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

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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*

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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, Tourneroché 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 μl 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: *Algिताlea*, *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. Staufenberg 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 psychophilic
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 (N₂), 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

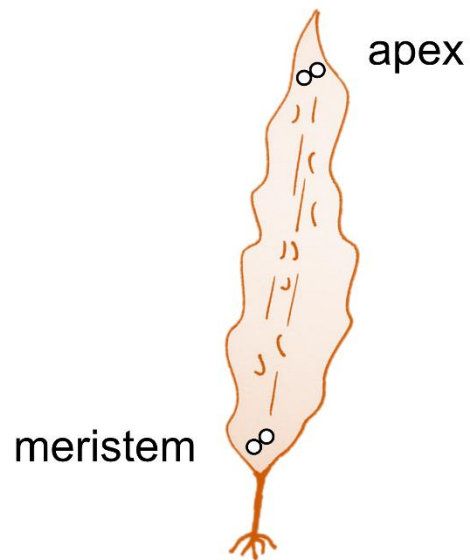
399 In conclusion, our study provides an extensive overview of the *S. latissima* microbiome
400 and highlights several factors driving its variability. In particular, the observation that the blade
401 part had a more profound impact on the microbial composition than region or season underlines
402 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

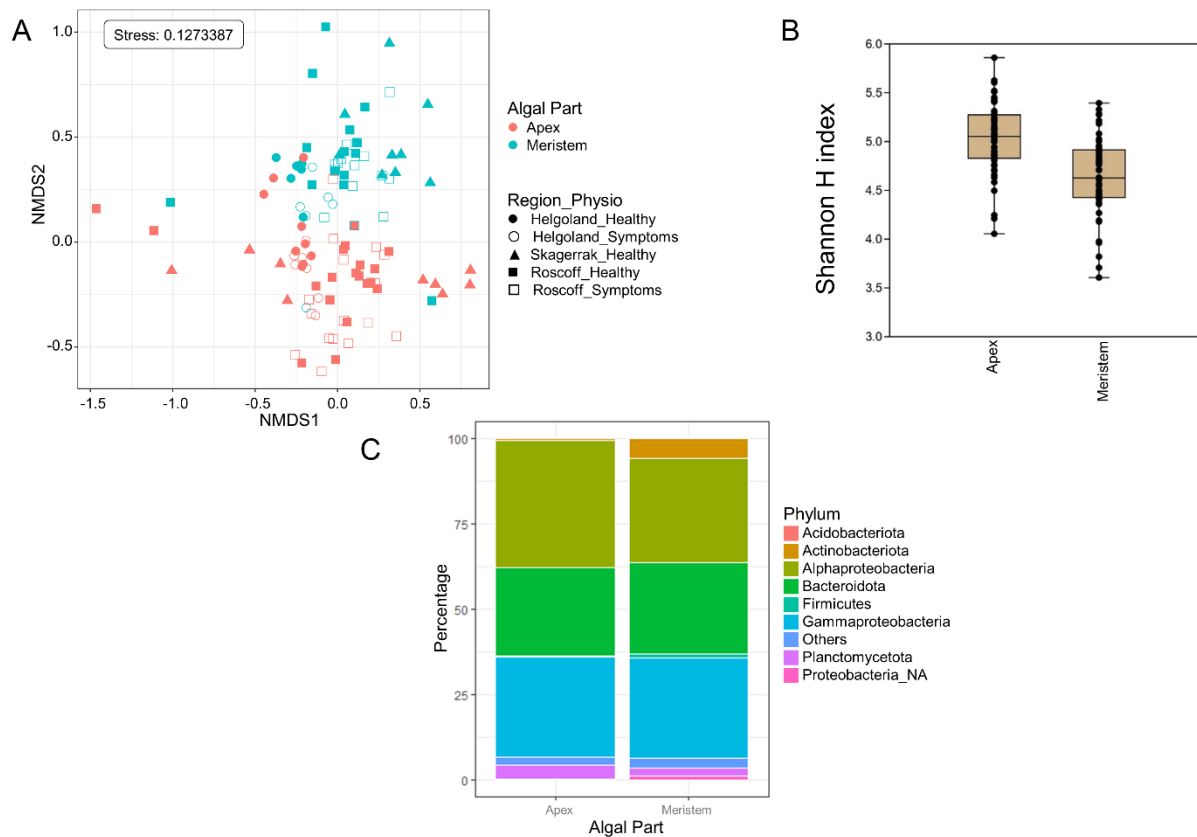


B

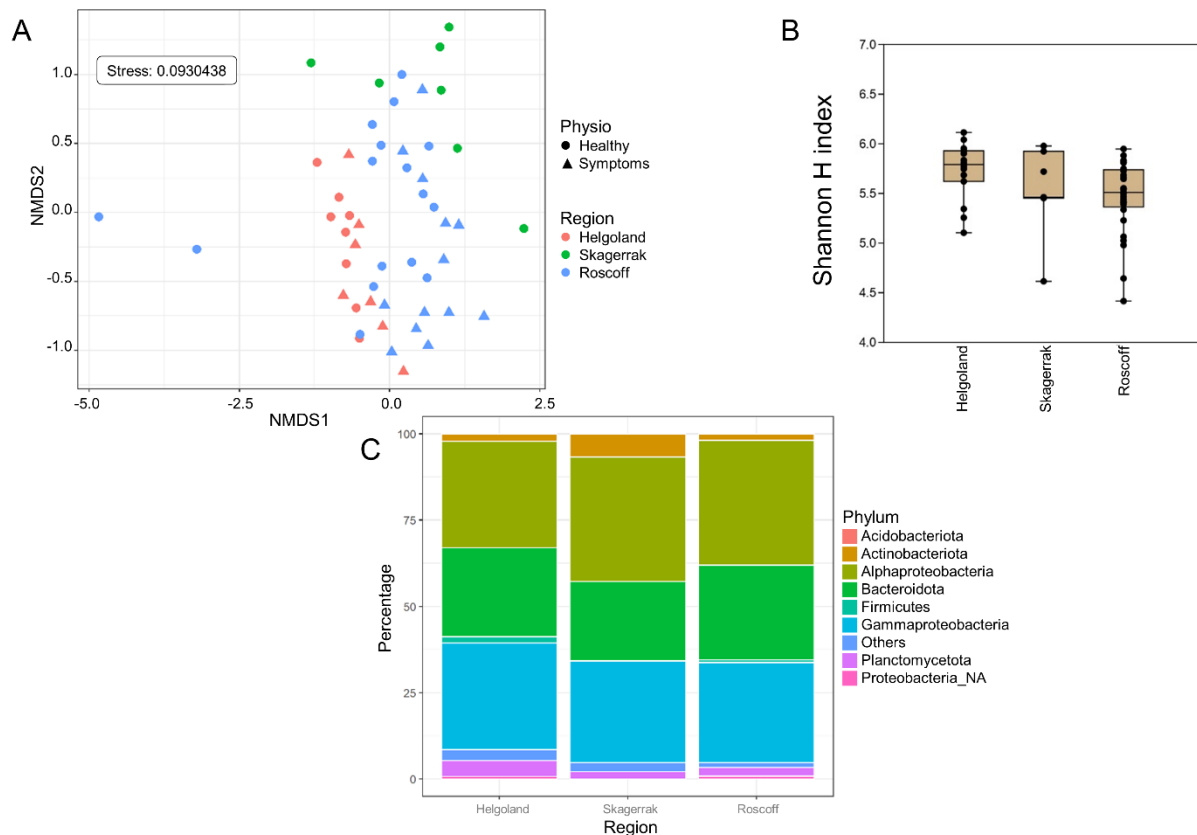


411
412 **Figure 1 – Sampling.** (A) Map of sampling locations and (B) Sampled parts of the *Saccharina*
413 *latissima* thalli (<1m). Two discs (Ø2cm) were punched out in immediate proximity for each part
414 of the blade and preserved in silica gel. Please refer to Table 1 for details on the sampling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

