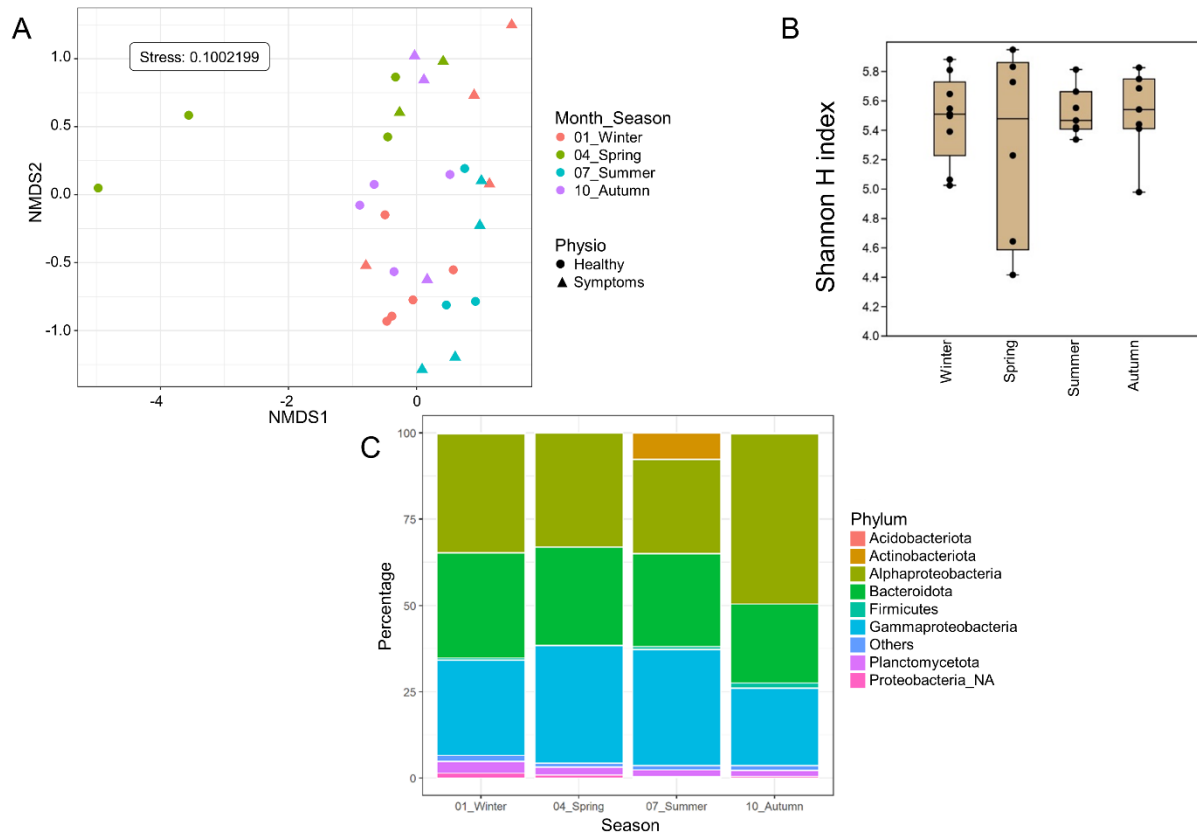
428 bars correspond to the range;  $n=51$ ; conditions do not differ significantly (Kruskal Wallis test,

429  $p>0.05$ ). **(C)** Microbiome composition of samples from Helgoland, Skagerrak, and Roscoff at the

430 phylum level. Bars show the relative abundance of 16S rRNA gene metabarcoding sequences; all

431 phyla were detected in all sample types, and only their relative abundance varied.

432 *Proteobacteria\_NA*: unclassified Proteobacteria.



433

434 **Figure 4 – Season analyses.** Only Roscoff samples were used to assess the impact of the season.

435 **(A)** NMDS analysis of the microbiome composition. Results show a separation of the samples

436 depending on the sampling season (PERMANOVA  $p=0.001$ ). **(B)** Box plot of alpha-diversity

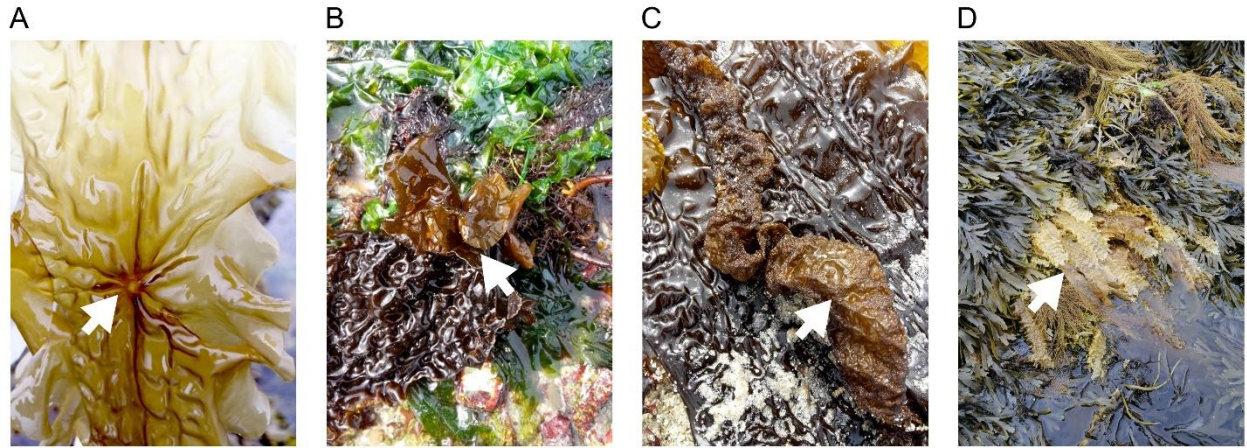
437 (Shannon H index) across different sample types calculated at the ASV level. Error bars correspond

438 to the range;  $n=30$ ; conditions do not differ significantly (Kruskal Wallis test,  $p>0.05$ ). **(C)** Seasonal

439 differences in microbiome composition in Roscoff at the phylum level. Bars show the relative

