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1 The *Saccharina latissima* microbiome: 2 algal tissue matters more than region, 3 season, and physiology

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11 ABSTRACT

12 *Saccharina latissima* is a canopy-forming species of brown algae and, as such, is
13 considered an ecosystem engineer. Several populations of this alga are exploited worldwide,
14 and a decrease in the abundance of *S. latissima* at its southern distributional range limits has
15 been observed. Despite its economic and ecological interest, only a few data are available on
16 the composition of microbiota associated with *S. latissima* and its role in algal physiology. We
17 studied the whole bacterial community composition associated with *S. latissima* samples from
18 three locations (Brittany, Helgoland, and Skagerrak) by 16S metabarcoding analyses at different
19 scales: algal blade part, regions, season, and physiologic state.

20 We have shown that the difference in bacterial composition is driven by factors of
21 decreasing importance: (i) the algal tissues (apex/meristem), (ii) the geographical area, (iii) the
22 seasons, and (iv) the algal host's condition (healthy vs. symptoms). Overall,
23 *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidota* dominated the general bacterial
24 communities. Almost all individuals hosted bacteria of the genus *Granulosicoccus*, accounting

25 for 12% of the total sequences, and eight additional core genera were identified. Our results
26 also highlight a microbial signature characteristic for algae in poor health independent of the
27 disease symptoms. Thus, our study provides a comprehensive overview of the *S. latissima*
28 microbiome, forming a basis for understanding holobiont functioning.

29 *Keywords: holobiont, brown macroalgae, microbiome, metabarcoding*

30 I. INTRODUCTION

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

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

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

51 Indeed, macroalgal functioning needs to be seen as the result of the interactions
52 between the algal hosts and their associated microbiota, constituting a singular entity termed
53 the algal holobiont (Egan et al., 2013). It has been shown that macroalgal health, fitness,
54 pathogen resistance (Wiese et al., 2009), acclimation to a changing environment (Dittami et al.,
55 2016), and metabolism (Burgunter-Delamare et al., 2020) are regulated and supported by
56 bacterial partners (Goecke et al., 2010). Considering the biofilm composition and deciphering
57 the interactions within the holobiont is thus essential to fully understand the biology of algae.

58 Previous studies were carried out on microbiota of different kelp species like *L. digitata* (Ihua et
59 al., 2020), *L. hyperborea* (Bengtsson et al., 2010), *L. religiosa* (Vairappan et al., 2001) and *L.*
60 *setchellii* (Lemay et al., 2021), but little is known about the *S. latissima* microbiota. Notably,
61 Staufenberg et al. (2008) analysed the bacterial composition of *Saccharina* from two locations
62 and seasons (Baltic and North Sea; January and April 2006) using denaturing gradient gel
63 electrophoresis (DGGE) and 16S rRNA gene clone libraries. Later, Tourneroc et al. (2020) used
64 16S metabarcoding and FISH to decipher the bacterial microbiota of young tissues of *S. latissima*
65 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 (Brittany, Helgoland, and Skagerrak) by 16S
68 metabarcoding analyses to decipher if the microbiota is specific to the area of origin,
69 seasonality, and algal blade part (apex/meristem). We examined the microbiota composition of
70 healthy and diseased algae, aiming to identify possible microbial signatures characteristic of
71 algae in poor health.

72 II. MATERIAL & METHODS

73 1. Biological material & Environmental Variables

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

82 Two parts of the blades were sampled: the basal meristem and the tip (**Figure 1**). A disc
83 with Ø2cm was punched out for each part of the blade and placed in a 15 ml Falcon tube
84 containing 5ml of clean silica gel (2-6mm; VWR). Tubes were stored at room temperature for up
85 to 15 days before DNA extraction.

86 For the samples from Brittany, corresponding environmental variables (temperature,
87 salinity and ammonium, nitrites, nitrates, and phosphate concentrations) were obtained from
88 the Service d'Observation en Milieu Littoral (SOMLIT) database
89 (<https://www.somlit.fr/mysomlit/>; Astan point; approximately 3.6km North-East of the
90 sampling point). They are available in **Figure S1**.

91 2. DNA extraction

92 DNA extraction was carried out with the silica-gel stored samples, according to the
93 protocol described by Bernard et al. (2017). Briefly, samples were freeze-dried, and ½ of a disk
94 was ground using a Qiagen TissueLyser II bead beater (3 sessions, 45sec, 30Hz, 3mm stainless
95 steel beads). Nucleic acids were then extracted using a 2% CTAB extraction buffer (100 mM Tris-
96 HCl [pH 7.5], 1.5 M NaCl, 2% CTAB, 50 mM EDTA [pH 8], 50 mM DTT; shaker 250 rpm at room
97 temperature). Supernatants were purified with one volume of chloroform/isoamyl alcohol
98 (24:1) followed by 15min centrifugation at 10 000 rpm (16°C). The upper phase was transferred
99 to a new tube, and ethanol (0.3 vol) was added drop by drop until polysaccharide precipitation
100 was visible, followed by a second chloroform/isoamyl alcohol extraction and recovery of the
101 aqueous phase. The pre-purified DNA was purified using Nucleospin plant II columns
102 (Macherey-Nagel, Germany) according to the manufacturer's instructions. Finally, DNA was
103 eluted in 50µl of elution buffer (Macherey-Nagel). Blank extractions were also performed, and
104 these extracts were used to identify potential contaminations introduced during the extraction
105 and downstream processing of the samples.

106 3. 16S Metabarcoding

107 The bacterial community composition associated with algal cultures was determined by
108 16S metabarcoding. A mock community comprising a mix of DNA from 26 cultivated bacterial
109 strains (Thomas et al., 2019) and negative control were run and treated in parallel to the DNA
110 extracts. For all of these samples, the V3 and V4 regions of the 16S rDNA gene were amplified
111 using the NOCHL primers including Illumina adapters (Thomas et al., 2019), to avoid plastid DNA
112 amplification. Then a standard Illumina protocol for metabarcoding (Illumina, 2013) was run
113 using the Q5® High-Fidelity PCR Kit (New England BioLabs, MA, USA), the AMPure XP for PCR
114 Purification Kit (Beckman Coulter, Brea, CA, USA), and the Nextera XT DNA Library Preparation

115 Kit (Illumina, San Diego, CA, USA). Libraries were quantified with a Quantifluor[®] ds DNA System
116 (Promega, WI, USA), and mean fragment size was determined using a LabChip[®] GX Touch™
117 (Perkin Elmer, MA, USA). An equimolar pool of all samples was generated at a concentration of
118 4 nM, diluted to 3 pM, spiked with 10% PhiX (Illumina), and sequenced on an Illumina MiSeq
119 sequencer at the Genomer platform (Station Biologique de Roscoff) using a MiSeq v3 kit
120 (2x300bp, paired-end). Raw Illumina reads were deposited at the European Nucleotide Archive
121 under project accession number PRJEB47035.

122 4. Analyses

123 Sequence analysis was performed using the DADA2 1.14.0 package (Callahan et al., 2016)
124 on R 3.6.2 following the protocol established by Benjamin Callahan
125 (<https://benjjneb.github.io/dada2/tutorial.html>). Sequences were filtered, allowing for a
126 maximum of 2 expected errors and reducing the read length to 291 bp for forward reads and
127 265 bp for reverse reads. An amplicon sequence variant (ASV) table was constructed, and
128 chimaeras were removed. The taxonomy of the remaining ASVs was assigned using the
129 Silva_SEED 138 database. The resulting abundance table and taxonomic classification were
130 analysed using Phyloseq 1.30.0 (McMurdie and Holmes, 2013). ASVs that were more abundant
131 in the blank samples than in the algal samples, organellar and eukaryote reads, rare ASVs
132 (<0.01% of total reads), and samples with less than 7688 remaining reads were removed. Non-
133 Metric Multidimensional Scaling analyses (NMDS) were carried out using the Bray-Curtis
134 dissimilarities and the Vegan R package. The most important factor separating the samples in
135 the NMDS was then further explored. The Shannon H diversity index was calculated using Past
136 version 4.02 (Hammer et al., 2001). Statistical analysis of differential abundance was performed
137 at the genus level using ANCOM-BC version 1.4.0 (Lin and Peddada, 2020) with default
138 parameters. Binomial tests followed by a Benjamini and Hochberg (BH) correction (Benjamini
139 and Hochberg, 1995) were carried out to determine the overrepresented genera among the
140 ASVs identified by ANCOM-BC. Then, the factor in question was eliminated from the dataset if
141 possible (grouping of apex and meristem sample, focus on specific region), and the analyses
142 were repeated to determine the next factor. The bacterial core was determined at the genus

143 level and defined as genera present in 90% of replicates for each algal part, season, and
144 location.

145 III. RESULTS

146 1. General taxonomy

147 16S metabarcoding analyses were carried out for all control and algal samples. A total of
148 4,028,372 raw sequences were generated and, after filtering, assembled into 1,658,746 merged
149 contigs. The taxonomic assignment of mock samples was consistent with the mock composition,
150 and a total of 18,028 ASVs were identified in the dataset. The sequences obtained corresponded
151 predominantly to *Alphaproteobacteria* (34,1% of total reads), followed by
152 *Gammaproteobacteria* (29,5% of total reads) and *Bacteroidota* (26% of total reads).

