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Abstract

In the Southern Ocean, natural iron fertilization in the wake of islands leads to annually occurring spring phytoplankton blooms associated with enhanced heterotrophic activity through the release of labile dissolved organic matter (DOM). The aim of this study was to investigate experimentally how diatom-derived DOM affects the composition of Southern Ocean winter water bacterial communities and to identify the most responsive taxa. A bacterial community collected in the naturally iron-fertilized region off Kerguelen Island (KEOPS2 October-November 2011) was grown onboard in continuous cultures, on winter water alone or amended with diatom-derived DOM supplied at identical DOC concentrations. 454 sequencing of 16S amplicons revealed that the two DOM sources sustained strikingly different bacterial communities, with higher relative abundances of *Sulfitobacter*, *Colwellia* and *Methylophaga* OTUs and lower relative abundances of *Polaribacter*, *Marinobacter*, NAC11-7 and SAR11 OTUs in diatom-DOM compared to winter water conditions. Using a modeling approach, we obtained growth rates for phylogenetically diverse taxa varying between 0.12 and 0.49 d^{-1} under carbon-limited conditions. Our results identify diatom-DOM as a key factor shaping Southern Ocean winter water bacterial communities and suggest a role for niche partitioning and microbial interactions in organic matter utilization.

Introduction

Bacteria are key players in the transformation of carbon present in marine dissolved organic matter (DOM). Understanding the mechanisms of carbon utilization by marine bacteria is

presently a major challenge because both the DOM pool (Hertkorn *et al.* 2007) and bacterial communities (Sogin *et al.* 2006; Sunagawa *et al.* 2015) are highly complex. Advances in the fields of analytical chemistry and constantly improving high-throughput sequencing technologies have enabled progress but much remains to be learned about the composition and interactions between the chemical and microbiological components of the ocean.

From an ecological point of view, understanding the factors that drive bacterial community composition would shed light on their roles in the biogeochemical cycling of carbon and other elements. Because DOM is the main source of substrates for marine bacteria, its characteristics, such as chemical composition and bioavailability, and its concentration are likely to play important roles in structuring bacterial communities. It has been suggested that individual bacterial taxa possess different metabolic properties, reflecting the vast diversity of compounds found in a given environment (Wawrik *et al.* 2005; Teeling *et al.* 2012; Satinsky *et al.* 2014). As a result, various taxa could be adapted to different lifestyles based on distinct resource exploitation strategies (Lauro *et al.* 2009). This hypothesis is supported by numerous studies showing biogeographic patterns as well as predictable bacterial community composition in recurring conditions (Fuhrman *et al.* 2006; Martiny *et al.* 2006; Nelson *et al.* 2008; Gilbert *et al.* 2012). However, there is also a large body of evidence for functional redundancy within and across bacterial communities (Langenheder, Lindström and Tranvik 2005, 2006; Sjöstedt *et al.* 2013; Beier *et al.* 2015), as well as generalist carbon consumption (Mou *et al.* 2008; Landa *et al.* 2014; Pedler, Aluwihare and Azam 2014; Sarmento, Morana and Gasol 2016), suggesting functional traits alone do not explain taxon distribution and abundance. Taken together, these observations indicate that the factors driving microbial community structure and activity are complex and vary in nature and intensity across space and time. The extent to which characteristics of the carbon pool used by heterotrophic bacteria act as shaping force on community composition remains to be determined.

Phytoplankton supplies labile DOM to heterotrophic bacteria, which results in a tight link between these components in all aquatic ecosystems (Cole, Findlay and Pace 1988). Numerous studies have investigated how pulses of bioavailable DOM released during phytoplankton blooms affect bacterial heterotrophic activity on seasonal (Church 2008 and references therein) and inter-annual scales (Kim and Ducklow 2016). The concurrent changes in the bacterial community were suggested to be driven by the quantity and quality of DOM released during bloom events (Kerkhof *et al.* 1999; Riemann, Steward and Azam 2000; Pinhassi *et al.* 2004; Rink *et al.* 2007; West *et al.* 2008; Tada *et al.* 2012; Delmont *et al.* 2014; Kim *et al.* 2014; Luria *et al.* 2016; Needham and Fuhrman 2016). However, few studies have supported their field observations with experimental studies, directly testing the effect of phytoplankton-derived DOM on bacterial community composition (Luria *et al.* 2017).

The Southern Ocean is the largest high-nutrient, low-chlorophyll (HNLC) region in the world's oceans. Iron-limited phytoplankton primary production (Boyd *et al.* 2000; Blain *et al.* 2007) in combination with permanent upwelling of deep water around Antarctica result in Southern Ocean surface waters characterized by low concentrations of dissolved organic carbon (DOC, about 50 µM, Hansell 2013), of rather refractory nature. This leads to a double constraint on heterotrophic bacteria, which require both iron and carbon for growth (Church, Hutchins and Ducklow 2000; Kirchman *et al.* 2000). While iron was identified as the limiting nutrient for heterotrophic microbes in only a few studies, the persistent enhancement of heterotrophic bacterial metabolism upon addition of labile organic carbon compounds (see for review Obernosterer, Fourquez and Blain 2015) clearly highlights that bacterial activity in the Southern Ocean is constrained by the bioavailability of DOM.

In the present study, we tested the hypothesis that the supply of labile DOM derived from diatoms, the dominant bloom forming phytoplankton in naturally iron-fertilized regions of the Southern Ocean (Blain *et al.* 2007; Quéguiner 2013), leads to shifts in the composition of a

natural, C-limited bacterial community. Continuous cultures were implemented, to actively select bacterial members most adapted either to the background DOM present in the winter waters, or to a mixture of background and diatom-derived DOM. Diversity analyses revealed striking differences in the identity of the taxa selected by each of the tested DOM conditions. We observe that, in this environment where labile substrates are scarce, bacterial community composition is strongly affected by qualitative differences in the DOM pool available to bacteria, demonstrating a tight link between the bioavailability of organic carbon and bacterial metabolism and diversity in the Southern Ocean.

Materials and Methods

Experimental design

The continuous culture experiment presented in this study was ran on board on the R/V *Marion Dufresne* during the KEOPS2 cruise conducted in the naturally iron-fertilized region around the Kerguelen Islands in the Southern Ocean in October and November 2011. Two experimental conditions were run in triplicates. In the first condition, or control, bacteria were supplied with 0.2 µm filtered seawater, while in the second condition, or diatom-DOM treatment, bacteria were supplied with 0.2 µm filtered seawater mixed with diatom-derived DOM.

For the control medium preparation, 60 l of seawater were sampled with the trace-metal clean rosette in the upper 80 m at Station A3 (Suppl. Table 1) located above the Kerguelen plateau (Suppl. Fig. 1), in the iron-fertilized region before the onset of the bloom (Landa *et al.* 2016). The 60 l were collected in acid-washed (10% HCl), Milli-Q water-rinsed polycarbonate (PC) carboys and immediately 0.2 µm-filtered. This water was stored at 4°C in acid-washed,

Milli-Q water-rinsed PC carboys for the entire duration of the experiment. Water for the bacterial inoculum was collected at 40 m and 4 l were filtered through a 0.8 μm PC filter (45 mm diameter, Nuclepore). The 0.8 μm filtrate was stored at 6°C for a few hours until the beginning of the experiment. Concurrently, 4.8 l of the < 0.8 μm filtrate were collected on a 0.2 μm cartridge (Sterivex, Millipore), 500 μl of lysis buffer (40 mM EDTA, 50 mM Tris, 0.75 M sucrose) were added, and the cartridge was stored at -80°C for further diversity analysis.

The diatom-derived DOM was prepared in the laboratory before the cruise. A non-axenic strain of the diatom *Chaetoceros debilis*, isolated from the Southern Ocean and provided by the Alfred Wegener Institute (Germany) was cultivated in artificial seawater containing low amounts of background organic carbon (DOC ~15 μM) and amended with nutrients according to Guillard's f/2 medium. The *C. debilis* cultures were grown at 4°C at a light:dark cycle of 16h:8h. Prior to the cruise, cells were removed from the culture by gentle filtration on two acid-washed, Milli-Q water-rinsed 0.2 μm PC filters (90 mm diameter, Nuclepore) placed on top of each other. The filtrate (5 l) was collected in acid-washed, Milli-Q water-rinsed PC carboys and stored at 4°C until the start of the experiment.

