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Differential responses of bacteria to diatom-derived dissolved organic matter in the Arctic Ocean

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ABSTRACT: The Arctic sea ice cover is undergoing an unprecedented decline due to climate change. This loss may result in the earlier start of ice algae blooms and more intense phytoplankton blooms, leading to higher concentrations of dissolved organic matter (DOM) derived from primary production. We investigated the response of early summer Arctic bacterial communities to the addition of Arctic diatom-derived DOM through biodegradation experiments in Baffin Bay. DOM produced by the planktonic diatom *Chaetoceros neogracilis* and the sea ice diatom *Fragilariopsis cylindrus* was added to seawater from 3 stations with different ice cover (2 ice zones and 1 open water zone) for 12 d. At the 3 stations, the addition of inorganic nutrients (PO_4 and NO_3) was not sufficient to stimulate bacterial growth compared to the controls, suggesting that bacteria were mainly limited by organic carbon. The addition of DOM from *C. neogracilis* stimulated bacterial abundance and production, with a more pronounced response in the ice zone compared to the open water zone. The enhanced bacterial metabolism was accompanied by changes in the bacterial community composition determined by 16S rRNA sequencing, driven by operational taxonomic units (OTUs) related to *Pseudoalteromonas* and *Polaribacter* that increased in relative abundance with DOM addition. Moreover, in the ice zone, DOM from *C. neogracilis* induced a priming effect on the bacterial utilization of ambient DOM. Our findings suggest that phytoplankton blooms, through the production of labile organic matter, will strongly affect bacterial heterotrophic activity, composition and dissolved organic carbon cycling in the Arctic Ocean.

KEY WORDS: Arctic Ocean · Sea ice · Diatom-derived organic matter · Biodegradation · Bacterial growth · Bacterial diversity · 16S rRNA sequencing

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INTRODUCTION

The Arctic Ocean is subject to unprecedented modifications triggered by environmental changes. For instance, the annual mean temperature has increased faster than in other parts of the world (Tingley & Huybers 2013). As a consequence, sea ice coverage decreases and retreats earlier (Lindsay & Hang 2005, Rothrock & Zhang 2005, Overland & Wang 2013, Arrigo et al. 2014, Peng & Meier 2017), leading to an increase in the amount of solar radiation, which in turn drives earlier, more intense and longer phytoplankton blooms (Arrigo et al. 2008, Palmer et al. 2014, Arrigo & van Dijken 2015). Indeed, a 30% increase in the Arctic Ocean annual net primary production was observed between 1998 and 2012 (Arrigo & van Dijken 2015). Moreover, current and future environmental changes may also affect the Arctic spring bloom through a shift of the dominant diatom groups toward smaller species (Quillfeldt 2000, Li et al. 2009, Poulin et al. 2011, Arrigo et al. 2012, Laney & Sosik 2014). These changes in the

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dynamics, extent and communities of the phytoplankton blooms may have dramatic consequences on the highly productive Arctic food web and biogeochemical cycles.

As primary production by-products contribute to the major part of the dissolved organic matter (DOM) pool (Thornton 2014), more intense phytoplankton blooms will lead to higher production of DOM. The composition and bioavailability of the DOM pool will depend on the dominant phytoplankton, as chemical characteristics are specific to species and growth phase (Biersmith & Benner 1998, Sarmento et al. 2013, Landa et al. 2014, Tada et al. 2017). Phytoplankton DOM is generally rich in carbohydrates, nitrogenous compounds, lipids and organic acids (Thornton 2014).

Therefore, as the Arctic spring phytoplankton blooms move and dominant species shift from under the sea ice to open-water deeper blooms (Degerlund & Eilertsen 2010, Arrigo et al. 2014), the phytoplankton DOM concentration and bioavailability is expected to change over time and space. In sea ice, 40% of the DOM is composed of extracellular polymeric substance (EPS) produced by diatoms, providing a rich carbohydrate source such as monosaccharides for bacteria (Underwood et al. 2013, Aslam et al. 2018). During the ice melt, this DOM is released into the water column, thus contributing to the carbon cycle (Vancoppenolle et al. 2013). In the Arctic Ocean, about 30% of the Arctic phytoplankton DOM, calculated between July and October 2014 during the ice-free period, is available for bacterial uptake (Paulsen et al. 2017). Thus, Arctic bacterial production is partly driven by this seasonal pulse of phytoplankton DOM. Variations in concentration, composition and bioavailability of the DOM can also affect bacterial community composition and diversity (Pinhassi et al. 2004, Niemi et al. 2014, Piontek et al. 2014, Landa et al. 2016, Tada et al. 2017). Consequently, not only bacterial productivity but also bacterial community structure may be affected by the changes in productivity occurring in the Arctic Ocean. Hence, as temperature rises, sea ice melts and bloom dynamics change, possible variations in bacterial utilization of available organic matter may affect ecosystem functioning.

In the present study, we sought to better understand the relationship between Arctic bacteria and available organic matter in a changing Arctic Ocean. The response of Arctic bacterial communities to the addition of phytoplankton DOM from 2 Arctic diatom species and sea-ice DOM was investigated through biodegradation experiments. The aims of this study were to (1) investigate the bacterial response to different sources of DOM and (2) compare the responses between communities from stations with different sea ice coverage.

MATERIALS AND METHODS

Study area

Sampling was conducted on board the Canadian icebreaker CCGS 'Amundsen' between 9 June and 10 July 2016 during the Green Edge cruise in Baffin Bay (Fig. 1). Three stations were sampled for this study. Stns G110 and G201 were located in the ice zone (IZ) and are referred to as Expts IZ-1 and IZ-2. IZ-1 and IZ-2 had a sea ice coverage (SIC) (Kaleshke & Tian-Kunze 2016) of 0.999 and 0.976, respectively, and the ice retreat was observed 23 d (IZ-1) and 18 d (IZ-2) after sampling. Stn G512 was located in the open water zone (Expt OWZ) with no sea ice (SIC = 0) (Fig. 1, Table 1). Seawater samples were collected at the surface (1 m depth) with 12 l Niskin bottles mounted on a CTD-rosette. The main characteristics of the stations sampled are presented in Table 1.