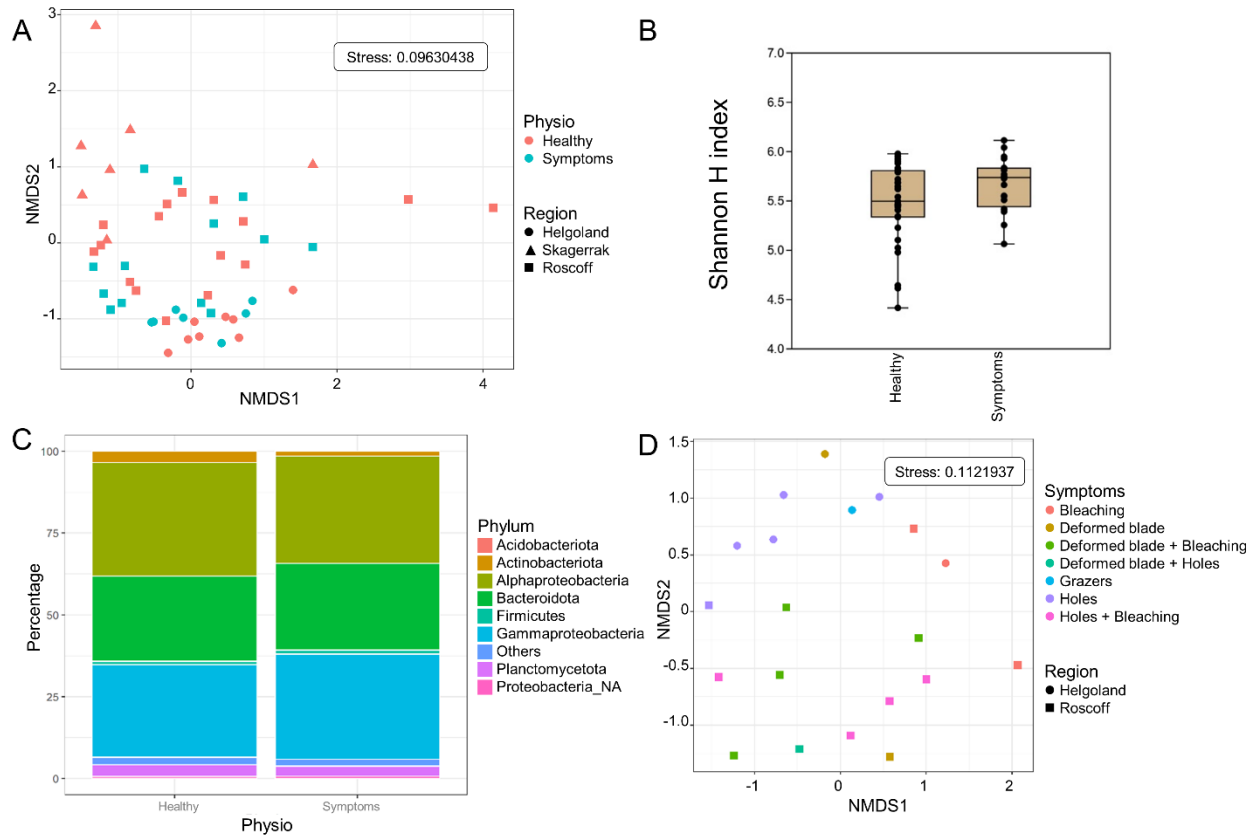
440 abundance of 16S rRNA gene metabarcoding sequences; all phyla were detected in all sample

441 types, and only their relative abundance varied. *Proteobacteria\_NA*: unclassified Proteobacteria.



442

443 **Figure 5 – Examples of symptoms observed on “diseased” *S. latissima* individuals. (A) hole, (B)**  
444 **twisted blade, (C) bubbling in blade, and (D) bleaching.**



446

447 **Figure 6 – Analysis of samples with symptoms** from Roscoff and Helgoland. **(A)** NMDS analysis of  
 448 the microbiome composition. Results do not show a separation of the healthy and symptomatic  
 449 samples (PERMANOVA  $p > 0.05$ ). **(B)** Box plot of alpha-diversity (Shannon H index) across different  
 450 sample types calculated at the ASV level. Error bars correspond to the range;  $n = 51$ , conditions do  
 451 not differ significantly (Mann-Whitney pairwise test,  $p > 0.05$ ). **(C)** Microbiome composition of  
 452 healthy and symptoms samples at the phylum level. Bars show the relative abundance of 16S  
 453 rRNA gene metabarcoding sequences; all phyla were detected in all sample types, and only their  
 454 relative abundance varied. *Proteobacteria\_NA*: unclassified *Proteobacteria*. **(D)** NMDS analysis of  
 455 the microbiome composition depending on the symptoms.

## TABLES

**Table 1 - Sampling dates and sites**

<i>Location</i>	<i>Latitude, Longitude</i>	<i>Date</i>	<i>Time</i>	<i>Types of samples</i>
Roscoff, Brittany, France	48°43'47.0 "N 4°00'17.1" W	23 January 2019 18 April 2019 31 July 2019 29 October 2019	At low tide mid-day for all	Healthy + Symptoms
Helgoland, North Sea, Germany	54°10'47.3 "N 7°54'59.4" E 54°11'27.1 "N 7°52'02.4" E	10 July 2019 11 July 2019	Low tide	Healthy + Symptoms
Skagerrak, Norway	58°15'15.5 "N 8°31'22.2" E 58°22'05.1 "N 8°44'04.7" E 58°05'39.3 "N 6°34'54.9" E	16 October 2018 18 October 2018 1 April 2019	n/a	Healthy samples No samples with symptoms

**Table 2 - Taxonomic affiliations of the ASVs characteristic for apex and meristem samples, compared with their occurrence in the entire dataset.**

n.c.: not calculated. P-values are the uncorrected results of a binomial test, and \*\*\*, \*\*, and \* indicate that these p-values were significant (*i.e.*, the genus is significantly overrepresented among the genera regulated by tissue type) even after a Benjamini and Hochberg correction (corrected  $p < 0.001$ ,  $<0.01$ , and  $<0.05$  respectively).

Taxa	APEX				MERISTEM				Entire dataset		
	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of ASVs	Total number of ASVs	ratio
<i>Kl89A_clade</i>	11	28	0.393	<0.00001 ***	0	94	0.0000	n.c.	100	16689	0.0060
<i>Litorimonas</i>	3	28	0.107	0.01132	13	94	0.1383	<0.00001 ***	530	16689	0.0318
<i>Maribacter</i>	0	28	0	n.c.	15	94	0.1596	<0.00001 ***	89	16689	0.0053
<i>Octadecabacter</i>	0	28	0	n.c.	5	94	0.0532	<0.00001 ***	73	16689	0.0044
<i>Sva0996_marine_group</i>	0	28	0	n.c.	14	94	0.1489	<0.00001 ***	115	16689	0.0069



<i>Proteobacteria_NA</i>	0	28	0	n.c.	4	94	0.0426	0.00004 ***	66	16689	0.0040
<i>Granulosicoccus</i>	3	28	0.107	0.05723	14	94	0.1489	0.00012 ***	878	16689	0.0526
<i>Rickettsiales_NA</i>	0	28	0	n.c.	1	94	0.0106	0.0053 ***	19	16689	0.0011

**Table 3 - Taxonomic affiliations of the ASVs characteristic for the Roscoff, Helgoland, or Skagerrak samples, compared with their occurrence in the entire dataset.** n.c.: not calculated. P-values are the uncorrected results of a binomial test, and \*\*\*, \*\*, and \* indicate that these p-values were significant (*i.e.*, the genus is significantly overrepresented among the genera regulated by tissue type) even after a Benjamini and Hochberg correction (corrected  $p < 0.001$ ,  $<0.01$ , and  $<0.05$  respectively).

