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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 coccolithophore 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 (Eastern North Atlantic),
35 exposed axenic *E. huxleyi* cultures to prokaryotic communities derived from bloom and non-
36 bloom conditions and followed the dynamics of their microbiome composition over one year.
37 Bloom-associated prokaryotic communities were dominated by SAR11, Marine group II
38 Euryarchaeota, Rhodobacterales and contained substantial proportions of known indicators of
39 phytoplankton bloom demises such as Flavobacteriaceae and Pseudoalteromonadaceae.
40 Taxonomic richness of bacteria derived from natural communities that associated with axenic
41 *E. huxleyi* rapidly shifted and then stabilized over time. The succession of microorganisms
42 recruited from the environment were consistently dependent on the composition of the initial
43 bacterioplankton community. Phycosphere-associated communities derived from the *E.*
44 *huxleyi* bloom were highly similar to one another, suggesting deterministic processes, whereas
45 cultures from non-bloom conditions show an effect of stochasticity. Overall, this work sheds
46 new light on the importance of the initial inoculum composition in microbiome recruitment
47 and elucidates the temporal dynamics of its composition and long-term stability.

48

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

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53

54 **Introduction**

55 In the surface ocean, marine phytoplankton generate up to 50% of global primary production
56 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). In addition, phytoplankton
68 release 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, possible strategies are to study the selection processes in
81 natural phytoplankton blooms (Zhou et al., 2019), in meso/microcosms or in cultures (Ajani et
82 al., 2018; Kimbrel et al., 2019; Sörenson et al., 2019; Fu et al., 2020; Mönnich et al., 2020),
83 when algal cells are at high concentrations. *Emiliania huxleyi* is the most abundant and
84 cosmopolitan coccolithophore species and is able to form massive annual blooms in temperate
85 and subpolar oceans mostly during Spring (Tyrrell & Merico, 2004). *E. huxleyi* blooms are
86 characterized by blue turquoise waters that can be observed from satellite images (Tyrrell &
87 Merico, 2004). These blooms have a critical importance for carbon and sulfur cycles due to the
88 ecological and biogeochemical roles of coccolithophores as primary producers, calcifiers, and
89 main contributors to the emission of dimethylsulfoniopropionate (DMSP) to the atmosphere
90 (Malin & Steinke, 2004; Rost et al., 2004). The potential role of viruses in bloom termination
91 has been thoroughly investigated (*e.g.* Bratbak, Egge & Heldal, 1993; Vardi et al., 2012;
92 Lehahn et al., 2014), but only few studies have targeted the microbial diversity associated with
93 *E. huxleyi* in natural environments (Gonzalez et al., 2000; Zubkov et al., 2001) and cultures
94 (Green et al., 2015; Orata et al., 2016; Rosana et al., 2016). The *Roseobacter*, SAR86 and
95 SAR11 lineages were identified as the main bacterial groups associated with natural *E. huxleyi*
96 blooms (Gonzalez et al., 2000; Zubkov et al., 2001). The co-occurrence of these groups could
97 be mediated by the presence of dimethylsulfoniopropionate (DMSP), produced and released
98 by *E. huxleyi* during blooms (Malin et al., 1993), which could be used as a sulfur compound
99 by bacteria (Miller & Belas, 2004; Tripp et al., 2008; Dupont et al., 2012). Meanwhile,
100 microbiomes of *E. huxleyi* in cultures are highly dominated by *Marinobacter* (Câmara dos Reis,
101 2021) and by Rhodobacteraceae (Green et al., 2015; Barak-Gavish et al., 2018).

102 In this study, we followed the dynamics of the prokaryotic community associated with
103 *E. huxleyi* along a natural bloom in the Celtic Sea (Eastern North Atlantic) and used natural
104 bloom and non-bloom samples from different depths to investigate the microbiome selection
105 by an axenic *E. huxleyi* culture. We hypothesized that microbiomes recruited from bloom
106 waters would be enriched in *Marinobacter* and Rhodobacteraceae, often associated with *E.*
107 *huxleyi* cultures. Since composition can differ between surface and deep chlorophyll maximum
108 (DCM) prokaryotic communities (Treusch et al., 2009; Allen et al., 2020; Mena et al., 2020)
109 and is influenced by phytoplankton-bacteria interactions (Seymour et al., 2017), we also
110 hypothesized that the recruited microbiomes would differ according to the initial prokaryotic
111 composition.

112

113 **Materials and Methods**

114 **Study site and sample collection**

115 Samples used in this study were collected aboard the schooner *Tara* (Sunagawa et al., 2020) in
116 the Celtic Sea (from 48°19'-48°24' N/6°28'-7°02' W; Fig. 1A and B), during the ‘*Tara*
117 BreizhBloom’ cruise from May 27 to June 2, 2019. To monitor the bacterial dynamics in an *E.*
118 *huxleyi* bloom formed in this area, an Argo float (<https://argo.ucsd.edu/>) was deployed in the
119 center of the bloom patch and its position was used twice a day (early morning and end of the
120 afternoon) for 5 days to determine the geographical locations of the sampling stations.

121 On the last sampling day, an additional site about 34 km apart from the bloom area was
122 also sampled (Fig. 1A). For each sampling event, surface to 50 m depth profiles of temperature,
123 salinity, turbidity, pressure, photosynthetic active radiation (PAR), chlorophyll *a* (chl_a)
124 fluorescence, oxygen concentrations and pH were conducted by deploying a SBE19+ profiler
125 (Sea-Bird Scientific). Bloom depth, determined as the maximum turbidity depth, was generally
126 very close to the DCM depth. Surface and bloom water samples were collected using an 8L

127 Niskin bottle for nutrient analyses. After collection, nutrient samples (125 mL) were stored at
128 -20°C for further analysis. Concentrations of nitrate, nitrite, phosphate and silicate were
129 measured using a AA3 auto-analyzer (Seal Analytical) following the methods described by
130 Tréguer & Le Corre (1975) and Aminot & Kerouel (2007). Samples for flow cytometry (FCM),
131 scanning electron microscopy (SEM), and metabarcoding analysis were collected at the bloom
132 depth by pumping and prefiltered through a 20 µm mesh to eliminate large microplankton. For
133 FCM analysis of photosynthetic eukaryotes and prokaryotic communities, two replicates (1.5
134 mL) were fixed using glutaraldehyde (0.25% final concentration) and Poloxamer 10% (0.1%
135 final concentration) and incubated for 15 min at 4°C before flash freezing in liquid nitrogen.
136 For SEM analysis, samples of morning sites (two replicates of 250 mL) were gently filtered
137 onto polycarbonate membranes (47 mm in diameter; 1.2 µm pore-size) (Millipore). Filters were
138 placed onto PetriSlides (Millipore), dried at least 2h at 50°C, and finally stored at room
139 temperature. For metabarcoding analysis, cell biomass from bloom depth was collected from
140 ~ 14 L of seawater by successive filtration onto large (142 mm in diameter) 3 µm pore-size and
141 then 0.2 µm pore-size polycarbonate membranes (Millipore). Filters were flash-frozen in liquid
142 nitrogen and stored at -80°C for later DNA analyses.