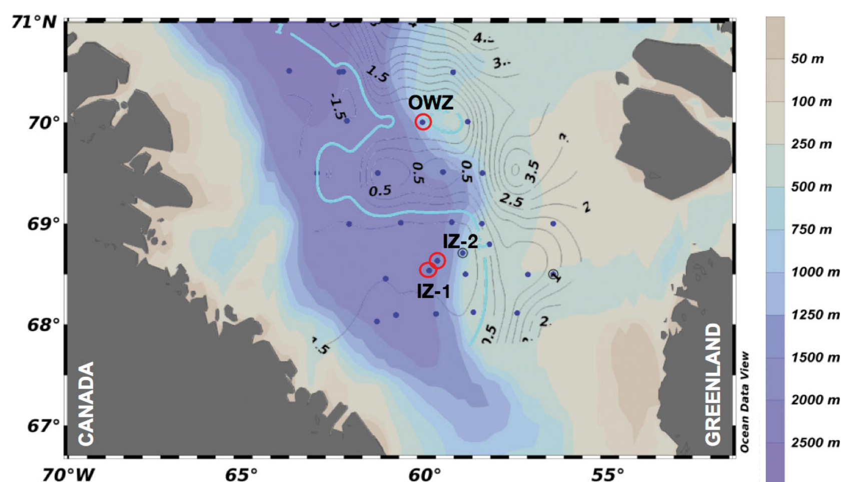

Fig. 1. Sampling stations (blue dots) during the Green Edge cruise in Baffin Bay. Stations sampled for this study are circled in red. Black isolines represent temperature (in °C). −1°C isoline indicates the limit for ice coverage (blue). Ice Zone 1 (IZ-1), Ice Zone 2 (IZ-2) and Open Water Zone (OWZ) correspond to Stns G110, G201 and G512, respectively

Table 1. *In situ* parameters of the 3 stations sampled for the different experiments. SIC: sea ice coverage (Kaleshke & Tian-Kunze 2016); IZ-1 (IZ-2): Ice Zone 1 (2); OWZ: Open Water Zone, DOC: dissolved organic carbon, BA: bacterial abundance, BP: bacterial production

	G110	G201	G512
Expt	IZ-1	IZ-2	OWZ
Sampling date	12 June 2016	14 June 2016	1 July 2016
Latitude (°N)	68.53	68.63	70.00
Longitude (°W)	60.17	59.95	60.36
SIC	0.999	0.976	0
Days of open water[a]	−23	−18	+3
Temperature (°C)	−1.65	−1.48	−0.78
Salinity	33.26	33.14	31.62
Nitrite + Nitrate (µM)	5.12	5.20	0.01
Phosphate (µM)	0.60	0.68	0.18
Chl *a* (µg l^{-1})	0.85	0.74	1.21
DOC (µM)	188	115	94
BA (cells ml^{-1})	3.9×10^5	6.1×10^5	2.2×10^6
BP (µgC l^{-1} d^{-1})	0.14	0.27	0.65
BP/DOC ((µg C l^{-1} d^{-1})/ µM DOC)	7.4×10^4	23×10^4	69×10^4

[a]Timing of ice retreat. Negative (positive) values indicate that the ice retreated after (before) the date of sampling

Preparation of DOM from the diatom cultures

Two species of Arctic marine diatoms were selected to investigate the bioavailability of organic matter released (Table S1 in the Supplement at www.int-res.com/articles/suppl/a082p059_supp.pdf). One planktonic diatom (*Chaetoceros neogracilis*, Chaetocerotaceae), and 1 sea-ice diatom (*Fragilariopsis cylindrus*, Bacillariaceae) were chosen because they are part of the dominant classes usually found in Arctic waters (Harvey et al. 1997, Tremblay et al. 2009, Balzano et al. 2012, Kilias et al. 2014). Both strains were provided by the Roscoff Culture Collection (www.roscoff-culture-collection.org). The non-axenic cultures were grown and maintained at 3°C, 10 µmol photons m^{-2} s^{-1} (continuous light) in L1 medium (Guillard & Hargraves 1993) and K/2 + Si medium (2× dilute K medium [Keller et al. 1987] with silica addition, 106 µM final concentration) for *F. cylindrus* and *C. neogracilis*, respectively (stock cultures).

Aliquots (15 ml) from each stock culture were diluted in fresh, modified K medium to a final volume of 1 l in 2 l polycarbonate carboys (Nalgene; previously rinsed with 10% HCl and washed thoroughly with Milli-Q water). Modified K medium consisted of K medium without Tris and EDTA to minimize the concentration of organic carbon, without ammonium and with silica. The cultures were incubated for 18 d in a temperature-controlled environment at the same conditions specified above for stock cultures. Cell growth was followed by flow cytometry every 3–4 d (data not shown).

At the onset of the stationary growth phase, each 1 l culture was centrifuged in 4 × 500 ml centrifuge tubes at 4000 rpm, 3200 × *g* (15 min at 4°C). The supernatant (1 l) was then filtered through a combusted GF/F filter (450°C, 6 h; Whatman) and through a 0.2 µm polycarbonate filter (Nuclepore) previously rinsed with 10% HCl and washed thoroughly with Milli-Q water. The filtrate was recovered in a combusted glass bottle (450°C, 6 h). Four replicate aliquots were sampled to determine dissolved organic carbon (DOC), nitrate and phosphate concentrations (Table S2), and 3 replicate aliquots were stored at −20°C until further use in the Arctic microcosm enrichment experiments.

Microcosm experiments with DOM from diatom exudates

At each station, surface seawater (1 m depth) was transferred to polycarbonate containers and filtered through a 0.2 µm capsule filter (Polycap TC 75 0.8/ 0.2 µm, Whatman) previously washed with 10% HCl and rinsed with Milli-Q water. The filtrate was collected in 10 l polycarbonate carboys. At each station, the inoculum of free-living bacteria was obtained after filtration of raw seawater through 0.65 µm polycarbonate membranes (Isopore, Millipore). The 0–0.65 µm size fraction was added to the 0.2 µm matrix of seawater (20% vol/vol of bacterial inoculum). After gentle mixing, aliquots (700 ml) were then distributed in 2 l polycarbonate carboys and divided into treatments in triplicate. Microcosms were incubated for 12 d on board at a temperature (1.5°C) close to that *in situ*, in the dark without agitation. All materials used were acid-washed with 10% HCl and triple-rinsed with Milli-Q water prior to use.

At each station, 3 control microcosms received no carbon addition (hereafter 'Control'), and 3 microcosms received 11–12 µM DOC (Table 2) from the same batch of *C. neogracilis* DOM (neoDOM). In

Table 2. Dissolved organic carbon (DOC) addition and consumption. Bacterial growth efficiency (BGE) was calculated from DOC consumption and bacterial production integrated over 12 d. Mean ± SD shown (n = 3). IZ-1 (IZ-2): Ice Zone 1 (2); OWZ: Open Water Zone, Nutrients: microcosms were enriched with nitrate and phosphate, DOM: dissolved organic matter, neoDOM: DOM produced by the planktonic diatom *Chaetoceros neogracilis*, iceDOM: DOM derived from diluted sea ice, cylDOM: DOM produced by the sea ice diatom *Fragilariopsis cylindrus*. Asterisks indicate significant differences from Controls (*p < 0.05, **p < 0.01, ***p < 0.001)

