RESEARCH ARTICLE

Hydrocarbon biodegradation by Arctic sea-ice and sub-ice microbial communities during microcosm experiments, Northwest Passage (Nunavut, Canada)

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One sentence summary: Ice-associated microorganisms of the Arctic Ocean can degrade petroleum at $-1.7°C$ within 15 days, and their response to oil varied whether they lived in ice or in underlying waters.

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ABSTRACT

The increasing accessibility to navigation and offshore oil exploration brings risks of hydrocarbon releases in Arctic waters. Bioremediation of hydrocarbons is a promising mitigation strategy but challenges remain, particularly due to low microbial metabolic rates in cold, ice-covered seas. Hydrocarbon degradation potential of ice-associated microbes collected from the Northwest Passage was investigated. Microcosm incubations were run for 15 days at $-1.7°C$ with and without oil to determine the effects of hydrocarbon exposure on microbial abundance, diversity and activity, and to estimate component-specific hydrocarbon loss. Diversity was assessed with automated ribosomal intergenic spacer analysis and Ion Torrent 16S rRNA gene sequencing. Bacterial activity was measured by ^{3}H-leucine uptake rates. After incubation, sub-ice and sea-ice communities degraded 94% and 48% of the initial hydrocarbons, respectively. Hydrocarbon exposure changed the composition of sea-ice and sub-ice communities; in sea-ice microcosms, *Bacteroidetes* (mainly *Polaribacter*) dominated whereas in sub-ice microcosms, the contribution of *Epsilonproteobacteria* increased, and that of *Alphaproteobacteria* and *Bacteroidetes* decreased. Sequencing data revealed a decline in diversity and increases in *Colwellia* and *Moritella* in oil-treated microcosms. Low concentration of dissolved organic matter (DOM) in sub-ice seawater may explain higher hydrocarbon degradation when compared to sea ice, where DOM was abundant and composed of labile exopolysaccharides.

Keywords: Arctic; sea ice; hydrocarbon biodegradation; bioremediation; 16S ribosomal RNA gene sequencing; microbial community fingerprinting

INTRODUCTION

The warming of the Arctic drives an accelerated decline in sea-ice cover extent and duration, which opens navigation routes and improves access to natural resources (Meier *et al.* 2014; Kwok and Cunningham 2015). Extensive offshore oil and gas activities have been taking place in the Arctic since the 1940s (AMAP 2010), but increased accessibility made possible by a longer open water season has created new opportunities for the petroleum industry (AMAP 2012; Meier *et al.* 2014). Higher risks of accidental hydrocarbon releases in the Arctic, either as oil spills or discharges from ships, are anticipated. One of the major transportation routes, the Northwest Passage in the Canadian Arctic Archipelago, has seen an increase in vessel transits in recent years (AMAP 2012; Michel 2013), thereby making the area particularly vulnerable to accidental leaks of hydrocarbons. Hydrocarbon pollution poses a major threat to Arctic marine ecosystems, particularly to the survival of seabirds and marine mammals (Meltofte *et al.* 2013). Oil may persist in the Arctic marine environment longer than in southern regions in part because of the challenges associated with oil spill clean-up in ice-infested waters (Meltofte *et al.* 2013).

While mechanical and chemical methods used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive, microbial degradation of hydrocarbons appears as a non-invasive and relatively cost-effective bioremediation technique (Das and Chandran 2011). This natural process is responsible for the removal of the last traces of oil along polluted shores (ITOPF 2011). In marine environments, oil biodegradation is mostly attributable to bacteria, with an efficiency ranging from 0.003% to 100%, whereas degradation by fungi and algae (Das and Chandran 2011) and archaea (Brakstad and Bonaunet 2006) are considered negligible. Hydrocarbon-degrading bacteria exist in very low abundance (usually less than 1%) in the world's marine environments (Atlas and Cerniglia 1995), but may reach 10% of the total bacterial population in contaminated sites, especially where the pollution is chronic such as in harbors (Hassanshahian and Cappello 2013). The complete degradation of hydrocarbon components requires a large consortium of indigenous bacteria with overall broad enzymatic capabilities (Leahy and Colwell 1990) and the presence of microorganisms possessing adequate metabolic potential (Das and Chandran 2011).

Rates of biodegradation depend on oil characteristics, temperature and the availability of oxygen and nutrients (mainly nitrogen in marine systems) (Das and Chandran 2011; ITOPF 2011). Temperature also directly affects oil contaminant solubility and viscosity, with degradation efficiency decreasing at low temperatures (Atlas 1981; Das and Chandran 2011). Nonetheless, cold-adapted bacteria that possess hydrocarbon-degrading genes have been found in Arctic soils (Whyte *et al.* 1999; Yergeau *et al.* 2012) and seawater (McFarlin *et al.* 2014). Sea ice is another distinctive Arctic ecosystem, characterized by very cold temperature and high brine content, and yet it harbors abundant and diverse microbial assemblages thriving in several distinct microhabitats (Bowman *et al.* 2012). Some studies have addressed the hydrocarbon degradation potential of ice-associated microbes (Atlas, Horowitz and Busdosh 1978; Deppe *et al.* 2005; Gerdes *et al.* 2005; Brakstad *et al.* 2008), but overall this area of research is rather unexplored, especially regarding first-year sea ice. The identification of key oil-degrading microorganisms that live in and below the sea ice is essential for the development of bioremediation strategies suitable for the ice-covered Arctic Ocean.

The Canadian Arctic Archipelago is a region of high biological production that supports large populations of seabirds and marine mammals (McLaughlin *et al.* 2004; Michel, Ingram

and Harris 2006). Bottom ice maintains the highest ice-algal biomass reported for the Arctic (Arrigo, Mock and Lizotte 2010) and contains elevated sea-ice bacterial abundances (Smith *et al.* 1989; Maranger, Bird and Juniper 1994). We studied the potential for hydrocarbon degradation of natural microbial communities thriving in first-year sea ice and sub-ice seawater, in the eastern part of the Northwest Passage during spring 2012. The objectives were to evaluate (i) the diversity of bacterial communities and the presence of oil-degrading taxa; (ii) the hydrocarbon degradation potential, including preferential degradation of certain components; and (iii) changes in communities induced by hydrocarbon exposure in terms of abundance, activity and diversity. We hypothesized that (i) the hydrocarbon degradation rates would be different in sea ice and sub-ice seawater because of variations in microbial abundance, activity and environmental conditions and that (ii) exposure to hydrocarbons would favor oil-degrading taxa. These hypotheses were tested in microcosms, in which natural microbial communities collected during the ice-algae bloom period were exposed to persistent hydrocarbons. Our study is one of the few that has combined data on hydrocarbon components and bacterial metabolic rates, as well as phylogenetic analysis and community fingerprinting, to investigate oil degradation by ice-associated bacteria.

MATERIALS AND METHODS

Study site and sample collection

Samples were collected on 1 May 2012 at a station located on the landfast ice of the Resolute Passage (74.7°N, 95.5°W), a subsection of the Northwest Passage in the Canadian Arctic Archipelago (Fig. 1A and B). The Northwest Passage is the main channel across the archipelago, linking Baffin Bay in the east to Beaufort Sea in the west (Fig. 1A). The region is extensively covered by first-year ice during most of the year, i.e. from mid/late November to the end of June. The Northwest Passage is influenced by different water masses, mainly Pacific waters to the west, Atlantic waters to the east and modified Arctic waters flowing from the northern channels (McLaughlin *et al.* 2004).

Two types of samples were collected: first-year sea-ice cores and samples of the seawater immediately under the ice cover, hereafter referred to as sub-ice seawater. Sub-ice seawater receives brine rejections during sea-ice formation from autumn to spring, at times making it more saline than the surface seawater found in ice-free areas (Petrich and Eicken 2009). Salinity in the sub-ice samples was 32.0 (Table 1), which is comparable to what is typically found at surface in Barrow Strait during open-water periods (McLaughlin *et al.* 2004). A total of 40 l of sub-ice seawater was collected with a pump submersed at the ice-water interface; 20 l was used for ancillary measurements and 20 l was used in microcosms (see below). The water was stored in acid-washed containers in the dark at −1°C until further processing (see below). A total of 13 sea-ice cores were collected using a manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprises, Roseburg, OR, USA). The ice cores were cut with a stainless steel saw to collect the bottom 3 cm, where most of the microbial biomass is found (e.g. Michel *et al.* 1996). Sterile gloves were worn at all times when handling the cores. All cores were kept in sterile Whirl-Pak® bags or in an acid-washed isothermal container in cold, dark conditions until further processing and analysis.

Initial conditions (T0) in sea ice and sub-ice seawater were characterized in terms of microbial variables, i.e. autotroph abundance, prokaryote abundance and heterotrophic activity (herein referred to as bacterial abundance and bacterial
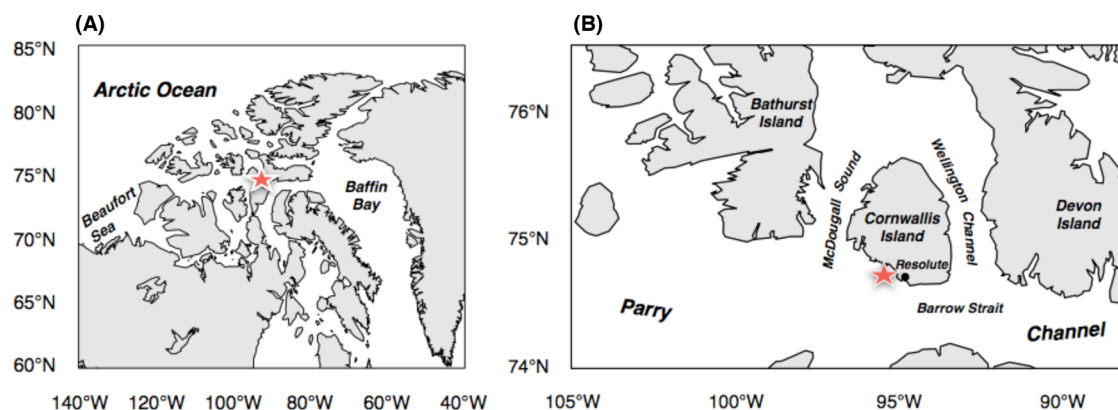
Figure 1. Canadian Arctic Archipelago (**A**) showing the sampling region in Resolute Passage, in the eastern part of the Northwest Passage (**B**). The star identifies the location of the sampling station.

Table 1. Environmental conditions and microbial biomass and production in sea ice and sub-ice water at a landfast ice station in Resolute Passage (Nunavut, Canada) on 1 May 2012. Values are average of duplicate measurements (± standard error), except for salinity, nitrate and total bacterial abundance (N = 1) and bacterial production (BP; N = 3). NO_3^- : nitrate; PO_4^{3-} : phosphate; $Si(OH)_4$: silicic acid; DOC: dissolved organic carbon; DN: dissolved nitrogen.