Taxa	Roscoff				Helgoland				Skagerrak				Entire dataset		
	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of ASVs	Total number of ASVs	ratio
<i>K189A_clade</i>	19	234	0.0812	<0.00001 ***	0	243	0	n.c.	2	18	0.111	0.00016 ***	100	16689	0.0060
<i>Litorimonas</i>	23	234	0.0983	<0.00001 ***	46	243	0.189	<0.00001 ***	0	18	0	n.c.	530	16689	0.0318
<i>Maribacter</i>	14	234	0.0598	<0.00001 ***	0	243	0	n.c.	0	18	0	n.c.	89	16689	0.00533
<i>Octadecabacter</i>	12	234	0.0513	<0.00001 ***	0	243	0	n.c.	0	18	0	n.c.	73	16689	0.00437
<i>Reichenbachiella</i>	19	234	0.0812	<0.00001 ***	0	243	0	n.c.	0	18	0	n.c.	258	16689	0.0155
<i>Tateyamaria</i>	7	234	0.0299	<0.00001 ***	0	243	0	n.c.	0	18	0	n.c.	49	16689	0.00294
<i>Yoonia-Loktanella</i>	10	234	0.0427	<0.00001 ***	0	243	0	n.c.	0	18	0	n.c.	91	16689	0.00545
<i>Algimonas</i>	4	234	0.0171	0.00002 ***	0	243	0	n.c.	0	18	0	n.c.	22	16689	0.00131
<i>Granulosicoccus</i>	23	234	0.0983	0.00150 **	17	243	0.070	0.0922	0	18	0	n.c.	878	16689	0.0526
<i>Proteobacteria_NA</i>	4	234	0.0171	0.00257 **	0	243	0	n.c.	0	18	0	n.c.	66	16689	0.00395
<i>Hypomonadaceae_NA</i>	11	234	0.0470	0.0064 **	0	243	0	n.c.	0	18	0	n.c.	369	16689	0.0221
<i>Algitalea</i>	8	234	0.0342	0.0871	17	243	0.070	0.00001 ***	0	18	0	n.c.	378	16689	0.0226

<i>Blastopirellula</i>	2	234	0.0085	0.687	14	243	0.0576	0.00001 ***	0	18	0	n.c.	252	16689	0.0151
<i>Thalassotalea</i>	0	234	0	n.c.	6	243	0.0247	0.00040 ***	0	18	0	n.c.	90	16689	0.00539
<i>Lewinella</i>	0	234	0	n.c.	7	243	0.0288	0.00084 ***	0	18	0	n.c.	133	16689	0.00797
<i>Rhodobacteraceae_NA</i>	1	234	0.0043	0.999	19	243	0.0782	0.00197 **	1	18	0.0556	0.160	666	16689	0.0399
<i>Paraglaciocola</i>	1	234	0.0043	0.0915	3	243	0.0123	0.00204 **	0	18	0	n.c.	36	16689	0.00216
<i>Sporolactobacillus</i>	1	234	0.0043	0.123	3	243	0.0123	0.00382 **	0	18	0	n.c.	43	16689	0.00258
<i>Arenicella</i>	5	234	0.0214	0.860	17	243	0.070	0.00399 **	3	18	0.167	0.00364 **	611	16689	0.0366
<i>Tenacibaculum</i>	6	234	0.0256	0.0866	9	243	0.0370	0.00661 **	0	18	0	n.c.	269	16689	0.0161
<i>Sva0996_marine_group</i>	2	234	0.0085	0.220	5	243	0.0206	0.00723 **	0	18	0	n.c.	115	16689	0.00689
<i>Robiginotomaculum</i>	2	234	0.0085	0.115	4	243	0.0165	0.00817 **	0	18	0	n.c.	84	16689	0.00503
<i>Rhizobiaceae_NA</i>	0	234	0	n.c.	0	243	0	n.c.	9	18	0.5	<0.00001 ***	97	16689	0.00581
<i>Zobellia</i>	0	234	0	n.c.	0	243	0	n.c.	1	18	0.0556	0.00178 **	58	16689	0.00348

**Table 4 - Taxonomic affiliations of the ASVs characteristic for each season from Roscoff samples, compared with their occurrence in the entire dataset.** n.c.: not calculated. P-values are the uncorrected results of a binomial test, and \*\*\*, \*\*, and \* indicate that these p-values were significant (*i.e.*, the genus is significantly overrepresented among the genera regulated by tissue type) even after a Benjamini and Hochberg correction (corrected  $p < 0.001$ ,  $<0.01$ , and  $<0.05$  respectively).

Taxa	Winter				Spring				Summer				Autumn				Entire dataset		
	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of over-expressed ASVs	Total number of over-expressed ASVs	ratio	p-value	Number of ASVs	Total number of ASVs	ratio
<i>Gammaproteobacteria_NA</i>	20	126	0.159	<0.00001 ***	5	85	0.0588	0.168	3	95	0.0316	0.600	2	115	0.0174	0.883	729	16689	0.0437
<i>Octadecabacter</i>	17	126	0.135	<0.00001 ***	1	85	0.0118	0.0538	0	95	0	n.c.	3	115	0.0261	0.00172 **	73	16689	0.00437
<i>Reichenbachiella</i>	10	126	0.0794	<0.00001 ***	0	85	0	n.c.	0	95	0	n.c.	0	115	0	n.c.	258	16689	0.0155