153 2. Comparison of apex and meristem samples

154 Global NMDS analysis of all samples demonstrated a clear separation between the apex
155 and meristem samples (**Figure 2A**). Overall, alpha diversity, as calculated using the Shannon H
156 index (**Figure 2B**), was higher in apex samples than in meristem samples (p -value <0.0001).
157 Several phyla were found to differ significantly in relative abundance between the apex and
158 meristem samples. The *Actinobacteriota*, *Firmicutes*, and unclassified *Proteobacteria* (p <0.0001)
159 were found in higher relative abundance in the meristem samples. The *Alphaproteobacteria*
160 (p =0.00016), *Bacteroidota* (p =0.00372), and *Planctomycetota* (p =0.004) phyla were relatively
161 more abundant in the apex samples (**Figure 2C**). ANCOM-BC analyses revealed a total of 122
162 ASVs to differ significantly (adjusted p -value < 0.05) in relative abundance between the apex and
163 meristem samples (28 ASVs were more abundant in apex and 94 in meristem samples; **Table**
164 **S1**). The taxonomic groups overrepresented (adjusted p -value < 0.05; BH correction) among
165 these significant ASVs are shown in **Table 2**: one genus was significantly overrepresented in the
166 apex samples (*Ki89A_clade*, 39%) and six in the meristem samples (including
167 *Gammaproteobacteria*, 70%; **Table 2**). The bacterial core in the apex and meristem samples
168 comprises the four genera *Granulosicoccus*, *Litorimonas*, *Hellea*, and *Blastopirellula*, accounting
169 for 32% of the total reads for all samples. Five additional genera were systematically present in

170 the apical part: *Algitalea*, *Arenicella*, *Portibacter*, *Tenacibaculum*, and *Bdellovibrio* and
171 accounted for 15% of the total reads.

172 3. Comparison of regions

173 For the following analyses, reads from the apex and meristem samples of the same alga
174 were pooled as individuals to remove the apex/meristem effect. On the NMDS plot, the samples
175 are now grouped according to their region of origin (**Figure 3A**). The alpha diversity did not
176 differ significantly between the regions (**Figure 3B**). However, at the phylum level, the
177 *Firmicutes* and unclassified *Proteobacteria* were underrepresented in the Norwegian samples
178 compared to Roscoff ($p=0.013$ and $p<0.0001$) and Helgoland ($p=0.004$ and $p<0.0001$; **Figure 3C**).
179 *Bacteroidota* and *Alphaproteobacteria* exhibited significantly higher relative abundance in
180 Roscoff than in Helgoland ($p=0.003$ for both phyla). At the ASV level, 234 ASVs were
181 represented in higher proportions in the Roscoff samples, 243 in the Helgoland samples, and 18
182 in the samples from Southern Norway (**Table S1**). The taxonomic affiliation of significantly over-
183 expressed ASVs (adjusted p -value < 0.05 ; BH correction) is shown in **Table 3**, and six genera were
184 significantly overrepresented in Helgoland samples, one genus in the Norwegian samples
185 (*Rhizobiaceae_NA*), and nine genera in Roscoff samples (*Proteobacteria* 77%; **Table 3**).

186 4. Seasonality

187 Only Roscoff samples were used to assess the impact of season on the microbiome
188 because these were the only samples with four sampling points from different seasons
189 available. The NMDS analysis shows a separation between the season's samples. The autumn
190 and winter samples clustered together, and the spring and summer samples were placed on the
191 sides (**Figure 4A**), even if the alpha diversity (**Figure 4B**) did not differ significantly between the
192 seasons. *Actinobacteria* were exclusively found in summer times. *Firmicutes* ($p=0.032$) were
193 more abundant in autumn samples than in spring samples ($p=0.032$). *Alphaproteobacteria* were
194 significantly more abundant in autumn than summer ($p=0.0017$) and winter ($p=0.014$).
195 *Gammaproteobacteria* were significantly more abundant in spring than in autumn ($p=0.032$)
196 and winter ($p=0.022$; **Figure 4C**). ANCOM-BC analyses revealed 422 ASVs with higher relative
197 abundance in one or several seasons. 126 ASVs were most abundant in winter samples, 85 ASVs
198 in spring samples, 95 ASVs in summer samples, and 115 ASVs in autumn samples (**Table S1**). The

199 taxa significantly overexpressed among these ASVs (adjusted p -value < 0.05; BH correction) are
200 shown in **Table 4**. Most ASVs with higher relative abundance in winter, spring, and autumn
201 samples belonged to the *Alphaproteobacteria* (17%, 24%, and 33% of ASVs). In summer, six
202 genera were over-represented, and 33% of ASVs belonged to the *Gammaproteobacteria*. Also,
203 the Sva0996_marine_group (*Actinobacteria*; 12%) was overrepresented only in these samples.

204 5. Comparison Healthy / Symptoms

205 Both healthy samples and samples with symptoms were found only in Roscoff and
206 Helgoland, and the symptoms were diverse: holes, twisted blade, and bubbling in the blade
207 (**Figure 5**). The NMDS shows no separation between the healthy individuals and those with
208 symptoms (**Figure 6A**), although the Shannon H index indicated slightly higher alpha diversity in
209 algal samples with symptoms than in healthy samples (p -value=0.046; **Figure 6B**). No phyla
210 significantly and systematically differed between healthy algae and algae with symptoms (**Figure**
211 **6C**). This observation also remains true when we distinguish samples between the different
212 types of symptoms (**Figure 6D**), and the samples are still separated depending on the region.
213 However, ANCOM-BC analyses revealed 9 ASVs that were characteristic in either of the groups:
214 four ASVs were more abundant in samples with symptoms (*Alteromonadaceae*_NA,
215 *Octadecabacter* sp., *Tenacibaculum* sp., *Yoonia-Loktanella* sp.) and five that were more
216 abundant in the healthy ones (*Escherichia/Shigella* sp., *Granulosicoccus* sp., *K189A_clade*,
217 *Rhodobacteraceae*_NA, *Zobellia* sp.; **Table S1**).

218 IV. DISCUSSION

219 Understanding holobiont functioning requires knowledge of the holobiont's bacterial
220 component. Here we studied the diversity and composition of bacterial communities of *S.*
221 *latissima* by 16S metabarcoding analysis. The impacts of several factors on bacterial
222 communities were examined: algal blade part, origin of the host, season, and host condition.

223 The blade part is the primary driver of samples separation

224 Distinct bacterial communities were demonstrated to be associated with different parts
225 of *S. latissima* by 16S metabarcoding, and this is the primary factor of separation regardless of
226 region, season, and physiology. Staufenberg et al. (2008) found the same dynamics when

227 working on several *S. latissima* tissue from the Baltic Sea and the North Sea, sampled in winter
228 and spring.

229 *S. latissima's* type of growth can explain this difference in bacterial communities. *S.*
230 *latissima* is a short-lived but perennial species, and growth occurs mainly in the meristem
231 region. From there, the proliferating cells form the thallus. Young algae only have short blades
232 and no access to the surrounding sediment as they only stand upright in the water column. As
233 the thallus grows, it becomes heavier, and the apex finally bends and touches the ground (Kain,
234 1979; Lüning, 1991). Water currents move the old blade, resulting in access to nearby substrates
235 and a broader environment. Mechanical stress also occurs in this part, which becomes
236 vulnerable to bacterial decomposition, offering new ecological niches for different bacteria
237 (Bengtsson and Øvreås, 2010). Therefore, the younger meristem tissues are typically less
238 colonised by bacteria and exhibit lower bacterial diversity, as previously found (Staufenberger et
239 al., 2008; Goecke et al., 2010; Ihua et al., 2020; Lemay et al., 2021). Furthermore, the synthesis
240 or release of compounds that either have an antimicrobial effect or act as nutrients for the
241 bacteria may vary between the different parts of the blade. This was described for phenolic
242 substances in the kelp *L. hyperborea* and likely contributed to differences in the microbial
243 composition (Bengtsson et al., 2012).

244 We found a higher proportion of *Planctomycetes* at the apex and *Actinobacteriota*
245 almost exclusively in the meristem. Both phyla are classically found in brown macroalgae
246 (Hollants et al., 2013) and on *S. latissima* [apex: (Staufenberger et al., 2008); meristem:
247 (Tourneroché et al., 2020)]. *Planctomycetes* contain many sulfatase genes (Wegner et al., 2013),
248 which help degrade sulphated polysaccharides. They may also be involved in degrading
249 polysaccharides from the extracellular matrix of microbial biofilms (Parrot et al., 2019), which
250 may explain their higher relative abundance in older tissues that exhibit first signs of
251 degradation. *Actinobacteriota*, on the other hand, is a diverse phylum that has successfully
252 colonised a wide range of habitats (Ul-Hassan and Wellington, 2009), but we currently do not
253 know what features make them successful colonisers of *the S. latissima* meristem.

254 The bacterial core also reveals the shift from a low to a higher diversity as the blade
255 ages, with four genera found in both algal parts and five additional genera found in >90% of
256 apex samples. Those taxa were also found on the meristem part of *L. digitata* (Ihua et al., 2020)
257 and on the blade of *L. setchellii* (Lemay et al., 2021), *Taonia atomaria* (Paix et al., 2021), and *U.*
258 *lactuca* (Comba González et al., 2021). Our results are furthermore consistent with the core
259 microbiota found in *S. latissima* and *L. hyperborea* in the United Kingdom (King et al., 2022).
260 *Granulosicoccus* sp. (*Gammaproteobacteria*) was one of the most abundant genera (12% of
261 total reads) and included several ASVs overexpressed in the meristem samples. This genus was
262 also found abundantly on the youngest parts of the sister species *S. japonica* (Balakirev et al.,
263 2012; Zhang et al., 2020) and other kelps like *L. setchellii* (Lemay et al., 2021), *L. hyperborea*
264 (Bengtsson et al., 2012), *Macrocystis pyrifera*, and *Nereocystis luetkeana* (Weigel and Pfister,
265 2019; Ramírez-Puebla et al., 2022), reinforcing the idea of a strong association between
266 *Granulosicoccus* and the kelp tissue. In the same vein, the genus *Algitalia* (*Flavobacteriaceae*;
267 3% of total reads) was one of the “apex” bacterial core genera. This genus belonged to the
268 pioneer bacterial communities found on the apical parts of *T. atomaria* (Paix et al., 2020). Also,
269 the *Flavobacterium* lineage has been recognised as necessary in the decomposition processes of
270 organic matter during algae blooms (Riemann et al., 2000; Pinhassi et al., 2004) and thus may
271 participate in the decay process occurring at the algal apices.

