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Exploring the phycosphere of *Emiliana huxleyi*: From bloom dynamics to microbiome assembly experiments

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Abstract

Coccolithophores have global ecological and biogeochemical significance as the most important calcifying marine phytoplankton group. The structure and selection of prokaryotic communities associated with the most abundant coccolithophore and bloom-forming species, *Emiliana huxleyi*, are still poorly known. In this study, we assessed the diversity of bacterial communities associated with an *E. huxleyi* bloom in the Celtic Sea (Eastern North Atlantic), exposed axenic *E. huxleyi* cultures to prokaryotic communities derived from bloom and non-bloom conditions, and followed the dynamics of their microbiome composition over one year. Bloom-associated prokaryotic communities were dominated by SAR11, Marine group II Euryarchaeota and Rhodobacterales and contained substantial proportions of known indicators of phytoplankton bloom demises such as Flavobacteriaceae and Pseudoalteromonadaceae. The taxonomic richness of bacteria derived from natural communities associated with axenic *E. huxleyi* rapidly shifted and then stabilized over time. The succession of microorganisms recruited from the environment was consistently dependent on the composition of the initial bacterioplankton community. Phycosphere-associated communities derived from the *E. huxleyi* bloom were highly similar to one another, suggesting deterministic processes, whereas cultures from non-bloom conditions show an effect of stochasticity. Overall, this work sheds new light on the importance of the initial inoculum composition in microbiome recruitment and elucidates the temporal dynamics of its composition and long-term stability.

KEYWORDS

Emiliana huxleyi, metabarcoding, microbiome assembly, phycosphere, phytoplankton–bacteria interactions

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1 | INTRODUCTION

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

Recent studies addressing the processes involved in bacterial community assembly in the phycosphere showed the influence of deterministic factors such as the place/time of isolation (Ajani et al., 2018) and the host species (Behringer et al., 2018; Jackrel et al., 2020; Kimbrel et al., 2019; Lawson et al., 2018; Mönnich et al., 2020; Sörenson et al., 2019). However, a combination between deterministic and stochastic effects in the microbiome recruitment process was also suggested (Kimbrel et al., 2019; Stock et al., 2022). To date, bacterial community composition and selection processes that influence the assembly of phycosphere microbiomes are not well known in many phytoplankton, in part because of the micrometer scale at which they take place (Kimbrel et al., 2019; Mönnich et al., 2020).

To overcome this challenge, possible strategies are to study the selection processes in natural phytoplankton blooms (Zhou et al., 2019), in meso-/microcosms or in cultures (Ajani et al., 2018; Fu et al., 2020; Kimbrel et al., 2019; Mönnich et al., 2020; Sörenson et al., 2019), when algal cells are at high concentrations. *Emiliania huxleyi* is the most abundant and cosmopolitan coccolithophore species and is able to form massive annual blooms in temperate and subpolar oceans mostly during Spring (Tyrrell & Merico, 2004). *E. huxleyi* blooms are characterized by blue turquoise waters that can be observed from satellite images (Tyrrell & Merico, 2004). These blooms have a critical importance for carbon and sulphur cycles due to the ecological and biogeochemical roles of coccolithophores as primary producers, calcifiers, and main contributors to the emission of dimethylsulfoniopropionate (DMSP) to the atmosphere (Malin & Steinke, 2004; Rost et al., 2004). The potential role of viruses in bloom termination has been thoroughly investigated (e.g., Bratbak et al., 1993; Lehahn et al., 2014; Vardi et al., 2012), but only a few studies have targeted the microbial diversity associated with *E. huxleyi* in natural environments (Gonzalez et al., 2000; Zubkov et al., 2001) and cultures (Green et al., 2015; Orata et al., 2016;

Rosana et al., 2016). The *Roseobacter*, SAR86 and SAR11 lineages were identified as the main bacterial groups associated with natural *E. huxleyi* blooms (Gonzalez et al., 2000; Zubkov et al., 2001). The co-occurrence of these groups could be mediated by the presence of DMSP, produced and released by *E. huxleyi* during blooms (Malin et al., 1993), which could be used as a sulphur compound by bacteria (Dupont et al., 2012; Miller & Belas, 2004; Tripp et al., 2008). Meanwhile, the microbiomes of *E. huxleyi* in cultures are highly dominated by *Marinobacter* (Câmara dos Reis, 2021) and by Rhodobacteraceae (Barak-Gavish et al., 2018; Green et al., 2015).

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

2 | MATERIALS AND METHODS

2.1 | Study site and sample collection

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

On the last sampling day, an additional site about 34 km apart from the bloom area was also sampled (Figure 1a). For each sampling event, surface to 50-m depth profiles of temperature, salinity, turbidity, pressure, photosynthetically active radiation (PAR), chlorophyll *a* (chl_a) fluorescence, oxygen concentrations and pH were conducted by deploying a SBE19+ profiler (Sea-Bird Scientific). Bloom depth, determined as the maximum turbidity depth, was generally very close to the DCM depth. Surface and bloom water samples were collected using an 8-L Niskin bottle for nutrient analyses. After collection, nutrient samples (125 ml) were stored at –20°C for further analysis. Concentrations of nitrate, nitrite, phosphate and silicate were measured using a AA3 auto-analyser (Seal Analytical) following the methods described by Tréguer and Le Corre (1975) and Aminot and Kérouel (2007). At the bloom depth, samples for flow cytometry (FCM) and scanning electron microscopy (SEM) were collected using the Niskin bottle while samples for metabarcoding