Expt	Treatment	Added DOC (µM)	Consumed DOC µM	Consumed DOC % of total DOC	BGE (%)
IZ-1	Control	0	10 ± 2	6 ± 1	1 ± 0.2
	Nutrients	0	8	4	1.5
	neoDOM	11	53 ± 3***	27 ± 1***	3.8 ± 0.5**
IZ-2	Control	0	20 ± 1	17 ± 1	6.9 ± 0.9
	Nutrients	0	15 ± 2	13 ± 2	8.6 ± 2
	neoDOM	11	26 ± 1**	20 ± 1**	9.2 ± 1.2
	iceDOM	3	18 ± 4	15 ± 4	8.5 ± 2.2
OWZ	Control	0	13 ± 2	14 ± 3	11.1 ± 2
	Nutrients	0	11	12	12.2
	neoDOM	12	19 ± 6*	18 ± 6*	9.6 ± 3.8
	cylDOM	16	29 ± 2*	18 ± 2*	9.2 ± 1
	iceDOM	1	15 ± 3	15 ± 4	12 ± 4.4

order to test the effect of inorganic nutrients added by the enrichment of *C. neogracilis* DOM (Table S2), 3 other microcosms received nitrate ($NaNO_3$) and phosphate (Na_2HPO_4) at a final concentration of 56 and 3 µM, respectively, similar to the nitrate and phosphate concentration added with *C. neogracilis* DOM (hereafter 'Nutrients'). These Nutrient microcosms did not receive additional carbon as for the Control microcosms.

In addition, for Expt OWZ, 3 microcosms received 16 µM DOC from *F. cylindrus* (cylDOM) (Table 2). These microcosms were enriched with nitrate and phosphate at the same final concentrations as for neoDOM and Nutrient treatments considering the residual concentrations of nutrients present in the *F. cylindrus* culture (Table S2).

Microcosm experiments with DOM from the bottom sea ice

In order to investigate the response of the bacterial community to DOM addition from sea ice, sea ice cores were collected using a manual ice corer at Stn G201. The bottom sea ice from the ice cores was subsampled using a metal cutter shape (diameter 1 cm, depth 3 cm). At the sampled station, the bottom of the sea ice was colonized by the centric diatom *Melosira* spp. (data not shown). One subsample was used immediately (Expt IZ-2) and one was kept at −80°C until Expt OWZ. In both cases, the ice was melted at ambient temperature. The volume (4–5 ml) was brought to 160 ml with 0.2 µm filtered surface water coming from Stn G201 for Expt IZ-2 and from Stn G512 for Expt OWZ. These mixes were subsampled for DOC analysis (see below). For both IZ-2 and OWZ experiments, triplicate microcosms (iceDOM) were prepared as described above and each received 50 ml of diluted sea ice. The nutrient concentrations in the ice were unknown at the experiment time, so no nutrients were added to those microcosms.

Nutrients and DOC analysis

Nitrate (NO_3^-) and phosphate (PO_3^-) concentrations were determined in the exudates collected from the diatom cultures after filtration. Samples (10 ml in duplicate) were stored at −20°C before analysis on a nutrient autoanalyzer (SEAL Analytical AA3HR). Samples for DOC concentration were taken at the beginning and at the onset of stationary growth phase to evaluate DOC production by each diatom strain. DOC samples were also taken at the beginning and at the end of the biodegradation experiments from each microcosm. All DOC samples were processed using combusted glassware. DOC samples (20 ml in duplicate) were filtered through 2 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 for diatom cultures. For biodegradation experiments, filtrate was transferred in high-density polyethylene bottles and stored at −80°C, and 85% H_3PO_4 was added before analysis. DOC concentrations were then measured by the high temperature catalytic oxidation technique (Cauwet 1994) using a Shimadzu TOC-V analyzer (Benner & Strom 1993).

Bacterial abundance and bacterial production

Samples for bacterial abundance (BA) were taken every 3 d from each microcosm. Samples (1.5 ml) were preserved with glutaraldehyde (1% final con-

centration) and stored at −80°C. Samples were stained with SYBR® Green I (ThermoFisher Scientific) and analyzed on a flow cytometer (FACSCanto, BD Biosciences) as previously described (Gasol & del Giorgio 2000). Specific growth rates were estimated from $(\ln N_t - \ln N_0)/t$, where N_t and N_0 are the final and initial BA during the exponential phase, respectively, and t is the period of exponential phase considered in days. The growth rate can be measured because grazers were removed by size fractionation during the preparation of the microcosms.

Bacterial production (BP) was measured every 3 d in each microcosm by $[H^3]$-leucine incorporation (Kirchman et al. 1985) modified for microcentrifugation (Smith & Azam 1992). Triplicate 1.7 ml aliquots were incubated with a mixture of 50/50 (v/v) $[H^3]$-leucine (Perkin Elmer) and nonradioactive leucine for 4 h at a temperature (1.5°C) close to that *in situ*. Samples with 5% trichloroacetic acid added prior to the isotope served as blank. Saturation and time course were performed beforehand to determine the concentration of leucine and minimum incubation time. Leucine incorporation was converted to carbon production using a conservative conversion factor of 1.5 kg C mol^{-1} leucine (Simon & Azam 1989).

Bacterial growth efficiency (BGE) was calculated for the different conditions in each experiment, using:

$$BGE = BP \text{ integrated } (0\text{–}12d) / (DOC \text{ consumption})$$

where DOC consumption is $DOC_{T0} - DOC_{T12}$. BGE was also calculated from integrated BA (Table S3) using a conversion factor of 12.4 fgC by bacteria (Fukuda et al. 1998).

Percentages of consumed DOC were calculated from the DOC measurements. For the total DOC, the following was used:

$$(DOC_{T0} - DOC_{T12}) / (DOC_{T0})$$

For the added DOC, we compared the consumption of DOC in the control microcosms and the consumption of DOC in microcosms amended with DOM (diatom exudates or sea-ice bottom) following the equation:

$$(\Delta DOC_{neoDOM} - \Delta DOC_{Control}) / DOC_{added}$$

DNA extraction and 16S rRNA amplicon sequencing

Samples (700 ml) at the start and end of the experiment were filtered by gentle vacuum filtration onto 0.2 µm pore size, 47 mm diameter polyethersulfone membrane filters (Supor, Pall) and stored at −80°C. DNA was extracted using a ZR Fungal/Bacterial DNA MiniPrep™ Kit (ZYMO research) following the manufacturer's guidelines but with an initial 45 min lysozyme digestion at 37°C and a 1 h Proteinase K digestion (20 mg ml^{-1} final concentration) at 55°C.

Bacterial 16S rRNA genes (V4−V5 region) were PCR amplified in separate triplicate PCR runs, using the KAPA2G Fast HotStart ReadyMix (Sigma) and primers 515F-Y (5'-GTG YCA GCM GCC GCG GTA A) and 926R (5'-CCG YCA ATT YMT TTR AGT TT) (Parada et al. 2016) complemented with Illumina-nextera index. Triplicate samples were pooled before amplification with sample-specific barcodes. After ExoSap clean-up (alkaline phosphatase and exonuclease I, Thermo-scientific) samples were quantified with PicoGreen (Invitrogen) and mixed in equimolar amounts before further purification with the Wizard® SV Gel and PCR Clean-Up System kit (Promega). Amplicons were sequenced with MiSeq Illumina 2 × 300 bp chemistry by Fasteris SA sequencing service (Switzerland).

