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Abstract

Global warming affects primary producers in the Arctic, with potential consequences on the bacterial community composition through the consumption of microalgae-derived dissolved organic matter. To determine the degree of specificity in the use of an exudate by bacterial taxa, we used simple microalgae-bacteria model systems. We isolated 92 bacterial strains from the sea ice bottom and the water column in spring-summer in the Baffin Bay (Arctic Ocean). The isolates were grouped into 42 species belonging to Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. 40 strains were tested for their capacity to grow on the exudate from two Arctic diatoms. Most of the strains tested (78 %) were able to grow on the exudate from the pelagic diatom *Chaetoceros neogracilis* and 33 % were able to use the exudate from the sea ice diatom *Fragilariopsis cylindrus*. 17.5% of the strains were not able to grow with any exudate while 27.5 % of the strains were able to use both types of exudates. All strains belonging to Flavobacteriia (n = 10) were able to use the DOM provided by *C. neogracilis*, and this exudate sustained a growth capacity of up to 100 times higher than diluted marine broth medium, of two *Pseudomonas* sp. strains and one *Sulfitobacter* strain. The variable bioavailability of exudates to bacterial strains highlights the potential role of microalgae in shaping the bacterial community composition.

Keywords: Arctic Ocean; diatoms; Dissolved organic exudates; Biodegradation; Bacterial isolation; Bacterial diversity.

1. Background

Phytoplankton are major contributors to the dissolved organic matter (DOM) pool in marine environments by different processes: grazing by zooplankton, viral lysis, autolysis and exudation [1].

Dissolved organic exudates from phytoplankton are considered as labile and semi labile molecules composed of sugars (monosaccharides, oligosaccharides and polysaccharides), nitrogen compounds (amino acids, polypeptides or proteins), lipids (fatty acids) and organic acids (vitamins) [2]. On average, DOM exudation accounts for 10-20 % of total primary production in the marine environment and for 2-10% (average 5 %) for exponentially growing cells [1]. However, different environmental factors may increase the percentage extracellular release (PER), such as suboptimal light intensity and temperature, nutrient limitation and high pCO_2 [2–4].

Phytoplankton primary production and PER are both affected by the unprecedented changes occurring in the Arctic Ocean over the past several decades due to climate variations and human activities. The Arctic air temperatures have increased by 2.7°C on the annual scale over the past 47 years (1971-2017), which is twice as fast as in the rest of the Northern Hemisphere [5]. This global warming led to dramatic modifications in the Arctic sea ice, both in the rates and the magnitude of change in extent, area, thickness, and spatial distribution [5]. This results in a greater transmission of light through the ice and into newly formed open ocean areas [6]. It is expected that the increase in light intensity in the water column will promote phytoplankton primary production and biological productivity throughout the Arctic Ocean [6,7]. In addition, while phytoplankton blooms were until recently considered negligible in the water column under the ice pack [8,9], recent studies have shown the existence of blooms under the sea ice in the marginal ice zone [10–13]. Diatoms are an important component of the primary production in both pelagic and under sea ice environments during spring blooms in Artic Ocean [14–17]. However, measurements of PER in the Arctic Ocean are scarce. Engel et al. [3] measured PER values between 11 % and 23 % during mesocosm experiments conducted from June to July in Kongsfjorden, Svalbard.

Bacteria are key organisms in carbon cycling in aquatic ecosystems, acting as a sink (mineralization of dissolved organic carbon to CO_2) or as a link (production of biomass which can be transferred through the microbial food web) [18]. Roughly 50% of carbon fixed by primary production in the ocean is passing through bacteria [19]. The quality of the DOM produced by phytoplankton constitutes an important regulator of both the metabolism and composition of bacterial communities [20]. Interactions between heterotrophic bacteria and diatoms are complex [21]. On the one hand bacteria exert positive (e.g., production of vitamins) or negative effects (e.g., production of algicidal metabolites) on diatom growth [22,23] and can also modify the composition of the diatom exudates [24]. These effects appear to be diatom species-specific, resulting in compositional shifts of diatom communities when different diatom species were grown together [25]. On other hand, DOM released during a diatom bloom can reshape the bacterial community by favoring some bacterial groups like Roseobacter, Sulfitobacter and Flavobacteriaceae, that are more adapted to the rapid use of substrates released by diatoms [20]. Consequently, the modification of the primary producers in the Arctic Ocean induced by the global

warming have potential consequences on the bacterial community composition through the consumption of microalgae-derived dissolved organic matter [26]. Some experimental studies have shown that bacterial community composition changes are dependent on microalgae species [27], whereas other studies observed a weak bacterial selectivity of exudates produced by specific algae species [28,29]. However, extrapolation of these results is complicated by temporal [30] and spatial [31] changes in the bacterial response to the same DOM exudate. Additionally, these different studies have been performed on natural bacterial communities where complex interactions can occur (e.g., competition, co-metabolism, antibiosis). By contrast, the capacity of distinct bacterial strains to use the exudates from phytoplankton is not well known. Only a simplified approach, combining one bacterial strain with the excretion product of one phytoplankton species allows to decipher more precisely the intrinsic capacity of bacteria to use diatom DOM. This type of study has thus far not been carried out and it complements those with natural microbial consortia.

To determine the degree of specificity in the use of an exudate by bacterial taxa, we used simple microalgae-bacteria model systems. We first identified different bacterial strains isolated from the sea ice bottom and water column during the spring phytoplankton bloom in the Baffin Bay. The field work for the isolation of the bacterial strains was carried out as part of the Green Edge project (http://greenedge-expeditions.com/) with its main objective to study the phytoplankton development in spring [32]. The study site, which is located in a marginal sea (360 m depth) of the Baffin Bay, is covered by a seasonally changing layer of ice. The ecological relevance of the bacterial strains isolated was evaluated by comparing them with the environmental 16S rRNA sequences retrieved during the same sampling period [33]. Then, we followed the capacity of the bacterial strains to grow on the exudates produced by two Arctic diatoms, *Chaetoceros neogracilis* and *Fragilariopsis cylindrus*, characteristic of planktonic and sea ice environments, respectively [17,34,35]. This experimental design allowed us to study the link between the phylogeny of isolated bacteria as well as their origin of their isolation and their capacity to degrade each type of exudate.

2. Methods

2.1. Sampling

A sampling campaign was conducted from April 20th to July 27th in 2016, on an ice camp located near Qikiqtarjuaq (Nunavut), in the Canadian Arctic at 67°28.784' N, 063°47.372' W (Fig. 1). This period was chosen in order to capture the dynamics of the sea-ice algae and phytoplankton spring bloom from initiation to termination. Water sampling was carried out through a 1x1 m hole in the ice pack shielded by a tent. Water samples were collected every week at 2 m and 40 m (or 60 m) depths using a Niskin bottle. The bottom of the first-year sea ice (first 10 cm) was subsampled from sea ice cores collected using a manual ice corer and melted at 4°C.

2.2. Bacterial strains isolation and identification

An aliquot of each sample (0.5 ml) was placed in cryotubes containing 500 µL of 70 % sterile glycerol and stored at –80°C before isolation at the laboratory. After thawing, 100 µL of each sample were spread in triplicate on Marine Agar (MA) medium ([36], Supplementary Table S1) and the petri dishes were incubated in the dark at 4°C to allow the development of aerobic heterotrophic psychrotolerant marine bacteria. At regular intervals (1, 2 and 3 weeks), the colonies were counted and categorized based on their morphological characteristics (morphotype). Representatives of each morphotype were selected for isolation by repeated streaking. Finally, we isolated 92 bacterial strains in total.

After isolation and purification, each strain was cultivated in Marine Broth (MB) medium ([36], Supplementary Table S1) at 4°C under agitation and darkness to identify the strains by partial sequencing of the 16S rRNA. Bacterial cells corresponding to each strain were recovered by centrifugation (3 min at 14000 g), from 2 mL of MB culture. The DNA was then extracted using the Wizard® Genomic DNA Purification kit (Promega) following the manufacturer's recommendations. The DNA was amplified by PCR using the primer pair 27f/1492r to target the 16S rRNA gene [37,38]. The complete PCR mix used contained ultrapure water (3.2 µL), KAPA2G mix (5 µL), forward primer (0.4 µM final concentration), reverse primer (0.4 µM final concentration) and DNA template (1 µL) for a total reaction volume of 10 µL. The PCR amplification conditions used were as follows: 5 min at 95°C; 35 cycles of 15s at 95°C, 15s at 50°C and 15s at 72°C; 5 min at 72°C and finally holding at 4°C in a VeritiTM Thermal Cycler (Applied Biosystems). The PCR products, previously controlled by electrophoresis, were then purified using the AmpliCleanTM Magnetic Bead-Based PCR Cleanup kit (Nimagen) according to the manufacturer's instructions. DNA was then sequenced by the Sanger method [39]. To do this, a sequence reaction was performed on the samples using the 907r primer [40]. The complete PCR mix used contained ultrapure water (5.75 µL), BDT buffer (1.75 µL), BigDye Terminator v3.1 kit (0.5 µL), primer (3.2 µM, 1 µL) and DNA matrix (1 µL) for a total reaction volume of 10 µL. The PCR amplification conditions used were as follows: 40 cycles of 10 s at 95°C, 5 s at 50°C and 2.5 min at 60°C in a VeritiTM Thermal Cycler (Applied Biosystems). The PCR reaction products were cleaned with the D-pure DyeTerminator Clean-up kit (Nimagen) before being sequenced using a Sanger 16 capillary sequencer AB3130XL (Applied Biosystems).

2.3. Sequences analysis

Partial 16S rRNA gene sequences were trimmed , quality controlled and dereplicated using the package Staden-GAP4 [41]. For bacterial strain identification, each FASTA file was uploaded in Ez Taxon-e [42] and compared with the cultured bacterial strain database using Basic Local Alignment Search Tool (BLAST). The sequences were aligned using the MUSCLE multiple alignment method [43]. A hierarchical Likelihood Ratio Test (hLRTs) was performed to determine the most appropriate substitution model for

our dataset. We used default parameters for these different tools. Finally, a phylogenetic tree was constructed from the model used according to the Maximum Likelihood (ML) criterion. To study the robustness of the tree nodes, a bootstrap analysis was performed with 1000 repetitions. All sequence processing was performed using MEGA X v10.0.1 software [44]. Sequences were deposited in Genbank (NCBI) under the following numbers: MK 224724-224815 for the 92 bacterial strains isolated during this study and MK 217878-217881 for the four bacterial strains isolated in August 2009 at 3 m depth in the Beaufort Sea and used in the microplate growth assay (see below). The strains were registered in the Banyuls Bacterial Culture Collection (BBCC) (https://collection.obs-banyuls.fr/index.php).