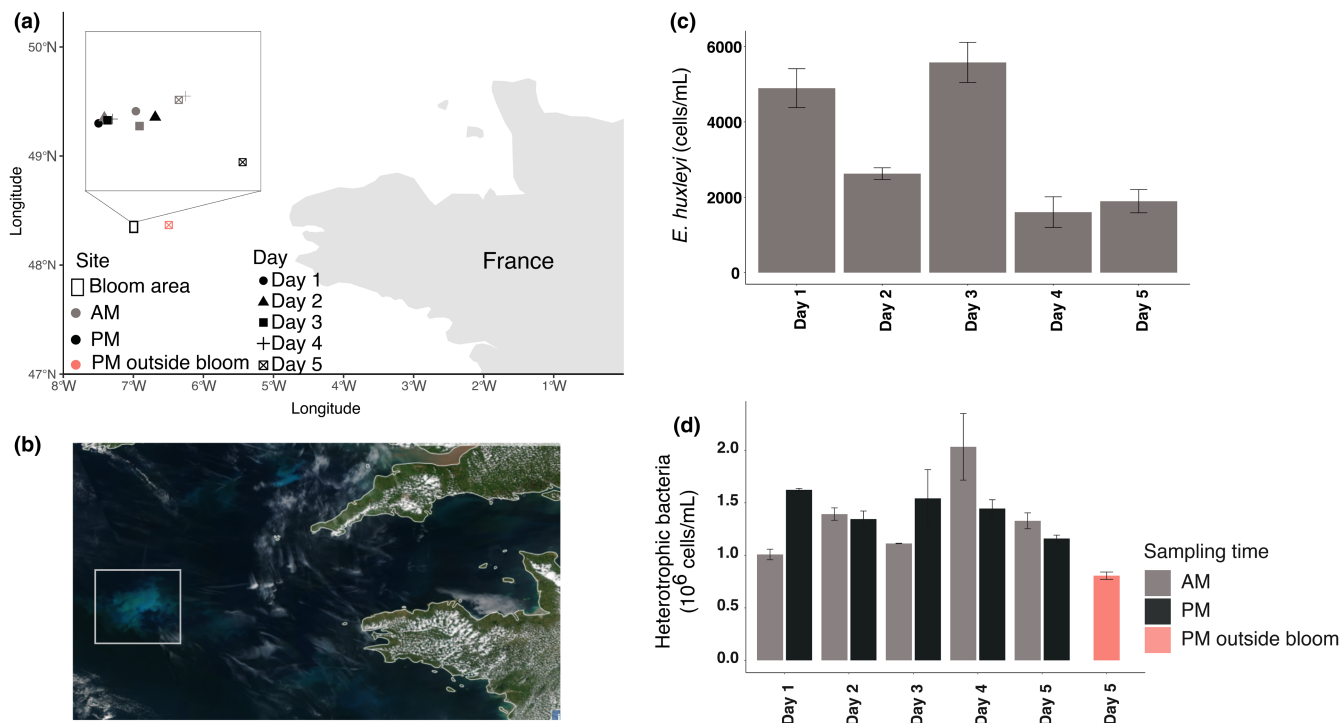


FIGURE 1 Sampling area and characteristics of the *E. huxleyi* bloom in the Celtic Sea. (a) Map showing the bloom area and spatio-temporal sampling strategy (AM and PM denote morning and afternoon samplings). (b) True-colour satellite image of the bloom area on May 21, 2019 (source: <https://www.star.nesdis.noaa.gov/sod/mecb/color/ocview/ocview.html>).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

analysis were collected by pumping and prefiltered through a 20- μ m mesh to eliminate large microplankton. For FCM analysis of photosynthetic eukaryotes and prokaryotic communities, two replicates (1.5 ml) were fixed using glutaraldehyde (0.25% final concentration) and Poloxamer 10% (0.1% final concentration) and incubated for 15 min at 4°C before flash freezing in liquid nitrogen. For SEM analysis, samples of morning sites (two replicates of 250 ml) were gently filtered onto polycarbonate membranes (47 mm in diameter; 1.2- μ m pore-size) (Millipore). Filters were placed onto PetriSlides (Millipore), dried for at least 2 hr at 50°C, and finally stored at room temperature. For metabarcoding analysis, cell biomass from bloom depth was collected from ~14 L of seawater by successive filtration onto large (142 mm in diameter) 3- μ m pore-size and then 0.2- μ m pore-size polycarbonate membranes (Millipore). Filters were flash-frozen in liquid nitrogen and stored at -80°C for later DNA analyses.

2.2 | Scanning electron microscopy analysis

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

2.3 | Community assembly experiments

2.3.1 | Axenization

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

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

2.3.2 | Sample preparation and inoculation

Four seawater samples were used in the bacterial community assembly experiment. They consisted of a surface and a DCM sample collected in the bloom area on day 5 (thereafter named inside bloom surface and inside bloom DCM) and a surface and a DCM sample collected the same day outside the bloom area (thereafter named outside bloom surface and outside bloom DCM) (Figure S1). To remove autotrophic picoeukaryotes and cyanobacteria from the inoculum, seawater samples were gently filtered through a 0.45- μ m pore size membrane (Millex-HV, PVFD; Millipore). To estimate the number

of prokaryotic cells lost during the filtration step, aliquots of total and filtered seawater samples were fixed for FCM analysis using the methods above. After filtration, 150 µl of each prokaryotic community (final cell concentration of about 6.8×10^3 cells ml⁻¹) were transferred in triplicates into 50-ml culture flasks filled with 15 ml of K/2 medium prepared as described in the Supplementary Materials and Methods. Finally, 150 µl of the axenic RCC1212 culture were added to each flask (final cell concentration of 3.8×10^3 cells ml⁻¹). Six flasks filled with 15 ml of K/2 medium and inoculated with 150 µl of the axenic RCC1212 culture were used as controls. In total, 18 cultures (three replicates of four treatments and six controls) were incubated at 15°C and a 12:12-hr photoperiod regime. Due to space limitation, only one thermostatic chamber with a light intensity of 20 µmol photons s⁻¹ m⁻² using a blue neutral density filter was available onboard for incubation.

2.3.3 | Survey of the culture microbiomes

Back in the laboratory and 10 days after inoculation, which corresponds to the time needed by *E. huxleyi* cultures to reach the end of exponential growth phase, the axenic status of controls was checked by FCM. Cultures (three replicates of four treatments and one axenic control) were transferred by inoculating 100 µl of the culture in 10 ml of fresh K/2 medium every 11–14 days for the first 176 days of the experiment and then every 3 weeks until its end (day 393). At each culture transfer and at the end of the experiment, treatments were sampled for FCM analyses and prokaryotic community composition analysis (3 replicates × 4 treatments × 8 DNA samplings = 96 samples) (Figure S1). The axenic control was regularly checked to ensure clean handling of the cultures. In addition, culture flasks were randomized daily in the incubator to minimize positional effects on growth.

For FCM, duplicate samples were fixed as previously described, and analyses, performed according to Marie et al. (1999), are detailed in the Supplementary Materials and Methods section. For community composition analysis, 2 ml of culture was centrifuged at 2000 g for 30 s to reduce the microalgal load. Preliminary tests showed that this procedure reduces microalgal load while keeping most of the bacterial cells (about 90%). The supernatants were transferred into new tubes containing 2 µl of Poloxamer 188 solution 10% (Sigma-Aldrich) and centrifuged at 5600 g for 5 min. The supernatants were discarded, and the pellets were stored at -80°C until DNA extraction.

2.4 | DNA extraction, PCR amplification and sequencing

DNA extraction from environmental and culture samples and amplification steps used to amplify the prokaryotic 16S rRNA gene using the universal prokaryote primers 515F-Y (5'-GTGYCAGCMGC CGCGGTAA-3') labelled with an eight-nucleotide tag unique to each

sample at the 5' end and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada et al., 2016) are detailed in the Supplementary Materials and Methods and in Romac (2022a, 2022b, 2022c, 2022d). The “tagged-PCR” approach was used (Bohmann et al., 2022). Briefly, DNA samples were PCR (polymerase chain reaction)-amplified using the above metabarcoding primers with 5' nucleotide tags and following PCR amplification, the individually tagged PCR products were purified and pooled. Pools were sent to Fasteris SA where ligation-based library preparation was carried out and high-throughput sequencing was performed using Illumina Miseq paired-end sequencing technology (2 × 250). In total, three DNA pools were sequenced. The two first, containing the environmental samples and the first 84 experiment samples (first seven time points) were sequenced in two independent Illumina runs (technical replicates). The last 12 experiment samples (day 393) were sequenced in another Illumina run without sequencing replicates.