	Sea ice	Sub-ice seawater
Salinity	8.0	32.0
NO_3^- (mmol m^{-3})	12.58 ± 0.2	7.08 ± 0.04
PO_4^{3-} (mmol m^{-3})	8.53 ± 0.09	1.07 ± 0.00
$Si(OH)_4$ (mmol m^{-3})	6.83 ± 0.23	12.00 ± 0.06
DOC (mmol m^{-3})	2030 ± 358	59.5 ± 2.8
DN (mmol m^{-3})	126 ± 29	10.4 ± 0.3
Chlorophyll *a* (mg m^{-3})	4563 ± 391	0.18 ± 0.05
Total protists (cells ml^{-1})	2.1×10^6	1.0×10^2
Bacteria (cells ml^{-1})	16×10^6	2.3×10^5
BP (pmol leu L^{-1} h^{-1})	441 ± 18	7.83 ± 0.72

production, respectively). Biochemical analyses were performed to determine concentrations of nutrients, dissolved organic carbon (DOC), dissolved nitrogen (DN) and salinity. Microbial variables in sea ice were measured on three ice cores that were previously combined in an acid-washed isothermal container and melted in filtered surface seawater (0.22 μm, 142 mm diameter GV Durapore® filter, Millipore, Darmstadt, Germany). The addition of 500 ml filtered seawater per core gave an average dilution factor of 3.7. One core was kept in a sterile Whirl-Pak bag for salinity, nutrients, DOC and DN analyses without the addition of filtered seawater to avoid a dilution effect of dissolved materials.

Salinity was measured with a salinometer (Portasal 8410A, Guildline Instruments Limited, Smiths Falls, Ontario, Canada) according to Müller (1999). Inorganic nutrients, i.e. nitrate (NO_3^-), phosphate (PO_4^{3-}) and silicic acid ($Si[OH]_4$), were analyzed on a SmartChem discrete analyzer (Westco Scientific Instruments Inc., Brookfield, CT, USA) according to Hansen and Koroleff (1999). DOC and DN concentrations were determined by high-temperature catalytic combustion on a Shimadzu TOC-VCPH analyzer with an ASI-V auto sampler and TNM-1 Total Nitrogen module (Knap *et al.* 1996). Chlorophyll *a* (chl *a*), a proxy for autotroph biomass, was measured by fluorometry using 10AU Turner Designs (San Jose, CA, USA) after filtration onto Whatman GF/F 25mm filters, according to Parsons, Maita and Lalli

(1984). Protist and bacterial cell numbers, as well as high nucleic acid content (HNA) bacteria, were stained with SYBR Green I and counted on an Epics Altra flow cytometer (Beckman Coulter, Brea, CA, USA) according to Belzile *et al.* (2008).

Microcosm experiments

The remaining 20 l of sub-ice seawater and nine ice cores were used in microcosm experiments, which started the day following sample collection. The ice cores were combined with 4.5 l of 0.2 μm filtered surface seawater (total volume of 6.2 l) into a 9 l acid-washed carboy (Nalgene, Rochester, NY, USA), and were left to melt slowly overnight in a cold room in the dark. The dilution factor was 3.6. Melted and diluted sea ice samples are convenient and routinely used because microbes in the liquid phase can be reached by stable isotope additions while at the same time be protected from osmotic shock and cell lysis caused by the fresher salinities of melt water (Deming 2010). The use of melted sea ice in the microcosms also ensured that the entire sea-ice bacterial community was exposed to hydrocarbons. This approach was based on previous experimental results showing that upward infiltration of hydrocarbons in sea-ice channels was very low in May (Karlsson, Petrich and Eicken 2011).

Control microcosms without oil were set up in duplicate for both sub-ice seawater and diluted ice samples. Triplicate microcosms of sub-ice seawater and diluted sea-ice were spiked with 25 mg L^{-1} of Arabian Light oil. The oil was previously weathered to remove volatiles. Arabian Light oil is a moderately waxy crude oil (McKetta 1997) and has a pour point of –40°C (ITOPF 2011). The API gravity (i.e. oil density in relation to pure water, a standard established by the American Petroleum Institute) of this oil varies between 31.8° and 33.4° (Environment Canada 1991), which indicates that it is lighter than water (which has an API gravity of 10°). The oil is classified in Group 3 (°API 17.5–35) and is considered persistent (ITOPF 2011).

Samples were incubated for 15 days in oil-treated and control microcosms and those were gently mixed by a custom-made rotating shaker. Incubations were run in sterile transparent Nalgene polycarbonate bottles at near *in situ* temperature (–1.7 ± 0.02°C; average ± standard error) and under light conditions comparable to those under the sea ice (7.8 ± 0.8 μE m^{-2} s^{-1}). Temperature was recorded every 10 min during the incubation period, and light was measured on 10 occasions. Average under-ice PAR at sampling stations visited was 9.4 μE m^{-2} s^{-1}. Water was collected from each microcosm and control at five time points (T), i.e. days 0, 3, 7, 11 and 15, using 250 ml Nalgene

bottles for sea ice and 250 ml or 1 l Nalgene bottles for sub-ice seawater, depending on the analytical volume required. Bacterial production and hydrocarbon composition were measured at each time point. Bacterial abundance was analyzed at the beginning (T0) and at the end (T15) of the experiment by flow cytometry (as mentioned above). Community composition was evaluated using automated ribosomal intergenic spacer analysis (ARISA) fingerprinting and 16S rRNA gene sequencing at T0 and T15.

Bacterial production

Bacterial production was measured from the incorporation rates of the titriated (^{3}H-) amino acid leucine (leu) according to the centrifugation protocol (Smith and Azam 1992). This approach measures the production rate of proteins (Kirchman and Ducklow 1993) in both bacteria and archaea (Herndl *et al.* 2005), although we assume a minor contribution of archaea in our samples, as observed on the Beaufort Sea shelf (< 1% to 7% of archaea in total DAPI counts; Garneau *et al.* 2006) and in the Northwest Passage (2% to 13%; Wells and Deming 2003). Triplicate subsamples of 1.2 ml were spiked with ^{3}H-leucine (specific activity, 60 Ci mmol^{-1}) to give a final concentration of 10 nM. Duplicate controls were immediately spiked with 50% trichloroacetic acid (TCA; 5% final concentration) to kill the bacteria. Vials for uptake measurement and killed control vials were incubated in the dark at $-1.7°$C for 4 h. At the end of the incubation, TCA (5% final concentration) was immediately added to the vials. All samples were frozen at $-80°$C until final analysis in our main laboratory. Samples were then thawed at 4°C and centrifuged at 14 000 rpm for 10 min. The supernatant was removed and pellets were rinsed by adding 1 ml of TCA (5% final concentration). This rinsing TCA was removed after a second centrifugation. Scintillation cocktail (Ecolume, MP Biomedicals, Santa Ana, CA, USA) was added to the vials, and bacterial cells were resuspended by vortex mixing. ^{3}H-Leucine incorporation was measured using a liquid scintillation counter (TRI-CARB 2100 TR, Packard Bio-Science, Meriden, CT, USA) after 48 h of incubation in the dark at 4°C. Data are given in pmol leu L^{-1} h^{-1}.

Hydrocarbon composition

Gas chromatography-mass spectroscopy (GC-MS) was used to quantify the different petroleum components at each experimental time point. Alkanes, polycyclic aromatic hydrocarbons and their alkylated homologs represent > 80% weight of the total oil in Arabian Light oil (Environment Canada 2001). Thus, these three major groups of hydrocarbon compounds were analyzed: (i) alkanes, including 28 linear *n*-alkanes ranging in carbon number from C_{10} to C_{35}, the branched alkanes pristane and phytane, and the cyclic alkane hopane; (ii) polyaromatic hydrocarbons (PAHs) consisting of the 2-, 3- and 4-ring aromatics (anthracene, chrysenes, dibenzothiophenes, fluorenes, fluoranthenes, naphthalenes, naphthobenzothiophenes, perylenes, phenanthrenes, pyrenes); and (iii) the methylated homologs of these aromatics (C_1- to C_4-methylated PAHs). These analytes were selected because they are most likely to contribute to toxicity in fish exposed to an oil spill, as the high-molecular-weight (HMW) resins and asphaltenes are not expected to cross fish tissue membranes. In parallel, we also analyzed 9 internal standards (5α-androstane, d_{10}-anthracene, d_{12}-chrysene, d_{12}-perylene, d_{22}-n-decane, d_{34}-hexadecane, d_{42}-n-eicosane, d_{62}-n-tricontane and d_8-naphthalene) and 10 surrogate standards (5β-cholestane, d_{10}-phenanthrene,

d_{10}-pyrene, d_{12}-benzo[a]pyrene, d_{12}-benzo[b]fluoranthene, d_{14}-dibenz[a,h]anthracene, d_{26}-dodecane, d_{36}-heptadecane, d_{50}-n-tetracosane and d_{66}-n-dotriacontane). A GC-MS detection of <100 ng L^{-1} was applied.

Sub-ice seawater and sea-ice samples (100 ml) were placed in clean amber glass bottles sealed with Teflon tape after adding dichloromethane (5% final concentration). The samples were refrigerated until analysis, which was performed within 4 months of collection. Hydrocarbons were extracted in dichloromethane and quantified using an Agilent 6890 Series GC with a 5973N MSD operated in the selected ion-monitoring mode. The column was a 30 m × 0.25 mm internal diameter, with 0.2 μm film thickness MS-5 or equivalent column (Supelco Pack (Supelco, Bellefonte, PA, USA)). Concentrations of the analytes at a given time point were divided by the concentration of the internal marker 17α(H),21β(H)-hopane to avoid any bias caused by physico-chemical losses of hydrocarbons (Prince *et al.* 1994). Hopane-normalized concentrations allow for the measurement of oil biodegradation as the internal marker biodegrade very slowly. The coefficients of variation on the measured concentrations (expressed in ng L^{-1}) between the triplicate filters confirmed that the precision and repeatability of the assay were good. The average coefficient of variation was 1% (N = 81) for the internal standards, 9% (N = 90) for the surrogate standards and 19% for the analytes (N = 429). The hydrocarbon loss (in %) was calculated as $HC_{loss} = (HC_{T0} - HC_{Tx}) \div HC_{T0} \times 100$, where HC_{T0} is the hydrocarbon concentration at T0 (initial concentration) and HC_{Tx} is the hydrocarbon concentration at sampling time x.

Hydrocarbon compound concentrations in seawater and melted ice cores were measured before the addition of Arabian Light oil. Methyl-PAH and PAH concentrations were below detection. Alkanes were detected but were, on average, at < 4% of initial concentrations in spiked bottles (5.3 ± 3.5 $\times10^4$ ng L^{-1}). Unfortunately, contamination occurred in T15 sub-ice samples during the preservation and results were excluded from the data analysis.