272 **Regional specificities: tides, seawater, and genetic background**

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

282 In our study, the regional differences were more pronounced than the seasonal
283 differences obtained for one sampling site, suggesting that region and not just variability
284 between sampling dates was the driving factor. The regional differences might be due to several
285 abiotic factors. The tidal ranges for the three regions, for instance, decrease going north: 10
286 meters in Roscoff, 3 meters in Helgoland, and less than 1 meter in Skagerrak. In the same vein,
287 winter seawater temperatures are lower in the north, which might favour psychrophilic strains
288 over mesophilic communities, as found in a culture-based study on the surface bacteria of *L.*
289 *longicruris* (Laycock, 1974). Also, increasing time exposure to rain, wind, or sunlight (UV), waves,
290 currents, hydrostatic pressure, pH, and salinity can lead to cellular stress and, by extension,
291 senescence. This changing environment can offer new ecological niches for different bacteria.
292 Lastly, bacterial communities can be altered by nutrient supply, interspecies competition, and
293 viral infection (Fuhrman et al., 2015; Stal and Cretoiu, 2016).

294 However, the regional differences might also be due, in part, to the genetic diversity of
295 the algae. Using single nucleotide polymorphisms (SNPs) and microsatellites, Guzinski et al.
296 (2016, 2020) determined that *S. latissima* individuals from Roscoff, Helgoland, and Norway are
297 genetically distinct, and this might lead to the attraction of different bacterial species (Griffiths
298 et al., 2019). Furthermore, *S. latissima* displays a unique lipidomic signature depending on its
299 geographic origin (Monteiro et al., 2020), as the content of chemical elements (C, H, N, S), fatty
300 acids, and lipids varies depending on the region. These molecules are common components of
301 membranes (Harwood, 2004) and might influence the attractiveness of the algal surface for
302 several bacterial strains.

303 Shifts in bacterial communities depending on the season

304 The third factor we examined was seasonality. Laycock (1974) observed that as the
305 seasonal temperatures decreased, the bacterial communities of *L. longicruris* shifted from
306 mesophilic to psychrophilic strains. When working with *L. hyperborea*, Bengtsson et al. (2010)
307 hypothesised that the seasonal succession in the bacterial communities might be explained by
308 abiotic factors like seawater temperature and biotic factors like seasonal changes in the kelp
309 substrate. Indeed, seawater temperature alone does not seem to have been the most important
310 factor in our data, as the seawater was coldest in winter and spring (<12°C), but the samples

311 from autumn and winter were more similar in their bacterial communities. Other
312 physicochemical parameters might also play a role. Nitrogen is an important element for
313 organisms, and microorganisms can take up nitrogen in different forms such as nitrate, nitrite,
314 ammonium, urea, organic nitrogen, and in some cases, dinitrogen gas (N₂), depending on the
315 organism (Zehr and Ward, 2002). Nitrate, nitrite, and ammonium concentrations follow
316 seasonal variations, with nitrates being lower in summer and higher in winter and nitrites at
317 their highest in autumn. Phosphorus is another essential nutrient for primary production in the
318 euphotic zone. Most of the phosphorus is present in the oxidised form as free phosphate or
319 bound to organic matter. The phosphate concentration was lowest in springtime. Several ASVs
320 overrepresented in spring belong to the *Roseobacter* clade, and Atlantic strains of this genus are
321 known to possess abundant high-affinity phosphorus uptake systems, constituting likely
322 adaptations to low environmental phosphate concentrations (Newton et al., 2010).

323 Lastly, seasonal variations may also be due to seasonal changes in the alga's chemical
324 composition. For instance, Schiener et al. (2015) demonstrated that in *S. latissima*, polyphenol
325 levels are higher between May and July and then decrease, reaching their lowest in March. This
326 could be interpreted as a defence against bacterial colonisation as the seawater temperature
327 rises, polyphenols being known for their wide range of antimicrobial properties (Zhang et al.,
328 2006; Daglia, 2012). Also, carbohydrate content (laminarin and mannitol) is higher in summer
329 (Schiener et al., 2015), and these are both substrates easy to degrade by the bacteria
330 (Alderkamp et al., 2007; Jeske et al., 2013; Groisillier et al., 2015). Similarly, algal iodine content
331 is generally lower in summer (Nitschke et al., 2018), and the algae's production of toxic iodine
332 compounds may control the surface biofilm and repulse microbial pathogens (Rodeheaver et al.,
333 1982; Gobet et al., 2017).

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

335 Some bacteria affect the alga in a deleterious manner by decomposing cell material, like
336 alginate and laminarin (Laycock, 1974; Dimitrieva and Dimitriev, 1997; Sawabe et al., 1998b;
337 Ivanova et al., 2003) or by causing diseases like *Alteromonas* species (Vairappan et al., 2001;
338 Peng and Li, 2013) and species of *Pseudoalteromonas* (Sawabe et al., 1998a). Ihua et al. (2019)
339 have shown that the microbial communities (phyla level) associated with intact *Ascophyllum*

340 differ from rotting algae, suggesting that the decay process might shape the associated bacterial
341 community. Similarly, the microbial communities of *Ecklonia* are strongly associated with the
342 algal condition (stressed or not) more than with other variables (Marzinelli et al., 2015).
343 Moreover, the core bacterial community characteristic of healthy algae may be lost when hosts
344 are subjected to stress, and the microbiota of stressed individuals of *Ecklonia* were more similar
345 to each other at a given location than those on healthy hosts (Marzinelli et al., 2015); which is
346 contrary to the so-called Anna Karenina principle (Zaneveld et al., 2017; Ma, 2020), stating that
347 all “healthy” microbiomes are alike and each “symptom” microbiome is “sick” in its own way.

348 These data reinforce our hypothesis of a characteristic microbial signature for algae in
349 poor health, regardless of the specific symptoms. In our study, the changes in bacterial
350 communities between healthy and diseased individuals are visible only at a lower taxonomic
351 scale, and we found 9 ASVs that were differentially expressed between the healthy (5 ASVs) and
352 diseased samples (4 ASVs). ASVs characteristic for the latter belong to the genus *Tenacibaculum*
353 and the *Alteromonadales*, known for their alginate lyase activities (Thomas et al., 2021), and to
354 the *Roseobacter* clade, known for their production of quorum-sensing molecules, a
355 phenomenon involved in virulence and pathogenicity (Buchan et al., 2005; Wagner-Döbler and
356 Biebl, 2006; Brinkhoff et al., 2008). One of the healthy specific ASVs is a *Granulosicoccus* sp.,
357 emphasising the importance of this genus in the algal microbiota. The fact that several ASVs
358 were found to differ indicates that, regardless of the type of disease, an alga that is not well will
359 undergo characteristic changes in the microbiome. Moreover, these ASVs signatures are
360 probably stable because they are derived from different places, times of the year, and
361 symptoms, as shown for *Ecklonia* (Marzinelli et al., 2015). Although this would require
362 additional developments, these signatures might also be helpful as bioindicators for kelp health.

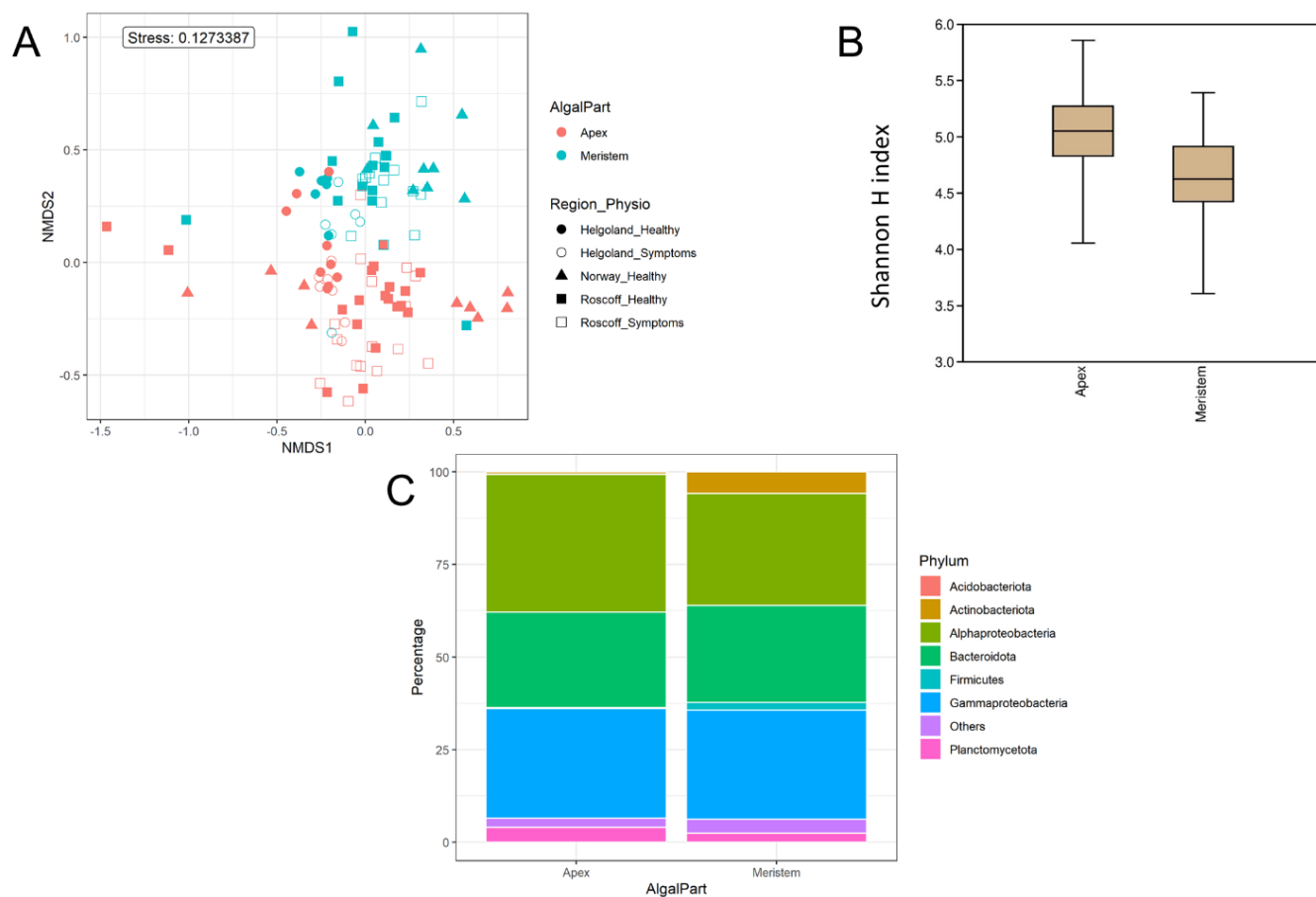
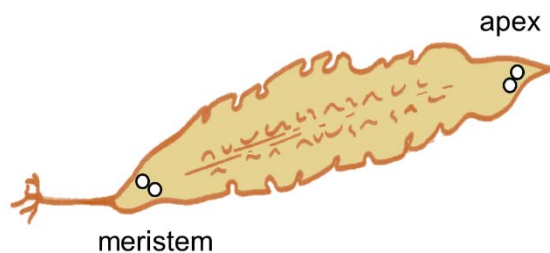
363 V. CONCLUSION