2.4. Culture of arctic microalgae and recovery of exuded dissolved organic matter

Two diatoms from the Roscoff Culture Collection (RCC) of the Roscoff Biological Station (http://roscoff-culture-collection.org/) were used: the pelagic strain RCC 2278 *Chaetoceros neogracilis* (Mediophyceae, [45]) and the ice strain RCC 4289 *Fragilariopsis cylindrus* (Bacillariophyceae, Grunow ex Cleve). Both strains were isolated from Arctic Ocean. Cultures were not axenic. These diatoms were cultured at 4°C with a continuous white light intensity of 10 µE in K/2 medium+Si [46] for *C. neogracilis* and L1 medium [47] for *F. cylindrus*. The composition of these different media is given on the website of the RCC (http://roscoff-culture-collection.org/). The diatoms were then transferred to a modified K culture medium in which (1) tris(hydroxymethyl)aminomethane [Tris-Base], Na_2-Glycerophosphate [$C_3H_7O_6PNa_2$, 5 to 6 H_2O], and ethylene diamine tetra acetic [EDTA] as organic molecules were removed, (2) ammonium was removed to reduce bacterial growth during culture and (3) iron concentration was reduced to 11.7 µM to limit iron precipitation due to the elimination of EDTA. The culture flasks containing 200 mL of modified K medium were incubated under the same conditions as before. Cell growth was measured by flow cytometry (BD Accuri C6 Plus, Becton Dickinson). The samples were fixed with glutaraldehyde (0.25 % final) and stored at –20°C before analysis. The diatoms were detected by chlorophyll fluorescence and side angle light scattering properties using blue laser (488 nm). Maximum growth rates (µmax) in exponential phase were calculated using cell counts according to the equation $\mu_{max} = \ln (N_2/N_1) / (t_2–t_1)$, where µ = doublings d^{-1}; N = algal abundance ml^{-1}; and t = time, d^{-1}. Bacteria were also counted after staining with SYBR Green (Invitrogen, 0.05 % final) for 10 min in the dark before to be run by flow cytometry. The bacteria were detected by green fluorescence and side angle light scattering properties using blue laser (488 nm). Flow cytometric results were processed using the BD CSamplerTM C6 v1.0.241.21 software (Becton Dickinson). Results were analyzed with the FlowJo v7.6.1 (FlowJo LLC) software.

The diatom DOM was harvested after 40-44 days of cultivation, corresponding to a prolonged stationary phase. This time period was chosen to assure the collection of a sufficient amount of organic exudates for the subsequent bioassay experiments. Therefore, triplicate culture flasks for each strain were pooled

and then centrifuged for 15 min at 5166 g at 4°C. The supernatant was then filtered through two GF/F filters (0.7 µm, 47 mm, Whatman), using a glass vacuum flask. To avoid any organic contamination, the filters and glassware were previously combusted at 450°C for 6 h. The filtrate of each strain was stored at –20°C in Nalgene vials, previously washed with 10% hydrochloric acid (HCl) and then rinsed with Milli-Q® water. Inorganic nutrients and dissolved organic carbon (DOC) were analyzed at the beginning and at the end of the culture for each diatom strain (see below). Particulate organic carbon (POC) and dissolved and total saccharides were analyzed at end of the culture (see below).

2.5. Chemical analyses

Samples for POC (10 ml aliquots of culture in duplicate) were filtered onto pre-combusted (450°C for 6 h) GF/F filters, which were then stored at −20°C. Before analysis, filters were placed in a desiccator for 48 h at room temperature. Samples were analysed with a Carlo Erba Instruments EA1108 elemental analyser using an acetanilide standard as reference. Samples (10 ml aliquots of culture in duplicate) for nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicate ($Si(OH)_4$) were stored at −20°C before analysis on a nutrient autoanalyzer (SEAL Analytical AA3HR). DOC samples (20 ml aliquots of culture in duplicate) were filtered through two overlaid combusted (450°C, 6 h) 25 mm GF/F filters. The filtrate was transferred into combusted glass tubes, poisoned with 85% H_3PO_4, closed with Teflon-lined screw caps and stored in the dark at room temperature until analysis. Samples (65 ml) for dissolved and total saccharides analyses were stored at −20°C.

Carbohydrates were determined in the filtrates of each strain by the TPTZ method [48,49]. This method detects the following categories of monosaccharides: hexoses (fructose, galactose, glucose, and mannose), pentoses (ribose, arabinose, and xylose), deoxy-sugars (fucose and rhamnose), and uronic acids (glucuronic, galacturonic, and mannuronic acids). Dissolved free monosaccharides (DFCHO) were determined without acid hydrolysis. Total dissolved monosaccharides (TDCHO) were determined after acid hydrolysis using 1 M HCl (final concentration) at 100°C for 20h [50]. Hydrolysis was stopped after placing the tubes in an ice bath for 5 min. Combined monosaccharides (i.e., dissolved polysaccharides or DPCHO) were then calculated as the difference between TDCHO and DFCHO. A standard calibration curve was obtained by the analysis of five glucose standards spanning a concentration 0.2 to 2 $mg.L^{-1}$. The response was linear over the above concentration range (R^2 = 0.999) and monosaccharides concentrations were converted to C-equivalents by using a molar conversion factor of six.

2.6. Microplate assay to measure bacterial growth with microalgae exudates

We studied the ability of 40 different bacterial strains, each corresponding to a different species, to degrade the DOM exuded by the two Arctic diatom strains. Among these bacterial strains, 36 were selected from the 92 bacterial strains isolated within the Green Edge project and 4 additional strains,

isolated in the Beaufort Sea at 3 m depth in August 2009 [51], were also selected. Bacterial strains were cultured in a Marine Broth at 4°C in the dark for 3 to 5 days, then 2 mL of culture were centrifuged at 8000 *g* for 5 min at 4°C. The cell pellets were washed 3 times with sterile artificial seawater [52], with a centrifugation cycle between each wash as before, in order to remove the DOM contained in the culture medium. The inoculum of each strain was resuspended in artificial seawater and the cell concentration was determined by flow cytometry as described above (Cytoflex, Beckman & Coulter).

Bacterial growth assays were conducted in 24-well microplates. Each well contained 2 ml of bacterial suspension adjusted to $2x10^5$ cell.mL^{-1} in artificial seawater. Dissolved organic exudates were added in triplicate at a final concentration of 375 µM DOC. NO_3^- and PO_4^{3-} were adjusted at 184 µM and 16 µM final concentrations, respectively, to avoid any effect of inorganic nutrients concentrations differences between the two exudates tested. Positive controls were prepared in triplicate for each strain by adding diluted MB (1/560) at the same DOC concentration than for exudates. Negative controls were also prepared in triplicate for each strain by adding a volume of artificial seawater equivalent to those of exudates and diluted MB. Microplates were incubated à 4°C in the dark with a slight agitation. Bacterial growth was monitored every 2 days for 6 days by flow cytometry using the same protocol described above, taking 50 or 100 µL samples in each well. Preliminary assays were performed with different MB dilutions to confirm that bacterial growth was measurable at this time scale of incubation with a concentration of 375 µM DOC (Fig. S1).

To estimate the growth capacity of the bacterial strains, we used a first index that is the ratio of the maximum cell abundance to the initial cell abundance: $\frac{[B]max-[B]T0}{[B]T0}$. $[B]_{T0}$ corresponds the initial cell concentration and $[B]_{max}$ to the maximum cell concentration. We considered that growth was positive when this index was greater than or equal to 4, which corresponds to two cell divisions.

In order to specify the growth capacity of the strains to use exudates comparatively to the MB, a second index was calculated that considers the intrinsic growth of each strain. For this purpose, we reported the growth obtained with exudates compared to that obtained with diluted MB (positive control). The mathematical formula for this index was: $\frac{\frac{[B]\max exudate-[B]T0\ exudate}{[B]T0\ exudate}}{\frac{[B]\max MB-[B]T0\ MB}{[B]T0\ MB}}$. With $[B]_{T0}$ exudate the initial cell concentration in the exudate, $[B]_{max\ exudate}$ the maximum cell concentration with the exudate, $[B]_{T0\ MB}$ the initial cell concentration with the diluted Marine Broth and $[B]_{max\ MB}$ the maximum cell concentration obtained with the diluted Marine Broth. This index was calculated only for strains showing positive growth with index 1.

3. Results

3.1. Viable counts and cultivable bacterial diversity

Percentages of cultivability varied between 0.002 % and 4.3 % (Table 1). The percentages of cultivability were not significantly different between the different habitats even if the sea ice samples were characterized by much higher concentrations of chlorophyll *a* compared to the samples from the water column (Table 1). No temporal trend was observed in the percentages of cultivability.

Sequence analysis of the 92 isolated bacterial strains allowed identification of 42 putatively different species, according to similarity criterion ≥ 99 % (1 % divergence) for the 16SrRNA gene sequence, belonging to 4 major phyla (Fig. 2, electronic supplementary material, figure S2A). The percentages of similarity of the 16S rRNA gene between the isolated strains and the closest reference sequences were between 95.72 and 100 %. The bacterial strains were mostly similar to species previously isolated from polar environments. They belong primarily to Proteobacteria (70 % of the 92 sequences identified) and more particularly to Gammaproteobacteria, which represented 53 % of all the sequences (electronic supplementary material, figure S2). The remaining Proteobacteria were Alphaproteobacteria (16 % of the total sequences). The two other phyla were Bacteroidetes and Actinobacteria, representing 16 % and 13 % of the sequences, respectively. Finally, one bacterial strain belonging to the Firmicutes phylum was isolated.

When the phylogeny of the bacterial strains was analyzed according to the sampling habitat, we observed only slight modifications in the relative proportion of each group (electronic supplementary material, figure S2B). Among Proteobacteria, Alphaproteobacteria were more present in sea ice (25 %) compared to the water column (13 % and 12 % at 5 m and 40-60 m, respectively). Inversely, the proportion of Flavobacteriia was higher in the water column (21 % and 15% at 2 m and 40 m, respectively) as compared to the sea ice (11 %). Twenty-nine bacterial species (69 %) were specific to a given environment. Only 5 bacterial isolates (12 %) were found in both the sea ice and the water column at both depths, with the closest relatives being *Alteromonas stellipolaris* (Gammaproteobacteria) [53], *Paraglaciecola chatamensis* (Gammaproteobacteria) [54,55], *Psychrobacter aquimaris* (Gammaproteobacteria) [56], *Leeuwenhoekiella aequorea* (Bacteroidetes) [57] and *Rhodococcus cerastii* (Actinobacteria) [58].

