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1 **Year-round quantification, structure and dynamics of epibacterial**
2 **communities from diverse macroalgae reveal a persistent core microbiota**
3 **and strong host specificities**

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5

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11 Running head: *Seasonal succession of macroalgae epiphytic microbiota*

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14 **Abstract**

15

16 Macroalgae-bacteria interactions play pivotal ecological roles in coastal ecosystems. Previous
17 characterization of surface microbiota from various macroalgae evidenced fluctuations based
18 on host tissues, physicochemical and environmental parameters. However, the dynamics and
19 degree of similarity of epibacterial communities colonizing phylogenetically distant algae from
20 the same habitat are still elusive. We conducted a year-long monthly epimicrobiota sampling
21 on five algal species inhabiting an English Channel rocky shore: *Laminaria digitata*,
22 *Ascophyllum nodosum*, *Fucus serratus* (brown algae), *Palmaria palmata* (red alga) and *Ulva*
23 sp. (green alga). To go beyond relative compositional data and estimate absolute variations in
24 taxa abundance, we combined qPCR measurements of 16S rRNA gene copies with amplicon
25 metabarcoding. A core microbiome composed of 10 genera was consistently found year-round
26 on all algae. Notably, the abundant genus *Granulosicoccus* stood out for being the only one
27 present in all samples and displayed an important microdiversity. Algal host emerged as the
28 primary driver of epibacterial community composition, before seasonality, and bacterial taxa
29 specifically associated with one or several algae were identified. Moreover, the impact of
30 seasons on the epimicrobiota varied depending on algal tissues. Overall, this study provides an
31 extensive characterization of the microbiota of intertidal macroalgae and enhances our
32 understanding of algal-bacteria holobionts.

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37 Introduction

38 Macroalgae play crucial roles in marine ecosystems, contributing significantly to primary
39 production, nutrient cycling, and habitat provision. Their surface harbors a dense, complex and
40 highly dynamic biofilm of bacteria, archaea, fungi and viruses. Bacteria dominate the
41 microbiota in abundance and diversity, representing up to 10^8 cells.cm⁻² (Egan *et al.*, 2013;
42 Martin *et al.*, 2015), and form intricate interactions with macroalgae. These associations were
43 reported to be either beneficial, detrimental or commensal (Burgunter-Delamare *et al.*, 2024)
44 and are at the basis of various essential processes in coastal oceans, including roles in nutrient
45 cycling (Brunet *et al.*, 2022) and in shaping host morphology, reproduction and defense against
46 pathogens (Wichard *et al.*, 2015; Singh and Reddy, 2016). The structure and composition of
47 the microbiota of various brown (eg. Bengtsson *et al.*, 2012; Florez *et al.*, 2019; Burgunter-
48 Delamare *et al.*, 2023), red (Miranda *et al.*, 2013; Zozaya-Valdés *et al.*, 2017) and green (Burke
49 *et al.*, 2011; van der Loos *et al.*, 2023) macroalgae has been largely investigated in the past
50 decades using culture-dependent and -independent approaches, allowing for the identification
51 of key members of the epiphytic microbiota. Macroalgae host specific bacterial communities
52 that largely differ from those occurring in the surrounding seawater (Weigel and Pfister, 2019;
53 Lu *et al.*, 2023). Among bacteria associated with macroalgae, the bacterial phyla
54 *Pseudomonadota* (mostly the classes *Alphaproteobacteria* and *Gammaproteobacteria*),
55 *Bacteroidota*, *Verrucomicrobiota*, *Actinomycetota* and *Planctomycetota* are the most
56 represented (Wahl *et al.*, 2012; Hollants *et al.*, 2013; Florez *et al.*, 2017). At the genus level,
57 the algal symbiont *Granulosicoccus* (*Gammaproteobacteria*) is commonly found as the most
58 abundant taxon on diverse algal hosts (Singh and Reddy, 2014), frequently reported to account
59 for more than 25% of the whole community on kelp tissue (Brunet *et al.*, 2021; Lemay *et al.*,
60 2021; Ramírez-Puebla *et al.*, 2022; Weigel *et al.*, 2022). It is now well established that bacterial
61 community composition and density is shaped by biotic and abiotic factors and varies
62 depending on biogeography, host tissue composition or seasonality (Lachnit *et al.*, 2011; Egan
63 *et al.*, 2013; Lemay *et al.*, 2018; Serebryakova *et al.*, 2018; Weigel and Pfister, 2019; Paix *et al.*
64 *et al.*, 2021; Ramírez-Puebla *et al.*, 2022). Algae release a wide range of bioactive compounds
65 with a broad spectrum of activities that participate in the control of bacterial colonization (Saha
66 and Fink, 2022). It was reported that host taxonomy (Kuba *et al.*, 2021; Chen *et al.*, 2022),
67 anatomy (Bengtsson *et al.*, 2012; Weigel and Pfister, 2019; Lemay *et al.*, 2021), genetics
68 (Wood *et al.*, 2022), health (Fernandes *et al.*, 2012; Marzinelli *et al.*, 2015; Burgunter-
69 Delamare *et al.*, 2023) or life cycle (Bengtsson *et al.*, 2010; Glasl *et al.*, 2021) influences its

70 surface microbiota. Studies investigating seasonal variations in bacterial communities
71 associated with healthy macroalgae have revealed dynamic patterns characterized by shifts in
72 community composition and diversity (Lachnit *et al.*, 2011; Burgunter-Delamare *et al.*, 2023).
73 These shifts can often be linked to seasonal changes in environmental factors such as
74 temperature, light or nutrient availability (Mancuso *et al.*, 2016; Florez *et al.*, 2019), which
75 influence the growth and metabolic activity of both macroalgae and associated bacteria.
76 Additionally, seasonal variations in macroalgal physiology, such as growth rates and
77 reproductive cycles, can indirectly impact bacterial communities by altering the tissues and
78 exudate composition (Pandey *et al.*, 2022).

79 Most of the works that characterized macroalgal-associated microbiota mainly focused on a
80 single algal species. Only a few recent studies compared algae from different phyla and of
81 distinct chemical composition revealing that algal phylum was a strong driver of epimicrobiota
82 composition (Kuba *et al.*, 2021) and identifying core taxa (Chen *et al.*, 2022; Lu *et al.*, 2023).
83 Moreover, temporal dynamics of epiphytic bacterial communities depending on the algal host
84 and type of tissue have been largely overlooked. In this study, the microbiota of three brown
85 (*Laminaria digitata* (Hudson) J.V.Lamouroux, 1813, *Fucus serratus* Linnaeus, 1753 and
86 *Ascophyllum nodosum* (Linnaeus) Le Jolis, 1863), one red (*Palmaria palmata* (Linnaeus)
87 F.Weber & D.Mohr, 1805) and one green (*Ulva* sp. Linnaeus, 1753) macroalgal species
88 predominant in the English Channel were investigated throughout a year. This selection of
89 species covers a large phylogenetic range, and different intertidal zonation (*A. nodosum* is
90 found on the upper-middle intertidal zone while *F. serratus*, *P. palmata* and *Ulva* sp. are in the
91 lower intertidal zone and *L. digitata* in the subtidal zone (Evans, 1957; Burel *et al.*, 2022)). The
92 sole use of metabarcoding amplicon sequencing has emerged as the preferred method to
93 investigate host-associated microbial communities. The resulting datasets are inherently
94 compositional as they only allow analysis based on relative proportions of microbial taxa
95 (Gloor *et al.*, 2017). Therefore, an increase in abundance of one taxon can partly be caused by
96 an equivalent decrease of another one, limiting the conclusions that can be drawn (Jian *et al.*,
97 2020). Despite the statistical approaches applied to overcome misinterpretations of bacterial
98 community structure (Gloor *et al.*, 2017; Shelton *et al.*, 2022), one of the most effective ways
99 to accurately compare microbial communities across samples and to identify patterns that may
100 be obscured when relying solely on relative abundance information, is to generate quantitative
101 microbial abundance data by combining amplicon sequencing with the cell or gene copy
102 density measurements. Several studies demonstrated an improved performance of quantitative
103 approaches over compositional data (Jian *et al.*, 2020; Lloréns-Rico *et al.*, 2021) and this has

104 been recently implemented to examine human (Vandeputte *et al.*, 2017; Barlow *et al.*, 2020),
105 freshwater (Props *et al.*, 2017) or soil (Camacho-Sanchez, 2023) microbiomes. Here we
106 coupled 16S rRNA gene amplicon sequencing with quantitative PCR to accurately estimate the
107 abundance of the different taxa in macroalgal epiphytic communities. We aimed to (i)
108 characterize the shared and specific patterns of epiphytic bacterial communities associated with
109 co-occurring macroalgae species and (ii) assess if seasonality equally impacts microbiomes
110 from phylogenetically distant species.