364 In conclusion, our study provides an extensive overview of the *S. latissima* microbiome
365 and highlights several factors driving its variability. In particular, the observation that the blade
366 part had a more profound impact on the microbial composition than season or region, both of
367 which are associated with changes in the abiotic environment, underlines the extent to which

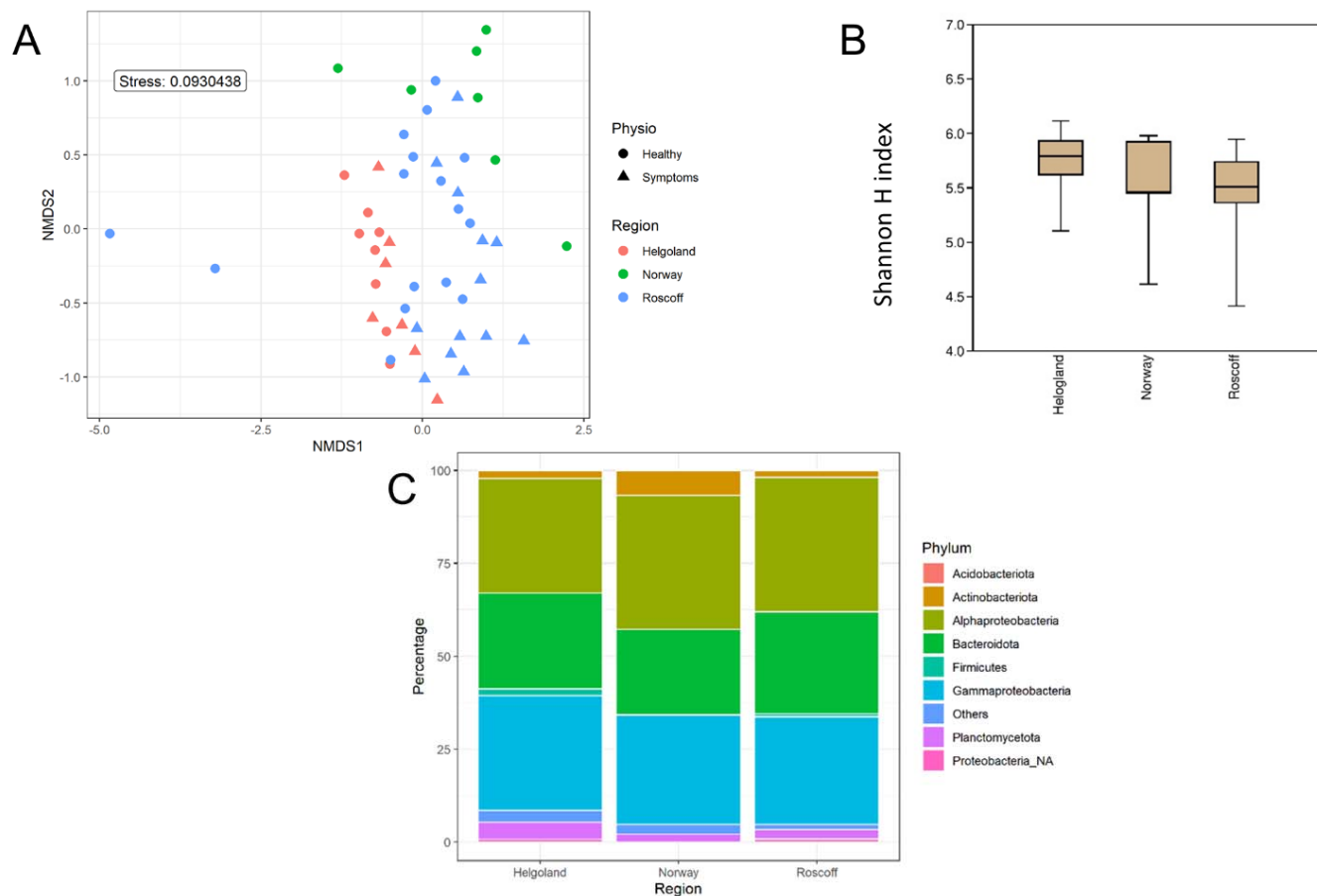
368 algal hosts select their associated microbiota. Our discovery of microbial signatures
369 characteristic for diseased *S. latissima* individuals that persist in our dataset independently of
370 the disease symptoms further supports this hypothesis. Given the variety of symptoms
371 observed in our samples, it is unlikely that the same bacteria could be the causative agents in all
372 cases. Rather, the different types of disease likely cause similar changes in the host, which
373 would lead to similar microbial changes. Understanding these signatures will be of interest for
374 fundamental research on the different algal diseases; in the long run, it may also help develop
375 molecular markers of host health to survey natural populations or aquacultures.

376 FIGURES

377
378 **Figure 1 - Sampled parts of the *Saccharina latissima* thallus.** Two discs (\varnothing 2cm) were punched out in immediate
379 proximity for each part of the blade.

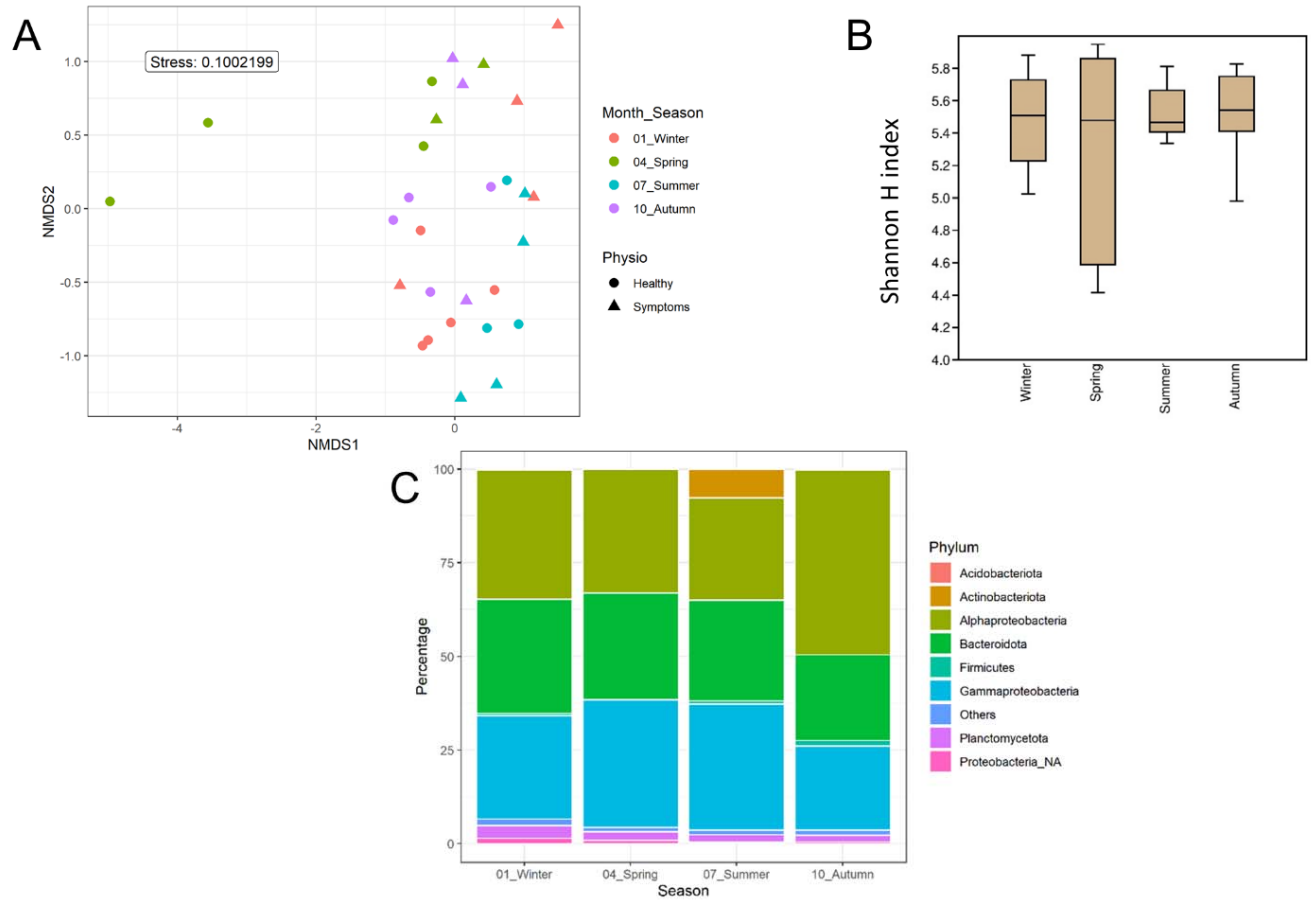


380
381 **Figure 2 – Algal blade part analysis.** A) NMDS analysis of the microbiome composition. Results show a clear
382 separation of the apex and meristem samples. B) Box plot of alpha-diversity (Shannon H index) across different
383 sample types. p -value < 0.0001 . C) Comparison of microbiome composition between apex and meristem samples.
384 Distribution of 16S rRNA gene metabarcoding sequences per phylum. *Proteobacteria_NA*: not classified as *Alpha*-
385 or *Gammaproteobacteria*.