The initial cultures (2 l final volume each) were prepared in acid-washed, Milli-Q water-rinsed 2 l PC carboys. For the control, the 0.2 μm-filtered *in situ* seawater was used as the medium, and for the diatom-DOM treatment the 0.2 μm-filtered *in situ* seawater was mixed with 0.2 μm-filtered diatom-derived DOM (see above). The volumes to mix were determined based on the DOC concentration of the diatom filtrate, so that diatom-DOM contributed to 10 μM of the final DOC concentration. The triplicate cultures of each condition were inoculated with 200 ml of 0.8 μm-filtered *in situ* seawater, corresponding to a 1:10 dilution. Inorganic phosphate and nitrate were added to the control to match the concentrations obtained in the diatom-DOM treatment.

The cultures were conducted in a cold room (6°C) in the dark. Each culture was bubbled with filter-sterilized air (Sartorious). A Milli-Q water purge was placed between the source and the filters to avoid potential DOC contamination by air. Cultures were homogenized by stirring. They were run as a batch for a few hours, to allow bacterial growth and avoid washing out the cells. Each medium, prepared in the same conditions without bacterial inoculum, was connected to the three replicate cultures of the corresponding condition by Teflon tubings, and the peristaltic pumps (Gilson) were switched on to start the inflow of input medium and the outflow of culture. The imposed flow rate was 0.2 day^{-1}, which was chosen based on the *in situ* growth rates determined during the first KEOPS cruise (Obernosterer *et al.* 2008) and resulted in a residence time of 5.6 days.

Sampling of cultures

The experiment was run for 19 days, during which the cultures were sampled every two to three days. The air purge was renewed every day. Input media were prepared every other day, after another 0.2 μm filtration of the required volume of phytoplankton exudate. Freshly prepared media were sampled for DOC concentration and inorganic nutrients. Sampling was performed without opening the cultures using glass syringes (Hamilton) connected with a luer-lock to Teflon tubes and glass cubes that plunged to the bottom of the cultures. The glass syringes were extensively rinsed with Milli-Q water prior to sampling. A maximum volume of 50 ml, including syringe rinsing, was taken from the cultures for the various analyses, representing less than 3% of the volume of the cultures.

DOC and inorganic nutrients

For the determination of DOC concentrations, duplicate 15 ml samples were filtered through two precombusted GF/F filters (Whatman) and the samples were stored acidified (H_3PO_4, pH = 2) in

combusted 20 mL glass ampoules in the dark at room temperature. DOC concentrations were measured in the home lab on a Shimadzu TOC-VCP analyzer with a Pt catalyst at 680°C (Benner and Strom 1993) as detailed in Tremblay *et al.* (2015). For the determination of nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations, a 10-ml subsample was fixed with mercuric chloride ($HgCl_2$, 20 mg l^{-1} final concentration) and analyzed onboard using continuous flow analysis (Aminot and Kérouel 2007) with a Skalar instrument.

Bacterial abundance and diversity

A 1.8 ml sample was taken for bacterial abundance analysis, fixed with formaldehyde (2% final concentration), kept at 4°C for 30 min, and then stored at -80°C until analysis. Bacterial abundances were determined in the lab on a FACSCalibur™ (BD20 Biosciences) equipped with an air-cooled laser, providing 15 mW at 488 nm with the standard filter setup. Heterotrophic bacteria were stained with SYBR Green I and determined by flow cytometry as described previously (Obernosterer *et al.* 2008).

Samples for bacterial diversity were collected in acid-washed, Milli-Q water-rinsed PC flasks from the outflow of the cultures at day 5, 10, 15 and 19. The tubes were placed in the flasks at night and between 135 and 216 ml of collected culture were filtered the next morning on 0.2 μm PC filters (Nuclepore), placed in tubes containing 500 μl of lysis buffer (40 mM EDTA, 50 mM Tris, 0.75 M sucrose) and stored at -80°C until the end of the cruise. The last day of the experiment, the volumes filtered for bacterial diversity were ~500 ml for each culture.

DNA extraction and pyrosequencing

After lysis of the cells and proteins as described in Landa *et al.* (2013), a combined DNA-RNA extraction using Qiagen's AllPrep DNA/RNA Micro Kit was performed on the 0.2 μm filters,

following the manufacturer's instruction. The molecular size and purity of the DNA were analyzed using agarose gel electrophoresis (1%). In total, 25 samples of genomic DNA were sent to the Molecular Research DNA laboratory (Shallowater, TX, USA) for 454 pyrosequencing. The sequencing was performed using an FLX-Titanium platform (Roche, Wolcott *et al.* 2009). Universal bacterial primers 27F (5'-AGRGTTTGATCMTGGCTCAG-3') and 530R (5'-CCGCNGCNGCTGGCAC-3') were used, targeting hypervariable regions V1 to V3 of the 16S rRNA gene. The raw sequences for this dataset were deposited in the Sequence Read Archive (SRA) database under accession number SRP041580. Sequences can be found on HUFUDAT01.sff, and the barcode corresponding to each sample can be found in Supplementary Table 2.

Bioinformatic analyses

Raw sequences were denoised using AmpliconNoise (Quince *et al.* 2011) in Mothur (Schloss *et al.* 2009). Chimeras were removed during clustering of reads into OTUs at 97% identity with Usearch (Edgar 2010) in QIIME (Caporaso *et al.* 2010). The most abundant sequence in each OTU was chosen as the representative sequence and used for further analyses. Taxonomy was assigned to each OTU using the RDP classifier (Wang *et al.* 2007) and the May 2013 Greengenes database as a reference. A matrix was constructed with the number of reads corresponding to each OTU in each sample. Relative abundances were calculated for each OTU, after subsampling of the data to 2930 reads per sample.

Phylogenetic analysis

Phylogenetic analysis was used to clarify the affiliation of the 21 abundant OTUs obtained in the present study. The partial 16S gene sequences were aligned using MUSCLE v3.8.31 (Edgar

2004). The phylogenetic inference was restricted to 242 sites that could be unambiguously aligned for the *Proteobacteria* tree, and 258 sites for the *Flavobacteria* and *Verrucomicrobia* tree. Phylogenetic analyses were performed using the Maximum Likelihood method implemented in MEGA7 (Kumar, Stecher and Tamura 2016). The Maximum Likelihood analysis was based on the General Time Reversible model, and initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Bootstrap proportions (BPs) were obtained from 1,000 pseudo-replicates. Bayesian Posterior Probabilities (BPPs) were calculated with the maximum likelihood method from 1,000 replicates for 1 million generations, until convergence (average standard deviation below 0.01), using MrBayes 3.2.6 (Ronquist, Huelsenbeck and van der Mark 2011).

Modeling approach

To obtain estimates of the growth rates of the abundant OTUs, we used the classical mathematical model of the chemostat (Monod 1950; Novick and Szilard 1950). We consider a multi-species population, represented here by the 21 most abundant OTUs (Dumont *et al.* 2008). The model assumes that these OTUs are in competition on a single limiting substrate. The application of this basic hypothesis to our data requires some considerations. The total pool of DOM is a complex mixture of heterogeneous substrates, each characterized by variable biological availability. In our experiment, only a small fraction of this DOM pool, representing the most labile fraction, is consumed during the time period of the imposed dilution rate. Because the composition and the concentration of the specific compounds used by bacteria, as well as the affinities and uptake kinetics by different taxa are not known, the ensemble of these labile compounds was presented in the model as one limiting resource.

On the long term (i.e. at steady state), it is well known that this model, that has no explicit intra- or inter-specific interaction terms between the populations of microorganisms, predicts the competitive exclusion. Here, we consider its non-equilibrium dynamics where a complex microbial consortium is present in the chemostat (Rapaport, Dochain and Harmand 2009). We recall from the mathematical analysis of this model that the species exclusion is an asymptotic result. It can take a long time before being observed, notably when species have nearby growth characteristics. Therefore, we consider here the transient dynamics of this model and not the steady state.

$$\begin{aligned} \frac{ds}{dt} &= D(s_{in} - s) - \sum_{i}^{n} \frac{\mu_i(s)}{Y_i} x_i \\ \frac{\mathrm{d}x_i}{dt} &= \mu_i(s)x_i - Dx_i \quad (1 \cdots n) \end{aligned} \qquad \text{(M1)}$$

1.