3.2. Growth of Arctic microalgae and characterization of the dissolved organic exudates

The two Arctic diatoms, *C. neogracilis* and *F. cylindrus,* were characterized by similar growth rates (0.25 ± 0.02 d^{-1} and 0.30 ± 0.002 d^{-1}, respectively) and reached stationary phase after 12 and 24 days,

265 respectively. Inorganic nutrient analyses showed that both diatoms cultures were limited mainly by
266 $Si(OH)_4$ in the stationary phase (Table 2).
267 The exudates were recovered from the cultures after 40 days of cultivation for *C. neogracilis* and after
268 44 days for *F. cylindrus*, corresponding to a stationary phase of 32 days for *C. neogracilis* and 20 days
269 for *F. cylindrus* (Fig. 3). In terms of diatom cell abundance, the stationary phase was stable for each strain
270 until this date, suggesting that cell lysis and the consequent release of DOM by this process were of
271 minor importance. DOC concentrations were 234 µM and 109 µM for *C. neogracilis* and *F. cylindrus*,
272 respectively, at the start of the cultures and they were significantly higher at the end of the experiment
273 due to DOM exudation (Table 2). This exudation ($DOC_{final} - DOC_{initial}$) represented ≈1800 µM for *C.*
274 *neogracilis* and ≈ 870 µM for *F. cylindrus*. The DOC exuded represented 45 % of the sum of POC and
275 DOC for *C. neogracilis* and 61 % for *F. cylindrus*. Carbohydrates accounted for 4 to 18 % of the DOC and
276 were dominated by polysaccharides in the *C. neogracilis* exudates (Table 2).

277 The diatom cultures were not axenic, and an increase in heterotrophic bacterial abundance over time
278 was observed in the cultures (electronic supplementary material, figure S3). We estimated the bacterial
279 consumption of DOC considering a carbon conversion factor of 20 fg C $cell^{-1}$ [59] and a bacterial growth
280 efficiency of 50 % for bacteria growing under high primary productivity conditions [60]. Integrated over
281 the 40-44 days, heterotrophic bacteria accounted for the processing of 0.01 % (0.26 µM DOC) and 6 %
282 (56 µM DOC) of the DOC produced by *C. neogracilis* and *F. cylindrus*, respectively. These results indicate
283 that DOM present in the diatom cultures after 44 days can be considered predominantly diatom-derived
284 and weakly reshuffled by bacteria.

285 ### 3.3. Bioavailability of dissolved organic exudates for the different bacterial strains

286 We observed different growth patterns among the strains, indicative for their capacity to use the
287 exudates (Fig. 4). According to the first index, we confirmed the lack of growth in the negative controls
288 (Artificial Sea Water (ASW) without DOM addition). Surprisingly, only 60 % of the strains were able to
289 grow in the diluted MB used as a positive control. When the exudates were used as growth substrates,
290 78 % of bacterial strains (31 out of 40) responded positively to the DOM from *C. neogracilis,* and 33 %
291 to the *F. cylindrus* exudates. The Venn diagram based on these growth capacities shows that only 9
292 strains (23%) were able to grow in all different media (i.e., diluted MB and the two exudates) (Fig. 5).
293 These strains belonged to the phyla Proteobacteria, Bacteroidetes and Actinobacteria. If we focus on the
294 use of exudates, we found that 55% of the strains were able to use only a single exudate and 27.5 %
295 grew on the two exudates. Finally, 17.5% of the strains tested did not grow in any of the two exudates
296 with the majority of bacteria belonging to the Gammaproteobacteria class. We did not find specific
297 relationships between the habitat of the bacterial strains (sea ice bottom versus water column) and their
298 capacity to use the different exudates. For instance, among the 13 bacterial strains able to grow on the

exudate of the ice diatom *F. cylindrus*, only 3 were isolated from the sea ice. Venn diagrams for each type of habitat are available in supplementary data, figure S4.

Several isolates belonging to the same genus, such as *Rhodococcus*, *Maribacter* and *Pseudoalteromonas*, had different growth patterns on the same type of exudate (Table 3). All Flavobacteriia strains succeeded in degrading the exudate of *C. neogracilis*. One third of the bacterial strains obtained an index higher than two using the exudate of *C. neogracilis* while this was observed for only 13 % of the strains for the *F. cylindrus* exudate. Index 2 highlights the large variability in the amplitude of growth on exudates relative to a common reference (diluted MB). The highest values (up to 100 times higher than in diluted MB medium) were measured with *C. neogracilis* exudates during the growth of two *Pseudomonas* sp. strains and one *Sulfitobacter* strain (Table 2).

4. Discussion

4.1. Cultivable bacterial diversity

Isolation of bacterial strains by culturing techniques is key for studying their physiology and functional capabilities. The environmental relevance of isolated strains remains, however, an important issue. Cultivation on a medium rich in organic matter leads to a well-known bias by selecting copiotrophic species that are not necessarily abundant in the marine environment, except during episodes of higher productivity or significant organic matter input [61–63]. Gammaproteobacteria are generally overrepresented in the cultivable fraction compared to the natural community [64]. In our case, they represented 53 % of the isolates, whereas *in situ* bacterial community composition determined by Illumina sequencing showed that the relative abundance of Gammaproteobacteria was 47 % in sea ice and 31 % in the water column [33]. Strains belonging to the Flavobacteriaceae family were well represented among the isolated strains (n=16; 17 % of the total strains isolated). Interestingly, the survey of the bacterial community composition by metabarcoding underlined the importance of this family during the phytoplankton bloom in this region [33]. Indeed, Flavobacteriaceae were well represented in the total *in-situ* bacterial community with 16% of the total DNA sequences. Furthermore, they reflected 19% of the sequences in the sea ice bottom and 16% of the sequences in the water column. Rhodobaceteraceae bacterial family was well represented in the isolates with 10 bacterial strains. As for Flavobacteriaceae, Rhodobaceteraceae members are frequently associated with phytoplankton blooms in marine ecosystems [20].

In the sea ice bottom, the cultivable genera belonging to *Paraglaciecola* (Gammaproteobacteria) represented 12*%* of the sequences obtained by Illumina sequencing from the *in-situ* community at the same period, *Polaribacter* (Flavobacteriia) represented 8%, *Pseudoalteromonas* (Gammaproteobacteria)

represented 6% and *Colwellia* (Gammaproteobacteria) 1% [33]. In the water column, the cultivable genera with the highest *in situ* abundance were *Polaribacter* (Flavobacteriia) with 7%, *Pseudoalteromonas* (Gammaproteobacteria) with 3 %, *Paraglaciecola* (Gammaproteobacteria) with 2%, *Rhodococcus* (Actinobacetria) with 2 % and *Pseudomonas* (Gammaproteobacteria) with 1% of total sequences. This is a rough estimate, because each genus may contain many different species, of which only a few representatives have been isolated. However, these results underline the fact that the isolated strains were overall representative of the *in situ* bacterial community, especially in the sea ice bottom.

Finally, comparison of the sequences of our isolated strains with sequences in the EZBiocloud database revealed that the lowest percentage of similarity (95.72 %) was obtained for *Antarcticibacterium flavum* [65] belonging to the class of Flavobacteriia (Bacteroidetes). This low percentage of similarity suggests the isolation of a new species belonging to the genus *Antarcticibacterium* which can only be confirmed by further genetic analysis.

4.2. Biodegradability of the microalgae exudates

To assess how phytoplanktonic blooms can shape bacterial community composition, we performed a biodegradation assay to identify the capacity of several isolated bacterial strains to use the DOM produced by two polar diatoms, *C. neogracilis* and *F. cylindrus.* The exudates used in the present study originated from non-axenic diatom cultures. We estimated that heterotrophic bacteria consumed up to 6 % of the DOC over the course of culturing period, and a fraction of this organic carbon could have been released by bacteria as DOM with a chemical composition different to the consumed compounds [66,67]. Therefore, part of the readily available substrates specific for each diatom species was likely utilized or transformed prior to the biodegradation experiments. Given the overall low fraction of bacterially processed organic carbon and exudation being a continuous process throughout the culturing period, we consider the DOM used here representative for the two diatoms. This idea is further supported by our biodegradation assays that revealed a preference of the bacterial strains for the use of DOM from *C. neogracilis*, the diatom with the longer stationary phase, as compared to *F. cylindrus*.

We investigated carbohydrates, major components of phytoplankton exudates to explore differences in the chemical composition of the two DOM sources. The relative abundance of carbohydrates in phytoplankton exudates appears variable among phytoplankton species (26-80% [68] ; 6-13% [28]) and tends to increase in the stationary phase [68]. These two facts can explain why *F. cylindrus* exudates had a lower contribution of carbohydrates to the DOM (4 %) as compared to *C. neogracilis* (18 %). Polymeric and monomeric carbohydrates were consistently shown to be utilized in short-term (days) biodegradation experiments [69,70]. The lower content of this labile source of organic carbon in the exudate of the sea ice diatom as compared to the water column diatom could explain the lower number

of bacterial strains growing on this DOM. Carbohydrates are present in high concentrations (80-1300 µMC) in sea-ice as compared to the water column in the Arctic ocean [71,72]. The high similarity observed in other studies between the carbohydrate composition of sea ice and the carbohydrates produced by sea ice diatoms, including *F. cylindrus,* confirm the microalgal origin of these compounds [72].

Furthermore, we did not find a specific relationship between the habitat of bacterial strains and their capacity to use the different exudates. As explained earlier, among the 13 bacterial strains able to grow on the *F. cylindrus* exudate, only 3 strains have been isolated from sea ice samples. This may indicate that a co-metabolim is important to degrade such exudates in sea ice environment. Indeed, some bacterial strains could be able to degrade the primary energy source given by the *F. cylindrus* DOM in the ice, producing secondary compounds which in turn would be used by other bacterial strains as potentially the ones which did not grow in our biodegradation assay. Thus, our results could complement previous observations with mixed communities where cross-feeding among taxa can occur [73]. However, 6 strains out of 13 (46%) which grew on this DOM were isolated at 2m and 3m depth. Thus, it means that 69% of the strains growing on *F. cylindrus* DOM were isolated in the ice or in the first meters of the water column. This is relevant with the fact that some interactions happen between the sea ice bottom and the water column. The microalgae living in the ice excrete DOM which will be used as a source of carbon by bacteria in the first few meters of the water column. Moreover, these interactions are reinforced as spring progresses with the beginning of the sea ice melt causing the release of a significant amount of DOM into the water column, which will serve as a source of energy and carbon for bacteria [33].

The growth of bacterial strains on the diatom exudates is in part linked to their capacity to produce extracellular enzymes to degrade polymers. In the present study, polysaccharides constituted a lower fraction of the carbohydrates of the *F. cylindrus* exudate (42 %) as compared to the *C. neogracilis* exudate (68 %) (Table 2). The incubation of phylogenetically different bacterial strains with a given DOM source allowed us to determine that the genetic potential to produce extracellular enzymes, more or less specific to DOM compounds, was a selective mechanism for the use of a given exudate. Phytoplankton-derived polysaccharide composition is highly diverse, may fluctuate between species [74] and is influenced by external factors such as nutrients, light and salinity stress [75]. Jain and Krishnan [76] showed that 52 % of a total of 215 cold-adapted heterotrophic bacteria (all belonging to Gammaproteobacteria) isolated in an open glacial fjord in the Arctic Ocean were able to produce extracellular polysaccharide enzymes at 4°C. Interestingly, these authors observed high diversity in terms of enzyme activities among strains belonging to a same genus and/or species. This result can be compared to the observation we made in the heterogeneity of the growth response within the same

399 genus for the same type of exudate. Whether a DOM source is biologically labile might therefore depend
400 on the bacterial taxa and their respective functional repertoire.