143

144 **Scanning electron microscopy analysis**

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

150

151 **Community assembly experiments**

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

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

159 **(ii) Sample preparation and inoculation.** Four seawater samples were used in the bacterial
160 community assembly experiment. They consisted of a surface and a DCM sample collected in
161 the bloom area on day 5 (thereafter named inside bloom surface and inside bloom DCM) and
162 a surface and a DCM sample collected the same day outside the bloom area (thereafter named
163 outside bloom surface and outside bloom DCM) (Supplementary Fig. 1). In order to remove
164 autotrophic picoeukaryotes and cyanobacteria from the inoculum, seawater samples were
165 gently filtered through a 0.45 μm pore size membrane (Millex-HV, PVFD, Millipore). To
166 estimate the number of prokaryotic cells lost during the filtration step, aliquots of total and
167 filtered seawater samples were fixed for FCM analysis using the methods above. After
168 filtration, 150 μL of each prokaryotic community (final cell concentration of about 6.8×10^3
169 cells/mL) were transferred in triplicates into 50 mL culture flasks filled with 15 mL of K/2
170 medium prepared as described in the Supplementary Materials and Methods. Finally, 150 μL
171 of the axenic RCC1212 culture were added to each flask (final cell concentration of 3.8×10^3
172 cells/mL). Six flasks filled with 15 mL of K/2 medium and inoculated with 150 μL of the
173 axenic RCC1212 culture were used as controls. In total, 18 cultures (3 replicates of 4 treatments
174 and 6 controls) were incubated at 15°C and a 12:12 photoperiod regime. Due to space
175 limitation, only one thermostatic chamber with a light intensity of 20 $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ using
176 a blue neutral density filter was available onboard for incubation.

177 **(iii) Survey of the culture microbiomes.** Back to the laboratory and 10 days after inoculation,
178 which corresponds to the time needed by *E. huxleyi* cultures to reach the end of exponential
179 growth phase, axenic status of controls was checked by FCM. Cultures (3 replicates of 4
180 treatments and one axenic control) were transferred by inoculating 100 μ L of the culture in 10
181 mL of fresh K/2 medium every 11-14 days for the first 176 days of experiment and then every
182 3 weeks until its end (day 393). At each culture transfer and at the end of the experiment,
183 treatments were sampled for FCM analyses and prokaryotic community composition analysis
184 (3 replicates x 4 treatments x 8 DNA samplings = 96 samples) (Supplementary Fig. 1). The
185 axenic control was regularly checked to ensure the clean handling of the cultures. In addition,
186 culture flasks were randomized daily in the incubator to minimize positional effect on growth.
187 For FCM, duplicate samples were fixed as previously described and analyses, performed
188 according to Marie et al., (1999), are detailed in the Supplementary Materials and Methods
189 section. For community composition analysis, 2 mL of culture was centrifuged at 2,000 g for
190 30 sec to reduce the microalgal load. Preliminary tests showed that this procedure reduces
191 microalgal load while keeping most of the bacterial cells (about 90%). The supernatants were
192 transferred into new tubes containing 2 μ L of Poloxamer 188 solution 10% (Sigma-Aldrich)
193 and centrifuged at 5,600 g for 5 min. The supernatants were discarded, and the pellets were
194 stored at -80°C until DNA extraction.

195

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

197 DNA extraction from environmental and culture samples and amplification steps used to
198 amplify the prokaryotic 16S rRNA gene using the universal prokaryote primers 515F-Y (5'-
199 GTGYCAGCMGCCGCGGTAA-3') labeled with eight-nucleotide tag unique to each sample
200 at the 5' end and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada et al., 2016) are
201 detailed in the Supplementary Materials and Methods and in Romac (2022a, 2022b, 2022c,

202 2022d). The “tagged-PCR” approach was used (Bohmann et al., 2022). Briefly, DNA samples
203 were PCR amplified using the above metabarcoding primers with 5' nucleotide tags and
204 following PCR amplification, the individually tagged PCR products were purified and pooled.
205 Pools were sent to Fasteris SA (Plan-les-Ouates, Switzerland) where ligation-based library
206 preparation was carried out and high-throughput sequencing was performed using Illumina
207 Miseq paired-end sequencing technology (2x250). In total, three DNA pools were sequenced.
208 The two first, containing the environmental samples and the first 84 experiment samples (first
209 7 time points) were sequenced in two independent Illumina runs (technical replicates). The last
210 12 experiment samples (day 393) were sequenced in another Illumina run without sequencing
211 replicates.

212

213 **Bioinformatics**

214 The steps of library separation, removal of Illumina adapters and first quality control were
215 performed by Fasteris SA (see Supplementary Materials and Methods). The detailed scripts
216 used in this study can be downloaded from
217 https://github.com/mcamarareis/microbiome_assembly. Briefly, raw reads from each
218 sequencing run were demultiplexed based on the 8 nucleotide tag sequences with cutadapt
219 (version 2.8.1) (Martin, 2011). The “tagged-PCR” approach we used generated half of both
220 forward and reverse reads containing a P5 Illumina adapter and the other half a P7 adapter
221 resulting in forward and reverse reads in both R1 and R2 files (mixed orientation). To deal with
222 the presence of reads in mixed orientation in the R1 and R2 raw files, the demultiplexing was
223 performed in two rounds (see details in Supplementary Materials and Methods). Then, primer
224 sequences were removed using cutadapt (version 2.8.1) (Martin, 2011). Because sequences
225 from different sequencing runs and from different sequencing cycles can have different error
226 rates, they were processed independently to obtain an amplicon sequence variant (ASV) table

227 using the DADA2 pipeline (version 1.14.0 in R 3.6.1) (Callahan et al., 2016; R Core Team,
228 2017). The overall read quality of the demultiplexed primer-free sequences was first
229 investigated with the DADA2 function *plotQualityProfile* to identify the position where the
230 quality distribution dropped for R1 and R2 reads. Then, forward and reverse reads were
231 trimmed where the quality decreased (i.e., at position 215 for R1 and at position 190 for R2
232 files) and reads with ambiguous nucleotides or with a maximum number of expected errors
233 (maxEE) superior to 2 were filtered out using the function *filterAndTrim*. For each combination
234 of runs and demultiplexing rounds, error rates were defined using the function *learnErrors* and
235 denoised using the *dada* function in pooled mode before being merged with *mergePairs* (which
236 generated ASVs of about 373 bp). Then, all the independent processed datasets were merged
237 in one sequence table (for each sample, reads from the two rounds of demultiplexing were
238 summed while reads coming from the two sequencing runs were kept separate) and processed
239 for chimera removal using the function *removeBimeraDenovo*, also performed in pooled mode
240 and with a “minFoldParentOverAbundance” of 8. The parameters used at each DADA2 step
241 are specified in the Supplementary Table 1.

242 ASVs shorter than 366 bp and longer than 377 bp were filtered out, and the remaining
243 ones were taxonomically assigned using IDTAXA (50% confidence threshold) with the Silva
244 database v138 (Quast et al., 2013; Murali et al., 2018). Chloroplasts and mitochondrial ASVs
245 were removed. ASVs not classified at the domain level by IDTAXA were assigned to the best
246 hit in Silva v138 by pairwise global alignment (*usearch_global* VSEARCH’s command)
247 (Rognes et al., 2016). These ASVs were removed if they could not be classified and/or were
248 classified as chloroplasts or mitochondrial sequences by VSEARCH (at 80% identity
249 threshold). The resultant ASV table was filtered to remove ASVs accounting for less than
250 0.001% of the total number of reads (abundance filter). Consistency of technical replicates was
251 evaluated by Procrustes analysis (function *procrustes* from *vegan* package), which measures

252 the similarity between two ordinations of the same objects, followed by a protest, which
253 measures the significance of the correlation (Oksanen et al., 2015). For this, we used a
254 comparative principal component analysis performed on the Hellinger transformed data. After
255 consistency was confirmed ($p < 0.001$ and correlation = 0.99), independent technical replicates
256 of each culture were merged by the sum of the number of reads of the ASVs present in the two
257 replicates of the same culture (prevalence filter). The abundance and prevalence filters
258 described above removed about 68% of the total number of ASVs while keeping 99% of the
259 number of reads. The final dataset (filtered ASV table used for further analysis) contained 107
260 samples (11 from the environment and 96 from cultures) for a total of 6,017,019 reads and 294
261 prokaryotic ASVs.