Here s and x_i stand for the substrate (that is the labile pool) and biomass concentrations of each OTU or "species" i in the cultures, s_{in} is the concentration of labile organic carbon in the input medium, and D the dilution rate. Our objective is to estimate from experimental data the specific growth rates $\mu_i(.): s \to \mu_i(s)$for each OTU.

The available data from the experiments are the operating parameters D and s_{in}, and the measured values $s(t_j)$ and $x_i(t_j)$ at four measurement instants t_j, for each replicate. As these data reveal that the four values of $s(t_j)$ are quite close, we have used two different estimation procedures, which reinforce the robustness of the estimations. The first method allows the concentration of the consumed substrate to vary over time, while the second method assumes explicitly the concentration of the consumed substrate to be constant over time.

Method 1: We identify the function $\mu_i(s)$ from the model (M1) allowing s to be time-varying but close to the observed value. We also assume that the bacterial growth efficiency, referred to as yield coefficient Y_i of each OTU is close to a common value Y. One can then observe from equations (M1) that under this assumption

$$\frac{}{dt}(Ys + b) = D(Ys_{in} - Ys - b)$$

where $b = \sum x_i$ is the total biomass. This equation predicts that the quantity $Ys + b$ changes exponentially towards s_{in} with a decay rate D. As the total biomass revealed only minor variation over time, we have considered the approximation $b = Y(s_{in} - s)$, allowing us to obtain an estimation of Y close to those found in the literature (in the present case, Y=0.42). We have made the generally applied assumption that the specific growth function $\mu_i(s)$ follows a Monod kinetics:

$$\mu_i(x) = \frac{\mu_{max,i}\, s}{K_{s,i} + s}$$

Nevertheless, from the magnitude of the values of the parameters μ_{max} and K_s found in the literature and the measured values $s(t_j)$, we expect it to be in the linear part of the Monod function. Consequently, we have identified the slopes $\mu'_i = \mu_{max,i}/K_{s,i}$ of the functions $\mu_i(s)$ at zero instead of two distinct parameters.

Method 2: We assume s to be constantly equal to $\bar{s}$ and we estimate parameters $\bar{\mu_i}$ as $\mu_i(\bar{s})$ and not the functions $\mu_i(s)$. This leads to a simplification of the model (M1) where the dynamic of s is ignored:

$$\frac{x_i}{dt} = \mu_i x_i - D x_i \quad (1 \cdots n)$$

As observed from the data that the total biomass does not vary much over time (about 10%), we have considered that the OTU proportions $p_i = x_i/b$ represent more reliable information than their abundances x_i. Then, under the assumption that the substrate concentration is constant at $\bar{s}$, one can give their explicit expressions as functions of time (Rapaport, Dochain and Harmand 2009):

$$p_i(t) = p_i(t_0) \frac{e^{\mu_i t}}{\sum_j p_j(t) e^{\mu_j t}} \qquad \text{(M2)}$$

under the constraint of the quasi-stationarity of the total biomass, that is:

$$\sum_j p_j(t) \mu_j = D$$

Note that this method allows an estimation of the value μ_i without requiring the knowledge of Y, s or s_{in}. Because the consumption of organic carbon in the cultures could not be quantified, we estimated the bacterial carbon demand, based on the bacterial production (BP= μ *x* bacterial biomass) and a bacterial growth efficiency of 10%. We assumed the μ to be equal to the dilution rate (0.2 d^{-1}) and we used the conversion factor of 12.4 fg C $cell^{-1}$ (Fukuda *et al.* 1998) to determine bacterial biomass from abundance. This estimate revealed a DOC consumption of 1 µmol and 3 µmol organic carbon in the control and the diatom-DOM treatments, respectively.

Both methods have used the least-square criterion for the estimation of the parameters. For each replicate and the average of replicates we have chosen estimations that provide the best R2. One

can then compare the values $\mu'_i . s(t_j)$ estimated by model (M1) with the values μ_i estimated by model (M2). Comparison of the biomass of the individual OTUs at 4 time points based on observations and model simulations validated M1 (Suppl. Fig. 2 and Suppl. Table 3).

Results

Dissolved organic matter and inorganic nutrients

DOC concentrations in the background seawater and the diatom-derived material used to prepare each culture medium were similar, resulting in equivalent DOC concentrations before and after the mixing. Measurements of DOC performed in each of the freshly prepared media over the course of the experiment were 49±1 µM for the winter water DOM (n=7), thereafter referred to as control, and 49±2 µM for the diatom-DOM condition (n=7). In the cultures, concentrations of DOC were slightly higher than in the respective media, in particular during the first few days of the experiment (range 50 – 56 µM) (Fig. 1). From day 7 (in the diatom-DOM treatment) and day 10 (in the control) to the end of the experiment, DOC concentrations remained relatively stable and they were significantly higher in the control (52±1, n=5) than in the diatom-DOM condition (50±1, n=6) (Fig. 1, Student test, $p \leq 0.05$). A possibly small DOC contamination during the setup of the cultures cannot be excluded. However, given the excellent reproducibility of our observations between the biological triplicates (i.e. DOC concentrations, bacterial abundance and community composition) after several complete renewals of the cultures, it is unlikely that it substantially affected the results of our experiment. Concentrations of nitrate and phosphate were identical (Student test, $p>0.05$) in the input medium (39±2 and 41±1 µM NO_3^- and 2±0.3 and 2±1 µM PO_4^{3-} (n=8) for the control and the diatom-DOM treatment, respectively) and the cultures (40±2 and 42±1 µM NO_3^- and 2±0.2 and 2±0.1 µM PO_4^{3-} (n=9) for the control and the diatom-

DOM treatment, respectively). This indicates that bacterial consumption of these nutrients in the cultures was lower than the precision of the analytical method. C:N and C:P ratios were similar in the two treatments (1.3:1 and 26:1, respectively), and below the C:N and C:P ratios of heterotrophic bacteria (5:1 and 45:1) (Goldman, Caron and Dennett 1987), indicating that heterotrophic bacteria were C-limited in these incubations.

Heterotrophic bacterial abundance

In the first days of the experiment, bacterial abundances increased in all cultures. They stabilized after 5 days in the diatom-DOM treatment, and after 10 days in the control treatment (Fig. 1). Bacterial abundances were significantly higher in the diatom-DOM treatment ($1.3\pm0.1 \times 10^6$ cells ml^{-1}, n=7) than in the control ($8.7\pm0.3 \times 10^5$ cells ml^{-1}, n=5) (Student test, $p<0.0001$).

Bacterial community composition

A 97% identity clustering of the reads obtained by 454 pyrosequencing of the 16S gene V1 to V3 regions yielded a total number of 544 OTUs for the 25 samples considered, after the denoising, cleanup and chimera removal steps. One sample (diatom-DOM replicate 2, after 15 days) only contained 476 sequences and was excluded from further analysis. The 24 remaining samples had 2930 to 11400 sequences, with an average of 5913±2000 sequences per sample. To enable comparison, the dataset was subsampled to 2930 sequences per sample.

A clustering of the 24 samples obtained from the two experimental conditions after 0, 5, 10, 15 and 19 days showed a clear separation between treatments, regardless of time (Fig. 2). A bootstrap analysis of the clustering indicated highly reproducible branching patterns. The communities growing in the control cultures were more similar to the inoculum than the

communities growing in the diatom-DOM cultures. The control cultures showed little change with time, with an overall difference between all control samples smaller than 8%. By contrast, the communities growing in the diatom-DOM cultures exhibited a larger range of change over time. At the end of the experiment, two of the triplicate cultures were 22% different to the other diatom DOM cultures (Fig. 2).