Sequence data analysis

Sequences were analyzed and processed with Mothur version 1.39.5 (Schloss et al. 2009) using default settings, excluding sequences <300 and >500 bp. Sequences were denoised following the MiSeq SOP pipeline (Kozich et al. 2013) updated on 10 May 2017. Chimeras were removed using the VSEARCH algorithm (Rognes et al. 2016). All singletons were removed. Every sequence was then classified using the Wang algorithm (Wang et al. 2007) by comparison with the SILVA (version 119) taxonomy. Those affiliated to chloroplasts and cyanobacteria were removed. Sequences were then classified into operational taxonomic units (OTUs) at 97% threshold using the OptiClust algorithm (Westcott & Schloss 2017). A representative sequence for each OTU was classified in order to get a consensus taxonomy for each OTU. For diversity analysis, samples were randomly resampled to the size of the sample containing the lowest number of sequences (n = 8673). All further analyses were performed on the randomly resampled OTU table in order to allow a comparison of bacterial communities without bias associated with varying sampling size. Raw sequence data were deposited in the NCBI Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession number SRP126160 and Bioproject PRJNA42109.

Statistical analysis

ANOVA followed by a Dunn post hoc test was performed on the integrated BP and growth rate in order to determine significant differences between treatments. Due to the loss of DOC replicates in the nutrient treatments of Expts IZ-1 and OWZ, DOC consumption and BGE variations were compared per experiment using Student's *t*-test after checking the normality of the data with the Shapiro-Wilk test. To compare BGE between the 3 stations, an ANOVA was performed. Those tests were performed in R software (www.R-project.org). A similarity profile test (SIMPROF) was performed using the clustsig package (Clarke et al. 2008) in R, with the null hypothesis that there was no *a priori* group structure. In order to define groups of samples that did not differ significantly in terms of bacterial community structure, an alpha of 0.05 was assigned for the SIMPROF test. To define bacterial clusters, branches with p-values >0.05 were used as a prerequisite. To identify which OTUs contributed most to the dissimilarity between the different treatments, a similarity percentage (SIMPER) analysis was performed using the vegan v2.4-2 package in R (Clarke 1993, Warton et al. 2012, R Core Team 2013). The inverse Simpson index and the Chao1 index for diversity and richness, respectively, were calculated with Mothur, and values were compared with an ANOVA and a post hoc test. Relative abundance combined at the genus level was tested between treatments using an ANOVA followed by a Dunn post hoc test after log transformation to approximate normal distributions.

RESULTS

Environmental context

The 3 stations were characterized by varying sea ice cover resulting in an increasing temperature gradient from the IZ towards the OWZ (Table 1). Concentrations of nitrate and phosphate were substantially higher at the IZ-1 and IZ-2 stations compared to the OWZ, while chlorophyll *a* (chl *a*) concentration showed the opposite pattern (Fig. S1 in the Supplement). A pronounced gradient from the IZ-1 to the OWZ was observed in bacterial parameters and for the DOM characteristics. BA and BP increased from the IZ-1 to OWZ, while the concentration of DOC followed the opposite trend. BP normalized to DOC indicated an increase in bioavailable DOM from the IZ-1 to the OWZ.

BA and BP in the enrichment experiments

Bacterial growth rates (calculated from BA) and BP in the microcosms showed different patterns for the 3 stations (Fig. 2). In IZ-1, bacterial growth and BP increased after a long lag phase (about 6 d), while growth in IZ-2 and OWZ started earlier (after 3 d). Inorganic nutrient additions did not show any effect on the integrated BP and the bacterial growth rates in any of the treatments ($p > 0.05$) (Fig. 2). The addition of neoDOM stimulated bacterial growth rates in IZ-2 and OWZ, and BP in IZ-1 and IZ-2. Integrated BP in the neoDOM treatment of IZ-1 and IZ-2 were significantly higher compared to the respective controls ($p < 0.05$, 19- and 1.7-fold, respectively). However, no significant differences were observed in OWZ ($p > 0.05$). Growth rates ranged between 0.22 and 0.37 d^{-1} at all sites for the control and the nutrient-amended microcosms, with lower growth rates in IZ-1 and IZ-2 (0.22–0.27 d^{-1}) than in OWZ (0.36–0.37 d^{-1}, $p < 0.05$). In the neoDOM treatments, growth rates increased up to 0.48, 0.41 and 0.47 d^{-1} in IZ-1, IZ-2 and OWZ, respectively. This increase was significant for IZ-2

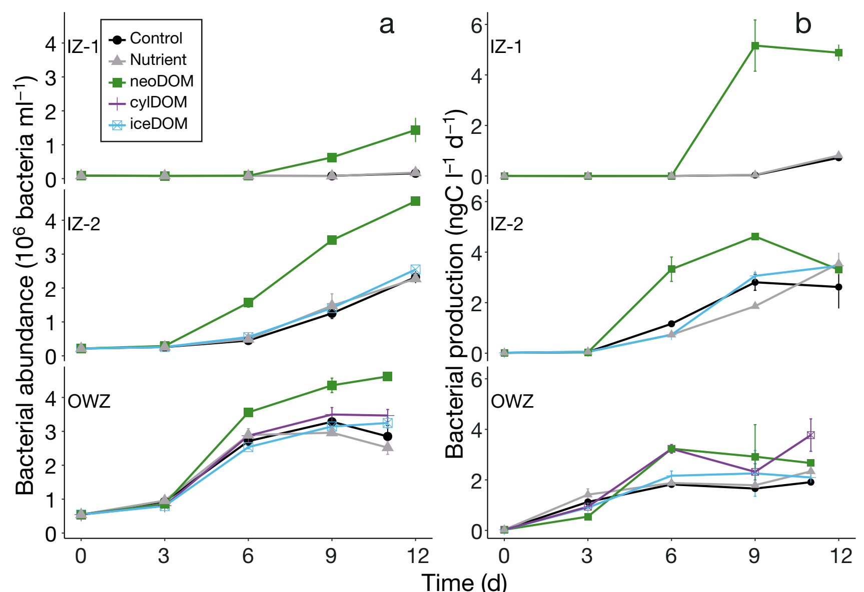

Fig. 2. Bacterial (a) abundance and (b) production in the Ice Zone (Expts IZ-1 and IZ-2) and the Open Water Zone (OWZ). Error bars represent standard deviations (n = 3). Treatments are as defined in Table 2

and OWZ ($p < 0.05$). In OWZ, the addition of cylDOM led to a small (1.3-fold), but significant increase in integrated BP ($p < 0.05$) and a higher growth rate (0.42 d^{-1}, $p < 0.05$).

In IZ-2 and OWZ, where iceDOM was added, no significant effects ($p > 0.05$) were observed on BA and integrated BP compared to the controls (Fig. 2). Overall, 3 and 1.2 µM DOC were added to Expts IZ-2 and OWZ, respectively (Table 2) through sea ice enrichment (which correspond to ~1500 µM of DOC in the sampled non-diluted sea ice).