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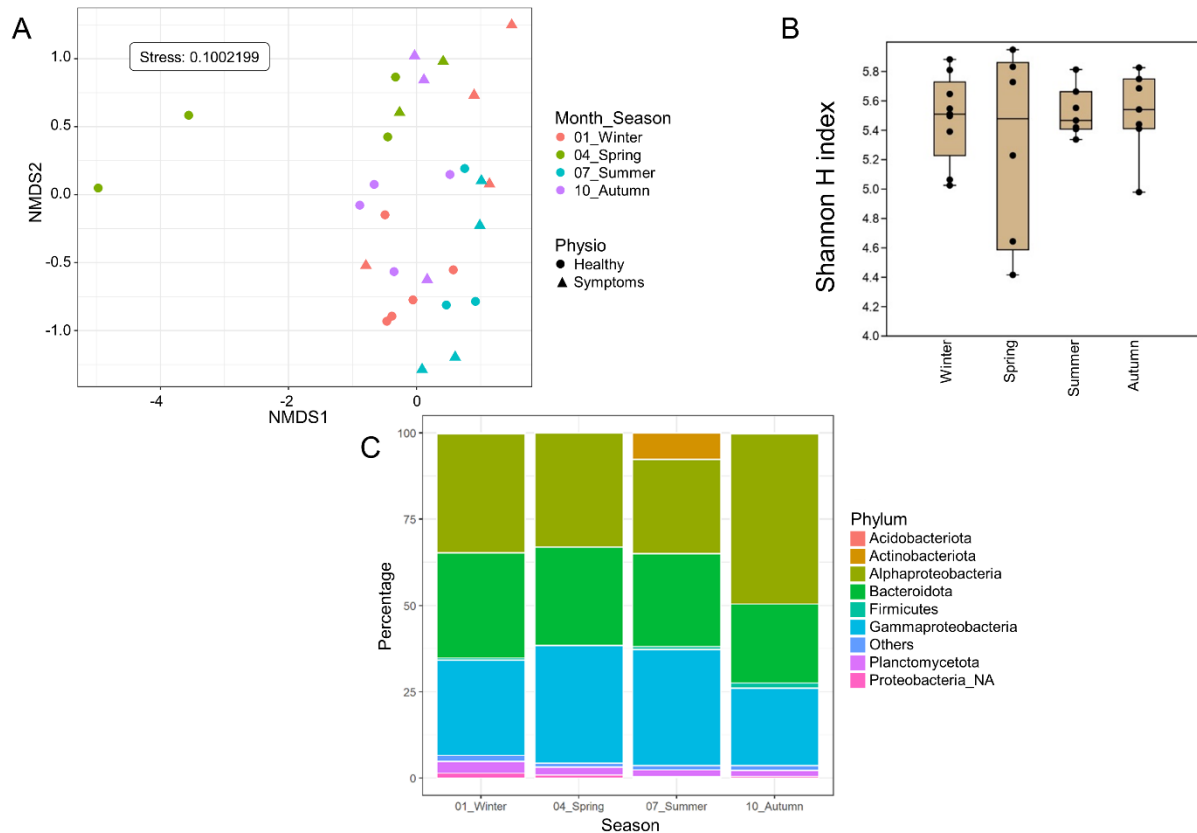


415
 416 **Figure 2 – Algal blade part analysis.** (A) NMDS analysis of the microbiome composition. Results
 417 show a clear separation of the apex and meristem samples (PERMANOVA $p=0.001$). (B) Box plot
 418 of alpha-diversity (Shannon H index) across different sample types calculated at the ASV level.
 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

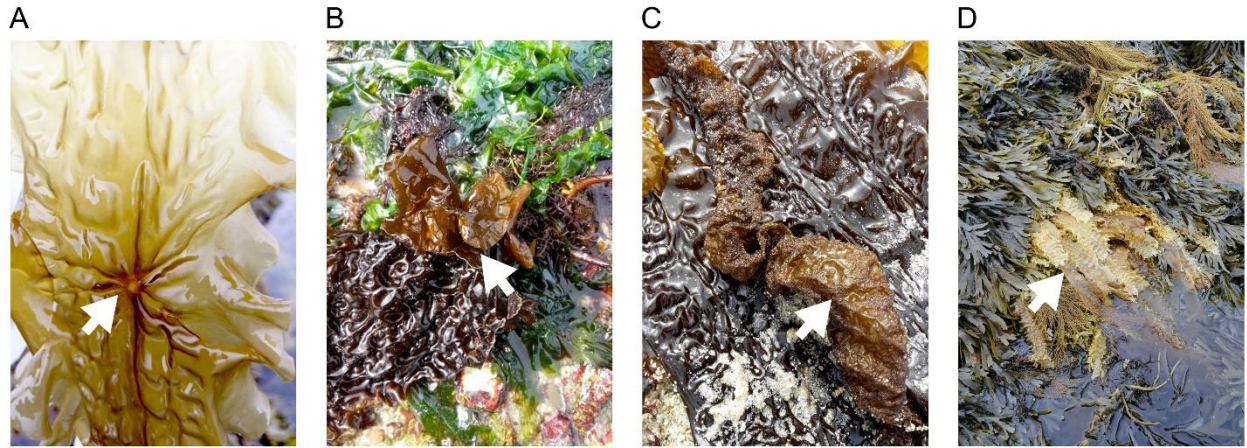
437 (Shannon H index) across different sample types calculated at the ASV level. Error bars correspond