<i>Yoonia-Loktanelia</i>	5	126	0.0397	0.00007 ***	5	85	0.0588	0.00001 ***	0	95	0	n.c.	4	115	0.0348	0.00045 ***	91	16689	0.00545
<i>Escherichia/Shigella</i>	2	126	0.0159	0.00043 ***	2	85	0.0236	0.00014 ***	0	95	0	n.c.	0	115	0	n.c.	19	16689	0.00114
<i>Litoreibacter</i>	2	126	0.0159	0.00248 **	1	85	0.0118	0.0140	0	95	0	n.c.	0	115	0	n.c.	35	16689	0.00210
<i>Granulosicoccus</i>	14	126	0.111	0.00266 **	2	85	0.0236	0.831	12	95	0.126	0.00143 **	4	115	0.0348	0.729	878	16689	0.0526
<i>Litorimonas</i>	4	126	0.0317	0.371	15	85	0.176	<0.00001 ***	7	95	0.0737	0.0109	26	115	0.226	<0.00001 ***	530	16689	0.0318
<i>Flavobacteriaceae_NA</i>	3	126	0.0238	0.311	7	85	0.0824	0.00065 ***	0	95	0	n.c.	1	115	0.0087	0.730	373	16689	0.0224
<i>Paraglaciecola</i>	0	126	0	n.c.	2	85	0.0236	0.00087 ***	0	95	0	n.c.	0	115	0	n.c.	36	16689	0.00216
<i>Robiginitomaculum</i>	1	126	0.00794	0.133	3	85	0.0353	0.00094 ***	0	95	0	n.c.	1	115	0.0087	0.115	84	16689	0.00503
<i>Portibacter</i>	1	126	0.00794	0.508	5	85	0.0588	0.00106 **	0	95	0	n.c.	0	115	0	n.c.	225	16689	0.0135
<i>Tenacibaculum</i>	0	126	0	n.c.	5	85	0.0588	0.00259 **	1	95	0.0105	0.454	1	115	0.0087	0.555	269	16689	0.0161
<i>Arenicella</i>	8	126	0.0635	0.0426	4	85	0.0471	0.201	14	95	0.147	<0.00001 ***	5	115	0.0435	0.246	611	16689	0.0366
<i>Sva0996_marine_group</i>	0	126	0	n.c.	0	85	0	n.c.	12	95	0.126	<0.00001 ***	0	115	0	n.c.	115	16689	0.00689
<i>K189A_clade</i>	0	126	0	n.c.	0	85	0	n.c.	5	95	0.0526	0.00003 ***	0	115	0	n.c.	100	16689	0.006
<i>Dokdonia</i>	2	126	0.0159	0.500	1	85	0.0118	0.539	9	95	0.0947	0.00004 ***	2	115	0.0174	0.440	353	16689	0.0212
<i>Hyphomonadaceae_NA</i>	5	126	0.0397	0.0618	0	85	0	n.c.	8	95	0.0842	0.00027 ***	6	115	0.0522	0.0143	369	16689	0.0221
<i>Algimonas</i>	0	126	0	n.c.	0	85	0	n.c.	2	95	0.0211	0.00029 ***	2	115	0.0174	0.00051 ***	22	16689	0.00132
<i>Planctomycetales_NA</i>	0	126	0	n.c.	0	85	0	n.c.	1	95	0.0105	0.00654 **	0	115	0	n.c.	21	16689	0.00126
<i>Tateyamaria</i>	1	126	0.00794	0.0534	1	85	0.0118	0.0262	1	95	0.0105	0.0321	8	115	0.0696	<0.00001 ***	49	16689	0.00294
<i>Algitalea</i>	4	126	0.0317	0.159	0	85	0	n.c.	0	95	0	n.c.	15	115	0.130	<0.00001 ***	378	16689	0.0226
<i>Cytophagales_NA</i>	0	126	0	n.c.	0	85	0	n.c.	0	95	0	n.c.	2	115	0.0174	0.00387 **	45	16689	0.0027

## 1    DECLARATIONS

2    **Funding:** This work was funded partially by ANR project IDEALG (ANR-10-BTBR-04)  
3    “Investissements d’Avenir, Biotechnologies-Bioressources”, the CNRS momentum call (2017), and  
4    by the European Union’s Horizon 2020 research and innovation program under grant agreement  
5    No 730984, ASSEMBLE Plus project. BBD was funded by a joint Ph.D. scholarship from the Brittany  
6    region (Project HOSALA) and Sorbonne University (ED227).

7    **Competing interests:** The authors declare that they have no competing interests.

8    **Data availability:** Raw sequence data were deposited at the European Nucleotide Archive under  
9    project accession number ENA: PRJEB47035.

10   **Code availability:** not applicable

11   **Authors’ contributions:** Designed study: BBD, SD; Sampling: BBD, SF, SD; Performed experiments:  
12   BBD, EL, GT; Analyzed data: BBD, SD; Wrote the manuscript: BBD, SD; Provided valuable input and  
13   corrected the manuscript: CB.

## 14    ACKNOWLEDGEMENTS

15    We thank François Thomas, Maéva Brunet, and Nolwen Le Duff for their primers and for providing  
16    the mock community; Sylvie Rousvoal for advice on sample preparation; Jonas Collén for advice  
17    and participating in the first sampling; Kai Bishof and Nora Diehl for providing samples from  
18    Svalbard, which unfortunately could not be included, and Catherine Leblanc for helpful  
19    discussions. This work benefited from access to the Station Biologique de Roscoff, an EMBRC-  
20    France and EMBRC-ERIC Site, and the Biologische Anstalt Helgoland AWI-BAH. We would like to  
21    thank the reviewers and editor for taking time and effort necessary to review the manuscript. We  
22    sincerely appreciate all valuable comments and suggestions, which helped us to improve the  
23    quality of the manuscript.

24

## 25 REFERENCES

- 26 Alderkamp, A.-C., Van Rijssel, M., and Bolhuis, H. (2007). Characterization of marine bacteria and  
27 the activity of their enzyme systems involved in degradation of the algal storage glucan  
28 laminarin: *FEMS Microbiol. Ecol.* 59, 108–117. doi: 10.1111/j.1574-6941.2006.00219.x.
- 29 Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance.  
30 *Austral Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x.
- 31 Araújo, R. M., Assis, J., Aguillar, R., Airoidi, L., Bárbara, I., Bartsch, I., et al. (2016). Status, trends  
32 and drivers of kelp forests in Europe: an expert assessment. *Biodivers. Conserv.* 25, 1319–  
33 1348. doi: 10.1007/s10531-016-1141-7.
- 34 Balakirev, E. S., Krupnova, T. N., and Ayala, F. J. (2012). Symbiotic associations in the  
35 phenotypically-diverse brown alga *Saccharina japonica*. *PLoS ONE* 7, e39587. doi:  
36 10.1371/journal.pone.0039587.
- 37 Bengtsson, M. M., and Øvreås, L. (2010). *Planctomycetes* dominate biofilms on surfaces of the  
38 kelp *Laminaria hyperborea*. *BMC Microbiol.* 10, 261. doi: 10.1186/1471-2180-10-261.
- 39 Bengtsson, M. M., Sjøtun, K., Lanzén, A., and Øvreås, L. (2012). Bacterial diversity in relation to  
40 secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. *ISME J.*  
41 6, 2188–2198. doi: 10.1038/ismej.2012.67.
- 42 Bengtsson, M., Sjøtun, K., and Øvreås, L. (2010). Seasonal dynamics of bacterial biofilms on the  
43 kelp *Laminaria hyperborea*. *Aquat. Microb. Ecol.* 60, 71–83. doi: 10.3354/ame01409.
- 44 Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and  
45 powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300. doi:  
46 10.1111/j.2517-6161.1995.tb02031.x.