Biodegradation of DOM

The amount of DOC consumed over the course of the biodegradation experiments varied between 10 µM (IZ-2) and 20 µM (IZ-2) in the control microcosms (Table 2). The amount of consumed DOC was smaller when inorganic nutrients were added as compared to the controls, but no statistical test could be done due to the loss of replicate DOC samples for the nutrient-amended microcosms. In IZ-1, added neoDOM represented 5.5% of the total DOC. This contribution increased in IZ-2 and OWZ (8.6 and 11.3% of DOC, respectively). The consumption of DOC in the diatom-derived DOM treatments was significantly higher compared to the controls at all sites (Table 2, $p < 0.05$). In IZ-1, DOC consumption in the presence of neoDOM exceeded the sum of the DOC added in the form of neoDOM and the DOC consumption determined in the control. This suggests that the addition of neoDOM stimulated the consumption of DOC from IZ-1. In contrast, the addition of iceDOM had no effect on DOC consumption compared to the control ($p > 0.05$).

BGE estimated in the controls were significantly different between stations ($p < 0.05$), with the lowest values determined for IZ-1 and the highest BGE for OWZ (Table 2). The neoDOM treatments had no effect on BGE ($p > 0.05$) compared to the controls in Expts IZ-2 and OWZ, but neoDOM induced a significantly higher BGE ($p > 0.05$) in Expt IZ-1 (Table 2).

Changes in bacterial diversity and community composition

Overall, 667489 reads were obtained for the 46 samples after quality filtering of the sequences, shared among 281 OTUs determined at 97% identity. Rarefaction curves can be found in Fig. S2. The taxonomic richness and diversity of the bacterial communities used as inoculum were highest in IZ-1 (12.7 for the inverse Simpson index and 174 for the Chao1 index) and decreased towards OWZ (7.7 and 103, respectively). Clustering of the samples based on the Bray-Curtis dissimilarity (Fig. 3) revealed that the initial bacterial communities from IZ-1 and IZ-2 were not significantly different from each other (73% similarity, $p > 0.05$) while the initial bacterial community from OWZ was significantly different from those of the other stations (with only 40% similarity, $p < 0.05$).

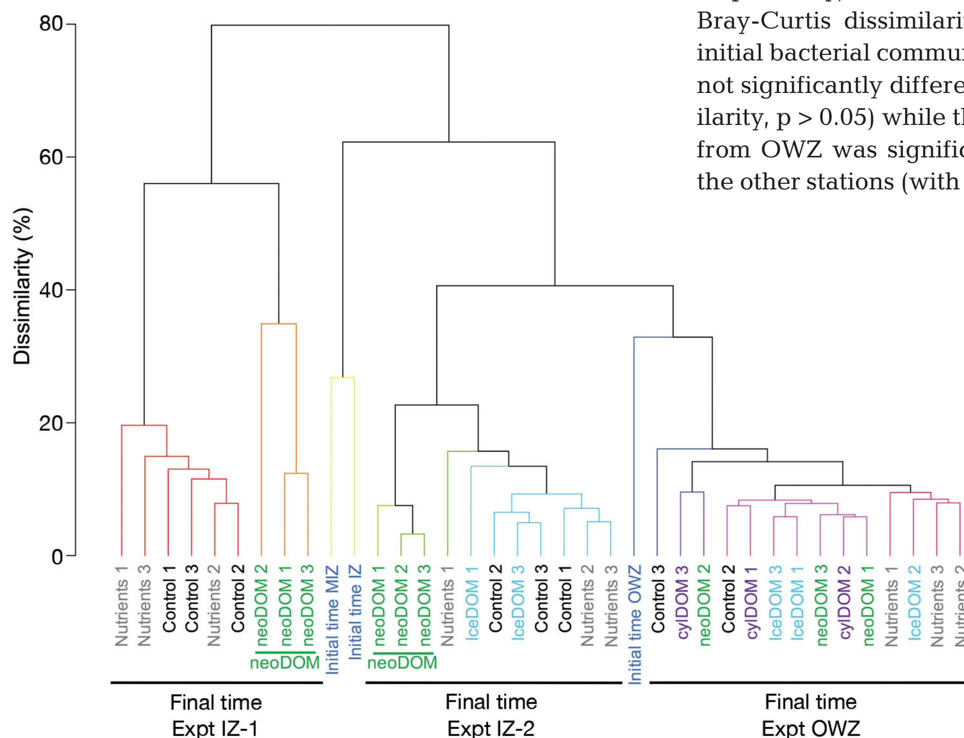

Fig. 3. Bacterial community similarity based on 16S rDNA sequencing. Sample clustering is based on Bray-Curtis dissimilarity distance matrix on subsampled sequences and the dendrogram was inferred with the weighted pair group average algorithm (UPGMA). Different branch colors correspond to statistically different groups (SIMPROF test, $p > 0.05$). Treatments are as defined in Table 2

A gradient of similarity between initial time and control microcosms is visible from IZ-1 to OWZ, with the lowest similarity for IZ-1 (13%), followed by IZ-2 (38%) and OWZ (65%). In IZ-1, the final bacterial community from the neoDOM treatment was significantly different from the communities in the controls and the nutrient-amended treatments (56% dissimilarity, $p < 0.05$). The same pattern was observed in Expt IZ-2, but with more similarity between the communities from the neoDOM treatment and the controls and nutrient-amended treatments (78%). No major difference was observed between DOM addition and control (with or without nutrients) in Expt OWZ. The community composition was not affected by the addition of sea ice in IZ-2 and OWZ.

Only minor differences in the relative abundance of *Proteobacteria* and *Bacteroidetes* were observed between the initial IZ-1 and IZ-2 communities, with a higher relative abundance of *Proteobacteria* in IZ-1 and more unclassified bacteria in IZ-2 (Fig. 4a–c). At the end of the experiment, major differences were observed between the incubations performed in IZ-1 and IZ-2. The dominant classes were *Gammaproteobacteria* and *Flavobacteria* in IZ-1 and IZ-2, respectively. In both experiments, the addition of neoDOM led to a significant decrease ($p < 0.05$) in the relative abundances of *Colwellia* and a significant increase ($p < 0.05$) of *Polaribacter* (Fig. 4d,e). Additionally, in IZ-1, the relative contribution of *Pseudoalteromonas* significantly increased ($p < 0.05$) and *Oleispira* significantly decreased ($p < 0.05$), while in IZ-2, the relative contribution of *Moritella* significantly increased ($p < 0.5$) and *Tenacibaculum* significantly decreased ($p < 0.5$).