111 **Experimental procedures**

112 **Sampling**

113 The collection of epibiota from algal specimens was previously described in (Brunet *et al.*,
114 2023). Briefly, the surface microbiota of intertidal algae was sampled monthly between
115 February 2020 and January 2021 (no sampling in April and May 2020 due to the Covid-19
116 pandemic). Microbiota were collected in triplicates using sterile flocked nylon swabs
117 (Zymobiomics) on healthy specimens of the brown algae *Laminaria digitata* (Ldig, 0.5-1 m
118 long), *Fucus serratus* (Fser) and *Ascophyllum nodosum* (Anod), the red alga *Palmaria palmata*
119 (Ppal) and a green alga *Ulva* sp. (Ulva) at the Bloscon site (48°43'29.982'' N, 03°58'8.27'' W)
120 in Roscoff (Brittany, France). Swabbed surface was standardized to 50 cm². Three different
121 regions of the kelp *L. digitata* were sampled: the basal meristem (young tissue, hereafter
122 LdigB), the medium frond (ca. 20 cm away from the meristem, hereafter LdigM) and the old
123 frond (the blade tip, hereafter LdigO). Only two replicates were retrieved for LdigB in February
124 and Ulva in March. Upon collection, swabs were immediately immersed in DNA/RNA Shield
125 reagent (ZymoBiomics) on ice and stored at -20 °C until DNA extraction. A total of 209
126 samples were collected (**SuppFile S1**), scattered into 70 different conditions (7 types of algal
127 tissues at 10 different months).

128 **Collection of environmental data**

129 A set of 15 environmental parameters measured at 1.3 km from the collection site (Estacade
130 sampling station) between February 2020 and January 2021 was used for correlation analyses.
131 It includes surface seawater temperature (T, °C), salinity (S), dissolved oxygen (O, ml*l⁻¹), pH,
132 ammonium (NH₄, μM), nitrate (NO₃, μM), nitrite (NO₂, μM), phosphate (PHO₄, μM), silicate
133 (SiOH₄, μM), particulate organic carbon (COP, μg*l⁻¹) and nitrogen (NOP, μg*l⁻¹), suspended
134 matter (MES, mg*l⁻¹), ¹⁵Nitrogen (DN15, ‰) and ¹³Carbon isotopes (DC13, ‰) and

135 Chlorophyll a (CHLA, $\mu\text{g}\cdot\text{l}^{-1}$). Data were downloaded from the SOMLIT database (Service
136 d'Observation en Milieu Littoral; <http://www.somlit.fr>, Cocquempot et al., 2019) on April 7th,
137 2022 and are listed in **SuppFile S1**.

138 **DNA extraction and qPCR assays**

139 DNA extraction and qPCR assays were performed as in (Brunet *et al.*, 2023). Briefly,
140 environmental DNA from swabs was extracted using the DNA/RNA Miniprep kit
141 (ZymoBiomics) following the manufacturer's instructions. DNA was quantified with the
142 QuantiFluor dsDNA System (Promega) kit and samples normalized at $0.5\text{ ng}\cdot\mu\text{l}^{-1}$ before qPCR
143 and library preparation. DNA concentrations obtained from each sample are listed in **SuppFile**
144 **S1**.

145 The number of total 16S rRNA gene copies was assessed using qPCR (primers 926F: 5'-
146 AAACTCAAAGGAATTGACGG-3' /1062R: 5'-CTCACRRCACGAGCTGAC-3',
147 (Bacchetti De Gregoris *et al.*, 2011) on a LightCycler 480 Instrument II (Roche). The global
148 predicted coverage of this primer pair is 94.1% of all bacterial sequences present in Silva SSU
149 r138.1 (Silva Testprime with only one mismatch allowed, analysis performed in June 2024).
150 All bacterial phylum-level taxa in the Silva database had predicted coverages above 90%,
151 except 10-bav-F6 (33%), *Candidatus* Bipolaricaulota (*Acetothermia* 50%), *Bdellovibrionota*
152 (85%), *Chrysiogenetota* (75%), *Candidatus* Dependientiae (88%), *Dictyoglomota* (88%),
153 *Candidatus* Edwardsbacteria (75%), NKB15 (86%) and *Patescibacteria* (46%). Among these,
154 only *Bdellovibrionota*, *Candidatus* Dependientiae and *Patescibacteria* were detected in our 16S
155 rRNA gene metabarcoding dataset, with average relative abundance of 1.2, 0.0001 and 0.09%,
156 respectively. qPCR results are listed in **SuppFile S1**, and MIQE information related to qPCR
157 experiments is available in **SuppFile S2**.

158 **Library preparation, sequencing and sequence processing**

159 The V3-V4 region of 16S rRNA gene was amplified using the primers S-D-Bact-0341-b-S-17
160 (5' CCTACGGGNGGCWGCAG 3') and 799F_rc (5' CMGGGTATCTAATCCKGTT 3'
161 (Thomas *et al.*, 2020). Sequencing was conducted on a MiSeq paired-end sequencing run (300
162 cycles \times 2, Illumina, San Diego, CA, USA) as described previously (Thomas *et al.*, 2020). 16S
163 rRNA gene amplicon sequences are available at NCBI under BioProject PRJNA1135191. The
164 R package *DADA2* (version 1.22.0, Callahan et al. 2016) was used to exclude primers
165 sequences, filter and trim low quality sequences (truncLen=c(280,210), trimLeft = c(10,10),
166 minLen = 150, maxN=0, maxEE=c(2,3), truncQ=2), for denoising, paired-read merging,

167 inferring Amplicon Sequence Variants (ASVs, length between and 400 and 452 nucleotides),
168 chimera removal and taxonomic assignment using the SILVA 138.1 (Quast *et al.*, 2013; Yilmaz
169 *et al.*, 2014) reference database. Default parameters were used unless otherwise specified. All
170 sequences affiliated to 16S rRNA gene from chloroplasts, mitochondria, cyanobacteria,
171 Eukaryota and *Archaea*, as well as sequences found in negative controls (PCR molecular grade
172 water) were removed using the R package *phyloseq* (version 1.38.0, McMurdie and Holmes,
173 2013).

174 **Multivariate and statistical analyses**

175 Relative ASV abundance was multiplied by the total number of 16S rRNA gene copies
176 quantified with qPCR for each sample, to obtain a table of abundance of each ASV that was
177 used in all subsequent analyses. To assess dissimilarities in community structure between algal
178 tissues, Bray–Curtis dissimilarity index (Bray *et al.*, 1957) was calculated before non-metric
179 multidimensional scaling (NMDS). Permutational analysis of variance (PERMANOVA, 999
180 permutations) was calculated using the *adonis* function to discriminate groups of samples
181 according to algal tissues or seasons (Anderson, 2001), followed by multivariate pairwise
182 comparisons using *pairwise.perm.manova*. Mean dispersions within groups were calculated
183 using *betadisper* (999 permutations). To identify environmental parameters that may be
184 associated with microbial community structure, we performed distance-based redundancy
185 analysis (dbRDA; Legendre and Anderson, 1999) on the Bray–Curtis dissimilarity matrix with
186 *dbrda* function. To avoid having highly correlated variables, pH, SiOH₄, NOP, PO₄, NO₃,
187 NH₄, DN15 were removed prior to the analysis. Only significant environmental factors were
188 selected for dbRDA visualization (ANOVA, $P < 0.05$). Months were grouped in 3 different
189 seasons (Winter: Feb, Mar, Jan; Summer: Jun, Jul, Aug; Autumn: Sep, Oct, Nov, Dec) prior to
190 PERMANOVA and *betadisper* analysis. Differential sequence abundance analysis between the
191 3 seasons (qPCR-corrected counts from months of the same season were averaged) was
192 performed at the ASV level using the GLM test with the ALDEx2 R package (Fernandes *et*
193 *al.*, 2014). To detect differential abundance, this test uses ratios among taxa, which are
194 conserved regardless of whether data are relative or absolute.

