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1 **Exploring the phycosphere of *Emiliana huxleyi*: from bloom dynamics to**
2 **microbiome assembly experiments**

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22 Running title: Microbiome assembly in *E. huxleyi* cultures

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29 **Abstract**

30 Cocolithophores have global ecological and biogeochemical significance as the most
31 important calcifying marine phytoplankton group. The structure and selection of prokaryotic
32 communities associated with the most abundant cocolithophore and bloom-forming species,
33 *Emiliana huxleyi*, are still poorly known. In this study, we assessed the diversity of bacterial
34 communities associated with an *E. huxleyi* bloom in the Celtic Sea, exposed axenic *E. huxleyi*
35 cultures to prokaryotic communities derived from bloom and non-bloom conditions and
36 followed the dynamics of their microbiome composition over one year. Bloom-associated
37 prokaryotic communities were dominated by SAR11, Marine group II Euryarchaeota,
38 Rhodobacterales and contained substantial proportions of known indicators of phytoplankton
39 bloom demises such as Flavobacteriaceae and Pseudoalteromonadaceae. Taxonomic richness
40 of replicated co-cultures resulting from natural communities with axenic *E. huxleyi* rapidly
41 shifted and then stabilized over time, presumably by ecological selection favoring more
42 beneficial populations. Recruited microbiomes from the environment were consistently
43 dependent on the composition of the initial bacterioplankton community. Phycosphere-
44 associated communities derived from the *E. huxleyi* bloom depth were highly similar to one
45 another, suggesting deterministic processes, whereas cultures from non-bloom conditions show
46 an effect of both deterministic processes and stochasticity. Overall, this work sheds new light
47 on the importance of the initial inoculum composition in microbiome recruitment and
48 elucidates the temporal dynamics of its composition and long-term stability.

49

50 **Key-words:** Phytoplankton-bacteria interactions, microbiome assembly, phycosphere,
51 metabarcoding, *Emiliana huxleyi*

52

53

54 **Introduction**

55 In the surface ocean, marine phytoplankton generate nearly up to 50% of global primary
56 production and at least half of this production is remineralized by marine heterotrophic bacteria
57 (Falkowski, 1994; Field et al., 1998; Pomeroy et al., 2007). From an ecological perspective,
58 interactions between these essential microbial groups are being increasingly recognized as a
59 major force shaping microbial communities (Amin et al., 2015, Seymour et al., 2017).
60 Phytoplankton-bacteria interactions are widespread in marine environments, in particular
61 within the phycosphere, the region immediately surrounding individual phytoplankton cell
62 (Bell & Mitchell, 1972; Smriga et al., 2016). This microscale region, analogous to the plant
63 root rhizosphere, serves as the interface for phytoplankton-bacteria associations.
64 Phytoplankton exudates fuel the activity of heterotrophic microorganisms, that in exchange can
65 stimulate microalgal growth through the provision of growth hormones and vitamins (Amin et
66 al., 2015, Croft et al., 2005), protection against pathogenic bacteria (Seyedsayamdost et al.,
67 2014) and through the facilitation of iron uptake (Amin et al., 2009). Phytoplankton release
68 broad chemical classes of metabolites (Cirri & Pohnert, 2019) which can influence the
69 taxonomy of phycosphere-associated bacteria (Buchan et al., 2014; Fu et al., 2020; Shibl et al.,
70 2020).

71 Recent studies addressing the processes involved in bacterial community assembly in
72 the phycosphere showed the influence of deterministic factors such as the place/time of
73 isolation (Ajani et al., 2018) and the host species (Behringer et al., 2018; Lawson et al., 2018;
74 Kimbrel et al., 2019; Sörenson et al., 2019; Jackrel et al., 2020; Mönnich et al., 2020). However,
75 a combination between deterministic and stochastic effects in the microbiome recruitment
76 process was also suggested (Kimbrel et al. 2019; Stock et al., 2022). To date, bacterial
77 community composition and selection processes that influence the assembly of phycosphere

78 microbiomes are not well known in many phytoplankton, in part because of the micrometer
79 scale at which they take place (Kimbrel et al., 2019; Mönnich et al., 2020).

80 To overcome this challenge, one strategy is to study the selection processes in natural
81 phytoplankton blooms (Zhou et al., 2019), in meso/microcosms or in cultures (Ajani et al.,
82 2018; Kimbrel et al., 2019; Sörenson et al., 2019; Fu et al., 2020; Mönnich et al., 2020), when
83 algal cells are at high concentrations. *Emiliania huxleyi* is the most abundant and cosmopolitan
84 coccolithophore species and is able to form massive annual blooms in temperate and subpolar
85 oceans mostly during Spring (Tyrrell & Merico, 2004). *E. huxleyi* blooms are characterized by
86 blue turquoise waters that can be observed from satellite images (Tyrrell & Merico, 2004).
87 These blooms have a critical importance for carbon and sulfur cycles due to the ecological and
88 biogeochemical roles of coccolithophores as primary producers, calcifiers, and main
89 contributors to the emission of dimethylsulfoniopropionate (DMSP) to the atmosphere (Malin
90 & Steinke, 2004; Rost et al., 2004). The potential role of viruses in bloom termination has been
91 thoroughly investigated (e.g. Bratbak, Egge & Heldal, 1993; Vardi et al., 2012; Lehahn et al.,
92 2014), but only few studies have targeted the microbial diversity associated with *E. huxleyi* in
93 natural environments (Gonzalez et al., 2000; Zubkov et al., 2001) and cultures (Green et al.,
94 2015; Orata et al., 2016; Rosana et al., 2016). The *Roseobacter*, SAR86 and SAR11 lineages
95 were identified as the main bacterial groups in natural *E. huxleyi* blooms (Gonzalez et al., 2000;
96 Zubkov et al., 2001). Meanwhile, microbiomes of *E. huxleyi* in cultures are highly dominated
97 by *Marinobacter* (Câmara dos Reis, 2021) and by Rhodobacteraceae (Green et al., 2015;
98 Barak-Gavish et al., 2018).

99 In this study, we followed the dynamics of the prokaryotic community associated with
100 *E. huxleyi* along a natural bloom in the Celtic Sea and used natural bloom and non-bloom
101 samples to investigate the microbiome selection by an axenic *E. huxleyi* culture. We
102 hypothesized that recruited microbiomes would be enriched in *Marinobacter* and

103 Rhodobacteraceae members often associated with *E. huxleyi* cultures and would differ
104 according to the initial prokaryotic composition.

105

106 **Materials and Methods**

107 **Study site and sample collection**

108 Samples used in this study were collected aboard the schooner *Tara* (Sunagawa et al., 2020) in
109 the Celtic Sea (from 48°19-48°24 N/6°28-7°02 W; Fig. 1A and B), during the ‘*Tara*
110 BreizhBloom’ cruise from May 27 to June 2, 2019. To follow an *E. huxleyi* bloom formed in
111 this area, an Argo float (<https://argo.ucsd.edu/>) was deployed in the center of the bloom patch
112 and its position was used twice a day (early morning and end of the afternoon) to determine
113 the geographical locations of the sampling stations.