2.5 | Bioinformatics

The steps of library separation, removal of Illumina adapters and first quality control were performed by Fasteris SA (see Supplementary Materials and Methods). The detailed scripts used in this study can be downloaded from https://github.com/mcamarareis/microbiome_assembly. Briefly, raw reads from each sequencing run were demultiplexed based on the eight-nucleotide tag sequences with CUTADAPT (version 2.8.1) (Martin, 2011). The “tagged-PCR” approach we used generated half of both forward and reverse reads containing a P5 Illumina adapter and the other half a P7 adapter resulting in forward and reverse reads in both R1 and R2 files (mixed orientation). To deal with the presence of reads in mixed orientation in the R1 and R2 raw files, the demultiplexing was performed in two rounds (see details in Supplementary Materials and Methods). Then, primer sequences were removed using CUTADAPT (version 2.8.1) (Martin, 2011). Because sequences from different sequencing runs and from different sequencing cycles can have different error rates, they were processed independently to obtain an amplicon sequence variant (ASV) table using the DADA2 pipeline (version 1.14.0 in R 3.6.1) (Callahan et al., 2016; R Core Team, 2017). The overall read quality of the demultiplexed primer-free sequences was first investigated with the DADA2 function *plotQualityProfile* to identify the position where the quality distribution dropped for R1 and R2 reads. Then, forward and reverse reads were trimmed where the quality decreased (i.e., at position 215 for R1 and at position 190 for R2 files) and reads with ambiguous nucleotides or with a maximum number of expected errors (maxEE) > 2 were filtered out using the function *filterAndTrim*. For each combination of runs and demultiplexing rounds, error rates were defined using the function *learnErrors* and denoised using the *dada* function in pooled mode before being merged with *mergePairs* (which generated ASVs of about 373 bp). Then, all the independent processed data sets were merged in one sequence table (for each sample, reads from the two rounds of demultiplexing were summed while reads coming from the two sequencing runs were

kept separate) and processed for chimera removal using the function *removeBimeraDenovo*, also performed in pooled mode and with a “minFoldParentOverAbundance” of 8. The parameters used at each DADA2 step are specified in the Table S1.

ASVs shorter than 366 bp and longer than 377 bp were filtered out, and the remaining ones were taxonomically assigned using ID-TAXA (50% confidence threshold) with the Silva database version 138 (Murali et al., 2018; Quast et al., 2013). Chloroplasts and mitochondrial ASVs were removed. ASVs not classified at the domain level by ID-TAXA were assigned to the best hit in Silva version 138 by pairwise global alignment (*usearch_global* VSEARCH's command) (Rognes et al., 2016). These ASVs were removed if they could not be classified and/or were classified as chloroplasts or mitochondrial sequences by VSEARCH (at 80% identity threshold). The resultant ASV table was filtered to remove ASVs accounting for <0.001% of the total number of reads (abundance filter). Consistency of technical replicates was evaluated by Procrustes analysis (function *procrustes* from the VEGAN package), which measures the similarity between two ordinations of the same objects, followed by a protest, which measures the significance of the correlation (Oksanen et al., 2015). For this, we used a comparative principal component analysis (PCA) performed on the Hellinger-transformed data. After consistency was confirmed ($p < .001$ and correlation = .99), independent technical replicates of each culture were merged by the sum of the number of reads of the ASVs present in the two replicates of the same culture (prevalence filter). The abundance and prevalence filters described above removed about 68% of the total number of ASVs while keeping 99% of the number of reads. The final data set (filtered ASV table used for further analysis) contained 107 samples (11 from the environment and 96 from cultures) for a total of 6,017,019 reads and 294 prokaryotic ASVs.

2.6 | Community composition and statistical analyses

2.6.1 | Environmental samples

All the analyses were conducted in R version 4.0.2 in RSTUDIO (1.1.442) and the plots were produced with GGLOT2 (R Core Team, 2017; RStudio Team, 2016; Wickham, 2016). Taxonomy treemaps of environmental samples were produced at the genus level considering the best hits classified by VSEARCH. To facilitate visualization, low-abundance genera (accounting for <3% of the relative abundance at each sample) present at the raw community table were grouped. To compare environmental samples and cultures, mean alpha diversity indices (richness and Shannon index) were measured after rarefying the ASV table 100 times at the minimum number of reads (2479) using the function *rtk* (Saary et al., 2017). Hierarchical cluster analysis (HCA) (method “ward.D2”) was used to identify differences in the free-living prokaryotic communities by sites using the Hellinger distance (Euclidean distance of the Hellinger-transformed matrix; Legendre & Gallagher, 2001) using the function *hclust* from the STATS package (R Core Team, 2017).

2.6.2 | Experiment

To analyse alpha diversity dynamics of the cultures, rarefaction was performed at a read depth of 5957 reads using the same approach as for the environmental samples. For beta diversity analysis, Jaccard dissimilarity was calculated on the rarefied table using the function *decostand* from the VEGAN package. Hellinger distances were calculated from the nonrarefied table also using the function *decostand* (Legendre & Gallagher, 2001). To explore beta diversity patterns, we performed a PCA on the Hellinger-transformed data using the function *rda* from VEGAN. A principal coordinates analysis (PCoA) was performed using the Jaccard dissimilarity using the function *pcoa* from the APE package (Paradis & Schliep, 2019). To test the influence of the treatments, replicates and time on the microbiome beta diversity, we performed a permutational analysis of variance (PERMANOVA) (Anderson, 2005). Before running the analysis, the functions *betadisper* from the package VEGAN and *anova*-like permutation test from the STATS package were used to identify significant deviations on the multivariate beta dispersion of the data for treatments, replicates, time, and of the interaction between treatments and time (Oksanen et al., 2015). The effect of treatments and replicates (nested within treatments) was tested using the function *nested.npmanova* from the package BIODIVERSITYR (Kindt & Coe, 2005). To test the effect of time and the interaction between treatments and time, we used the function *adonis* from VEGAN including treatments, replicates and time (number of days) as fixed variables in the model, with permutations restricted to the replicates level. HCA was done using the Hellinger distance as previously described. Taxonomy barplots were produced by showing the three most abundant genera (considering the best hits classified by VSEARCH), while the less abundant genera were merged as “others.” *IndVal* analyses were run with the rarefied table to identify indicative species of the three groups of treatments evidenced in the beta diversity analysis (inside and outside bloom DCM and both surface samples) using the function *multipatt* from the package INDICESPECIES version 1.7.9 with 10,000 permutations (De Cáceres & Legendre, 2009). The *p*-values were adjusted for multiple comparisons using the false discovery rate method using the function *p.adjust* from the package STATS.