195 A standardized approach based on occupancy–redundancy distribution was applied to identify
196 core genera on each algal tissue (Shade and Stopnisek, 2019). Using a presence/absence matrix
197 (1 for presence, 0 for absence), an index was calculated for each genus, as the sum of the
198 occupancy term and the redundancy term for each month. The occupancy term is the replicate

199 average of the presence/absence matrix. The redundancy term is equal to 1 if the genus is
200 present in all three replicates and to 0 otherwise. The index was scaled to an upper limit of 1
201 and calculated separately for each algal tissue.

202 **Phylogenetic analysis**

203 Phylogenetic analysis was conducted using phylogeny.fr (Dereeper *et al.*, 2008) for the most
204 abundant *Granulosicoccus*-affiliated ASVs in the dataset (representing at least 5% of the
205 *Granulosicoccus* community in at least 5 samples) together with 16S rRNA gene sequences
206 from cultured *Granulosicoccus* strains and uncultured clones retrieved from Genbank.
207 Sequences were aligned using Muscle (full mode) and the resulting alignment was curated
208 using Gblocks. The 405 conserved positions were used for neighbor-joining tree reconstruction
209 with K2P substitution model and 1,000 bootstraps. The tree was visualized using iTOL
210 (Letunic and Bork, 2024) and rooted at midpoint.

211 **Results**

212 The structure and dynamic of the bacterial community associated with five phylogenetically
213 distant macroalgae from a temperate rocky shore has been investigated over one year. The ASV
214 relative abundance was multiplied by the total number of 16S rRNA gene copies to consider
215 absolute taxa count and obtain a more precise understanding of the algal epimicrobiome
216 structure.

217 **Algal host as a major driver of epiphytic bacterial community composition**

218 The quantified number of bacterial 16S rRNA gene copies (**SuppFile S1**) varied significantly
219 along the year (2-way ANOVA, ‘sampling month’ effect $F_{9,158} = 5.0$, $p < 0.001$) but not across
220 macroalgal species ($F_{4,158} = 0.8$, $p = 0.531$) and with no interactions between the two factors
221 ($F_{36,158} = 1.1$, $p = 0.3$). This proxy for total bacterial abundance was relatively homogeneous
222 for all algae, with year-round average ranging between 7×10^6 to 1×10^7 16S rRNA gene
223 copies.cm⁻², depending on the algal species. Yet, we could detect a large range of variations
224 spanning more than two orders of magnitude, from a minimum of 1.04×10^5 (Ldig-base in
225 February 2020) to a maximum of 5.02×10^7 16S rRNA gene copies.cm⁻² (Ldig-old in
226 November 2020). The structure of the epiphytic bacterial communities was strongly impacted
227 by the algal host (**Figure 1A**; PERMANOVA, $F_{4,203} = 22.7$, $P < 0.001$), showing that
228 communities from different algal species sharing the same habitat are distinct from one another.

229 This observation is supported by the fact that 80% of the total number of ASVs (8 316 out of
230 10 243) are specific to an algal species (**SuppFigure S1**) and only 1% (103) are present on all
231 algae. However, 83% of these specific ASVs were only found in one sample. Despite their
232 closer phylogenetic distance, brown algae (Ldig, Anod and Fser, all belonging to the class
233 Phaeophyceae) did not share more ASVs or genera than with green (Ulva) and red (Ppal) algae.
234 Besides host taxonomy, the age of algal tissue also influenced the surface density and
235 composition of the microbiota. Indeed, the younger meristematic tissues of *L. digitata* (Ldig-
236 base) consistently harbored less 16S rRNA gene copies than the medium frond (Ldig-medium,
237 paired t-test, $P = 0.002$) and the older apical tissues (Ldig-old, paired t-test, $P < 0.001$).
238 Multivariate analysis also separated Ldig-base epibacterial communities from Ldig-medium
239 and Ldig-old (**Figure 1B**; PERMANOVA, $F_{2,86} = 12.4$, $P < 0.001$).

240 *Gammaproteobacteria*, *Alphaproteobacteria*, *Bacteroidia*, *Acidimicrobiia* and
241 *Planctomycetes* were the most abundant bacterial classes on all algae, representing on average
242 93-98 % of the communities, depending on the algal host (**SuppFigure S2**). After pooling the
243 ASVs by genus, we observed that 28% of all genera (93 out of 331) were shared between all
244 algae, suggesting they might be ubiquitous algal epibionts (**Figure 2** and **SuppFile S3**). These
245 generalists were mainly *Bacteroidia* (including *Flavobacteriales*), *Gammaproteobacteria* and
246 *Alphaproteobacteria* and represented on average 50-75 % of all sequences depending on algae.
247 An occupancy-redundancy index was calculated following Shade and Stopnisek, 2019 method
248 to identify core genera that were consistently found associated with the five studied algal
249 species all year round (**Figure 2B**). Genera that had an index > 0.65 on all algae were defined
250 as core genera: *Granulosicoccus* (*Gammaproteobacteria*), *Litorimonas*, *Hellea*, *Fretibacter*
251 (*Alphaproteobacteria*), *Portibacter*, *Algitalea*, *Rubidimonas*, *Lewinella* (*Bacteroidota*),
252 *Blastopirellula* (*Planctomycetota*) and Sva0996 marine group (*Actinomycetota*). These
253 represented only 3% of all genera, yet accounted for 46% of the bacterial abundance on
254 average. The genus *Granulosicoccus* was the only one to have an index equal to 1 on all algal
255 tissues, meaning it was present in all 209 samples (see below). On the contrary some genera
256 were specifically associated with particular algae. Especially, the genus *Arenicella*
257 (*Gammaproteobacteria*) was strongly associated with brown algae all year round (index > 0.78 ,
258 2.8×10^5 copies.cm⁻² on average) while scarce on Ppal (index = 0.27, 3.4×10^4 copies.cm⁻²)
259 and Ulva (index = 0.2, 4.1×10^3 copies.cm⁻²). The opposite was observed for *Truepera*
260 (*Deinococcota*; index < 0.3 on brown algae, > 0.9 on the red and green algae). Some genera
261 were only found strongly associated (index > 0.8) with one algal species, namely *Tateyamaria*
262 (*Alphaproteobacteria* on Anod, *Pibocella* (*Bacteroidota*) on Fser, *Sulfitobacter*

263 (*Alphaproteobacteria*), *Polaribacter* (*Bacteroidota*) and *Jannaschia* (*Alphaproteobacteria*) on
264 *Ulva*. Moreover, important differences were observed between the different parts of the Ldig
265 frond (**Figure 2**). In particular, members of the Sva0996 marine group and of the family
266 *Microtrichaceae* (*Actinomycetota*) accounted on average for only 3×10^4 16S rRNA gene
267 copies.cm⁻² (1%) on the meristem compared to 2×10^6 (15%) and 4×10^6 (21%) on the medium
268 and old frond respectively. These taxa were also overrepresented on Fser (2×10^6 , 21%) and
269 Ppal (2×10^6 , 20%).

270 **Seasonal succession of epiphytic bacterial communities**

271 The number of 16S rRNA gene copies.cm⁻² varied differently through seasons depending on
272 the algal host, and no common pattern to all algae could be highlighted (**Figure 3**). The number
273 of total bacteria tended to reach a peak in late summer / autumn on Ldig, Fser, Ppal and *Ulva*.
274 Ldig-old, Anod, Fser, Ppal and *Ulva* samples from March 2020 showed higher bacterial
275 abundances than neighboring months (February and June). The absence of data in April and
276 May (COVID lockdown) prevents us from assessing if this abundance peak was an isolated
277 event caused by specific environmental conditions in March or if it was characteristic of
278 through the entire spring season.

279 Globally, the number of observed ASVs did not significantly differ through the year
280 (**SuppFigure S3**). A 3-fold decrease of the ASV number was only observed in June compared
281 to October (Ldig-medium), December (Ldig-old) or September (*Ulva*).
282 Distance-based Redundancy Analysis (dbRDA) and PERMANOVA (**Figure 4**) showed clear
283 dissimilarities between the three identified seasons (winter, summer and autumn) for all algae
284 (except between winter and autumn with Anod and *Ulva*) and suggested these seasonal
285 variations might be partly explained by environmental parameters, especially seawater
286 temperature, salinity and chlorophyll A concentration.