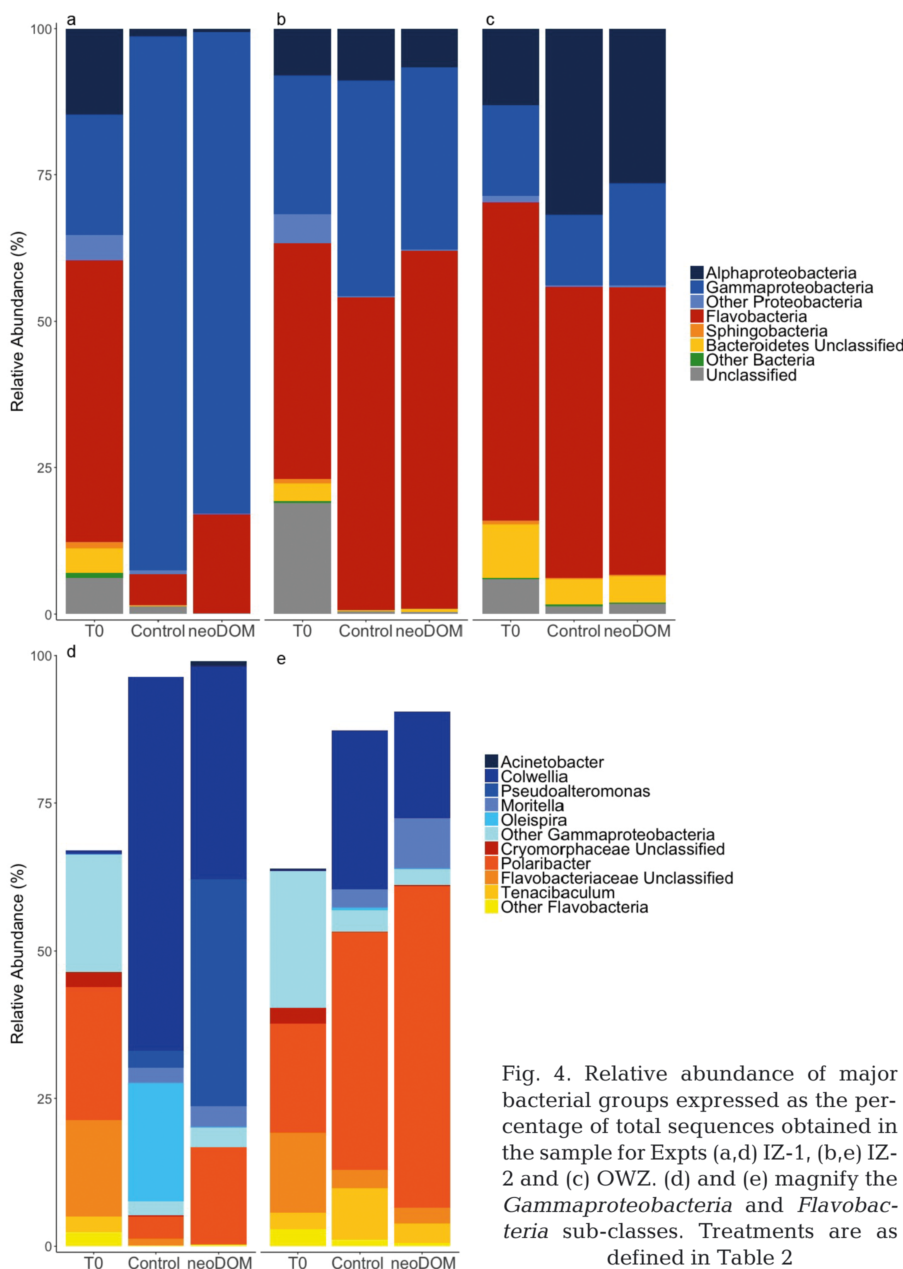

Fig. 4. Relative abundance of major bacterial groups expressed as the percentage of total sequences obtained in the sample for Expts (a,d) IZ-1, (b,e) IZ-2 and (c) OWZ. (d) and (e) magnify the *Gammaproteobacteria* and *Flavobacteria* sub-classes. Treatments are as defined in Table 2

Some OTUs were common and abundant for all experiments (e.g. OTU001 related to *Polaribacter* spp.), while others appeared more specific to one location (e.g. OTU13 closely related to *Oleispira* spp. in IZ-1) (Fig. 5). The relative contribution of some OTUs seemed to increase along the ice to open water gradient (e.g. OTU002 related to *Rhodobacteraceae*), when others seemed to decrease along this gradient (e.g. OTU007 related to *Colwellia*).

Based on a SIMPER analysis, we determined that only 5 and 9 OTUs explained up to 85% of the dissimilarity observed in the bacterial community composition between the controls and the neoDOM addition in IZ-1 and IZ-2, respectively (Table 3). Four of these OTUs were positively affected by DOM addition and 6 were negatively affected. Among these OTUs, 2 were closely related to *Colwellia* spp. Surprisingly, 1 *Colwellia*-related OTU (OTU003) was common to

