Viability and stress state of bacteria associated with primary production or zooplankton-derived suspended particulate matter in summer along a transect in Baffin Bay (Arctic Ocean)

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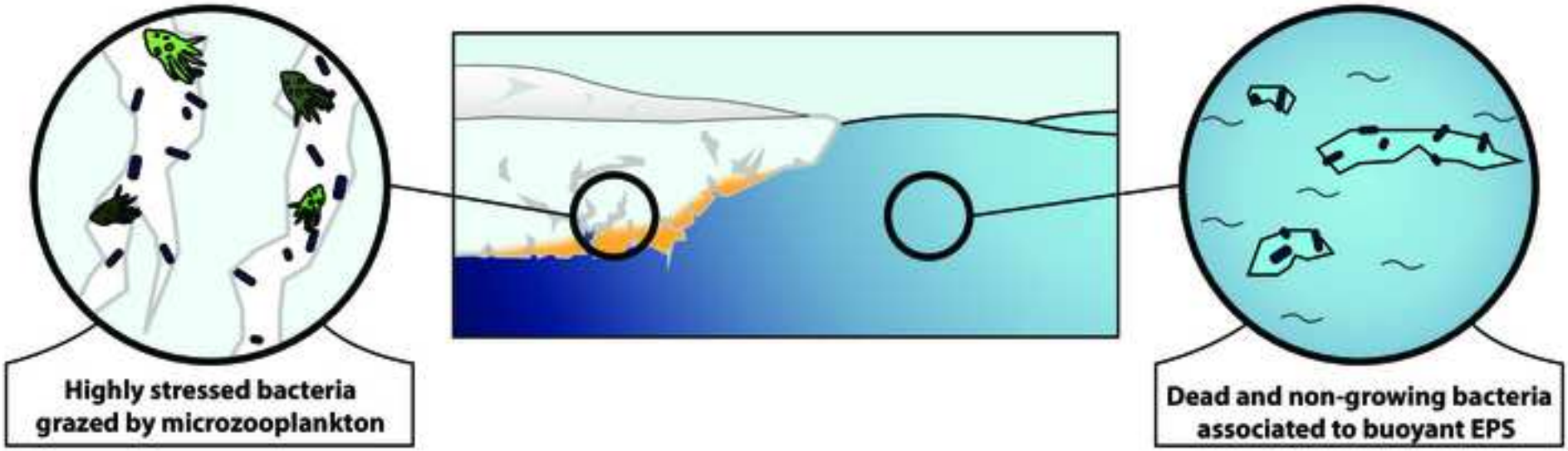
Abstract

In the framework of the GreenEdge Project (whose the general objective is to understand the dynamic of the phytoplankton spring bloom in Arctic Ocean), lipid composition and viability and stress state of bacteria were monitored in sea ice and suspended particulate matter (SPM) samples collected in 2016 along a transect from sea ice to open water in Baffin Bay (Arctic Ocean). Lipid analyses confirmed the dominance of diatoms in the bottommost layer of ice and suggested (i) the presence of a strong proportion of micro-zooplankton in SPM samples collected at the western ice covered St 403 and St 409 and (ii) a high proportion of macro-zooplankton (copepods) in SPM samples collected at the eastern ice covered St 413 and open water St 418. The use of the propidium monoazide (PMA) method allowed to show a high bacterial mortality in sea ice and in SPM material collected in shallower waters at St 409 and St 418. This mortality was attributed to the release of bactericidal free fatty acids by sympagic diatoms under the effect of light stress. A strong *cis-trans* isomerization of bacterial MUFAs was observed in the deeper SPM samples collected at the St 403 and St 409. It was attributed to the ingestion of bacteria stressed by salinity in brine channels of ice by sympagic bacterivorous microzooplankton (ciliates) incorporating *trans* fatty acids of their preys before to be released in the water column during melting. The high *trans/cis* ratios also observed in SPM samples collected in the shallower waters at St 413 and St 418 suggest the presence of positively or neutrally buoyant extracellular polymeric substances (EPS)-rich particles retained in sea ice and discharged (with bacteria stressed by salinity) in seawater after the initial release of algal biomass. Such EPS particles, which are generally considered as ideal vectors for bacterial horizontal distribution in the Arctic, appeared to contain a high proportion of dead and non-growing bacteria.

Keywords: Sea ice algae; Bacterial viability; Salinity stress; *Cis-trans* isomerase; EPS; Micro- and macro-zooplankton.

Graphical Abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

- Grazing of salinity-stressed bacteria by microzooplankton in brine channels of ice
- Incorporation of the dietary bacterial isomerized fatty acids by microzooplankton
- Induction of high bacterial mortality by free fatty acid producing ice algae
- Dead or non-growing bacteria associated to buoyant EPS particles in surface waters
- Good viability of bacteria associated to zooplanktonic material in deeper waters

1. Introduction

Arctic sea ice shelters a huge diversity of organisms particularly well-adapted to the harsh living conditions in this ecosystem (Boetius et al., 2015). They include bacteria, viruses, archaea and diatoms (von Quillfeldt et al., 2003; Junge et al., 2004; Sazhin et al., 2018). One of the numerous ecosystem services that this particular biota fulfil is the production of organic matter (Boras et al., 2010; Boetius et al., 2015). Sympagic diatoms (diatoms inhabiting the ice matrix) are responsible for much of this production. Their contribution to annual primary production (PP) varies widely depending on the season and the region (<1–60%, e.g. Dupont, 2012; Fernández-Méndez et al., 2015), but it represents a crucial food source for the marine food web, especially in winter (Søreide et al., 2010).

It has been shown that Arctic sea ice contains large amounts of extracellular polymeric substances (EPSs) (Krembs et al., 2002), one order of magnitude higher than in the surface water (Krembs and Engel, 2001; Meiners et al., 2003). These substances are produced by both bacteria and algae, with sympagic diatoms being their primary source in sea ice (Meiners et al., 2003; Mancuso Nichols et al., 2004). EPSs can help protect cells against harsh environmental conditions (e.g. salinity fluctuations) and assist cell adhesion (Cooksey and Wigglesworth-Cooksey, 1995). The release of exopolymers by the sympagic biota further influences carbon cycling by (i) providing a carbon-rich substrate that will support bacterial production and metabolic activity (Simon et al., 2002), (ii) bypassing microbially mediated POC production by the abiotic formation of large EPS-containing particles or aggregates (Passow, 2002; Thornton, 2002), (iii) directly contributing to the organic carbon pool, with concentrations potentially equivalent to those of particulate organic carbon (POC) in pelagic environments (Mari, 1999; Engel and Passow, 2001), and (iv) increasing the sympagic biota sedimentation rates through aggregation (Riebesell et al., 1991; Azetsu-Scott and Passow, 2004). From their high sedimentation rates and their good preservation during their travel toward the seafloor (Boetius

et al., 2013; Rontani et al., 2016; Amiraux et al., 2017), it has been suggested that sympagic PP contributes significantly to the total PP source in sea waters, especially deep ones (Glud and Rysgaard, 2007; Krause-Jensen et al., 2007). The fate of sympagic biota in sea ice and as they sink in the water column depends mostly on grazing by zooplankton and mineralization by their attached bacteria. In the Arctic, it is estimated that the zooplankton graze about 66–79% of the new PP (including sympagic algae; Forest et al., 2011), and since their fecal pellets generally increase the sinking rates of their food, it is estimated that zooplankton probably form most of the PP export to the aphotic zone and seafloor (Forest et al., 2011). By contrast, bacterial activity, which is higher for attached than for free bacteria (Hoppe, 1991; Karner and Herndl, 1992), allows cleavage of the POM into smaller pieces by extracellular enzymatic hydrolysis (Cho and Azam, 1988). Such processes enhance the further enzymatic digestion of the matter and ultimately reduce its potential to reach the seafloor. However, while heterotrophic bacteria and the rest of the microbial loop process about half of the PP in low-latitude oceans (Ducklow, 2000), their contribution at higher latitudes is assumed to be smaller. Based on bacterial activity measurements, Howard-Jones et al. (2002) suggest that a significant fraction (25–80%) of Arctic bacterioplankton is dormant or inactive in the marginal ice zone of the Barents Sea.

Recently, Amiraux et al. (2017) suggested that the weaker activity of bacteria in the Arctic could result from the involvement of some stress factors in ice, such as salinity. During the early stage of ice melting in spring, brine inclusions (where salinity may reach up to 150 in some ice sections collected in early spring 2015 at the GreenEdge Ice Camp, Galindo, unpublished data) become interconnected in channels and are expelled from the sea ice into the underlying seawater (Wadhams and Martin, 2001). The ice algal bacterial community is therefore exposed to a salinity stress, which occurs over relatively short timescales (e.g. hours). In this ecosystem, prokaryotic cells subjected to high osmotic pressure have developed mechanisms to live in these extreme conditions. Various strategies are used: (i) implementation

of active Na^+ and K^+ ion transport systems (Thompson and MacLeod, 1971), (ii) accumulation of osmocompatible compounds such as glycine betaine or proline (Piuri et al., 2003) or (iii) production of EPSs, which can act as a diffusion barrier (Kim and Chong, 2017). Another major adaptive response of many microorganisms, including bacteria, is to maintain membrane fluidity through 'homeoviscous adaptation' (Sinensky, 1974). The shifts in fatty acid composition of membrane lipids, and most notably by enzymatic conversion of *cis*- to *trans*-unsaturated fatty acids (Loffeld and Keweloh, 1996; Heipieper et al., 2003) through the activity of *cis-trans* isomerases (CTIs) can be an important bacterial mechanism for modifying membrane fluidity. It has been previously suggested that *trans/cis* ratios > 0.1 in environmental samples may be indicative of bacterial stress (Guckert et al., 1986). Previous analyses of sea ice and sinking particles collected in the water column during the vernal melting period showed a relatively strong CTI activity, suggesting the occurrence of salinity stress during the early stages of ice melt (Amiraux et al., 2017). The high *trans/cis* ratios observed in sinking particles was attributed to the flush of bacteria associated with ice algae from internal hypersaline ice brines (Amiraux et al., 2021b). The relative stability of these ratios with depth also suggested that bacterial communities associated with sinking sympagic algae were non-growing.