287 No single ASV was found to vary consistently through seasons on all algae (**SuppFile S4**).
288 This might be partly explained by the low proportion of ASVs present on all algae, as
289 mentioned above. Anod-associated communities were the least impacted by seasons with only
290 9 ASVs differentially abundant (**SuppFile S4, Figure 2A**). Bacterial communities' seasonal
291 dynamics varied depending on the part of the Ldig blade. The basal part exhibited less
292 variations than the older ones (15 differentially abundant ASVs compared to 55 and 92 on
293 medium and old fronds, respectively). On the basal tissues, 2 ASVs from the genus
294 *Peredibacter* (*Bdellovibrionota*) were more abundant during summer months (3×10^4 16S
295 rRNA gene copies.cm⁻², against 2×10^2 in winter and 6×10^3 in autumn, respectively) and 3

296 ASVs from the family *Rhodobacteraceae* (*Alphaproteobacteria*) less abundant in winter. The
297 most outstanding abundance variations on the older tissues were observed for ASVs from the
298 classes *Acidimicrobiia* (Sva0996 marine group and the family *Micotrichaceae*) and
299 *Bacteroidia* (orders *Chitinophagales* and *Flavobacteriales*), that peaked in autumn on the
300 medium frond and in autumn and winter at the tip part. The community succession on Fser was
301 characterized by a significantly higher abundance of *Micavibrionales* (*Alphaproteobacteria*)
302 in summer (2×10^4 16S rRNA gene copies.cm⁻², against 0 in winter and 2×10^3 in autumn).
303 Also, the abundance of 28 *Chitinophagales* (*Bacteroidota*) ASVs significantly varied between
304 seasons but exhibited distinct abundance patterns. Only few variations were observed with the
305 red alga Ppal (24 differentially abundant ASVs). The most notable succession was a peak of
306 the *Aquimarina* (*Flavobacteriales*) population in winter (1×10^5 16S rRNA gene copies.cm⁻²
307 compared to none in summer and 4×10^3 autumn). Summer communities displayed high
308 specificity at the surface of *Ulva*. Thirty-one ASVs were differentially abundant, in particular,
309 members of the genus *Truepera* (*Deinococcales*), of the families *Micotrichaceae*
310 (*Actinomycetota*) and *Saprospiraceae* (*Bacteroidota*) exhibited a sharp decrease in summer
311 while alphaproteobacteria from orders *Rhodobacterales* and *Caulobacterales* (*Litorimonas* and
312 *Fretibacter*) observed an opposite trend.

313 ***Granulosicoccus* are ubiquitous members of macroalgal epibiota**

314 The genus *Granulosicoccus* (*Gammaproteobacteria*) was the only one present in all 208
315 samples, covering the 5 different algal species and the 10 sampling months. It was also the
316 most abundant genus, representing 15.4% of the communities on average (up to 42% on Ldig-
317 base), and accounting for 1.2×10^6 copies.cm⁻² on average (up to 7.7×10^6 copies.cm⁻² on Ppal).
318 It comprised 464 different ASVs, the highest number of ASVs for all genera in our dataset,
319 suggesting a large micro-diversity. Half of these ASVs (236) were only found in one condition
320 (ie. on one type of tissue at one specific time point). Phylogenetic analysis of the most abundant
321 *Granulosicoccus* ASVs (representing at least 5% of the *Granulosicoccus* community in at least
322 5 conditions) confirmed this large diversity and separated three main clades (**Figure 4**), likely
323 representing new species compared to the 4 validly described species. Clades 2 and 3 clustered
324 with reference sequences from cultured *Granulosicoccus* strains and uncultured clones. This
325 notably included other marine host-associated strains, retrieved from the brown alga *Undaria*,
326 the red alga *Gracilaria*, the seagrass *Zostera marina*, sponge and plankton (Kurilenko *et al.*,
327 2010; Park *et al.*, 2014; Heins and Harder, 2023). Clade 1 comprised 5 ASVs that clustered
328 away from other sequences. In addition, all ASVs were not equally distributed on the different

329 algal species. Only two closely related *Granulosicoccus* ASVs were consistently detected on
330 all algal species (ASV11 and to a lesser extent ASV62). Most of the ASVs were specific or
331 strongly linked to a unique algal species, e.g. ASV1, ASV7, ASV61, ASV208 and ASV49 to
332 Ldig, ASV34, ASV70, ASV123 ASV91 and ASV57 to Anod, ASV73 to Fser and ASV31 to
333 Ppal (**Figure 4A**). While clade 2 encompassed ASVs detected on multiple algae, clade 1 and
334 clade 3 comprised ASVs more abundant on red and brown algae, respectively.
335 *Granulosicoccus* ASVs associated with medium and old Ldig tissues exhibited stronger
336 significant seasonal variations than *Granulosicoccus* ASVs associated with the other algal
337 species (**Figure 4B**). Moreover, even ASVs found on the same tissue displayed distinct
338 variations patterns, e.g. for Ldig-medium where ASV1, 7 and 61 peaked in summer and
339 ASV66, 208 and 139 in autumn.

340

341 **Discussion**

342

343 Next-generation sequencing methods have become indispensable tools in microbial ecology,
344 opening new avenues for studying microbial communities. However, these approaches are
345 limited, as they yield relative abundance profiles of microbial taxa, which may not accurately
346 reflect true variations in the actual taxon abundances in the environment (Gloor *et al.*, 2017;
347 Lloréns-Rico *et al.*, 2021). In this study, we estimated actual ASV abundance by multiplying
348 the ASV relative abundance matrix by the corresponding number of 16S rRNA gene copies
349 estimated through qPCR (Barlow *et al.*, 2020; Jian *et al.*, 2020). This approach has been
350 previously applied to soil microbiota (Lou *et al.*, 2018; Azarbad *et al.*, 2022), revealing that
351 quantification provides a more comprehensive understanding of bacterial community dynamics
352 than compositional data, which are more prone to false-positive changes. Absolute abundance
353 of bacterial taxa associated with macroalgae has been previously characterized but focusing
354 only on a few targeted taxa using specific qPCR primers or FISH probes (Ramírez-Puebla *et al.*,
355 2022; Brunet *et al.*, 2023). Here we implemented this method to quantitatively characterize
356 the whole macroalgal epimicrobiota composition for the first time, aiming to assess true taxa
357 variations across different algal hosts and seasons. Although providing valuable improvements
358 over sole amplicon sequencing to estimate absolute abundance of different taxa, coupling with
359 qPCR still has some limitations. At least three limitations are common with the metabarcoding-
360 only approach: (1) potential saturation of the swabs during sampling that could lead to
361 underestimate bacterial abundance on densely colonized algal surfaces; (2) variation in

362 extraction efficiencies depending on algal tissue composition (e.g. polysaccharide content); (3)
363 unequal lysis of different bacterial taxa. Here, we used flocked nylon swabs, previously shown
364 to outperform other swab types in terms of collection efficiency and DNA recovery from
365 microbiomes (Bruijns *et al.*, 2018; Wise *et al.*, 2021). The large variation in DNA
366 concentrations retrieved from swabs (minimum 0.11 ng. μ l⁻¹, median 7.57 ng. μ l⁻¹, maximum
367 41.46 ng. μ l⁻¹, 92% of samples below 24 ng. μ l⁻¹, **SuppFile S1**) further suggests that if saturation
368 occurred, it only affected a limited number of samples. Moreover, DNA recovery from swabs
369 did not differ depending on algal species (on average Anod: 9.3 ng. μ l⁻¹; Fser 8.9 ng. μ l⁻¹; Ldig
370 10.3 ng. μ l⁻¹; Ppal 10.6 ng. μ l⁻¹; Ulva 6.9 ng. μ l⁻¹, **SuppFile S1**), suggesting tissue composition
371 did not largely affect extraction efficiency. An additional bias lies in the use of different sets
372 of primers for qPCR and amplicon sequencing. Indeed, qPCR requires short amplicon size for
373 optimal efficiency and specificity (< 200 bp, Debode *et al.*, 2017), whereas optimal primers
374 for 16S metabarcoding cover a region of at least approximately 450 bp after merging, to
375 encompass both conserved and hypervariable regions. Yet, this bias is minimal since the qPCR
376 primers covered most of the taxa found in the 16S metabarcoding dataset (see Methods).