114 On the last sampling day, a site about 34 km apart from the bloom area was also sampled
115 (Fig. 1A). For each sampling event, surface to 50 m depth profiles of temperature, salinity,
116 turbidity, pressure, photosynthetic active radiation (PAR), chlorophyll *a* (chl_a) fluorescence,
117 oxygen concentrations and pH were conducted by deploying a SBE19+ profiler (Sea-Bird
118 Scientific). Water samples were collected using an 8L Niskin bottle for nutrient analyses. After
119 collection, nutrient samples (125 mL) were stored at -20°C for further analysis. Concentrations
120 of nitrate, nitrite, phosphate and silicate were measured using a AA3 auto-analyzer (Seal
121 Analytical) following the methods described by Tréguer & Le Corre, (1975) and Aminot &
122 Kerouel (2007). Samples for flow cytometry (FCM), scanning electron microscopy (SEM),
123 and metabarcoding analysis were collected at the bloom depth and prefiltered through a 20 µm
124 mesh to eliminate large microzooplankton. For FCM analysis of photosynthetic eukaryotes and
125 prokaryotic communities, two replicates (1.5 mL) were fixed using glutaraldehyde (0.25% final
126 concentration) and Poloxamer 10% (0.1% final concentration) and incubated for 15 min at 4°C
127 before flash freezing in liquid nitrogen. For SEM analysis, samples of morning sites (two

128 replicates of 250 mL) were gently filtered onto PC membranes (47 mm in diameter; 1.2 μ m
129 pore-size) (Millipore). Filters were placed onto Petri slides, dried at least 2h at 50°C, and finally
130 stored at room temperature. For metabarcoding analysis, cell biomass was collected from ~ 14
131 L of seawater by successive filtration onto large (142 mm in diameter) 3 μ m pore-size and then
132 0.2 μ m pore-size polycarbonate membranes (Millipore). Filters were flash-frozen in liquid
133 nitrogen and stored at -80°C for later DNA analyses.

134

135 **Scanning electron microscopy analysis**

136 Representative filter portions were fixed in aluminum stubs and sputter coated with gold–
137 palladium (20 nm) (Keuter et al., 2019). Quantitative assessment of *E. huxleyi* cells was
138 performed using a Phenom Pro scanning electron microscope. Cells were counted in twenty
139 random screens (area analyzed = 0.16 mm²) and cell concentrations were calculated based on
140 the filtered sample volume corresponding to the area analyzed (0.042 mL).

141

142 **Community assembly experiments**

143 **(i) Axenization.** The *E. huxleyi* strain RCC1212, obtained from the Roscoff Culture Collection,
144 was axenized following a sequence of washing and centrifugation steps, and variable
145 incubation periods with increasing concentrations of an antibiotic solution mixture (ASM) as
146 detailed in the original protocol developed at the Scottish Association for Marine Science
147 (Oban, UK) available at: [https://www.ccap.ac.uk/wp-](https://www.ccap.ac.uk/wp-content/uploads/2020/06/KB_Antibiotic_treatment.pdf)
148 [content/uploads/2020/06/KB_Antibiotic_treatment.pdf](https://www.ccap.ac.uk/wp-content/uploads/2020/06/KB_Antibiotic_treatment.pdf).

149 This method is briefly detailed in the Supplementary Materials and Methods section.

150 **(ii) Sample preparation and inoculation.** Four seawater samples were used in the bacterial
151 community assembly experiment. They consisted of a surface and a DCM sample collected in
152 the bloom area on day 5 (thereafter named inside bloom surface and inside bloom DCM) and

153 a surface and a DCM sample collected the same day outside the bloom area (thereafter named
154 outside bloom surface and outside bloom DCM) (Supplementary Fig. 1). In order to remove
155 autotrophic picoeukaryotes and cyanobacteria from the inoculum, seawater samples were
156 gently filtered through a 0.45 μm pore size membrane (Millex-HV, PVFD, Millipore). To
157 estimate the number of prokaryotic cells lost during the filtration step, aliquots of total and
158 filtered seawater samples were fixed for FCM analysis using the methods described in the
159 Supplementary Materials and Methods. After filtration, 150 μL of each prokaryotic community
160 were transferred in triplicates into 50 mL culture flasks filled with 15 mL of K/2 medium
161 prepared as described in the Supplementary Materials and Methods. Finally, 150 μL of the
162 axenic RCC1212 culture were added to each flask. Six flasks filled with 15 mL of K/2 medium
163 and inoculated with 150 μL of the axenic RCC1212 culture were used as controls. In total, 18
164 cultures (12 treatments and 6 controls) were incubated at 15°C and a 12:12 photoperiod regime.
165 Due to space limitation, only one thermostatic chamber with a light intensity of 20 μmol
166 photons $\text{s}^{-1}\text{m}^{-2}$ using a blue neutral density filter was available onboard for incubation.

167 **(iii) Survey of the culture microbiomes.** Back to the laboratory and 10 days after inoculation,
168 which corresponds to the time needed by *E. huxleyi* cultures to reach the end of exponential
169 growth phase, axenic status of controls was checked by FCM. Then, each treatment was
170 sampled for FCM analyses and prokaryotic community composition. Cultures (12 treatments
171 and one axenic control) were transferred by inoculating 100 μL of the culture in 10 mL of fresh
172 K/2 medium every 11-14 days for the first 176 days of experiment and then every 3 weeks until
173 its end (day 393). The axenic control was regularly checked to ensure the clean handling of the
174 cultures. In addition, culture flasks were randomized daily in the incubator to minimize
175 positional effect on growth.

176 For FCM, duplicate samples were fixed as previously described and analyses performed
177 according to Marie et al., (1999) are detailed in the Supplementary Materials and Methods

178 section. For community composition analysis, 2 mL of culture was centrifuged at 2,000 g for
179 30 sec to reduce the microalgal load. Preliminary tests showed that this procedure reduces
180 microalgal load while keeping most of the bacterial cells (about 90%). The supernatants were
181 transferred into new tubes containing 2 µL of Poloxamer 188 solution 10% (Sigma-Aldrich)
182 and centrifuged at 5,600 g for 5 min. The supernatants were discarded, and the pellets were
183 stored at -80°C until DNA extraction.

184

185 **DNA extraction, PCR amplification and sequencing**

186 DNA extraction from environmental and culture samples and amplification steps used to
187 amplify the prokaryotic 16S rRNA gene using the universal prokaryote primers 515F-Y 5'-
188 GTGYCAGCMGCCGCGGTAA-3' (eight-nucleotide tag unique to each sample) and 926R
189 5'-CCGYCAATTYMTTTRAGTTT-3' (Parada et al., 2016) are detailed in the Supplementary
190 Materials and Methods and in (Romac, 2022a, 2022b, 2022c, 2022d). Library preparation and
191 high-throughput sequencing using Illumina technology were performed at Fasteris SA (Plan-
192 les-Ouates, Switzerland). Pooled amplicons were sent to Fasteris SA (Plan-les-Ouates,
193 Switzerland) for Illumina high throughput sequencing.

194

195 **Bioinformatics**

196 The steps of library separation, removal of Illumina adapters and first quality control were
197 performed by Fasteris SA (see Supplementary Materials and Methods for details). The detailed
198 scripts in this study (from bioinformatic treatment to statistical analysis) can be downloaded
199 from https://github.com/mcamarareis/microbiome_assembly. Briefly, raw reads from each
200 sequencing run were demultiplexed based on the 8 nucleotide tag sequences with cutadapt
201 (version 2.8.1) (Martin, 2011). To deal with the presence of reads in mixed orientation in the
202 R1 and R2 raw files (corresponding to each cycle of paired-end sequencing), the

203 demultiplexing was performed in two rounds (see details in Supplementary Materials and
204 Methods). Then, primer sequences were removed using cutadapt (version 2.8.1) (Martin,
205 2011). The demultiplexed primer-free sequences from different sequencing runs and from
206 different sequencing cycles were processed independently to obtain an amplicon sequence
207 variant (ASV) table using the DADA2 pipeline (version 1.14.0 in R 3.6.1) (Callahan et al.,
208 2016; R Core Team, 2017). The ASV tables obtained independently for R1 and R2 were
209 merged. Then, all the independent processed datasets were merged in one sequence table for
210 chimera removal, also performed in pooled mode. The parameters used at each DADA2 step
211 are specified in the Supplementary Table 1.