401 The commonly observed rapid utilization of phytoplankton exudates by mixed microbial communities is
402 likely due to the activity of the most successful taxa. In this context, it was interesting to note that the
403 highest growth was observed for one *Sulfitobacter* strain on the *C. neogracilis* exudate. Two *Sulfitobacter*
404 Operational Taxonomic Units (OTUs) were identified as most responsive among a Southern Ocean
405 natural bacterial community to the exudate of *Chaetoceros debilis* [77]. This bacterial group is known
406 for its capacity to use organic sulfur compounds that could be the basis for this distinct diatom-bacteria
407 association. *Sulfitobacter* could have responded positively to the dimethylsulfide produced in the *C.*
408 *neogracilis* culture, even if this diatom has been shown to emit this compound at a lower rate as
409 compared to *Emiliania huxleyi* [78].

410 All Flavobacteriia, including a representative of the genus *Polaribacter*, had the capacity to use the *C.*
411 *neogracilis* exudate. Flavobacteriia and more precisely the genus *Polaribacter* was identified as a
412 bacterial group responding positively to diatom blooms in polar regions [30,33]. Recently, we confirmed
413 the responsiveness of *Polaribacter* after the addition of *C. neogracilis* exudates to a natural bacterial
414 community present in the Baffin Bay at two ice zone stations [31]. Marine Flavobacteriia are known as
415 efficient degraders of biopolymers, such as proteins and polysaccharides [79].
416 Over the last decade, next generation sequencing (NGS) technologies have enabled the emergence of
417 adetailed analysis of the response of several cultivable bacterial strains, representative of
418 Flavobacteriia, to organic matter, in particular through proteomic and transcriptomic approaches [80].
419 This approach was used to explore the metabolic pathways of two *Polaribacter* strains isolated in the
420 North Sea during their growth with various models of organic molecules like carbohydrates and
421 polysaccharides [80]. This study showed that both *Polaribacter* strains were able to degrade algal
422 polysaccharides while they each occupy different ecologic niches. However, they observed that
423 one strain was feeding on proteins and a small subset of algal polysaccharides while the other was able
424 to degrade a wider range of polysaccharides, making it a more flexible strain [80]. More recently,
425 proteomic approaches have also been used to explore the response of *Formosa* strains
426 (Flavobacteriaceae) isolated in the North Sea to laminarin as a model of polysaccharides [81]. The authors
427 observed that laminarin was used as a major source of energy, and as signal molecule, which induced
428 transporters and digestive enzymes to use also other compounds released from the lysis of diatom cells.
429 These studies confirm the link between Flavobacteria and microalgal blooms with their capacity to
430 degrade and use algal DOM. It's consistent with the fact that all the bacterial strains belonging to
431 Flavobacteria tested in our biodegradation assay were able to grow
432 on the pelagic microalgae *C. neogracilis* exudate. However, depending on their genetic

material, bacterial strains differ in their ability to degrade DOM according to its composition. It implies the occurrence of different ecological niches in which bacterial strains use the energy source for which they are the most adapted to.

Even though environmental relevance of isolated strains remains an important issue, our study underlines the fact that the isolated strains from the ice camp were overall representative of the *in situ* bacterial community, especially in the sea ice bottom. We showed through a biodegradation experiment a preference for the DOM degradation from the pelagic diatom *Chaetoceros neogracilis* among 40 different bacterial strains isolated from ice and several depths. Indeed, most of the strains tested (78 %) were able to grow on the exudate from the pelagic diatom *C. neogracilis* and 33 % were able to use the exudate from the sea ice diatom *F. cylindrus*. 17.5% of the strains were not able to grow with any exudate while 27.5% of the strains were able to use both types of exudates. Nevertheless, we observed highly diverse response patterns among the bacterial strains even between strains with the same genus. We did not find specific relationships between the habitat of the bacterial strains (sea ice bottom versus water column) and their capacity to use the different exudates. One hypothesis for this lack of relationship between microbial habitat and microalgal DOM could be cross-feeding, meaning the production of secondary compounds based on the degradation of microalgal DOM by specific bacterial strains which allows less specific strains to use these secondary compounds for their growth. All strains belonging to Flavobacteriia (n = 10) were able to use the DOM provided by *C. neogracilis*, and this exudate sustained a growth capacity of up to 100 times higher than diluted marine broth medium, of two *Pseudomonas* sp. strains and one *Sulfitobacter* strain.

In conclusion, using a simple model of interaction (one microalgal exudate / one bacterial strain) to avoid complex biotic interactions which occur in microbial communities, we were able to confirm that dissolved exudates from different microalgae differed in their bioavailability to bacterial strains. Our results reinforce the fact that DOM can shape the bacterial community composition by selecting the most responsive species having the right genetic material to degrade the DOM at their disposal in their specific environment.

Additional Information

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Data Accessibility

All DNA sequences were deposited in Genbank (NCBI) with accession nos.: MK 224724-224815 for bacterial strains isolated during this study and MK 217878-217881 for four bacterial strains isolated in the Beaufort Sea. Additional data are available within the electronic supplementary material.

Competing Interests

We have no competing interests.

Authors' Contributions

F.J. and L.T. conceived and designed the research, L.T., L.D., L.I., P.C., C.P. performed the experimental work, L.T., L.D., L.I., P.C., C.P., I.O., F.J. contributed to the interpretation of the data and the discussion of the results presented in the manuscript. F.J. wrote the first draft of the manuscript and all the authors made comments and amendments, and approved the final version.

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Tables

Table 1: Cultivable bacteria counts for sea ice bottom and water column samples collected at an ice camp (67°28.784' N, 063°47.372' W) located near Qikiqtarjuaq (Nunavut) in the Baffin Bay. CFU: Colony-Forming Unites, ND: No data.

Table 2: Parameters measured in the microalgae cultures at beginning (T0) and after 44 days (T44d). DOC: dissolved organic carbon; POC: particulate organic carbon; DFCHO-C: dissolved free monosaccharides; DPCHO-C: dissolved polysaccharides; TDCHO-C: total dissolved saccharides. ND: not determined.

Table 3: Indices characterizing the growth of bacterial strains according to the substrate added. (A) Index 1: Distinction between strains which have shown significant growth using different substrates (in red) and those which have not shown visible growth (in blue). (B) Index 2: Results of cell growth on different exudates expressed in relation to those measured in diluted MB. See the material and methods for the explanations of the calculation of the indexes. * Bacterial strains isolated in 2009 in the Beaufort Sea, Arctic Ocean.

Figures

Figure 1. Location of Qikiqtarjuaq Ice Camp (67°28.784' N, 063°47.372' W). The position is shown with a white dot (courtesy of R. Amiraux).

Figure 2. Phylogenetic tree of bacterial strains isolated from ice camp samples and closely related species obtained from GenBank. The codes indicate the environmental Arctic strains isolated (see Table 3). The GenBank accession number follows specific names of the closely related species found in the database. The phylogenetic tree was made using the maximum likelihood method ("Maximum Likelihood", ML). The phylogeny obtained was statistically analyzed by the bootstrap method, with 1000 repetitions (values >0.5 are shown at each node). In red: Gammaproteobacteria ; in orange: Alphaproteobacteria ; in green: Flavobacteriia; in blue: Actinobacteria; in grey: Firmicutes. The *Chloroflexus islandicus* sequence has been used as outgroup to root the tree. Scale bar represents 2% estimated substitutions.

Figure 3. Growth of the microalgae (A) *Chaetoceros neogracilis* and (B) *Fragilariopsis cylindrus*. Values stand for means of triplicate cultures and error bars show ±SD.

Figure 4. Growth of (A) Strain BBCC 770, *Polaribacter filamentus* (3 m depth, Beaufort Sea) ; (B) Strain BBCC 2586, *Idiomarina abyssalis* (40 m depth, Green Edge ice camp) ; (C) Strain BBCC 2589, *Mesonia algae* (40 m depth, Green Edge ice camp) ; (D) Strain BBCC 2637, *Stenotrophomonas chelatiphaga* (sea ice, Green Edge ice camp) ; with different sources of dissolved organic matter (DOM): absence of DOM (ASW); diluted Marine Broth (MB); exudates from *C. neogracilis* and *F. cylindrus*. Values stand for means of triplicate treatments and error bars show ±SD.

Figure 5. Venn diagram showing the number of bacterial strains growing with the different microalgae exudates (*C. neogracilis*, *F. cylindrus*) and diluted Marine Broth (MB).

Supplementary information

Table S1. Marine agar 2216 and Marine broth 2216 composition

Figure S1. Composition of the cultivable bacterial community. (A) Proportion of phyla represented (92 bacterial strains). (B) Proportion of phyla as a function of different habitats (sea ice bottom: 28 strains; water column2 m: 38 strains; water column 40-60 m: 26 strains). For a better representation, the phylum of Proteobacteria was divided into two classes: γ-Proteobacteria and α-Proteobacteria.

Figure S2. Bacterial growth in the (A) *Chaetoceros neogracilis* and (B) *Fragilariopsis cylindrus* cultures. Values stand for means of triplicate cultures and error bars show ±SD.

Figure S3. Venn diagram showing the number of bacterial strains growing with the different microalgae exudates (*C. neogracilis, F. cylindrus*) and diluted Marine Broth (MB). (A) Bacterial strains isolated from sea ice. (B) Bacterial strains isolated at 2-3m depth. (C) Bacterial strains isolated at 40-60m depth.

Figure S4. Bacterial growth of (A) Strain BBCC770, *Polaribacter filamentus* and (B) Strain BBCC808, *Maribacter arcticus* with different dilutions of Marine broth (MB) with or without stirring. The different dilutions of MB contained the following dissolved organic carbon (DOC) concentrations: 1/25 = 8400 µM ; 1/50 = 4200 µM ; 1/100 = 2100 µM ; 1/250 = 840 µM ; 1/500 = 420 µM ; 1/1000 = 210 µM.