377 **Common core of the epiphytic microbiome**

378 The large number of samples collected in this study, encompassing 7 types of algal tissues
379 among 5 algal species at 10 sampling months, allow for the identification of a core epiphytic
380 microbiome that would be consistently found on algae all year round. The identification of
381 these core microbes is crucial as they likely have more substantial impact on the host's biology
382 than other members of the microbiome (Neu *et al.*, 2021). Here the core microbiota was
383 identified based on occupancy and redundancy criteria as recently used in soil microbial
384 ecology studies (eg. Hodgson *et al.*, 2024). The identified core genera, especially
385 *Granulosicoccus*, *Litorimonas* and *Hellea*, concur with previous works that revealed the
386 preponderance of these taxa at the surface of macroalgae (Weigel and Pfister, 2019; Lemay *et al.*,
387 2021; Wood *et al.*, 2022; Burgunter-Delamare *et al.*, 2023; Lu *et al.*, 2023).
388 *Granulosicoccus* was the dominant genus on all algae, both in terms of presence/absence and
389 abundance. Predominance of *Granulosicoccus* on the surface of other brown algae was
390 previously suggested using relative compositional data (eg. Lemay *et al.*, 2021; Weigel *et al.*,
391 2022; Burgunter-Delamare *et al.*, 2023) and quantitative cell counts using FISH probes
392 (Ramírez-Puebla *et al.*, 2022). Here we quantify this ubiquity on a larger temporal scale and
393 on other algal species, extending this observation to red and green algae. Using an estimate of
394 3 rRNA operons per cell (as seen in the complete reference genome of *Granulosicoccus*

395 *antarcticus* (Kang *et al.*, 2018)), the maximum observed density of 7.7×10^6 16S rRNA gene
396 copies.cm⁻² on the red algae *P. palmata* would correspond to ca. 2 millions *Granulosicoccus*
397 cell.cm⁻². Using FISH counts on the kelp *Nereocystis luetkeana* in summer, Ramírez-Puebla
398 *et al.*, 2022 reported 5.5×10^4 and 6.5×10^6 *Granulosicoccus* cells.cm⁻² on basal and old tissues,
399 respectively. Comparable values can be inferred from our dataset on the basal and old tissues
400 of the kelp *L. digitata* in summer (5×10^5 and 2.3×10^6 *Granulosicoccus* cells.cm⁻²,
401 respectively), confirming qPCR correction of metabarcoding compositional data is a useful
402 approach to estimate absolute abundances of specific taxa. *Granulosicoccus* sequences were
403 scattered into multiple ASVs that fall within distinct clades whose abundance patterns across
404 algal hosts and seasons differ. These clades exhibit distinct tissue specificity and could be
405 characterized either as generalist (clade 2) for containing taxa colonizing all types of tissue or
406 specialist for clades 1 and 3 that encompass taxa that are more specific to particular hosts.
407 These data indicate that ASVs from a same genus colonize distinct ecological niches and
408 underscore important genetic and functional diversity within core bacterial genera associated
409 with macroalgae. Similar observations were made in recent studies where different genomes
410 of core epiphytic genera, especially *Granulosicoccus*, were differentially abundant at the
411 surface of the bull kelp *N. luetkeana* depending on its location (Weigel *et al.*, 2022) or on *Fucus*
412 sp. depending on season and geographical location (Park *et al.*, 2022). The analysis of
413 *Granulosicoccus* genomes revealed strong abilities for an epiphytic lifestyle, that would allow
414 fast colonization and stable associations with the host. In particular, genes involved in motility
415 and chemotaxis, B12 vitamin biosynthesis, DMSP metabolism, transport and utilization of
416 sugars, were found abundant in *Granulosicoccus* metagenomes (Kang *et al.*, 2018; Weigel *et*
417 *al.*, 2022).

418 **Host algal tissue is a major driver of epiphytic community composition**

419 Our study revealed that the type of algal tissue, whether it is different algal species or different
420 blade parts, was a stronger driver of epiphytic community composition than seasonality. This
421 observation is consistent with previous reports stating that host characteristics may have a
422 greater impact on shaping the structure of macroalgae-associated microbial communities than
423 environmental variables (Marzinelli *et al.*, 2015; Burgunter-Delamare *et al.*, 2023). Chemical
424 composition of algal tissues varies depending on algal taxonomy (Mišurcová, 2011) or tissue
425 age (Küpper *et al.*, 1998). Significant differences of concentration of nutritional factors such as
426 proteins, minerals, lipids or sugars were largely shown. Moreover, polyphenol content was
427 reported to be 10-20 times higher in *A. nodosum* and *F. serratus* compared to the other algae

428 collected in this study (Pandey *et al.*, 2022). Species-specific differences were also reported
429 for iodine, as its concentration was shown to be approximately 5 and 20 times higher in *L.*
430 *digitata* compared to *A. nodosum* or *F. serratus* and *P. palmata* or *Ulva* sp., respectively
431 (Nitschke *et al.*, 2018). Both iodine and polyphenols are known to be algal defense compounds
432 involved in the regulation of microbial colonization (eg. Cosse *et al.*, 2009; Besednova *et al.*,
433 2020). Such chemical variations were also observed across seasons but were much less
434 pronounced than between algal species (Nitschke *et al.*, 2018; Pandey *et al.*, 2022). Therefore,
435 if these compounds are drivers of community composition, their limited annual variations
436 within the tissues could partly explain why seasons had a smaller impact on community
437 composition than algal host. Interestingly, microbiota composition similarities were not higher
438 between brown algae (*L. digitata*, *A. nodosum* and *F. serratus*) than with the red or green algae.
439 Similarly, no common pattern was found among species inhabiting the same intertidal zone.
440 Then, despite the important discrepancies observed in the microbiota of distinct algal species,
441 neither higher taxonomic level (algal phylum) nor algae location on the foreshore appeared to
442 be strong community drivers, corroborating observations made by Chen *et al.*, 2022 and
443 Selvarajan *et al.*, 2019. Nevertheless, the abundance of certain taxa displayed important
444 differences between algal phyla, *Arenicella* was consistently associated only with brown algae,
445 whereas *Truepera* was consistently found only with red and green algae. *Truepera* phylum
446 specificity was also reported in (Lu *et al.*, 2023) with different algal species.

447 This work shows that different parts of the *L. digitata* blade support distinct bacterial
448 communities and that total bacteria abundance and diversity are higher on older tissues, in
449 accordance with studies carried out on the same species (Ihua *et al.*, 2020) and on other kelp
450 species (Bengtsson *et al.*, 2012; Weigel and Pfister, 2019; Lemay *et al.*, 2021; Ramírez-Puebla
451 *et al.*, 2022; Burgunter-Delamare *et al.*, 2023). Moreover, the specific colonization of older *L.*
452 *digitata* tissues by members of the Sva0996 marine group, in comparison to the meristem,
453 concur with the one observed in (Brunet *et al.*, 2021) where the abundance of this taxon was
454 linked with tissue decomposition and degradation. Then, the presence of such taxa on old
455 damaged tissues might be related to their capacity to use complex algal derived organic matter
456 such as polysaccharides (Brunet *et al.*, 2022).

457 Seasonal variations of the surface microbiota have received unequal attention depending on the
458 algal host. To our knowledge, to date, there is no report of these fluctuations on *Ascophyllum*
459 sp. or *Palmaria* sp., while a few studies were conducted on *Fucus* sp. (Lachnit *et al.*, 2011;
460 Park *et al.*, 2022), *Ulva* sp. (Tujula *et al.*, 2010; Lachnit *et al.*, 2011) and *Laminaria* sp. (Corre

461 and Prieur, 1990; Bengtsson *et al.*, 2010; Brunet *et al.*, 2021). Seasonal microbial variations
462 are known to be influenced by a combination of biotic and abiotic factors. Here we showed
463 that seasons affect epiphytic community composition for all algae but in different ways.
464 Community richness decreased in summer for *L. digitata* and *Ulva* sp. Temperature and salinity
465 were environmental parameters that could explain the observed seasonal discrepancies.
466 Seawater temperature shapes microbial community variations, in particular, it was already
467 shown that increasing temperatures lead to a decrease in community richness on kelp (Paix *et*
468 *al.*, 2021) and in seawater (Sunagawa *et al.*). Salinity was also reported as an important
469 structuring variable of *Ulva*-associated bacterial communities (van der Loos *et al.*, 2023).
470 Anod-associated microbiota was the most stable across seasons among all five algae. *A.*
471 *nodosum* is known to observe monthly epidermal shedding, a way for longer-lived marine
472 macroalgae to rid themselves of epibionts via the removal of the outer cell layer of the thallus
473 (Halat *et al.*, 2015; Garbary *et al.*, 2017). Therefore, it is likely that the newly exposed thallus,
474 free of epibionts, does not present any alterations or damages and is monthly colonized by the
475 same early bacterial epibionts.