212 Sequences shorter than 366 bp and longer than 377 bp were filtered out, and the
213 remaining sequences were taxonomically assigned using IDTAXA (50% confidence threshold)
214 with the Silva database v138 (Quast et al., 2013; Murali et al., 2018). Chloroplasts and
215 mitochondrial sequences were removed. Sequences not classified at the domain level by
216 IDTAXA were assigned to the best hit in Silva v138 by pairwise global alignment
217 (`usearch_global VSEARCH's command`) (Rognes et al., 2016). These sequences were
218 removed if they could not be classified and/or were classified as chloroplasts or mitochondrial
219 sequences by VSEARCH (at 80% identity threshold). The resultant ASV table was filtered to
220 remove ASVs accounting for less than 0.001% of the total number of reads. Consistency of
221 technical replicates was evaluated by procrustes analysis, which measures the similarity
222 between two ordinations of the same objects, followed by a protest, which measures the
223 significance of the correlation. For this, we used a comparative principal components analysis
224 performed on the Hellinger transformed data. After consistency was confirmed ($p < 0.001$ and
225 correlation = 0.99), independent technical replicates of each culture were merged by the sum
226 of the number of reads of the ASVs present in the two replicates of the same culture. The
227 abundance and prevalence filters applied removed about 68% of the total number of ASVs

228 while keeping 99% of the number of reads. The final dataset (filtered ASV table used for further
229 analysis) contained 107 samples (11 from the environment and 96 from cultures) for a total of
230 6,017,019 reads and 294 prokaryotic ASVs.

231

232 **Community composition and statistical analyses**

233 **(i) Environmental samples.** All the analyses were conducted in R version 4.0.2 in Rstudio
234 (1.1.442) and the plots were produced with ggplot2 (RStudio Team, 2016; Wickham, 2016; R
235 Core Team, 2017). Taxonomy treemaps of environmental samples were produced at the genus
236 level considering the best hits classified by VSEARCH. To facilitate visualization, low
237 abundant genera (accounting to less than 3% of relative abundance at each sample) present at
238 the raw community table were grouped. To compare environmental samples and cultures, mean
239 alpha diversity indices (richness and Shannon index) were measured after rarefying the ASV
240 table 100 times at the minimum number of reads (2,479) (Saary et al., 2017). Hierarchical
241 cluster analysis (HCA) (method “ward.D2”) was used to identify differences in the free-living
242 prokaryotic communities by sites using the Hellinger distance (Euclidean distance of the
243 Hellinger-transformed matrix) (Legendre & Gallagher, 2001; Oksanen et al., 2015).

244 **(ii) Experiment.** To analyze alpha diversity dynamics of the cultures, rarefaction was
245 performed at a reads depth of 5,957 reads using the same approach as for the environmental
246 samples. For beta diversity analysis, the Jaccard dissimilarity was calculated on the rarefied
247 table (Oksanen et al., 2015). Hellinger distance was calculated from the non rarefied table
248 (Legendre & Gallagher, 2001; Oksanen et al., 2015). To test the influence of the treatments,
249 replicates and time on the microbiome beta diversity, we performed a permutational analysis
250 of variance (PERMANOVA) (Anderson, 2005). Before running the analysis, the functions
251 *betadisper* and *anova*-like permutation test from the package *vegan* were used to identify
252 significant deviations on the multivariate beta dispersion of the data for treatments, replicates,

253 time and of the interaction between treatments and time (Oksanen et al., 2015). The effect of
254 treatments and replicates (nested within treatments) was tested using the function
255 *nested.npmanova* from the package BiodiversityR (Kindt & Coe, 2005). To test the effect of
256 time and the interaction between treatments and time, we used the function *adonis* (Oksanen
257 et al., 2015) including treatments, replicates, and time (number of days) as fixed variables in
258 the model, with permutations restricted to the replicates level. HCA was done using the
259 Hellinger distance (Oksanen et al., 2015). Taxonomy barplots were produced by showing the
260 three most abundant genera (considering the best hits classified by VSEARCH), while the less
261 abundant were merged as “others”. *IndVal* analyses were run with the rarefied table to identify
262 indicative species of the three groups of treatments evidenced in the beta diversity analysis
263 (inside and outside bloom DCM and both surface samples) using the package *indicspecies*
264 v1.7.9 with 10,000 permutations (De Cáceres and Legendre, 2009). P-values were adjusted for
265 multiple comparisons using the false discovery rate method (Oksanen et al., 2015).

266

267 **Results**

268 **Physico-chemical parameters and bacterial community structure dynamics in the *E.***

269 ***huxleyi* bloom**

270 Coccolithophore blooms occur seasonally from April to June in the Bay of Biscay along the
271 continental shelf to the Celtic Sea (Holligan et al., 1983; Poulton et al., 2014; Perrot et al.,
272 2018). Here, we followed and sampled an *E. huxleyi* bloom for a week from end of May to
273 early June 2019 in the Celtic Sea (Fig. 1A and 1B) using near-real time interpolated images of
274 non-algal suspended particulate matter (SPM) derived from MERIS and MODIS satellite
275 reflectance data (Perrot et al., 2018; Gohin et al., 2019) as provided by Ifremer
276 (<http://marc.ifremer.fr/en>).

277 During the 5-day sampling period, temperature and salinity ranged from 12.4°C to 15.4°C and
278 from 35.4 to 35.5 PSU, respectively (Supplementary Table 2). Nutrient concentrations were
279 low with NO₂ + NO₃ and PO₄ ranging from the detection thresholds to 1.25 μmol and 0.05 to
280 0.2 μmol/L, respectively. These low values were typical of a bloom event where cells consume
281 most of the nutrients. *E. huxleyi* whose cell densities ranged from 1.6 x 10³ to 5.6 x 10³ cells/mL
282 within the DCM layer of bloom waters (Fig. 1C) dominated the total photosynthetic eukaryotic
283 community (2.5 x 10⁴ cells/mL on average). In these samples, total numbers of heterotrophic
284 bacteria varied from 8.1 x 10⁵ to 2.0 x 10⁶ cells/mL (Fig. 1D) whereas the lowest prokaryotic
285 cell concentration was measured in the outside bloom sample (Fig. 1D and Supplementary
286 Table 2).

287 Overall, the inside and outside bloom DCM samples displayed a prokaryotic richness of about
288 140 ± 29 ASVs (mean ± SD, n=11) (Fig. 2A). Richness increased over the course of the bloom,
289 reaching a maximum at day 4. The DCM samples collected on day 5 inside and outside the
290 bloom for the community assembly experiments contained 148 and 133 ASVs, respectively.
291 The Shannon diversity index displayed homogeneous values (mean 4.1 ± 0.2) across samples
292 (Fig. 2B).