3 | RESULTS

3.1 | Physicochemical parameters and bacterial community structure dynamics in the *E. huxleyi* bloom

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

During the 5-day sampling period, temperature and salinity ranged from 12.4 to 15.4°C and from 35.4 to 35.5 PSU, respectively (Table S2). In both inside and outside bloom waters, nutrient concentrations were low with $\text{NO}_2 + \text{NO}_3$ and PO_4 ranging from the detection thresholds to 1.25 μmol and 0.05 to 0.2 $\mu\text{mol L}^{-1}$, respectively. These low values were typical of a bloom event where cells consume most of the nutrients. *E. huxleyi*, whose cell densities ranged from 1.6×10^3 to 5.6×10^3 cells ml^{-1} within bloom waters (Figure 1c), dominated the total photosynthetic eukaryotic community (2.5×10^4 cells ml^{-1} on average). In these samples, total numbers of heterotrophic bacteria varied from 8.1×10^5 to 2.0×10^6 cells ml^{-1} (Figure 1d) whereas the lowest prokaryotic cell concentration was measured in the outside bloom sample (Figure 1d and Table S2).

Overall, the inside and outside bloom DCM samples displayed a prokaryotic richness of about 140 ± 29 ASVs (mean \pm SD, $n = 11$) (Figure 2a). Richness increased over the course of the bloom, reaching a maximum on day 4. The DCM samples collected on day 5 inside and outside the bloom for the community assembly experiments

contained 148 and 133 ASVs, respectively. The Shannon diversity index displayed homogeneous values (mean 4.1 ± 0.2) across samples (Figure 2b).

Hierarchical clustering revealed three groups of sampling periods, the first one comprising samples from day 1 to day 2 a.m., the second grouping those of day 2 p.m. and day 3 a.m., and the third clustering those of day 3 p.m. to day 5 p.m. These successive sampling periods reflected the distinct shifts in the bacterial community during the bloom and may be related to the bloom development (Figure S2). During the whole bloom survey, mean abundances of prokaryotic communities inside the bloom showed that Proteobacteria (64% of the total reads) and Bacteroidota (15%) were the two major dominant phyla, followed by Cyanobacteria (7%), Thermoplasmata (4%) and Verrucomicrobiota (4%). Pelagibacteraceae (15%), Pseudoalteromonadaceae (12%) and Rhodobacteraceae (12%) were the most abundant proteobacterial families while Flavobacteriaceae (11%) dominated within the Bacteroidota. Among the dominant genera, abundances of SAR11 clade Ia (about 10% of the total

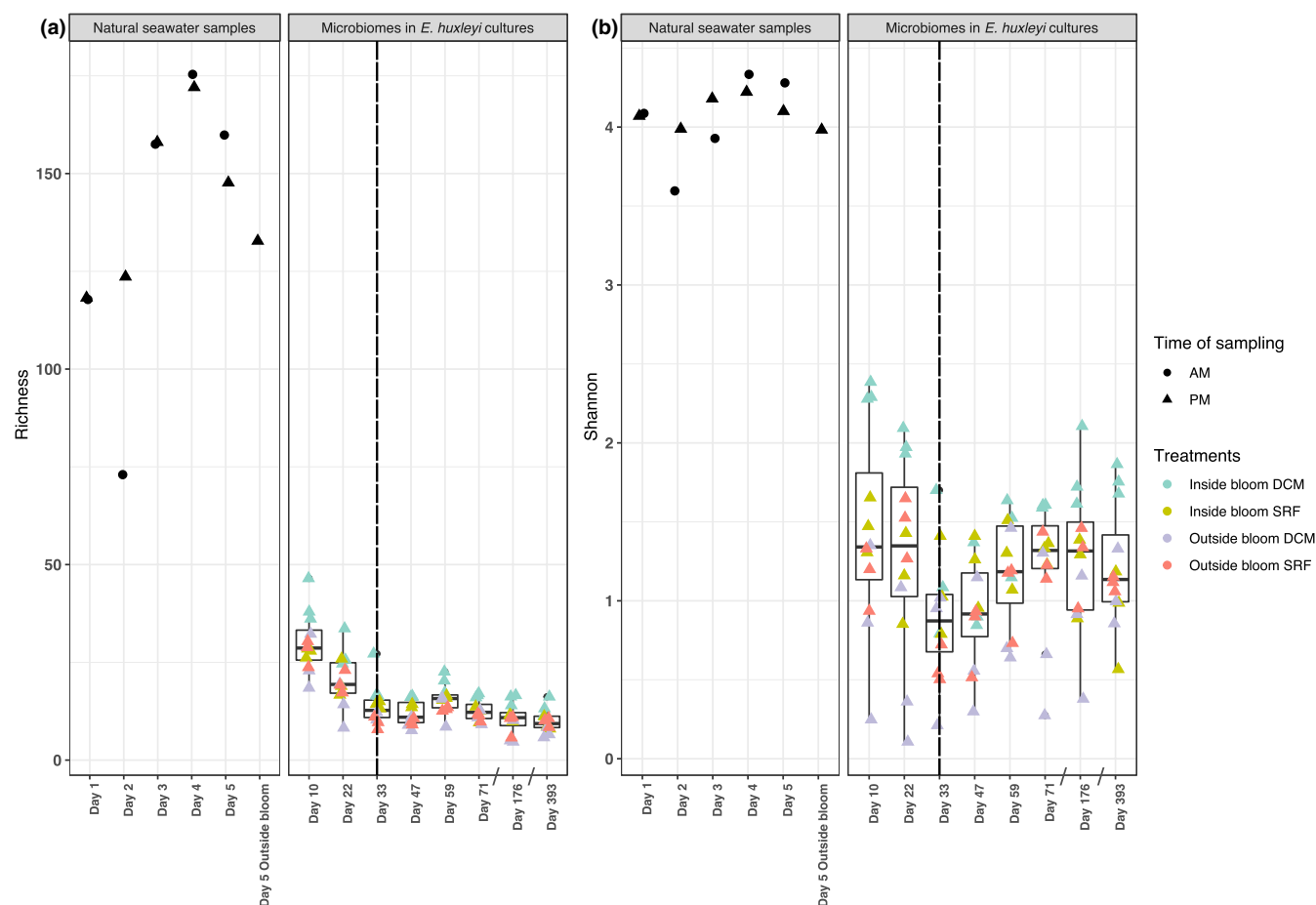


FIGURE 2 Composite representation of the dynamics of the prokaryotic richness (a) and Shannon (b) indexes in natural samples (0.2–3 μm DCM samples only) and culture experiments. ASV tables were rarefied to 2479 (minimum number of reads of the environmental samples). The boxes represent the interquartile range. The thin horizontal lines represent the 25th and 75th percentiles and the thick horizontal line represents the median. The vertical lines indicate the minimum and maximum values (using 1.5 coefficients above and below the percentiles). The dots represent the values measured for each culture. Dots further than the vertical lines represent potential outliers. The x-axis in the experiment plot are not proportional to the length of time between samplings. The black dotted line represents the time where the cultivation conditions changed.

reads), *Synechococcus* sp. (7%), SAR86 clade (5%) and uncultured Rhodobacteraceae members (4%) remained stable along the bloom survey (Figure S3). Several other genera demonstrated substantial abundance shifts. *Asciadiaceihabitans* and *Sulfitobacter*, accounting for 7% in the first sampling period, decreased in the second and third sampling periods. *Nitrosopumilus* (Thaumarchaeota), Thermoplasmata (Marine group II euryarchaeota), *Vibrio* and *Pseudoalteromonas* exhibited an inverse pattern. As low as 2% or less in the first sampling period, their abundance increased significantly (9%–14%) in the second sampling period. *Pseudoalteromonas* proportions persisted (14%) in the third sampling period while those of other taxa decreased to their initial numbers. Not detected or at very low abundance in the first sampling periods, *Alteromonas* peaked (up to 4%) only at the end of the bloom survey.