Bacterial community fingerprinting

ARISA fingerprinting (Fisher and Triplett 1999) was used to determine bacterial community structure under the experimental conditions. Sub-ice seawater (1400 ml) and bottom sea-ice (75 ml) samples that were taken at T0 and T15 were filtered onto 0.2 μm sterile filters (Millipore, 47 mm diameter). DNA was extracted by bead-beating and purified using a PowerWater DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). The amount of DNA was then determined by spectrofluorometry (Tecan Safire, Tecan Group Ltd, Männedorf, Switzerland) using Quant-iT PicoGreen dsDNA assay (Invitrogen, Carlsbad, CA, USA). The PCR amplification of the 16S–23S rRNA intergenic transcribed spacer (ITS) region was conducted in triplicate for each DNA extract and according to Ranjard *et al.* (2000). We used the universal bacterial primers SDBact (eubacterial rRNA gene small subunit, 5′-TGC GGC TGG ATC CCC TCC TT-3′) and LDBact (eubacterial rRNA gene large subunit, 5′-CCG GGT TTC CCC ATT CGG-3′) (Normand *et al.* 1996). The primers were synthesized by Molecular Probes Invitrogen. The primer SDBact was labeled at the 5′ end with the phosphoramidite dye 5-FAM fluorochrome for subsequent analysis by ARISA. The three-step thermal PCR amplification protocol was as follows: (i) 94°C for 5 min; (ii) 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min; and (iii) 72°C for 5 min. The 25 μl reaction mixtures contained 1× PCR buffer, 1.5 mM MgCl$_2$, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.1 μM of each primer, 1U of *rTaq* polymerase

(GE Healthcare, Little Chalfont, UK) and 10 ng of DNA template. Triplicate amplified ITS fragments were pooled together and then purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) to eliminate primer surplus that could interfere during electrophoresis. The bacterial ITS fragments were quantified by spectrofluorometry.

Bacterial ITS products were denatured using Hi Di formamide followed by a heat treatment at 95°C for 5 min. An internal fluorescent size marker (LIZ1200) was added to each sample and 10 ng of bacterial product were run on a 3130XL Capillary Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using a 50 cm capillary. ARISA electrophoresis profiles were analyzed with Genemapper software (Applied Biosystems). Peak lengths and absolute areas that characterized the different operational taxonomic units and their abundances were used in data analysis. Peaks with a length inferior to 200 bp and superior to 1200 bp were considered noise and were thus excluded from further analysis. The area of each peak was divided by the total area of the sample to obtain relative abundance (%). Background and electrophoresis internal deviation were eliminated using the variable percentage threshold method (Osborne *et al.* 2006) and the binning procedure under R environment (interactive and automatic binning algorithms; Ramette 2009). Data were transformed to abundance matrices, from which Bray–Curtis dissimilarities (BC_{diss}) between samples were calculated using the Primer software (Clarke and Warwick 2001). The complement of the Bray–Curtis measure was used to calculate the similarities.

Ion Torrent 16S rRNA gene sequencing

At the beginning of the experiment (T0), triplicate samples of sub-ice seawater (1400 ml) and melted sea ice (75 ml) were filtered onto 0.2 μm sterile filters (Millipore, 47 mm diameter) for DNA extraction using hexadecyltrimethylammonium bromide (CTAB), following a slightly modified version of the method published by Ausubel *et al.* (2002). A phenol/chloroform extraction was first performed and was followed by a chloroform/isoamyl alcohol (24:1) extraction. This extraction method gives superior DNA purity than the bead-beating method we used for DNA fingerprinting, but it produces toxic phenol–chloroform wastes. The DNA was precipitated overnight at –20°C. The DNA pellet was resuspended in 100 μl of sterile deionized water and quantified using the Quant-iT PicoGreen dsDNA assay (Invitrogen). The water from triplicate oiled microcosms was harvested at T15. The control treatments at T0 and T15 were in duplicate. These samples were filtered and extracted as described above for the initial sub-ice seawater and melted sea-ice samples.

Partial 16S ribosomal (rRNA) gene amplicons were produced using the universal primers F343 (5′-TAC GGR AGG CAG CAG-3′) and R534 (5′-ATT ACC GCG GCT GCT GGC-3′) as in Yergeau *et al.* (2014). The primers contained 10 bp multiplex identifiers (MIDs) and adaptor sequences for Ion Torrent sequencing (Yergeau *et al.* 2012; Bell *et al.* 2013). Reactions were performed in 25 μl volumes containing 10 ng of DNA, 0.3 μM of each primer, 0.4 mg ml^{-1} of bovine serum albumin, 0.2 μM of dNTPs and 0.05 U μl^{-1} of r*Taq* DNA polymerase (GE Healthcare). The PCR cycling conditions involved an initial denaturation step at 95°C for 5 min, followed by (i) 30 cycles of 30 s at 95°C, (ii) 30 s at 55°C, (iii) 45 s at 72°C and (iv) a final elongation step of 3 min at 72°C. The PCR products were purified on agarose gel using the QIAquick gel extraction kit from QIAGEN and quantified with the Quant-iT PicoGreen dsDNA assay (Invitrogen). All of the 20 purified products were pooled in equimolar ratios. A total of 39.15 $\times 10^7$ molecules was used in the emulsion PCR using the Ion PGM Template 0T2 200 and the One-Touch instrument (Life Technologies, Carlsbad, CA, USA). Community composition assessment was performed using the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/); sequences were separated based on their adapter, as well as deconvoluted and binned according to their respective MIDs.

Sequencing was performed on an Ion Torrent personal genome machine using the Ion 314 chip and the Ion PGM Sequencing 200 kitV2 (Life Technologies) following the manufacturer's instructions. Sequences with an average Phred quality score below 20 or a length of less than 150 were filtered out of the data set. Taxonomic identities were assigned by classifying sequences in parallel with a boostrap value of 80% using the MultiClassifier tool from the Ribosomal Database Project Pipeline Classifier (Release 9; Wang *et al.* 2007). Number of reads before and after quality control generated during the 16Sr RNA gene sequencing analysis are presented in Table S1 (Supporting Information). The results of the replicate sequences belonging to a given taxonomic level were averaged and expressed as a percentage of all sequences. Raw sequence data produced in this study was deposited in NCBI under the Bioproject accession PRJNA321190.

Statistical analysis

Differences between two groups (i.e. control *versus* oil-treated microcosms; ice *versus* sub-ice seawater) were tested with the R software (R Core Team 2013). Student's *t*-test was used since data distributions were not far from a normal distribution and variances were equal. Differences between incubation days were tested with the Kruskal–Wallis test (KW) and multiple comparison post-hoc tests were run using the kruskalmc function in the pgirmess package of R software (Giraudoux 2014). Mean values are presented with standard error (SE).

Bray–Curtis similarities were illustrated using non-metric multidimensional scaling (MDS), where each data point represents a bacterial assemblage, and the distance between data points represents the level of similarity in bacterial assemblages; the longer the distance, the lower the similarity. Bacterial community composition was assessed using ANalysis Of SIMilarity (ANOSIM), based on a random Monte-Carlo test of 100 000 permutations (Primer V06 software; Clarke and Warwick 2001). The test compares ranks of distances between groups with ranks of distances within groups. Resulting R values > 0.75 are interpreted as well distinct, R > 0.5 as different but overlapping and R < 0.25 as barely different (Clarke and Gorley 2001). The Mann–Withney *U* test (MW) was performed to test difference in assemblage similarity between (i) sub-ice seawater and sea-ice microcosms, (ii) controls and oil-treated microcosms and (iii) T0 and T15.

RESULTS

Environmental parameters and initial experimental conditions

Environmental conditions in sub-ice seawater and in sea ice at the time of the sampling were typical of the spring period (Table 1). Nutrient inventories were high in sub-ice seawater but bacterial (2.3 $\times 10^5$ cells ml^{-1}) and phytoplankton biomass (< 0.2 mg chl*a* m^{-3}) were low, indicating pre-bloom conditions. Pre-bloom nitrate concentrations in the surface waters of the Canadian Archipelago are typically ca. 10 mmol m^{-3} (Codispoti

Table 2. Initial (T0) bacterial abundances in microcosms. Values are averages of duplicate control microcosms (± standard error). The asterisks indicate significant difference (t-test, $P < 0.01$) in sea ice when compared to sub-ice seawater. HNA: high nucleic acid.

	Sea ice	Sub-ice seawater
Bacteria ($\times 10^5$ cells ml^{-1})	4.2* ± 0.7	2.1 ± 0.0
HNA bacteria (% cells)	92* ± 1	64 ± 1
Bacterial production (pmol leu L^{-1} h^{-1})	21* ± 2	12.7 ± 2.3

et al. 2013), which is concordant with our nitrate concentrations (Table 1). Comparatively, sea ice had more nutrients and dissolved matter, and had higher bacterial (16 $\times 10^6$ cells ml^{-1}) and algal (> 4000 mg chl a m^{-3}) biomasses (Table 1). Concentrations of DOC and chl a, and protist abundance were three to five orders of magnitude higher in the ice than in sub-ice seawater (Table 1), indicating that the ice-algal bloom was well underway.

For microcosm experiments, sea ice was diluted to reach a bacterial abundance comparable to that measured in sub-ice seawater (Table 2). Initial (T0) bacterial abundance and production in the microcosms, as well as the proportion of HNA bacteria, were significantly higher in the sea ice than in sub-ice seawater (t-test, $P < 0.01$; Table 2). Nonetheless, values were of the same order of magnitude (Table 2).