293 Hierarchical clustering revealed 3 groups of samples that reflected the evolution of the
294 prokaryotic communities across the bloom in a time-dependent manner (Supplementary Fig.
295 3). Free-living prokaryotic communities in and outside the bloom were mainly composed of
296 members of Proteobacteria (66% of the total of reads), Bacteroidota (14%), Cyanobacteria
297 (7%), Thermoplasmatota (4%), and Verrucomicrobiota (4%). Pelagibacteraceae (15%),
298 Pseudoalteromonadaceae (13%) and Rhodobacteraceae (12%) were the most abundant
299 proteobacterial families while Flavobacteriaceae (11%) dominated within the Bacteroidota. A
300 diverse and rather stable prokaryotic community was observed at the genus level
301 (Supplementary Fig. 4). Present in all samples, *Pseudoalteromonas* was the most abundant

302 genus (13% of the total reads, n=11) and overdominated SAR11 inside (in several samples)
303 and outside the *E. huxleyi* bloom. Abundances of SAR11 clade Ia (12% of the total reads),
304 *Synechococcus* sp. (7%), SAR86 (5%), Marine group II Euryarchaeota (4%), uncultured
305 Rhodobacteraceae members (4%), *Asciadiaceihabitans* (4%), and *Sulfitobacter* (4%) were also
306 consistent along the study period. The Flavobacteriaceae dominated by the NS4 group (3%)
307 comprised 11% of the total reads. Some genera dominated in only one sample like *Vibrio* (12%
308 in day 2 PM sample) and *Nitrosopumilus* (18% in day 3 AM sample) or were substantial such
309 as the members of OCS116 clade (4% in day 2 PM sample). Relative abundance of
310 *Alteromonas* increased at the end of the bloom survey.

311 Prokaryotic communities outside the bloom grouped with those collected a few hours before
312 inside the bloom area. The main compositional differences these samples that served as inocula
313 for the community assembly experiment were the relative abundance of *Lentimonas* (5% inside
314 vs 2% outside bloom), and of the OM60 (NOR5) clade and Marine group II members (each of
315 them accounting for 2% inside vs 4% outside bloom).

316

317 **Community assembly experiment**

318 ***(i) Dynamics of cell concentrations and alpha diversity patterns***

319 Seawater samples used to inoculate axenic *E. huxleyi* cultures were filtered through 0.45 μm
320 membranes to remove phototrophs (autotrophic eukaryotes and *Synechococcus* populations)
321 and overcome their effects on microbiome assembly. FCM analysis demonstrated that about
322 40% of the initial bacterial cell concentration was lost after this filtration step. Due to limited
323 incubation space onboard, cultures were incubated at low light (20 $\mu\text{mol photons s}^{-1}\text{m}^{-2}$) and
324 these light conditions were maintained during the first weeks of incubation. However, a drastic
325 decrease ($\sim 80\%$) of *E. huxleyi* cell concentrations was observed in all the treatments between
326 the first (day 10) and the third (day 33) culture transfers (Supplementary Fig. 2A). To avoid

327 culture crash, we increased the light intensity to $70 \pm 20 \mu\text{mol photons s}^{-1}\text{m}^{-2}$ and larger
328 microalgal inocula (10% of the final culture volume instead of 1%) were used to transfer the
329 cultures at day 33. *E. huxleyi* cell densities gradually increased at each subsequent transfer until
330 day 71. At that point, they reached the highest cell concentration ($9.9 \times 10^5 \pm 1.2 \times 10^5$ cells/mL)
331 and remained stable up to the end of the experiment (Supplementary Fig. 2A). Bacterial cell
332 concentrations followed an opposite trend during the first weeks of incubation. After a rapid
333 increase (~94%) from day 10 to 47, they decreased once *E. huxleyi* abundance became higher
334 and remained relatively stable up to the end of the experiment (Supplementary Fig. 2B).

335 Regarding the structure of the bacterial community, a severe loss of richness was observed
336 between the environmental and culture samples (Fig. 2A). At day 10, the bacterial richness in
337 the cultures was about one fifth of the richness in the natural samples (30 ± 8 SD, $n=12$) (Fig.
338 2A). This reflected a parallel decrease in the Shannon index, which at day 10, was about one
339 third the values recorded in environmental samples (1.4 ± 0.6 SD, $n=12$, Fig. 2B). Over the
340 course of the experiment, we observed a decrease in richness along the first five weeks (mean
341 decrease 25 ± 7 SD, $n=12$, until day 47) (Supplementary Fig. 2C). After an increase at day 59
342 that corresponded to the period of culture recovery, the richness values decreased again and
343 remained stable until day 393 (12 ASVs ± 2). The decrease of richness was mainly associated
344 with the loss of low abundance ASVs, while the dominant ones remained over the course of
345 the experiment (Supplementary Fig. 5). In general, the Shannon index also decreased over the
346 first three time points (mean decrease 0.5 ± 0.6 SD, $n=12$) and then gradually increased to
347 values similar to that from day 22 (day 393: 1.2 ± 0.4 , $n=12$). The highest richness and Shannon
348 indexes were obtained in the treatments amended with the inside bloom DCM sample (richness:
349 26 ± 12 ASVs; Shannon 1.6 ± 0.4 , $n=24$).

350

351

352 ***ii) Dynamics of beta diversity patterns in recruited microbiomes***

353 In order to identify the influence of the different initial prokaryotic community
354 composition and to follow the changes in the microbiome beta diversity with time, we used
355 two metrics, the Hellinger distance (Euclidean distance of Hellinger-transformed data) and the
356 Jaccard dissimilarity. Principal coordinates analysis using Jaccard dissimilarity demonstrated
357 that *E. huxleyi* cultures inoculated with surface samples grouped together (Fig. 3A), while those
358 inoculated with inside and outside bloom DCM samples formed two other independent
359 clusters.

360 Statistical significance of the effect of treatments, replicates and time, as well as the
361 interaction of treatment and time on the diversity of the microbiomes was assessed by
362 PERMANOVA and nested PERMANOVA. Before performing PERMANOVA analysis we
363 tested the beta-dispersion (variance) of the microbiomes grouped by treatments, time,
364 treatments over time, and replicates. The dispersions (variance) of treatments as well as the
365 interaction of treatments over time were not homogeneous for both metrics tested ($p < 0.05$).
366 On the other hand, dispersions were likely homogeneous over time and across replicates ($p >$
367 0.05). Still, PERMANOVA results are robust to dispersion for balanced designs like ours
368 (Anderson & Walsh, 2013). PERMANOVA results of Hellinger and Jaccard dissimilarities
369 showed that significant proportions of the variance in microbiome composition among samples
370 were explained by treatments (25-31%), replicates (15-31%), and time (6-7%) ($p < 0.01$) (Fig.
371 3B, Supplementary Tables 3 and 4). Although the interaction between treatments and time was
372 significant using Hellinger distance ($F = 2.23$; $p = 0.016$), it explained a small proportion of
373 the variance (2.5%).

374 Clustering using Hellinger distance revealed that the prokaryotic community
375 composition of all the cultures grouped into four main clusters according to the inoculum origin
376 (Fig. 3C). The outside bloom DCM treatment was formed by two clusters (a and b),

377 highlighting the compositional differences between the 3 replicates. Replicate 1 (cluster a) was
378 dominated by *Alcanivorax* (78%, n=8), while *Erythrobacter* prevailed in replicate 2 (cluster b,
379 88%) and 3 (cluster a, 45%). Cluster (c) mainly consisted of microbiomes recruited from both
380 surface water samples. Surface treatments were dominated by bacteria related to OM43,
381 KI89A, and SAR92 clades, and to *Luminiphilus*. The third main cluster (d) entirely formed by
382 microbiomes from the inside bloom DCM treatment was dominated by members of the OM43
383 (29%), KI89A (28%), SAR92 clades, and *Polaribacter* (11%). ASVs that dominate ($\geq 1\%$) in
384 the cultures amended with inside and outside bloom DCM samples were generally low in
385 abundance or not detected in the initial bacterioplankton community. However, relative
386 abundance of cultured ASVs related to *Sulfitobacter*, *Luminiphilus* and the OM43 clade were
387 also substantial in the field samples (Supplementary Fig. 6).