Diverse bacterial taxa belonging mostly to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes were observed in both experimental conditions (Fig. 3). Interestingly, distinct, sometimes closely related taxa within these broad groups showed strong association to either condition. For instance, the Roseobacter clade subgroups NAC11-7 and *Sulfitobacter* were comparatively abundant in the control and in the diatom-DOM cultures, respectively. Likewise, in the order of Alteromonadales, the genera *Marinobacter* and *Shewanella* were found abundant in the control whereas *Colwellia* was highly abundant in the diatom-DOM cultures. Most Bacteroidetes sequences belonged to the *Polaribacter* genus, which had high relative abundances in the control (50±10% of total communities, n=12) compared to the diatom-DOM cultures (4±2% of total communities, n=11). By comparison, the *Ulvibacter* genus was not abundant in the first three time points in either condition, and reached an average of 7±2% of the total communities in both experimental treatments after 19 days (n=3). The SAR11 clade, making up 51% of the inoculum, persisted in both treatments over the entire course of the experiment, with higher relative abundances in the control cultures (24±7% of the total communities, n=12) than in the diatom-DOM cultures (9±4% of the total communities, n=11). In the diatom-DOM cultures, the final time point was characterized by a striking increase in the relative abundance of the *Lentimonas* group in two out of three replicate cultures, making up to 51% of the total community in replicate 3 (Fig. 3). Overall, the communities developing in the two conditions were widely different.

To determine how many and which OTUs were driving the differences observed in the composition of these communities, we examined the distribution patterns of abundant OTUs (relative abundance ≥ 1% in at least one time point in all the replicates of a treatment). We found 21 abundant OTUs (Fig. 4), together comprising 64 to 97% (average of 89±8%, n=23) of the total communities. Out of these 21 abundant OTUs, 16 had significantly different relative abundances between conditions in at least one time point (Fig. 5), including 8 for which the differences were significant throughout the entire experiment (Student test, $p \leq 0.05$, Fig. 5A). Two *Polaribacter* (OTUs 1 and 6) and one NAC11-7 (OTU 13) OTUs had significantly higher relative abundances in the control compared to the diatom-DOM cultures at all time points. Conversely, one *Sulfitobacter* (OTU 1348), two *Methylophaga* (OTUs 44 and 202) and two *Colwellia* (OTUs 8 and 24) OTUs had significantly higher relative abundances in the diatom-DOM cultures at all time points (Fig. 5A). Taken together, these 8 OTUs, constituting the core differences between the two conditions, represented 22 to 89% of the total communities in the different samples, with an average of 56±10% of the total communities in the control cultures (n=12) and 60±23% of the total communities in the diatom-DOM cultures (n=11).

Three other abundant OTUs, a SAR11 (OTU 1582) and two *Sulfitobacter* (OTUs 2 and 31) OTUs had significantly different relative abundances between treatments, but in two or three time points only (Fig. 5B). The SAR11 OTU had higher relative abundance in the control cultures, but it was significant only for 3 out of 4 time points. The *Sulfitobacter* OTUs had higher relative abundances in the diatom-DOM cultures, and the difference with the control was significant after 5 and 19 days for both OTUs, and also after 10 days for OTU 2 (Fig. 5B). Interestingly, the four OTUs associated with the control were also detected in the diatom-DOM cultures, but the seven OTUs with higher relative abundances in the diatom-DOM cultures were not detected in the control cultures, with some exceptions (≤0.1% of the total community in the control).

Two abundant OTUs, a *Marinobacter* (OTU 41) and a *Lentimonas* (OTU 15) had higher relative abundances in the control and in the diatom-DOM amended cultures, respectively (Fig. 5B). Because of large variability among replicates, these differences were not significant according to a Student test. One unknown Alphaproteobacteria, 2 SAR11 and 2 SAR86 OTUs only had significantly higher relative abundances in the control after 5 days (Suppl. Fig. 3). Finally, 3 abundant OTUs belonging to the betaproteobacterial clade OM43 (OTU 1288), the gammaproteobacterial family of Oceanospirillaceae (OTU 51) and the Bacteroidetes genus *Ulvibacter* (OTU 17) had similar relative abundances in the two treatments (Fig. 5B, $p \geq 0.06$).

Modeled growth rates of abundant OTUs

Because of the small number of cells inoculated into the cultures, bacterial growth was not limited by organic carbon during the first days of the experiment. After the first 5 days, the supply of organic carbon and the growth rate were constrained by the dilution rate. Using the classical chemostat model (Monod 1950; Novick and Szilard 1950) allowed us to simulate growth rates for abundant OTUs in mixed communities under carbon-limited growth conditions. For a robust estimation, we used two methods of the model that are both based on the changes in biomasses of the abundant OTUs between 5 and 19 days, representing a transient phase towards equilibrium. The first method (method 1) allows the concentration of the consumed substrate to vary over time, while the second method (method 2) assumes explicitly the concentration of the consumed substrate to be constant over time. This second approach represents a simplification of the first method, because knowledge of the concentration of the consumed substrate is not required. The aim of considering these two methods is to verify a posteriori the hypothesis that the communities have reached a quasi-constant level of substrate consumption. The estimated growth rates for the 21 OTUs ranged between 0.12 and 0.49 d^{-1} for method 1 and between 0.14

and 0.33 d^{-1} for method 2 (Fig. 6 and Suppl. Table 4). Even though the 2 methods revealed variable absolute values for some OTUs, the ranking of OTUs from highest to lowest growth rates exhibited coherent patterns. Because all 21 OTUs were abundant in the diatom-DOM treatment, we illustrate the modeled growth rates for this treatment (Fig. 6). Our model results suggest 3 groups of OTUs characterized by growth rates that are high, intermediate and low. Highest growth rates (0.28-0.49 d^{-1}) were attributed by both methods to OTUs belonging to *Lentimonas*, *Ulvibacter*, Oceanospirillaceae and the OM43 clade. Intermediate growth rates (0.21 – 0.31 d^{-1}) were attributed to the NAC11-7, 2 *Polaribacter*, 1 *Sulfitobacter*, 3 SAR11 and 1 SAR86 OTUs. Lowest growth rates (0.12-0.18 d^{-1}) were determined for OTUs belonging to *Methylophaga*, *Colwellia* and *Marinobacter*. Interestingly, the two methods suggested overall similar growth rates for a given OTU under different DOM resource regimes (Suppl. Table 4), except for *Marinobacter* that had higher growth rates in the control as compared to the DOM treatment.

Discussion

We observed major differences in the identity and the abundance of the bacterial taxa selected by continuous cultures supplied with DOM from Southern Ocean winter water alone or amended with diatom-derived organic matter. Concentrations of DOC were identical in both conditions, but DOC bioavailability was higher in the diatom-DOM condition, as shown by increased bacterial abundances and DOC consumption, revealing that differences in origin and chemical composition of the organic matter sources were the main drivers of the observed patterns in bacterial community composition. The response to each of our experimental conditions was phylogenetically diverse, as it involved OTUs from various marine groups commonly observed in polar regions, including the Southern Ocean (Bano and Hollibaugh 2002; Gentile *et al.* 2006;

West *et al.* 2008; Ghiglione and Murray 2012; Thiele *et al.* 2012; Wilkins *et al.* 2013; Luria *et al.* 2016). Differences were already detectable after 5 days, suggesting the mechanisms that allowed the successful groups to develop in each condition were fast. Our results indicate that in the carbon-limited Southern Ocean, changes in the DOM bioavailability such as induced by an input of diatom-derived organic matter can act as powerful selective constraints on bacterial communities through the rapid growth of most adapted members.

Ecology and functional traits of taxa selected by winter water conditions

A common trait for taxa reaching high abundances in the cultures supplied with winter water alone is the ability to grow on DOM of overall low biological availability. The most abundant OTUs in the cultures supplied with winter water belonged to the *Polaribacter* genus in the class of Flavobacteria, and to the SAR11 and NAC11-7 alphaproteobacterial clades. Flavobacteria are known to be efficient degraders of complex polymers, such as polysaccharides, proteins or chitin, and tend to be less active than other bacterial groups in taking up simple sugars or amino acids (Cottrell and Kirchman 2000; González *et al.* 2008; Straza *et al.* 2010; Simon *et al.* 2012; Wietz *et al.* 2015; Fourquez *et al.* 2016). These characteristics are supported by proteomic and genomic studies showing that Flavobacteria possess numerous genes involved in hydrolytic activities as well as high molecular weight compounds transporters such as TonB-dependent receptors, and comparatively low numbers of genes involved in the uptake of low molecular weight compounds (González *et al.* 2008; Gómez-Pereira *et al.* 2012; Fernández-Gómez *et al.* 2013; Williams *et al.* 2013). Such metabolic traits could have given *Polaribacter* OTUs an advantage in the cultures maintained with organic matter from winter water.