Bacterial production

Bacterial production during the incubation reached values that were about 10 times higher in sea ice (Fig. 2). Bacterial production in sub-ice microcosms at T0 was significantly higher in the controls than in the oil-treated microcosms (13 versus 2 pmol leu L^{-1} h^{-1}; t-test, $P < 0.01$; Fig. 2B). This situation was reversed during the remainder of the incubation (i.e. excluding T0) with a mean of 6.5 ± 1.0 pmol leu L^{-1} h^{-1} in oil treatment compared to a mean of 3.7 ± 0.8 pmol leu L^{-1} h^{-1} in controls (t-test, $P < 0.05$; Fig. 2B).
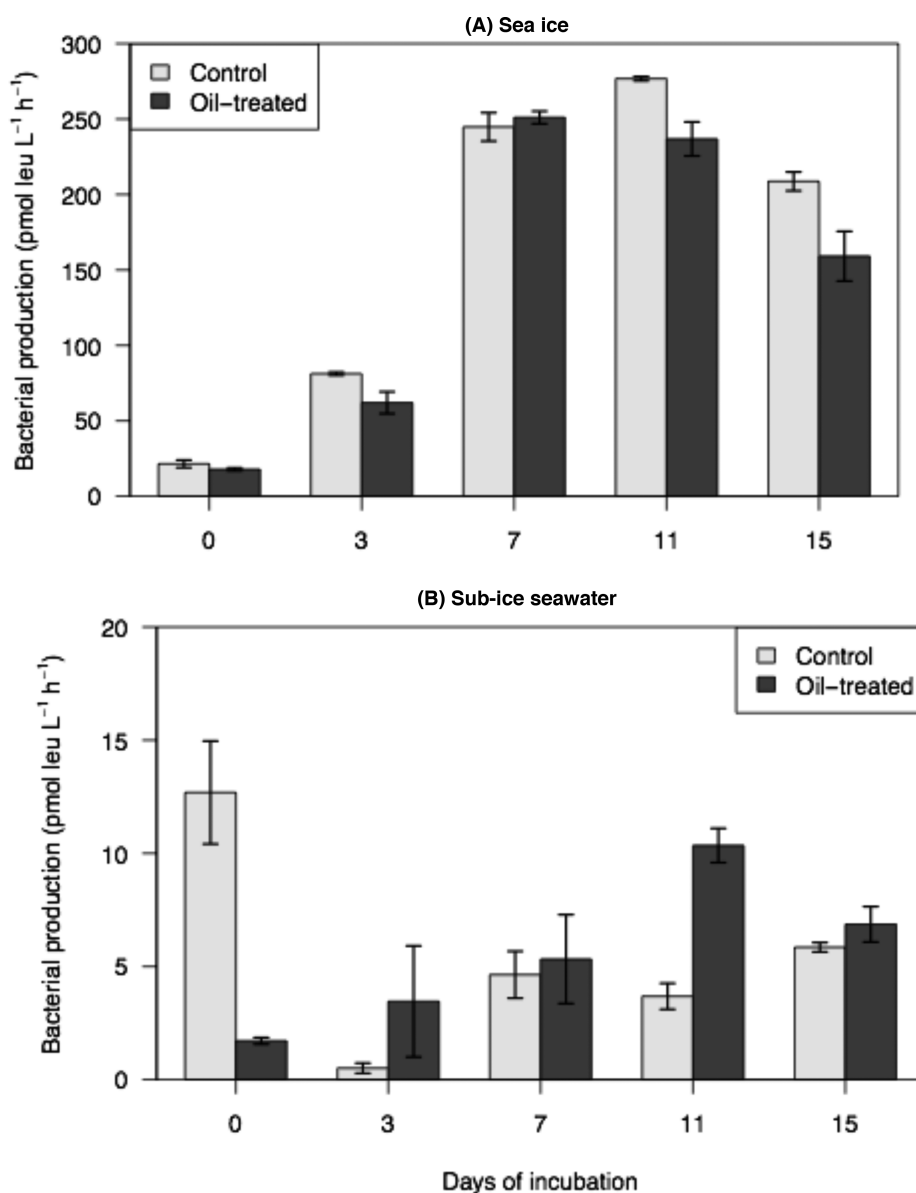

Figure 2. Time series of bacterial production in sea ice (**A**) and sub-ice seawater (**B**) during microcosm experiments. Bars and vertical lines represent averages and standard errors, respectively, for oil-exposed (N = 3) or control (N = 2) microcosms. Note different scales.
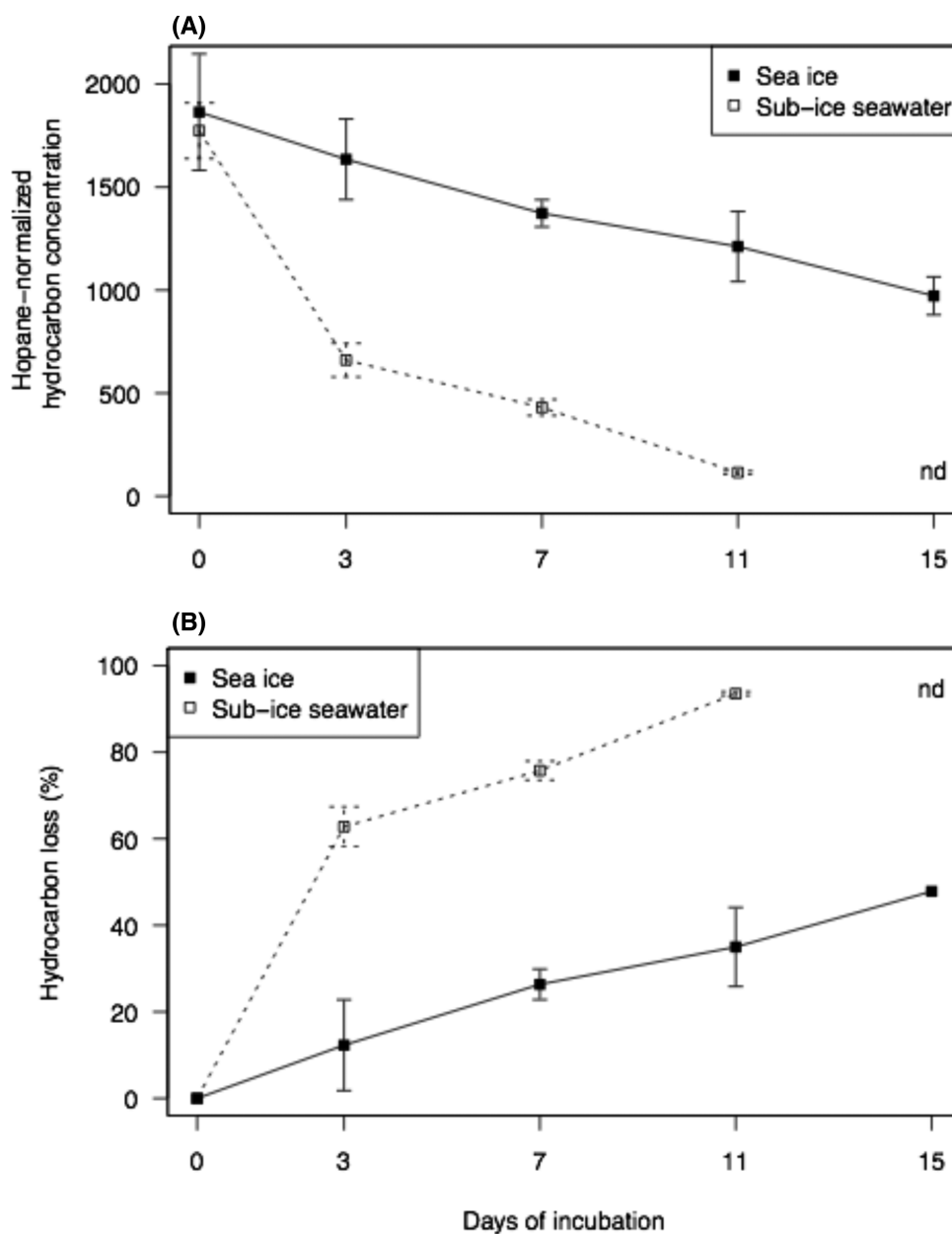
Figure 3. Time series of total hydrocarbon concentrations normalized to hopanes (**A**) and cumulative percent loss of total hydrocarbons (**B**) in sea ice and sub-ice seawater during microcosm experiments. Squares and vertical lines represent averages and standard error, respectively, for oil-exposed microcosms (N = 3). nd: no data.

In the experiments with sea ice, however, there was no significant difference in bacterial production between the oil-treated and control microcosms (t-test, $P > 0.05$; Fig. 2A). The overall bacterial production in the sea-ice microcosms increased significantly over the course of the experiment, with a 4-fold increase from T0 to T7 (19 ± 1 *versus* 248 ± 4 pmol leu L^{-1} h^{-1}; KW, $P < 0.05$, Fig. 2A).

Hydrocarbon degradation in oil-treated microcosms

Total hydrocarbon concentrations at the beginning of the incubation (T0) were not significantly different between sea ice and sub-ice seawater, averaging 1819 ± 141 hopane-normalized units (t-test, $P > 0.05$; Fig. 3A). The 28 alkanes analyzed were de-tected in both microcosms at T0 (data not shown). Three low-molecular-weight (LMW) PAHs (i.e. two or three rings; Canadian Council of Ministers of the Environment 1999) were also detected. They were phenanthrene, naphthalene and dibenzothio-phene, but there was no PAH compound of HMW (four to seven rings; Canadian Council of Ministers of the Environment 1999). A total of 26 methyl-PAHs were detected in the microcosms at T0 (data not shown).

Total concentrations decreased in both sea-ice and sub-ice seawater microcosms during the experiment (Fig. 3). Concentration in sea-ice microcosms was 972 ± 92 hopane-normalized after 15 days, but there was no significant difference between T0 and T15 (t-test, $P > 0.05$). In sub-ice seawater, there was a significant difference between T0 and T11, at which time the

concentration was 115 ± 8 hopane-normalized units remaining after 11 days (t-test, $P < 0.005$).

The hydrocarbon loss rate in sea-ice microcosms was constant over the duration of the experiment, as shown by the consistent slope of percent hydrocarbon loss by incubation days (Fig. 3B). In these microcosms, 1635 ± 195 hopane-normalized units remained at T3 (Fig. 3A), corresponding to a loss of 12 ± 11% (Fig. 3B). Comparatively, in sub-ice seawater microcosms most of the total hydrocarbon loss (63 ± 5% of initial concentrations) occurred during the first days, with 660 ± 82 hopane-normalized units remaining at T3 (Fig. 3A). The estimated loss rate of hydrocarbons between T0 and T3 was significantly higher in sub-ice seawater than in the sea-ice microcosms (371 *versus* 76 hopane-normalized unit d^{-1}, respectively; test on slopes, $P < 0.01$). On average, the loss of hydrocarbon components by the end of the incubation was 94% in sub-ice seawater and 48% in sea ice (Fig. 3B). Based on the time series of hydrocarbon percent loss, the overall half-life of crude oil in Resolute Passage would be ca. 3 days in seawater under ice cover, but at least 2 weeks when in contact with sea-ice bacteria under similar environmental conditions.

There was no significant difference in the initial (T0) amounts of the major groups of hydrocarbon compounds between sea-ice and sub-ice seawater microcosms (Fig. S1, Supporting Information), which consisted mainly of alkanes (85 ± 1%), followed by methylated PAHs (15 ± 1%) and PAHs (0.50 ± 0.03%). These proportions remained unchanged from T0 to T15

in sea-ice (Fig. S1A, Supporting Information) microcosms (KW, $P > 0.05$), but slightly changed in sub-ice seawater microcosms during the incubation although the difference was not statistically significant (Fig. S1B, Supporting Information). The final hydrocarbon composition in sub-ice seawater consisted of 65 ± 4% alkanes, 34 ± 4% methylated PAHs and 1.8 ± 0.3% PAHs (T11).

The three majors groups of hydrocarbon compounds showed different rates of decrease during the incubation period (Fig. 4). Alkanes showed the highest loss in terms of absolute concentrations, followed by methyl-PAHs and PAHs in both sea ice and sub-ice seawater, and this trend is especially evident in sub-ice microcosms (Fig. 4A and C). This also applied to the relative loss values (in %) measured in sub-ice water microcosms (Fig. 4D), but not to sea-ice microcosms where the PAHs experienced higher loss (43 ± 5%) than methyl-PAHs (36 ± 9%) throughout the experiment (Fig. 4B). Loss rates (in % per day) of alkanes and methyl-PAHs were significantly higher in sub-ice seawater than in sea ice (t-test, $P < 0.01$, Fig. 4).