388 The number of indicative ASVs for each treatment varied widely and was significantly
389 higher (21 out of 29) in cultures amended with inside bloom DCM waters (Supplementary
390 Table 5). Flavobacteriales, with *Aurantivirga* and *Polaribacter* in particular, was the order
391 containing the most indicative ASVs of microbiomes recruited from the inside bloom DCM
392 sample. Members of SAR92 and KI89 clades displayed high Indval indexes in both inside
393 bloom DCM and surface samples. The indicator ASVs of outside bloom DCM treatment were
394 related to *Erythrobacter*, *Alcanivorax*, and OM60(NOR5) clade. Besides the compositional
395 differences among treatments, we observed a somehow cyclic pattern of the beta-diversity over
396 time using Hellinger distance (Fig. 4). Microbiome community compositions clearly differed
397 from each other from days 10, 22 and 33 for all treatments, but they gradually tended to become
398 similar to their initial status at the following time-points, particularly in the inside bloom DCM
399 treatment (Fig. 4B). This pattern occurred at the replicate level in the outside bloom DCM
400 cultures, and only for replicates 2 and 3 (Fig. 4D). This dynamic was mainly driven by the

401 transient dominance of the OM43 clade (ASV1) during the alga crash and the increased
402 abundance of *Luminiphilus* after the algal growth recovery (Supplementary Fig. 7).

403

404 **Discussion**

405 In this study, we monitored the diversity of bacterial communities associated with an *E. huxleyi*
406 bloom in the Celtic Sea, and collected bacterioplankton samples for conducting a microbiome
407 selection experiment in axenic *E. huxleyi* cultures.

408

409 **Prokaryotic communities associated to the demise phase of the *E. huxleyi* bloom**

410 Satellite images and post-cruise analyses indicate that the *E. huxleyi* bloom development had
411 entered the decaying phase when we started the sampling. First, the high reflectance patch
412 visible on the satellite images (Fig. 1A) and the daily vanishing of the coccolith-derived
413 turbidity signal observed from the interpolated images of non-algal SPM were both indicative
414 of detached coccoliths from dead *E. huxleyi* cells (Neukermans & Fournier, 2018; Perrot et al.,
415 2018). This assumption was confirmed by the complete disappearance of the coccolith-derived
416 turbidity signal a couple of days after we left the sampling area. Second, a suite of ongoing
417 experiments on the bloom samples using diagnostic lipid- and gene-based molecular
418 biomarkers (Vardi et al., 2009; Hunter et al., 2015; Ziv et al., 2016; Vincent et al., 2021)
419 revealed the detection of specific viral polar lipids and visualized *E. huxleyi* infected cells
420 during bloom succession, suggesting that Coccolithovirus infections may have partially
421 participated in the demise of *E. huxleyi* bloom (F. Vincent, C. Kuhlisch, G. Schleyer, pers.
422 comm.) as often proposed (Bratbak et al., 1993; Vardi et al., 2012; Laber et al., 2018). Third,
423 the composition of the bacterial community, *i.e.* the presence of Flavobacteriaceae,
424 Pseudoalteromonadaceae, Alteromonadaceae and members of the genus *Sulfitobacter*, is
425 another indicator of bloom demise (Lovejoy et al., 1998; Buchan et al., 2014). Flavobacteriia,

426 are reported amongst the main bacteria present in the declining phase of phytoplankton blooms
427 (Teeling et al., 2012, 2016; Landa et al., 2016), which seems linked to their capacity to degrade
428 high molecular weight substrates such as proteins and polysaccharides (Cottrell & Kirchman,
429 2000; Kirchman, 2002; Fernández-Gomez et al., 2013; Kappelmann et al., 2019; Francis et al.,
430 2021). Finally, the algicidal effects of *Pseudoalteromonas*, *Alteromonas*, and *Sulfitobacter*
431 strains and species have been documented in many microalgae including *E. huxleyi*
432 (Holmström & Kjelleberg, 1999; Meyer et al., 2017; Li et al., 2018; Barak-Gavish et al., 2018),
433 which calls attention to their potential role in the *E. huxleyi* bloom termination (Lovejoy et al.,
434 1998; Barak-Gavish et al., 2018).

435 Our results evidenced a relative stability of the prokaryotic community during the study
436 period and agree with reports of the co-occurrence of SAR11, *Roseobacter* and SAR86 clades
437 in *E. huxleyi* blooms (Gonzalez et al., 2000; Zubkov et al., 2001). The co-occurrence of these
438 groups could be mediated by the presence of dimethylsulfoniopropionate (DMSP), produced
439 and released by *E. huxleyi* during blooms (Malin et al., 1993), which could be used as a sulfur
440 compound by bacteria (Miller & Belas, 2004; Tripp et al., 2008; Dupont et al., 2012). DMSP
441 and senescence compounds from decaying *E. huxleyi* cells probably also fueled members of
442 the genus *Asciidiaceihabitans* (formerly *Roseobacter* OCT lineage) (Wemheuer et al., 2015),
443 whose relative abundances typically fluctuate during phytoplankton blooms (Hahnke et al.,
444 2015; Lucas et al., 2016; Chafee et al., 2018; Choi et al., 2018).

445

446 **Community composition of environmentally recruited *E. huxleyi* microbiomes**

447 Since our primary objective was to study the bacterial community selection and
448 assembly by a single phytoplankton host, we used a filtration step to discard autotrophic
449 phytoplankton cells, such as *Synechococcus* and picoeukaryotes abundantly represented in the
450 initial planktonic communities (Supplementary Table 2). We acknowledge that this filtration

451 strategy has removed large and particle-attached prokaryotes, the latter probably being
452 abundant in the demise phase of the bloom, and has induced substantial modifications in the
453 initial community composition of the inocula and finally in recruited microbiomes. Indeed,
454 some of the main taxonomic groups recruited in the treatments were not previously reported in
455 *E. huxleyi* and other phytoplankton cultures or in low abundance, notably *Luminiphilus*, and
456 the clades SAR92, KI89A and OM43 (Green et al., 2015; Câmara dos Reis, 2021). Except
457 members of the OM43 clade (Yang et al., 2016), these bacteria are known as important groups
458 of oligotrophic marine Proteobacteria that do not usually grow in the rich organic matter
459 conditions provided in phytoplankton-derived cultures (Cho & Giovannoni, 2004; Spring &
460 Riedel, 2013). Another unexpected result of our study is the very low representation of the
461 genus *Marinobacter* in the recruited microbiomes whereas previous studies have reported their
462 dominance in cultures of worldwide *E. huxleyi* isolates (Green et al., 2015; Câmara dos Reis,
463 2021). We cannot fully exclude the possibility that the filtration step impacted the abundance
464 of *Marinobacter* in the inocula and finally in the *E. huxleyi* cultures. A more likely hypothesis
465 however is that low light conditions might have induced algal cell death promoting the release
466 of methylated compounds (Reese et al., 2019; Fisher et al., 2020). The release of methylated
467 compounds by *E. huxleyi* may have provided a selective advantage to the specialist OM43
468 clade methylotrophs (Neufeld et al., 2008), dominant in all the cultures, and to other less
469 common bacterial taxa in the absence of strong competitors such as *Marinobacter*. This
470 hypothesis is in line with the opposite dynamics of *E. huxleyi* and bacteria coupled to the sharp
471 decrease of the bacterial alpha diversity during the first month of culture, indicating that a few
472 bacterial taxa were outcompeting others.

473 Despite the above limitations, the high reproducibility of microbiome community
474 composition across the biological replicates suggests that they did not alter the general
475 conclusions raised from our study.