On day 5, prokaryotic communities outside the bloom grouped with those collected inside the bloom area (Figure S2). In both samples, the fourth most abundant taxa making up to >44% of the total reads were identical and displayed very close proportions (*Pseudoalteromonas* 19% and 21% in inside and outside bloom, respectively, *Synechococcus* 9% and 7%, SAR11 clade Ia 8% and 11%, and *Alteromonas* 8% in both samples). The main compositional differences in these samples, which served as inocula for the community assembly experiment, were the abundance of *Lentimonas* (5% inside vs. 2% outside bloom), and of the OM60 (NOR5) clade and Marine group II members (each of them accounting for 2% inside vs. 4% outside bloom). Relative abundances of the clades that were recruited from these samples during the experiment were lower and close in both samples (OM43 clade, 1.5% and 2.3% in inside and outside bloom, respectively; KI89A clade, 0.6% and 0.4%; *Luminiphilus*, 0.7% and 0.3%; *Aurantivirga*, 0.1% and 0.3%; *Polaribacter*, 0.5% and 0.2%) or similar (SAR92 clade, 1.5%).

3.2 | Community assembly experiment

3.2.1 | Dynamics of cell concentrations and alpha diversity patterns

Seawater samples used to inoculate axenic *E. huxleyi* cultures were filtered through 0.45- μm membranes to remove phototrophs (autotrophic eukaryotes and *Synechococcus* populations) and overcome their effects on microbiome assembly. FCM analysis demonstrated that about 40% of the initial bacterial cell concentration was lost after this filtration step. As addressed in the Discussion, we acknowledge that this step may have also biased the bacterial composition in the inocula. Due to limited incubation space onboard, cultures were incubated at low light (20 $\mu\text{mol photons s}^{-1} \text{m}^{-2}$) and these light conditions were maintained during the first weeks of incubation. However, a drastic decrease (~80%) of *E. huxleyi* cell concentrations was observed in all the treatments between day 10 and 33 (Figure S4a). To avoid culture crash, we increased the light intensity to $70 \pm 20 \mu\text{mol photons s}^{-1} \text{m}^{-2}$ and larger microalgal inocula (10% of the final culture volume instead of 1%) were used to transfer

the cultures at day 33. *E. huxleyi* cell densities gradually increased at each subsequent transfer until day 71. At that point, they reached the highest cell concentration ($9.9 \times 10^5 \pm 1.2 \times 10^5 \text{ cells ml}^{-1}$) and remained stable up to the end of the experiment (Figure S4a). Bacterial cell concentrations followed an opposite trend during the first weeks of incubation. After a rapid increase (~94%) from day 10 to 47, they decreased once *E. huxleyi* abundance became higher and remained relatively stable up to the end of the experiment (Figure S4b).

Regarding the structure of the bacterial community, a severe loss of richness was observed between the environmental and culture samples (Figure 2a). At day 10, the bacterial richness in the cultures was about one-fifth of the richness in the natural samples ($30 \pm 8 \text{ SD}$, $n = 12$) (Figure 2a). This reflected a parallel decrease in the Shannon index, which at day 10 was about one-third the values recorded in environmental samples ($1.4 \pm 0.6 \text{ SD}$, $n = 12$, Figure 2b). Over the course of the experiment, we observed a decrease in richness along the first 3 weeks (mean decrease $25 \pm 7 \text{ SD}$, $n = 12$, until day 47) (Figure 2a and Figure S4c). After an increase at day 59 that corresponded to the period of culture recovery, the richness values decreased again and remained stable until day 393 ($12 \pm 2 \text{ ASVs}$). The decrease of richness was mainly associated with the loss of low-abundance ASVs, while the dominant ones remained over the course of the experiment (Figure S5). In general, the Shannon index also decreased from day 10 to day 33 (mean decrease of $0.5 \pm 0.6 \text{ SD}$, $n = 12$) and then gradually increased to values (i.e., 1.2 ± 0.4 at day 393, $n = 12$) similar to that from day 22. The highest richness and Shannon indexes were obtained in the treatments amended with the inside bloom DCM sample (richness, $26 \pm 12 \text{ ASVs}$; Shannon, 1.6 ± 0.4 , $n = 24$).

3.2.2 | Dynamics of beta diversity patterns in recruited microbiomes

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

Statistical significance of the effect of treatments, replicates and time, as well as the interaction of treatment and time on the diversity of the microbiomes was assessed by PERMANOVA and nested PERMANOVA. Before performing PERMANOVA, we tested the beta-dispersion (variance) of the microbiomes grouped by treatments, time, treatments over time, and replicates. The dispersions (variance) of treatments as well as the interaction of treatments over time were not homogeneous for both metrics tested ($p < .05$). On the other hand, dispersions were probably homogeneous over time and across replicates ($p > .05$). Still, PERMANOVA results are robust to dispersion for balanced designs like ours (Anderson &