262

263 **Community composition and statistical analyses**

264 (i) *Environmental samples.* All the analyses were conducted in R version 4.0.2 in Rstudio
265 (1.1.442) and the plots were produced with ggplot2 (RStudio Team, 2016; Wickham, 2016; R
266 Core Team, 2017). Taxonomy treemaps of environmental samples were produced at the genus
267 level considering the best hits classified by VSEARCH. To facilitate visualization, low
268 abundant genera (accounting to less than 3% of relative abundance at each sample) present at
269 the raw community table were grouped. To compare environmental samples and cultures, mean
270 alpha diversity indices (richness and Shannon index) were measured after rarefying the ASV
271 table 100 times at the minimum number of reads (2,479) using the function *rtk* (Saary et al.,
272 2017). Hierarchical cluster analysis (HCA) (method “ward.D2”) was used to identify
273 differences in the free-living prokaryotic communities by sites using the Hellinger distance
274 (Euclidean distance of the Hellinger-transformed matrix; Legendre & Gallagher, 2001) using
275 the function *hclust* from stats package (R Core Team, 2017).

276 **(ii) Experiment.** To analyze alpha diversity dynamics of the cultures, rarefaction was
277 performed at a read depth of 5,957 reads using the same approach as for the environmental
278 samples. For beta diversity analysis, the Jaccard dissimilarity was calculated on the rarefied
279 table using the function *decostand* from *vegan* package. Hellinger distance was calculated from
280 the non rarefied table also using the function *decostand* (Legendre & Gallagher, 2001). To
281 explore beta diversity patterns, we performed a principal component analysis (PCA) on the
282 Hellinger transformed data using the function *rda* from *vegan*. A principal coordinates analysis
283 (PCoA) was performed using the Jaccard dissimilarity using the function *pcoa* from *ape*
284 package (Paradis & Schliep, 2019). To test the influence of the treatments, replicates and time
285 on the microbiome beta diversity, we performed a permutational analysis of variance
286 (PERMANOVA) (Anderson, 2005). Before running the analysis, the functions *betadisper* from
287 the package *vegan* and *anova*-like permutation test from *stats* package were used to identify
288 significant deviations on the multivariate beta dispersion of the data for treatments, replicates,
289 time and of the interaction between treatments and time (Oksanen et al., 2015). The effect of
290 treatments and replicates (nested within treatments) was tested using the function
291 *nested.npmanova* from the package *BiodiversityR* (Kindt & Coe, 2005). To test the effect of
292 time and the interaction between treatments and time, we used the function *adonis* from *vegan*
293 including treatments, replicates, and time (number of days) as fixed variables in the model,
294 with permutations restricted to the replicates level. HCA was done using the Hellinger distance
295 as previously described. Taxonomy barplots were produced by showing the three most
296 abundant genera (considering the best hits classified by VSEARCH), while the less abundant
297 were merged as “others”. *IndVal* analyses were run with the rarefied table to identify indicative
298 species of the three groups of treatments evidenced in the beta diversity analysis (inside and
299 outside bloom DCM and both surface samples) using the function *multipatt* from the package
300 *indicspecies* v1.7.9 with 10,000 permutations (De Cáceres and Legendre, 2009). P-values were

301 adjusted for multiple comparisons using the false discovery rate method using the function
302 *p.adjust* from the package stats.

303

304 **Results**

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

306 ***huxleyi* bloom**

307 Cocolithophore blooms occur seasonally from April to June in the Bay of Biscay along
308 the continental shelf to the Celtic Sea (Holligan et al., 1983; Poulton et al., 2014; Perrot et al.,
309 2018). Here, we followed and sampled an *E. huxleyi* bloom for a week from end of May to
310 early June 2019 in the Celtic Sea (Fig. 1A and 1B) using near-real time interpolated images of
311 non-algal suspended particulate matter (SPM) derived from MERIS and MODIS satellite
312 reflectance data (Perrot et al., 2018; Gohin et al., 2019) as provided by Ifremer
313 (<http://marc.ifremer.fr/en>) .

314 During the 5-day sampling period, temperature and salinity ranged from 12.4°C to
315 15.4°C and from 35.4 to 35.5 PSU, respectively (Supplementary Table 2). In both inside and
316 outside bloom waters, nutrient concentrations were low with NO₂ + NO₃ and PO₄ ranging from
317 the detection thresholds to 1.25 μmol and 0.05 to 0.2 μmol/L, respectively. These low values
318 were typical of a bloom event where cells consume most of the nutrients. *E. huxleyi* whose cell
319 densities ranged from 1.6 x 10³ to 5.6 x 10³ cells/mL within bloom waters (Fig. 1C) dominated
320 the total photosynthetic eukaryotic community (2.5 x 10⁴ cells/mL on average). In these
321 samples, total numbers of heterotrophic bacteria varied from 8.1 x 10⁵ to 2.0 x 10⁶ cells/mL
322 (Fig. 1D) whereas the lowest prokaryotic cell concentration was measured in the outside bloom
323 sample (Fig. 1D and Supplementary Table 2).

324 Overall, the inside and outside bloom DCM samples displayed a prokaryotic richness
325 of about 140 ± 29ASVs (mean ± SD, n=11) (Fig. 2A). Richness increased over the course of

326 the bloom, reaching a maximum at day 4. The DCM samples collected on day 5 inside and
327 outside the bloom for the community assembly experiments contained 148 and 133 ASVs,
328 respectively. The Shannon diversity index displayed homogeneous values (mean 4.1 ± 0.2)
329 across samples (Fig. 2B).

330 Hierarchical clustering revealed 3 groups of sampling periods, the first one comprising
331 samples from day 1 to day 2 AM, the second grouping those of day 2 PM and day 3 AM, and
332 the third clustering those of day 3 PM to day 5 PM. These successive sampling periods reflected
333 the distinct shifts in the bacterial community during the bloom and may be related to the bloom
334 development (Supplementary Fig. 2). During the whole bloom survey, mean abundances of
335 prokaryotic communities inside the bloom showed that Proteobacteria (64% of the total of
336 reads) and Bacteroidota (15%) were the two major dominant phyla, followed by Cyanobacteria
337 (7%), Thermoplasmatota (4%), and Verrucomicrobiota (4%). Pelagibacteraceae (15%),
338 Pseudoalteromonadaceae (12%) and Rhodobacteraceae (12%) were the most abundant
339 proteobacterial families while Flavobacteriaceae (11%) dominated within the Bacteroidota.
340 Among the dominant genera, abundances of SAR11 clade Ia (about 10% of the total reads),
341 *Synechococcus* sp. (7%), SAR86 clade (5%), and uncultured Rhodobacteraceae members (4%)
342 remained stable along the bloom survey (Supplementary Fig. 3). Several other genera
343 demonstrated substantial abundance shifts. *Asciidiaceihabitans* and *Sulfitobacter*, accounting
344 for 7% in the first sampling period, decreased in the second and third sampling periods.
345 *Nitrosopumilus* (Thaumarchaeota), Thermoplasmata (Marine group II euryarchaeota), *Vibrio*
346 and *Pseudoalteromonas* exhibited an inverse pattern. As low as 2% or less in the first sampling
347 period, their abundance increased significantly (9 to 14%) in the second sampling period.
348 *Pseudoalteromonas* proportions persisted (14%) in the third sampling period while those of
349 other taxa decreased to their initial numbers. Not detected or at very low abundance in the first
350 sampling periods, *Alteromonas* peaked (up to 4%) only at the end of the bloom survey.