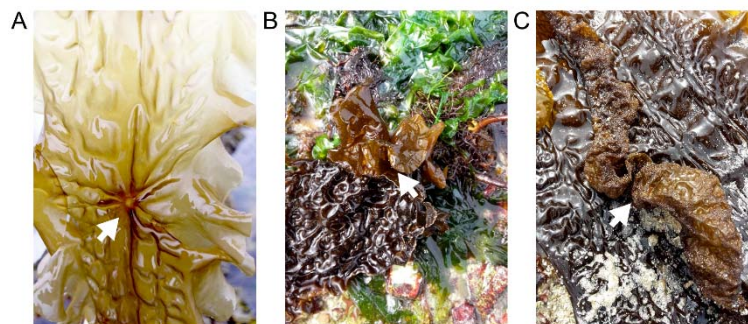


386

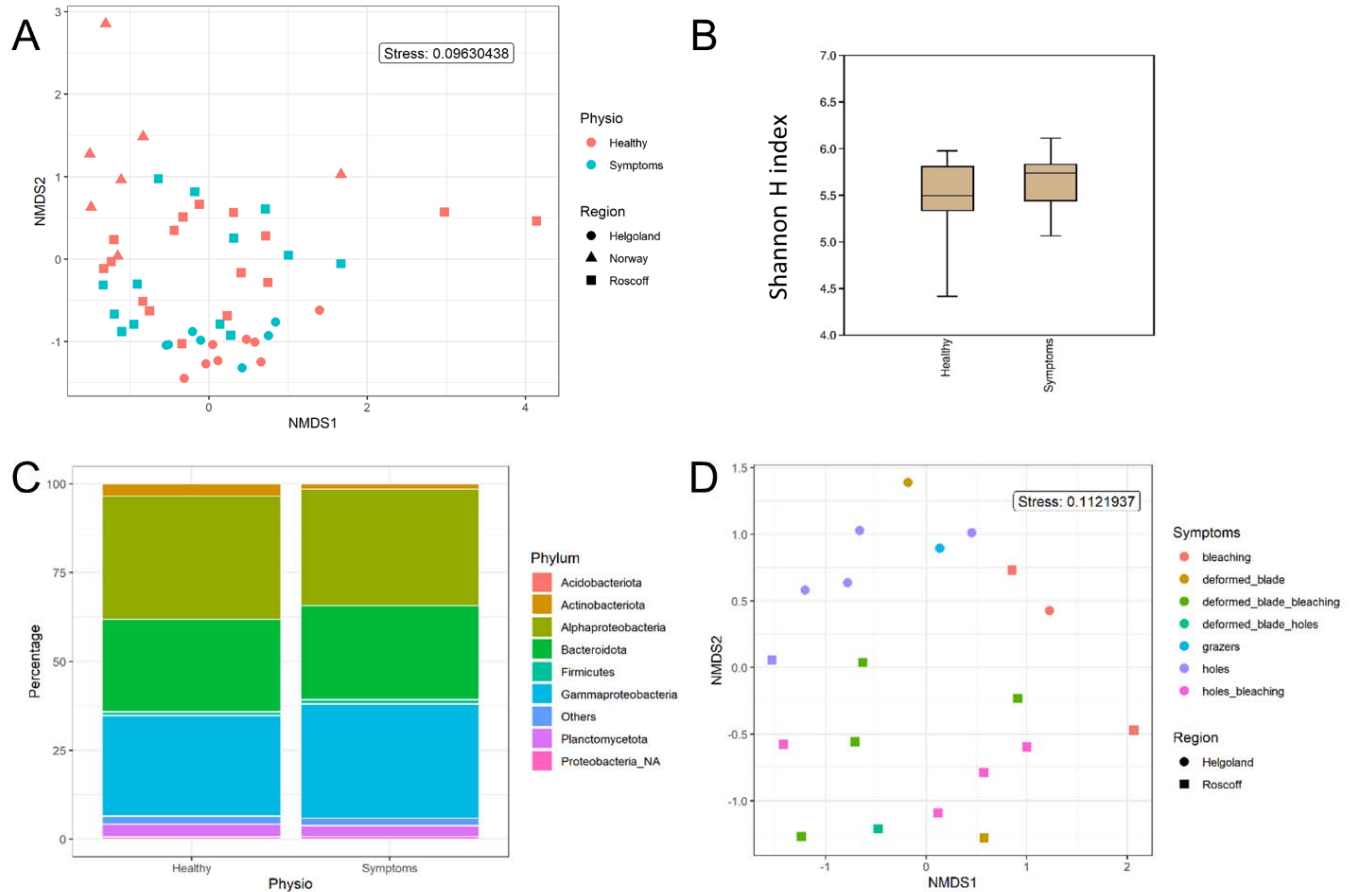
387 **Figure 3 – Regions analysis.** A) NMDS analysis of the microbiome composition. Results show a clear separation of
388 the samples according to their origin. B) Box plot of alpha-diversity (Shannon H index) across different sample
389 types. $p\text{-value} > 0.05$. C) Microbiome composition of samples from Helgoland, Norway, and Roscoff. Distribution of
390 16S rRNA gene metabarcoding sequences per phylum. *Proteobacteria_NA*: not classified as *Alpha*- or
391 *Gammaproteobacteria*.



392
 393 **Figure 4 – Season analyses.** A) NMDS analysis of the microbiome composition. Results show a separation of the
 394 samples depending on the sampling's season. B) Box plot of alpha-diversity (Shannon H index) across different
 395 sample types. $p\text{-value} > 0.05$. C) Seasonal microbiome composition in Roscoff. Distribution of 16S rRNA gene
 396 metabarcoding sequences per phylum. *Proteobacteria_NA*: not classified as *Alpha*- or *Gammaproteobacteria*.



397
 398 **Figure 5 – Examples of symptoms observed on “diseased” *S. latissima* individuals.** A) hole, B) twisted blade, and
 399 C) bubbling in blade



400

401 **Figure 6 – Analysis of samples with symptoms.** A) NMDS analysis of the microbiome composition. Results do not
 402 show a separation of the healthy and symptomatic samples. B) Box plot of alpha-diversity (Shannon H index) across
 403 different sample types. $p\text{-value}=0.046$. C) Microbiome composition of healthy and symptoms samples. Distribution
 404 of 16S rRNA gene metabarcoding sequences per phylum. *Proteobacteria_NA*: not classified as Alpha- or
 405 Gammaproteobacteria. D) NMDS analysis of the microbiome composition depending on the symptoms.

TABLES

Table 1 - Sampling dates and sites

Places	GPS coordinates	Dates	Time	Types of samples
Roscoff, Brittany, France	48°43'47.0 "N 4°00'17.1" W	10 October 2018 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	Diving 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		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 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.

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>K189A_clade</i>	11	28	0.39286	<0.00001 ***	0	94	0.00000	n.c.	100	16689	0.00599
<i>Maribacter</i>	0	28	0.00000	n.c.	15	94	0.15957	<0.00001 ***	89	16689	0.00533
<i>Sva0996_marine_group</i>	0	28	0.00000	n.c.	14	94	0.14894	<0.00001 ***	115	16689	0.00689
<i>Litorimonas</i>	3	28	0.10714	0.05815	13	94	0.13830	0.00001 ***	530	16689	0.03176
<i>Octadecabacter</i>	0	28	0.00000	n.c.	5	94	0.05319	0.00006 ***	73	16689	0.00437
<i>Granulosicoccus</i>	3	28	0.10714	0.18075	14	94	0.14894	0.00040 ***	878	16689	0.05261
<i>Proteobacteria_NA</i>	0	28	0.00000	n.c.	4	94	0.04255	0.00056 ***	66	16689	0.00395

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Table 3 - Taxonomic affiliations of the ASVs characteristic for the Roscoff, Helgoland, or Norway samples, compared with their occurrence in the entire dataset. n.c.: not calculated. P-values are the 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.**