Habitat	Sampling date (d/m/y)	Julian day	Temperature (°C)	Salinity (psu)	Chl*a* ($\mu g.L^{-1}$)	CFU ($10^2.mL^{-1}$)	Total bacteria ($10^5.mL^{-1}$)	CFU/Total (%)	Number of isolated strains
Sea ice	09/05/2016	130	ND	ND	30	17.2	1.08	1.59%	8
Sea ice	23/05/2016	144	ND	ND	95.87	8.7	4.87	0.18%	7
Sea ice	30/05/2016	151	ND	ND	164.88	0.1	5.26	0.00%	ND
Sea ice	06/06/2016	158	ND	ND	61.89	0.9	4.6	0.02%	ND
Sea ice	20/06/2016	172	ND	ND	138.01	17.5	ND	ND	8
Sea ice	27/06/2016	179	ND	ND	39.68	27.1	6.24	4.35%	5
2 m	09/05/2016	130	-1.73	32.22	0.06	11.5	2.01	0.57%	7
2 m	16/05/2016	137	-1.74	32.24	ND	20.8	2.62	0.80%	ND
2 m	23/05/2016	144	-1.73	32.22	0.07	32.6	2.64	1.23%	6
2 m	30/05/2016	151	-1.71	32.26	0.16	15.5	2.47	0.63%	ND
2 m	06/06/2016	158	-1.7	32.28	0.88	12.7	2.38	0.53%	ND
2 m	13/06/2016	165	-1.69	32.29	0.36	23.3	2.71	0.86%	6
2 m	20/06/2016	172	-1.62	32.16	0.4	19.5	3.51	0.56%	6
2 m	27/06/2016	179	-1.51	32.1	1.06	14.5	5.09	0.29%	7
2 m	11/07/2016	193	-1.29	31.6	1.44	4.6	6.81	0.07%	6
40 m	09/05/2016	130	-1.67	32.28	0.13	5.7	2.09	0.27%	8
40 m	16/05/2016	137	-1.68	32.35	ND	2.9	2.35	0.12%	ND
40 m	23/05/2016	144	-1.69	32.27	0.06	3	2.43	0.12%	4
40 m	30/05/2016	151	-1.65	32.36	0.05	0.7	2.38	0.03%	ND
40 m	06/06/2016	158	-1.68	32.31	0.08	3.8	2.56	0.15%	ND
40 m	13/06/2016	165	-1.63	32.44	0.06	13.2	2.59	0.51%	5
60 m	27/06/2016	179	-1.58	32.51	0.32	10.5	3.16	0.33%	6
60 m	11/07/2016	193	-1.57	32.38	0.59	1.6	3.62	0.04%	3

Table 1

	C. neogracilis		*F. cylindrus*	
	T0	T44d	T0	T44d
Cells $x10^4.mL^{-1}$	8.7 (± 2.9)	122 (± 13)	0.6 (± 0.2)	31.2 (± 2.2)
NO_3 (µM)	864.9 (± ND)	224.3 (± 118.2)	859.4 (± ND)	674.5 (± 60.2)
PO_4 (µM)	81.5 (± ND)	20.7 (± 8.7)	79.9 (± ND)	62.1 (± 0.8)
$Si(OH)_4$ (µM)	35.2 (± ND)	3.6 (± 0.7)	36.0 (± ND)	2.7 (± 1.2)
DOC (µM)	234.1 (± 0.8)	2007.2 (± 778.4)	108.8 (± 6.8)	977 (± 70.2)
POC (µM)	ND	2500 (± 423)	ND	612 (± ND)
DOC/(POC+DOC) (%)	ND	45	ND	61
DFCHO-C (µM)	ND	118.2	ND	20.2
PCHO-C (µM)	ND	246.4	ND	14.6
TCHO-C (µM)	ND	364.6	ND	34.8
TCHO-C/DOC (%)	ND	18	ND	4

Table 2

Habitat	Strain	Class	Family	Closest Relative Species	A. MB	A. *C. neogracilis*	A. *F. cylindrus*	B. *C. neogracilis*/MB	B. *F. cylindrus*/MB
Sea ice	BBCC 2678	Actinobacteria	Microbacteriaceae	*Salinibacterium amurskyense*				0.97	NA
Sea ice	BBCC 2851	Alphaproteobacteria	Erythrobacteraceae	*Porphyrobacter sanguineus*				0.62	NA
Sea ice	BBCC 2680	Alphaproteobacteria	Rhodobacteraceae	*Loktanella salsilacus*				1.58	0.57
Sea ice	BBCC 2856	Gammaproteobacteria	Pseudoalteromonadaceae	*Pseudoalteromonas distincta*				NA	1.76
Sea ice	BBCC 2592	Gammaproteobacteria	Pseudoalteromonadaceae	*Pseudoalteromonas neustonica*				1.81	NA
Sea ice	BBCC 2584	Gammaproteobacteria	Pseudoalteromonadaceae	*Pseudoalteromonas nigrifasciens*				0.47	2.48
Sea ice	BBCC 2635	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas paralactis*				8.27	NA
Sea ice	BBCC 2597	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas pelagia*				45.79	NA
Sea ice	BBCC 2637	Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas chelatiphaga*				0.40	NA
Sea ice	BBCC 2850	Flavobacteria	Flavobacteriaceae	*Antarticibacterium flavum*				0.35	NA
Sea ice	BBCC 2681	Flavobacteria	Flavobacteriaceae	*Flavobacterium gelidilacus*				0.81	NA
2m	BBCC 2625	Actinobacteria	Microbacteriaceae	*Cryobacterium arcticum*				1.19	NA
2m	BBCC 2611	Actinobacteria	Nocardiaceae	*Rhodococcus fascians*				4.13	13.24
2m	BBCC 2639	Actinobacteria	Nocardiaceae	*Rhodococcus kyotonensis*				7.11	4.22
2m	BBCC 2602	Alphaproteobacteria	Hyphomonadaceae	*Litorimonas haliclonae*				11.06	NA
2m	BBCC 2676	Alphaproteobacteria	Rhodobacteraceae	*Paracoccus hibiscisoli*				NA	NA
3m	BBCC 810*	Alphaproteobacteria	Rhodobacteraceae	*Sulfitobacter marinus*				0.63	0.11
2m	BBCC 2638	Gammaproteobacteria	Alteromonadaceae	*Alteromonas stellipolaris*				2.89	9.17
2m	BBCC 2601	Gammaproteobacteria	Alteromonadaceae	*Paraglaciecola chathamensis*				NA	NA
3m	BBCC 757*	Gammaproteobacteria	Colwelliaceae	*Colwellia polaris*				0.48	1.42
2m	BBCC 2603	Gammaproteobacteria	Moraxellaceae	*Psychrobacter aquimaris*				NA	NA
2m	BBCC 2599	Gammaproteobacteria	Moraxellaceae	*Psychrobacter glaciei*				NA	NA
2m	BBCC 2579	Gammaproteobacteria	Moraxellaceae	*Psychrobacter okhotskensis*				NA	NA
2m	BBCC 2626	Gammaproteobacteria	Pseudoalteromonadaceae	*Pseudoalteromonas translucida*				NA	NA
2m	BBCC 2854	Flavobacteria	Flavobacteriaceae	*Bizionia berychis*				0.93	NA
2m	BBCC 2578	Flavobacteria	Flavobacteriaceae	*Leeuwenhoekiella aequorea*				0.34	NA
2m	BBCC 2613	Flavobacteria	Flavobacteriaceae	*Maribacter dokdonensis*				3.62	NA
2m	BBCC 2624	Flavobacteria	Flavobacteriaceae	*Maribacter orientalis*				0.61	NA
3m	BBCC 808*	Flavobacteria	Flavobacteriaceae	*Maribacter arcticus*				0.82	NA
3m	BBCC 770*	Flavobacteria	Flavobacteriaceae	*Polaribacter filamentus*				1.37	NA
60m	BBCC 2632	Actinobacteria	Nocardiaceae	*Rhodococcus cerastii*				NA	NA
40m	BBCC 2605	Alphaproteobacteria	Rhodobacteraceae	*Paracoccus homiensis*				3.95	0.18
40m	BBCC 2855	Alphaproteobacteria	Rhodobacteraceae	*Sulfitobacter litoralis*				86.19	NA
40m	BBCC 2586	Gammaproteobacteria	Idiomarinaceae	*Idiomarina abyssalis*				0.48	NA
40m	BBCC 2617	Gammaproteobacteria	Moraxellaceae	*Psychrobacter marincola*				7.82	4.49
40m	BBCC 2590	Gammaproteobacteria	Moraxellaceae	*Psychrobacter nivimaris*				NA	NA
40m	BBCC 2848	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas sabulinigri*				98.61	NA
40m	BBCC 2585	Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas zhaodongensis*				NA	NA
40m	BBCC 2589	Flavobacteria	Flavobacteriaceae	*Mesonia algae*				1.11	0.86
60m	BBCC 2643	Flavobacteria	Flavobacteriaceae	*Gillisia hiemivivida*				0.44	NA

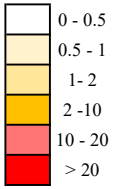
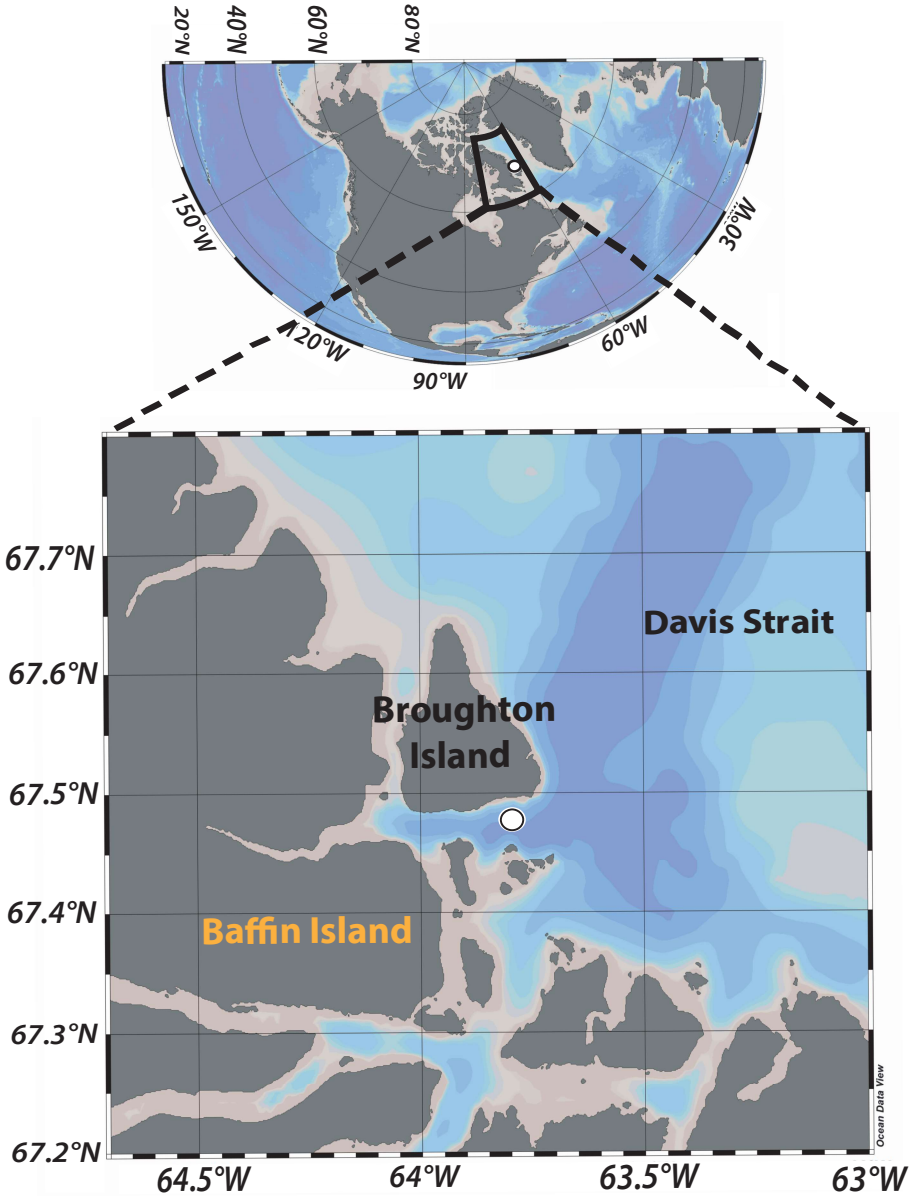