These *Polaribacter* OTUs were also detected in the diatom-DOM cultures, however, in much lower abundances. While Flavobacteria have been described as associated with

phytoplankton, many studies examining bacterial community succession over the course of natural or experimental phytoplankton blooms show different timing of the response from Bacteroidetes groups compared to taxa belonging to Alpha- and Gammaproteobacteria (Tada *et al.* 2012; Teeling *et al.* 2012; Luria *et al.* 2016). Our investigation of bacterial community composition during a five-month diatom bloom at the study site showed a late peak in *Polaribacter* abundances, associated with bloom decay (Landa *et al.* 2016). These results point to weak competitiveness on fresh, labile phytoplankton material and a preference for more complex, detrital algal products for these taxa. *Polaribacter* were abundant in mesoscosms performed with DOM from coastal Antarctic waters under pre-bloom conditions (Luria *et al.* 2017). In that study, *Polaribacter* dominated not only in unamended mesocosms, but also in mesocosms amended with DOM from the diatom *Thalassiosira weissflogii*. However, their final abundances in these mesocosms seemed to depend on their abundances in the bacterial communities used as inoculum, and were consistently lower when *Colwellia* OTUs became abundant (Luria *et al.* 2017). In our study, low initial abundances of *Polaribacter* coupled to higher competitiveness of other groups, such as *Colwellia*, on the fresh diatom DOM, collected during exponential growth, are possible factors contributing to *Polaribacter* OTUs being outcompeted in this experimental condition.

SAR11 are abundant in oligotrophic waters across the world (Morris *et al.* 2002; Giovannoni 2017). Recent genomic and proteomic information obtained from both cultivation-independent methods and cultivated representatives of this diverse group confirms genetic and physiological adaptation to life in nutrient-poor waters for most members of the group (Sowell *et al.* 2009; Thompson *et al.* 2013). SAR11 are characterized by unusual nutrient utilization profiles, including carbon (Carini *et al.* 2013; Tripp 2013). Single cell approaches have shown that SAR11 efficiently take up labile, low molecular weight compounds such as glucose and free amino acids,

preferentially over high molecular weight compounds such as proteins (Malmstrom *et al.* 2004, 2005; Alonso and Pernthaler 2006), a pattern that was also observed in our study region (Fourquez *et al.* 2016). The high abundance of SAR11 in the presence of winter-water DOM is likely due to their efficient uptake of labile compounds present in low concentration (Tremblay *et al.* 2015) or of substrates derived from organic matter degradation performed by other organisms (Morris, Papoulis and Lenski 2014). Evidence for this type of cross-feeding involving SAR11 has been reported in the Atlantic Ocean (Wietz *et al.* 2015) and in the Southern Ocean (Williams *et al.* 2013). In this latter study, a metaproteomics approach revealed that SAR11 expressed transporters for molecules released by other microorganisms, in particular Flavobacteria.

The Roseobacter clade is abundant across the world ocean and is characterized by high phylogenetic, functional and ecological diversity (Selje, Simon and Brinkhoff 2004; Brinkhoff, Giebel and Simon 2008; Newton *et al.* 2010; Buchan *et al.* 2014). The NAC11-7 cluster is detected during phytoplankton bloom events (González *et al.* 2000), but was also reported in other environmental conditions (Buchan, González and Moran 2005). Recent genomic evidence based on the one cultivated representative of this cluster (HTCC2255) suggests a more streamlined genome and adaptation to more oligotrophic conditions than what is commonly observed for most Roseobacter clade members (Luo *et al.* 2013). Such adaptation could explain the success of one NAC11-7 OTU in the cultures provided with non-amended winter water.

Ecology and functional traits of taxa selected by diatom-DOM

Three other Roseobacters were abundant in the diatom-DOM cultures, which is in agreement with previous work showing the opportunistic lifestyle of many members of this clade and their frequently observed associations with phytoplankton. A substantial fraction of their genomic content allows high versatility in carbon utilization compared to other abundant marine taxa, a characteristic that facilitates this ecological strategy (Buchan, González and Moran 2005; Newton *et al.* 2010). All three Roseobacter OTUs belonged to *Sulfitobacter*, a group known for their

capacity to use organic sulfur compounds, such as dimethylsulfoniopropionate (Moran *et al.* 2007; Ankrah *et al.* 2014), a common osmolyte produced by phytoplankton, including the diatom species used in this study (Speeckaert *et al.* 2018). Roseobacters have been shown to use a variety of other sulfur compounds produced by phytoplankton (Durham *et al.* 2015). Sulfur compounds, among other modes of nutrition, could have contributed to maintain the high abundances of the three *Sulfitobacter* OTUs in the diatom-DOM amended cultures.

Four OTUs belonging to the gammaproteobacterial genera *Colwellia* and *Methylophaga* were also abundant in the diatom-DOM cultures. Members of the genus *Colwellia* are commonly found in polar environments and are well adapted to life in cold environments through a number of extracellular enzymes that are most active at low temperatures (Methé *et al.* 2005). High *Colwellia* abundances were observed in mesocosms performed in the Western Antarctic Peninsula during winter (Luria *et al.* 2017)and in environments experiencing important pulses in organic matter, such as oil spills (Redmond and Valentine 2012; Kleindienst *et al.* 2015). In an experimental study *Colwellia* became a dominant taxon at an intermediate stage of hydrocarbon degradation (Hu *et al.* 2017). The increase in abundance of this group was concurrent with an increased number of aromatic degradation genes as shown by metagenomic analyses and the decrease in the concentration of one – to three - ring aromatic compounds (Hu *et al.* 2017). These findings and the results from the present study point to a potentially widespread ability among members of the group to quickly use inputs of organic carbon. In particular, the capacity of *Colwellia* to degrade small aromatic compounds could have provided an ecological advantage to this group in the DOM-amended treatment.

Several *Methylophaga* strains have been suggested to associate with marine algae (Li *et al.* 2007) and members of this group are known to utilize C1 compounds (Methé *et al.* 2005; Neufeld *et al.* 2007), a metabolic trait that could favor their growth in the presence of phytoplankton DOM. A recent transcriptomic study conducted in the McMurdo Sound showed

utilization by *Methylophaga* of phytoplankton-derived C1 compounds such as methanol, methylamine and dimethylsufide (Bertrand *et al.* 2015). C1 compounds are common end-products of microbial metabolism and could have been present in our cultures, either directly in the diatom-derived DOM, or as a result of bacterial processing of diatom-DOM, providing a functional niche for these four OTUs (Krause *et al.* 2017).

Ecological implications for DOM processing

Our results from an experimental system point to interesting roles of niche occupation as well as interactions among members of a microbial community in DOM decomposition. Genomic data predict that *Colwellia* and *Polaribacter* share the ability to cleave polymeric substances. In our cultures, the presence of *Polaribacter* OTUs in both treatments suggests they were using polymers present in winter water, while *Colwellia* OTUs present in the diatom-DOM condition were almost undetectable in the non-amended winter water, indicating a preference for phytoplankton-derived polymers. Specialized functional niches seem to exist at a fine phylogenetic level, as suggested by the co-existence in a given treatment of closely related OTUs, such as the two OTUs in the *Polaribacter, Colwellia* and *Methylophaga* groups, or by the preferential association of the various Roseobacter OTUs within a specific treatment. These observations are consistent with recent functional, genomic and transcriptional studies demonstrating the diversity of metabolisms existing among members of the same genus or clade (Kimes *et al.* 2014; Neumann *et al.* 2015; Xing *et al.* 2015).

The distributions of similar or complementary traits in co-occurring bacterial taxa further suggest that interactions among microbes such as competition and cooperation are important for the degradation of organic matter, and play a role in community structure. Decreasing relative abundances over time for some OTUs in the diatom-DOM cultures, as illustrated in the low

growth rates obtained by the model approach, could reflect competition for the freshly supplied phytoplankton-derived material and replacement over time of initially successful members by functionally similar taxa. As discussed above, C1 compounds produced by organic matter degradation performed by some bacterial groups such as Roseobacter and Flavobacteria could have benefited other members in our cultures, such as the *Colwellia*, *Methylophaga*, OM43 and SAR11 OTUs who all share this metabolic trait (Tripp *et al.* 2008; Sun *et al.* 2011; Carini *et al.* 2013; Gifford *et al.* 2016). These interactive processes have been demonstrated in other environmental contexts (Datta *et al.* 2016; Krause *et al.* 2017). These observations highlight that interactions among taxa are driven by both the chemical nature of the available substrates and by the traits present in the microbial members, ultimately impacting community structure and organic matter decomposition.