476

477 This study, through the extensive number of collected samples and the analysis of absolute taxa
478 abundance, revealed true microbiota variations based on hosts and seasons, providing novel
479 insights in the structure and fluctuations of bacterial communities associated with macroalgae.
480 Future studies should focus on deciphering the ecological and metabolic functions of the
481 identified core epiphytic taxa to gain further insights into epiphyte–host interactions.

482

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490 BTBR-04).

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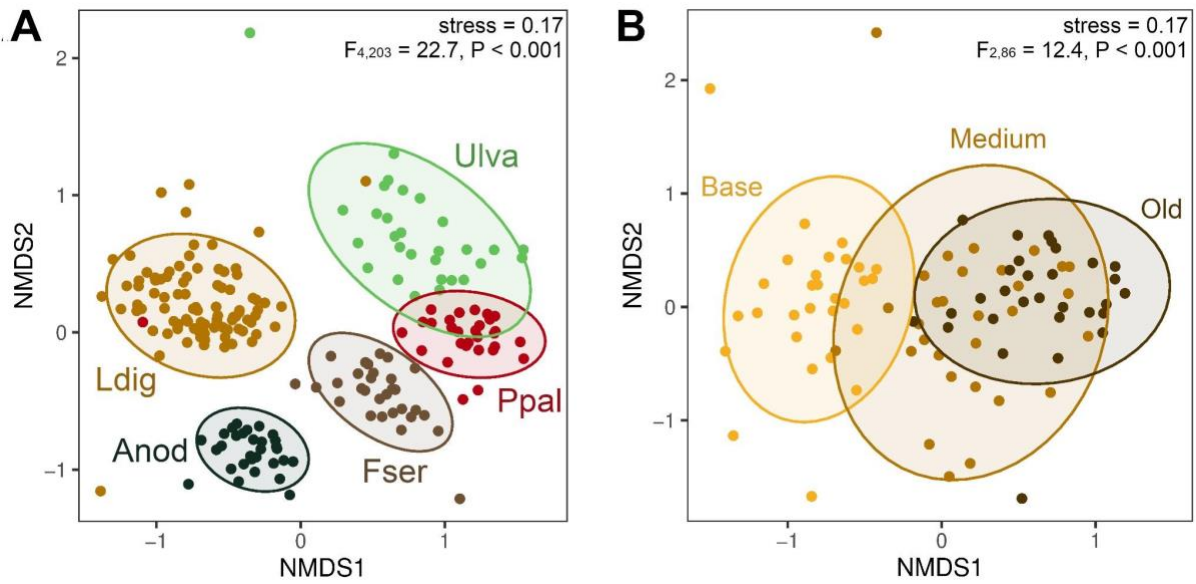
492

493 **Contributions (following CRediT taxonomy)**

494 Conceptualization, Funding acquisition, Project administration: FT; Data curation, Formal
495 analysis, Visualization, Writing-Original draft preparation: MB, FT; Investigation: MB, NLD,
496 FT; Supervision: FT, TB. Writing-Review & Editing: MB, NLD, TB, FT.

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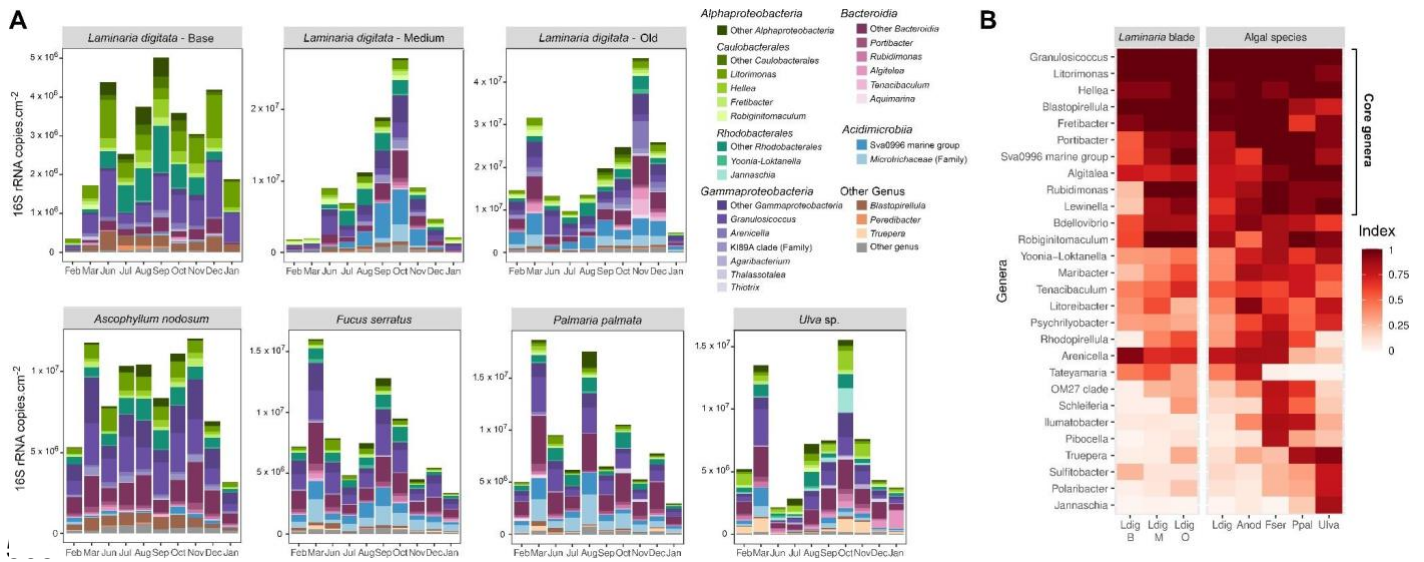
498 **Figures**



499

500 **Figure 1:** NMDS ordination plots of abundance data of all samples (A) or *L. digitata* samples
501 (B), based on Bray-Curtis dissimilarity. 95% confidence ellipses for a multivariate t-
502 distribution are depicted for the different type of algal tissues (either algal species or *L. digitata*
503 blade part). PERMANOVA F-statistic and p-value are displayed.