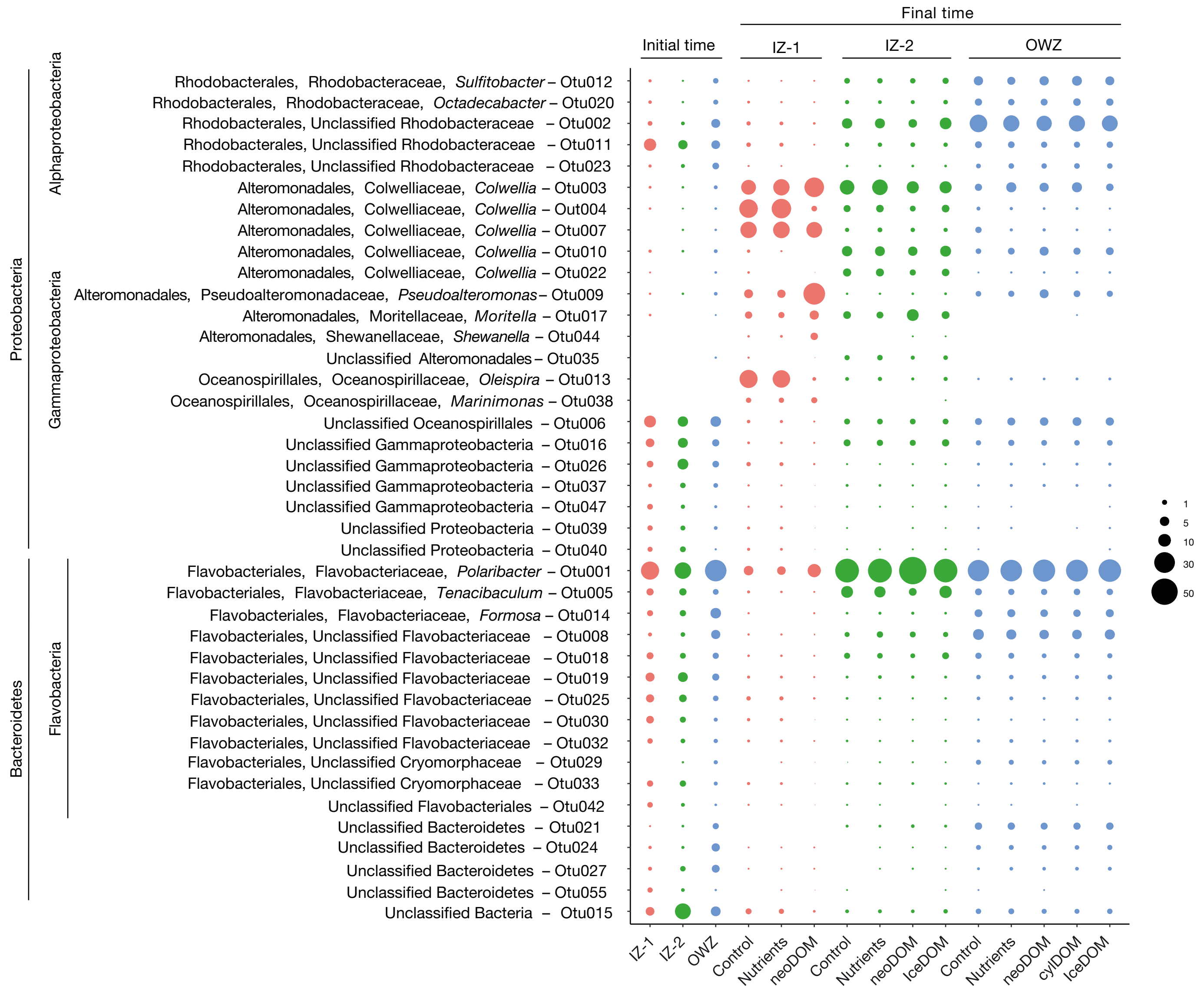

Fig. 5. Relative abundance of operational taxonomic units (OTUs) with a contribution higher than 1% in at least 1 sample (Final time is an average of triplicate microcosms). Circle sizes represent relative abundance (%). Treatments are as defined in Table 2

both experiments but shifted either positively (IZ-1) or negatively (IZ-2) with neoDOM addition.

DISCUSSION

Nutrient and substrate limitation of Arctic bacteria

Studies conducted in the Arctic Ocean show that nutrient and carbon limitation of bacterial activity vary in space and time. For instance, in the Western Arctic, bacteria were limited by carbon availability in spring, while a co-limitation by carbon and inorganic nutrients (ammonium and phosphate) was observed in late summer (Kirchman et al. 2005). A similar carbon–inorganic nutrient co-limitation was observed in the subarctic Norwegian Sea in spring (Cuevas et al. 2011). In the Beaufort Sea, nitrogen sources appeared to be the limiting nutrient in offshore surface water in late summer (Ortega-Retuerta et al. 2012). In the present study, the addition of inorganic nitrate and phosphate had no effect on bacterial growth and production, suggesting that bacteria were not nutrient limited in early summer in Baffin Bay. Nevertheless, all of our incubations received nutrients, and the sole addition of carbon was not tested. Thus, we cannot overlook the possibility that the observed stimulation of bacterial activity with diatom-derived DOM may be

Table 3. SIMPER analysis showing the contribution of operational taxonomic units (OTUs) explaining 85% of the dissimilarity between control and neoDOM microcosms after 12 d in Expts IZ-1 and IZ-2 (abbreviations are as defined in Table 2). Relative abundance is expressed as the percentage (mean ± SD, n = 3) of total sequences obtained in the sample

Taxa	Relative abundance (%) Control	neoDOM	Ratio neoDOM: Control	Contribution to the dissimilarity (%)
IZ-1				
Gammaproteobacteria, *Alteromonadales*, *Pseudoalteromonadaceae*, *Pseudoalteromonas* – OTU009	4.4 ± 1.8	33.1 ± 1.1	7.52	26.2
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU004	23.5 ± 5.5	1.3 ± 0.2	0.06	20.2
Gammaproteobacteria, *Oceanospirillales*, *Oceanospirillaceae*, *Oleispira* – OTU013	22.3 ± 2.2	0.4 ± 0.3	0.02	20.0
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU003	14.3 ± 1.5	26.7 ± 4.7	1.87	11.8
Bacteroidetes, *Flavobacteria*, *Flavobacteriales*, *Flavobacteriaceae*, *Polaribacter* – OTU001	5.2 ± 1.3	11.1 ± 2.7	2.13	6.1
IZ-2				
Bacteroidetes, *Flavobacteria*, *Flavobacteriales*, *Flavobacteriaceae*, *Polaribacter* – OTU001	40.2 ± 2.6	54.5 ± 2.3	1.36	32.7
Bacteroidetes, *Flavobacteria*, *Flavobacteriales*, *Flavobacteriaceae*, *Tenacibaculum* – OTU005	8.5 ± 0.2	3.1 ± 0.3	0.36	12.4
Gammaproteobacteria, *Alteromonadales*, *Moritellaceae*, *Moritella* – OTU017	3.1 ± 1.3	8.5 ± 0.4	2.74	12.3
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU003	13.9 ± 1.3	9.1 ± 1.5	0.65	11.0
Alphaproteobacteria, *Rhodobacterales*, *Rhodobacteraceae* – OTU002	6.2 ± 0.3	4.0 ± 0.5	0.65	5.2
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU022	3.6 ± 0.2	1.7 ± 0.3	0.47	4.2
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU010	6.2 ± 1.1	5.0 ± 0.4	0.81	3.2
Gammaproteobacteria, *Alteromonadales*, *Colwelliaceae*, *Colwellia* – OTU004	2.5 ± 0.6	1.5 ± 0.2	0.60	2.3
Gammaproteobacteria unclassified – OTU016	2.4 ± 0.8	1.6 ± 0.4	0.67	2.0

a result of a carbon and nutrient co-limitation, as has been observed in other regions of the Arctic Ocean (Kirchman et al. 2005, Cuevas et al. 2011).

Spatial gradient of bacterial responses, from ice-influenced to open waters

The measured ambient DOC concentration and bacterial activities suggest a spatial gradient of carbon availability from IZ-1 to OWZ. The dynamic nature of bioavailable DOM variability (as measured with amino acid yields) is associated with the extent of ecosystem productivity in the Arctic Ocean (Shen et al. 2018). A distinct bacterial response to the neoDOM addition was observed at the 3 studied sites. In IZ-1, characterized by the highest DOC concentration and lowest bioavailable DOM, the bacterial response to diatom-derived DOM input was most important. IZ-1 was probably sampled during a pre-bloom phase, as indicated by high nitrate and low chl *a* concentrations, while OWZ may correspond to a later bloom phase as indicated by the peak of chl *a* at 20 m depth (Fig. S1). The decrease in the fraction of diatom DOC consumed, and the increase in BP, growth rates and BGE from IZ-1 to OWZ further support this idea. In OWZ, the low bacterial stimulation induced by DOM addition compared to the controls and the high bacterial activity even in the controls could indicate that bacteria were less carbon-limited. During this later phase of the phytoplankton bloom, higher amounts of bioavailable DOC were probably released by *in situ* phytoplankton, sustaining the observed bacterial growth.