Table 3

Fig. 1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67.3°N
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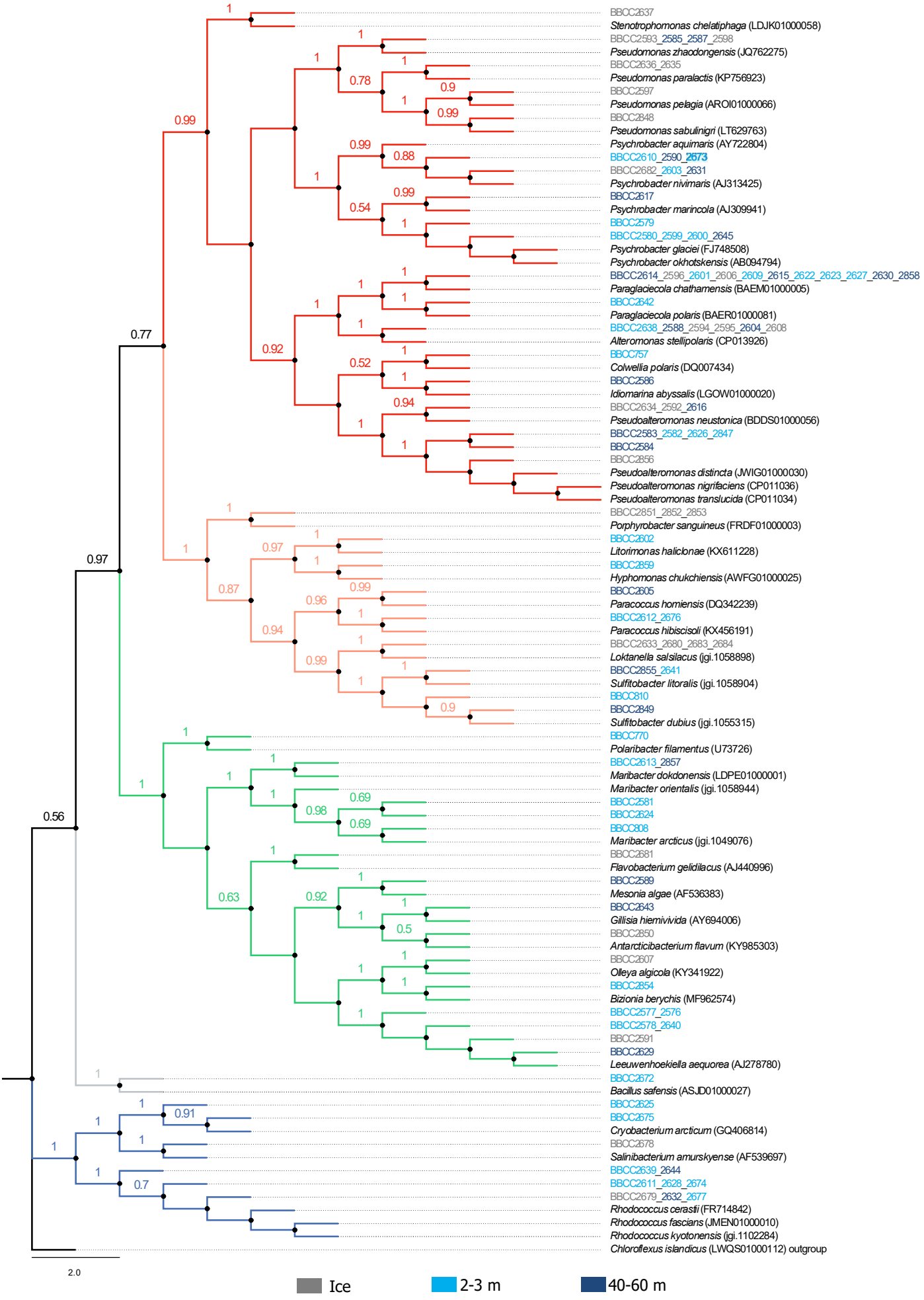
Baffin Island
Ocean Data View

Fig. 2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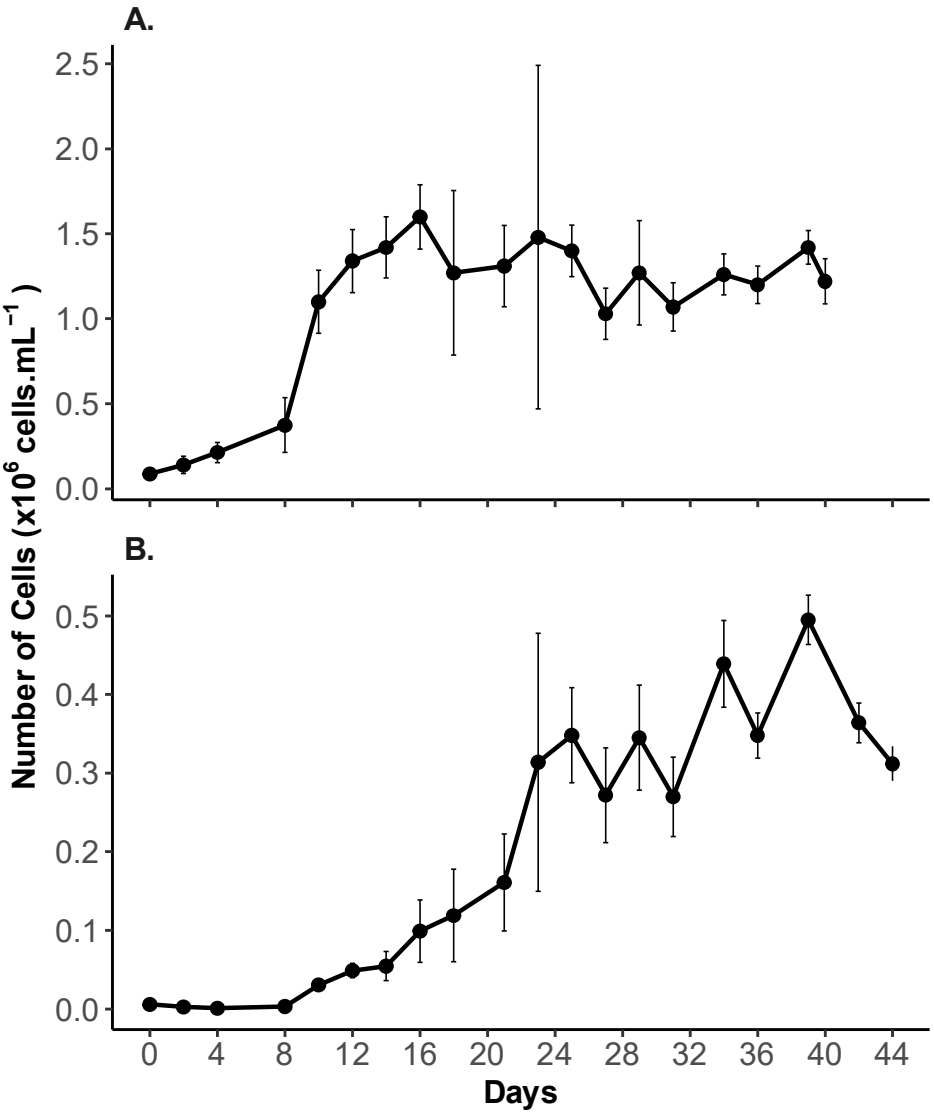
2-3 m
40-60 m

Fig. 3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0.3
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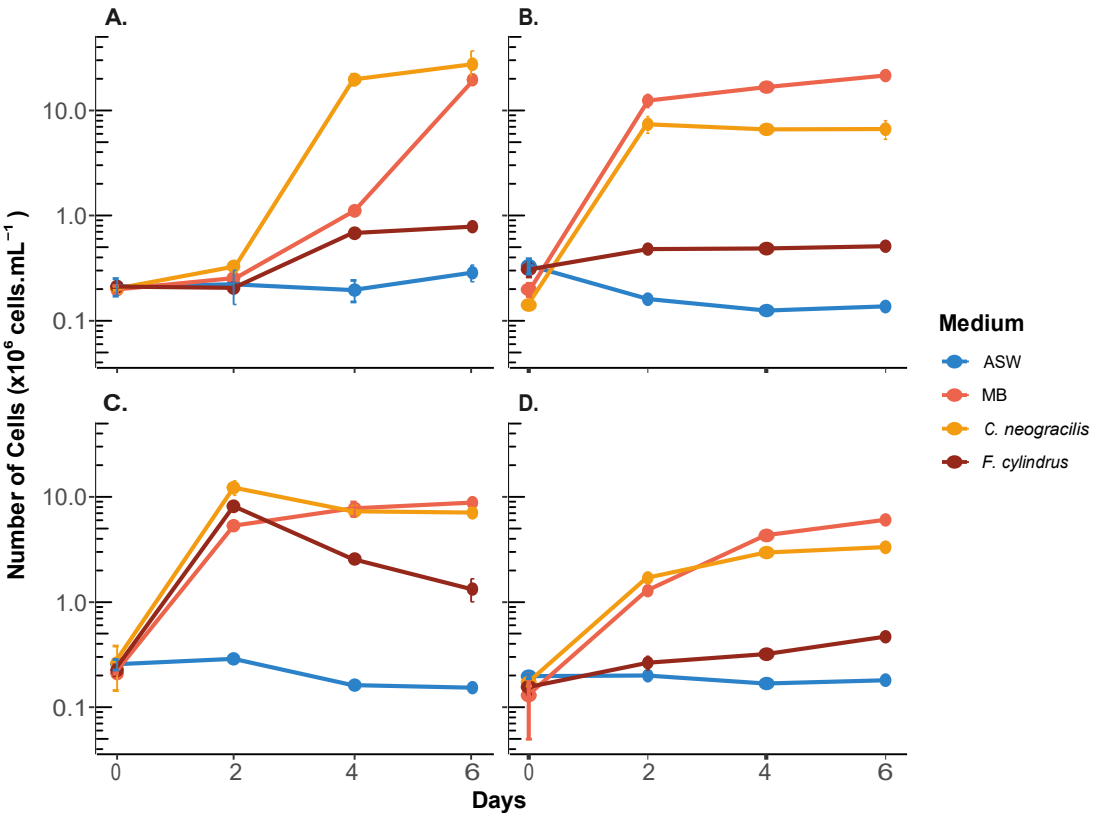
20
24
28
32
36
40
44
Days