Few aromatics were completely biodegraded in sea-ice microcosms (Fig. 5A); only C_1-methylated fluorene, phenanthrene and C_2-methylated pyrene were fully degraded. The loss of alkanes varied between 50% and 56%, whereas the loss of other compounds was below the average total hydrocarbon loss of 48% for sea ice (Fig. 5A). Comparatively, many aromatics were completely biodegraded (i.e. 100% loss) in sub-ice seawater: C_1- to C_3-methylated fluorenes, phenanthrene and its C_1- to
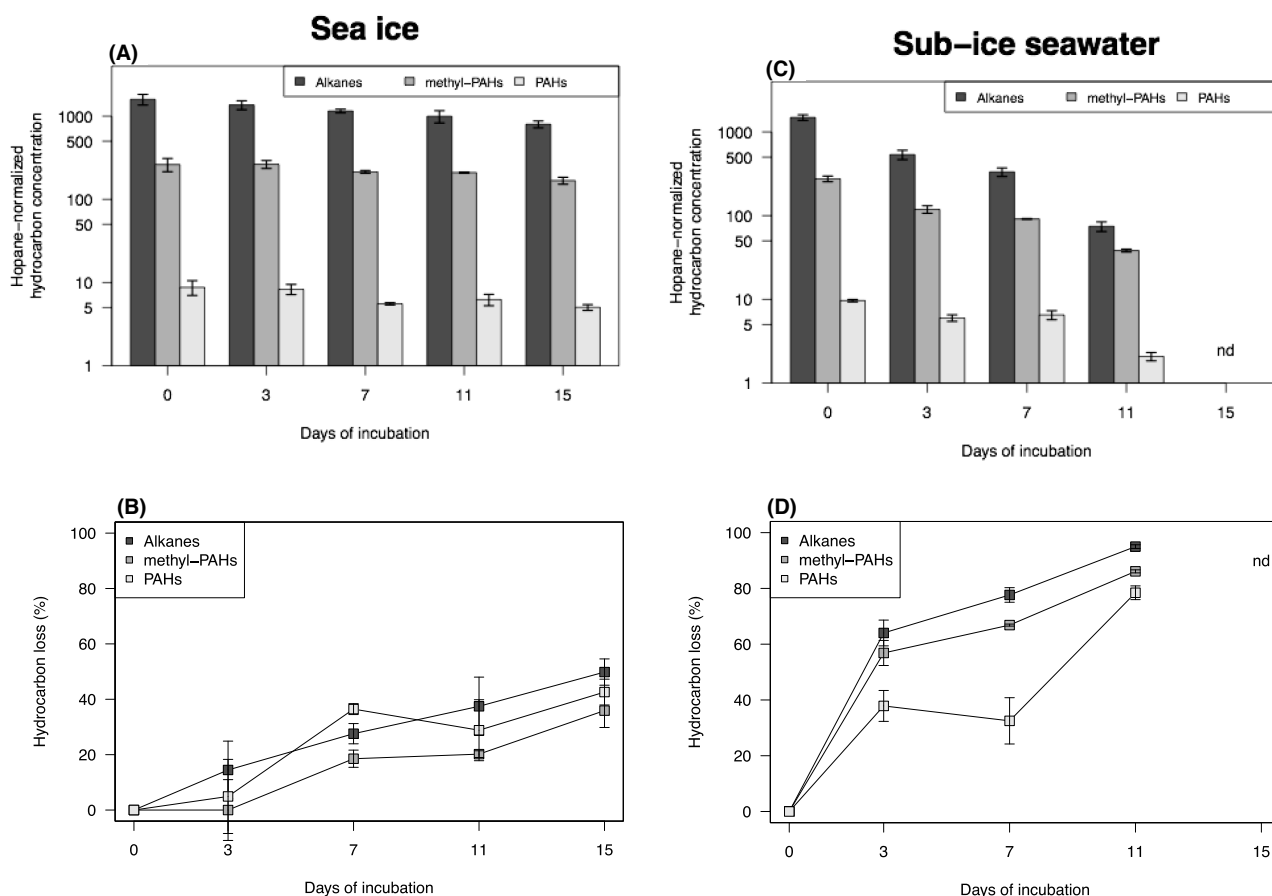

Figure 4. Time series of hopane-normalized concentrations of hydrocarbons and percent loss of main hydrocarbon compounds in sea ice (**A** and **B**) and sub-ice seawater (**C** and **D**) during microcosm experiments. Bars (or symbols) and vertical lines represent averages and standard deviations, respectively, for oil-exposed microcosms (N = 3). Note logarithm scale on y-axis in (A) and (C). nd: no data.
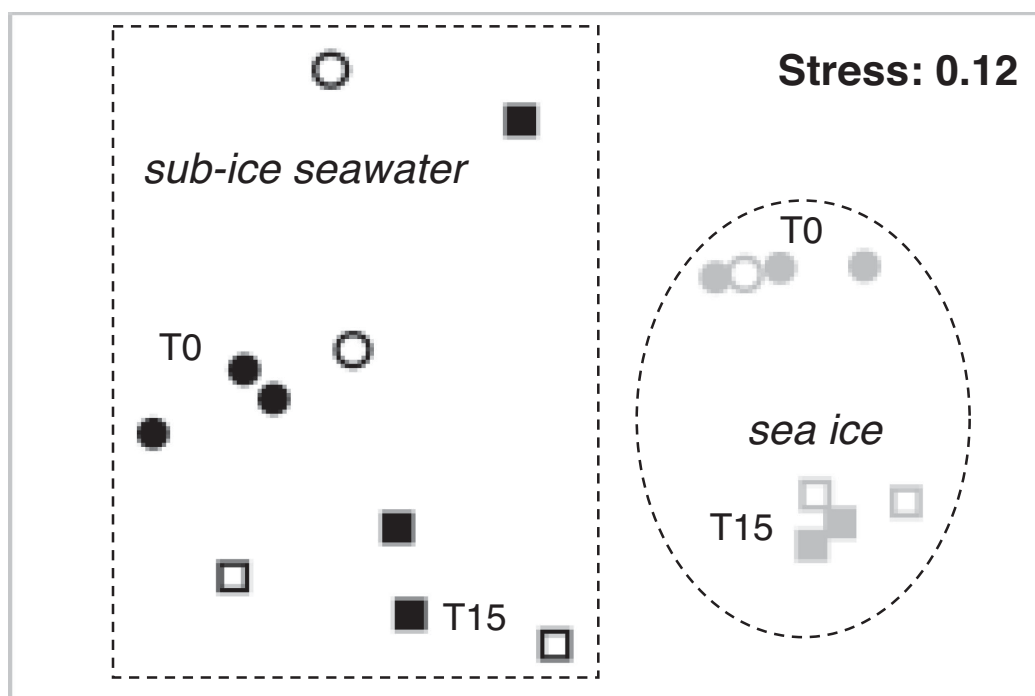
Figure 5. Loss of hydrocarbon compounds in sea ice (**A**) and sub-ice seawater (**B**) after 15 and 11 days in microcosms, respectively.

C_4-methylated forms, C_1- to C_4-methylated naphthobenzothiophenes, as well as C_4-methylated naphthalenes, and the dibenzothiophene and its C_3- and C_4-methylated forms (Fig. 5B). The loss of linear *n*-alkanes (C_{10} to C_{35}) was close to the average total hydrocarbon loss of 94%, whereas the loss of the small methylated aromatics C_0-C_2 naphthalenes and C_1-C_2 dibenzothiophenes was below that average (Fig. 5B).

Bacterial community composition

Differences in bacterial community composition were observed between sub-ice seawater and sea-ice microcosms (ANOSIM, R = 0.77, P < 0.01; Fig. 6). The non-metric MDS plot had a 2D stress of 0.12, indicating good representations of the similarity matrices (Fig. 6). The initial (T0) bacterial community structure was similar in oil-enriched and control microcosms for a given

Figure 6. 2D MDS configuration of microcosms based on Bray–Curtis similarities of bacterial community composition in sub-ice seawater (black) and sea ice (gray) at the beginning (T0, circles) and end (T15, squares) of the incubation. Full symbols represent controls, and open symbols the oil-exposed microcosms. The 2D stress of 0.12 indicated good representations of the Bray–Curtis similarity matrices. Data points positioned closer together are similar. Microbial community structure was different in sub-ice and sea-ice microcosms (ANOSIM, R = 0.77, P < 0.01).

sample type; similarity was 75 ± 6% (MW, P < 0.01) in sea ice and 69 ± 5% (MW, P < 0.01) in sub-ice seawater. There was a significant difference in bacterial community between T0 and T15 in sea ice (ANOSIM, R = 1, P < 0.05), but at T15 the control and oil-treated microcosms were similar (MW, P > 0.05; Fig. 6). Significant difference between T0 and T15 was also observed in oil-treated microcosms for the sub-ice seawater (ANOSIM, R = 0.61, P < 0.05). The similarity in community structure was significantly higher between T0 and T15 in the controls (38 ± 6%, MW, P < 0.01) when compared to the similarity in oil-treated microcosms over the same period (26 ± 7%, MW, P < 0.01). In other terms, the bacterial community composition in sub-ice seawater changed more in oil-enriched microcosms than in controls after the incubation time.

In sea ice at the beginning of the experiment, an average of 5474 ± 970 16S rRNA gene sequences were obtained from each replicate sample (Table S1, Supporting Information), and chloroplasts (a proxy for algal sequences) represented 72 ± 2% of the total sequences. After 2 weeks of incubation, less than 0.1% of the sequences were chloroplast for both the oiled-enriched microcosms and the controls (data not shown). In sub-ice seawater microcosms, an average of 5964 ± 540 16S rRNA gene sequences were obtained (Table S1, Supporting Information). Of these, 1.9% ± 0.1 were affiliated to chloroplasts in both treatments at T0 and T15 (not shown). This is consistent with the higher algal biomass (chl *a*) measured in our sea-ice samples when compared to sub-ice seawater (Table 1). Sea-ice bacterial community composition at T0 consisted of, on average, 77 ± 1% of *Proteobacteria* (*alpha*: 0.3%; *gamma*: 49% and others: 28%), 23 ± 1% of *Bacteroidetes* (*Flavobacteria*: 9%) and >1% of *Actinobacteria, Acidobacteria* and *Firmicutes* (data not shown). In sub-ice seawater, the bacterial community at T0 was composed of, on average, 72 ± 1% of *Proteobacteria* (*alpha*: 7%; *epsilon*: 0.1%; *gamma*: 58% and others: 6%), 28 ±

1% of *Bacteroidetes* (*Flavobacteria*: 17%) and >2% of *Actinobacteria, Verrucomicrobia, Fusobacteria, Firmicutes* and *Planctomycetales* (not shown).