476 Our results illustrate the importance of niche differentiation in natural communities.
477 Indeed, although no major differences were observed between environmental inside and
478 outside bloom bacterial communities, *E. huxleyi* microbiomes recruited from these samples
479 differed. Similarly, although we did not analyze the initial bacterial composition of epipelagic
480 surface samples (collected 34 km apart), they converged towards similar compositions,
481 dominated by *Luminiphilus*, SAR92, KI89A and OM43 clades. Other microbiome studies of
482 phytoplankton cultures have highlighted the impact of the initial community composition on
483 microbiomes after short (Ajani et al., 2018; Sörenson et al., 2019; Jackrel et al., 2020), and
484 long-term selection (Behringer et al., 2018). Remarkable features were found in the
485 microbiomes resulting from inside bloom DCM waters where several indicative flavobacterial
486 ASVs, mainly assigned to *Polaribacter* and *Aurantivirga*, were initially selected and remained
487 among the most prevalent and abundant ASVs after growth recovery of the host. Both genera
488 were identified as the main degraders of polysaccharides during diatom blooms (Krüger et al.,
489 2019) and showed clear successions along the bloom stages (Teeling et al., 2012; Landa et al.,
490 2016; Krüger et al., 2019; Liu et al., 2020). This may be related to the differential capacity of
491 these bacteria to degrade phytoplankton-derived polysaccharides during blooms (Teeling et al.,
492 2012; Krüger et al., 2019; Avci et al., 2020; Francis et al., 2021). Interestingly, SAR92 and
493 *Luminiphilus* were also identified as important degraders of algal polysaccharides in bloom
494 conditions (Francis et al., 2021), suggesting their potential functional role in our cultures.

495 We assume that exopolysaccharides/exudates of axenic *E. huxleyi* cultures have
496 strongly influenced the initial microbiome composition and its long-term stability. This was
497 exemplified by the contrasting results obtained with the inside and outside bloom DCM
498 samples. The bacterioplankton communities associated with the inside bloom DCM sample
499 displayed a higher diversity than that of the outside bloom DCM sample, and resulted in more
500 diverse recruited microbiomes and higher number of indicative ASVs. We hypothesize that the

501 bacterial assemblages adapted to *E. huxleyi* natural bloom conditions favored the recruitment
502 of greater diversity microbiomes that may likely explain the almost complete cyclic pattern
503 they followed (Fig. 4A). Such cyclic patterns were shown in the mucus microbiome of the coral
504 *Porites astreoides* (Glasl et al., 2016) and the surface microbiome of the seaweed *Delisea*
505 *pulchra* (Longford et al., 2019) after experimental disturbances. This pattern was consistently
506 observed in the four separate treatments, with some variability. With the exception of the
507 outside bloom DCM treatment, we observed lower levels of between-replicate variability at all
508 time points, indicating a higher degree of uniformity among cultures at corresponding time-
509 points.

510 Our study suggests the combined effect of deterministic processes and stochasticity on
511 the microbiome assembly. The significant imprint of the original community in the inside
512 bloom DCM treatments suggests that deterministic processes (*e.g.* assemblages adapted to *E.*
513 *huxleyi* bloom exudates) influenced the final microbiome composition. On the other hand,
514 variable communities selected from outside bloom DCM treatment show an effect of both
515 deterministic processes and stochasticity. The latter microbiomes also contained commonly
516 reported phytoplankton-associated bacterial groups (*Erythrobacter* and *Alcanivorax*),
517 suggesting that the initial communities were exposed to other phytoplankton-mediated
518 chemical conditions in the field. Variability in the community composition of biological
519 replicates in this treatment suggests differential selection pressures, resulting in divergent
520 communities. A similar pattern was found from enrichment experiments in the phycosphere of
521 *Phaeodactylum tricornutum* (Kimbrel et al., 2019).

522

523 **Conclusions**

524 In this work, we showed that the source of the initial bacterioplankton communities influences
525 the resulting composition of *E. huxleyi* microbiomes. Our experimental approach demonstrated

526 the stability of *E. huxleyi* microbiomes agreeing with previous reports for other phytoplankton
527 microbiomes (Geng et al., 2016; Camp et al., 2020). Although species losses still occurred in
528 the last sampled microbiomes of the survey, they were associated with low abundance taxa and
529 did not induce major restructuring of the community, as previously shown in long-term
530 experiments by Behringer et al., (2018). Overall, we bring evidence that microbiomes
531 associated with phytoplankton cultures represent a valuable resource to explore phytoplankton-
532 bacteria interactions. Isolation of indicative ASVs will be necessary to investigate the role and
533 functions of stable core bacterial members interacting with *E. huxleyi*. Future co-culture
534 experiments coupled with transcriptomic and metabolomic analyses will provide valuable
535 information about the genes and molecules involved in these ecologically key interactions.

536

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546 and to Lydia White for her help in the statistical analysis.

547

548

549

550

551 **Data Accessibility**

552 **Genetic data and sample metadata:**

553 Environmental samples are deposited in the bioproject PRJEB50692. Reads are deposited
554 under the accession numbers XXXX and biosamples under the accession numbers
555 ERS10466567-ERS10466596.

556 Samples from the assembly experiment are deposited in the bioproject PRJEB48747. Reads
557 are deposited under the accession numbers XXXX and biosamples under the accession
558 numbers ERS10539058-ERS10539153.

559 *Unfortunately, we were unable to submit the reads of both bioprojects because of a problem*
560 *on the ENA server. Sequences will be submitted once the server recovers.*

561

562 **Author Contributions**

563 CJ, MCdR, CdV designed the research. MCdR, CJ and SR participated on the sampling cruise.
564 MCdR and CJ collected the DNA and transferred the cultures. FLG, SR, CJ produced the
565 genetic data. MJF and GK analyzed the SEM filters. DM and MCdR analyzed the FCM
566 samples. TC analyzed the nutrients samples. MCdR and NH analyzed the results. MCdR, CJ
567 and CdV wrote the paper. All authors contributed to the discussions that led to the final
568 manuscript, revised it and approved the final version.

569

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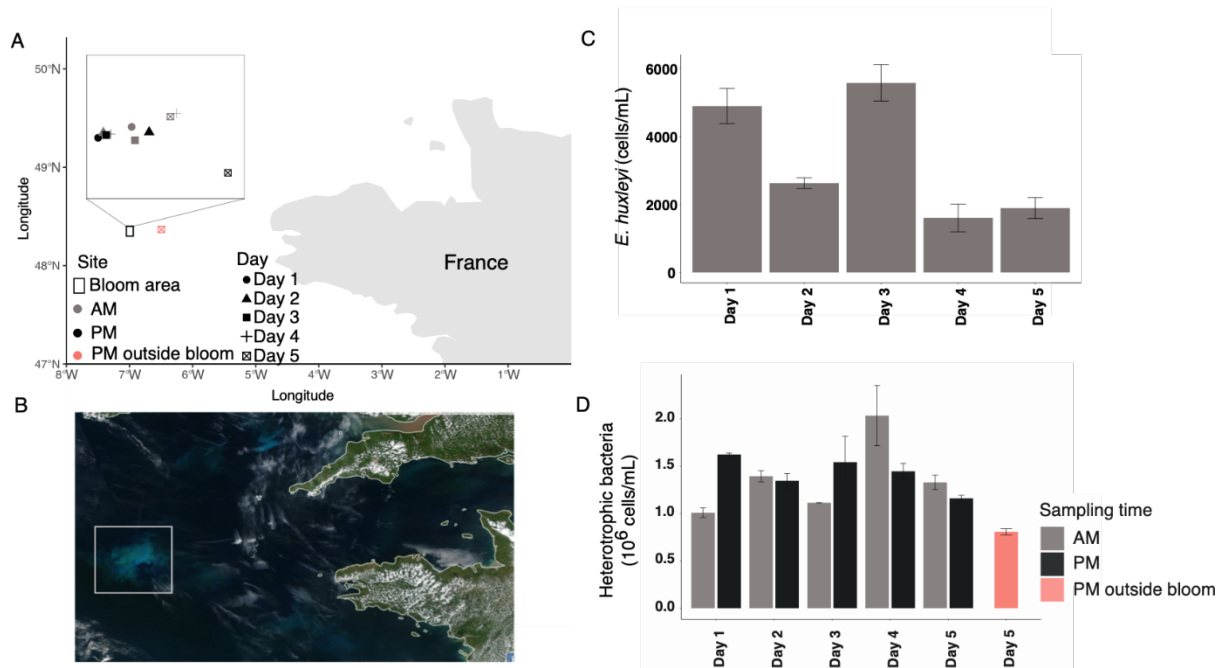
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976 **Figures**