351 On day 5, prokaryotic communities outside the bloom grouped with those collected inside the
352 bloom area (Supplementary Fig. 2). In both samples, the fourth most abundant taxa making up
353 to >44% of the total reads were identical and displayed very close proportions
354 (*Pseudoalteromonas* 19% and 21% in inside and outside bloom, respectively, *Synechococcus*
355 9% and 7%, SAR11 clade Ia 8% and 11%, and *Alteromonas* 8% in both samples). The main
356 compositional differences these samples that served as inocula for the community assembly
357 experiment were the abundance of *Lentimonas* (5% inside vs 2% outside bloom), and of the
358 OM60 (NOR5) clade and Marine group II members (each of them accounting for 2% inside vs
359 4% outside bloom). Relative abundances of the clades that were recruited from these samples
360 during the experiment were lower and close in both samples (OM43 clade, 1.5% and 2.3% in
361 inside and outside bloom, respectively; KI89A clade, 0.6% and 0.4%; *Luminiphilus*, 0.7% and
362 0.3%; *Aurantivirga*, 0.1% and 0.3%; *Polaribacter*, 0.5% and 0.2%) or similar (SAR92 clade,
363 1.5%).

364

365 **Community assembly experiment**

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

367 Seawater samples used to inoculate axenic *E. huxleyi* cultures were filtered through
368 0.45 μm membranes to remove phototrophs (autotrophic eukaryotes and *Synechococcus*
369 populations) and overcome their effects on microbiome assembly. FCM analysis demonstrated
370 that about 40% of the initial bacterial cell concentration was lost after this filtration step. As
371 addressed in the Discussion, we acknowledge that this step may have also biased the bacterial
372 composition in the inocula. Due to limited incubation space onboard, cultures were incubated
373 at low light (20 $\mu\text{mol photons s}^{-1}\text{m}^{-2}$) and these light conditions were maintained during the
374 first weeks of incubation. However, a drastic decrease ($\sim 80\%$) of *E. huxleyi* cell concentrations
375 was observed in all the treatments between the day 10 and day 33 (Supplementary Fig. 4A).

376 To avoid culture crash, we increased the light intensity to $70 \pm 20 \mu\text{mol photons s}^{-1}\text{m}^{-2}$ and
377 larger microalgal inocula (10% of the final culture volume instead of 1%) were used to transfer
378 the cultures at day 33. *E. huxleyi* cell densities gradually increased at each subsequent transfer
379 until day 71. At that point, they reached the highest cell concentration ($9.9 \times 10^5 \pm 1.2 \times 10^5$
380 cells/mL) and remained stable up to the end of the experiment (Supplementary Fig. 4A).
381 Bacterial cell concentrations followed an opposite trend during the first weeks of incubation.
382 After a rapid increase (~94%) from day 10 to 47, they decreased once *E. huxleyi* abundance
383 became higher and remained relatively stable up to the end of the experiment (Supplementary
384 Fig. 4B).

385 Regarding the structure of the bacterial community, a severe loss of richness was
386 observed between the environmental and culture samples (Fig. 2A). At day 10, the bacterial
387 richness in the cultures was about one fifth of the richness in the natural samples ($30 \pm 8 \text{ SD}$,
388 $n=12$) (Fig. 2A). This reflected a parallel decrease in the Shannon index, which at day 10, was
389 about one third the values recorded in environmental samples ($1.4 \pm 0.6 \text{ SD}$, $n=12$, Fig.
390 2B). Over the course of the experiment, we observed a decrease in richness along the first five
391 weeks (mean decrease $25 \pm 7 \text{ SD}$, $n=12$, until day 47) (Fig. 2A and Supplementary Fig. 4C).
392 After an increase at day 59 that corresponded to the period of culture recovery, the richness
393 values decreased again and remained stable until day 393 ($12 \text{ ASVs} \pm 2$). The decrease of
394 richness was mainly associated with the loss of low abundance ASVs, while the dominant ones
395 remained over the course of the experiment (Supplementary Fig. 5). In general, the Shannon
396 index also decreased over the from day 10 to day 33 (mean decrease of $0.5 \pm 0.6 \text{ SD}$, $n=12$)
397 and then gradually increased to values (i.e. 1.2 ± 0.4 at day 393, $n=12$) similar to that from day
398 22. The highest richness and Shannon indexes were obtained in the treatments amended with
399 the inside bloom DCM sample (richness, $26 \pm 12 \text{ ASVs}$; Shannon, 1.6 ± 0.4 , $n=24$).

400

401 **Dynamics of beta diversity patterns in recruited microbiomes**

402 In order to identify the influence of the different initial prokaryotic community
403 composition and to follow the changes in the microbiome beta diversity with time, we used
404 two metrics, the Hellinger distance (Euclidean distance of Hellinger-transformed data) and the
405 Jaccard dissimilarity. PCoA using Jaccard dissimilarity demonstrated that *E. huxleyi* cultures
406 inoculated with surface samples grouped together (Fig. 3A), while those inoculated with inside
407 and outside bloom DCM samples formed two other independent clusters.

408 Statistical significance of the effect of treatments, replicates and time, as well as the
409 interaction of treatment and time on the diversity of the microbiomes was assessed by
410 PERMANOVA and nested PERMANOVA. Before performing PERMANOVA analysis we
411 tested the beta-dispersion (variance) of the microbiomes grouped by treatments, time,
412 treatments over time, and replicates. The dispersions (variance) of treatments as well as the
413 interaction of treatments over time were not homogeneous for both metrics tested ($p < 0.05$).
414 On the other hand, dispersions were likely homogeneous over time and across replicates ($p >$
415 0.05). Still, PERMANOVA results were robust to dispersion for balanced designs like ours
416 (Anderson & Walsh, 2013). PERMANOVA results of Hellinger and Jaccard dissimilarities
417 showed that significant proportions of the variance in microbiome composition among samples
418 were explained by treatments (31% and 25%, respectively), replicates (31% and 15%,
419 respectively), and time (6% and 7%, respectively) ($p < 0.01$) (Fig. 3B, Supplementary Tables
420 3 and 4). Although the interaction between treatments and time was significant using Hellinger
421 distance ($F = 2.23$; $p = 0.016$), it explained a small proportion of the variance (2.5%).

422 Clustering using Hellinger distance revealed that the prokaryotic community
423 composition of all the cultures grouped into three major clusters (Fig. 3C) supporting the PCoA
424 using Jaccard dissimilarity results. Based on the Hellinger distance, the outside bloom DCM
425 treatment samples (cluster a) formed two subclusters, highlighting the compositional

426 differences between the 3 replicates. Replicate 1 was dominated by *Alcanivorax* (78%, n=8),
427 while *Erythrobacter* prevailed in replicate 2 (88%) and 3 (45%). Cluster b mainly consisted of
428 microbiomes recruited from both surface water samples. Surface treatments were dominated
429 by bacteria related to OM43, KI89A, and SAR92 clades, and to *Luminiphilus*. The third main
430 cluster (c) entirely formed by microbiomes from the inside bloom DCM treatment was
431 dominated by members of the OM43 (29%), KI89A (28%), SAR92 clade and *Polaribacter*
432 (11% each). With the exception of those related to *Sulfitobacter*, *Luminiphilus*, OM43 and the
433 OM60(NOR5) clades, ASVs that dominate ($\geq 1\%$) in the cultures amended with inside and
434 outside bloom DCM samples were generally low in abundance or not detected in the initial
435 bacterioplankton community (Supplementary Fig. 6).