Amplicon sequencing analyses of the 16S rRNA gene also revealed that the bacterial community composition changed significantly over the course of the experiment (i.e. between T0 and T15) in the control and oil-enriched microcosms for both sea ice and sub-ice seawater (Fig. 7). All sea-ice microcosms experienced an increase in the relative contribution of *Flavobacteria/Bacteroidetes* and a decrease in *Proteobacteria* (Fig. 7A). The reduction in *Proteobacteria* was less important in oil-enriched microcosms. Community composition evolved differently in the sub-ice seawater microcosms; the relative contribution of *Bacteroidetes* decreased and relative contribution of *Proteobacteria* increased, especially the *Gammaproteobacteria* (Fig. 7B). Notably, in the oil-enriched microcosms, the contribution of *Epsilonproteobacteria* increased (T15: 4 ± 2% *versus* T0: 0.1 ± 0.0%; t-test, P < 0.01) whereas the contribution of *Alphaproteobacteria* decreased significantly (T15: 3 ± 1% *versus* T0: 7 ± 1%; t-test, P < 0.01).

Resolution at lower taxonomic ranks revealed finer changes in sea ice (Fig. 8). Increases in the relative contribution of the genera *Polaribacter* and *Moritella* were observed at T15 in control and oil-treated microcosms, as well as a decrease in the relative contribution of *Glaciecola* (Fig. 8C and D). *Polaribacter* contributed for 99 ± 1% of the *Bacteroidetes*, whereas *Moritella* and *Glaciecola* contributed 69 ± 7% and 1.2 ± 0.4% of the *Proteobacteria*, respectively. *Moritella* was the only genus that was more abundant in oil-treated microcosms (41%) than in the controls (24%). The most striking change was in oil-treated microcosms where the community diversity decreased because minor taxa (i.e. *Arenicella, Octadecabacter, Lewinella, Ulvibacter* and *Perscivirga*) were below detection (Fig. 8D). Sequences from the genus *Algicola* were only detected after incubation with crude oil (Fig. 8D).
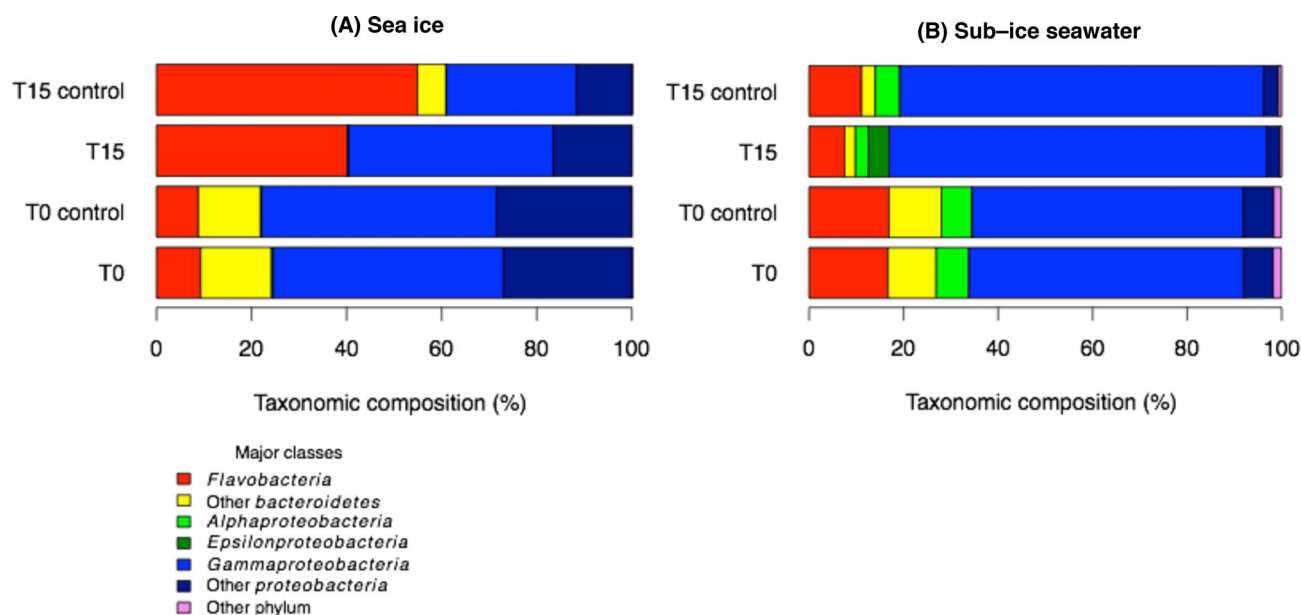
(A) Sea ice

(B) Sub–ice seawater

Major classes
- ■ *Flavobacteria* (red)
- ■ Other *bacteroidetes* (yellow)
- ■ *Alphaproteobacteria* (green)
- ■ *Epsilonproteobacteria* (dark green)
- ■ *Gammaproteobacteria* (blue)
- ■ Other *proteobacteria* (dark blue)
- ■ Other phylum (pink)

Figure 7. Bacterial community taxonomic composition based on 16S rRNA gene sequences in sea ice (**A**) and sub-ice seawater (**B**) at the beginning (T0) and end (T15) of the incubation in control and experimental microcosms.

Interestingly, the sub-ice seawater at T0 in both the oil-treated and controls microcosms consisted of ∼25% unclassified bacteria at the class level, which increased to 97% unclassified bacteria at the genus level (Fig. 9A and B). The presence of oil stimulated the growth of *Colwellia,* which constituted 33% of the population at the end of the incubation period (Fig. 9D). Three genera were only obtained after 2 weeks in the oil-treated microcosms; *Alcanivorax*, *Sulfitobacter* and *Oleispira* contributed to 0.52%, 0.20% and 0.05%, respectively (Fig. 9D). Even though new genera were detected after 2 weeks in the oil-treated microcosms, the overall diversity had decreased; the contribution of unclassified bacteria was to 61% in oil-treated microcosms at T15, whereas it was 96% in controls (Fig. 9C and D).

DISCUSSION

Contrasting hydrocarbon degradation within and under first-year sea ice

Ice-associated microorganisms are able to substantially degrade typical crude oil components at temperatures as low as –1.7°C, without additional nutrients or oil dispersant. The biodegradation capacity of the indigenous ice-associated microbes of the eastern Canadian Arctic Archipelago was markedly distinct whether they lived in the ice matrix or in the underlying waters. Bacterial communities in sub-ice seawater were much more efficient in degrading hydrocarbons than those living in sea ice, as the former degraded the bulk (94%) of added hydrocarbons within 11 days (Fig. 3B). Sea ice bacteria removed less than 40% of the hydrocarbons over a similar period (Fig. 3B), although these cells were two times more abundant at the beginning of the experiment (Table 2).

Sub-ice seawater bacteria depleted almost all the resolvable alkanes (C_9 to C_{40}, pristane and phytane; *sensu* Atlas and Bragg 2009) within 11 days at –1.7°C (Fig. 5B). Alkanes are major components of crude oil, and are typically utilized as carbon and energy sources by bacteria (Rojo 2009), including Arctic populations growing at 4°C (Deppe *et al.* 2005) down to 1°C (Gerdes *et al.* 2005),

and as low as –1°C (McFarlin *et al.* 2014). Interestingly, the methylated forms of PAHs were degraded almost as much as the alkanes by the sub-ice seawater bacteria (Fig. 4B). This contrasts with recent experimental results obtained with Arctic seawater bacteria where parent PAHs were degraded before their methylated homologs (McFarlin *et al.* 2014). These compounds are considerably toxic and bioaccumulate more than the non-methyl forms because the addition of a methyl group decreases water solubility (Seo, Keum and Li 2009). Methylated PAHs are nonetheless biodegradable under aerobic conditions, but few studies have addressed this topic (Seo, Keum and Li 2009). Sea-ice bacteria were not as efficient in degrading methyl-PAHs; only 2 compounds out of the 26 detected were fully metabolized (Fig. 5A), whereas the remaining ones were partially degraded (24% to 64%).

LMW PAHs are highly toxic to most aquatic organisms (Peng *et al.* 2008), naphthalenes being the most noxious (International Association of Oil and Gas Producers 2002), but they can be metabolized by microorganisms (van Agteren, Keuning and Oosterhaven 1998). Phenanthrene, a biodegradable and relatively water-soluble PAH (Head, Jones and Röling 2006), was completely degraded by all ice-associated microbes, whereas naphthalene and dibenzothiophene were degraded to a lesser extent (Fig. 5). Nonetheless, naphthalene-degrader microbes in cold Arctic ecosystems are as efficient as their temperate counterparts (Bagi *et al.* 2014). Assuming the degradation rate observed during our experiment would persist, 15 more days would be needed for the complete degradation of these components. Our results indicate that sub-ice microbial communities have the physiological capabilities to degrade crude oil and could possibly biodegrade naphthalenes from an accidental spill within a month, even under seasonal sea-ice cover.

Microcosms to study hydrocarbon degradation

The bacterial communities studied in microcosms are representative, in terms of abundance, production and diversity, of
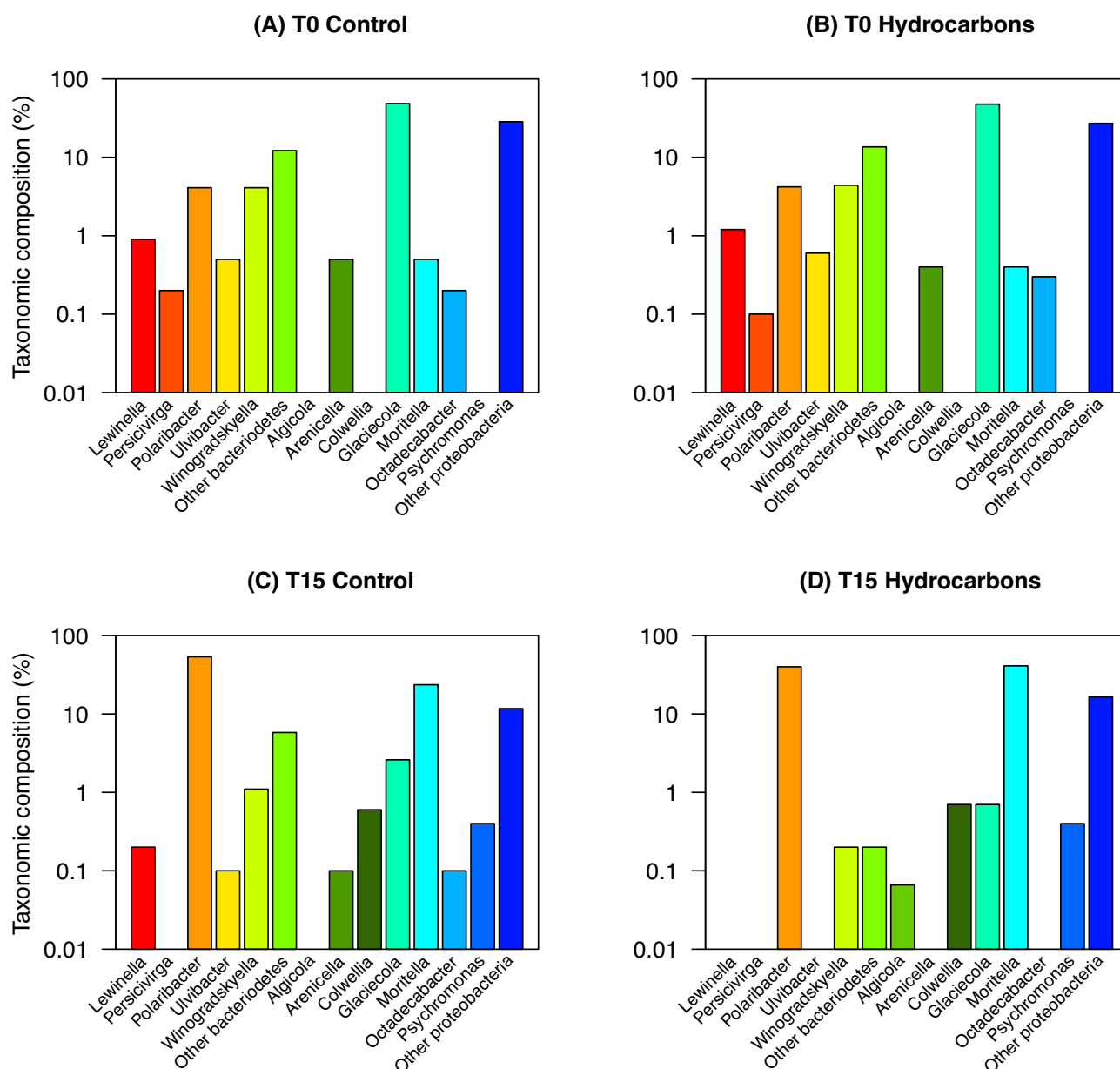
Figure 8. Bacterial community taxonomic composition (in %) based on 16S rRNA gene sequences in sea ice at the beginning (T0) and end (T15) of the incubation period in control and experimental (hydrocarbons) microcosms. Note logarithm scale on y-axis.