Taxa	Roscoff				Helgoland				Norway				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	0.08120	<0.00001 ***	0	243	0.00000	n.c.	2	18	0.11111	0.00515	100	16689	0.00599
<i>Litorimonas</i>	23	234	0.09829	<0.00001 ***	46	243	0.18930	<0.00001 ***	0	18	0.00000	n.c.	530	16689	0.03176
<i>Maribacter</i>	14	234	0.05983	<0.00001 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	89	16689	0.00533
<i>Octadecabacter</i>	12	234	0.05128	<0.00001 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	73	16689	0.00437
<i>Reichenbachiella</i>	19	234	0.08120	<0.00001 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	258	16689	0.01546
<i>Yoonia-Loktanelia</i>	10	234	0.04274	<0.00001 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	91	16689	0.00545
<i>Tateyamaria</i>	7	234	0.02991	0.00001 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	49	16689	0.00294
<i>Algimonas</i>	4	234	0.01709	0.00029 ***	0	243	0.00000	n.c.	0	18	0.00000	n.c.	22	16689	0.00132
<i>Granulosicoccus</i>	23	234	0.09829	0.00318 **	17	243	0.06996	0.14341	0	18	0.00000	n.c.	878	16689	0.05261
<i>Algitalea</i>	8	234	0.03419	0.16425	17	243	0.06996	0.00005 ***	0	18	0.00000	n.c.	378	16689	0.02265
<i>Blastopirellula</i>	2	234	0.00855	0.86957	14	243	0.05761	0.00003 ***	0	18	0.00000	n.c.	252	16689	0.01510
<i>Rhodobacteraceae_NA</i>	1	234	0.00427	0.99993	19	243	0.07819	0.00441 **	1	18	0.05556	0.51956	666	16689	0.03991
<i>Lewinella</i>	0	234	0.00000	n.c.	7	243	0.02881	0.00365 **	0	18	0.00000	n.c.	133	16689	0.00797
<i>Thalassotalea</i>	0	234	0.00000	n.c.	6	243	0.02469	0.00223 **	0	18	0.00000	n.c.	90	16689	0.00539
<i>Rhizobiaceae_NA</i>	0	234	0.00000	n.c.	0	243	0.00000	n.c.	9	18	0.50000	<0.00001 ***	97	16689	0.00581

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 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.

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	0.15873	<0.00001 ***	5	85	0.05882	0.31399	3	95	0.03158	0.78951	2	115	0.01739	0.96324	729	16689	0.04368
<i>Octadecabacter</i>	17	126	0.13492	<0.00001 ***	1	85	0.01176	0.31107	0	95	0.00000	n.c.	3	115	0.02609	0.01437	73	16689	0.00437
<i>Reichenbachiella</i>	10	126	0.07937	0.00003 ***	0	85	0.00000	n.c.	0	95	0.00000	n.c.	0	115	0.00000	n.c.	258	16689	0.01546
<i>Yoonia-Loktanelia</i>	5	126	0.03968	0.00068 ***	5	85	0.05882	0.00011 ***	0	95	0.00000	n.c.	4	115	0.03478	0.00378 **	91	16689	0.00545
<i>Litorimonas</i>	4	126	0.03175	0.56997	15	85	0.17647	<0.00001 ***	7	95	0.07368	0.03193	26	115	0.22609	<0.00001 ***	530	16689	0.03176
<i>Sva0996_marine_group</i>	0	126	0.00000	n.c.	0	85	0.00000	n.c.	12	95	0.12632	<0.00001 ***	0	115	0.00000	n.c.	115	16689	0.00689
<i>Arenicella</i>	8	126	0.06349	0.09228	4	85	0.04706	0.37827	14	95	0.14737	0.00001 ***	5	115	0.04348	0.41291	611	16689	0.03661
<i>Dokdonia</i>	2	126	0.01587	0.74823	1	85	0.01176	0.83752	9	95	0.09474	0.00019 ***	2	115	0.01739	0.70182	353	16689	0.02115
<i>K189A_dade</i>	0	126	0.00000	n.c.	0	85	0.00000	n.c.	5	95	0.05263	0.00029 ***	0	115	0.00000	n.c.	100	16689	0.00599
<i>Hyphomonadaceae_NA</i>	5	126	0.03968	0.14802	0	85	0.00000	n.c.	8	95	0.08421	0.00126 **	6	115	0.05217	0.04303	369	16689	0.02211
<i>Granulosicoccus</i>	14	126	0.11111	0.00669	2	85	0.02353	0.94214	12	95	0.12632	0.00420 **	4	115	0.03478	0.86030	878	16689	0.05261
<i>Tateyamaria</i>	1	126	0.00794	0.30960	1	85	0.01176	0.22115	1	95	0.01053	0.24371	8	115	0.06957	<0.00001 ***	49	16689	0.00294
<i>Algitalea</i>	4	126	0.03175	0.31968	0	85	0.00000	n.c.	0	95	0.00000	n.c.	15	115	0.13043	<0.00001 ***	378	16689	0.02265

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10 **Code availability:** not applicable

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21 REFERENCES

- 22 Alderkamp, A.-C., Van Rijssel, M., and Bolhuis, H. (2007). Characterization of marine bacteria
23 and the activity of their enzyme systems involved in degradation of the algal storage
24 glucan laminarin: *FEMS Microbiol. Ecol.* 59, 108–117. doi: 10.1111/j.1574-
25 6941.2006.00219.x.
- 26 Araújo, R. M., Assis, J., Aguillar, R., Airoidi, L., Bárbara, I., Bartsch, I., et al. (2016). Status, trends
27 and drivers of kelp forests in Europe: an expert assessment. *Biodivers. Conserv.* 25, 1319–
28 1348. doi: 10.1007/s10531-016-1141-7.
- 29 Balakirev, E. S., Krupnova, T. N., and Ayala, F. J. (2012). Symbiotic associations in the
30 phenotypically-diverse brown alga *Saccharina japonica*. *PLoS ONE* 7, e39587. doi:
31 10.1371/journal.pone.0039587.
- 32 Bengtsson, M. M., and Øvreås, L. (2010). *Planctomycetes* dominate biofilms on surfaces of the
33 kelp *Laminaria hyperborea*. *BMC Microbiol.* 10, 261. doi: 10.1186/1471-2180-10-261.
- 34 Bengtsson, M. M., Sjøtun, K., Lanzén, A., and Øvreås, L. (2012). Bacterial diversity in relation to
35 secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. *ISME*
36 *J.* 6, 2188–2198. doi: 10.1038/ismej.2012.67.
- 37 Bengtsson, M., Sjøtun, K., and Øvreås, L. (2010). Seasonal dynamics of bacterial biofilms on the
38 kelp *Laminaria hyperborea*. *Aquat. Microb. Ecol.* 60, 71–83. doi: 10.3354/ame01409.
- 39 Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and
40 powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300. doi:
41 10.1111/j.2517-6161.1995.tb02031.x.
- 42 Bernard, M., Rousvoal, S., Jacquemin, B., Ballenghien, M., Peters, A. F., and Leblanc, C. (2017).
43 qPCR-based relative quantification of the brown algal endophyte *Laminarionema elsbetiae*
44 in *Saccharina latissima*: variation and dynamics of host - endophyte interactions. *J. Appl.*
45 *Phycol.* 30, 2901–2911. doi: 10.1007/s10811-017-1367-0.
- 46 Bolton, J. J. (2010). The biogeography of kelps (*Laminariales*, *Phaeophyceae*): a global analysis
47 with new insights from recent advances in molecular phylogenetics. *Helgol. Mar. Res.* 64,
48 263–279. doi: 10.1007/s10152-010-0211-6.
- 49 Bonthond, G., Bayer, T., Krueger-Hadfield, S. A., Barboza, F. R., Nakaoka, M., Valero, M., et al.
50 (2020). How do microbiota associated with an invasive seaweed vary across scales? *Mol.*
51 *Ecol.* 29, 2094–2108. doi: 10.1111/mec.15470.
- 52 Bosch, T. C. G., and Miller, D. J. (2016). “Bleaching as an obvious dysbiosis in corals,” in *The*
53 *Holobiont Imperative* (Vienna: Springer Vienna), 113–125. doi: 10.1007/978-3-7091-1896-
54 2_9.
- 55 Bourne, D., Iida, Y., Uthicke, S., and Smith-Keune, C. (2008). Changes in coral-associated
56 microbial communities during a bleaching event. *ISME J.* 2, 350–363. doi:
57 10.1038/ismej.2007.112.

- 58 Brinkhoff, T., Giebel, H.-A., and Simon, M. (2008). Diversity, ecology, and genomics of the
59 Roseobacter clade: a short overview. *Arch. Microbiol.* 189, 531–539. doi: 10.1007/s00203-
60 008-0353-y.
- 61 Buchan, A., González, J. M., and Moran, M. A. (2005). Overview of the Marine Roseobacter
62 Lineage. *Appl. Environ. Microbiol.* 71, 5665–5677. doi: 10.1128/AEM.71.10.5665-
63 5677.2005.
- 64 Burgunter-Delamare, B., KleinJan, H., Frioux, C., Fremy, E., Wagner, M., Corre, E., et al. (2020).
65 Metabolic complementarity between a brown alga and associated cultivable bacteria
66 provide indications of beneficial interactions. *Front. Mar. Sci.* 7, 85. doi:
67 10.3389/fmars.2020.00085.
- 68 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.
69 (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat.*
70 *Methods* 13, 581–583. doi: 10.1038/nmeth.3869.
- 71 Comba González, N. B., Niño Corredor, A. N., López Kleine, L., and Montoya Castaño, D. (2021).
72 Temporal changes of the epiphytic bacteria community from the marine macroalga *Ulva*
73 *lactuca* (Santa Marta, Colombian-Caribbean). *Curr. Microbiol.* 78, 534–543. doi:
74 10.1007/s00284-020-02302-x.
- 75 Corre, S., and Prieur, D. (1990). Density and morphology of epiphytic bacteria on the kelp
76 *Laminaria digitata*. *Bot. Mar.* 33. doi: 10.1515/botm.1990.33.6.515.
- 77 Daglia, M. (2012). Polyphenols as antimicrobial agents. *Curr. Opin. Biotechnol.* 23, 174–181. doi:
78 10.1016/j.copbio.2011.08.007.
- 79 Dimitrieva, G. Y., and Dimitriev, S. M. (1997). Symbiotic microflora of brown algae of the genus
80 *Laminaria* as a bioindicator of the ecological condition of coastal laminarian biocenoses.
81 *Oceanogr. Lit. Rev.* 11, 1330.
- 82 Dittami, S. M., Duboscq-Bidot, L., Perennou, M., Gobet, A., Corre, E., Boyen, C., et al. (2016).
83 Host-microbe interactions as a driver of acclimation to salinity gradients in brown algal
84 cultures. *ISME J.* 10, 51–63. doi: 10.1038/ismej.2015.104.
- 85 Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., and Thomas, T. (2013). The seaweed
86 holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol. Rev.* 37, 462–
87 476. doi: 10.1111/1574-6976.12011.
- 88 Fuhrman, J. A., Cram, J. A., and Needham, D. M. (2015). Marine microbial community dynamics
89 and their ecological interpretation. *Nat. Rev. Microbiol.* 13, 133–146. doi:
90 10.1038/nrmicro3417.
- 91 Gobet, A., Corre, E., Correc, G., Delage, L., Dittami, S., KleinJan, H., et al. (2017). Characterization
92 of the epiphytic bacterial community associated with the kelp *Laminaria digitata*.
93 *Phycologia* 56, 64.
- 94 Goecke, F., Labes, A., Wiese, J., and Imhoff, J. (2010). Chemical interactions between marine
95 macroalgae and bacteria. *Mar. Ecol. Prog. Ser.* 409, 267–299. doi: 10.3354/meps08607.