47 Bernard, M., Rousvoal, S., Jacquemin, B., Ballenghien, M., Peters, A. F., and Leblanc, C. (2017).  
48 qPCR-based relative quantification of the brown algal endophyte *Laminarionema elsbetiae*  
49 in *Saccharina latissima*: variation and dynamics of host - endophyte interactions. *J. Appl.*  
50 *Phycol.* 30, 2901–2911. doi: 10.1007/s10811-017-1367-0.

51 Bolton, J. J. (2010). The biogeography of kelps (*Laminariales*, *Phaeophyceae*): a global analysis  
52 with new insights from recent advances in molecular phylogenetics. *Helgol. Mar. Res.* 64,  
53 263–279. doi: 10.1007/s10152-010-0211-6.

54 Bonthond, G., Bayer, T., Krueger-Hadfield, S. A., Barboza, F. R., Nakaoka, M., Valero, M., et al.  
55 (2020). How do microbiota associated with an invasive seaweed vary across scales? *Mol.*  
56 *Ecol.* 29, 2094–2108. doi: 10.1111/mec.15470.

57 Bosch, T. C. G., and Miller, D. J. (2016). “Bleaching as an obvious dysbiosis in corals,” in *The*  
58 *Holobiont Imperative* (Vienna: Springer Vienna), 113–125. doi: 10.1007/978-3-7091-1896-  
59 2\_9.

60 Bourne, D., Iida, Y., Uthicke, S., and Smith-Keune, C. (2008). Changes in coral-associated microbial  
61 communities during a bleaching event. *ISME J.* 2, 350–363. doi: 10.1038/ismej.2007.112.

62 Brinkhoff, T., Giebel, H.-A., and Simon, M. (2008). Diversity, ecology, and genomics of the  
63 Roseobacter clade: a short overview. *Arch. Microbiol.* 189, 531–539. doi: 10.1007/s00203-  
64 008-0353-y.

65 Buchan, A., González, J. M., and Moran, M. A. (2005). Overview of the Marine Roseobacter  
66 Lineage. *Appl. Environ. Microbiol.* 71, 5665–5677. doi: 10.1128/AEM.71.10.5665-5677.2005.

67 Burgunter-Delamare, B., KleinJan, H., Frioux, C., Fremy, E., Wagner, M., Corre, E., et al. (2020).  
68 Metabolic complementarity between a brown alga and associated cultivable bacteria

69 provide indications of beneficial interactions. *Front. Mar. Sci.* 7, 85. doi:  
70 10.3389/fmars.2020.00085.

71 Burgunter-Delamare, B., Tanguy, G., Legeay, E., Boyen, C., and Dittami, S. M. (2022). Effects of  
72 sampling and storage procedures on 16S rDNA amplicon sequencing results of kelp  
73 microbiomes. *Mar. Genomics* 63, 100944. doi: 10.1016/j.margen.2022.100944.

74 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016).  
75 DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13,  
76 581–583. doi: 10.1038/nmeth.3869.

77 Capistrant-Fossa, K. A., Morrison, H. G., Engelen, A. H., Quigley, C. T. C., Morozov, A., Serrão, E.  
78 A., et al. (2021). The microbiome of the habitat-forming brown alga *Fucus vesiculosus*  
79 (*Phaeophyceae*) has similar cross-Atlantic structure that reflects past and present drivers. *J.*  
80 *Phycol.*, jpy.13194. doi: 10.1111/jpy.13194.

81 Comba González, N. B., Niño Corredor, A. N., López Kleine, L., and Montoya Castaño, D. (2021).  
82 Temporal changes of the epiphytic bacteria community from the marine macroalga *Ulva*  
83 *lactuca* (Santa Marta, Colombian-Caribbean). *Curr. Microbiol.* 78, 534–543. doi:  
84 10.1007/s00284-020-02302-x.

85 Corre, S., and Prieur, D. (1990). Density and morphology of epiphytic bacteria on the kelp  
86 *Laminaria digitata*. *Bot. Mar.* 33. doi: 10.1515/botm.1990.33.6.515.

87 Daglia, M. (2012). Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* 23, 174–181. doi:  
88 10.1016/j.copbio.2011.08.007.

89 Dimitrieva, G. Y., and Dimitriev, S. M. (1997). Symbiotic microflora of brown algae of the genus  
90 *Laminaria* as a bioindicator of the ecological condition of coastal laminarian biocenoses.  
91 *Oceanogr. Lit. Rev.* 11, 1330.

92 Dittami, S. M., Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., et al. (2016). Host-  
93 microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures.  
94 *ISME J.* 10, 51–63. doi: 10.1038/ismej.2015.104.

95 Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., and Thomas, T. (2013). The seaweed  
96 holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol. Rev.* 37, 462–  
97 476. doi: 10.1111/1574-6976.12011.

98 Florez, J. Z., Camus, C., Hengst, M. B., Marchant, F., and Buschmann, A. H. (2019). Structure of the  
99 epiphytic bacterial communities of *Macrocystis pyrifera* in localities with contrasting  
100 nitrogen concentrations and temperature. *Algal Res.* 44, 101706. doi:  
101 10.1016/j.algal.2019.101706.

102 Fuhrman, J. A., Cram, J. A., and Needham, D. M. (2015). Marine microbial community dynamics  
103 and their ecological interpretation. *Nat. Rev. Microbiol.* 13, 133–146. doi:  
104 10.1038/nrmicro3417.

105 Gobet, A., Corre, E., Correc, G., Delage, L., Dittami, S., KleinJan, H., et al. (2017). Characterization  
106 of the epiphytic bacterial community associated with the kelp *Laminaria digitate*. *Phycologia*  
107 56, 64.

108 Goecke, F., Labes, A., Wiese, J., and Imhoff, J. (2010). Chemical interactions between marine  
109 macroalgae and bacteria. *Mar. Ecol. Prog. Ser.* 409, 267–299. doi: 10.3354/meps08607.



110 Griffiths, S. M., Antwis, R. E., Lenzi, L., Lucaci, A., Behringer, D. C., Butler, M. J., et al. (2019). Host  
111 genetics and geography influence microbiome composition in the sponge *Ircinia campana*.  
112 *J. Anim. Ecol.* 88, 1684–1695. doi: 10.1111/1365-2656.13065.

113 Groisillier, A., Labourel, A., Michel, G., and Tonon, T. (2015). The mannitol utilization system of  
114 the marine bacterium *Zobellia galactanivorans*. *Appl. Environ. Microbiol.* 81, 1799–1812.  
115 doi: 10.1128/AEM.02808-14.