436 The number of indicative ASVs for each treatment varied widely and was significantly
437 higher (21 out of 29) in cultures amended with inside bloom DCM waters (Supplementary
438 Table 5). Flavobacteriales, with *Aurantivirga* and *Polaribacter* in particular, was the order
439 containing the most indicative ASVs of microbiomes recruited from the inside bloom DCM
440 sample. Members of SAR92 and KI89 clades displayed high *Indval* indexes in both inside
441 bloom DCM and surface samples. The indicator ASVs of outside bloom DCM treatment were
442 related to *Erythrobacter*, *Alcanivorax*, and OM60(NOR5) clade.

443 Besides the compositional differences among treatments, we observed a somewhat
444 cyclic pattern of the beta-diversity over time using Hellinger distance (Fig. 4). Microbiome
445 community compositions clearly differed from each other from days 10, 22 and 33 for all
446 treatments, but they gradually tended to become similar to their initial status at the following
447 time-points. This cyclic pattern was observed for inside and outside bloom surface treatments
448 (Fig. 4A and C) and was particularly evident in the inside bloom DCM treatment (Fig. 4B). In
449 these treatments, the dynamic was mainly driven by the dominance of the OM43 clade (ASV1)
450 during the alga crash. In the algal growth recovery phase, the increased abundance of

451 *Luminiphilus* (ASV6) in surface treatments and of *Aurantivirga* (ASV12) and *Polaribacter*
452 (ASV15) in inside bloom DCM cultures were also involved (Supplementary Fig. 7). The
453 contributions of these ASVs were also supported by the PCAs species loadings. No cyclic
454 pattern was observed for outside bloom DCM cultures (Fig. 4D). Instead, in line with
455 hierarchical clustering results (Fig. 3C), dissimilarities in these cultures were higher between
456 replicates than over time.

457

458 **Discussion**

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

462

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

464 The composition of the bacterial community, *i.e.* the presence of Flavobacteriaceae,
465 Pseudoalteromonadaceae, Alteromonadaceae and members of the genus *Sulfitobacter*,
466 indicated that the *E. huxleyi* bloom had already entered the decaying phase when we started the
467 sampling (Lovejoy et al., 1998; Buchan et al., 2014). Indeed, Flavobacteriia are reported
468 amongst the main bacteria present in the declining phase of phytoplankton blooms (Teeling et
469 al., 2012, 2016; Landa et al., 2016), which seems linked to their capacity to degrade high
470 molecular weight substrates such as proteins and polysaccharides (Cottrell and Kirchman,
471 2000; Kirchman, 2002; Fernández-Gomez et al., 2013; Kappelmann et al., 2019; Francis et al.,
472 2021). Furthermore, the algicidal effects of *Pseudoalteromonas*, *Alteromonas*, and
473 *Sulfitobacter* strains and species have been documented in many microalgae including *E.*
474 *huxleyi* (Holmström & Kjelleberg, 1999; Meyer et al., 2017; Li et al., 2018; Barak-Gavish et
475 al., 2018), which calls attention to their potential role in the *E. huxleyi* bloom termination

476 (Lovejoy et al., 1998; Barak-Gavish et al., 2018). Satellite images and post-cruise analyses
477 support that we started sampling the bloom in its decline phase. First, the high reflectance patch
478 visible on the satellite images (Fig. 1A) and the daily vanishing of the coccolith-derived
479 turbidity signal observed from the interpolated images of non-algal SPM were both indicative
480 of detached coccoliths from dead *E. huxleyi* cells (Neukermans & Fournier, 2018; Perrot et al.,
481 2018). This assumption was confirmed by the complete disappearance of the coccolith-derived
482 turbidity signal a couple of days after we left the sampling area. Second, a suite of ongoing
483 experiments on the bloom samples using diagnostic lipid- and gene-based molecular
484 biomarkers (Vardi et al., 2009; Hunter et al., 2015; Ziv et al., 2016; Vincent et al., 2021)
485 revealed the detection of specific viral polar lipids and visualized *E. huxleyi* infected cells
486 during bloom succession, suggesting that Coccolithovirus infections may have partially
487 participated in the demise of *E. huxleyi* bloom (F. Vincent, C. Kuhlisch, G. Schleyer, pers.
488 comm.) as often proposed (Bratbak et al., 1993; Vardi et al., 2012; Laber et al., 2018).

489 As the bloom decline progressed, rapid and important shifts of the prokaryotic
490 community were observed, probably reflecting a direct response by certain bacterial taxa to
491 specific *E. huxleyi*-derived organic matter. Senescence compounds from decaying *E. huxleyi*
492 cells probably fueled members of the genus *Asciidiaceihabitans* (formerly *Roseobacter* OCT
493 lineage) (Wemheuer et al., 2015), whose relative abundances typically fluctuate during
494 phytoplankton blooms (Hahnke et al., 2015; Lucas et al., 2016; Chafee et al., 2018; Choi et al.,
495 2018) and promoted other functionally different transient taxa that represent key prokaryotic
496 members during bloom decline. Among them, Thermoplasmata are generally associated with
497 protein and lipid degradation (Orellana et al., 2019) while *Nitrosopumilus* may be favored by
498 nitrite accumulation caused by algal release (Kim et al., 2019). The opportunistic *Vibrio* was
499 among the most rapidly responding bacterial heterotrophs in the bloom termination conditions,
500 likely degrading organic matter released from algal cells (Eiler *et al.*, 2007) while *Alteromonas*

501 sp. have the metabolic capacity to degrade a diverse set of complex compounds like
502 polysaccharides during the later stages of the bloom (Reintjes et al., 2019).

503

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

505 Since our primary objective was to study the bacterial community selection and
506 assembly by a single phytoplankton host, we used a filtration step to discard autotrophic
507 phytoplankton cells, such as *Synechococcus* and picoeukaryotes abundantly represented in the
508 initial planktonic communities (Supplementary Table 2). We acknowledge that this filtration
509 strategy has removed large and particle-attached prokaryotes, the latter probably being
510 abundant in the demise phase of the bloom, and has induced substantial modifications in the
511 initial community composition of the inocula and finally in recruited microbiomes. Indeed,
512 some of the main taxonomic groups recruited in the treatments were not previously reported in
513 *E. huxleyi* and other phytoplankton cultures or in low abundance, notably *Luminiphilus*, and
514 the clades SAR92, KI89A and OM43 (Green et al., 2015; Câmara dos Reis, 2021). Except
515 members of the OM43 clade (Yang et al., 2016), these bacteria are known as important groups
516 of oligotrophic marine Proteobacteria that do not usually grow in the rich organic matter
517 conditions provided in phytoplankton-derived cultures (Cho & Giovannoni, 2004; Spring &
518 Riedel, 2013). Another unexpected result of our study is the very low representation of
519 *Marinobacter* sp. in the recruited microbiomes whereas previous studies have reported their
520 dominance in cultures of worldwide *E. huxleyi* isolates (Green et al., 2015; Câmara dos Reis,
521 2021). We found them in low abundance in inside and outside bloom waters (0.15-0.2%),
522 similar to multiyear average values (0.18%) in the Western English Channel (Gilbert et al.,
523 2012). Since *Marinobacter* can be closely associated with particulate organic matter, including
524 the eukaryotic phytoplankton population (Sonnenschein et al., 2012; Thompson et al., 2020),
525 we cannot fully exclude the possibility that the filtration step impacted the abundance of

526 *Marinobacter* in the inocula and finally in the *E. huxleyi* cultures. A more likely hypothesis
527 however is that low light conditions might have induced algal cell death promoting the release
528 of methylated compounds (Reese et al., 2019; Fisher et al., 2020). The release of methylated
529 compounds by *E. huxleyi* may have provided a selective advantage to the specialist OM43
530 clade methylophs (Neufeld et al., 2008), dominant in all the cultures, and to other less
531 common bacterial taxa in the absence of strong competitors such as *Marinobacter*. This
532 hypothesis is in line with the opposite dynamics of *E. huxleyi* (decrease) and bacteria (increase)
533 coupled to the sharp decrease of the bacterial alpha diversity during the first month of culture,
534 indicating that a few bacterial taxa were outcompeting others. Despite the above limitations,
535 the high reproducibility of microbiome community composition across the biological replicates
536 suggests that they did not alter the general conclusions raised from our study.