In a previous study, Amiraux et al. (2021a) studied the stress state of bacteria attached to sinking sympagic algae during a vernal melting season at a landfast ice station in Davis Strait. Their results emphasized the impact of salinity, limiting the growth state of attached bacteria at the beginning of sea ice melting, subsequently giving way to an intense free fatty acid (FFAs) stress. Indeed, the production of bactericidal FFAs by sympagic algae is enhanced by the increase in light transmittance through the ice (due to the advance melting of sea ice) resulting in a high bacterial mortality. If this study gave us valuable information on the interactions between sympagic algae and their associated bacteria in sinking samples, data on those interactions after ice melting and on suspended particles are still lacking.

In the present work, we thus monitored the salinity stress and mortality of bacteria associated with sea ice and suspended particulate matter (SPM) samples collected in 2016 along a transect from sea ice to open water in Baffin Bay (Arctic Ocean). We intend to determine if the bacteria associated with these suspended particles are also weakly active or in a poor physiological state, thus impacting the preservation of this material.

2. Materials and methods

2.1. Sampling

Samples were taken at three ice stations (St 403, St 409 and St 413) and one open water station (St 418) (Fig. 1) from the Canadian icebreaker CCGS *Amundsen* along a longitudinal transect from 68° 4' 25.32"N and 61° 36' 30.54" W to 68° 6' 52.14" N and 57° 46' 7.14" W between 25 and 28 June 2016 as part of the GreenEdge project. At the time of sampling, this transect was under the influence of the Arctic current from the North acting practically perpendicular to the transect (A. Randelhoff, Personal communication). Consequently, advection along the transect (East-West) should be relatively limited.

The sea ice sampling was carried out using a Kovacs Mark V 14 cm diameter corer, focusing on the bottom-most 10 cm of sea ice (sub-sectioned into two further intervals: 0–3 and 3–10 cm) where most ice biota are found (Smith et al., 1990). To compensate for biomass heterogeneity, common in sea ice (Gosselin et al., 1986), three or four equivalent core sections were pooled for each sampling day in isothermal containers. Pooled sea ice sections were then melted in the dark with 0.2 μm filtered seawater (FSW; 3:1 v:v) to minimize osmotic stress on the microbial community during melting (Bates and Cota, 1986; Garrison and Buck, 1986).

Suspended particulate matter (SPM) samples were collected at seven depths in the first 100 m of the water column using large (20 L) Niskin bottles to accommodate any within-sample heterogeneity.

For both sea ice and SPM, samples were collected in pentaplicate (a sample for lipid analyses and four for PMA analyses) as follows. Lipid, chlorophyll *a*, and total particulate carbon samples were obtained by filtration through pre-weighed Whatman glass fiber filters (Buckinghamshire, UK; GF/F, porosity 0.7 µm, combusted 4 h at 450°C) and kept frozen (< -80°C). Bacterial viability samples were obtained by filtration on 0.8 µm Whatman nucleopore filters (24 mm, autoclaved 1 h at 110°C) and kept frozen (< -80°C) prior to analysis. Owing to the porosity of the filters, the analyses concerned mainly algae, particles and their attached bacteria. Bacterial abundance and productivity were measured directly onboard the CCGS *Amundsen* by cytometry (see *Bacterial abundance*) and ^{3}H-leucine incorporation (see *Bacterial productivity*).

2.2. Treatment

2.2.1. Chlorophyll a

Concentration of chlorophyll *a* and phaeopigments retained on the GF/F filters were measured before and after acidification (5% HCl) using a TD-700 Turner Design fluorometer, after 18–24 h extraction in 90% acetone at 4°C in the dark (Parsons et al., 1984). The fluorometer was calibrated with a commercially available chlorophyll *a* standard (*Anacystis nidulans*, Sigma).

2.2.2. Total particulate carbon

At Université Laval, filters were dried for 24 h at 60°C, weighed again for dry weight determination and then analyzed using a Perkin Elmer carbon-hydrogen-nitrogen-sulfur (CHNS) 2400 Series II instrument to measure TPC. Calibration was done using accurately weighed samples of acetanilide (C_8H_9NO).

2.2.3. Bacterial abundance

Samples were analyzed directly on board the CCGS *Amundsen* using an Accuri C6 flow cytometer equipped with 488 nm and 633 nm lasers and the standard filter setup. For enumeration of phototrophs, cells were detected on the base of their red fluorescence (FL3) and unfixed samples were analyzed 3 min at a flow rate around 65 µL min^{-1}. Samples were then fixed with 0.25% glutaraldehyde (final concentration) and stained for a minimum of 15 min with SYBR Green I at 1/10 000 of the commercial solution for enumeration of heterotrophic cells (Marie et al. 1999). Trigger was set on the green fluorescence of the SYBR and samples were analyzed 2 min at a flow rate of about 35 µL min^{-1}.

2.2.4. Bacterial productivity

Bacterial production (BP) was measured for 8 to 10 depths per station, distributed in the first 350 m, by [^{3}H]-leucine incorporation (Kirchman et al., 1985) modified for microcentrifugation (Smith and Azam, 1992). Triplicate 1.7 mL aliquots were incubated with a mixture of 50/50 (v/v) [^{3}H]- 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 and Azam, 1989).

2.2.5. Bacterial viability analysis

The bacterial viability analysis was conducted using a method based on propidium monoazide (PMA). PMA is a photoreactive dye that binds to DNA, inhibiting its replication by PCR. Live cells have intact membranes and are impermeable to PMA, which only influxes into cells with disrupted membranes. The combination of PMA use and PCR provides a rapid and reliable method for discriminating live and dead bacteria. The viability analysis requires two sets of the same sample, one treated with PMA (that gives the quantity of living organisms in the sample) and an untreated one (that gives the total amount of organisms in the sample).

The first step of this method consists of a treatment of the concentrated and filtrated samples with PMA, the filters are then exposed to light, allowing PMA to bind with DNA. For a detailed protocol see Amiraux et al. (2021a).

Nucleic acids were extracted using a chloroform-based method. Filters were placed in 2 mL Eppendorf ® tubes and heat-shocked (+80°C then -80°C alternately, twice) to improve cell lysis. 100 µL of lysis solution (Tris 20 mM, EDTA 25 mM, lysozyme 1 $\mu g.\mu L^{-1}$) was added and the samples were incubated at 37°C for 15 min. 900 µL of sterile ice-cold water and 900 µl of chloroform were then added and the samples were vortexed five times for 5 s and then centrifuged for 5 min at 10,000 × *g*. 700 µL of the aqueous phase was collected, transferred to a new tube and any traces of remaining chloroform removed in speed vacuum concentrator (Savant DNA 120, Thermo Scientific ™) for 15 min. Finally, 10 µL of RNase (10 mg. mL^{-1}) was added to the samples, and they were incubated for 30 min at 37°C or overnight at 4°C. The DNA obtained was kept frozen at -20°C for further use.

Absolute quantification of bacterial SSU ribosomal RNA (rRNA) gene was carried out by qPCR with SsoAdvanced™ Sybr Green Supermix on a CFX96 Real-Time System (C1000

Thermal Cycler, Bio-Rad Laboratories, CA, USA) according to the procedure described in Fernandes et al. (2016). For more details about the qPCR program, see Amiraux et al. (2021a).

2.2.6. Lipid extraction

Samples (GF/F filters) were reduced with excess $NaBH_4$ after adding MeOH (25 mL, 30 min) to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols, which are more amenable to analysis using gas chromatography-mass spectrometry (GC-MS). Water (25 mL) and KOH (2.8 g) were then added and the resulting mixture saponified by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2 N) to pH 1 and extracted with dichloromethane (DCM; 3 × 20 mL). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered and concentrated by rotary evaporation at 40°C to give total lipid extracts (TLEs). Aliquots of TLEs were either silylated and analyzed by gas chromatography-electron impact quadrupole time-of-flight mass spectrometry (GC-QTOF) for sterol quantification, or methylated, and then treated with dimethyldisulfide (DMDS) and analyzed by GC-MS/MS for the determination of monounsaturated fatty acid double-bond stereochemistry as previously described by Amiraux et al. (2017). *Cis* and *trans* isomers of monounsaturated fatty acid (MUFA) methyl esters react with DMDS, stereospecifically, to form *threo* and *erythro* adducts, which exhibit similar mass spectra but are well-separated by gas chromatography, allowing unambiguous double-bond stereochemistry determination (Buser et al., 1983).

2.2.7. Gas chromatography-tandem mass spectrometry

GC-MS and GC-MS/MS analyses were performed using an Agilent 7890A/7010A tandem quadrupole gas chromatograph system (Agilent Technologies, Parc Technopolis - ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Agilent; HP-5MS ultra inert, 30 m × 0.25 mm, 0.25 μm film thickness) capillary column was used. Analyses

were performed with an injector operating in pulsed splitless mode set at 270°C. Oven temperature was ramped from 70°C to 130°C at 20°C min^{-1}, then to 250°C at 5°C min^{-1} and then to 300°C at 3°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program and then ramped from 0.69×10^5 Pa to 1.49×10^5 Pa at 0.04×10^5 Pa min^{-1}. The following mass spectrometer conditions were used: electron energy 70 eV, source temperature 230°C, quadrupole 1 temperature 150°C, quadrupole 2 temperature 150°C, collision gas (N_2) flow 1.5 mL min^{-1}, quench gas (He) flow 2.25 mL min^{-1}, mass range 50–700 Dalton, cycle time 313 ms. DMDS derivatives were quantified in multiple reaction monitoring (MRM) mode. Precursor ions were selected from the most intense ions (and specific fragmentations) observed in electron ionization (EI) mass spectra. *Trans/cis* ratios were obtained directly from peak area measurement of *threo* and *erythro* DMDS adducts after analyses, which were carried out three times.