Growth rates of marine bacterial taxa

We used the classical chemostat model (Monod 1950; Novick and Szilard 1950) to simulate the growth rates for abundant OTUs in a mixed community and under carbon-limited conditions. From the modeling point of view, the equilibrium in the chemostat model corresponds to the exclusion of all taxa except for the most competitive ones. However, when taxa have similar growth rates their dynamics over time in the chemostat can be slow even at stationary substrate levels (Rapaport, Dochain and Harmand 2009). As a diverse microbial community was present over the course of our experiment, we consider non-equilibrium dynamics. In this transient stage, the growth rates are not necessarily reflected in the relative abundance of taxa.

Even though our model estimates are in the range of previously determined values in the oligotrophic ocean for bacterial groups at broad phylogenetic levels (see review by (Kirchman 2016), we discuss our findings in relative rather than absolute terms. Interestingly,

phylogenetically diverse taxa had similar growth rates in our experimental conditions. According to the model simulations, the SAR11 OTUs observed in this experimental system grew as fast as OTUs belonging to *Polaribacter* and Roseobacter. OTUs belonging to *Colwellia* and *Methylophaga* were among those with slowest growth rates. These observations provide a complementary view on the thus far poorly documented growth rates of bacterial groups at fine phylogenetic level (Campbell and Kirchman 2013; Lankiewicz, Cottrell and Kirchman 2016). An issue that was debated in this context is whether the growth rates of SAR11 are lower (Teira *et al.* 2009; Ferrera *et al.* 2011; Campbell and Kirchman 2013) or similar (Malmstrom *et al.* 2004; Laghdass *et al.* 2012; Salter *et al.* 2015; Lankiewicz, Cottrell and Kirchman 2016) as compared to other bacterial groups or the average community. Our model simulations support the idea that some SAR11 can have growth rates similar to other taxa under carbon-limited conditions, coherent with the dominance of this clade in the oligotrophic ocean.

Conclusion

We demonstrate here the tight link between changes in the composition of DOM and that of the bacterial community in the severely C-limited Southern Ocean. The rapid emergence in the diatom-DOM treatment of bacterial groups that were also abundant in Southern Ocean surface waters during an intense diatom bloom (Landa *et al.* 2016) emphasizes the relevance of our findings to help explain the ecosystem response to a supply in fresh, labile phytoplankton material. Our experimental system enabled us to simulate the growth rates of several bacterial taxa under constrained resource supply. These simulations in combination with present knowledge on the metabolic strategies of the co-occurring abundant taxa highlight important roles for ecological interplays of marine microbes in the regulation of DOM degradation. Future studies combining detailed community composition and metabolic profiling with chemical

characterization of organic matter will help understand the interactions that exist between specific metabolites and microbial taxa, and how these interactions in return shape DOM processing and bacterial community structure in the ocean.

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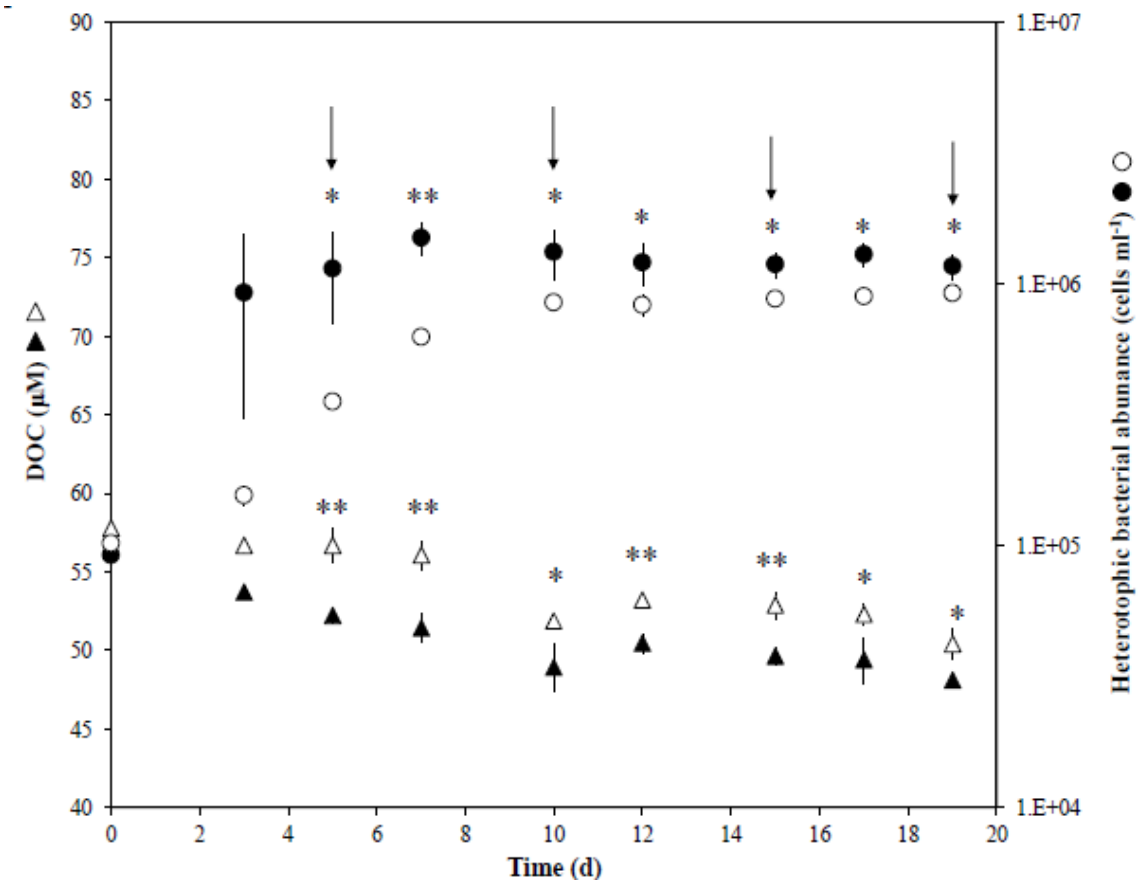

Figure 1

Abundance of heterotrophic bacteria (circles) and DOC concentration (triangles) in the control (white) and the diatom-DOM (black) cultures over time. Arrows indicate time points sampled for

bacterial diversity analyses. Mean values ± SD of biological triplicates are shown for each time point. * and ** indicate significant ($p<0.05$) and highly significant ($p<0.005$) differences between conditions, according to a Student test.

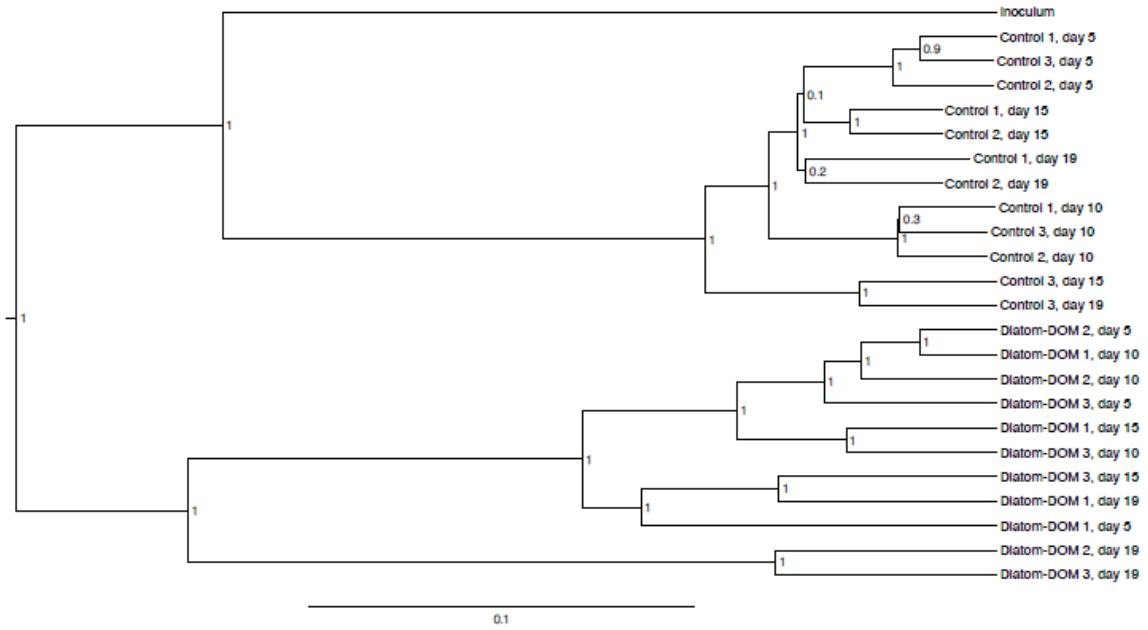

Figure 2

UPGMA clustering of bacterial communities in the control and the diatom-DOM cultures after 5, 10, 15 and 19 days, based on a weighted UNIFRAC similarity matrix. The biological triplicates are annotated 1, 2 and 3 for each treatment. The scale indicates percent dissimilarity. Bootstrap values are indicated on each node.