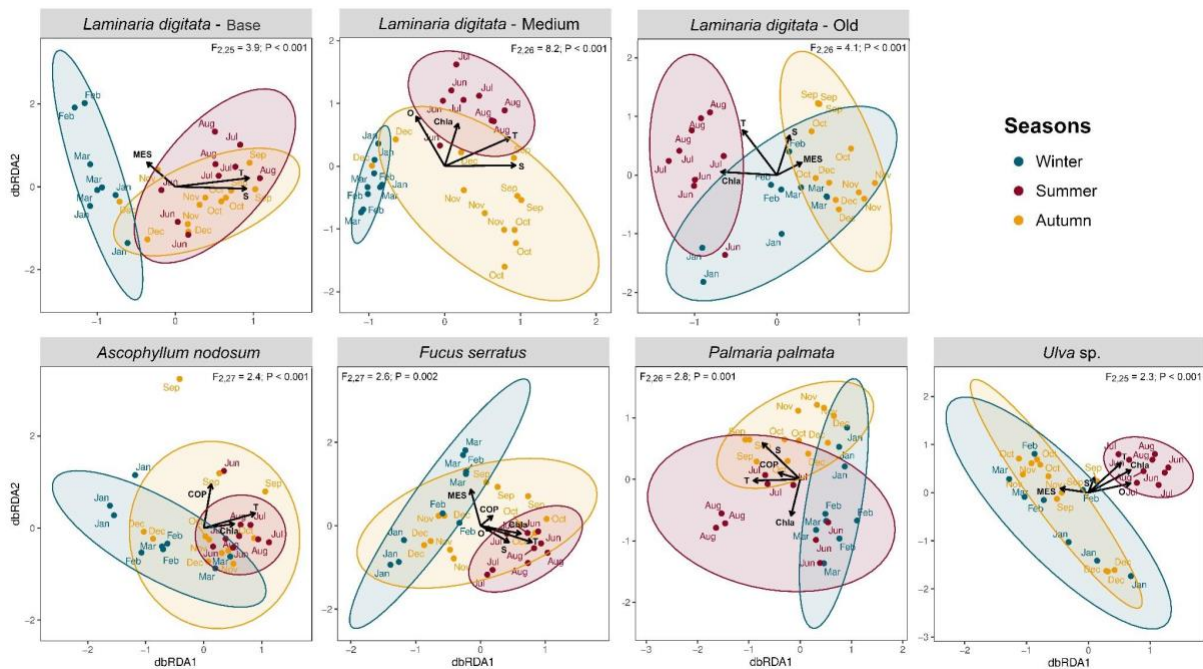
504



506 **Figure 2:** (A) Estimated abundance of the main bacterial genera. Only genera representing
 507 more than 5% (triplicate average) of the communities for at least one condition (ie. at one time
 508 point for one algae) are shown. (B) Approach to identify core macroalgal genera. Indices are
 509 calculated based on abundance-occupancy distributions (see Methods for index calculation).
 510 Only genera that have an index > 0.5 on at least one type of tissue are represented. For the Ldig
 511 column, the index is an average of the indices calculated on the base, medium and old Ldig
 512 tissues.

513

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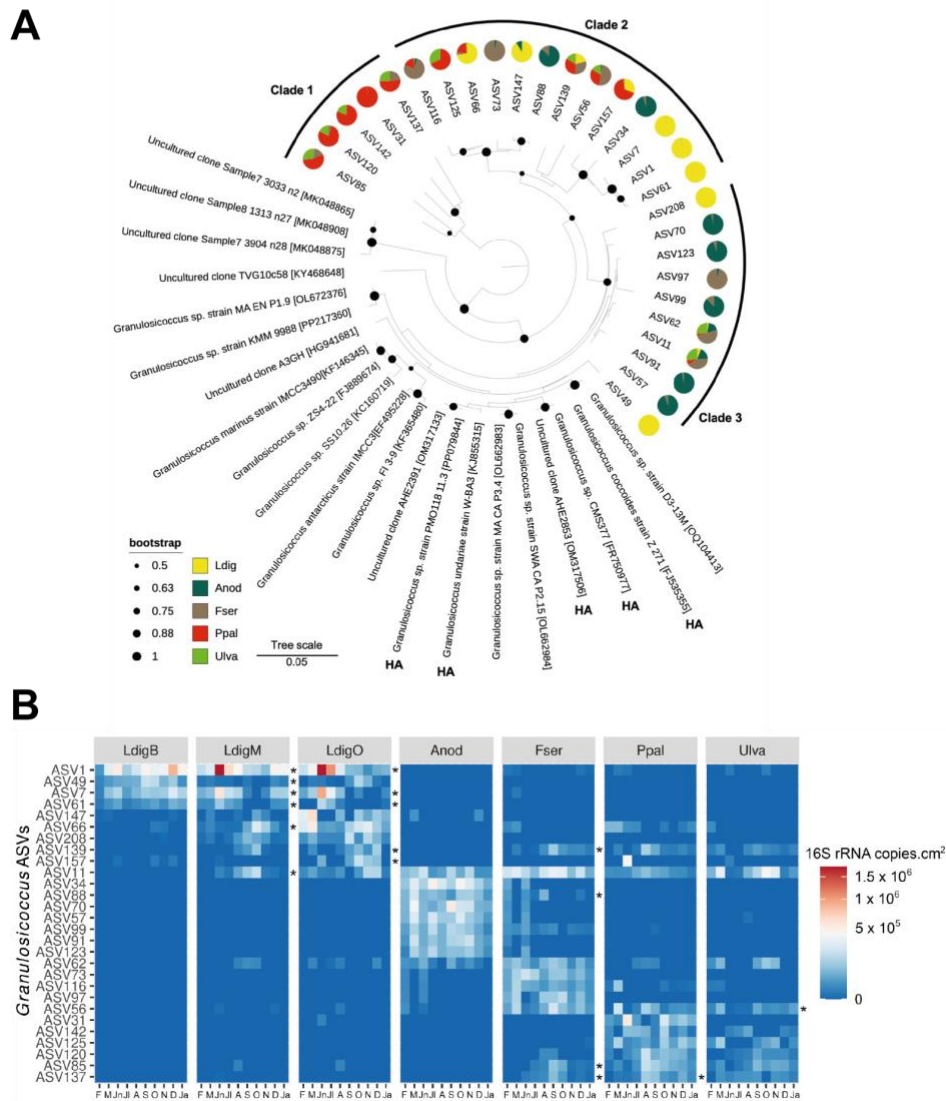


515

516 **Figure 3:** db-RDA plots with vectors representing significant contextual predictors. 95%
517 confidence ellipses for a multivariate t-distribution are depicted for the different seasons.
518 PERMANOVA F-statistic and p-value are displayed. T: Temperature; O: Dissolved oxygen;
519 S: Salinity; ChlA: Chlorophyll A; MES: Suspended matter; COP: particulate organic carbon.