2.2.8. Gas chromatography-EI quadrupole time-of-flight mass spectrometry

Accurate mass measurements were made in full scan mode using an Agilent 7890B/7200 GC/QTOF system (Agilent Technologies, Parc Technopolis – ZA Courtaboeuf, Les Ulis, France). A cross-linked 5% phenyl-methylpolysiloxane (Macherey-Nagel; OPTIMA-5MS Accent, 30 m × 0.25 mm, 0.25 μm film thickness) capillary column was used. Analyses were performed with an injector operating in pulsed splitless mode set at 270°C. Oven temperature was ramped from 70°C to 130°C at 20°C min^{-1} and then to 300°C at 5°C min^{-1}. The pressure of the carrier gas (He) was maintained at 0.69×10^5 Pa until the end of the temperature program. Instrument temperatures were 300°C for transfer line and 230°C for the ion source. Nitrogen (1.5 mL min^{-1}) was used as collision gas. Accurate mass spectra were recorded across the range *m/z* 50–700 at 4 GHz with the collision gas opened. The QTOF-MS instrument provided a typical resolution ranging from 8009 to 12252 from *m/z* 68.9955 to 501.9706.

Perfluorotributylamine (PFTBA) was used for daily MS calibration. Compounds were identified by comparing their TOF mass spectra, accurate masses and retention times with those of standards. Quantification of each compound involved extraction of specific accurate fragment ions, peak integration and determination of individual response factors using external standards.

2.2.9. Statistical analysis

The collected data were analyzed using the XLStat version 22.05 software (Adinsoft™). Kruskal-Wallis tests associated with a pairwise multiple comparison (using the Conover-Iman procedure) were performed on *trans*/*cis* ratios and on bacterial mortality data, at significance level $\alpha = 5\%$.

3. Results

Chlorophyll *a* concentration in the bottommost layer of ice (0–3 cm) was 0.44, 3.93 and 11.76 µg L^{-1} at St 403, St 409 and St 413, respectively. Chlorophyll *a*, phaeopigments and particulate organic carbon (POC) concentrations, bacterial abundance (BA) and bacterial production (BP) were measured in the upper 100 m of the water column at each station. Though relatively weak along the whole transect, pelagic chlorophyll *a* concentrations were found to be highest (up to 0.58 µg L^{-1}) in the upper 30 m of St 409, St 413 and St 418 (Fig. 2A). The highest phaeopigment concentrations (up to 0.50 µg L^{-1}) were observed between 20 m and 40 m at St 413 (Fig. 2B). The highest POC concentrations were measured in the topmost waters of St 409, St 413 and St 418 (values reaching 200 mg L^{-1} at the surface of St 418 and at 15 m in the case of St 413) (Fig. 2C). BA was found to be relatively low at St 403 and St 409, but increased from St 413 to St 418, reaching 1.9×10^6 cells mL^{-1} in the upper 30 m of water of St 418 (Fig.

3A). The highest values of BP (up to 0.33 µgC L^{-1} d^{-1}) were observed at the surface of St 409 and at 10 m for St 413 (Fig. 3B).

To learn more about the nature of the organisms present in the different ice and SPM samples, the relative proportions of the main monounsaturated fatty acids (MUFAs) ($C_{16:1\Delta9}$, $C_{16:1\Delta11}$, $C_{18:1\Delta9}$, $C_{18:1\Delta11}$, $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$), and alcohols ($C_{20:1\Delta11}$ and $C_{22:1\Delta11}$) were measured (Fig. 4). All the ice samples collected at St 403, St 409 and St 413 were dominated by $C_{16:1\Delta9}$ (palmitoleic) acid. By contrast, $C_{18:1\Delta9}$ (oleic) acid appeared to be the dominant MUFA of most of the SPM samples, except for those collected in the upper 30 m at St. 413 and at the surface and between 25 m and 30 m at St 418 containing a high abundance of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ acids and alcohols (Fig. 4).

The main algal sterols – cholest-5,24-dien-3β-ol (desmosterol), 24-methylcholesta-5,22*E*-dien-3β-ol (brassicasterol), 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol), and 24-ethylcholest-5-en-3β-ol (sitosterol) – were quantified in ice and water samples at the different stations to confirm the nature of the material present in SPM. Cholesterol, a contaminant often introduced in the samples during their withdrawal and treatment, was excluded from this comparison. Sea ice was found to be dominated by brassicasterol and 24-methylenecholesterol at St 403 and by 24-methylenecholesterol at St 409 and St 413 (Fig. 5). The deeper SPM samples collected at St 403 showed a dominance of desmosterol, while most of those collected at St 409 and at St 413 were dominated by brassicasterol. Relatively high proportions of desmosterol were also observed in the surface and 20 m samples of St 413 and in the surface and 30 m samples of St 418 (Fig. 5).

To estimate the stress state of bacteria induced by salinity in brine channels of ice, *trans/cis* ratios of $C_{16:1\Delta11}$ (hexadec-11-enoic), oleic and $C_{18:1\Delta11}$ (vaccenic) acids were measured in all the samples (Fig. 6). The results obtained showed a very strong isomerization of hexadec-11-enoic (*trans/cis* ratios reaching 2.1), and vaccenic (*trans/cis* ratios reaching 0.95) acids, but not of

oleic acid in the deeper SPM samples of St 403 and St 409 (Fig. 7). A strong isomerization of hexadec-11-enoic (*trans/cis* ratio 0.8 and 0.78) and vaccenic acids (*trans/cis* ratios 0.31 and 0.18) was also observed in the 0–3 cm layer of ice at St 409 and St 413, respectively. At St 418, the three fatty acids were strongly isomerized in the upper SPM samples (*trans/cis* ratio reaching 0.6, 1.0 and 0.45 for hexadec-11-enoic, oleic and vaccenic acids, respectively) (Fig. 7).

The viability of bacteria was estimated with PMA in sea ice and SPM samples collected at St 409 and in SPM samples collected at St 418. At St 409, a high bacterial mortality was observed in sea ice (88.7 and 84.3% in the 3–10 and 0–3 cm layers, respectively) and in the 10 m SPM sample (62.3%), but not in the 20 m sample (Table 1). In the deeper (≥ 30 m) SPM samples collected at St 418, bacterial mortality was found to be very low, but it was relatively high in the surface and 20 m samples (23.3 and 67.5%, respectively) (Table 1).

4. Discussion

4.1. Composition of sea ice and SPM samples

The low chlorophyll *a* concentrations measured in the bottommost ice samples corresponded to only one-tenth of the values previously observed at the time of the sympagic algal bloom at the GreenEdge ice camp located near Broughton Island in Baffin Bay (Fig. 1) (Amiraux et al., 2019). It thus seems that the bloom of sympagic algae took place before the start of sampling. The increase in chlorophyll *a* concentration observed in this layer from St 403 to St 413 was thus attributed to the growth of the epiphytic diatom *Melosira arctica*, whose presence was noted during the sampling (Amiraux, unpublished data). The fatty acid profiles of all the sea ice samples were dominated by $C_{16:1\Delta9}$ (palmitoleic) acid (Fig. 4) well-known to be the main fatty acid component of sea ice-associated (sympagic or epiphytic) diatoms (Fahl and Kattner, 1993; Falk-Petersen et al., 1998; Leu et al., 2010). The algal community present

in the 0–3 cm sample at St 403 seemed to be mainly composed of pennate and centric (*M. arctica*?) diatoms and Thalassiosirales as suggested by the dominance of brassicasterol and 24-methylenecholesterol (Rampen et al., 2010) (Fig. 5). The increasing proportions of 24-methylenecholesterol observed at St 409 and St 413 were attributed to the presence of increasing amounts of *M. arctica* (containing a significant proportion of this sterol, Smik, unpublished data).

It is well known that sea ice retreat controls the timing of summer plankton blooms in the Arctic Ocean (Janout et al., 2016). From visual observation and knowledge of bloom dynamics, a chlorophyll *a* threshold of 0.5 µg L^{-1} was defined by Perrette et al. (2011) to identify the blooms in the Arctic. The chlorophyll *a* concentrations measured in the water column along the transect investigated (up to 0.58 µg L^{-1}) only exceeded this threshold between 0 and 20 m at St 409 and between 15 m and 35 m at St 413 (Fig. 2A), suggesting the presence of a weak ice-edge bloom at these stations. In the ice-covered water column, chlorophyll may result from: (i) the growth of pelagic phytoplankton or (ii) the release of non-aggregated sympagic or epiphytic algae during melting. The former hypothesis is well supported by the dominance of brassicasterol observed in surface SPM samples (brassicasterol/24-methylenecholesterol ratio 1.0–1.8) (Fig. 5), which contrasts with the dominance of 24-methylenecholesterol in the bottommost layer of sea ice at these stations (brassicasterol/24-methylenecholesterol ratio 0.07–0.7) (Fig. 5). This dominance of brassicasterol probably results from the presence of the prymnesiophyte *Phaeocystis pouchetii*, whose sterol fraction is known to consist of almost 100% brassicasterol (Nichols et al., 1991) and which is a main component of under-ice and spring blooms across the Arctic (Riisgaard et al., 2015). Microscopic examination of some SPM samples supported this explanation (e.g. percentage of *Phaeocystis* to protists at 30 m at St 413 ≈ 50%) (Babin, unpublished data). However, during sea ice melting the species composition of dispersed and aggregated algae may differ significantly (Riebesell et al., 1991). Proportionately

more cells of weakly aggregated pennate diatoms containing high proportions of brassicasterol (Rampen et al., 2010) may thus stay suspended, while other more aggregated ice algae (e.g. Thalassiosirales or *M. arctica*) incorporated into sinking particles should rapidly sink out of the euphotic zone (Riebesell et al., 1991).