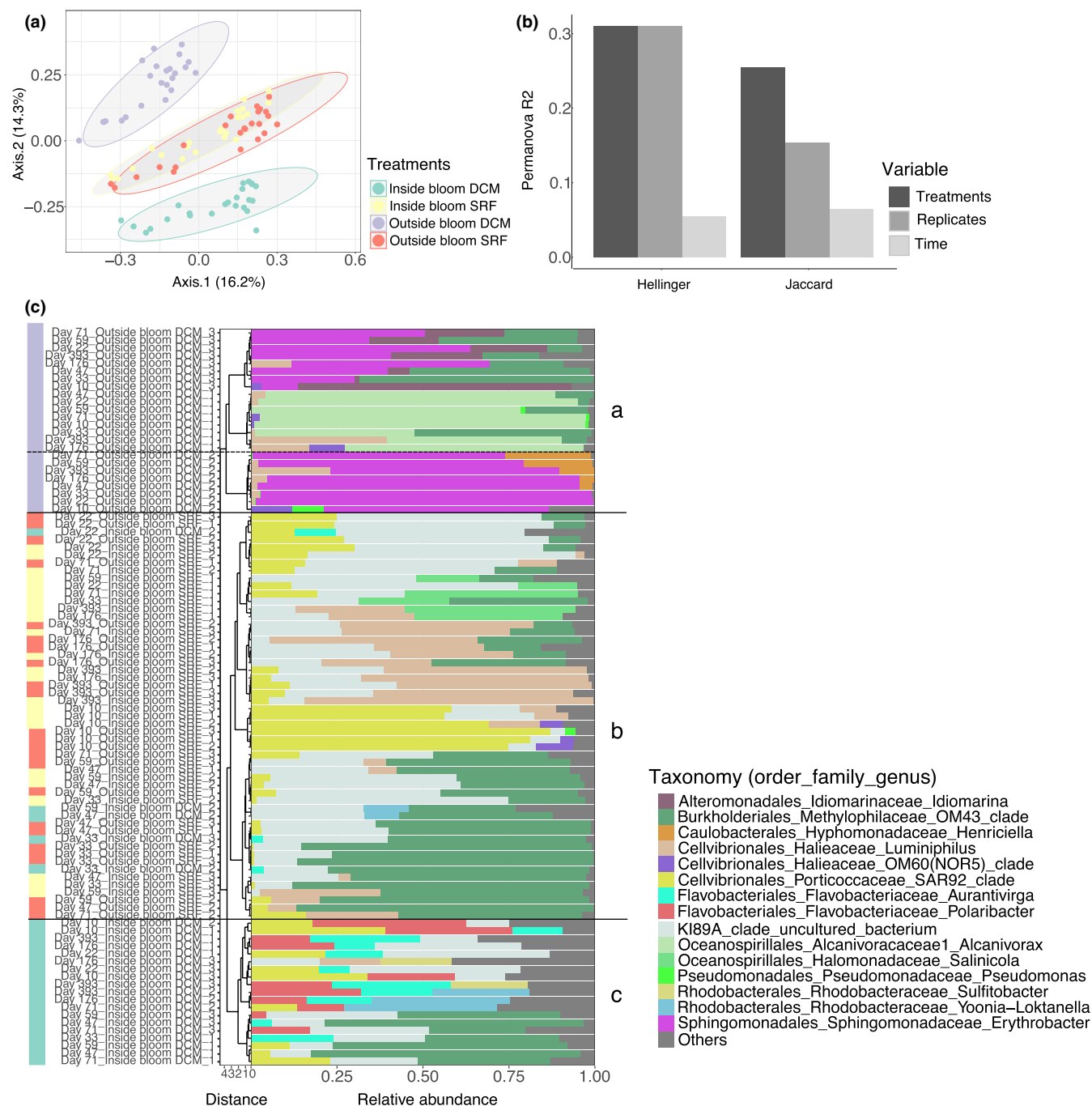


FIGURE 3 Beta diversity patterns of *E. huxleyi* microbiomes across treatments and time. (a) Principal coordinates analysis (PCoA) using a Jaccard dissimilarity matrix of the presence–absence-transformed rarefied table. Colours correspond to each treatment that received prokaryotic communities from different water samples: Green, inside bloom DCM; yellow, inside bloom surface (SRF); purple, outside bloom DCM; red, outside bloom SRF. Ellipses represent 95% confidence. (b) Permutational multivariate analysis of variance (PERMANOVA) and nested PERMANOVA r^2 of significant variables ($p < .01$) using both metrics (see Tables S3 and S4 for detailed results). (c) Hierarchical clustering produced with the Hellinger distance matrix using “ward.D2” method. Codes of each microbiome are experiment sampling day_ treatment_replicate. On the left, colours used for each treatment are the same as in (a). Bar plots indicate the taxonomy of the three most abundant genera. The other genera were merged as “others”.

Walsh, 2013). PERMANOVA results of Hellinger and Jaccard dissimilarities showed that significant proportions of the variance in microbiome composition among samples were explained by treatments (31% and 25%, respectively), replicates (31% and 15%, respectively) and time (6% and 7%, respectively) ($p < .01$) (Figure 3b; Tables S3 and

S4). Although the interaction between treatments and time was significant using Hellinger distance ($F = 2.23$; $p = .016$), it explained a small proportion of the variance (2.5%).

Clustering using Hellinger distance revealed that the prokaryotic community composition of all the cultures grouped into three

major clusters (Figure 3c) supporting the PCoA using Jaccard dissimilarity results. Based on the Hellinger distance, the outside bloom DCM treatment samples (cluster a) formed two subclusters, highlighting the compositional differences between the three replicates. Replicate 1 was dominated by *Alcanivorax* (78%, $n = 8$), while *Erythrobacter* prevailed in replicate 2 (88%) and replicate 3 (45%). Cluster b mainly consisted of microbiomes recruited from both surface water samples. Surface treatments were dominated by bacteria related to OM43, K189A and SAR92 clades, and to *Luminiphilus*. The third main cluster (c) formed entirely by microbiomes from the inside bloom DCM treatment was dominated by members of the OM43 (29%), K189A (28%) and SAR92 clades and *Polaribacter* (11% each). With the exception of those related to *Sulfitobacter*, *Luminiphilus*, OM43 and the OM60(NOR5) clades, ASVs that dominate ($\geq 1\%$) in the cultures amended with inside and outside bloom DCM samples were generally low in abundance or not detected in the initial bacterioplankton community (Figure S6).

The number of indicative ASVs for each treatment varied widely and was significantly higher (21 out of 29) in cultures amended with inside bloom DCM waters (Table S5). Flavobacteriales, with

Aurantivirga and *Polaribacter* in particular, was the order containing the most indicative ASVs of microbiomes recruited from the inside bloom DCM sample. Members of the SAR92 and K189 clades displayed high *IndVal* indexes in both inside bloom DCM and surface samples. The indicator ASVs of outside bloom DCM treatment were related to *Erythrobacter*, *Alcanivorax* and the OM60(NOR5) clade.

Besides the compositional differences among treatments, we observed a somewhat cyclical pattern of beta-diversity over time using Hellinger distances (Figure 4). Microbiome community compositions clearly differed from each other from days 10, 22 and 33 for all treatments, but they tended to become gradually similar to their initial status at the following time points. This cyclical pattern was observed for inside and outside bloom surface treatments (Figure 4a,c) and was particularly evident in the inside bloom DCM treatment (Figure 4b). In these treatments, the dynamic was mainly driven by the dominance of the OM43 clade (ASV1) during the alga crash. In the algal growth recovery phase, the increased abundance of *Luminiphilus* (ASV6) in surface treatments and of *Aurantivirga* (ASV12) and *Polaribacter* (ASV15) in inside bloom DCM cultures were also involved (Figure S7). The contributions of these ASVs were