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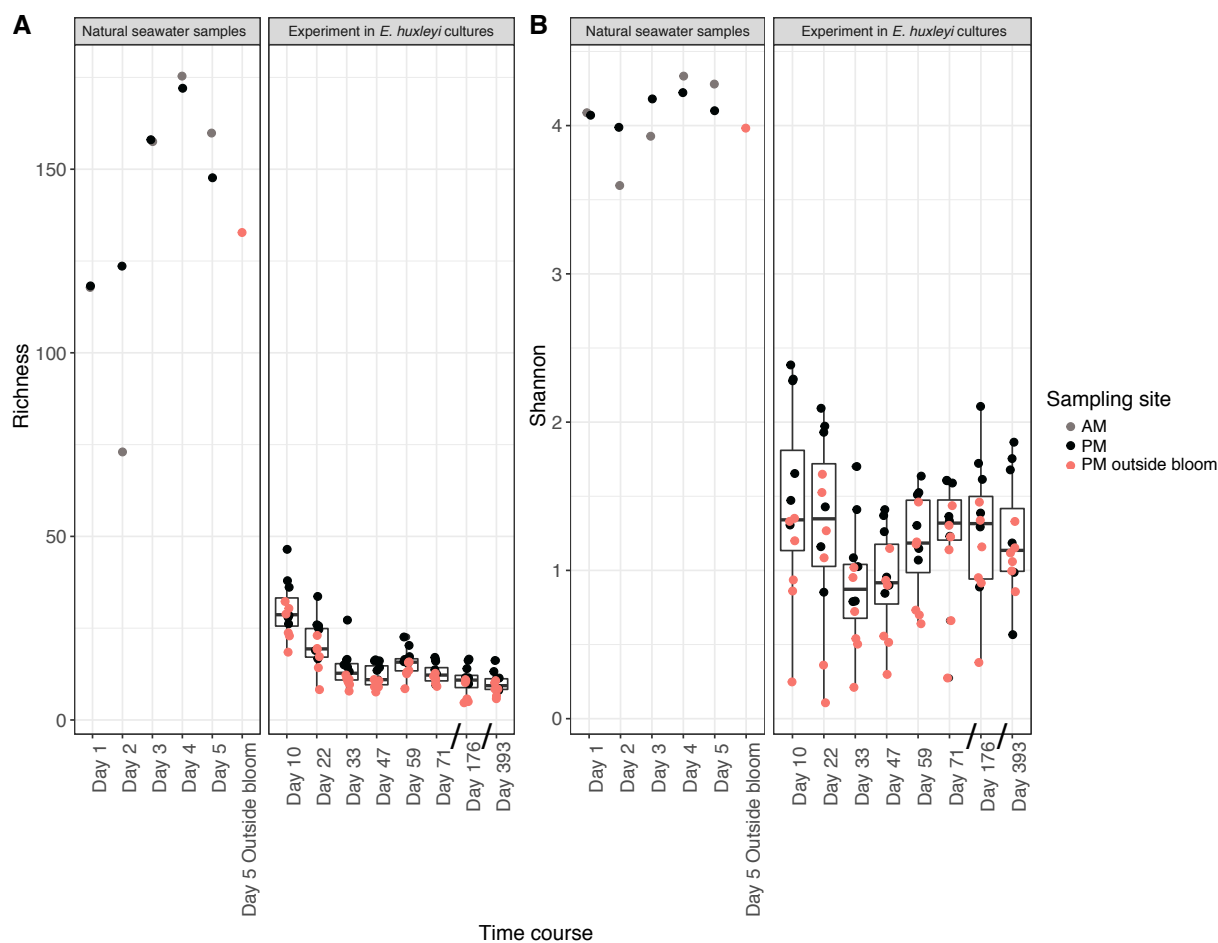


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980 **Fig. 1.** Sampling area and characteristics of the *E. huxleyi* bloom in the Celtic Sea. **A)** Map
981 showing the bloom area and spatio-temporal sampling strategy. **B)** True-color satellite image
982 of the bloom area on May 21, 2019 (source:
983 <https://www.star.nesdis.noaa.gov/sod/mecb/color/ocview/ocview.html>. **C)** *Emiliana huxleyi*
984 cell concentrations at morning bloom sites during the survey, measured from duplicate filters
985 using scanning electron microscopy. **D)** Heterotrophic bacterial cell concentrations at morning
986 and afternoon sites during the survey, measured from duplicate samples by flow cytometry.

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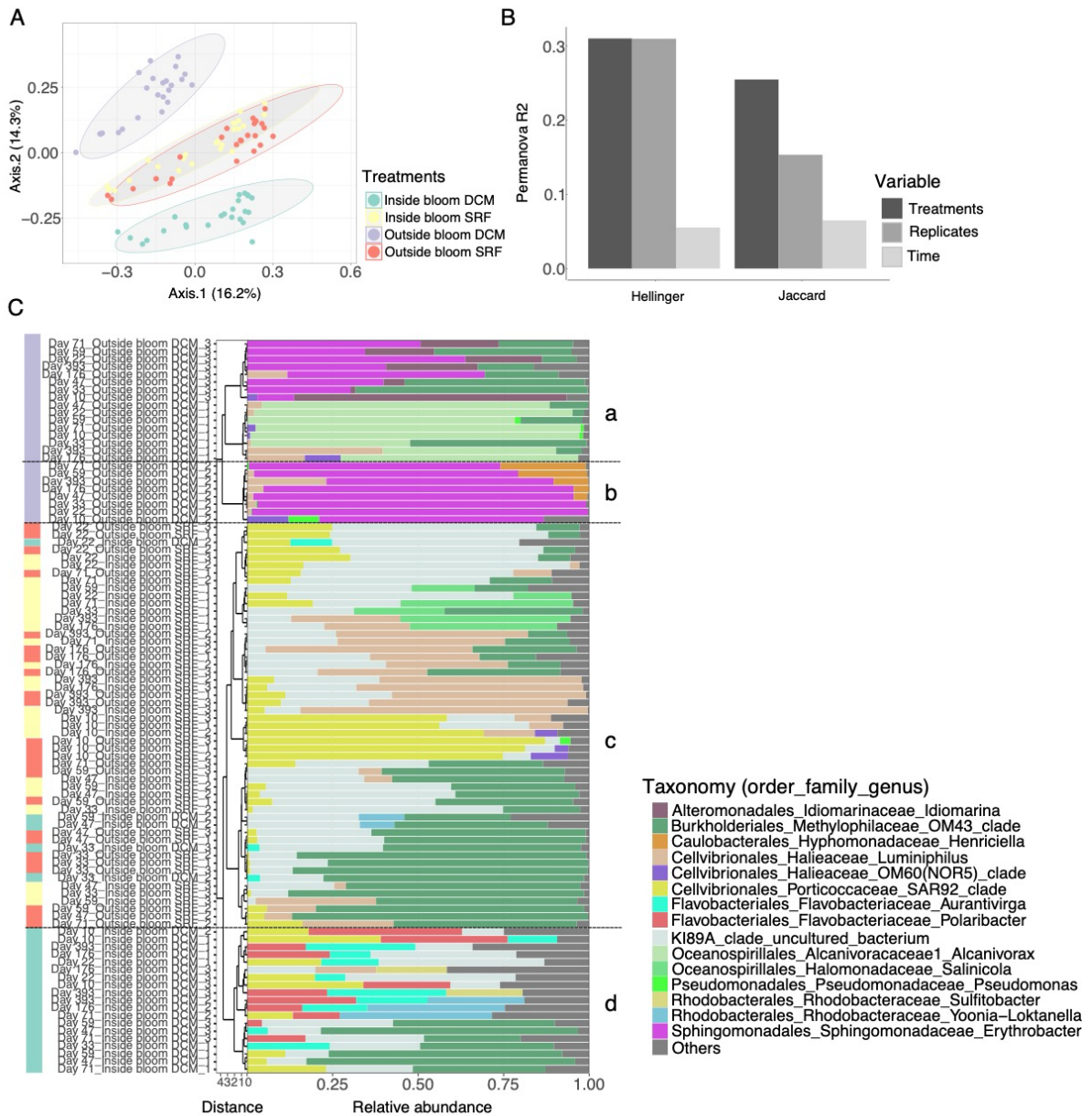