- 96 Griffiths, S. M., Antwis, R. E., Lenzi, L., Lucaci, A., Behringer, D. C., Butler, M. J., et al. (2019). Host
97 genetics and geography influence microbiome composition in the sponge *Ircinia campana*.
98 *J. Anim. Ecol.* 88, 1684–1695. doi: 10.1111/1365-2656.13065.
- 99 Groisillier, A., Labourel, A., Michel, G., and Tonon, T. (2015). The mannitol utilization system of
100 the marine bacterium *Zobellia galactanivorans*. *Appl. Environ. Microbiol.* 81, 1799–1812.
101 doi: 10.1128/AEM.02808-14.
- 102 Guzinski, J., Mauger, S., Cock, J. M., and Valero, M. (2016). Characterization of newly developed
103 expressed sequence tag-derived microsatellite markers revealed low genetic diversity
104 within and low connectivity between European *Saccharina latissima* populations. *J. Appl.*
105 *Phycol.* 28, 3057–3070. doi: 10.1007/s10811-016-0806-7.
- 106 Guzinski, J., Ruggeri, P., Ballenghien, M., Mauger, S., Jacquemin, B., Jollivet, C., et al. (2020).
107 Seascape genomics of the sugar kelp *Saccharina latissima* along the North Eastern Atlantic
108 Latitudinal Gradient. *Genes* 11, 1503. doi: 10.3390/genes11121503.
- 109 Hammer, Ø., Harper, D. A., and Ryan, P. D. (2001). PAST: Paleontological statistics software
110 package for education and data analysis. *Palaeontol. Electron.* 4, 9.
- 111 Harwood, J. L. (2004). “Membrane lipids in algae,” in *Lipids in Photosynthesis: Structure,*
112 *Function and Genetics Advances in Photosynthesis and Respiration.*, eds. S. Paul-André and
113 M. Norio (Dordrecht: Kluwer Academic Publishers), 53–64. doi: 10.1007/0-306-48087-5_3.
- 114 Hollants, J., Leliaert, F., De Clerck, O., and Willems, A. (2013). What we can learn from sushi: a
115 review on seaweed–bacterial associations. *FEMS Microbiol. Ecol.* 83, 1–16. doi:
116 10.1111/j.1574-6941.2012.01446.x.
- 117 Ihua, M., Guihéneuf, F., Mohammed, H., Margassery, L., Jackson, S., Stengel, D., et al. (2019).
118 Microbial population changes in decaying *Ascophyllum nodosum* result in macroalgal-
119 polysaccharide-degrading bacteria with potential applicability in enzyme-assisted
120 extraction technologies. *Mar. Drugs* 17, 200. doi: 10.3390/md17040200.
- 121 Ihua, M. W., FitzGerald, J. A., Guihéneuf, F., Jackson, S. A., Claesson, M. J., Stengel, D. B., et al.
122 (2020). Diversity of bacteria populations associated with different thallus regions of the
123 brown alga *Laminaria digitata*. *PLOS ONE* 15, e0242675. doi:
124 10.1371/journal.pone.0242675.
- 125 Illumina (2013). 16S Metagenomic sequencing library preparation. *Prep. 16S Ribosomal RNA*
126 *Gene Amplicons Illumina MiSeq Syst.*, 1–28.
- 127 Ivanova, E. P., Bakunina, I. Yu., Nedashkovskaya, O. I., Gorshkova, N. M., Alexeeva, Y. V.,
128 Zelepuga, E. A., et al. (2003). Ecophysiological variabilities in ectohydrolytic enzyme
129 activities of some *Pseudoalteromonas* species, *P. citrea*, *P. issachenkonii*, and *P.*
130 *nigrifaciens*. *Curr. Microbiol.* 46, 6–10. doi: 10.1007/s00284-002-3794-6.
- 131 Jeske, O., Jogler, M., Petersen, J., Sikorski, J., and Jogler, C. (2013). From genome mining to
132 phenotypic microarrays: *Planctomycetes* as source for novel bioactive molecules. *Antonie*
133 *Van Leeuwenhoek* 104, 551–567. doi: 10.1007/s10482-013-0007-1.

- 134 Kain, J. M. (1979). A view of the genus *Laminaria*. *Oceanogr. Mar. Biol. Annu. Rev.* 17, 101–161.
- 135 King, N. G., Moore, P. J., Thorpe, J. M., and Smale, D. A. (2022). Consistency and Variation in the
136 Kelp Microbiota: Patterns of Bacterial Community Structure Across Spatial Scales. *Microb.*
137 *Ecol.* doi: 10.1007/s00248-022-02038-0.
- 138 Lachnit, T., Blümel, M., Imhoff, J., and Wahl, M. (2009). Specific epibacterial communities on
139 macroalgae: phylogeny matters more than habitat. *Aquat. Biol.* 5, 181–186. doi:
140 10.3354/ab00149.
- 141 Laycock, R. A. (1974). The detrital food chain based on seaweeds. I. Bacteria associated with the
142 surface of *Laminaria* fronds. *Mar. Biol.* 25, 223–231. doi: 10.1007/BF00394968.
- 143 Lemay, M. A., Davis, K. M., Martone, P. T., and Parfrey, L. W. (2021). Kelp-associated microbiota
144 are structured by host anatomy. *J. Phycol.* 57, 1119–1130. doi: 10.1111/jpy.13169.
- 145 Lin, H., and Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction.
146 *Nat. Commun.* 11, 3514. doi: 10.1038/s41467-020-17041-7.
- 147 Lüning, K. (1991). *Seaweeds: their environment, biogeography, and ecophysiology*. New York,
148 NY: Wiley.
- 149 Ma, Z. (Sam) (2020). Testing the Anna Karenina Principle in Human Microbiome-Associated
150 Diseases. *iScience* 23, 101007. doi: 10.1016/j.isci.2020.101007.
- 151 Marzinelli, E. M., Campbell, A. H., Zozaya Valdes, E., Vergés, A., Nielsen, S., Wernberg, T., et al.
152 (2015). Continental-scale variation in seaweed host-associated bacterial communities is a
153 function of host condition, not geography. *Environ. Microbiol.* 17, 4078–4088. doi:
154 10.1111/1462-2920.12972.
- 155 Mazure, H. G. F., and Field, J. G. (1980). Density and ecological importance of bacteria on kelp
156 fronds in an upwelling region. *J. Exp. Mar. Biol. Ecol.* 43, 173–182. doi: 10.1016/0022-
157 0981(80)90024-6.
- 158 McHugh, D. J. (2003). *A guide to the seaweed industry*. Rome: Food and Agriculture Organization
159 of the United Nations Available at: <https://www.fao.org/3/y4765e/y4765e.pdf>.
- 160 McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive
161 analysis and graphics of microbiome census data. *PLoS ONE* 8, e61217. doi:
162 10.1371/journal.pone.0061217.
- 163 Monteiro, J. P., Rey, F., Melo, T., Moreira, A. S. P., Arbona, J.-F., Skjermo, J., et al. (2020). The
164 unique lipidomic signatures of *Saccharina latissima* can be used to pinpoint their
165 geographic origin. *Biomolecules* 10, 107. doi: 10.3390/biom10010107.
- 166 Newton, R. J., Griffin, L. E., Bowles, K. M., Meile, C., Gifford, S., Givens, C. E., et al. (2010).
167 Genome characteristics of a generalist marine bacterial lineage. *ISME J.* 4, 784–798. doi:
168 10.1038/ismej.2009.150.
- 169 Nitschke, U., Walsh, P., McDaid, J., and Stengel, D. B. (2018). Variability in iodine in temperate
170 seaweeds and iodine accumulation kinetics of *Fucus vesiculosus* and *Laminaria digitata*
171 (Phaeophyceae, Ochrophyta). *J. Phycol.* 54, 114–125. doi: 10.1111/jpy.12606.