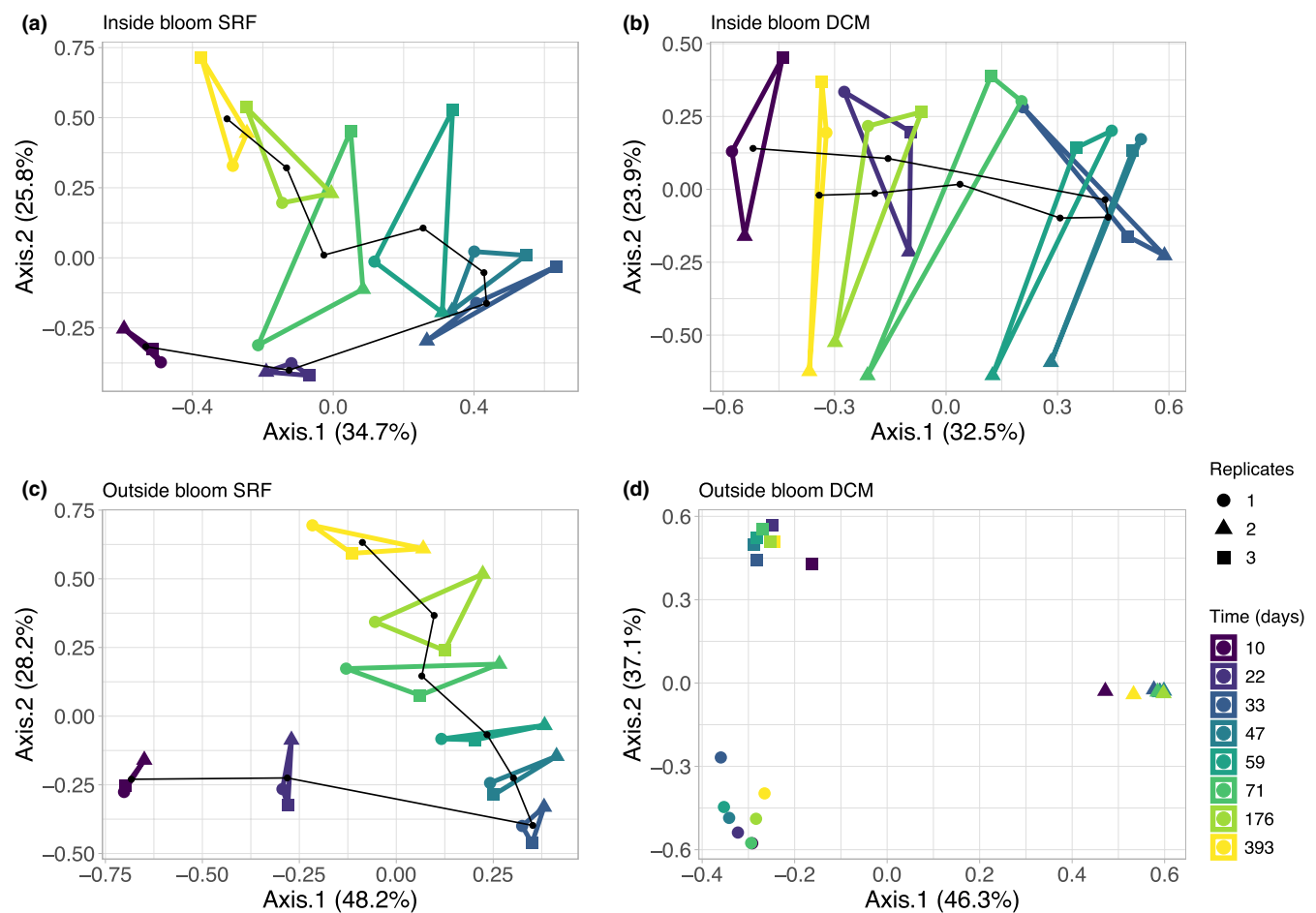


FIGURE 4 Principal component analysis (PCA) showing the cyclical patterns of microbiome beta diversity. Community distances (Euclidean distances of Hellinger-transformed data) are shown for microbiomes from inside bloom surface (SRF) (a), inside bloom DCM (b), outside bloom SRF (c) and outside bloom DCM (d). The polygons link replicates (shape coded) at each DNA sampling (colour coded). The black line links the barycenters of the replicates.

also supported by the PCA species loadings. No cyclical pattern was observed for outside bloom DCM cultures (Figure 4d). Instead, in line with hierarchical clustering results (Figure 3c), dissimilarities in these cultures were higher between replicates than over time.

4 | DISCUSSION

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

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

The composition of the bacterial community, that is the presence of Flavobacteriaceae, Pseudoalteromonadaceae, Alteromonadaceae and members of the genus *Sulfitobacter*, indicated that the *E. huxleyi* bloom had already entered the decaying phase when we started the sampling (Buchan et al., 2014; Lovejoy et al., 1998). Indeed, Flavobacteriia are reported among the main bacteria present in the declining phase of phytoplankton blooms (Landa et al., 2016; Teeling et al., 2012, 2016), which seems linked to their capacity to degrade high-molecular-weight substrates such as proteins and polysaccharides (Cottrell & Kirchman, 2000; Fernández-Gomez et al., 2013; Francis et al., 2021; Kappelmann et al., 2019; Kirchman, 2002). Furthermore, the algicidal effects of *Pseudoalteromonas*, *Alteromonas* and *Sulfitobacter* strains and species have been documented in many microalgae including *E. huxleyi* (Barak-Gavish et al., 2018; Holmström & Kjelleberg, 1999; Li et al., 2018; Meyer et al., 2017), which calls attention to their potential role in termination of the *E. huxleyi* bloom (Barak-Gavish et al., 2018; Lovejoy et al., 1998). Satellite images and post-cruise analyses support that we started sampling the bloom in its decline phase. First, the high-reflectance patch visible on the satellite images (Figure 1b) and the daily vanishing of the coccolith-derived turbidity signal observed from the interpolated images of nonalgal SPM were both indicative of detached coccoliths from dead *E. huxleyi* cells (Neukermans & Fournier, 2018; Perrot et al., 2018). This assumption was confirmed by the complete disappearance of the coccolith-derived turbidity signal a couple of days after we left the sampling area. Second, a suite of ongoing experiments on the bloom samples using diagnostic lipid- and gene-based molecular biomarkers (Hunter et al., 2015; Vardi et al., 2009; Vincent et al., 2021; Ziv et al., 2016) revealed the detection of specific viral polar lipids and visualized *E. huxleyi*-infected cells during bloom succession, suggesting that Coccolithovirus infections may have partially participated in the demise of the *E. huxleyi* bloom (F. Vincent, C. Kuhlisch, G. Schleyer, pers. comm.) as often proposed (Bratbak et al., 1993; Laber et al., 2018; Vardi et al., 2012).

As the bloom decline progressed, rapid and important shifts of the prokaryotic community were observed, probably reflecting a direct

response by certain bacterial taxa to specific *E. huxleyi*-derived organic matter. Senescence compounds from decaying *E. huxleyi* cells probably fuelled members of the genus *Asciadiaceihabitans* (formerly the *Roseobacter* OCT lineage) (Wemheuer et al., 2015), whose relative abundances typically fluctuate during phytoplankton blooms (Chafee et al., 2018; Choi et al., 2018; Hahnke et al., 2015; Lucas et al., 2016) and promoted other functionally different transient taxa that represent key prokaryotic members during bloom decline. Among them, Thermoplasmata are generally associated with protein and lipid degradation (Orellana et al., 2019) while *Nitrosopumilus* may be favoured by nitrite accumulation caused by algal release (Kim et al., 2019). The opportunistic *Vibrio* was among the most rapidly responding bacterial heterotrophs in the bloom termination conditions, probably degrading organic matter released from algal cells (Eiler et al., 2007) while *Alteromonas* spp. have the metabolic capacity to degrade a diverse set of complex compounds such as polysaccharides during the later stages of the bloom (Reintjes et al., 2019).