116 Guzinski, J., Mauger, S., Cock, J. M., and Valero, M. (2016). Characterization of newly developed  
117 expressed sequence tag-derived microsatellite markers revealed low genetic diversity within  
118 and low connectivity between European *Saccharina latissima* populations. *J. Appl. Phycol.*  
119 28, 3057–3070. doi: 10.1007/s10811-016-0806-7.

120 Guzinski, J., Ruggeri, P., Ballenghien, M., Mauger, S., Jacquemin, B., Jollivet, C., et al. (2020).  
121 Seascape genomics of the sugar kelp *Saccharina latissima* along the North Eastern Atlantic  
122 Latitudinal Gradient. *Genes* 11, 1503. doi: 10.3390/genes11121503.

123 Hammer, Ø., Harper, D. A., and Ryan, P. D. (2001). PAST: Paleontological statistics software  
124 package for education and data analysis. *Palaeontol. Electron.* 4, 9.

125 Harwood, J. L. (2004). “Membrane lipids in algae,” in *Lipids in Photosynthesis: Structure, Function*  
126 *and Genetics* Advances in Photosynthesis and Respiration., eds. S. Paul-André and M. Norio  
127 (Dordrecht: Kluwer Academic Publishers), 53–64. doi: 10.1007/0-306-48087-5\_3.

128 Hollants, J., Leliaert, F., De Clerck, O., and Willems, A. (2013). What we can learn from sushi: a  
129 review on seaweed–bacterial associations. *FEMS Microbiol. Ecol.* 83, 1–16. doi:  
130 10.1111/j.1574-6941.2012.01446.x.

131 Ihua, M., Guihéneuf, F., Mohammed, H., Margassery, L., Jackson, S., Stengel, D., et al. (2019).  
132 Microbial population changes in decaying *Ascophyllum nodosum* result in macroalgal-  
133 polysaccharide-degrading bacteria with potential applicability in enzyme-assisted extraction  
134 technologies. *Mar. Drugs* 17, 200. doi: 10.3390/md17040200.

135 Ihua, M. W., FitzGerald, J. A., Guihéneuf, F., Jackson, S. A., Claesson, M. J., Stengel, D. B., et al.  
136 (2020). Diversity of bacteria populations associated with different thallus regions of the  
137 brown alga *Laminaria digitata*. *PLOS ONE* 15, e0242675. doi:  
138 10.1371/journal.pone.0242675.

139 Illumina (2013). 16S Metagenomic sequencing library preparation. *Prep. 16S Ribosomal RNA Gene*  
140 *Amplicons Illumina MiSeq Syst.*, 1–28.

141 Ivanova, E. P., Bakunina, I. Yu., Nedashkovskaya, O. I., Gorshkova, N. M., Alexeeva, Y. V., Zelepuga,  
142 E. A., et al. (2003). Ecophysiological variabilities in ectohydrolytic enzyme activities of some  
143 *Pseudoalteromonas* species, *P. citrea*, *P. issachenkonii*, and *P. nigrifaciens*. *Curr. Microbiol.*  
144 46, 6–10. doi: 10.1007/s00284-002-3794-6.

145 Jeske, O., Jogler, M., Petersen, J., Sikorski, J., and Jogler, C. (2013). From genome mining to  
146 phenotypic microarrays: *Planctomycetes* as source for novel bioactive molecules. *Antonie*  
147 *Van Leeuwenhoek* 104, 551–567. doi: 10.1007/s10482-013-0007-1.

148 Kain, J. M. (1979). A view of the genus *Laminaria*. *Oceanogr. Mar. Biol. Annu. Rev.* 17, 101–161.

149 Kang, I., Lim, Y., and Cho, J.-C. (2018). Complete genome sequence of *Granulosicoccus antarcticus*  
150 type strain IMCC3135T, a marine gammaproteobacterium with a putative  
151 dimethylsulfoniopropionate demethylase gene. *Mar. Genomics* 37, 176–181. doi:  
152 10.1016/j.margen.2017.11.005.

153 King, N. G., Moore, P. J., Thorpe, J. M., and Smale, D. A. (2022). Consistency and Variation in the  
154 Kelp Microbiota: Patterns of Bacterial Community Structure Across Spatial Scales. *Microb.*  
155 *Ecol.* doi: 10.1007/s00248-022-02038-0.

156 Lachnit, T., Blümel, M., Imhoff, J., and Wahl, M. (2009). Specific epibacterial communities on  
157 macroalgae: phylogeny matters more than habitat. *Aquat. Biol.* 5, 181–186. doi:  
158 10.3354/ab00149.

159 Laycock, R. A. (1974). The detrital food chain based on seaweeds. I. Bacteria associated with the  
160 surface of *Laminaria* fronds. *Mar. Biol.* 25, 223–231. doi: 10.1007/BF00394968.

161 Lemay, M. A., Davis, K. M., Martone, P. T., and Parfrey, L. W. (2021). Kelp-associated microbiota  
162 are structured by host anatomy. *J. Phycol.* 57, 1119–1130. doi: 10.1111/jpy.13169.

163 Li, J., Majzoub, M. E., Marzinelli, E. M., Dai, Z., Thomas, T., and Egan, S. (2022). Bacterial controlled  
164 mitigation of dysbiosis in a seaweed disease. *ISME J.* 16, 378–387. doi: 10.1038/s41396-021-  
165 01070-1.

166 Lin, H., and Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction.  
167 *Nat. Commun.* 11, 3514. doi: 10.1038/s41467-020-17041-7.

168 Lüning, K. (1991). *Seaweeds: their environment, biogeography, and ecophysiology*. New York, NY:  
169 Wiley.

170 Ma, Z. (Sam) (2020). Testing the Anna Karenina Principle in Human Microbiome-Associated  
171 Diseases. *iScience* 23, 101007. doi: 10.1016/j.isci.2020.101007.

172 Marzinelli, E. M., Campbell, A. H., Zozaya Valdes, E., Vergés, A., Nielsen, S., Wernberg, T., et al.  
173 (2015). Continental-scale variation in seaweed host-associated bacterial communities is a

174 function of host condition, not geography. *Environ. Microbiol.* 17, 4078–4088. doi:  
175 10.1111/1462-2920.12972.

176 Mazure, H. G. F., and Field, J. G. (1980). Density and ecological importance of bacteria on kelp  
177 fronds in an upwelling region. *J. Exp. Mar. Biol. Ecol.* 43, 173–182. doi: 10.1016/0022-  
178 0981(80)90024-6.