520

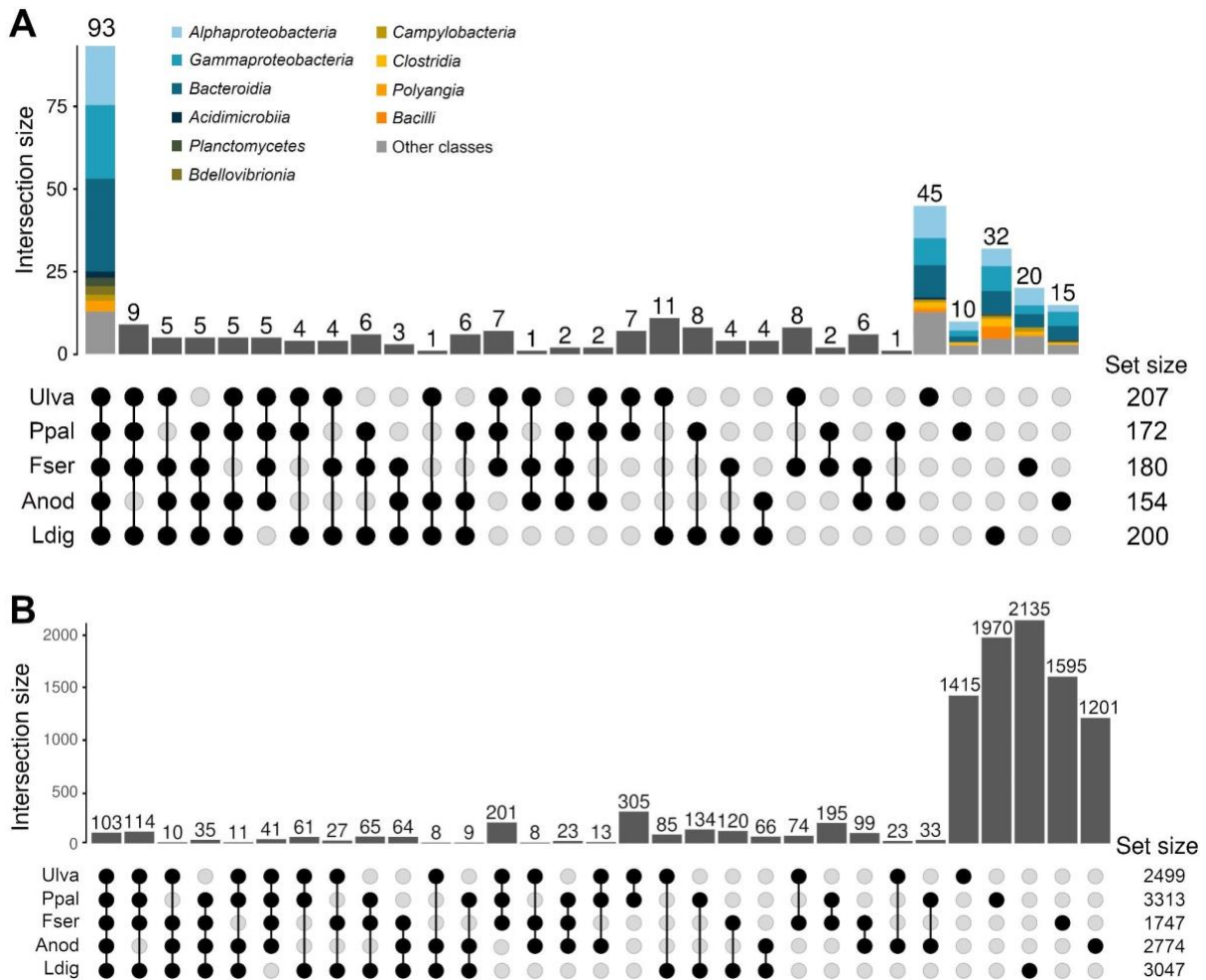
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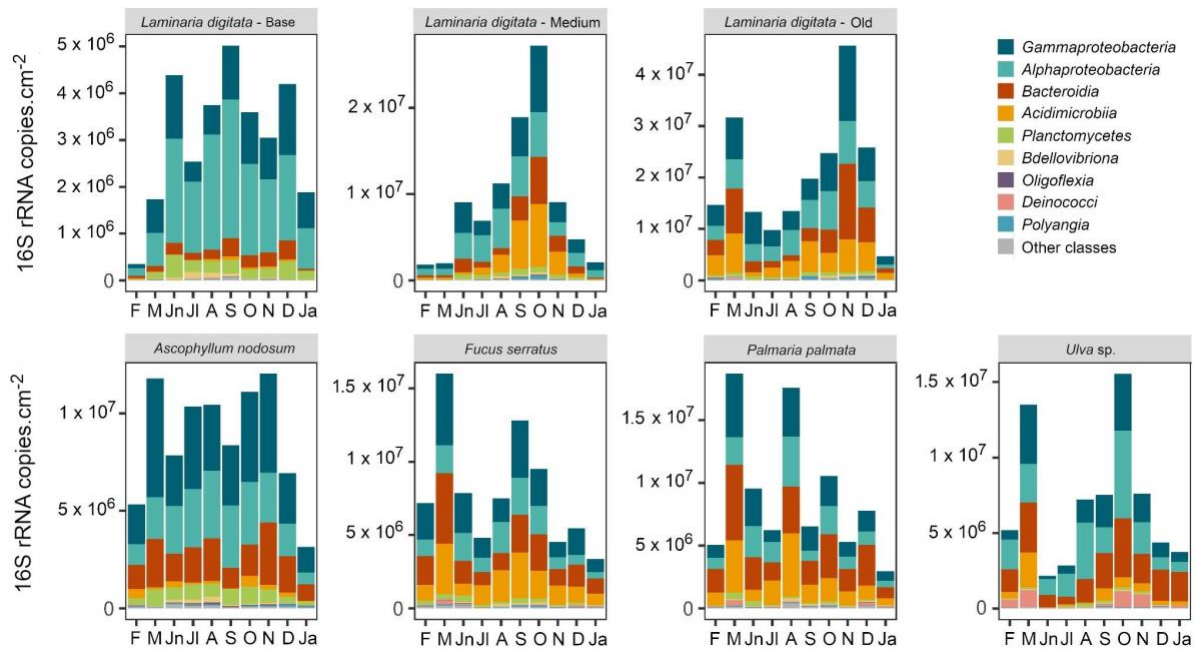
524 **Figure 4:** (A) Phylogenetic tree of the main 16S rRNA ASVs affiliated to *Granulosiccoccus*
 525 and reference sequences (Genbank accession in brackets). Pie charts represent year-round
 526 average abundance of each ASV on different algal species. HA: host-associated. (B) Heatmap
 527 of the abundance of the main ASVs assigned to the genus *Granulosiccoccus*. Only
 528 *Granulosiccoccus* ASVs representing at least 5% of the *Granulosiccoccus* community in at least
 529 5 conditions (out of 70) are displayed. Abundances of triplicates were averaged and square root
 530 transformation was applied. Asterisks on the right panel of each alga denote differentially
 531 abundant ASVs through seasons. F: February; M: March; Jn: June; Jl: July; A: August; S:
 532 September; O: October; N: November; D: December; Ja: January.



533

534 **Figure S1:** (A) Upset plot of the bacterial genera found in the algal microbiota. Set size
 535 represents the total amount of genera present on each alga. Taxonomy is displayed at the class
 536 level for the genera shared by all algae and the ones specific to one algal species. (B) Upset
 537 plot of the 10 243 ASVs found in the algal microbiota. Set size represents the total amount of
 538 ASVs present on each alga.

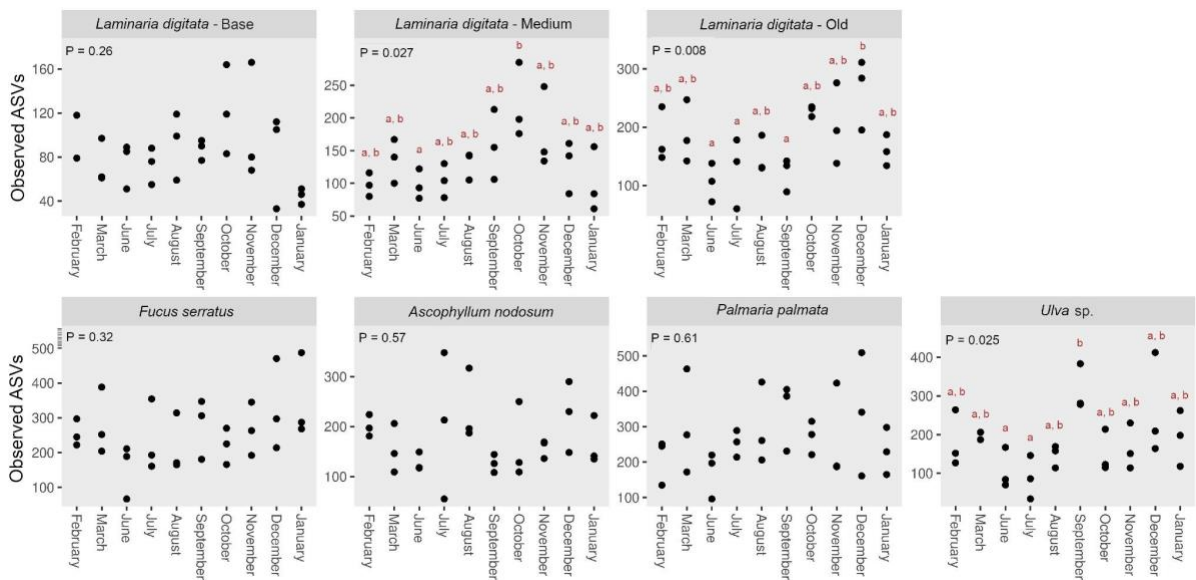
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540

541 **Figure S2:** Estimated abundance of the main bacterial classes. Only classes representing more
 542 than 3% (triplicate average) of the communities for at least one condition (ie. at one time point
 543 for one algae) are shown.

544



545

546 **Figure S3:** Fluctuation of the number of observed ASVs. When significant ANOVA results
 547 were found ($P < 0.05$), a post hoc Tukey HSD test was calculated. Accordingly, different letters
 548 indicate significant differences between sampling months.

549

550 **File S1:** Characteristic of the 209 samples of macroalgal epibiota collected during this study.

551 **File S2:** Details of the qPCR assay on the environmental samples, following the Minimum
552 Information for publication of Quantitative real-time PCR Experiments (MIQE) guidelines.

553 **File S3:** List of the 331 assigned taxonomic genera. The number of samples in which they were
554 found is indicated for each alga.

555 **File S4:** List of differentially abundant ASVs through seasons. A red excel cell indicates the
556 abundance of the ASV varies significantly through seasons on the associated algal tissue. The
557 different sheets named after each algal tissue display the season average abundance of the
558 differentially abundant ASVs. The genus (or the deepest known taxonomic level) assigned to
559 each ASV is mentioned.

560

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