4.2 | Community composition of environmentally recruited *E. huxleyi* microbiomes

Since our primary objective was to study the bacterial community selection and assembly by a single phytoplankton host, we used a filtration step to discard autotrophic phytoplankton cells, such as *Synechococcus* and picoeukaryotes abundantly represented in the initial planktonic communities (Table S2). We acknowledge that this filtration strategy has removed large and particle-attached prokaryotes, the latter probably being abundant in the demise phase of the bloom, and has induced substantial modifications in the initial community composition of the inocula and finally in the recruited microbiomes. Indeed, some of the main taxonomic groups recruited in the treatments were not previously reported in *E. huxleyi* and other phytoplankton cultures or were in low abundance, notably *Luminiphilus*, and the clades SAR92, KI89A and OM43 (Câmara dos Reis, 2021; Green et al., 2015). Except members of the OM43 clade (Yang et al., 2016), these bacteria are known as important groups of oligotrophic marine Proteobacteria that do not usually grow in the rich organic matter conditions provided in phytoplankton-derived cultures (Cho & Giovannoni, 2004; Spring & Riedel, 2013). Another unexpected result of our study is the very low representation of *Marinobacter* spp. in the recruited microbiomes whereas previous studies have reported their dominance in cultures of worldwide *E. huxleyi* isolates (Câmara dos Reis, 2021; Green et al., 2015). We found them in low abundance in inside and outside bloom waters (0.15%–0.2%), similar to multiyear average values (0.18%) in the Western English Channel (Gilbert et al., 2012). Since *Marinobacter* can be closely associated with particulate organic matter, including the eukaryotic phytoplankton population (Sonnenschein et al., 2012; Thompson et al., 2020), we cannot fully exclude the possibility that the filtration step impacted the abundance of *Marinobacter* in the inocula and finally in the *E. huxleyi* cultures. A more likely hypothesis however is that low light conditions might have induced algal

cell death, promoting the release of methylated compounds (Fisher et al., 2020; Reese et al., 2019). The release of methylated compounds by *E. huxleyi* may have provided a selective advantage to the specialist OM43 clade methylotrophs (Neufeld et al., 2008), dominant in all the cultures, and to other less common bacterial taxa in the absence of strong competitors such as *Marinobacter*. This hypothesis is in line with the opposite dynamics of *E. huxleyi* (decrease) and bacteria (increase) coupled to the sharp decrease of the bacterial alpha diversity during the first month of culture, indicating that a few bacterial taxa were outcompeting others. Despite the above limitations, the high reproducibility of microbiome community composition across the biological replicates suggests that they did not alter the general conclusions raised from our study.

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

We observed contrasting results between the inside and outside bloom DCM recruited microbiomes that can be linked to the origin of the samples. First, recruited microbiomes from inside bloom DCM samples were more diverse and displayed a higher number of indicative ASVs, probably reflecting the higher diversity observed in the original seawater (Figure 2). We assume that exopolysaccharides/exudates of axenic *E. huxleyi* cultures have strongly influenced the recruited microbiomes and their long-term stability. We hypothesize that the higher diversity conferred stability to the microbiomes allowing the recovery after disturbance. This may explain the almost complete cyclical pattern they followed (Figure 4b). Such cyclical patterns were shown in the surface microbiome of the seaweed *Delisea pulchra* (Longford et al., 2019) after experimental

disturbances. These authors hypothesized that the production of halogenated furanones by the red algae exerts a selective force for the establishment and persistency of early-colonizing bacteria, which may protect the host against the colonization of pathogenic bacteria in later successional stages (Longford et al., 2019). In line with our data, this study favoured the view that higher diversity in disturbed microbiomes may be a source of stability and resilience against perturbation (Longford et al., 2019). We consistently observed cyclical patterns in treatments having a high degree of uniformity in the replicates. This was not the case for the outside bloom DCM cultures whose bacterial communities displayed high levels of between-replicate variability.

Our study suggests the combined effect of deterministic processes and stochasticity on the microbiome assembly. The significant imprint of the original community in the inside bloom DCM treatments suggests that deterministic processes (e.g., pre-exposure to algal exudates in the *E. huxleyi* bloom assemblages) influenced the final microbiome composition. On the other hand, variable communities grown from outside bloom DCM treatment are consistent with stochastic assembly overwhelming any signal of ecological selection. In line with our results, both deterministic and stochastic processes were found to influence community assembly in both the surface and DCM waters of the South Pacific Gyre (Allen et al., 2020). Homogeneous selection was the dominant community assembly process at both depths. However, stochastic processes had more effect at the DCM than in the temporally stable surface waters, presumably due to the greater influence of vertical nutrient supply and higher productivity and lower influence of horizontal dispersal (Allen et al., 2020 and references therein).

5 | CONCLUSIONS

In this work, we combined an observational and an experimental approach to reveal the bacterial community structure in an *E. huxleyi* bloom and to address whether different microbial composition could influence microbiome assembly in an *E. huxleyi* culture. Our environmental data showed that the *E. huxleyi* bloom created unique ecological conditions favouring the combination of bacterial and archaeal groups that followed a clear successional trajectory. This trajectory suggests both potential algicidal bacteria–algae interactions and niche specialization by different taxa possibly corresponding to different stages in the successive degradation of *E. huxleyi*-derived organic compounds. Our experimental approach showed that the compositional homogeneity of the prokaryotic community of an *E. huxleyi* bloom in the demise phase influenced the community assembly through deterministic processes. We showed that the source of the initial bacterioplankton communities influences the resulting composition of *E. huxleyi* microbiomes. Further studies using diverse phytoplankton cultures isolated from a variety of oceanic regions and different trophic regimes could be useful to disentangle deterministic and stochastic factors driving microbiome assembly in the

marine environment. Axenic phytoplankton cultures also represent a valuable resource to explore phytoplankton–bacteria interactions. Co-cultivation of isolates corresponding to indicative ASVs and *E. huxleyi* will be helpful to decipher how they interact. Future analyses combining transcriptomic and metabolomic analyses will provide valuable information about the genes and molecules involved in these ecologically key interactions.

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Environmental samples are deposited in the bioproject PRJEB50692. Reads are deposited under accession nos. ERX9644183–ERX9644242 and biosamples under accession nos. ERS10466567–ERS10466596. Samples from the assembly experiment are deposited in the bioproject PRJEB48747. Reads are deposited under accession nos. ERX9767825–ERX9768004 and biosamples under accession nos. ERS10539058–ERS10539153.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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