A high zooplanktonic grazing activity, well supported by the high abundance of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols, was observed in the upper 30 m at St 413 and at the surface and between 25 m and 30 m at St 418 (Fig. 4). Wax esters are generally the main storage lipids of marine zooplankton in high-latitude species (Lee et al., 2006) and the most common alkan-1-ols of the wax esters found in herbivorous zooplankton are $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols (Lee and Nevenzel, 1979; Albers et al., 1996). These alcohols are only known to occur in copepods that undergo diapause (Graeve et al., 1994), which are widely distributed in the Arctic.

In this area, dominant herbivorous zooplankton are the three large *Calanus hyperboreus, C. glacialis and C. finmarchicus* in addition to the smallest *Pseudocalanus spp. (*Sameoto, 1984; Forest et al., 2012). At this period of the year, naupliis and copepodits depend on the bloom to develop into adults, and diapausing adults metabolize primary production into highly rich esters stocks (Conover and Huntley, 1991; Falk-Petersen et al., 2009). A study on the surface copepod community of Baffin Bay from Underwater Vision Profiler (UVP) data revealed that a lot a small copepods were actively feeding in the eastern and ice-free waters (including St 418) during the GreenEdge cruise (Vilgrain et al., submitted). Complementary data from net sampling showed that ice-free stations are dominated by young stages of *C. finmarchicus* and *C. glacialis* (naupliis, and stages CI to CVI) in addition with CII to CIV stages of *C. hyperboreus* (Fig. S1). All stages of *Pseudocalanus spp.* were also more abundant in St 413 and 418 with naupliis in particular (Fig. S1). The large proportion of young herbivorous stages was expected in ice-free stations according to their life cycle strategies (Hirche and Niehoff 1996; Soreide et al., 2010). Adults of all these species were generally distributed all

over the Bay, but diapausing species such as *Calanus spp.*, are probably metabolizing esters from phytoplanktonic precursors, which could explain the presence of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols at St 413 and St 418.

Although the degradation of chlorophyll *a* to phaeopigments occurs in the guts of both large and small macro-zooplankton (Nelson, 1989), these compounds could be detected in significant proportions only at 30 m at St 413 (Fig. 2B). The low concentrations of phaeopigments observed in the upper SPM samples of St 413 may be attributed to photooxidation processes, well known to degrade such pigments quickly (Welschmeyer and Lorenzen, 1985) and strongly favored at St 413 due to the lack of snow cover and the relatively thin ice (limited to 40 cm). Despite the very high copepod activity present at 30 m at St 418 (Fig. 5), phaeopigment concentration was found to be very weak (Fig. 2B), probably owing to a particularly intense photooxidation at this open water station. As expected, in the samples where the presence of high proportions of copepods was indicated by $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols, large proportions of their two main sterols (Harvey et al., 1987) cholesterol (not shown) and desmosterol (Fig. 5) could be observed.

The deepest SPM samples collected at St 403 were characterized by (i) very low chlorophyll *a* concentrations (Fig. 2A), (ii) high proportions of oleic acid (Fig. 4) and desmosterol (Fig. 5) and (iii) lack of $C_{20:1\Delta11}$ and $C_{22:1\Delta11}$ alkan-1-ols. Oleic acid is often enriched in secondary producers (Falk-Petersen et al., 1999) and thus commonly interpreted as a marker of heterotrophic feeding (Graeve et al., 1997; Tolosa et al., 2004). Moreover, desmosterol is produced by zooplankton during the conversion of dietary phytosterols to cholesterol (Harvey et al., 1987). Given the absence of alkanols, the presence of copepods in these samples was excluded and that of micro-zooplankton suspected. In Baffin Bay, it is well known that the ice microfauna is dominated by dinoflagellates and ciliates (Michel et al., 2002), rarely observed in the water column, probably because prey are too scarce. These SPM samples

thus seem to contain herbivorous micro-zooplankton feeding in ice and then released in the water column during melting. The presence of *trans* MUFAs, typical of stressed sympagic bacteria (see 4.2.), suggests the simultaneous presence of sympagic herbivorous and bacterivorous micro-zooplankton in these samples. In the deepest samples of St 409 characterized by very low amounts of sterols (Fig. 5), only bacterivorous micro-zooplankton, generally incapable of synthesizing or incorporating sterols (Breteler et al., 2004), seem present.

SPM material collected between 10 m and 20 m of the open water station St 418 exhibited relatively high proportions of hexadec-11-enoic and vaccenic acids (Fig. 4), well known to be specific to bacteria (Lambert and Moss, 1983; Sicre et al., 1988). Most of the oleic acid present in these samples also arises from bacteria (see Section 4.2.). These samples thus contained a large proportion of bacteria (Fig. 4). These observations are consistent with the highest BA measured in these samples (Fig. 3A), which is similar to those previously measured in spring in sea ice of the Chukchi Sea (0.7–2.5 10^6 cells mL^{-1}; Meiners et al., 2008). Arctic sea ice harbors large amounts of extracellular polymeric substances (EPS) in both the dissolved and particulate fractions (Krembs and Engel, 2001; Krembs et al., 2002; Meiners et al., 2003). Some authors (Riedel et al., 2006; Juhl et al., 2011) previously demonstrated that EPS retained within the melting sea ice in the Arctic could supply a pulse of organic carbon to surface waters after most of the sea-ice algal biomass has been released into the water column. In the pelagic realm, EPS-rich particles, which have been observed to be positively or neutrally buoyant (Azetsu-Scott and Passow, 2004; Meiners et al., 2008), are densely colonized by attached bacteria and are ideal vectors for their horizontal distribution (Meiners et al., 2008). Bacteria use these particles as sites of attachment, possibly to protect them from grazers (Salcher et al., 2005), or as a carbon-rich substrate, which could enhance bacterial production (Riedel et al., 2006). These exopolymers could thus ascend in association with attached bacteria (Azetsu-Scott and Passow, 2004). The highest abundances of bacteria observed at 10 m and 20 m at St 418 (Fig. 3A) were

thus attributed to the release of EPS particles heavily colonized by bacteria during sea ice melting after previous loss of the sympagic algal biomass.

4.2. Stress state of bacteria in sea ice and SPM particles

Cis-trans isomerization of MUFAs has been shown to serve as an adaptive response to chemical or osmotic stress in strains of the widespread genera *Pseudomonas* and *Vibrio* (Okuyama et al., 1991; Heipieper et al., 1992; Molina- Santiago et al., 2017). High *trans/cis* vaccenic acid ratios were previously observed in sinking particles collected during the 2015 and 2016 GreenEdge ice camps (Amiraux et al., 2017; Amiraux et al., 2021a). These high values were attributed to release of non-growing bacteria attached to sympagic algae stressed by salinity in internal brine channels during the early stages of sea ice melting. To determine whether bacteria attached to suspended particles were also stressed by salinity, *trans/cis* ratios of the main monounsaturated fatty acids present in *Pseudomonas sp*. and *Vibrio sp*. (hexadec-11-enoic, oleic and vaccenic acids) (Lambert et al., 1983; Holsmtröm et al., 1998; Jia et al., 2014) were measured in sea ice and in SPM samples along the transect investigated. Although present in some *Vibrio sp*. (Lambert et al., 1983; Jia et al., 2014), palmitoleic acid was excluded from these measurements owing to its lack of specificity (strong dominance in sympagic and pelagic diatoms) (Fahl and Kattner, 1993; Leu et al., 2010).

Very high *trans/cis* ratios of hexadec-11-enoic and vaccenic acids were observed in the deepest SPM samples of St 403 and St 409 (Fig. 7), which seemed to be dominated by micro-zooplankton (see Section 4.1.). It was previously observed that the lipid composition (fatty acids and neutral lipids) of bacterivorous ciliates resembled that of their prey (Harvey et al., 1987; Boëchat and Adrian, 2005). The high *trans/cis* values observed in these samples were thus attributed to (i) the ingestion of bacteria stressed by salinity in internal brines of sea ice by sympagic ciliates, (ii) the direct incorporation of highly isomerized dietary fatty acids and

(iii) the release of these bacterivorous ciliates in the water column during ice melting. The well-known biosynthesis of *cis*-oleic acid during the metabolism of ciliates (Erwin and Bloch, 1963) is consistent with the relatively weak *trans/cis* ratio of this acid observed in these SPM samples (Fig. 7). Brine salinity, which could not be measured during the cruise, are expected to be low at the time of sampling (summer). However, high brine salinity values (ranging from 50 to 70) were measured in May 2015 and 2016 in the upper part of the ice at Qikiqtarjuaq (GreenEdge fixed station relatively close to the transect investigated, Fig. 1) (Amiraux et al., 2019; 2021a). Non-halophilic bacteria strongly affected by these hypersaline conditions in spring could thus have been ingested by sympagic ciliates and trapped in the ice before to be released in the water column during the summer melting period.

It is well known that the uppermost section of the ice experiences the most drastic changes in brine salinity (Ewert and Deming, 2013). As a consequence, bacteria attached to sympagic algae in the bottommost ice are generally not highly affected by osmotic stress (Rontani et al., 2018; Amiraux et al., 2021a). The high *trans/cis* ratios of hexadec-11-enoic and vaccenic acids measured in the bottommost 3 cm of ice of St 409 and St 413 (Figs. 6 and 7) were thus surprising. Given the relative similarity of these ratios with those observed in the deepest samples of St 403 and St 409 (Fig. 7), this isomerization was attributed to the presence of ciliates fed on salinity-stressed bacteria in internal brine channels and trapped during their discharge at the bottom of ice.

It is generally considered that suspended particles, which constitute most of the standing stock of particulate matter in the ocean (Wakeham and Lee, 1989), sink very slowly through the water column. However, aggregation processes, the extent of which remains to be estimated (Wakeham and Lee 1989; Hill 1998), can strongly increase the settling velocity of these particles and thus their contribution to the seafloor. Sympagic microzooplankton can thus

contribute to the transfer of the signature of bacteria stressed by hypersaline conditions in brine channels of sea ice to the sediments.