179 McHugh, D. J. (2003). *A guide to the seaweed industry*. Rome: Food and Agriculture Organization  
180 of the United Nations Available at: <https://www.fao.org/3/y4765e/y4765e.pdf>.

181 McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive  
182 analysis and graphics of microbiome census data. *PLoS ONE* 8, e61217. doi:  
183 10.1371/journal.pone.0061217.

184 Monteiro, J. P., Rey, F., Melo, T., Moreira, A. S. P., Arbona, J.-F., Skjermo, J., et al. (2020). The  
185 unique lipidomic signatures of *Saccharina latissima* can be used to pinpoint their geographic  
186 origin. *Biomolecules* 10, 107. doi: 10.3390/biom10010107.

187 Neiva, J., Paulino, C., Nielsen, M. M., Krause-Jensen, D., Saunders, G. W., Assis, J., et al. (2018).  
188 Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina*  
189 *latissima*. *Sci. Rep.* 8, 1112. doi: 10.1038/s41598-018-19620-7.

190 Newton, R. J., Griffin, L. E., Bowles, K. M., Meile, C., Gifford, S., Givens, C. E., et al. (2010). Genome  
191 characteristics of a generalist marine bacterial lineage. *ISME J.* 4, 784–798. doi:  
192 10.1038/ismej.2009.150.

193 Nitschke, U., Walsh, P., McDaid, J., and Stengel, D. B. (2018). Variability in iodine in temperate  
194 seaweeds and iodine accumulation kinetics of *Fucus vesiculosus* and *Laminaria digitata*  
195 (Phaeophyceae, Ochrophyta). *J. Phycol.* 54, 114–125. doi: 10.1111/jpy.12606.

196 Paix, B., Carriot, N., Barry-Martinet, R., Greff, S., Misson, B., Briand, J.-F., et al. (2020). A multi-  
197 omics analysis suggests links between the differentiated surface metabolome and epiphytic  
198 microbiota along the thallus of a Mediterranean seaweed holobiont. *Front. Microbiol.* 11,  
199 494. doi: 10.3389/fmicb.2020.00494.

200 Paix, B., Layglon, N., Le Poupon, C., D'Onofrio, S., Misson, B., Garnier, C., et al. (2021). Integration  
201 of spatio-temporal variations of surface metabolomes and epibacterial communities  
202 highlights the importance of copper stress as a major factor shaping host-microbiota  
203 interactions within a Mediterranean seaweed holobiont. *Microbiome* 9, 201. doi:  
204 10.1186/s40168-021-01124-8.

205 Parrot, D., Blümel, M., Utermann, C., Chianese, G., Krause, S., Kovalev, A., et al. (2019). Mapping  
206 the surface microbiome and metabolome of brown seaweed *Fucus vesiculosus* by amplicon  
207 sequencing, integrated metabolomics and imaging techniques. *Sci. Rep.* 9, 1061. doi:  
208 10.1038/s41598-018-37914-8.

209 Peixoto, R. S., Rosado, P. M., Leite, D. C. de A., Rosado, A. S., and Bourne, D. G. (2017). Beneficial  
210 Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience.  
211 *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00341.

212 Peng, Y., and Li, W. (2013). A bacterial pathogen infecting gametophytes of *Saccharina japonica*  
213 (*Laminariales, Phaeophyceae*). *Chin. J. Oceanol. Limnol.* 31, 366–373. doi: 10.1007/s00343-  
214 013-2136-9.

215 Peteiro, C. (2018). "Alginate production from marine macroalgae, with emphasis on kelp  
216 farming," in *Alginates and their biomedical applications* Springer Series in Biomaterials

217 Science and Engineering., eds. B. H. A. Rehm and M. F. Moradali (Singapore: Springer  
218 Singapore), 27–66. doi: 10.1007/978-981-10-6910-9\_2.

219 Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., et al. (2004). Changes in  
220 bacterioplankton composition under different phytoplankton regimens. *Appl. Environ.*  
221 *Microbiol.* 70, 6753–6766. doi: 10.1128/AEM.70.11.6753-6766.2004.

222 Ramírez-Puebla, S. T., Weigel, B. L., Jack, L., Schlundt, C., Pfister, C. A., and Mark Welch, J. L. (2022).  
223 Spatial organization of the kelp microbiome at micron scales. *Microbiome* 10, 52. doi:  
224 10.1186/s40168-022-01235-w.

225 Riemann, L., Steward, G. F., and Azam, F. (2000). Dynamics of bacterial community composition  
226 and activity during a mesocosm diatom bloom. *Appl. Environ. Microbiol.* 66, 578–587. doi:  
227 10.1128/AEM.66.2.578-587.2000.

228 Risely, A. (2020). Applying the core microbiome to understand host–microbe systems. *J. Anim.*  
229 *Ecol.* 89, 1549–1558. doi: 10.1111/1365-2656.13229.

230 Rodeheaver, G., Bellamy, W., Kody, M., Spatafora, G., Fitton, L., Leyden, K., et al. (1982).  
231 Bactericidal activity and toxicity of iodine-containing solutions in wounds. *Arch. Surg.* 117,  
232 181–186. doi: 10.1001/archsurg.1982.01380260051009.

233 Roth-Schulze, A. J., Pintado, J., Zozaya-Valdés, E., Cremades, J., Ruiz, P., Kjelleberg, S., et al. (2018).  
234 Functional biogeography and host specificity of bacterial communities associated with the  
235 Marine Green Alga *Ulva* spp. *Mol. Ecol.* 27, 1952–1965. doi: 10.1111/mec.14529.

236 Roussel, S., Caralp, C., Leblanc, C., Le Grand, F., Stiger-Pouvreau, V., Coulombet, C., et al. (2019).  
237 Impact of nine macroalgal diets on growth and initial reproductive investment in juvenile

238 abalone *Haliotis tuberculata*. *Aquaculture* 513, 734385. doi:  
239 10.1016/j.aquaculture.2019.734385.

240 Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., et al. (1998a).  
241 *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent  
242 of red spot disease of *Laminaria japonica*. *Int. J. Syst. Bacteriol.* 48, 769–774. doi:  
243 10.1099/00207713-48-3-769.

244 Sawabe, T., Sawada, C., Suzuki, E., and Ezura, Y. (1998b). Intracellular alginate-oligosaccharide  
245 degrading enzyme activity that is incapable of degrading intact sodium alginate from a  
246 marine bacterium *Alteromonas* sp. *Fish. Sci.* 64, 320–324. doi: 10.2331/fishsci.64.320.

247 Schiel, D., and Lilley, S. (2007). Gradients of disturbance to an algal canopy and the modification  
248 of an intertidal community. *Mar. Ecol. Prog. Ser.* 339, 1–11. doi: 10.3354/meps339001.