537 Our results illustrate the importance of niche differentiation in natural communities.
538 Indeed, although no major differences were observed between environmental inside and
539 outside bloom bacterial communities, *E. huxleyi* microbiomes recruited from these samples
540 differed. Similarly, although we did not analyze the initial bacterial composition of epipelagic
541 surface samples (collected 34 km apart), they converged towards similar compositions,
542 dominated by *Luminiphilus*, SAR92, KI89A and OM43 clades. Other microbiome studies of
543 phytoplankton cultures have highlighted the impact of the initial community composition on
544 microbiomes after short (Ajani et al., 2018; Sörenson et al., 2019; Jackrel et al., 2020), and
545 long-term selection (Behringer et al., 2018). Remarkable features were found in the
546 microbiomes resulting from inside bloom DCM waters where several indicative flavobacterial
547 ASVs, mainly assigned to *Polaribacter* and *Aurantivirga*, were initially selected and remained
548 among the most prevalent and abundant ASVs after growth recovery of the host. Both genera
549 were identified as the main degraders of polysaccharides during diatom blooms (Krüger et al.,
550 2019) and showed clear successions along the bloom stages (Teeling et al., 2012; Landa et al.,

551 2016; Krüger et al., 2019; Liu et al., 2020). This may be related to the differential capacity of
552 these bacteria to degrade phytoplankton-derived polysaccharides during blooms (Teeling et al.,
553 2012; Krüger et al., 2019; Avci et al., 2020; Francis et al., 2021). Interestingly, SAR92 and
554 *Luminiphilus* were also identified as important degraders of algal polysaccharides in bloom
555 conditions (Francis et al., 2021), suggesting their potential functional role in our cultures.

556 We observed contrasting results between the inside and outside bloom DCM recruited
557 microbiomes that can be linked to the origin of the samples. First, recruited microbiomes from
558 inside bloom DCM samples were more diverse and displayed higher number of indicative
559 ASVs, probably reflecting the higher diversity observed in the original seawater (Fig.2). We
560 assume that exopolysaccharides/exudates of axenic *E. huxleyi* cultures have strongly
561 influenced the recruited microbiomes and their long-term stability. We hypothesize that the
562 higher diversity conferred stability to the microbiomes allowing the recovery after disturbance.
563 This may likely explain the almost complete cyclic pattern they followed (Fig. 4B). Such cyclic
564 patterns were shown in the surface microbiome of the seaweed *Delisea pulchra* (Longford *et*
565 *al.*, 2019) after experimental disturbances. These authors hypothesized that the production of
566 halogenated furanones by the red algae exert a selective force for the establishment and
567 persistency of early-colonizing bacteria which may protect the host against the colonization of
568 pathogenic bacteria in later successional stages (Longford et al., 2019). In line with our data,
569 this study favored the view that higher diversity in disturbed microbiomes may be a source of
570 stability and resilience against perturbation (Longford et al., 2019). We consistently observed
571 cyclic patterns in treatments having a high degree of uniformity in the replicates. This was not
572 the case for the outside bloom DCM cultures whose bacterial communities displayed high
573 levels of between-replicate variability.

574 Our study suggests the combined effect of deterministic processes and stochasticity on
575 the microbiome assembly. The significant imprint of the original community in the inside

576 bloom DCM treatments suggests that deterministic processes (*e.g.* pre-exposition to algal
577 exudates in the bloom assemblages adapted to *E. huxleyi* bloom exudates) influenced the final
578 microbiome composition. On the other hand, variable communities grown from outside bloom
579 DCM treatment are consistent with stochastic assembly overwhelming any signal of ecological
580 selection. In line with our results, both deterministic and stochastic processes were found to
581 influence community assembly in both the surface and DCM waters of the South Pacific Gyre
582 (Allen et al., 2020). Homogeneous selection was the dominant community assembly process
583 at both depths. However, stochastic processes had more effect at the DCM than in the
584 temporally stable surface waters, presumably due to the greater influence of vertical nutrient
585 supply and higher productivity and lower influence of horizontal dispersal (Allen et al., 2020
586 and references therein).

587

588 **Conclusions**

589 In this work, we combined an observational and an experimental approach to reveal
590 the bacterial community structure in an *E. huxleyi* bloom and to address whether different
591 microbial composition could influence microbiome assembly in a *E. huxleyi* culture. Our
592 environmental data showed that the *E. huxleyi* bloom created unique ecological conditions
593 favoring the combination of bacterial and archaeal groups that followed a clear successional
594 trajectory. This trajectory suggests both potential algicidal bacteria-algae interactions and niche
595 specialization by different taxa possibly corresponding to different stages in the successive
596 degradation of *E. huxleyi*-derived organic compounds. Our experimental approach showed that
597 the compositional homogeneity of the prokaryotic community of an *E. huxleyi* bloom in the
598 demise phase influenced community assembly through deterministic processes. We showed
599 that the source of the initial bacterioplankton communities influences the resulting composition
600 of *E. huxleyi* microbiomes. Further studies using diverse phytoplankton cultures isolated from

601 a variety of oceanic regions and different trophic regimes could be useful to disentangle
602 deterministic and stochastic factors driving microbiome assembly in the marine environment.
603 Axenic phytoplankton cultures also represent a valuable resource to explore phytoplankton-
604 bacteria interactions. Co-cultivation of isolates corresponding to indicative ASVs and *E.*
605 *huxleyi* will be helpful to decipher how they interact. Future analyses combining transcriptomic
606 and metabolomic analyses will provide valuable information about the genes and molecules
607 involved in these ecologically key interactions.

608

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623

624 **Data Accessibility**

625 **Genetic data and sample metadata:**

626 Environmental samples are deposited in the bioproject PRJEB50692. Reads are deposited
627 under the accession numbers ERX9644183 - ERX9644242 and biosamples under the accession
628 numbers ERS10466567-ERS10466596.

629 Samples from the assembly experiment are deposited in the bioproject PRJEB48747. Reads
630 are deposited under the accession numbers ERX9767825 - ERX9768004 and biosamples under
631 the accession numbers ERS10539058-ERS10539153.