SPM material collected in the topmost waters of St 418 seems to be composed of EPS particles retained in sea ice and discharged in seawater after the initial release of algal biomass (Riedel et al., 2006; Juhl et al., 2011). Such EPS particles contain high amounts of bacteria (Meiners et al., 2008) that may be of sympagic or pelagic origin. The very high *trans/cis* ratios of hexadec-11-enoic, vaccenic and oleic acids observed in these SPM samples (Fig. 7) demonstrate that the bacteria attached to EPS particles are strongly stressed by salinity and thus arise from sea ice. The strong isomerization of oleic acid observed also attests to the bacterial origin of this acid. In the presence of osmotic stress, CTI activity is used by bacteria as an urgent response to guarantee survival, before other adaptive mechanisms (Heipieper et al., 2007). Consequently, with no osmotic stress (as is the case in the water column) the *trans/cis* ratio of bacteria stressed by salinity in brine channels of sea ice should decrease to the basic level (Fischer et al., 2010). Since conversion of *tran*s to *cis* fatty acids is not catalyzed (Eberlein et al., 2018), recovery of the regularly low *trans/cis* ratio needs *de novo* synthesis of *cis* fatty acids and thus depends on bacterial growth rates. The very high values of the *trans/cis* ratio observed in the topmost waters of St 418 (Fig. 7) are thus indicative of the non-growing state of bacteria attached to EPS particles. This assumption is well supported by the relatively weak BP measured in these samples (Fig. 3B) exhibiting the highest BA (Fig. 3A).

4.3. Viability of attached bacteria in sea ice and SPM particles

PMA treatment showed that most of the bacteria associated with sympagic algae at St 409 had disrupted membranes and so were dead (Table 1). These results are consistent with the high mortality of attached bacteria measured in sea ice at the end of the 2016 GreenEdge ice camp (Amiraux et al., 2021a) and attributed to the bactericidal properties of free fatty acids (FFAs)

released by sympagic algae under the effect of light stress. The toxicity of FFAs results from their insertion into the bacterial inner membrane, increasing its permeability and letting internal contents leak from the cell, which can result in death (Boyaval et al., 1995; Shin et al., 2007). A high mortality was also observed in the 10 m SPM sample (Table 1). This suggests that the algal material present in this sample (Fig. 5) results from the release of non-aggregated and FFA-producing sympagic diatoms during ice melting rather than from the growth of pelagic algae. By contrast, the viability of bacteria attached to suspended particles collected at 20 m and dominated by micro-zooplankton was found to be very good (Table 1).

Concerning SPM samples collected at St 418, PMA revealed a very low mortality of attached bacteria in the deeper (≥30 m) samples (Table 1). This good viability is probably due to the presence of unstressed bacteria associated with copepod or micro-zooplankton material, which dominated these samples (see Section 4.1.). By contrast, a lower viability was observed in the samples collected at the surface and at 20 m (23.3 and 67.5% of mortality, respectively) (Table 1). The 20 m sample mainly composed of EPS particles thus contained a mixture of dead bacteria (in which the integrity of cell membranes could not be maintained) and non-growing bacteria (where *cis-trans* isomerization of monounsaturated fatty acids ensured membrane stiffness but not growth). The presence of a significant proportion of zooplanktonic material (potential supports of unstressed bacteria) in the surface sample (Fig. 4) could explain the lower mortality (relative to the 20 m sample) observed (Table 1).

4.4. Considerations about the preservation and transfer of sympagic material to the seafloor

The preservation of sympagic algae during their transfer in the water column depends mostly on grazing by zooplankton and mineralization by their attached bacteria. We previously demonstrated that during the early stages of ice melting, bacteria associated to sinking sympagic material have been strongly stressed in hypersaline brine channels and are thus mainly non-

growing in these particles (Amiraux et al., 2017). In contrast, during the advances stages of melting most bacteria associated to sinking ice algae appeared to be stressed by free fatty acids and dead (Amiraux et al., 2021a).

Whereas only a small part of the sympagic material is released during the early stages of ice melting, i.e. when bacteria are stressed by hypersaline conditions (Amiraux et al., 2021a, 2021b), a strong *cis-trans* isomerization of MUFAs was previously observed in Arctic sediments (Rontani et al., 2012; Amiraux et al., 2017). The results obtained during the present study allow to propose an explanation to this paradox. Indeed, during the early stages of ice melting feeding of sympagic microzooplankton on stressed bacteria results to the incorporation and transfer of that stress signal (after aggregation) to the deeper waters, whereas at the advanced stages of melting copepods intensively assimilate the sympagic material (EPS-rich particles and sea ice algae) released in the water column. Due to the good healthy state of bacteria associated to the resulting copepod fecal pellets, this material should be degraded intensively within the water column contributing only weakly to the sediments. It thus appears that trophic relationships between sea-ice algae, their associated bacteria and zooplanktonic grazers are strongly intricate and need to be more investigated.

5. Conclusions

Lipid analyses and propidium monoazide (PMA) method allowed the monitoring of the stress and viability of attached bacteria in sea ice and SPM samples collected during the GreenEdge 2016 cruise in Baffin Bay, along a transect from sea ice to open water. Our results are summarized in a conceptual trophic network scheme (Fig. 8).

At the western stations ice covered St 403 and St 409 lipid analyses showed a strong *cis-trans* isomerization of MUFAs attributed to the presence of sympagic bacterivorous microzooplankton (ciliates) incorporating *trans* fatty acids after ingestion of bacteria

osmotically stressed in hypersaline brine channels of ice (Fig. 8). At the St 409, the high bacterial mortality measured in sea ice is consistent with that previously observed during the GreenEdge 2016 ice camp (Amiraux *et al*., 2021a) and is likely due to the release of bactericidal FFAs by sympagic algae under the effect of light stress (Fig. 8).

At the eastern ice-covered station St 413 and open water station St 418 the lipid analysis showed a high proportion of macro-zooplankton (copepods) (Fig. 8). SPM material collected in shallower waters at the open water St 418 seems to be mainly composed of EPS-rich particles retained in sea ice and discharged in seawater after the initial release of algal biomass. In those waters, most of the bacteria associated to this material appeared to be either dead or in a non-growing state, while these attached to deeper SPM of St 418 (dominated by zooplanktonic material) were in good healthy state (Fig. 8).

2021a2021b

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Figure captions

Figure 1. Map of the study area with location of the stations investigated in Baffin Bay. Blue circles on the enlarged map of western Baffin Bay indicate the stations investigated during the transect. The orange circle indicates the GreenEdge ice camp station investigated by Amiraux et al. (2019, 2021a). White color indicates the sea ice cover during the sampling.

Figure 2. Chlorophyll *a* (A), phaeopigments (B) and total particulate carbon (C) in seawater at the different stations investigated. Data were interpolated and plotted using Ocean Data View v4.7.8 (Schlitzer, 2015).

Figure 3. Bacterial abundance (BA) (A) and bacterial production (BP) (B) in seawater at the different stations investigated. Data were interpolated and plotted using Ocean Data View v4.7.8 (Schlitzer, 2015).

Figure 4. Relative proportion of the main monounsaturated fatty acids (FA) and alcohols (ol) in sea ice and underlying seawater at the different stations investigated.

Figure 5. Relative proportions of brassicasterol, desmosterol, sitosterol and 24-methylenecholesterol in sea ice and underlying water column at the different stations investigated.

Figure 6. MRM chromatograms (*m/z* 217 → 185 and *m/z* 245 → 213) of DMDS derivatives of MUFAs in the bottommost layer of ice (0-3 cm) at St 409.

Figure 7. Mean *Trans/cis* ratio of vaccenic ($C_{18:1\Delta11}$), oleic ($C_{18:1\Delta9}$), and hexadec-11-enoic ($C_{16:1\Delta11}$) acids in sea ice and the underlying water column at the different stations investigated

(n = 3, analytical triplicates). For each station and each acid, significantly different values are annotated with different letters ($P < 0.05$).

Figure 8. Conceptual scheme summarizing the main results obtained.

Table 1. Mean percentage of dead attached bacteria in sea ice and SPM samples collected at St 409 and St 418 (n = 3, 2 samples + an analytical replicate). For each station, significantly different values are annotated with different letters ($P < 0.05$).

Supplementary material

Fig. S1: Concentrations (number of individuals per m^3) of dominant copepod species at the different stations investigated, according to their development stages. Main feeding modes are indicated -her: herbivorous, -omn: omnivorous, -car: carnivorous. Herbivorous species, and young development stages in particular, show high variations in abundances along sea ice gradients, while it is less clear for omnivorous species.

Table 1

Mean percentage of dead attached bacteria in sea ice and SPM samples collected at St 409 and St 418 (n = 3). For each station, significantly different values are annotated with different letters (P < 0.05).