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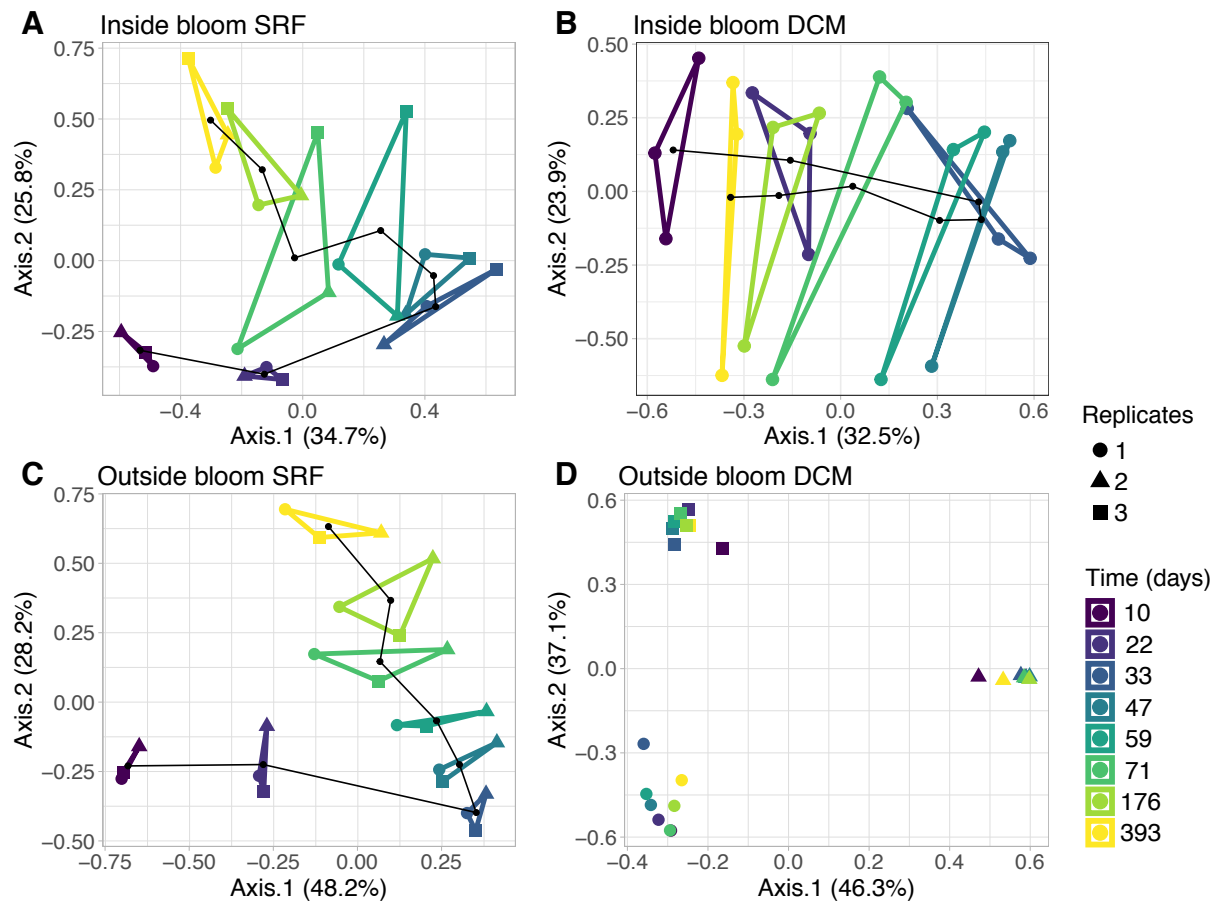
991 **Fig. 2.** Composite representation of the dynamics of the prokaryotic richness (**A**) and Shannon
 992 (**B**) indexes in natural samples (0.2 - 3 μm DCM samples only) and culture experiments. ASV
 993 tables were rarefied to 2,479 (minimum number of reads of the environmental samples. The
 994 boxes represent the interquartile range. The thin horizontal lines represent the 25th and 75th
 995 percentiles and while the thick horizontal line represents the median. The vertical lines indicate
 996 the minimum and maximum values (using 1.5 coefficients above and below the percentiles).
 997 The dots represent the values measured for each culture. Dots further than the vertical lines
 998 represent potential outliers. X-axes in the in the experiment plot are not proportional to the time
 999 length between samplings.

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1003 **Fig. 3.** Beta diversity patterns of *E. huxleyi* microbiomes across treatments and time. (A)
1004 Principal coordinates analysis (PCoA) using Jaccard dissimilarity matrix of the presence-
1005 absence transformed rarefied table. Colors correspond to each treatment that received
1006 prokaryotic communities from different water samples: green - Inside bloom DCM; yellow -
1007 Inside bloom SRF; purple Outside bloom DCM; red - Outside bloom SRF. Ellipses represent
1008 95% confidence. (B) r^2 of permutational multivariate analysis of variance (PERMANOVA)
1009 and nested PERMANOVA using two metrics (see material and methods for details). (C)
1010 Hierarchical clustering produced with the Hellinger distance matrix using “ward.D2” method.
1011 Codes of each microbiome are experiment sampling day_treatment_replicate. Bar plots
1012 indicate the taxonomy of the 3 most abundant genera. The other genera were merged as
1013 “others”.
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Fig. 4. Principal components analysis (PCA) showing the cyclic patterns of the microbiome beta diversity. Community distances (Euclidean distances of Hellinger-transformed data) are shown for microbiomes from inside bloom SRF (A), inside bloom DCM (B), outside bloom SRF (C), and outside bloom DCM (D). The polygons link replicates (shape coded) at each time point (color coded). The black line links the barycenters of the replicates. In D., the cyclic pattern is observed at the replicate level for replicates 2 and 3.