632

633 **Author Contributions**

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

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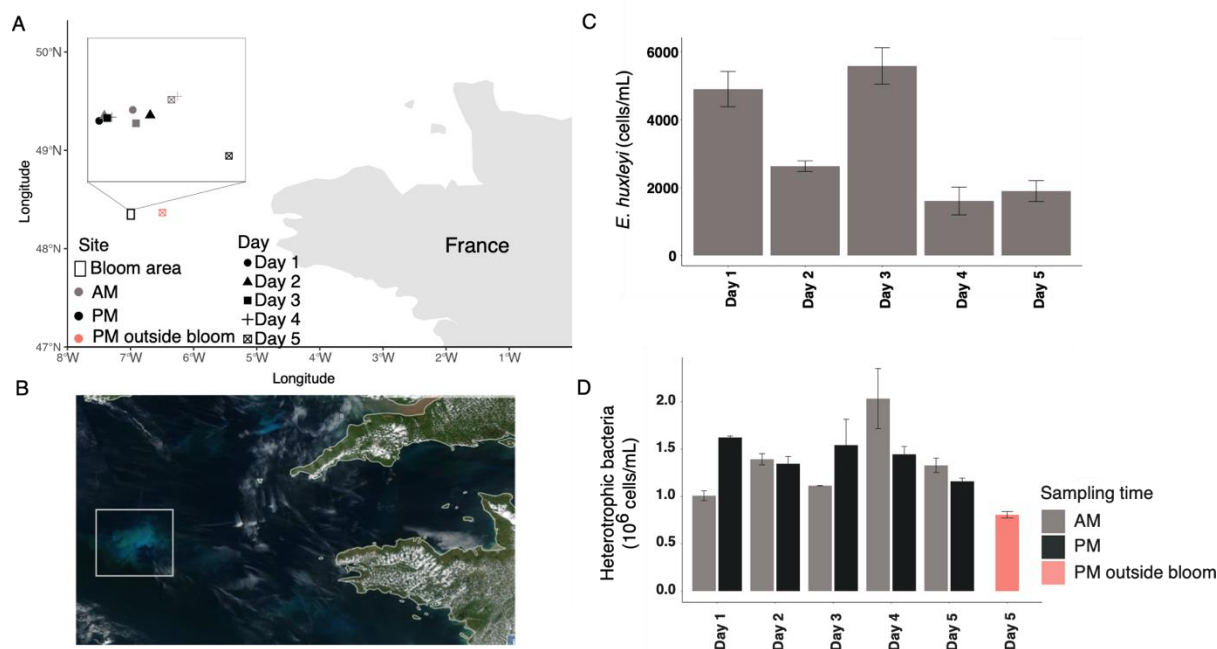
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Figures and Captions

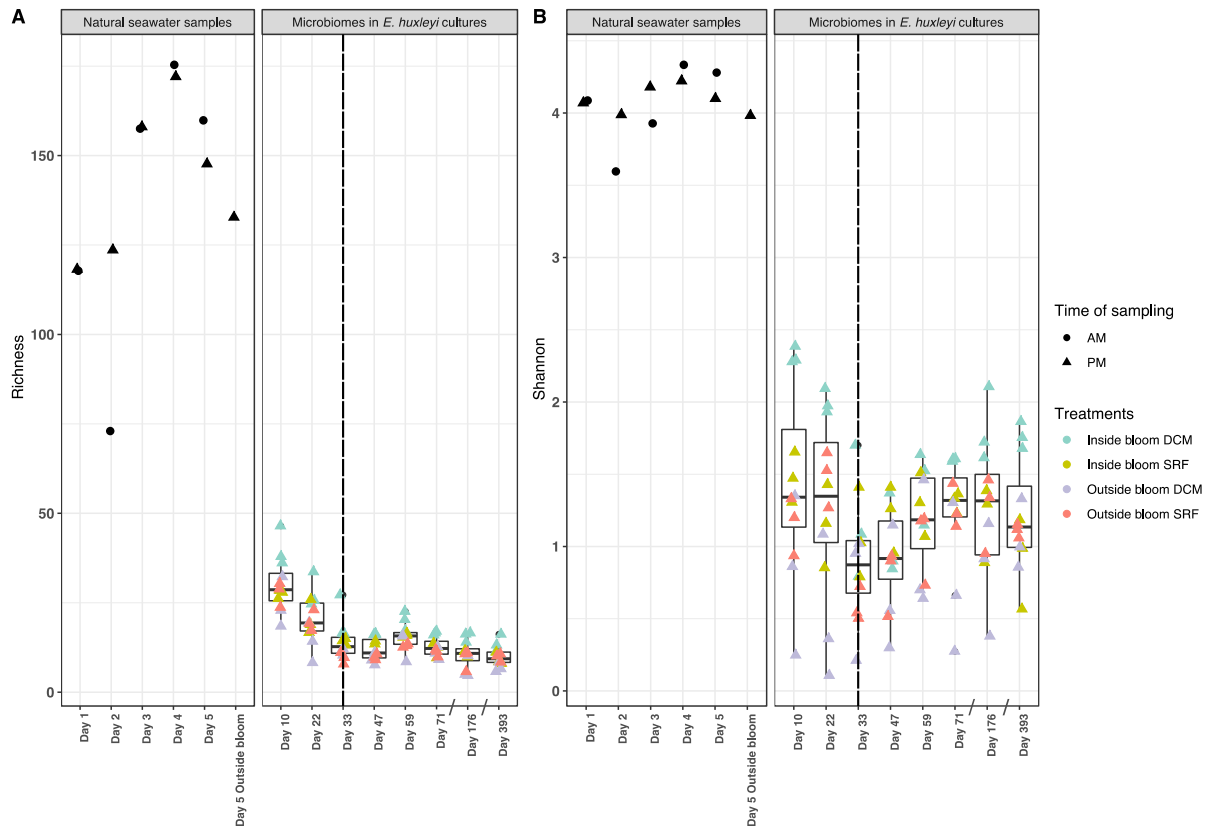
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1100 **Fig. 1.** Sampling area and characteristics of the *E. huxleyi* bloom in the Celtic Sea. **A)** Map
1101 showing the bloom area and spatio-temporal sampling strategy (AM and PM denote morning
1102 and afternoon samplings). **B)** True-color satellite image of the bloom area on May 21, 2019
1103 (source: <https://www.star.nesdis.noaa.gov/sod/mecb/color/ocview/ocview.html>. **C)** *E. huxleyi*
1104 cell concentrations at morning bloom sites during the survey, measured from duplicate filters
1105 using scanning electron microscopy. **D)** Heterotrophic bacterial cell concentrations at morning
1106 and afternoon sites during the survey, measured from duplicate samples by flow cytometry.