The high DOC concentrations observed in IZ-1 and IZ-2 waters could suggest that most of the DOC in these pre-bloom waters was derived from sea-ice melt. Sea ice can contain high concentrations of DOC (669–2000 µM) (Underwood et al. 2010, Garneau et al. 2016), as was measured in our sea ice samples (~1500 µM DOC). DOM in sea ice is mainly composed of algal-derived EPS rich in carbohydrates such as glucose and galactose (Krembs & Deming 2008, Underwood et al. 2010, Ewert & Deming 2013). Indeed, in melted sea ice cores, dissolved carbohydrates represented 40% of the total DOC, and up to 83% of these carbohydrates were derived from EPS (Underwood et al. 2010). Carbohydrates are a good source of carbon for marine bacteria (Elifantz et al. 2005). Nevertheless, the low bacterial activity observed in the control treatments and the IZ stations and the marked response of bacteria to the added diatom-derived DOM suggest a low bioavailability of

the ambient DOM in the ice-influenced water. This could be due to the rapid utilization of the labile DOM fraction by sea-ice bacteria. Despite the bioavailability of sea-ice DOM to bacteria (Amon et al. 2001), the addition of this DOM source in our experiment did not result in higher bacterial growth or production. The low concentration of added sea-ice DOM may explain the lack of bacterial response, as it is highly correlated to the amount of DOM as suggested by Niemi et al. (2014).

Priming effect of diatom exudates in ice-covered waters

In IZ-1, the DOC consumed in the neoDOM treatment exceeded the sum of diatom-DOC added and the DOC consumed in the control treatment, suggesting that more ambient DOM was consumed compared to the control treatment. The increase in microbial degradation of ambient DOM by addition of labile organic matter to the system (coined priming effect, PE) has been previously observed (Blagodatskaya & Kuzyakov 2008). In the present study, the PE is based on the assumption that the diatom-derived organic carbon is biologically more labile than the *in situ* organic carbon and is therefore consumed more rapidly. Even if only part of the added diatom-derived DOM was consumed, this would still support our conclusions on the PE. In this latter scenario, our calculations would only underestimate the extent of the PE. Whereas the PE has been repeatedly observed in soil ecosystems, reports in aquatic ecosystems occurred later. A first hypothesis for the importance of the PE in aquatic ecosystems was made by Guenet et al. (2010), who suggested that the release of labile DOM (e.g. mono- and polysaccharides, amino acids) by phytoplankton increased the remineralization rate of more recalcitrant DOM in aquatic ecosystems (Guenet et al. 2014, Blanchet et al. 2017). The present results suggest that the release of diatom-derived DOM in sea-ice covered water increases the recycling of less available ambient DOM, highlighting the importance of the sea ice bloom dynamics on carbon cycling in the Arctic.

Response of the bacterial community composition to diatom DOM exudates at ice-influenced stations

While a number of studies have investigated the bioavailability of terrestrial DOM (Paulsen et al. 2017) and sea-ice DOM (Niemi et al. 2014) to bacteria in the Arctic Ocean, the question of how DOM originating from Arctic phytoplankton and sea-ice diatoms affects bacterial community composition has not been investigated thus far. We observed a pronounced effect of diatom-derived DOM on the bacterial community composition, similar to observations made in experimental studies conducted in Antarctic waters (Landa et al. 2016, Luria et al. 2017) and in temperate seas (Pinhassi et al. 2004, Landa et al. 2013, Tada et al. 2017).

Polaribacter (*Flavobacteria*) related OTUs appeared to be stimulated by the DOM addition in IZ-1 and IZ-2. This genus is abundant in sea ice and polar waters (Teeling et al. 2012, Boetius et al. 2015, Torstensson et al. 2015, Landa et al. 2016), especially in the Arctic (Galand et al. 2008, Yergeau et al. 2017). *Polaribacter* was frequently observed during experimental and *in situ* phytoplankton blooms (Williams et al. 2013, Luria et al. 2016), and this group is reported to respond to phytoplankton DOM additions (Luria et al. 2017, Tada et al. 2017). This could be due to their high ability to degrade polymeric DOM such as algal polysaccharides (Klindworth et al. 2014). During a spring phytoplankton bloom in coastal Antarctic waters, *Polaribacter* dominated during the later bloom stage, while *Colwellia* (*Gammaproteobacteria*) was dominant at the beginning of the bloom (Luria et al. 2016).

In the present study, the relative abundance decrease of taxa related to *Colwellia* in the DOM treatment was lower compared to the control at both ice-influenced stations, suggesting that *Colwellia* were not the most competitive taxa in the presence of diatom DOM. *Colwellia* were outcompeted by *Pseudoalteromonas* (*Gammaproteobacteria*) in IZ-1 and by *Moritella* (*Gammaproteobacteria*) in IZ-2. *Pseudoalteromonas* produce extracellular compounds and have bacteriolytic activity that could increase their competitiveness (Holmström & Kjelleberg 1999). Interestingly, at the OTU level, a specific OTU related to *Colwellia* increased in IZ-1 and decreased in the IZ microcosms amended with diatom DOM. These 2 opposite responses for the same OTU could be explained by complex interaction or competition occurring between the species present in each bacterial community (Hibbing et al. 2010).

In IZ-1, 1 *Oleispira* (*Gammaproteobacteria*) related OTU was negatively affected by the addition of neoDOM. This OTU was not detected in the initial water, but was well represented at the end of the experiment in the control treatment (Table 3). Interestingly, *Oleispira* was reported to be stimulated by the addition of methane during incubation of seawater

from Utqiagvik, Alaska (Uhlig et al. 2018), and has been identified as highly active in the uptake of bicarbonate in Arctic seawater (Alonso-Sáez et al. 2010). *Oleispira* seems to be less competitive when DOM released by diatoms is present in the environment.

CONCLUSIONS

Increased temperatures in the Arctic have a dramatic impact on the sea ice cover (Screen & Simmonds 2010). The reduced ice cover affects the timing and the intensity of spring phytoplankton blooms (Arrigo et al. 2008, Palmer et al. 2014, Arrigo & van Dijken 2015) and potentially bacterial heterotrophic activity and community composition through quantitative and compositional changes in DOM. Our results show a clear response of heterotrophic bacteria to diatom-derived DOM in early summer in Baffin Bay (Arctic Ocean); however, the magnitude of the response varies with sea-ice cover. Heterotrophic bacteria present in the sea-ice covered Arctic Ocean were stimulated by diatom-derived DOM, and increased bacterial activity was associated with changes in the bacterial community composition. The addition of this labile DOM source further resulted in the enhanced bacterial degradation of *in situ* DOM. To our knowledge, this could be the first evidence of PE in the Arctic Ocean. This last finding needs further study to understand the importance of this process in an ocean receiving a large amount of recalcitrant terrestrial DOM from rivers (Peterson et al. 2002) and becoming more productive due to a decrease in sea ice coverage.

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