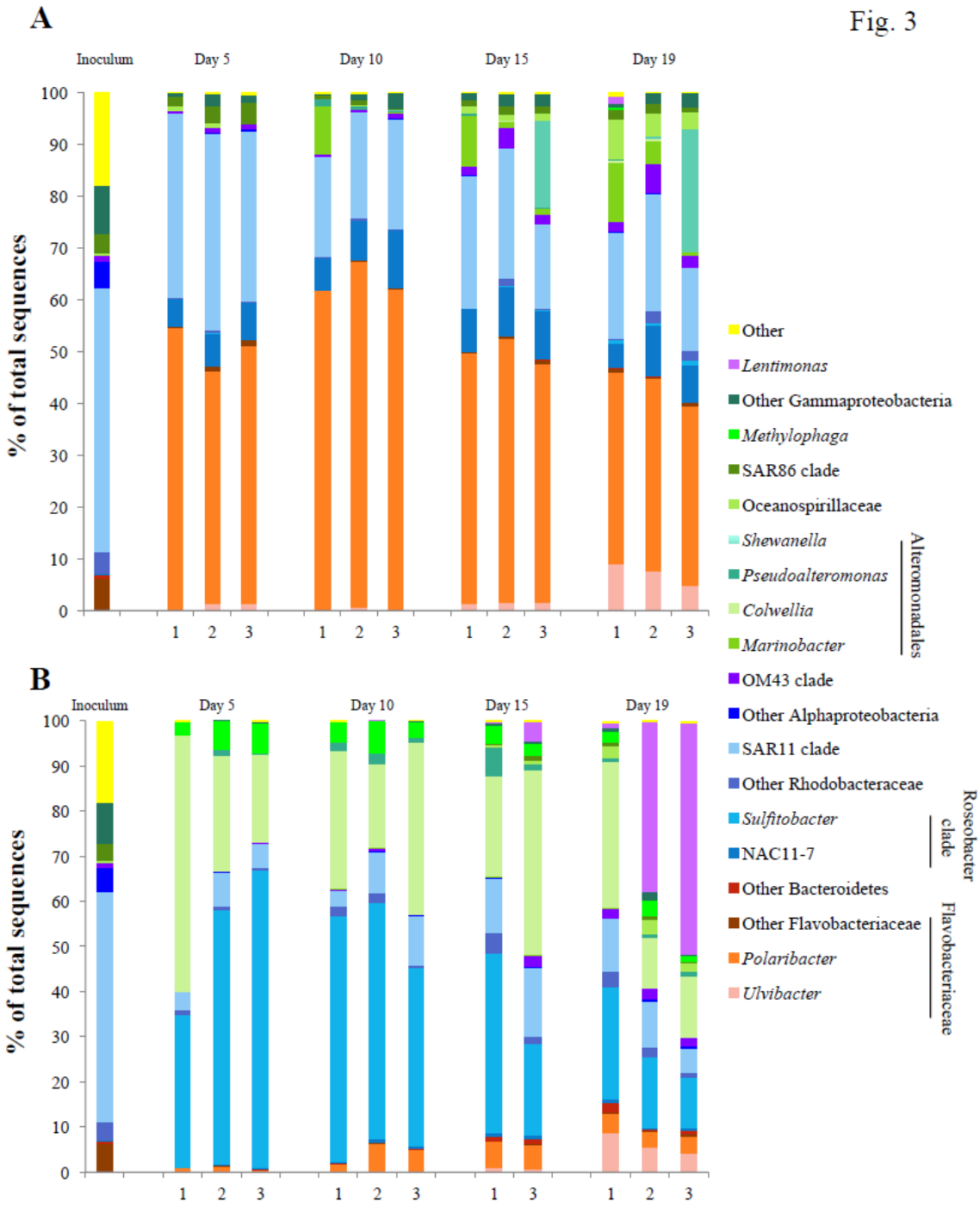

Fig. 3

Figure 3

Bacterial community composition in the control cultures (A) and the diatom-DOM cultures (B), in percent of total sequences. The natural community sampled in the winter waters on the plateau east of the Kerguelen Islands and used as an inoculum for these cultures is shown on the left in

both graphs. Bacterial groups belonging to Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes are shown in blue, green and red, respectively.

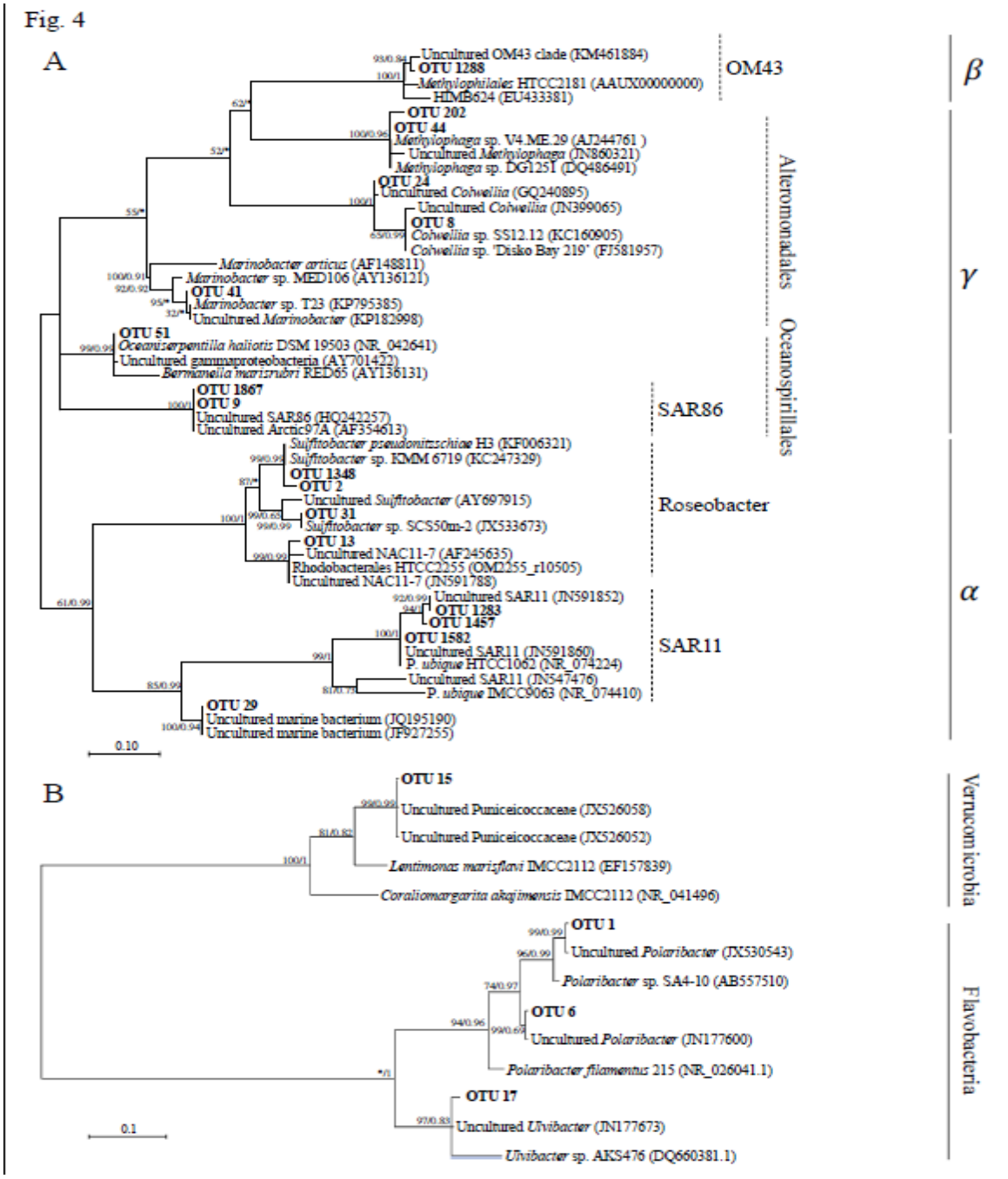

Figure 4

Unrooted maximum likelihood distance tree based on 16S gene sequences clarifying affiliation of (A) 17 OTUs belonging to the group of *Proteobacteria* and (B) 4 OTUs belonging the groups