Station	Sample	Dead bacteria (%)
St 409	Ice (3-10 cm)	88.7 ± 4.7 (b)
St 409	Ice (0-3 cm)	84.3 ± 9.7 (b)
St 409	SPM 10 m	62.3 ± 22.06 (ab)
St 409	SPM 20 m	0 ± 11.9 (a)
St 418	SPM surface	23.3 ± 11.3 (b)
St 418	SPM 20 m	67.5 ± 4.1 (c)
St 418	SPM 30 m	3.8 ± 39.0 (ab)
St 418	SPM 40 m	0.6 ± 15.5 (ab)
St 418	SPM 100 m	0 ± 4.6 (a)

Figure 1

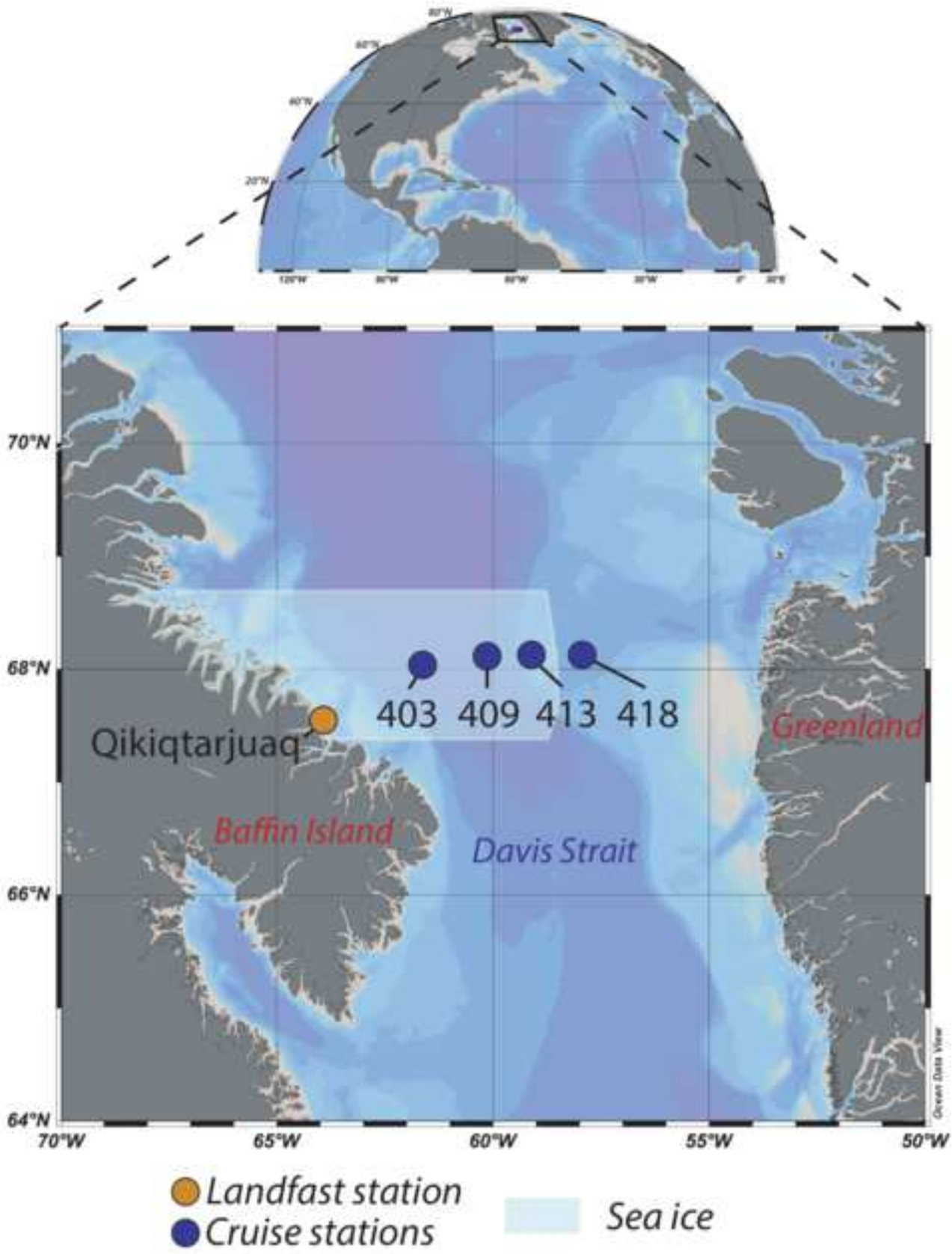

Figure 2

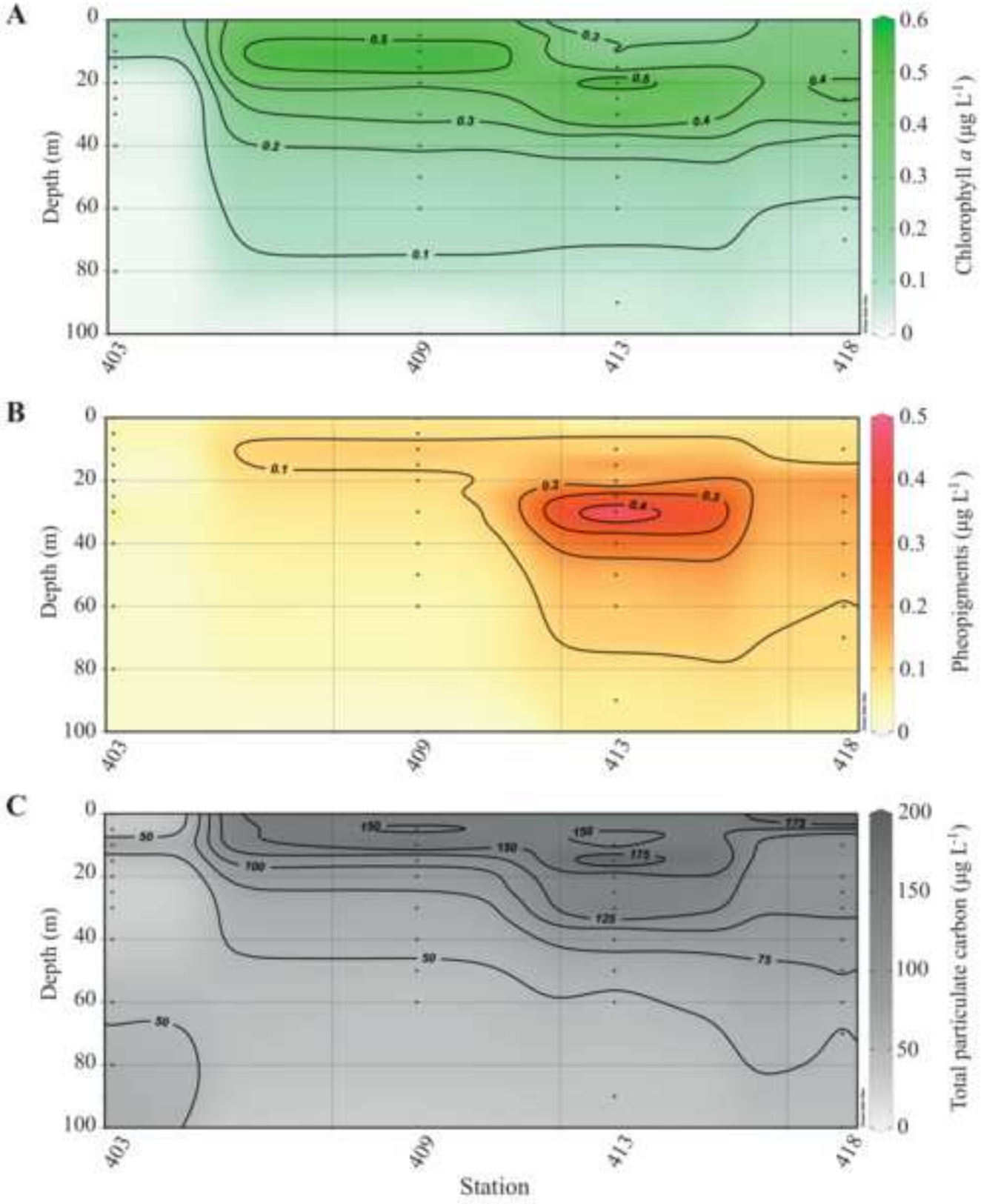

Figure 3

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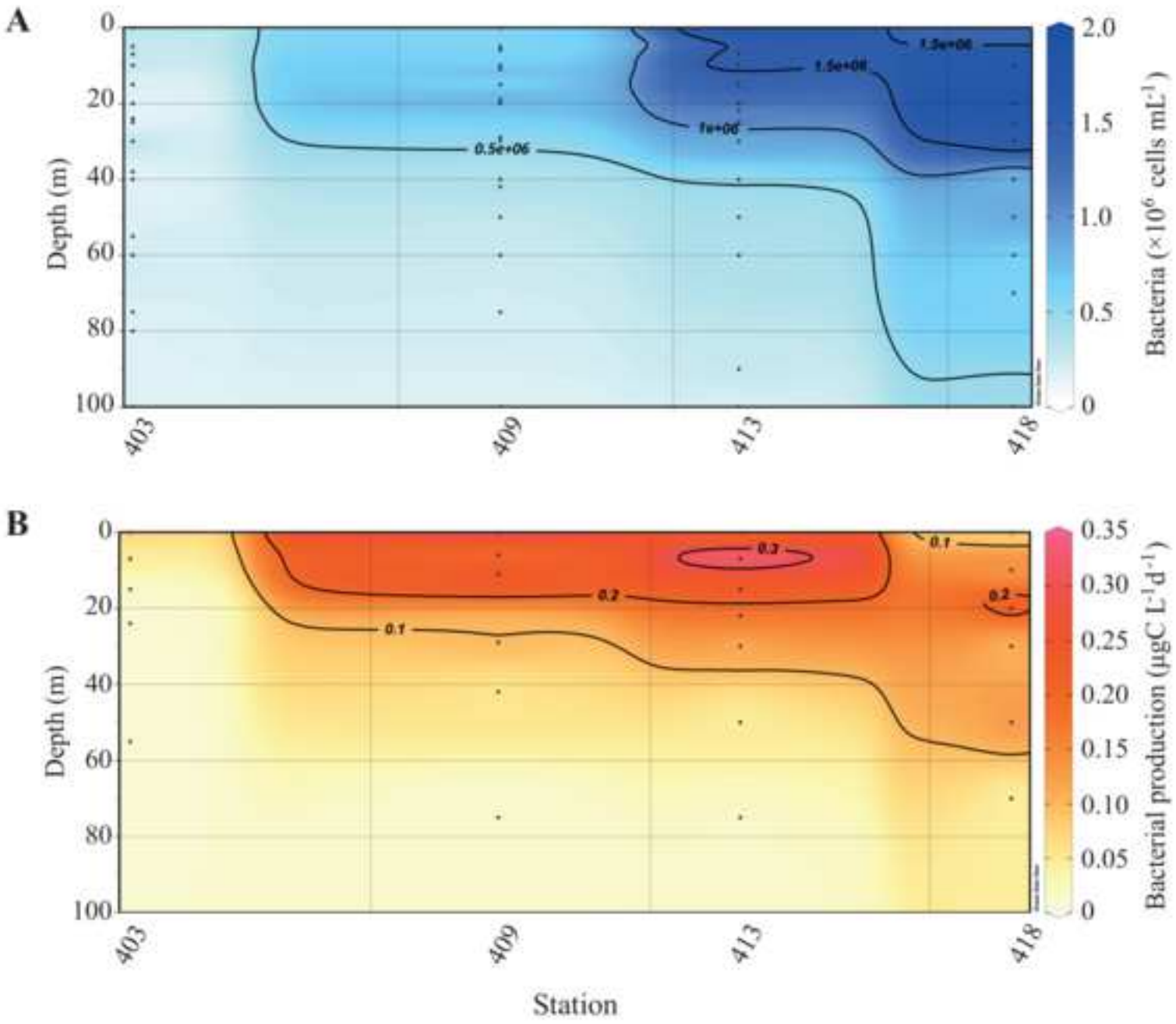