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.

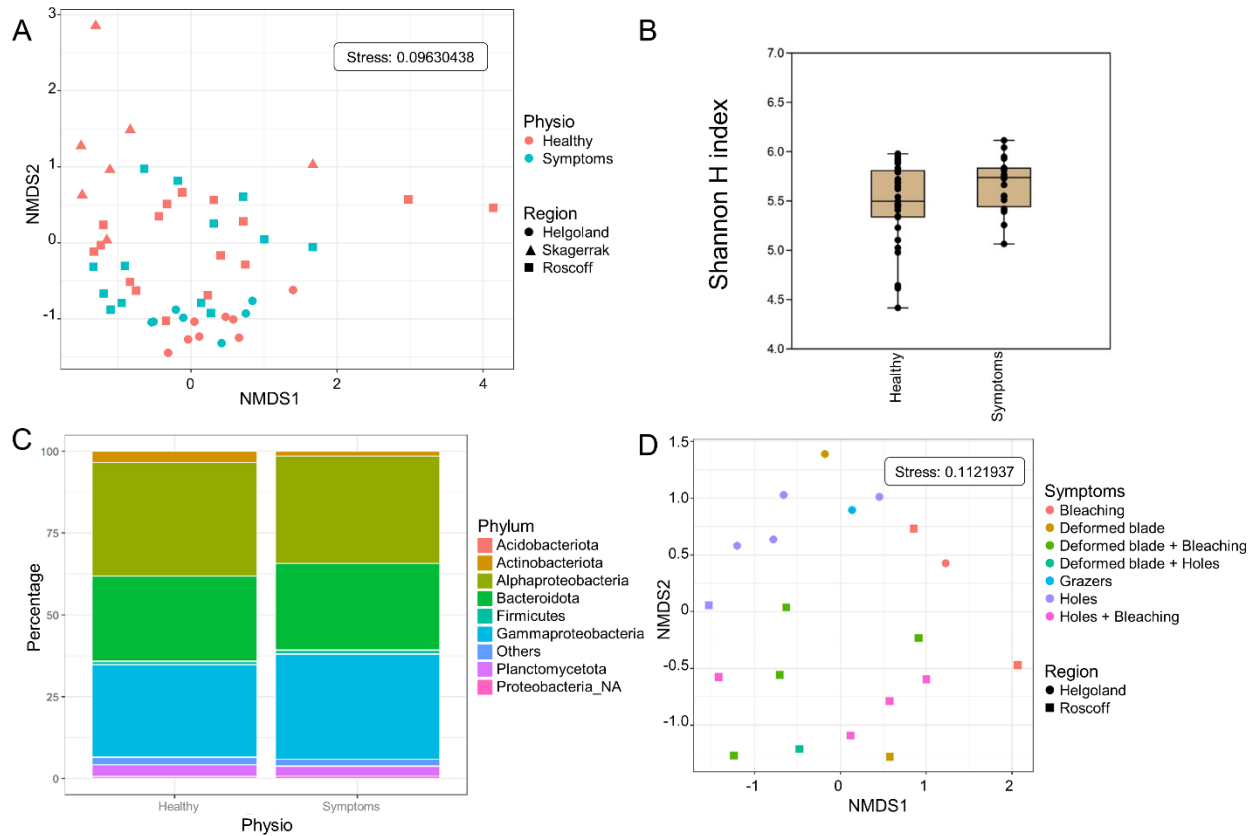


442

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.**

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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>Kl189A_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

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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
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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
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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 1st and 3rd quartiles, bold line: median value, dashed error bars: 1st and 9th deciles,
318 red point: value for the selected year.

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