- 172 Paix, B., Carriot, N., Barry-Martinet, R., Greff, S., Misson, B., Briand, J.-F., et al. (2020). A multi-
173 omics analysis suggests links between the differentiated surface metabolome and
174 epiphytic microbiota along the thallus of a Mediterranean seaweed holobiont. *Front.*
175 *Microbiol.* 11, 494. doi: 10.3389/fmicb.2020.00494.
- 176 Paix, B., Layglon, N., Le Poupon, C., D’Onofrio, S., Misson, B., Garnier, C., et al. (2021).
177 Integration of spatio-temporal variations of surface metabolomes and epibacterial
178 communities highlights the importance of copper stress as a major factor shaping host-
179 microbiota interactions within a Mediterranean seaweed holobiont. *Microbiome* 9, 201.
180 doi: 10.1186/s40168-021-01124-8.
- 181 Parrot, D., Blümel, M., Utermann, C., Chianese, G., Krause, S., Kovalev, A., et al. (2019). Mapping
182 the surface microbiome and metabolome of brown seaweed *Fucus vesiculosus* by amplicon
183 sequencing, integrated metabolomics and imaging techniques. *Sci. Rep.* 9, 1061. doi:
184 10.1038/s41598-018-37914-8.
- 185 Peixoto, R. S., Rosado, P. M., Leite, D. C. de A., Rosado, A. S., and Bourne, D. G. (2017). Beneficial
186 Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience.
187 *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.00341.
- 188 Peng, Y., and Li, W. (2013). A bacterial pathogen infecting gametophytes of *Saccharina japonica*
189 (*Laminariales, Phaeophyceae*). *Chin. J. Oceanol. Limnol.* 31, 366–373. doi: 10.1007/s00343-
190 013-2136-9.
- 191 Peteiro, C. (2018). “Alginate production from marine macroalgae, with emphasis on kelp
192 farming,” in *Alginates and their biomedical applications* Springer Series in Biomaterials
193 Science and Engineering., eds. B. H. A. Rehm and M. F. Moradali (Singapore: Springer
194 Singapore), 27–66. doi: 10.1007/978-981-10-6910-9_2.
- 195 Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., et al. (2004). Changes
196 in bacterioplankton composition under different phytoplankton regimens. *Appl. Environ.*
197 *Microbiol.* 70, 6753–6766. doi: 10.1128/AEM.70.11.6753-6766.2004.
- 198 Ramírez-Puebla, S. T., Weigel, B. L., Jack, L., Schlundt, C., Pfister, C. A., and Mark Welch, J. L.
199 (2022). Spatial organization of the kelp microbiome at micron scales. *Microbiome* 10, 52.
200 doi: 10.1186/s40168-022-01235-w.
- 201 Riemann, L., Steward, G. F., and Azam, F. (2000). Dynamics of bacterial community composition
202 and activity during a mesocosm diatom bloom. *Appl. Environ. Microbiol.* 66, 578–587. doi:
203 10.1128/AEM.66.2.578-587.2000.
- 204 Rodeheaver, G., Bellamy, W., Kody, M., Spatafora, G., Fitton, L., Leyden, K., et al. (1982).
205 Bactericidal activity and toxicity of iodine-containing solutions in wounds. *Arch. Surg.* 117,
206 181–186. doi: 10.1001/archsurg.1982.01380260051009.
- 207 Roth-Schulze, A. J., Pintado, J., Zozaya-Valdés, E., Cremades, J., Ruiz, P., Kjelleberg, S., et al.
208 (2018). Functional biogeography and host specificity of bacterial communities associated
209 with the Marine Green Alga *Ulva* spp. *Mol. Ecol.* 27, 1952–1965. doi: 10.1111/mec.14529.

- 210 Roussel, S., Caralp, C., Leblanc, C., Le Grand, F., Stiger-Pouvreau, V., Coulombet, C., et al. (2019).
211 Impact of nine macroalgal diets on growth and initial reproductive investment in juvenile
212 abalone *Haliotis tuberculata*. *Aquaculture* 513, 734385. doi:
213 10.1016/j.aquaculture.2019.734385.
- 214 Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., et al. (1998a).
215 *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent
216 of red spot disease of *Laminaria japonica*. *Int. J. Syst. Bacteriol.* 48, 769–774. doi:
217 10.1099/00207713-48-3-769.
- 218 Sawabe, T., Sawada, C., Suzuki, E., and Ezura, Y. (1998b). Intracellular alginate-oligosaccharide
219 degrading enzyme activity that is incapable of degrading intact sodium alginate from a
220 marine bacterium *Alteromonas* sp. *Fish. Sci.* 64, 320–324. doi: 10.2331/fishsci.64.320.
- 221 Schiel, D., and Lilley, S. (2007). Gradients of disturbance to an algal canopy and the modification
222 of an intertidal community. *Mar. Ecol. Prog. Ser.* 339, 1–11. doi: 10.3354/meps339001.
- 223 Schiel, D. R., and Foster, M. S. (2006). The population biology of large brown seaweeds:
224 ecological consequences of multiphase life histories in dynamic coastal environments.
225 *Annu. Rev. Ecol. Evol. Syst.* 37, 343–372. doi: 10.1146/annurev.ecolsys.37.091305.110251.
- 226 Schiener, P., Black, K. D., Stanley, M. S., and Green, D. H. (2015). The seasonal variation in the
227 chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*,
228 *Saccharina latissima* and *Alaria esculenta*. *J. Appl. Phycol.* 27, 363–373. doi:
229 10.1007/s10811-014-0327-1.
- 230 Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytol.* 225,
231 1447–1454. doi: 10.1111/nph.16107.
- 232 Smit, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *J.*
233 *Appl. Phycol.* 16, 245–262. doi: 10.1023/B:JAPH.0000047783.36600.ef.
- 234 Stal, L. J., and Cretoiu, M. S. eds. (2016). *The Marine Microbiome*. Cham: Springer International
235 Publishing doi: 10.1007/978-3-319-33000-6.
- 236 Staufenberger, T., Thiel, V., Wiese, J., and Imhoff, J. F. (2008). Phylogenetic analysis of bacteria
237 associated with *Laminaria saccharina*. *FEMS Microbiol. Ecol.* 64, 65–77. doi:
238 10.1111/j.1574-6941.2008.00445.x.
- 239 Thomas, F., Dittami, S. M., Brunet, M., Le Duff, N., Tanguy, G., Leblanc, C., et al. (2019).
240 Evaluation of a new primer combination to minimize plastid contamination in 16S rDNA
241 metabarcoding analyses of alga-associated bacterial communities. *Environ. Microbiol. Rep.*
242 12, 30–37. doi: 10.1111/1758-2229.12806.
- 243 Tournerocche, A., Lami, R., Burgaud, G., Domart-Coulon, I., Li, W., Gachon, C., et al. (2020). The
244 bacterial and fungal microbiota of *Saccharina latissima* (Laminariales, Phaeophyceae).
245 *Front. Mar. Sci.* 7, 587566. doi: 10.3389/fmars.2020.587566.
- 246 Ul-Hassan, A., and Wellington, E. M. (2009). “Actinobacteria,” in *Encyclopedia of Microbiology*
247 (Elsevier), 25–44. doi: 10.1016/B978-012373944-5.00044-4.

- 248 Vairappan, C. S., Suzuki, M., Motomura, T., and Ichimura, T. (2001). Pathogenic bacteria
249 associated with lesions and thallus bleaching symptoms in the Japanese kelp *Laminaria*
250 *religiosa* Miyabe (Laminariales, Phaeophyceae). *Hydrobiologia* 445, 183–191. doi:
251 10.1023/A:1017517832302.
- 252 Wagner-Döbler, I., and Biebl, H. (2006). Environmental Biology of the Marine *Roseobacter*
253 Lineage. *Annu. Rev. Microbiol.* 60, 255–280. doi:
254 10.1146/annurev.micro.60.080805.142115.
- 255 Wegner, C.-E., Richter-Heitmann, T., Klindworth, A., Klockow, C., Richter, M., Achstetter, T., et
256 al. (2013). Expression of sulfatases in *Rhodopirellula baltica* and the diversity of sulfatases
257 in the genus *Rhodopirellula*. *Mar. Genomics* 9, 51–61. doi: 10.1016/j.margen.2012.12.001.
- 258 Weigel, B. L., and Pfister, C. A. (2019). Successional dynamics and seascape-level patterns of
259 microbial communities on the canopy-forming kelps *Nereocystis luetkeana* and
260 *Macrocystis pyrifera*. *Front. Microbiol.* 10, 346. doi: 10.3389/fmicb.2019.00346.
- 261 Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., and Imhoff, J. F. (2009). Diversity of antibiotic-
262 active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea.
263 *Mar. Biotechnol.* 11, 287–300. doi: 10.1007/s10126-008-9143-4.
- 264 Zaneveld, J. R., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the Anna
265 Karenina principle to animal microbiomes. *Nat. Microbiol.* 2, 17121. doi:
266 10.1038/nmicrobiol.2017.121.
- 267 Zehr, J. P., and Ward, B. B. (2002). Nitrogen Cycling in the Ocean: New Perspectives on
268 Processes and Paradigms. *Appl. Environ. Microbiol.* 68, 1015–1024. doi:
269 10.1128/AEM.68.3.1015-1024.2002.
- 270 Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., and Barrow, C. J. (2006). A simple 96-well
271 microplate method for estimation of total polyphenol content in seaweeds. *J. Appl. Phycol.*
272 18, 445–450. doi: 10.1007/s10811-006-9048-4.
- 273 Zhang, R., Chang, L., Xiao, L., Zhang, X., Han, Q., Li, N., et al. (2020). Diversity of the epiphytic
274 bacterial communities associated with commercially cultivated healthy and diseased
275 *Saccharina japonica* during the harvest season. *J. Appl. Phycol.* 32, 2071–2080. doi:
276 10.1007/s10811-019-02025-y.
- 277

278 SUPPLEMENTARY DATA

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

281 **Figure S1 - Seasonal variations in A) temperature, B) salinity and C) ammonium, D) nitrites, E)**
282 **nitrites, and F) phosphate concentrations.** Roscoff, 2019. Legend for each month, rectangle:
283 region inside 1st and 3rd quartiles, bold line: median value, dashed error bars: 1st and 9th deciles,
284 red point: value for the selected year.