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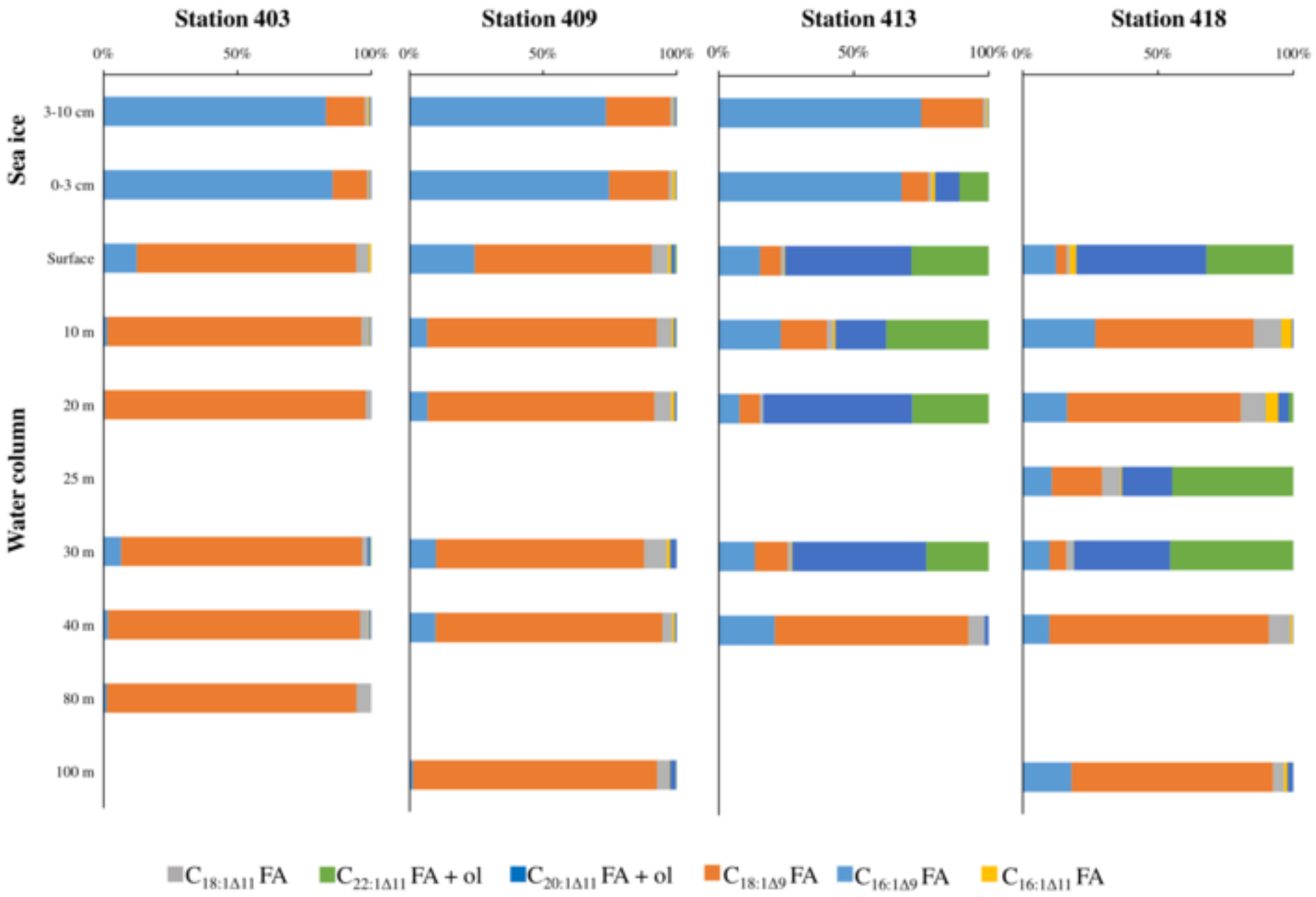