Fig. 4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B.
C.
D.
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Medium
ASW
MB
C. neogracilis
F. cylindrus

Fig. 5

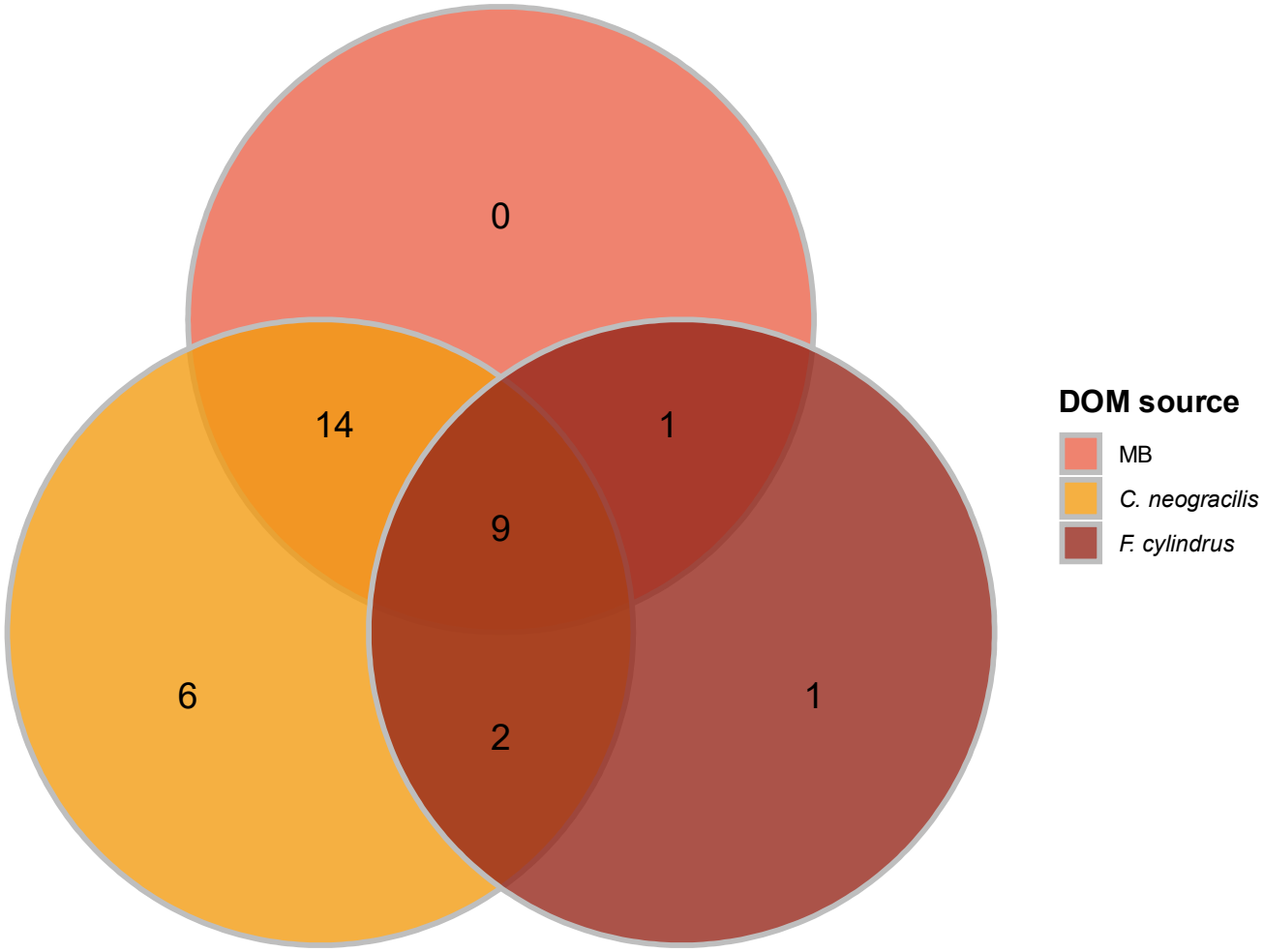
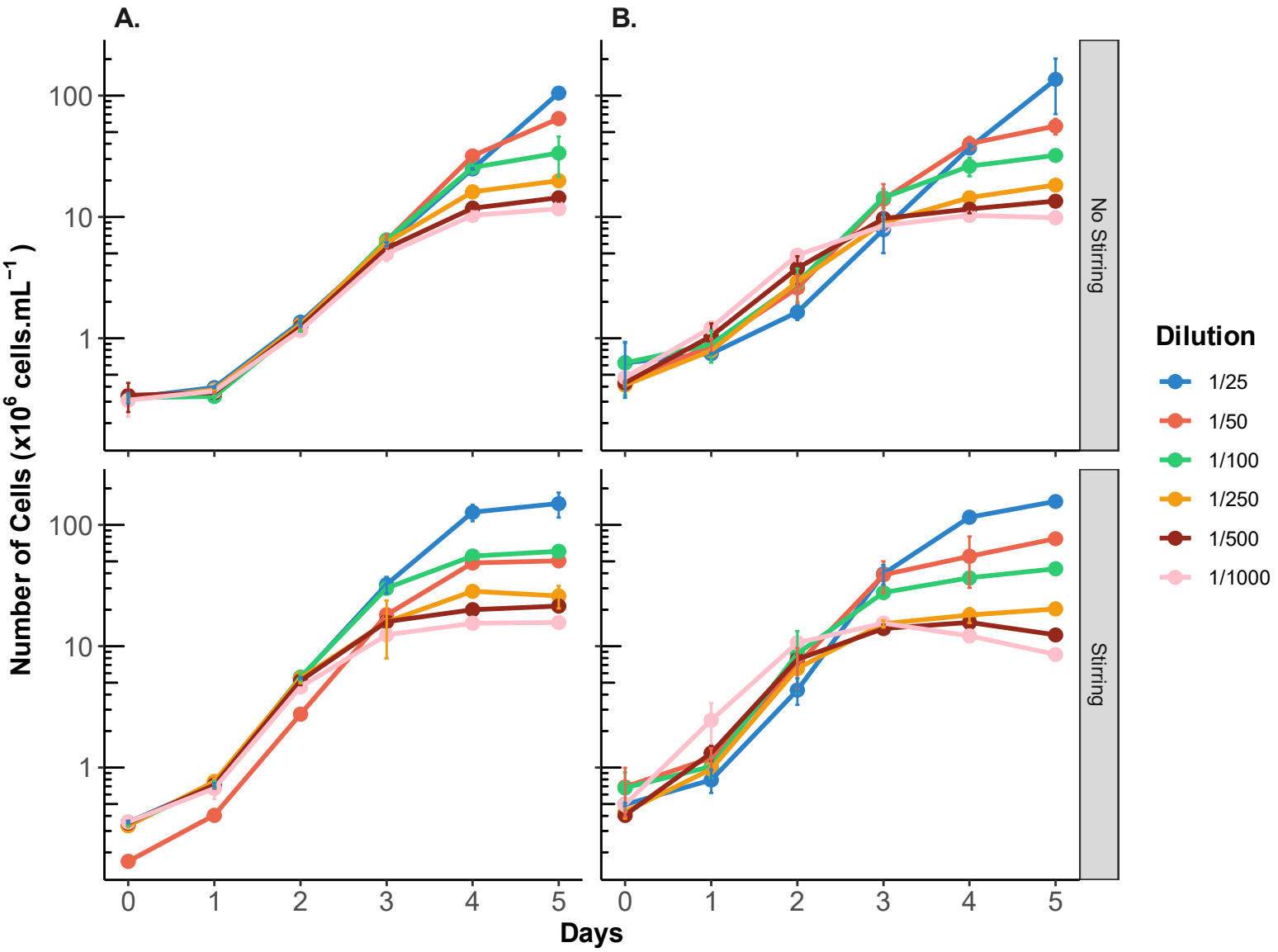


Table S1

Component	Quantity
Peptone	5.0 g
Yeast Extract	1.0 g
Ferrice Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	8.8 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	34.0 mg
Boric Acid	22.0 mg
Sodium Silicate	4.0 mg
Sodium fluoride	2.4 mg
Ammonium Nitrate	1.6 mg
Disodium Phosphate	8.0 mg
Agar*	15.0 g

* Omit Agar for Broth medium

Fig. S1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B.
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10
1
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Stirring
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2
3
4
5
Days
Dilution
1/25
1/50
1/100
1/250
1/500
1/1000