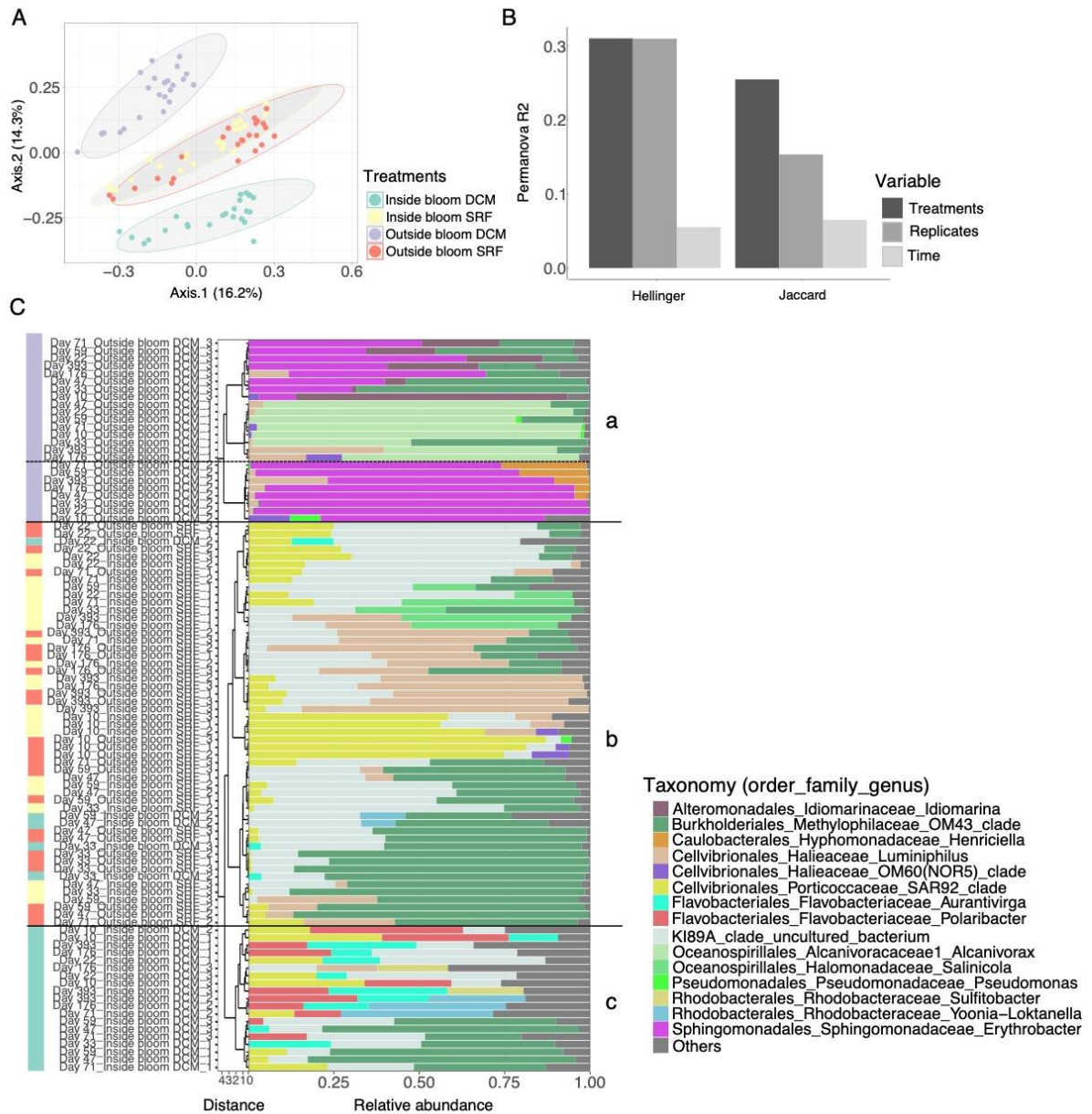
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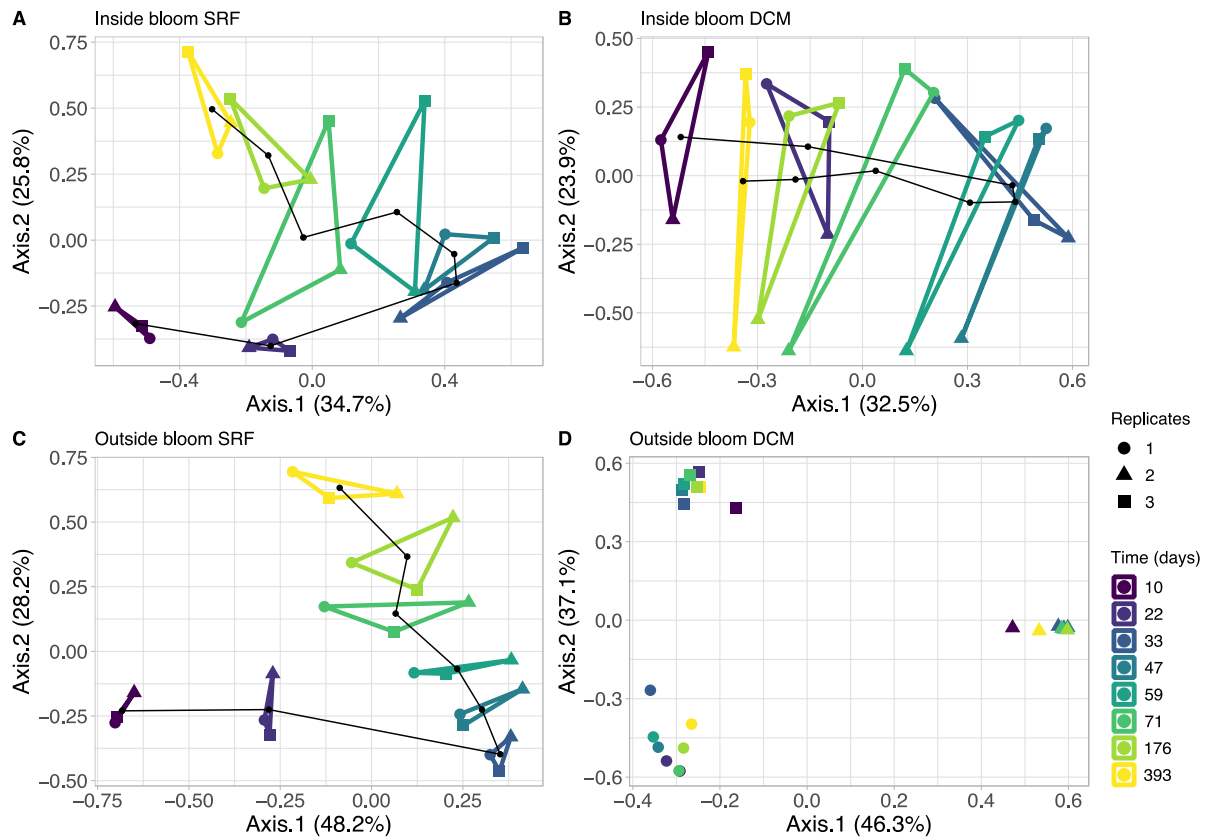
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1110 **Fig. 2.** Composite representation of the dynamics of the prokaryotic richness (A) and Shannon
 1111 (B) indexes in natural samples (0.2 – 3 μ m DCM samples only) and culture experiments. ASV
 1112 tables were rarefied to 2,479 (minimum number of reads of the environmental samples. The
 1113 boxes represent the interquartile range. The thin horizontal lines represent the 25th and 75th
 1114 percentiles and while the thick horizontal line represents the median. The vertical lines indicate
 1115 the minimum and maximum values (using 1.5 coefficients above and below the percentiles).
 1116 The dots represent the values measured for each culture. Dots further than the vertical lines
 1117 represent potential outliers. X-axis in the in the experiment plot are not proportional to the time
 1118 length between samplings. The black dotted line represents the time where the cultivation
 1119 conditions changed.

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1121 **Fig. 3.** Beta diversity patterns of *E. huxleyi* microbiomes across treatments and time. (A)
 1122 Principal coordinates analysis (PcoA) using Jaccard dissimilarity matrix of the presence-
 1123 absence transformed rarefied table. Colors correspond to each treatment that received
 1124 prokaryotic communities from different water samples: green - Inside bloom DCM; yellow -
 1125 Inside bloom surface (SRF); purple - Outside bloom DCM; red - Outside bloom SRF. Ellipses
 1126 represent 95% confidence. (B) Permutational multivariate analysis of variance
 1127 (PERMANOVA) and nested PERMANOVA r^2 of significant variables ($p < 0.01$) using both
 1128 metrics (see Supplementary Tables 3 and 4 for detailed results). (C) Hierarchical clustering
 1129 produced with the Hellinger distance matrix using “ward.D2” method. Codes of each
 1130 microbiome are experiment sampling day_treatment_replicate. On the left, colors used for each
 1131 treatment are the same than in Fig. 3A. Bar plots indicate the taxonomy of the 3 most abundant
 1132 genera. The other genera were merged as “others”.
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Fig. 4. Principal component 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 surface (SRF) (A), inside bloom DCM (B), outside bloom SRF (C), and outside bloom DCM (D). The polygons link replicates (shape coded) at each DNA sampling (color coded). The black line links the barycenters of the replicates.