natural communities found in the Northwest Passage in spring. In May–June, the area is typically covered with first-year ice that supports high ice algal production (Cota *et al.* 1991), which was reflected by the very high bottom-ice chl *a* concentrations at the time of our experiment (Table 1). Bacterial abundances in sub-ice seawater and in sea ice (Table 1) were comparable to previous records for the same region; 1.0×10^5 cells ml^{-1} were found at the ice-water interface, and from 1.5 up to 100×10^5 cells ml^{-1} in sea ice of Resolute Passage during April–May (Maranger, Bird and Juniper 1994). When applying the conservative conversion factor of 1.5×10^6 mg C mol^{-1} leucine (Ducklow 2003) to our leucine uptake rates at T0, daily bacterial production was 0.77 mg C m^{-3} d^{-1} in sea ice and 0.46 mg C m^{-3} d^{-1} in sub-ice seawater. These rates are consistent with rates in sea ice of 0.48 to 3 mg C m^{-3} d^{-1}, and

a mean of 0.72 mg C m^{-3} d^{-1} in sub-ice waters of the Resolute Passage (Maranger, Bird and Juniper 1994).

Gammaproteobacteria is typically the predominant group in Arctic sea ice (Junge *et al.* 2002; Brinkmeyer *et al.* 2003; Bowman *et al.* 2012) followed by *Bacteroidetes* (Deming 2010), whereas *Alphaproteobacteria* dominates in Arctic surface waters (Garneau *et al.* 2006; Alonso-Sáez *et al.* 2008). Accordingly, our results showed a dominance of *Gammaproteobacteria* and *Bacteroidetes* in the marine communities on the underside of landfast ice but no *Alphaproteobacteria* were detected (Fig. 7A), which is concordant with observed low abundance (ca. 4% of 16S rRNA gene sequence tags) in Arctic multiyear ice (Bowman *et al.* 2012). Bacterial composition in sub-ice seawater at the beginning of our experiment showed the presence of the same three dominant groups,

(A) T0 Control

(B) T0 Hydrocarbons
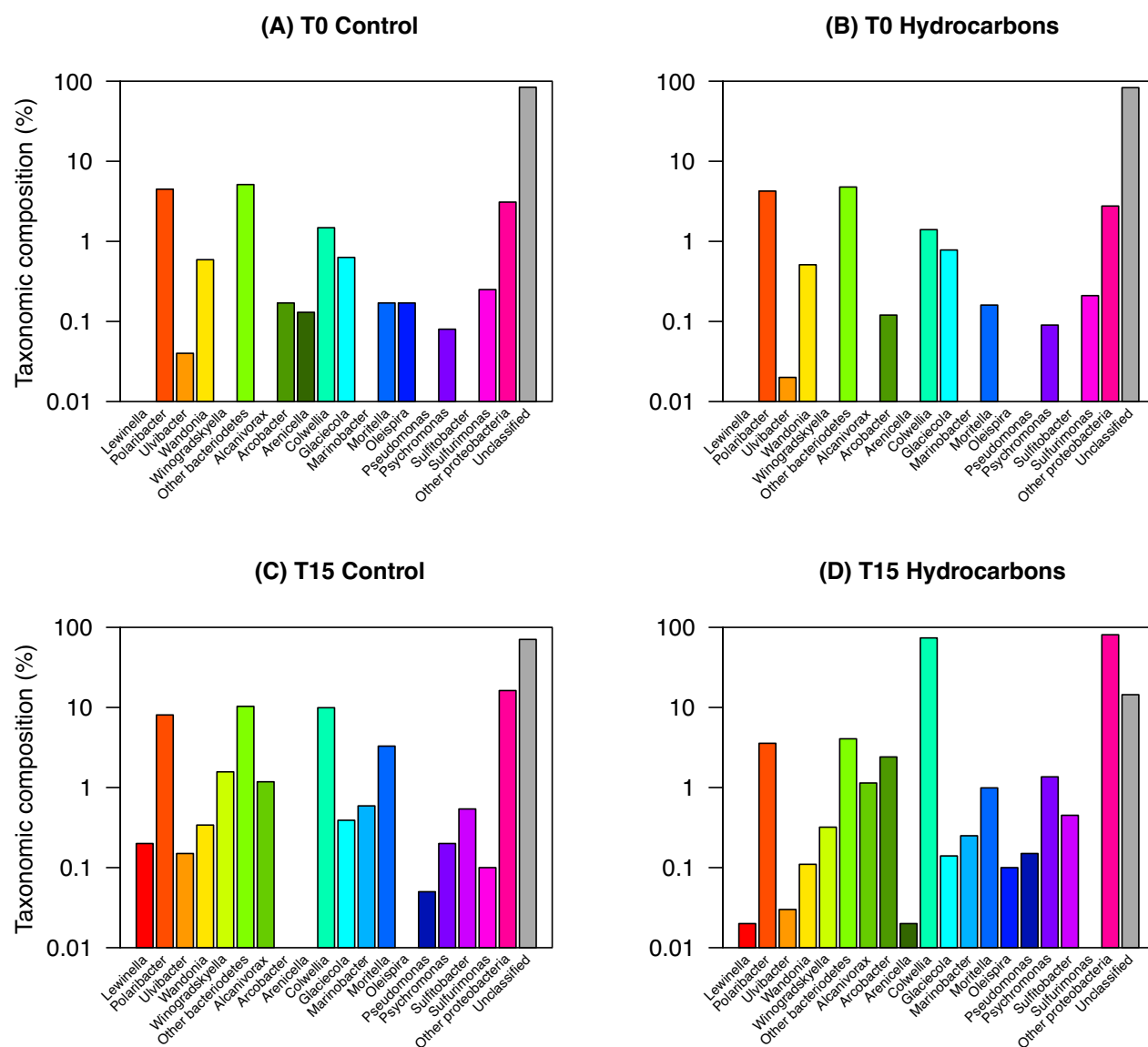

(C) T15 Control

(D) T15 Hydrocarbons

Figure 9. Bacterial community taxonomic composition (in %) based on 16S rRNA gene sequences in sub-ice seawater at the beginning (T0) and end (T15) of the incubation period in control and experimental (hydrocarbons) microcosms. Note logarithm scale on y-axis.

but with a predominance of the *Gammaproteobacteria* over the *Alphaproteobacteria* (Fig. 7B). This could be related to the phytoplankton pre-bloom conditions, sub-ice conditions or exchange of ice bacteria with sub-ice seawater. Given that our experiment was run in oxic conditions, as would be expected in the diffusive interfacial sea-ice layer, sequencing results did not reveal the presence of anaerobic bacteria. However, anoxic microsites have been found in sea-ice brine channels in the Arctic (Rysgaard and Glud 2004), where anaerobic bacteria were detected (Petri and Imhoff 2001; Bowman *et al.* 2012). Biodegradation of hydrocarbon is also possible in anoxic pockets when NO_3^-, Fe^{3+} and SO_4^{2-} are used as electron acceptors (Widdel and Rabus 2001). The extent of anaerobic oil biodegradation in ice could not be estimated in our oil-treated microcosms, and would require further investigation.

One cannot rule out that part of naphthalene losses may be due to volatilization in the headspace of the incubation microcosms. Irwin and colleagues (1997) showed that volatiliza-

tion in soils accounted for about 20% and 30% of the loss of C_1-methylnaphthalene and naphthalene, respectively, but for the 12 remaining HMW PAHs, volatilization was not an important loss mechanism. Given that at low temperature the volatilization of short-chain alkanes is reduced (Yang *et al.* 2009), volatilization of naphthalenes in our microcosms was probably smaller than what was observed in soils and therefore most losses were likely due to biodegradation.

The overall half-life of low-pour point oil spilled in Resolute Passage would be ca. 3 days in seawater under ice cover. This is faster than other recent experimental results, which suggest that the half-life of crude oil would be ca. 60 days in the Chukchi Sea at sub-zero temperatures (McFarlin *et al.* 2014). No bacterial activity was measured during that experiment, but nutrient concentrations were below the detection limit in the Chukchi Sea, suggesting that bacterial growth may have been limited and explaining the comparatively shorter half-life measured in our microcosms. In contrast, a half-life of 14 days was suggested for

crude oil in temperate (8°C) seawater offshore of New Jersey, USA (Prince *et al.* 2013).

Microbial response to oil exposure

Initial bacterial abundances in microcosms did not have much impact on the overall degradation of oil because even though cell abundance in sub-ice seawater microcosms was half the count in ice (Table 2), sub-ice microbes degraded three times more hydrocarbons in 3 days (Fig. 3). Results rather support the idea that difference in the starting community composition and/or environmental conditions (e.g. DOC concentrations) were key factors. The relative contribution of *Gammaproteobacteria* to the total bacteria was higher in sub-ice seawater than in the ice matrix, where the contribution of other *Proteobacteria* was also important (Fig. 7). *Gammaproteobacteria* were confirmed to be the main players of oil biodegradation in cold marine environments (Yakimov *et al.* 2004; Gerdes *et al.* 2005; Brakstad and Bonaunet 2006; Brakstad *et al.* 2008). Bacterial diversity also changed in the presence of hydrocarbons (Figs 7–9) and shifted toward a higher contribution of *Gammaproteobacteria* in both sub-ice seawater and sea ice, further supporting the contention that oil-degrading bacteria are affiliated to this class. Interestingly, the *Epsilonproteobacteria* in sub-ice seawater also seemed to have benefited from the presence of oil, as their contribution increased in oil-enriched microcosms but not in controls (Fig. 7B). Several genera of this class that are able to oxidize sulfur compounds dominated the microbial biomass in groundwater contaminated by crude-oil extraction activities (Kodama and Watanabe 2003; Hubert *et al.* 2011). This may explain why dibenzothiophene, a sulfur-containing compound, and its methylated forms were highly oxidized (average of 97% loss) in sub-ice waters but not in sea ice (< 40% loss; Fig. 5).