Fig. S2

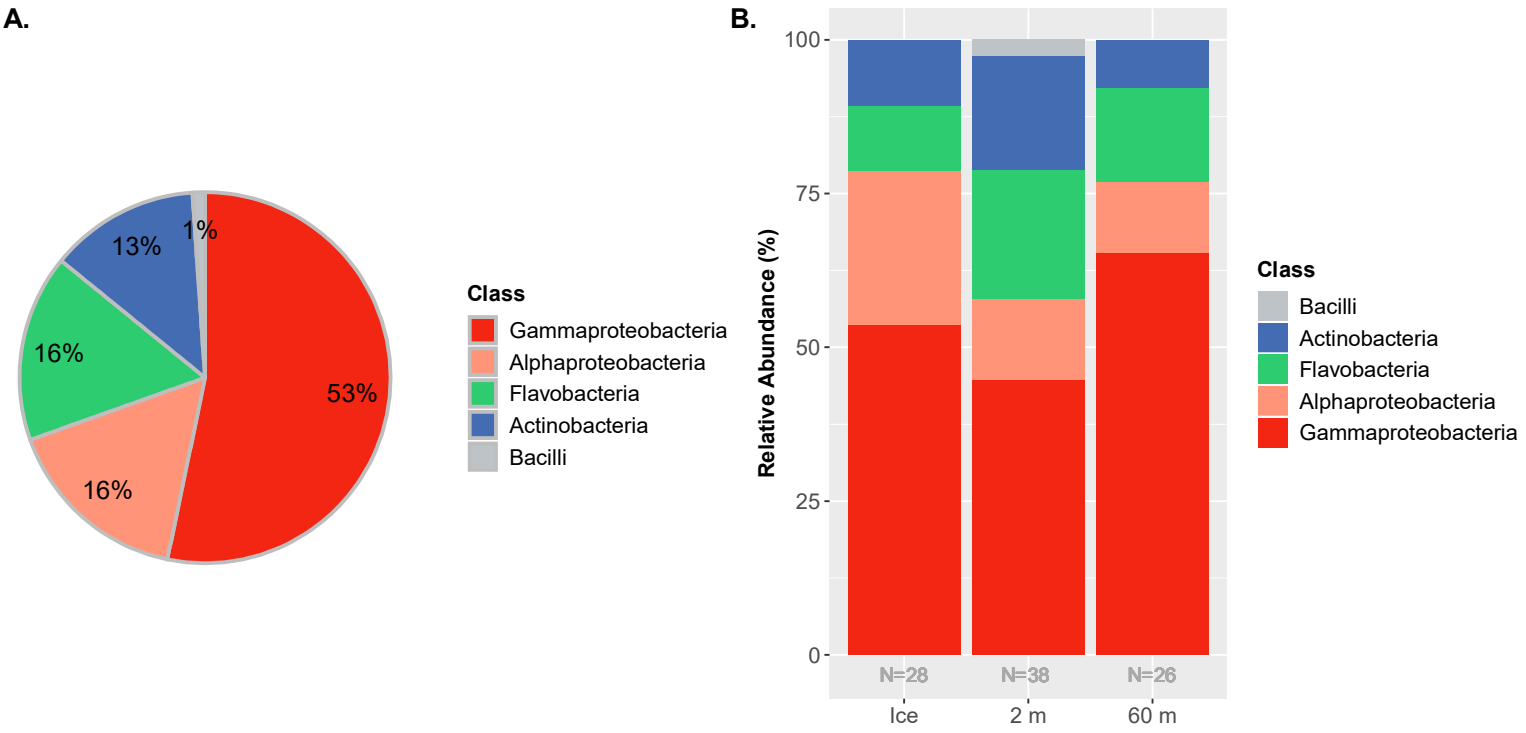

A.
1%
13%
16%
53%
16%
Class
Gammaproteobacteria
Alphaproteobacteria
Flavobacteria
Actinobacteria
Bacilli
B.
Relative Abundance (%)
100
75
50
25
0
N=28
N=38
N=26
Ice
2 m
60 m
Class
Bacilli
Actinobacteria
Flavobacteria
Alphaproteobacteria
Gammaproteobacteria

Fig. S3

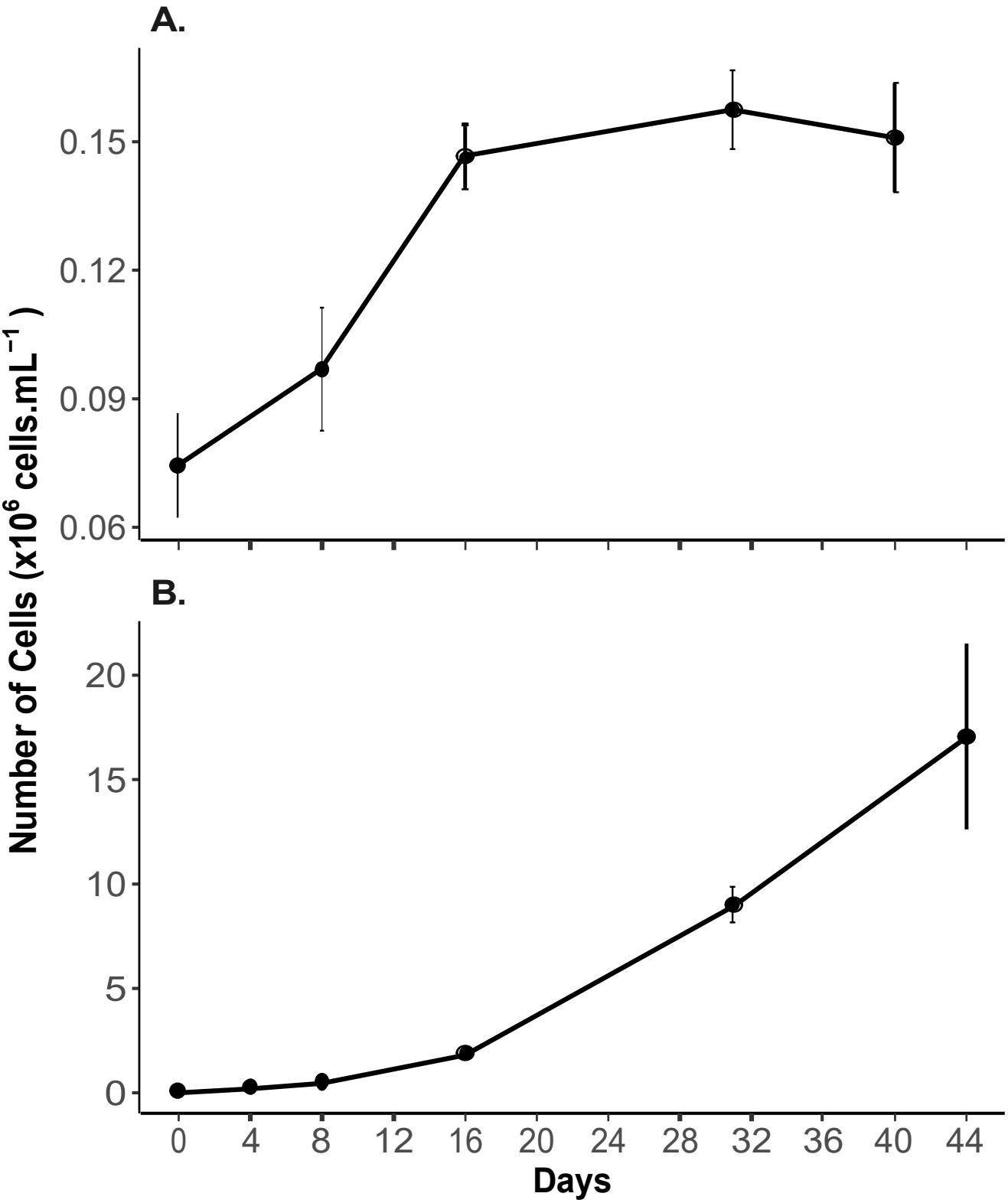
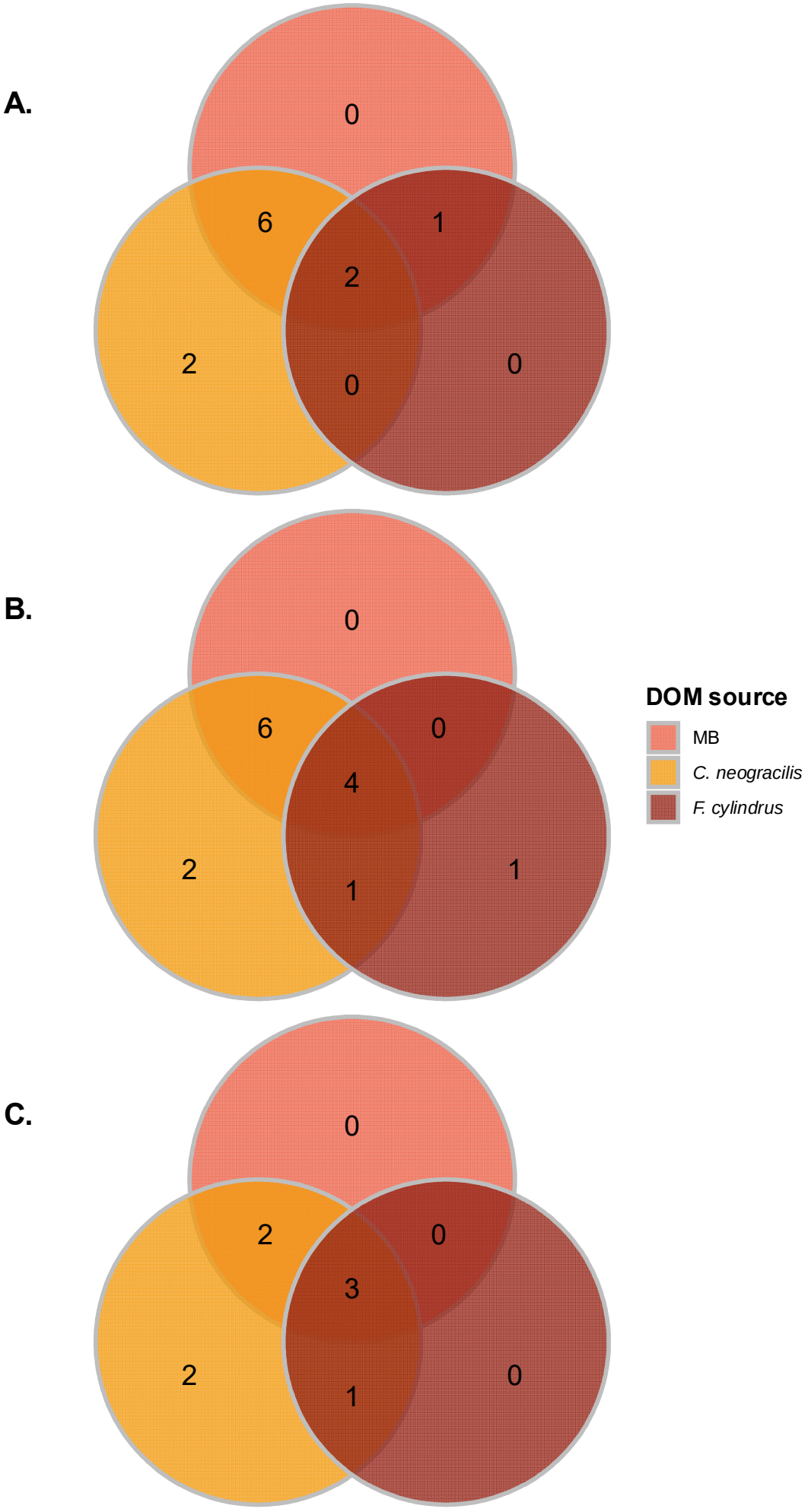

A.
0.15
0.12
0.09
0.06
B.
20
15
10
5
0
0
4
8
12
16
20
24
28
32
36
40
44
Days
Number of Cells (x10^6 cells.mL^-1)

Fig. S4


A.
0
6
1
2
2
0
0
B.
0
6
0
4
2
1
1
DOM source
MB
C. neogracilis
F. cylindrus
C.
0
2
0
3
2
1
0