Flavobacteria and *Verrucomicrobia*. The sequences obtained in this study are shown in bold. The numbers at the node indicate BPs and BPPs respectively, given by ML and Bayesian analyses with 1,000 replicates. The asterisks designate nodes with BPs or BPPs below 50% or 0.5.

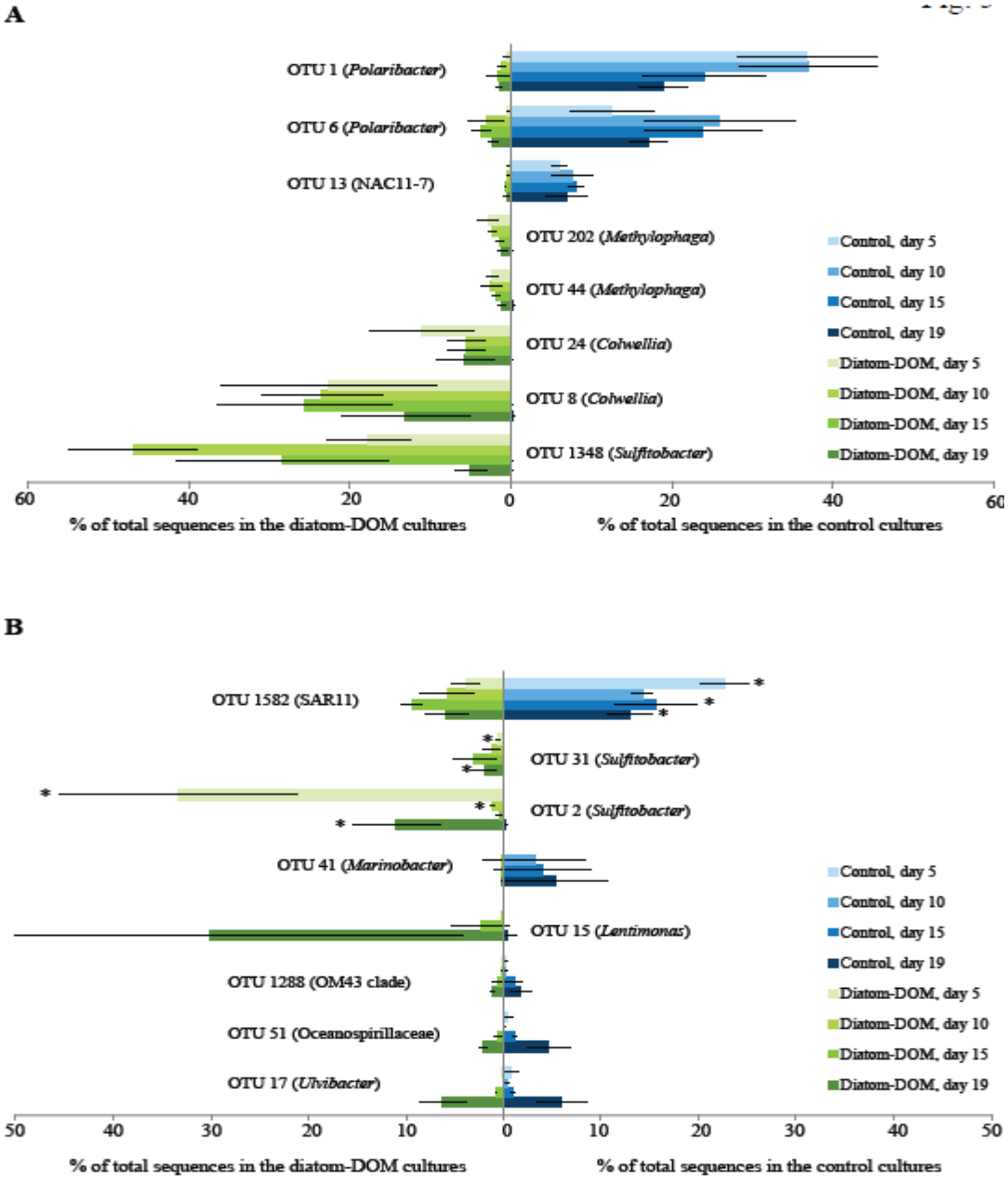

Figure 5

Relative abundances of dominant OTUs with significant differences between control and diatom-DOM cultures at all time points (A). Relative abundances of dominant OTUs with significant differences between control and diatom-DOM amended cultures at certain time points, or without

significant differences (B). Relative abundances in the control cultures are shown in blue on the right side of the graphs, relative abundances in the diatom-DOM cultures are shown in green on the left side of the graphs. Light to dark blue and green represent successive early (day 5) to late (day 19) time points. * represent statistical differences ($p \leq 0.05$) between the two conditions according to a Student test, except for graph A where all the OTUs have significantly different relative abundances at all time points.

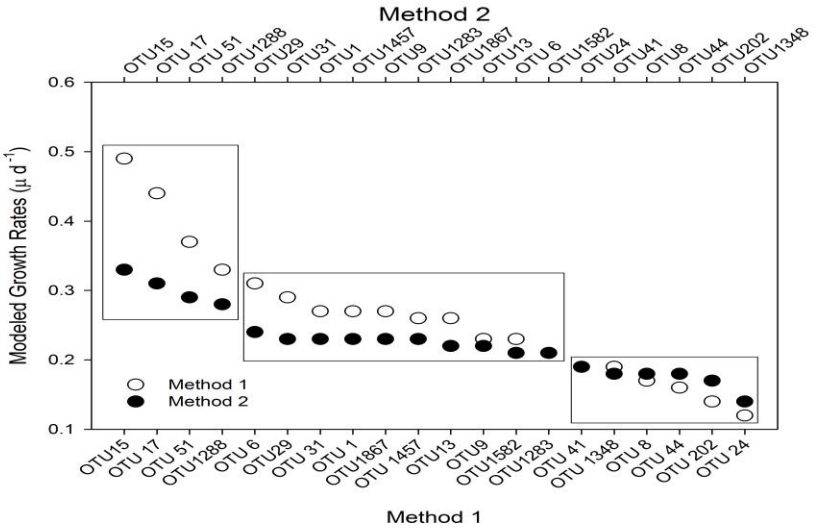

Fig. 6

Figure 6

Growth rates of the abundant OTUs in the diatom-DOM treatment obtained by 2 methods of the chemostat model. OTUs are grouped according to high, intermediate and low growth rates as

described in the text. Respective growth rates of the control are given in Supplementary Table S1. The relative abundances of the OTUs over the course of the experiment are presented in Figs. 5 and S1. OTU 1 –*Polaribacter;* OTU 2-*Sulfitobacter;* OTU 6-*Polaribacter*; OTU 8 –*Colwellia*; OTU 9-SAR86; OTU 13 –NAC11-7; OTU 15-*Lentimonas;* OTU 17-*Ulvibacter;* OTU 24-*Colwellia*; OTU 29-Alphaproteobacteria; OTU 41-*Marinobacter*; OTU 44 –*Methylophaga*; OTU 51-Oceanospirillaceae; OTU 202-*Methylophaga*; OTU 1457-SAR11; OTU 1283-SAR11; OTU 1288-OM43; OTU 1348 –*Sulfitobacter*; OTU 1582 – SAR11; OTU 1867-SAR86; OTU 31-*Sulfitobacter*.