Bacteroidetes increased the most after 15 days of incubation in sea-ice microcosms, regardless of the presence of hydrocarbons (Fig. 7A) and following bacterial production rates (Fig. 2), i.e. *Bacteroidetes* increased in sea ice, but decreased in seawater. These changes agree with previous observations of a *Bacteroidetes* predominance associated with high bacterial biomass and production (Teira *et al.* 2008). *Bacteroidetes* are the main degraders of complex polymeric organic compounds (Cottrell and Kirchman 2000; Fernández-Gómez *et al.* 2013); therefore, the presence of extracellular polymeric substances (EPS) in the sea ice of the Canadian Arctic Archipelago (Aslam *et al.* 2016) could have provided a competitive advantage for resources to the *Bacteroidetes* over the *Gammaproteobacteria*. Conversely, the low organic matter content in sub-ice seawater (Table 1) could have been more advantageous for the *Gammaproteobacteria*.

The characterization at the genus level indicated that crude-oil addition reduced microbial diversity (Figs 8 and 9), as previously shown for oil-polluted fjord ice (Brakstad *et al.* 2008) and Arctic multiyear ice (Gerdes *et al.* 2005). Three genera benefited from the oil enrichment in sea ice, the *Gammaproteobacteria* genera *Moritella*, *Algicola* and *Colwellia* (Fig. 8). *Moritella* was recently reported to be the second most abundant genus in naphthalene-spiked Arctic seawater (Bagi *et al.* 2014), whereas the genus *Algicola* has not been previously described as oil degrader. It is unclear if *Algicola* strains metabolized the added hydrocarbons or if they were rather secondary consumers that profited from other compounds released by oil-degrading bacteria.

Members of the genus *Colwellia*, which are oil-degrading *Gammaproteobacteria* (Yakimov *et al.* 2004; Redmond and Valentine 2012), were also stimulated by the presence of oil in sub-ice seawater (Fig. 9). Like *Polaribacter* (*Bacteroidetes*), *Colwellia* are known psychrophiles and may have benefited from the low incubation temperature (< −1°C), as previously observed in cold marine environments (Yakimov *et al.* 2004; Brakstad *et al.* 2008). Three typical marine hydrocarbonoclastic bacteria, i.e. *Alcanivorax* and *Oleispira* (Yakimov *et al.* 2007), only grew in oil-treated sub-ice seawater microcosms, as well as *Sulfitobacter*, a sulfite-oxidizing *Alphaproteobacteria* bacterium. This genera was also obtained in beach sands in the Gulf of Mexico after the Deepwater Horizon oil spill (Kostka *et al.* 2011). The very high percentage of unclassified bacteria in the sub-ice seawater (97%) at the genus level indicates that the majority of bacteria in cold Arctic waters have yet to be discovered.

The oil treatment in sea-ice microcosms did not induce an increase in bacterial production as it did in sub-ice microcosms after 3 days and until the end of incubation (Fig. 2). This lag period was similar to the 5 to 8 day lag observed during other carbon enrichment experiments on polar bacterial communities (Dyda *et al.* 2009; Ducklow *et al.* 2011). Bacterial communities usually need time to activate the pathway of enzymes required for hydrocarbon degradation (Rojo 2009). The fast response to hydrocarbon exposure observed in our study may be due to a subpopulation of alkane degraders that is maintained by alkanes, which are ubiquitous in the marine environment, being continuously produced by many organisms (plants, green algae, bacteria and animals; Rojo 2009). The sub-ice carbon:nitrogen (DOC:DN, mol:mol) ratio of 6:1 (Table 1) suggests a carbon-limited community because it is below the ratio of 10:1 for optimal hydrocarbon degradation (Alexander 1999). The addition of hydrocarbons very likely stimulated the activity of the carbon-limited sub-ice bacteria. Likewise, the addition of ^{3}H-leucine may have induced an artificial stimulation of the uptake rates, explaining why bacterial production in control microcosms at T0 were much higher than at any other time point (Fig. 2). Toxic stress on bacteria after oil addition (e.g. Siron *et al.* 1993) may have impeded such stimulation in the oil-treated microcosms.

In sea ice, the DOC:DN ratio of 16:1 (Table 1) was above the optimal ratio, indicating that the biodegradation of spilled hydrocarbons would rather be limited by nutrients. Accordingly, the addition of hydrocarbons did not lead to a sharp increase in bacterial production (Fig. 2A). Nutrient limitation after oil spills is common in marine environments because hydrocarbons have high organic carbon content and no bioavailable nitrogen (Das and Chandran 2011). The limited biodegradation in sea ice may also be explained by the availability of highly labile dissolved organic matter produced by sea-ice algae (Amon, Fitznar and Benner 2001). Algal-derived carbon supports substantial bacterial growth (Riedel *et al.* 2008), and most alkane-degrading bacteria were shown to prefer such labile compounds than alkanes when available (Rojo 2009). The very high DOC and chl *a* concentrations found in sea ice as well as the apparent absence of (or a short) lag period in sea-ice bacterial activity would agree with a bacterial production supported by sea-ice organic carbon.

Factors governing hydrocarbon degradation in ice-covered Arctic waters

Among the 59 crude oils classified in ITOPF's technical paper (2011), about 61% have a pour point < −2°C (including the Arabian Light used in this study), implying that a majority of oils would still behave like fluids and spread in droplets under wave action even if the Arctic seawater is at its coldest. Arabian Light oil was

efficiently degraded in this study in part because the oil was still fluid and accessible to microorganisms, even at sub-zero temperatures. High degradation rates could be found in the field for several other low pour-point oils, although in some cases oil composition could lead to divergent results. In ice-covered areas, however, where temperature in first-year ice varies between –2°C and –20°C (Junge, Eicken and Deming 2004), degradation rates would potentially be much lower than what was observed in ice microcosms because only 39% of the ITOPF-classified oils would exhibit liquid characteristics below –15°C. At temperature as low as –25°C in multiyear ice (Perovich and Elder 2001), biodegradation rates would be negligible, if not null, because most oils will solidify (ITOPF 2011) and because very cold ice has little brine volume fraction and thus, fewer microhabitats to support an active bacterial community (Deming 2010).

The 'law of five' states that sea ice that has a brine volume fraction of 5% and the typical salinity of 5 ppt will be impermeable at temperatures below –5°C (Golden, Ackley and Lytle 1998). Conversely, at temperatures warmer than −5°C, the sea ice will be permeable because brine inclusions become connected, allowing heat, brine, nutrients and seawater to move through ice (Golden, Ackley and Lytle 1998). Thus, whether an oil spill occurs in winter or during the warmer ice melting season has important implications for the biodegradation of hydrocarbons. In winter, chances are that sea ice would be impermeable because the temperature can drop below −5°C (Junge, Eicken and Deming 2004). This means that oil spilled on the ice cover would not percolate and be in contact with bottom-ice bacterial communities, thereby limiting biodegradation. However, if some oil was floating at the ice–water interface, it could reach the lowermost part of the ice where the temperature remains near to the seawater freezing point of –1.8°C (Notz and Worster 2009).

Field observations indicate that oil spilled in calm seas spreads out under the ice where it gradually fills depressions (Fingas and Hollebone 2003). Our results imply that bacteria in these cavities and growing close to the ice-water interface should be capable of biodegradation during winter. Bacteria were shown to be active in the upper water column even in winter, but rates were low at < 0.05 mg C m^{-3} d^{-1} (Garneau *et al.* 2008). Assuming the oil reaches the ice–water interface, biodegradation rates could be higher than the ones measured in the microcosms because bottom-ice DOC and EPS concentrations are very low in winter (Riedel *et al.* 2008), as well as the primary producer biomass (< 0.04 mg chl *a* m^{-3}; Garneau *et al.* 2008). In such carbon-limiting conditions, oil additions could enhance bacterial production.

It is unclear if under-ice biodegradation during cold seasons could be significant. Limited oil biodegradation was found under a winter ice cover in the St. Lawrence River, a temperate marine system, and was attributed to the low productivity of the microbial communities (Siron *et al.* 1993). Another study in the Beaufort Sea investigated the movement of oil under first-year ice and showed that oil spilled in October was encapsulated in ice within days, and remained in the bottom 5–10 cm of the first-year ice until February, without apparent degradation (NORCOR Engineering Research Limited 1975). Recent results show that full-depth connectivity between the top and bottom of first-year ice can happen in spring, well before summer melt (Jardon *et al.* 2013), potentially favoring bacterial degradation in the event of oil release during spring. Therefore, remediation strategies for oil mitigation in the Arctic likely need to vary according to seasonal conditions. Finally, the biodegradation potential of microbial communities in melt ponds formed at the ice-cover surface remains to be determined.

CONCLUDING REMARKS

The experimental results demonstrate great potential for bioremediation techniques in the Arctic Ocean, whether in sea ice or in the water beneath it. Nutrient concentrations should not be a limiting factor if a small amount of contamination occurs during the pre-bloom period, considering that 94% of the initial hydrocarbons were lost within 2 weeks in our oil-treated microcosms. So far, biostimulation (i.e. supply of limiting resources; Brakstad 2008) has been more successful than bioaugmentation in cold environments because the added microorganisms were stressed by the polar conditions and were outcompeted by indigenous species (Yang *et al.* 2009). Biostimulation in winter or early spring, when nutrient concentrations in surface water are elevated, will likely have little effect. However, such a technique could be beneficial if used after the phytoplankton bloom period, when the surface water is depleted of nutrients. Likewise, biostimulation could be efficiently used in case of an accidental release of large amounts of hydrocarbons, which would result in rapid depletion of the available nutrients.

Our results highlight the influence of the natural, ice-algal carbon resources on the activity of oil-degrading bacteria. Hydrocarbon degradation by sub-ice planktonic bacteria under high DOC concentration (i.e. during the phytoplankton bloom), as well as by sea-ice bacteria under low DOC concentration (before the ice-algal bloom), could provide a better understanding of bioremediation during the spring-summer transition. Further studies could also target the oil-degradation functional genes of ice-associated bacteria, as was previously reported for Arctic soils (Yergeau *et al.* 2012). All this information will provide a finer comprehension of natural oil degradation and help in developing bioremediation strategies for the Arctic.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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