249 Schiel, D. R., and Foster, M. S. (2006). The population biology of large brown seaweeds: ecological  
250 consequences of multiphase life histories in dynamic coastal environments. *Annu. Rev. Ecol.*  
251 *Evol. Syst.* 37, 343–372. doi: 10.1146/annurev.ecolsys.37.091305.110251.

252 Schiener, P., Black, K. D., Stanley, M. S., and Green, D. H. (2015). The seasonal variation in the  
253 chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*,  
254 *Saccharina latissima* and *Alaria esculenta*. *J. Appl. Phycol.* 27, 363–373. doi: 10.1007/s10811-  
255 014-0327-1.

256 Shade, A., and Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome.  
257 *Environ. Microbiol.* 14, 4–12. doi: 10.1111/j.1462-2920.2011.02585.x.

258 Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytol.* 225, 1447–  
259 1454. doi: 10.1111/nph.16107.

260 Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *J.*  
261 *Appl. Phycol.* 16, 245–262. doi: 10.1023/B:JAPH.0000047783.36600.ef.

262 Stal, L. J., and Cretoiu, M. S. eds. (2016). *The Marine Microbiome*. Cham: Springer International  
263 Publishing doi: 10.1007/978-3-319-33000-6.

264 Staufenberg, T., Thiel, V., Wiese, J., and Imhoff, J. F. (2008). Phylogenetic analysis of bacteria  
265 associated with *Laminaria saccharina*. *FEMS Microbiol. Ecol.* 64, 65–77. doi: 10.1111/j.1574-  
266 6941.2008.00445.x.

267 Thomas, F., Dittami, S. M., Brunet, M., Le Duff, N., Tanguy, G., Leblanc, C., et al. (2019). Evaluation  
268 of a new primer combination to minimize plastid contamination in 16S rDNA metabarcoding  
269 analyses of alga-associated bacterial communities. *Environ. Microbiol. Rep.* 12, 30–37. doi:  
270 10.1111/1758-2229.12806.

271 Tourneroc, A., Lami, R., Burgaud, G., Domart-Coulon, I., Li, W., Gachon, C., et al. (2020). The  
272 bacterial and fungal microbiota of *Saccharina latissima* (Laminariales, Phaeophyceae). *Front.*  
273 *Mar. Sci.* 7, 587566. doi: 10.3389/fmars.2020.587566.

274 Ul-Hassan, A., and Wellington, E. M. (2009). “Actinobacteria,” in *Encyclopedia of Microbiology*  
275 (Elsevier), 25–44. doi: 10.1016/B978-012373944-5.00044-4.

276 Vairappan, C. S., Suzuki, M., Motomura, T., and Ichimura, T. (2001). Pathogenic bacteria  
277 associated with lesions and thallus bleaching symptoms in the Japanese kelp *Laminaria*  
278 *religiosa* Miyabe (Laminariales, Phaeophyceae). *Hydrobiologia* 445, 183–191. doi:  
279 10.1023/A:1017517832302.

280 Wagner-Döbler, I., and Biebl, H. (2006). Environmental Biology of the Marine *Roseobacter*  
281 Lineage. *Annu. Rev. Microbiol.* 60, 255–280. doi: 10.1146/annurev.micro.60.080805.142115.



282 Wegner, C.-E., Richter-Heitmann, T., Klindworth, A., Klockow, C., Richter, M., Achstetter, T., et al.  
283 (2013). Expression of sulfatases in *Rhodopirellula baltica* and the diversity of sulfatases in  
284 the genus *Rhodopirellula*. *Mar. Genomics* 9, 51–61. doi: 10.1016/j.margen.2012.12.001.

285 Weigel, B. L., Miranda, K. K., Fogarty, E. C., Watson, A. R., and Pfister, C. A. (2022). Functional  
286 Insights into the Kelp Microbiome from Metagenome-Assembled Genomes. *mSystems*,  
287 e01422-21. doi: 10.1128/msystems.01422-21.

288 Weigel, B. L., and Pfister, C. A. (2019). Successional dynamics and seascape-level patterns of  
289 microbial communities on the canopy-forming kelps *Nereocystis luetkeana* and *Macrocystis*  
290 *pyrifera*. *Front. Microbiol.* 10, 346. doi: 10.3389/fmicb.2019.00346.

291 Wiese, J., Thiel, V., Nagel, K., Staufenberg, T., and Imhoff, J. F. (2009). Diversity of antibiotic-  
292 active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea.  
293 *Mar. Biotechnol.* 11, 287–300. doi: 10.1007/s10126-008-9143-4.

294 Zaneveld, J. R., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the Anna  
295 Karenina principle to animal microbiomes. *Nat. Microbiol.* 2, 17121. doi:  
296 10.1038/nmicrobiol.2017.121.

297 Zehr, J. P., and Ward, B. B. (2002). Nitrogen Cycling in the Ocean: New Perspectives on Processes  
298 and Paradigms. *Appl. Environ. Microbiol.* 68, 1015–1024. doi: 10.1128/AEM.68.3.1015-  
299 1024.2002.

300 Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., and Barrow, C. J. (2006). A simple 96-well  
301 microplate method for estimation of total polyphenol content in seaweeds. *J. Appl. Phycol.*  
302 18, 445–450. doi: 10.1007/s10811-006-9048-4.

303 Zhang, R., Chang, L., Xiao, L., Zhang, X., Han, Q., Li, N., et al. (2020). Diversity of the epiphytic  
304 bacterial communities associated with commercially cultivated healthy and diseased  
305 *Saccharina japonica* during the harvest season. *J. Appl. Phycol.* 32, 2071–2080. doi:  
306 10.1007/s10811-019-02025-y.

307 Zhu, G., Ebbing, A., Bouma, T. J., and Timmermans, K. R. (2021). Morphological and physiological  
308 plasticity of *Saccharina latissima* (Phaeophyceae) in response to different hydrodynamic  
309 conditions and nutrient availability. *J. Appl. Phycol.* 33, 2471–2483. doi: 10.1007/s10811-  
310 021-02428-w.

311

Accepted Manuscript

312 SUPPLEMENTARY DATA

313 **Table S1 - Taxonomic affiliations of over-expressed ASVs for each comparison** (algal part,  
314 regions, seasons, and symptoms)

315 **Figure S1 - Seasonal variations in (A) temperature, (B) salinity and (C) ammonium, (D) nitrites,**  
316 **(E) nitrates, and (F) phosphate concentrations.** Roscoff, 2019. Legend for each month, rectangle:  
317 region inside 1<sup>st</sup> and 3<sup>rd</sup> quartiles, bold line: median value, dashed error bars: 1<sup>st</sup> and 9<sup>th</sup> deciles,  
318 red point: value for the selected year.

Accepted Manuscript