Figure 5

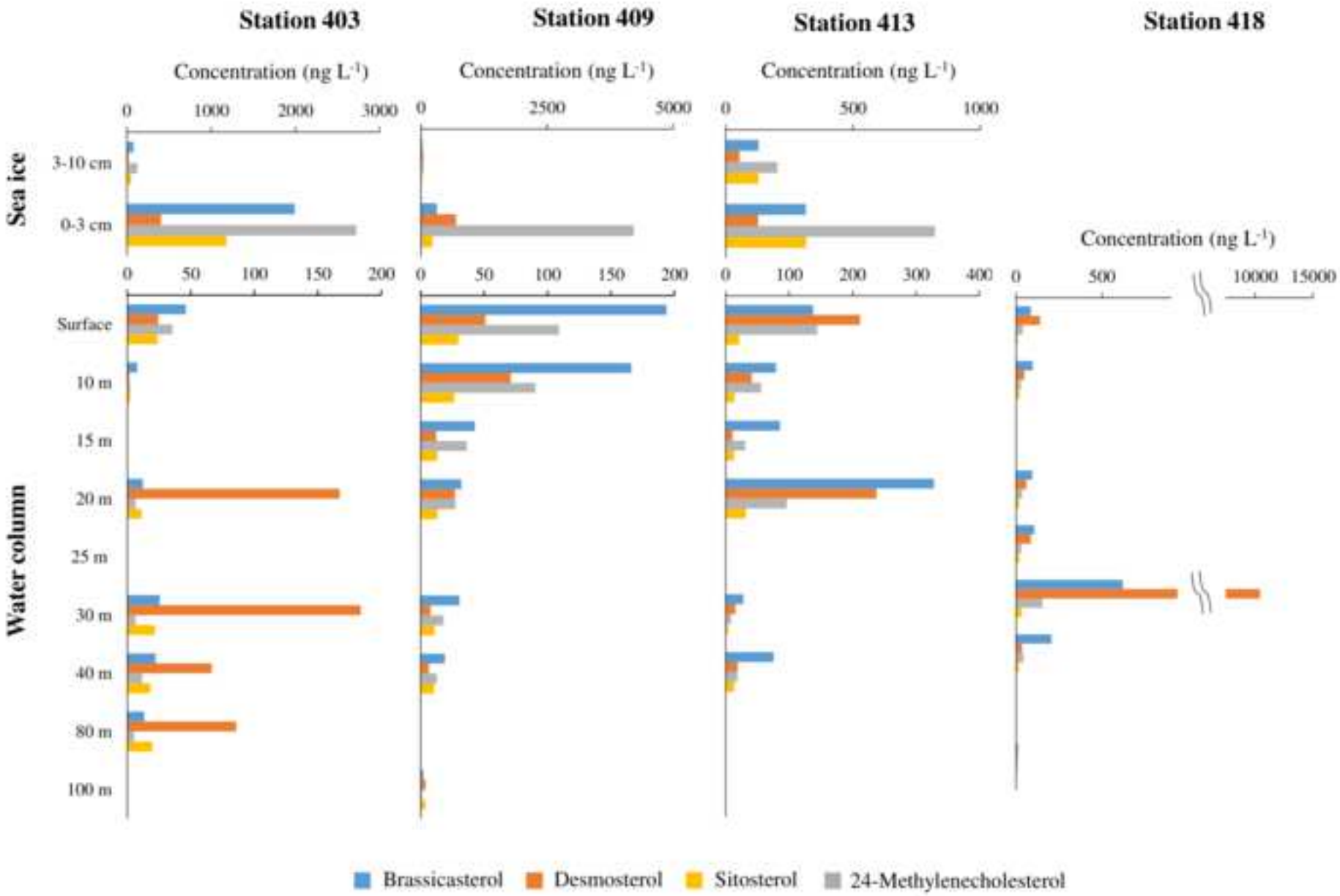

Figure 6

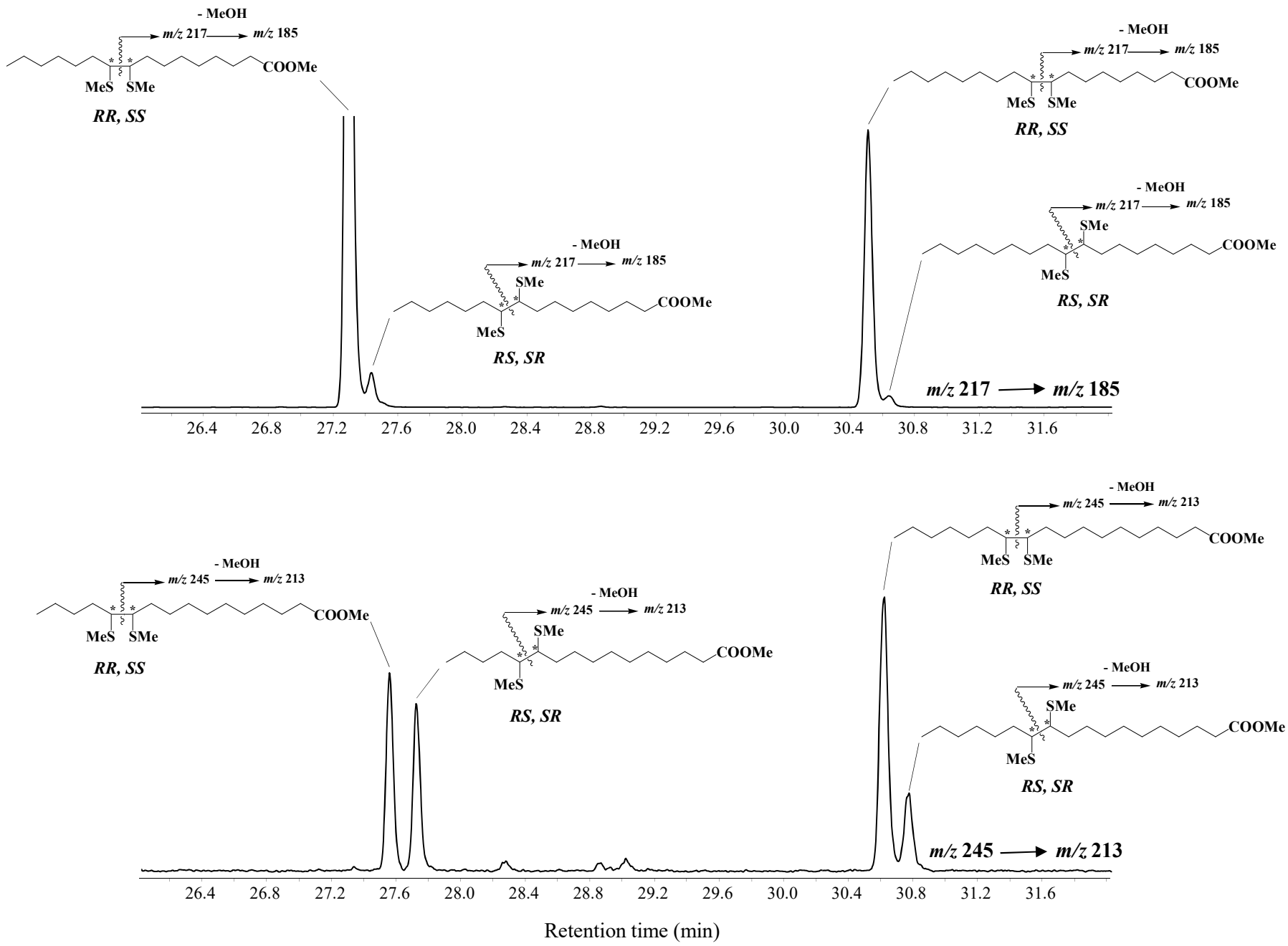

Figure 7

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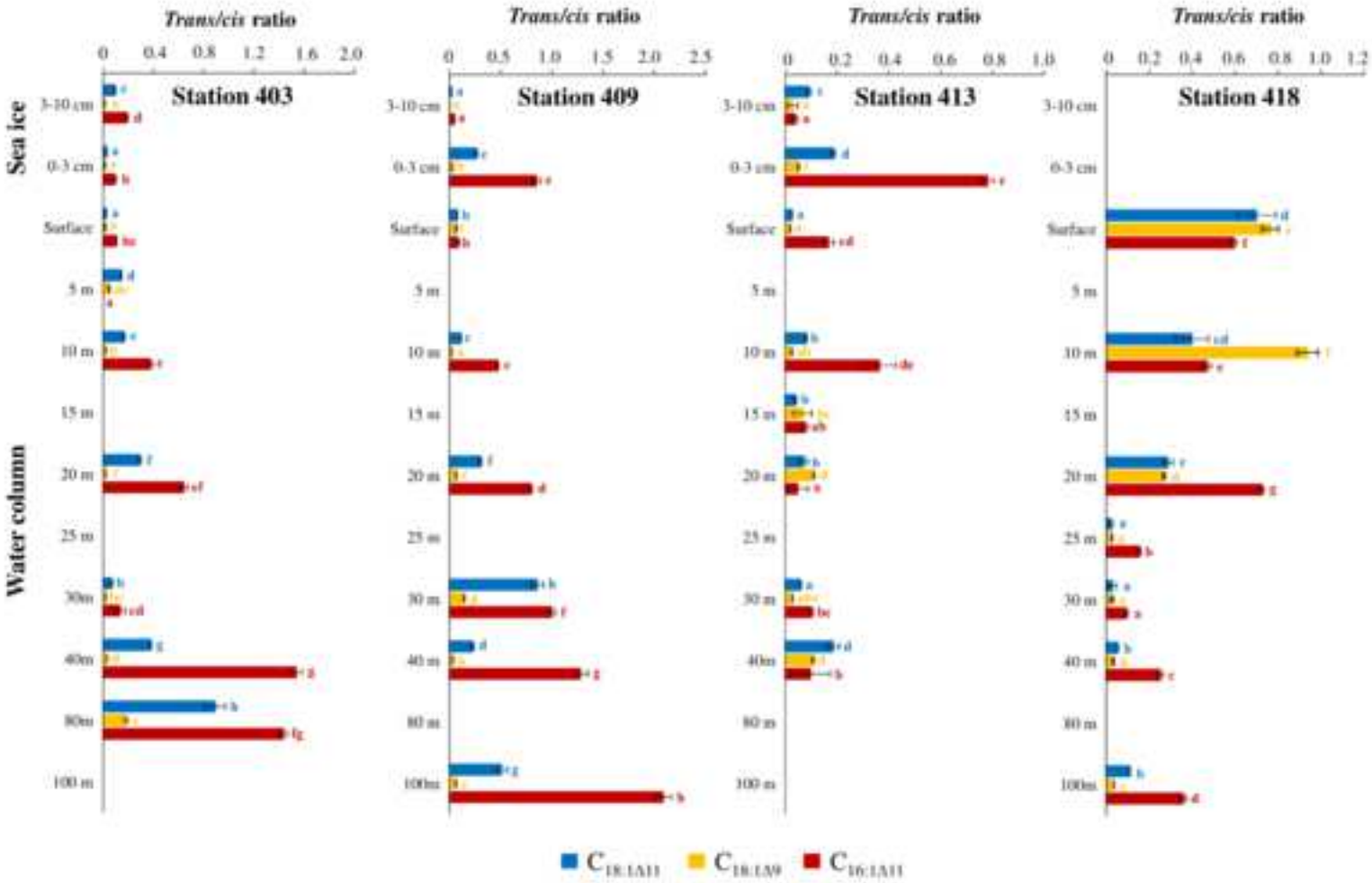

Figure 8

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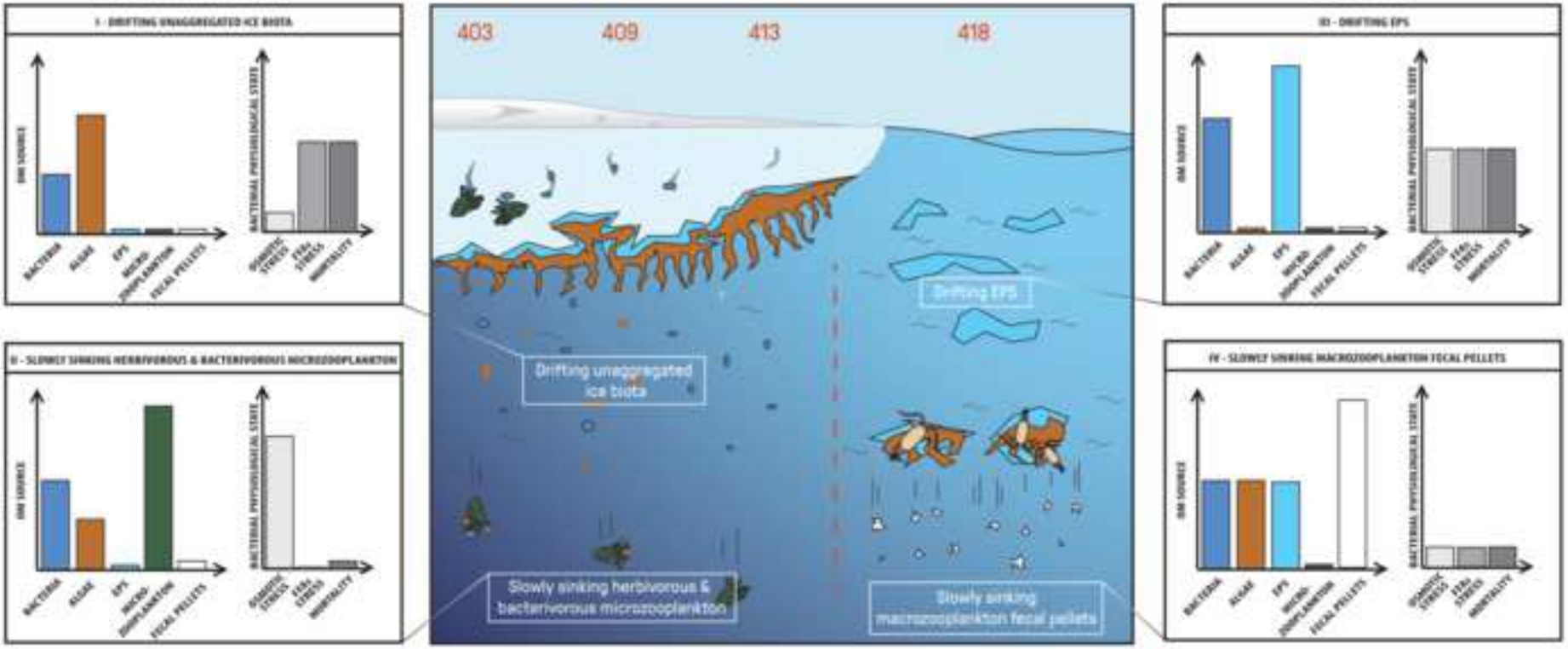

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: