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Biodegradation of weathered crude oil by microbial communities in solid and melted sea ice

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ABSTRACT

Oil spilled in the Arctic may drift into ice-covered areas and become trapped until the ice melts. To determine if exposure to oil during freezing may have a priming effect on degradation of the oil, weathered dispersed oil (2-3 mg/L) was frozen into solid ice for 200 days at -10 °C, then melted and incubated for 64 days at 4 °C.

No degradation was measured in oil frozen into ice prior to melting. Both total amount of oil and target compounds were biotransformed by the microbial community from the melted ice. However, oil released from melted ice was degraded at a slower rate than oil incubated in fresh seawater at the same temperature (4 °C), and by a different microbial community. These data suggest negligible biodegradation of oil frozen in sea ice, while oil-degrading bacteria surviving in the ice may contribute to biodegradation when the ice melts.

1. Introduction

Oil exploration, production and transport in the Arctic are expected to increase due to declining ice coverage (Serreze and Meier, 2019), resulting in elevated risks of oil discharges to the marine environment. The estimated occurrence of undiscovered oil and gas north of the Arctic Circle may be as much as 90 billion barrels of oil and 47 trillion cubic meters of natural gas, most of it in offshore areas (Bird et al., 2008). In the case of an oil spill in the Arctic, oil may drift with wind and prevailing currents into ice-covered areas, become trapped in the ice, and drift with the ice until released when the ice melts.

Oil spilled into the marine environment undergoes a number of weathering processes which alter oil properties and behaviour over time. These processes include evaporation, water-in-oil emulsification, oil-in-water dispersion, dissolution, photo-oxidation and biodegradation (National Research Council (NRC), 2003; Daling et al., 2014; Lee et al., 2015). Weathering of oil typically complicates oil spill response (OSR) operations, as the viscosity, density, flash point and pour point increase, making OSR operations more challenging (Lee et al., 2015). It is generally accepted that OSR-operations become impossible after the oil has frozen into sea ice, and these should therefore be executed when the oil is still in open water (Dickins, 2017). Use of dispersants may be a relevant OSR method for oil spills in remote areas like the Arctic (Lewis and Prince, 2018), because of a relatively short response time compared to common mechanical recovery methods. Dispersants are used in an effort to generate small oil droplet dispersions, which will transfer oil from the sea surface to the water column (Lee et al., 2013). It is generally accepted that dispersion of oil is favourable for biodegradation, since small oil droplets with large surface-to-volume ratios are formed. Dispersion of oil increases dissolution and gives oil-degrading microbes a large oil-water interphase to attach to (Prince et al., 2013; Brakstad et al., 2015a; Hazen et al., 2016).

If oil is spilled in Arctic winter, dispersions may be trapped in growing sea ice together with bacteria, algae and other particles (Garrison et al., 1983). Studies of oil frozen into sea ice have shown that water-soluble oil compounds can dissolve into brine channels, and that the dissolved oil compounds can migrate through the brine channels in the ice to the underlying water (Faksness and Brandvik, 2008a; Faksness and Brandvik, 2008b), while studies of chemically dispersed oil frozen into ice have shown that the oil may maintain partial dispersibility after ice thawing, when compared to non-treated oil (Nedwed et al., 2017).

Microbes trapped in sea ice are exposed to an extreme environment with sub-zero temperatures and high salinity in the liquid brine channels formed when the ice grows. Microorganisms surviving in the brine must therefore adapt to low temperature and high salinity (Krembs et al., 2002; Junge et al., 2017). Psychrophilic (cold-loving) microbes have a

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variety of adaption mechanisms for these environments, involving protein and enzyme stabilisation (Groudieva et al., 2004; Collins et al., 2008), and production of substances with cryo- and osmo-protective properties like extracellular polymeric substances (EPS) (Krembs et al., 2002). It has also been suggested that EPS has an influence on the microstructures and the salt retention in growing ice, creating larger brine pockets with lower salinity (Krembs et al., 2011).

Few field studies have investigated oil biodegradation in solid sea ice, and significant degradation of hydrocarbons was not measured in any of these studies (Brakstad et al., 2008; Boccadoro et al., 2018; Vergeynst et al., 2019). However, results from the same studies showed that oil effects the microbial communities in ice, increasing the relative abundances of bacteria associated with oil biodegradation, particularly the Gammaproteobacteria class. Within the Gammaproteobacteria, the genera *Colwellia, Marinomonas,* and *Glaciecola* were observed in oil polluted ice cores by Brakstad et al. (2008), whereas Vergeynst et al. (2019) found *Oleispira* in biofilms growing on oil coated adsorbents frozen into ice. *Oleispira* and *Colwellia* were also observed in the bottom layers of sea ice with dispersed oil (Boccadoro et al., 2018). *Glaciecola* is a common genus in sea ice (Boetius et al., 2015), while members of *Oleispira* represent obligate psychrophilic hydrocarbonoclastic bacteria associated with alkane degradation (Yakimov et al., 2003; Lofthus et al., 2018). *Colwellia* are primarily psychrophilic heterotrophic bacteria (Bowman et al., 1998; Methé et al., 2005), with the potential to degrade a variety of hydrocarbons (Chakraborty et al., 2012; Mason et al., 2014).

Microbial communities from melted sea ice have also been shown to degrade hydrocarbons (Deppe et al., 2005; Gerdes et al., 2005; Garneau et al., 2016). However, in these studies, oil was added after melting. How oil dispersions in natural seawater that are frozen over a time period equivalent to an Arctic winter, and then melted to represent Arctic summer conditions, affects biodegradation potentials of oil and microbial community structures, have to our knowledge, never been investigated.

While previous biodegradation studies of oil in sea ice have used fresh or light oils, the current study focused on biodegradation of weathered chemically dispersed oil, relevant for an oil spill in open seawater, where the oil drifts in the open sea and subsequently freezes into sea ice as dispersed oil. The aim of the current laboratory study was therefore to (i) investigate biodegradation of dispersed weathered oil frozen into solid sea ice from surface and subsurface seawater; (ii) to study the degradation potentials of bacterial communities from melted ice after exposed to oil during 200 days of freezing, and (iii) compare oil biodegradation and microbial communities in melted ice to seawater not previously frozen and exposed to oil.

2. Materials and methods

2.1. Seawater, frazil ice and oil

2.1.1. Seawater and frazil ice

Seawater was collected (March 21, 2018) fromsurface seawater and subsurface seawater of a Norwegian fjord (Trondheimsfjorden; 63°26′N, 10°23′E). Seawater was collected at 80 m depth and transported to the laboratories of SINTEF Sealab (Trondheim) through a pipeline system (continuous flow). Seawater was passed a through a sand filter for removal of course particles before entering the laboratory facilities. The seawater location is considered to be non-polluted, with a salinity of 34 ppt. Mineral nutrient conditions have previously been reported (Brakstad et al., 2015a; Brakstad et al., 2018a). Microbial community analyses from the seawater pipeline inlet and laboratory outlet showed comparable community abundance profiles, differing from the SSW profiles (Supplementary Information (SI) 1, Fig. S1). Sterilized seawater was prepared for abiotic controls by filtration (0.22 μm; Sterivex filters, Millipore), and added $HgCl_2$ (final concentration 100 mg/L). Frazil ice was prepared before the start of the experiment by mechanical stirring of seawater in a tank (800 L) at -20 °C. Both surface- and subsurface seawater were acclimated for two days at 0 °C before the start of the experiment. No additional nutrient amendment was supplied to any of the treatments.

2.1.2. Oil

A weathered residue of the Norwegian crude *in-reservoir* biodegraded naphthenic oil Troll B was used in the experiment. Fresh Troll B was heated at 250 °C (Troll B 250+), corresponding to evaporation after 2-5 days of weathering at sea (Daling et al., 1990). The weathered oil had a density of 0.922 g/mL and pourpoint -3 °C. Physical and chemical properties are described in SI 2 (Table S1 and Fig. S2). The Troll B 250+ oil was premixed with Corexit 9500A dispersant (Nalco Environmental Solutions LLC, Sugar Land, TX, USA) at a dispersant-to-oil ratio (DOR) of 1:100. Stock oil dispersions were prepared using an oil droplet generator, as previously described (Nordtug et al., 2011). The median oil droplet size of the stock dispersions was 10-20 μm (measured by Coulter Counter), and the nominal oil concentration was 616 mg/L (calculated from measured oil droplet concentration and oil density).

2.2. Biodegradation experiments and sampling

2.2.1. Experiment with dispersed oil in solid ice

To investigate biodegradation of oil in solid ice, an experimental ice-brine system was prepared in beakers (approx. 1.8 L; Schott AG, Mainz, Germany). Ice samples with dispersed oil were prepared by adding oil stock dispersions to a mix of frazil ice and seawater to a final concentration of 2-3 mg/L. Frazil ice was added to speed up the freezing process. Samples were incubated at -10 °C for 181 days.

Replicates of samples were sacrificed for microbial and chemical analyses at the start of the experiment (day 0), and after 7, 61, 120, or 181 days (see Table 1).

Sampling was conducted by separating ice from brine, using sterile ice screw. Brine was drained from the ice core for 10 min, and the ice core emptied of brine was then transferred to a sterile beaker. Ice temperature was measured with a thermometer with a sterile temperature probe. Sterile-filtered seawater (0.2 μm; Sterivex filters, Millipore) was added to the ice cores to avoid osmotic shock of microorganisms during melting. The ice was then slowly melted at 4 °C. Aliquots of both ice and brine were taken for measurement of salinity, microbial analyses, and oil compound quantification. An aliquot for measurement of sea ice salinity was taken separately before sterile seawater was added. Sample volumes for used in each analysis are presented in SI 3, Table S2.

The experiment was conducted with seven treatments: (i) samples with surface seawater added oil (SSW+oil); (ii) samples with subsurface seawater added oil (SW+oil); (iii) samples with subsurface seawater added oil and the sea ice diatom *Chaetoceros gelidus* (10^4 cells/mL; SW+oil+algae); (iv) biotic controls with surface seawater without oil (SSW); (v) biotic controls with subsurface seawater without oil (SW); (vi) abiotic controls with sterilized surface water added oil and $HgCl_2$ (abiotSSW+oil); and (vii) abiotic controls with sterilized subsurface

Table 1
Experimental setup of solid ice-brine systems. Samples were incubated in darkness at -10 °C. Samples were prepared with either surface seawater or subsurface sweater from 80 m below sea level (bsl).

Label	Seawater source	Treatment	Replicates at each sampling day				
			0	7	61	120	181
SSW+oil	Surface seawater	Oil	3	3	3	3	3
SSW	Surface seawater	SSW only	3	1	1	1	3
abiotSSW+oil	Surface seawater	Oil; $HgCl_2$	–	1	1	1	1
SW+oil+algae	Seawater, 80 m bsl	Oil; algae	1	1	3	3	3
SW+oil	Seawater, 80 m bsl	Oil	1	1	–	–	3
SW	Seawater, 80 m bsl	SW only	Only sampled at day 200, the start of the experiment with melted ice				
abiotSW+oil	Seawater, 80 m bsl	Oil; $HgCl_2$					

seawater added oil and $HgCl_2$ (100 mg/L; abiotSW+oil). An overview of all treatments is shown in Table 1.

Different seawater sources were included to determine if surface seawater, subsurface seawater and seawater added algae affected the distribution of oil compounds between ice and brine, since both particles and algae in sea ice have been suggested to influences on the microstructure and salt retention of sea ice (Krembs and Deming, 2008). The surface seawater was not filtered, retaining coarse particles and algae present in the source seawater. Controls without oil were included to account for the changes in the microbial communities in the absence of oil, while abiotic controls were added to account for abiotic losses of oil compounds.

2.2.2. Experiment with dispersed oil in melted ice and fresh seawater

To investigate biodegradation of weathered dispersed oil in melted ice, solid ice incubated with oil was melted and incubated at 4 °C.

Melted ice samples were prepared by adding oil stock dispersions to a mix of frazil ice and seawater to a final concentration of 2-3 mg/L (2 L borosilicate flasks; Schott AG). Samples were frozen and incubated at -10 °C. After 200 days of incubation, ice was melted slowly at 4 °C. Flasks with melted ice were filled completely (no headspace) with sterile-filtered seawater (0.22 μm; Sterivex filters, Millipore).

On the same day as the ice samples were melted, fresh oil stock dispersions of were prepared (as described above) and mixed with fresh seawater to a final concentration of 2-3 mg/L (in 2 L borosilicate flasks; Schott AG, no headspace). The dispersions in the fresh seawater were included to determine if the exposure to oil during freezing had a positive impact on the degradation, compared to seawater communities in water not exposed to oil contaminated sea ice.

Samples, both flasks with melted ice and fresh seawater, were mounted on slowly rotating carousels designed to keep oil dispersions in suspension (Brakstad et al., 2015a), and incubated at 4 °C for 64 days.

Replicate samples were sacrificed for microbial and chemical analyses at day 0 (ice melting day), and after 7, 15, 30 and 64 days of incubation after melting, as shown in Table 2. Aliquots of each sample were collected for measurement of dissolved oxygen, salinity, microbial analyses, and oil compound quantification. Sample volumes used in each analysis are presented in SI 3 Table S2.

The experiment was conducted with six treatments: (i) samples with melted sea ice and oil (MI+oil); (ii) biotic controls with melted ice without oil (MI); (iii) abiotic controls with sterilized melted ice and oil (abiotMI+oil); (iv) samples with fresh subsurface seawater added oil (freshSW+oil); (v); biotic controls with fresh subsurface seawater without oil (freshSW); and (vi) abiotic controls with fresh sterilized subsurface seawater added oil and $HgCl_2$ (abiotfreshSW+oil). An overview of all treatments is shown in Table 2.

2.3. Analysis and data processing

2.3.1. Chemical analyses

Samples for chemical analysis were acidified with HCl (pH < 2), stored at 4 °C, and solvent-solvent extracted with dichloromethane (DCM). Quantification of C_{10}-C_{36} total petroleum hydrocarbons (TPH) was performed by gas chromatography coupled to a flame ionization detector (GC-FID; Agilent 6890 N with 30 mDB1 column; Agilent Technologies, Inc. CA, USA) with *o*-Terphenyl (100 μg/mL) used as surrogate internal standard (SIS), and 5a-androstane (100 μg/mL) as recovery internal standard (RIS). Target analytes were quantified by gas chromatography coupled to a mass spectrometer (GC–MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector), operated in Selected Ion Monitoring [SIM] mode (Agilent Technologies, Inc. CA, USA). GC–MS analyses of targeted *n*C10-*n*C36 alkanes, 2-6 ring polycyclic aromatic hydrocarbons (PAH; $n = 49$; listed in SI 4, Table S3) and the biomarker 17α(H),21β(H)-hopane (30ab hopane) were performed as previously described (Brakstad et al., 2014; Brakstad et al., 2015a).

2.3.2. Calculations of degradation rates and statistical analyses

Biotransformation rate coefficients and half-lives were determined as first-order rates by non-linear regression analyses (Brakstad et al., 2015a), with determination of lag-phases included, using the option "plateau followed by one-phase decay" in GraphPad Prism *vs.* 7.02 (GraphPad Software Inc., La Jolla, CA, USA). Statistical analysis of the degradation of hydrocarbons was performed using built-in functions in GraphPad Prism 7.02.

2.3.3. Microbial community analysis

Samples for microbial community analyses were filtered through 47 mm polyethersulphone membranes (pore size 0.22 μm; Millipore) and stored in sterile tubes (-18 °C) until DNA extraction. DNA was extracted from frozen filters using ZymoBiomics MagBead DNA kit (Zymo Research, CA, USA) and further purified using KingFisher Duo Prime (ThermoFisher Scientific), as described in the Supplementary Information (SI 5). Assessment of DNA qualities and yields, as well as the preparation of libraries for sequencing of the 16S rRNA gene amplicons, were performed as recently described (Lofthus et al., 2020). Amplicons covering the V3 and V4 region of the 16S rRNA gene were generated using the primers Ill338F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNCCTACGGGWGGCAGCAG-3') and Ill805R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNGACTACNVGGGTATCTAAKCC-3') (Sigma Life Science, TX, USA). The pooled and concentrated amplicon libraries were sequenced on a MiSeq lane with V3 reagents employing 300 bp paired end reads (illumina, Inc.), at the Norwegian Sequencing Centre (NSC, Oslo, Norway). Sequence data were analysed using the USEARCH pipeline (version 11). For details, see SI 5. Operational Taxonomic Units (OTUs) were classified using the USEARCH command Sintax script with a confidence threshold of 0.8 and the RDP reference data set (v16). The OTU table was rarefied to 12,000 reads per sample, and this rarefied OTU table was used for further analyses. The USEARCH command *Sintax_summary* was used to generate taxa summary tables, and the USEARCH command *Alpha_div* was used to calculate alpha diversity indices (Chao1, Equilibrity, Richness and Shannon index). The Bray-Curtis and Jaccard similarity metrics were computed using the USEARCH command *beta_div*, computing beta diversity metrics from the filtered OUT table. Further analysis of OTU tables, alpha and beta diversity were performed using R-studio v. 1.1.463 (2016) and R software version 3.5.3 (see more details in SI 5). OTUs that were not classified in the pipeline were identified using the

Table 2
Experimental setup of carousel experiment with melted ice and fresh seawater. Samples were incubated in darkness at 4 °C. Both melted ice and fresh seawater were made with subsurface seawater from 80 m below sea level (bsl). The sampling days are after freezing (AF) and after melting (AM) are both included.

Label	Seawater source	Treatment	Replicates at each sampling day				
			200 (AF)	207	215	230	264
			0 (AM)	7	15	30	64
MI+oil	Frozen and melted seawater, 80 m bsl	Oil	3	3	3	3	3
MI	Frozen and melted seawater, 80 m bsl	SW only	1	–	1	1	1
abiotMI+oil	Frozen and melted seawater, 80 m bsl	Oil; $HgCl_2$	1	–	1	1	1
freshSW+oil	Seawater, 80 m bsl	Oil	3	1	2	3	3
freshSW	Seawater, 80 m bsl	SW only	1	–	–	–	1
abiot freshSW+oil	Seawater, 80 m bsl	Oil; $HgCl_2$	–	–	–	1	1

Silva ACT search tool (Pruesse et al., 2012).

2.3.4. Microbial enumeration

Total cell concentrations were determined by epifluorescence microscopy (1250 times magnification) of samples stained with the nucleic acid stain 4',6-diamidino-2-phenylindol (DAPI) (Porter and Feig, 1980). Most probable numbers (MPN) of viable heterotrophic (HM) and oil degrading microorganisms (ODM) were determined in 24-well sterile culture well plates (Brown and Braddock, 1990). MPN of HM were determined in Difco Marine Broth 2216 (Becton, Dickinson and Company, NJ, USA), and ODM in Difco Bushnell-Haas Broth (Becton, Dickinson and Company) supplemented with 30 g/L NaCl and 0.1% (vol/vol) of 250 °C+ Troll B oil. The plates were incubated at 4 °C for a total of 14 and 35 days for HM and ODM, respectively, and checked for positive wells after days 3, 7, 14, 21, 28 and 35.

2.3.5. Other analyses

Oil droplet concentrations and size distributions in stock dispersions were determined by a Coulter Counter (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, USA), fitted with a 100 µm aperture for measurement of droplets within a diameter range of 2–60 µm. Salinity measurements were determined refractometrically (S/Mill-E refractometer; ATAGO CO., LTD., Tokyo, Japan). Dissolved oxygen (DO) was determined using a DO meter connected to a BOD probe (YSI, Yellow Springs, OH, USA).

3. Results

3.1. Physical parameters

3.1.1. Solid ice

In the experiment with dispersed weathered oil in solid ice, oil was mixed into cold seawater and allowed to freeze at an air temperature averaging -10.7 °C (max/min air temperatures of -9.0 °C and -11.6 °C; SI 6, Fig. S3) over 181 days. The ice temperature was constant and equal to the air temperature (-10.8 °C). During the first 7 days of the experiment, brine was rejected from the growing sea ice, forming a fraction of high salt concentration liquid in the bottom of the beakers. The average volume of brine was 355±39 ml, corresponding to 19.7% of the total volume in the ice-brine system (SI 6, Table S4). The amount of brine was not significantly different between the seawater sources (SSW, SW and SW+algae; two-way ANOVA, $p > 0.1$). Desalination of ice resulted in measured salinities of 93-103 ppt in the brine fraction at day 181, with corresponding ice salinities of 5-7 ppt (Table S4).

3.1.2. Melted ice and fresh seawater

The experiment with dispersed weathered oil in melted ice and fresh seawater was performed with an average water temperature of 4.8±0.4 °C during the incubation period of 64 days. The melted ice (MI+oil) had a bulk salinity of 33.6±1.6 ppt, after mixing of ice and brine. Fresh seawater (freshSW+oil) had an average salinity of 36.3±0.5 ppt.

Dissolved oxygen (DO) in MI+oil was 10.3±0.2 mg/L at day 0 after melting and decreased to an average concentration of 7.3±0.1 mg/L at day 64. Biotic controls without oil (MI) had a similar decrease in DO from 10.1 mg/L at day 0 to 6.8 mg/L at day 64. Sterilized controls (abiotMI+oil) also showed some depletion of oxygen, from 9.2 mg/L to 7.8 mg/L during the experimental period. In freshSW+oil the average DO was 10.7±0.0 mg/L at day 0, which decreased to 6.9±0.1 mg/L at the end of the experiment. DO in sterilized (abiotfreshSW+oil) and biotic controls (freshSW) also showed some depletion, having a DO concentration of 8.55 and 6.88 mg/L, respectively, after day 64.

3.2. Oil compound distribution in ice and brine

Since sea ice can be considered impermeable in this experiment (temperature less than -5 °C, and bulk salinity of 5), transport of oil compounds to the brine must have occurred during the freezing process (before day 7). Oil compound distributions in ice and brine at the end of the experiment in ice (181 days) varied with their solubility in the seawater (Fig. 1A-D).

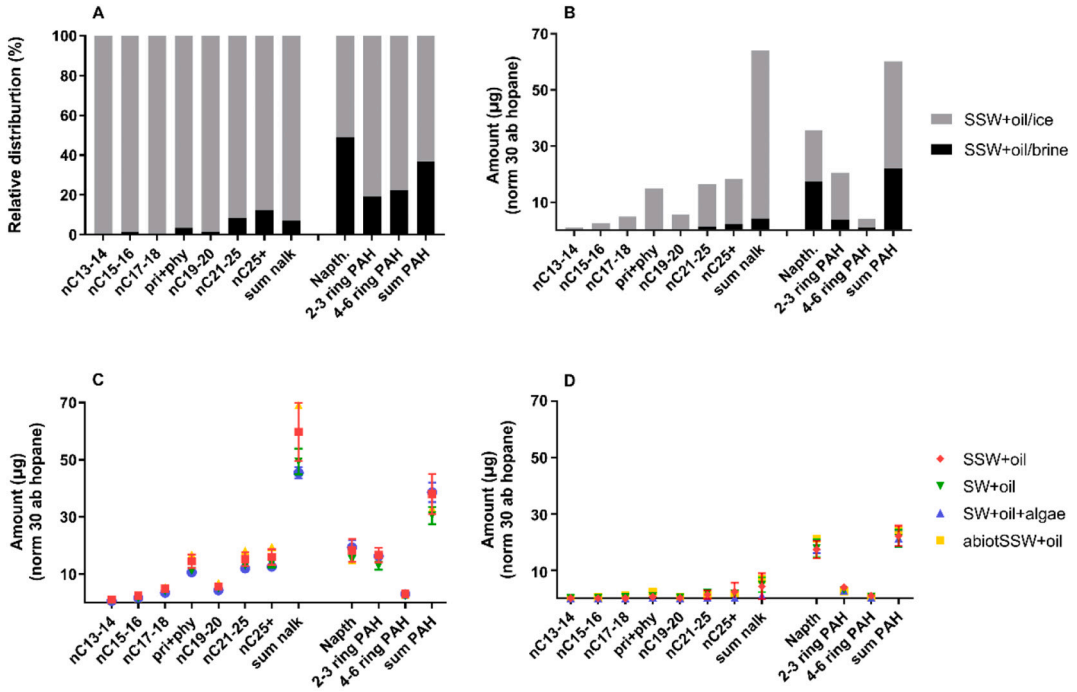

Fig. 1. The relative distribution in percent (A) and the total amount in µg (B) of oil compounds in ice and brine in surface seawater with oil (SSW+oil) at day 181. Fig. C and D shows the oil compound distribution into ice (C) and brine (D) between the different seawater sources; surface seawater added oil (SSW+oil); subsurface seawater added oil (SW+oil); subsurface seawater added algae and oil (SW+oil+algae); and abiotic surface seawater control added oil (abiotSSW+oil) at day 181 of the experiment.

Different seawater sources were included in the experimental setup to investigate if the presence of particles and algae affected the distribution of oil compounds into brine. After 6 months of incubation, no significant differences (two-way ANOVA, $P > 0.05$) were measured between the relative oil compound distributions into brine from the different seawater sources (SSW+oil, SW+oil and SW+oil+algae). A comparison of the amounts of oil in ice and brine in each treatment is shown in Fig. 1 C—D.

The total amount of oil (TPH) in the ice systems (sum of oil in ice and brine) showed an average amount of 3.6±0.9 mg, corresponding to a concentration of 2.1±0.5 mg/L. Since there was no effect of seawater source on the distribution of oil into ice and brine, samples from the different treatments are treated as replicates at each sampling day when calculating averages. Most of the oil (average 83.3±7.9%) was trapped in the ice lattice during the experiment. *n*-Alkanes were associated with the oil fraction, with 92.9±6.6% (58.1±14 μg) of these compounds remaining in ice at day 181. Larger fractions of the more water-soluble aromatic hydrocarbons were distributed into the brine (40.9±8.0% of the average sum of PAHs, corresponding to 52.0±9.2 μg). The distributions of PAHs in brine were mainly associated with naphthalenes, being the most water-soluble compounds of the measured PAHs. While 48.8±1.1% of the naphthalenes were present in the brine at day 181, 19.1±1.6% and 24±4.6%, of 2-3 ring PAHs and 4-6 ring PAHs, respectively, were measured in the brine.

The total amount (sum of ice and brine) of the recalcitrant biomarker 30ab hopane was used to normalise the amount of oil in each sample. The total amounts of hopane were relatively stable during the experiment (SI7, Fig. S4), and the biomarker was mainly detected in the ice fraction (84.4±12.1%) being associated with the oil, in accordance with the low water-solubility of the compound.

3.3. Oil biodegradation

3.3.1. Fate of oil and oil compounds in solid ice

During the 181 days of incubation at -10 °C, no oil was lost by biodegradation in any of the treatments (SSW+oil, SW+oil or SW+oil+algae). The amounts of TPH and target oil compounds (*n*-alkanes, naphthalenes, 2-3 ring PAHs and 4-6 ring PAHs) were negligible during the incubation period (Fig. 2 and SI 7, Fig. S5). The amount of *n*-alkanes in SSW+oil were reduced after 181 days compared to the sterilized controls (abiotSSW+oil; Fig. S5). However, the differences were not significant (two-way ANOVA, $P > 0.5$), and the loss of *n*-alkanes can therefore not be considered being caused by biodegradation.

3.3.2. Oil depletion in melted ice and fresh seawater

After 64 days of incubation at 4 °C, oil was degraded in both melted ice (MI+oil) and fresh seawater (freshSW+oil). Degradation of TPH and targeted oil compounds was determined after normalization against 30ab hopane. TPH were degraded to an extent of 13.9±2.0% in MI+oil and 67.1±2.9% in freshSW+oil at the end of the experiment (as a percentage of sterilized controls). Depletion of TPH was also observed in abiotMI+oil, although at insignificant levels compared to the TPH depletion in MI+oil. The TPH depletion determined in MI+oil could therefore be considered to be a result of biodegradation (SI 8, Fig. S6).

Biotransformation of *n*-alkanes in MI+oil was observed after 30 days, whereas substantial degradation of *n*-alkanes occurred after 15 days in freshSW+oil, as shown in Fig. 3. Although, initial degradation was faster in freshSW+oil, *n*-alkanes were degraded to a similar extent in both treatments at the end of the incubation period of 64 days. *n*-Alkanes were degraded to an extent of 88.0±3.9% in MI+oil and 98.1±0.4% in freshSW+oil. Calculations of first-order biotransformation kinetics of the sum of *n*-alkanes resulted in half-lives (as sum of lag-periods and half-lives) of 34.7 and 15.3 days in MI+oil and freshSW+oil, respectively (SI 8, Table S5). The typical pattern of decreased degradation with increasing *n*-alkane chain length was observed (Fig. 3), which was also reflected by increasing half-life in both treatments. Delayed onset of biotransformation of the isoprenoids pristane and phytane compared to their *n*-alkane analogues (*n*C17 and *n*C18) was also observed (Fig. 3).

Differences in biotransformation between treatments (MI+oil and freshSW+oil) were more extensive for the sum of PAHs (naphthalenes, 2-3 ring PAHs and 4-6 ring PAHs) than *n*-alkanes. PAH biotransformation was 54.6±3.3% and 95.9±0.6% in MI+oil and freshSW+oil, respectively, after 64 days of incubation at 4 °C. Naphthalenes and unsubstituted 2-3 ring PAHs were completely biotransformed between day 15 and 30 in freshSW+oil, while biotransformation of target PAHs was not measured before day 30 in MI+oil (Fig. 4). Half-lives (sum of lag-period and half-life) for the sum of PAH were estimated to be 47.3 days in MI+oil and 17.9 days in freshSW+oil. Biotransformation rate coefficients of the different compound groups decreased with increasing molecular weight in both treatments, with naphthalenes having shorter half-lives than 2-to 3 ring and 4-to 6 ring PAHs (Table S5). Biotransformation of PAHs was also related to the degree of alkyl substitution. Non-alkylated PAHs were biotransformed faster than their alkyl-substituted homologues, which were transformed at slower rates with increasing degrees of alkyl substitution (Fig. 4).

In summary, the biotransformation patterns of *n*-alkanes and PAHs were similar in both treatments. However, the extent of degradation was significantly less in MI+oil than freshSW+oil (two-way ANOVA, $P < 0.01$) for both the sum of *n*-alkanes and PAHs, as well as TPH.

3.4. Microbial community compositions and dynamics

3.4.1. Microbial communities in oil polluted solid ice

After 181 days of incubation at -10 °C, no changes in the microbial communities were observed in response to oil in solid ice. The bacterial community compositions were assessed separately in brine and ice from each sample. However, community compositions in ice and brine were not significantly different from each other (Permanova; $P > 0.05$) and

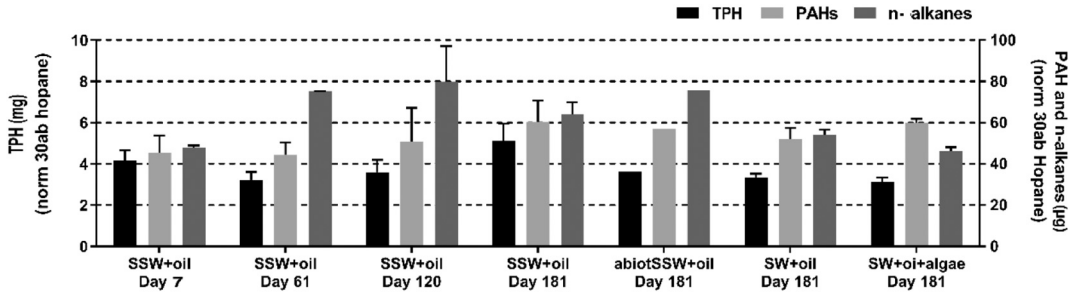

Fig. 2. Normalized amounts of TPH, PAHs and *n*-alkanes in the ice-brine system during the 181 days of incubation.

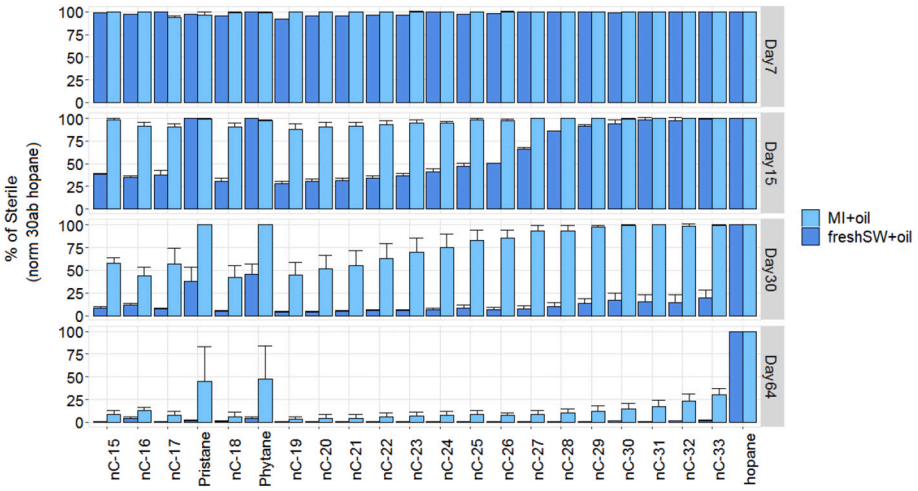

Fig. 3. Degradation of *n*-alkanes in weathered oil dispersed in seawater from melted sea ice (MI+oil) and fresh seawater (freshSW+oil) that had not been frozen. Loss of target compoundsare presented as percentage of abiotic controls.

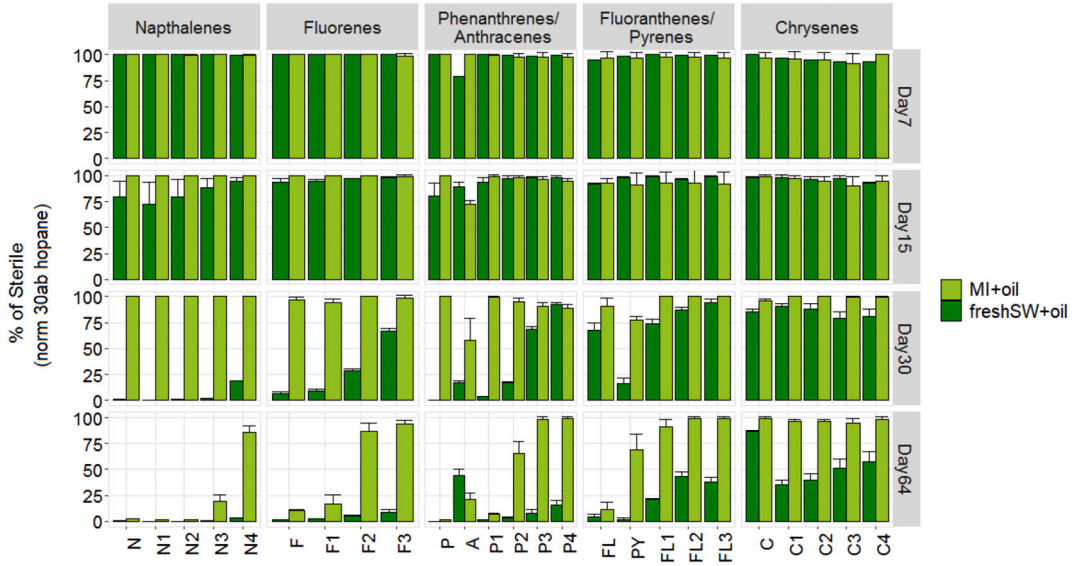

Fig. 4. Degradation of targeted PAHs in weathered oil dispersed in seawater from melted sea ice (MI+oil) and fresh seawater (freshSW+oil) that had not been frozen. Loss of target compounds are presented as percentage of abiotic controls.

are therefore treated as replicates at each sampling day. PCoA plots based on Bray-Curtis dissimilarities (Fig. 5) showed that the microbial community structures did not change over time in response to the presence of oil in any of the seawater sources (Permanova; $P > 0.05$). However, significant differences were observed between the microbial community compositions in the different seawater sources, where SSW+oil, SW+oil and SW+oil+algae clustered differently in the PCoA plot (Fig. S7; Permanova; $P < 0.01$).

The relative abundances of bacterial classes remained similar during the 181 days of incubation at -10 °C (SI 9, Fig. S7). At day 181 the microbial communities in ice made from surface seawater, both with and without oil (SSW+oil and SSW) were dominated by the bacterial classes Gammaproteobacteria (41.7±9.1% and 33.9±7.2%), Flavobacteriia (23.9±6.2% and 25.4±6.3%) and Alphaproteobacteria (22.0±5.3% and 27.4±5.5%), while higher abundances of Alphaproteobacteria (40.1±9.0%) than Gammaproteobacteria (27.9±8.3%) and Flavobacteriia (10.9±4.7) were determined in SW+oil samples (Fig. S7). Different from the other treatments, the relative abundance of Gammaproteobacteria in SW+oil+algae changed from day 0 to day 7, from 64.7% to 82.4±14.5%. Examination of the OTU table revealed that the predominance of Gammaproteobacteria was caused mainly by one OTU, classified as the genus *Glaciecola.* This change was also observed in the alpha-diversity metrics, with a decline in both OTU richness and evenness between days 0 and 7 (SI 9, Fig. S8).

3.4.2. *Microbial communities in melted sea ice and fresh seawater*

3.4.2.1. Microbial responses to ice melting and presence of oil. After 200 days of incubation at -10 °C (corresponding to day 0 at 4 °C), the total concentration of microbial cells in MI+oil was $7.7\pm1.4 \times 10^4$ cells/mL, as determined by epifluorescence microscopy. This concentration was comparable to the cell concentrations in freshSW+oil ($5.5\pm1.2 \times 10^4$ cells/mL). The cell concentrations increased during the 64 days of incubation at 4 °C, being $1.2\pm0.1 \times 10^5$ and $9.2\pm3.3 \times 10^4$ cells/mL in MI+oil and freshSW+oil, respectively (SI 10, Fig. S9A). An increase was also observed in the most probable number (MPN) of viable

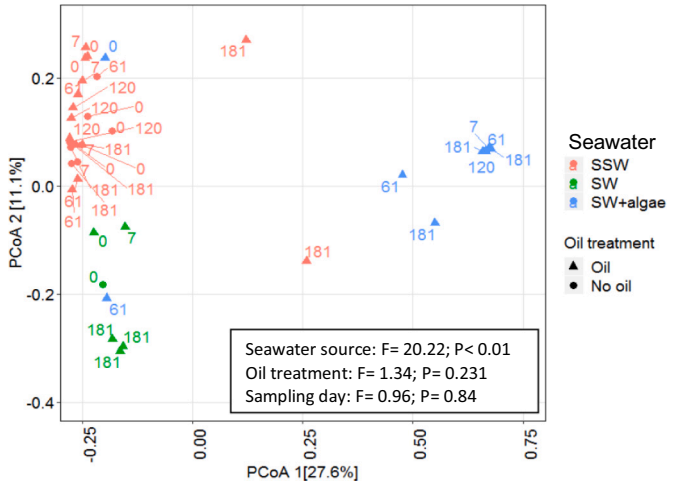

Fig. 5. Changes within the microbial community structures in solid ice over time, based on Bray-Curtis dissimilarity computed from the rarefied and normalized OTU table. Principal component (PCoA) 1 explains 27.6% of the variance between samples, whereas PCoA 2 accounts for 11.1%. Permanova was performed using the 'adonis' function in the R package 'vegan'. Surface seawater (SSW); seawater (SW); seawater added algae (SW+algae).

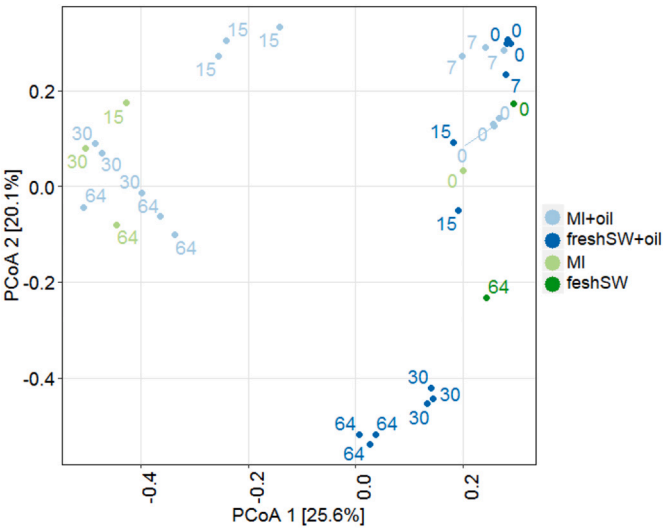

Fig. 6. Changes of the microbial community structures over time in melted ice and fresh seawater, with and without oil, based on Bray-Curtis dissimilarity. Principal component (PCoA) 1 explains 25.6% of the variance between samples, whereas PCoA 2 accounts for 20.1%. Melted ice added oil (MI+oil); melted ice without oil (MI); fresh seawater added oil (freshSW+oil); fresh sweater without oil (freshSW).

heterotrophic microbes in both MI+oil and freshSW+oil (SI 10, Fig. S9B). MPN of viable heterotropic microbes was $1.61\pm0.7 \times 10^1$ cells/mL for MI+oil at day 0, while the coresponding concentration was 4.6×10^2 cells/mL in fresh seawater. MPN reached a concentration of $6.3\pm4.8 \times 10^5$ MPN/mL in MI+oil (day 64), which was higher than the total cell concentrations. MPN in freshSW+oil reached a concentration of $5.7\pm5.4 \times 10^5$ MPN/mL on day 30. MPN concentrations of oil-degrading microbes remained low (<10 MPN/mL) in all treatments (MI+oil, MI and freshSW+oil; SI 10, Fig. S9C).

After approximately 40 days of incubation at 4 °C, formations of fast sinking aggregates were observed in MI+oil, and visualization by fluorescence microscopy showed aggregates with attached microbes (SI 10, Fig. S10). In freshSW+oil, only a few loosely filamentous aggregates were observed on the last day of sampling, with different appearances than those observed in MI+oil, which were small, numerous (SI 10, Fig. S11) and had high sinking velocities.

Although there were no observed changes in total cell concentrations, initial loss of OTU richness was observed in both melted ice (MI+oil and Ml) and fresh seawater (freshSW+oil and freshSW) from day 0 to day 7 (SI 11, Fig. S12). OTU richness decreased with time, from an average of 325 to less than 100 OTUs in MI+oil and MI samples, and from 345 to 150 in freshSW+oil, at day 30 (Fig. S12). FreshSW without oil did only show a slight decrease in OTU richness compared to the other treatments, to about 300 OTUs at day 30. Shannon's diversity analyses also showed an initial decrease, from day 0 to day 15, in both melted ice (MI+oil and MI) and fresh seawater (freshSW+oil). For the MI+oil and MI samples, Shannon's diversity and evenness increased at the end of the experiment (between day 30 and 64), without a corresponding increase in richness. In freshSW+oil, both richness, evenness and Shannon diversity increased between 30 and 64 days of incubation at 4 °C.

3.4.2.2. Microbial community dynamics in melted ice and fresh seawater. The microbial communities in melted ice (MI+oil and MI) and fresh seawater (freshSW+oil and freshSW) appeared to be shaped by both time and treatment (presence of oil, and whether seawater had been frozen or not prior to incubation at 4 °C), as indicated by PCoA plots based on Bray-Curtis dissimilarities (Fig. 6). PCoA showed that while the communities from all treatments clustered together at day 0, differences between the communities in melted ice (MI+oil and MI) and fresh seawater (freshSW+oil and freshSW) became evident with time (Fig. 6). Permanova analysis showed significant differences between microbial communities at day 0 and day 64 in both MI+oil ($R2 = 0.60$, $p < 0.001$) and freshSW+oil ($R2 = 0.73$, $p < 0.001$). Even though the microbial communities in MI+oil and freshSW+oil appeared to become more dissimilar by time (Fig. 6), the differences between the communities were not statistically significant (Permanova, $R2 = 0.24$, $p = 0.61$).

During the experimental period of 64 days at 4 °C, changes within microbial communities in MI+oil and MI followed the same trajectory in the PCoA plot (Fig. 6), indicating that the presence of oil was not a significant factor in determining the difference in the beta-diversity of the microbial communities in melted ice. Even though the Bray-Curtis dissimilarities between the communities in the two treatments were not significant, differences in the relative abundances of some of the most abundant OTUs were observed. Examination of the community compositions in MI+oil showed that three OTUs, classified as *Piscirickettsiaceae* (OTU9), *Paraglaciecola* (OTU19) and *Sulfitobacter* (OTU21) each increased 10-fold in relative abundance from day 0 to 15 day. The same OTUs were present in MI without oil, but in lower abundance (SI 11, Fig. S13 and Fig. S14). SIMPER analysis of the entire OTU table showed that OTU3, OTU9, OTU21 and OTU19 accounted for 40% of the difference between the microbial communities in melted ice with and without oil.

3.4.2.3. Microbial community compositions in melted ice and fresh seawater. The initial microbial communities in MI+oil were predominated by Gammaproteobacteria, having an average relative abundance of 52.6±6.3%, whereas Alphaproteobacteria (16.8±0.9%), Actinobacteria (14.5±1.7%) and Flavobacteriia (1.2±0.3%) were present to a lesser extent. FreshSW+oil was also dominated by Gammaproteobacteria 55.4±9.5%, but with a higher relative abundance of Actinobacteria (20.0±5.3%) compared to the melted ice, and a lower relative abundance of the Alphaproteobacteria (5.0±0.9%) and Flavobacteriia (0.3±0.0%). The changes in the microbial community structures at class level are shown in SI11, Fig. S15. The relative abundances of Gammaproteobacteria increased in both MI+oil and freshSW+oil during the experimental period. Gammaproteobacteria accounted for over 80% relative sequence abundances at day 15 in MI+oil, and 75% in freshSW+oil. The same predominances of Gammaproteobacteria were not determined in the controls without oil (MI and freshSW). After 30 days of incubation at 4 °C, the evenness of the microbial communities increased in both MI+oil

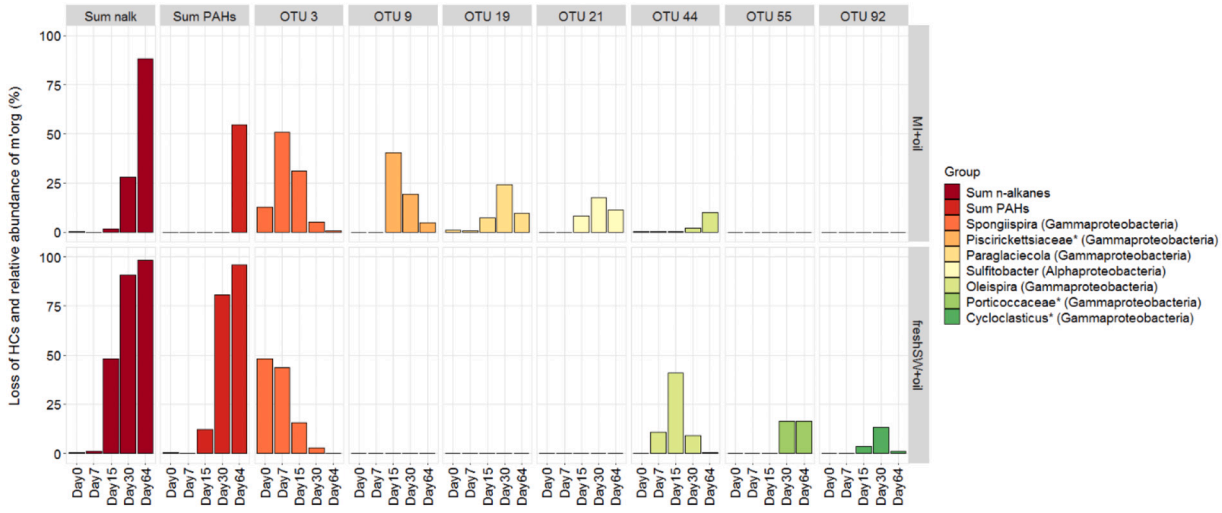

Fig. 7. Comparison of biotransformation (%) of the sum of *n*-alkanes and PAHs, and the relative abundance of dominant OTUs in melted ice with oil (MI+oil) and fresh seawater with oil (freshSW+oil). *Indicates that taxa were not classified and are subsequently identified through the Silva ACT search tool.

and freshSW+oil (Fig. S12), coinciding with an increase in relative abundance of Alphaproteobacteria in MI+oil, and Alphaproteobacteria and Flavobacteriia in freshSW+oil.

Classification was poor at lower taxonomic levels, with high abundances of unclassified OTUs. This was particularly evident in freshSW+oil, where 90% of the OTUs could not be classified at genus levels. Examination of the community composition at OTU level (SI 11, Fig. S13 and Fig. S14) showed that MI+oil and freshSW+oil differed in the relative abundances of specific OTUs. The increases in the relative abundances of Gammaproteobacteria in MI+oil samples were mainly caused by the three OTUs, *Spongiispira* (OTU3), *Piscirickettsiaceae* (OTU9) and *Paraglaciecola* (OTU19). These OTUs accounted together for relative abundances of 51.3% after 7 days, 78.9% after 15 days, and 49.0% after 30 days of incubation. Alphaproteobacteria, were mainly represented by *Sulfitobacter* (OTU21) which increased in abundance from 8% at day 15 to 21-22% at days 30 and 64 (Fig. 7). In freshSW+oil, *Oleispira* (OTU44), *Porticoccaceae* (OTU55) and *Cycloclasticus* (OTU92), as well as *Spongiispira* (OTU3) were predominant, with combined relative abundances of 54.4% at day 7, 60.0% at day 15 and 41.4% at day 30. At day 30, members of the families *Rhodobacteraceae* and *Flavobacteriaceae* also increased in abundances (10-13% and 13%, respectively; Fig. S13).

3.4.2.4. Oil depletion and the relation to changes in the microbial community. Biotransformation of *n*-alkanes and PAHs were compared to the most abundant OTUs in both MI+oil and freshSW+oil (Fig. 7).

In MI+oil, biotransformation of *n*-alkanes occurred mainly between day 15 and 64 of incubation at 4 °C. *n*-alkanes shorter than *n*C25 were biotransformed between day 15 and 30, while *n*C25–*n*C33 alkanes were not significantly transformed until between day 30 and 64 (two-way ANOVA; $p < 0.001$). The biotransformation coincided with increased abundances of mainly *Piscirickettsiaceae* (OTU9; day 15-30; 41 - 20%) in MI+oil, but also with the later emergence of *Paraglaciecola* (OTU19) and *Sulfitobacter* (OTU21) having peak abundances of 18% and 24% (day 30), respectively (Fig. 7).

Biotransformation of PAHs was not evident in MI+oil, before the last sampling at day 64, when non-alkylated and C1-alkylated naphthalenes and 2-3 ring PAHs were almost completely transformed. Biotransformation of PAHs could not be linked to increased abundances of any specific OTUs in MI+oil samples. However, the relative abundance of the family *Rhodobacteraceae* (21-22%) increased at the end of the experiment (Fig. S13).

Biotransformation of oil compounds was faster in freshSW+oil than in MI+oil, and the microbial communities in these treatments were predominated by different OTUs. During the period of extensive loss of *n*-alkanes (between days 7 and 30) in freshSW+oil, *Oleispira* (OTU44) increased in relative abundances, from less than 1% at day 0 to an average abundance of 41% at day 15, followed by a decrease to 10% at day 30 (Fig. 7 and Fig. S13).

Biotransformation of naphthalenes in freshSW+oil was almost complete between days 15 and 30, in addition to the 2-3 ring PAHs and their C1- and C2-alkyl homologues (Fig. 4), with later onset of biotransformation of C3- and C4- alkylated PAH and 4-6 ring PAHs. Biotransformation coincided with increased relative abundances of *Porticoccaceae* (OTU55; 16% at both days 30 and 64), and *Cycloclasticus* (OTU92; peak abundance of 13% at day 30). PAHs transformation could also be related to a general increases of the families *Rhodobacteraceae* (10-13%) and *Flavobacteriaceae* (13%) towards the end of the experiment (Fig. S13).

Spongiispira (OTU3) was abundant in both oil treatments (MI+oil and freshSW+oil) early in the experiment. The average relative abundance of OTU3 was 48.1±10% in freshSW+oil at the start of the experiment, with declining abundance to 31.4±8% at day 15. In MI+oil the average relative abundance of OTU3 was 12.8±1% at day 0, which increased to 50.8±11.9% at day 7. At day 15 the relative abundance was 21.0±3.9% in MI+oil, with a similar abundance in the no oil control (MI; 14.0%).

SIMPER analyses were performed to identify the OTUs responsible for the differences between the microbial communities in MI+oil and freshSW+oil. SIMPER analysis showed that the same OTUs that increased in abundances in relation to decreases in concentration of oil compounds, caused the main differences (40.2%) between the communities in MI+oil and freshSW+oil treatments (OTU3, OTU9, OTU44, OTU19, OTU21, OTU18 and OTU55; Table S6).

4. Discussion

In this study, two experiments were performed to assess biodegradation of weathered dispersed oil and the changes within the microbial communities in response to the oil degradation. Dispersed weathered oil was first frozen into sea ice with a temperature of -10 °C. The ice was then melted, and biodegradation of oil compounds was assessed in melted sea ice, as well as in fresh seawater at a temperature of 4 °C. A low oil concentration was used in all oil treatments, 2-3 mg/L, which is considered to be environmentally relevant when studying biodegadation of dispersed oil (Lee et al., 2013).

4.1. Biodegradation of oil in solid ice

After ice formation, oil droplets were entrained mainly in the solid ice matrix, while the more soluble oil compounds were measured in the brine fractions. This was in agreement with field studies of oil in ice, showing that mainly the water-soluble fraction (WAF) of oil dissolved into brine (Faksness and Brandvik, 2008a). Faksness and Brandvik (2008a) observed that naphthalene and C1-naphthalene migrated downward through the brine channels, whereas *n*-alkanes and PAHs with low water solubility were associated with bulk oil that were trapped in the upper part of the ice. At temperatures lower than -5 °C, and bulk salinity around 5 ppt, the porosity of sea ice is 5% which does not allow for interconnection of brine channels (Golden et al., 1998; Pringle et al., 2009). As the temperature of ice in this experiment was below -10 °C, the ice is considered impermeable. Transport of oil compounds into the brine therefore most likely occurred during the freezing process, before the ice became impermeable.

Over time, there was no observed effect of seawater source (surface seawater, subsurface seawater and seawater added algse) on the distribution of oil compounds into brine, brine volume, or brine salinity. The volume of brine drained from ice in this experiment, was within the volumes ranging from 5 to 30%, as previously reported for sea ice (Krembs et al., 2001). The presence of algae seemed to generate an immediate higher brine volume during ice formation (at sampling after 7 days incubation) compared to the other treatments. However, the relative brine volumes were similar in all treatments later in the experiment. The temperature of -10 °C may have been too low to allow for interconnected brine channels over the 181 days of incubation, even with the presence of EPS producing algae which are hypothesized to increase brine volume and the connection between brine pockets in sea ice (Krembs et al., 2011). The porosity of sea ice, and the distribution of oil compounds into brine may have ecological implications, as slow releases of low concentrations of oil compounds to seawater from porous ice may expose ice fauna to toxic compounds over a prolonged time period (Faksness and Brandvik, 2008a; Faksness and Brandvik, 2008b). Less porous ice with closed brine channels may, however, release oil compounds over short periods during brine channel formation in the spring, with elevated exposure to the ice-associated organisms (Toxværd et al., 2018).

Chemical analyses showed that weathered dispersed oil were not biodegraded when frozen into sea ice (SSW+oil, SW+oil and SW+oil+algae), confirming previous data from field experimets with sea ice (Gerdes et al., 2005; Brakstad et al., 2008; Boccadoro et al., 2018; Vergeynst et al., 2019). However, experiments with chemically dispersed diesel in artificial brines suggested that both target *n*-alkanes and PAHs were biodegraded at -6 °C, although to a lesser degree compared to biotransformation of the same oil at -2 °C (Dang et al., 2020). Diesel has a lower pour point than the weathered crude oil used in this study, which affects bioavailability, and thus biodegradation of oil compounds at low temperature.

Contrary to expectations, no changes in the bacterial community structures were determined as a response to dispersed oil in solid ice in this experiment. Changes during the 181 days of incubation at -10 °C were insignificant both looking at community composition and beta diversity analysis based on Bray-Curtis dissimilarities. The lack of response in the bacterial communities differed to some extent from field data, in which microbial communities within oil polluted ice responded to the presence of oil, although hydrocarbon degradation was insignificant (Brakstad et al., 2008, Boccadoro et al., 2018, Vergeynst et al., 2019). It is important to emphasise that DNA based analysis may reflect the presence of both dormant and dead cells, as well as viable bacteria (Collins et al., 2010), and minute changes in community composition may therefore be masked by the abundance of the non-viable cells.

In this study, the microbial communities in the oil-polluted sea ice resembled the communities of non-polluted ice, emphasising that oil as a substrate was not bioavailable to the bacteria at low temperature, and selection for oil degrading communities did therefore not occur. Pristine first year sea ice is typically dominated by the bacterial classes Flavobacteriia, Gammaproteobacteria and Alphaproteobacteria (Boetius et al., 2015). Whereas previous experiments with oil polluted ice have shown increases in the relative abundances of oil degrading Gammaproteobacteria (Brakstad et al., 2008, Boccadoro et al., 2018, Vergeynst et al., 2019). Although the presence of oil in ice did not select for specific oil-degrading bacterial communities in our study, taxa present in the ice such as the genera *Colwellia, Glaciecola, Polaribacter* and the family *Rhodobacteriaceae,* have previously been enriched in oil contaminated ice (Brakstad et al., 2008, Boccadoro et al., 2018, Vergeynst et al., 2019). These taxa are also among the most common genera observed in non-polluted ice (Boetius et al., 2015), and it is possible that they have capacities to use dissolved oil compounds as substrates. Biogenic alkane production by cyanobacteria and algae (Gelpi et al., 1970) is hypothesized to be sufficient to sustain populations of alkane-degrading bacteria (Lea-Smith et al., 2015) and it is likely that several of the genera known to be enriched in ice possess oil degrading genes.

4.2. Biodegradation of oil in melted ice and fresh seawater

Since biodegradation of oil in solid sea ice was shown to be limited both in this and previous studies (Brakstad et al., 2008, Boccadoro et al., 2018, Vergeynst et al., 2019), the fate of the oil will be determined when the ice melts. The capacities of microbes to degrade oil when released from sea ice have, to our knowledge, not been investigated. After 200 days of incubation at -10 °C, oil compounds in melted ice (MI+oil) were biotransformed at an incubation temperature of 4 °C, although at slower rates compared to samples in which dispersed oil was incubated in fresh seawater (freshSW+oil). Both onset of degradation (lag-time) and biotransformation rates were slower in MI+oil than in freshSW+oil. Degradation of oil compounds in freshSW+oil was comparable to other relevant degradation experiments with dispersed oil in cold seawater from a Norwegian fjord (Brakstad et al., 2015a; Brakstad et al., 2018a; Brakstad et al., 2018b; Lofthus et al., 2018). While biotransformation rates were different between the two treatments, the degradation pattern of *n*-alkanes and PAHs was similar in both MI+oil and freshSW+oil. Biotransformation of *n*-alkanes occurred with decreasing rates in relation to increasing chain lengths and branching, while PAHs were transformed with decreasing rates increased degree of alkyl substitution and molecular weights, in agreement with several other biodegradation experiments in cold seawater (Prince et al., 2013; Brakstad et al., 2015a; Brakstad et al., 2018a; Dang et al., 2020). In our study, the total amount of oil was depleted by 67% in fresh seawater (freshSW+oil) and by 14% in melted ice (MI + oil) at the end of the experiment (64 days). A similar pattern was observed in an experiment where the bacterial communities from pristine melted sea ice degraded 40% of Arabian Light oil, compared the sub-ice seawater community that degraded 94% of the bulk oil (Garneau et al., 2016).

The differences in the extent of degradation and biotransformation rates between the MI+oil and freshSW+oil showed that freezing and subsequent melting altered the microbial communities in a way that reduced degradation. However, the results also showed that bacteria capable of degrading oil compounds survived in the sea ice.

At the start of the experiment at the carousels at 4 °C, the microbial communities in the melted ice (MI and MI+oil) and in fresh subsurface seawater (freshSW and freshSW+oil) were similar, as shown by PCoA analyses. This is presumably caused by a preservation of the pre-ice communities during the 200 days incubation at -10 °C. However, during the 64 days of incubation at 4 °C, the differences between MI+oil and freshSW+oil became evident. While the total cell concentrations in melted ice (both MI and MI+oil) and in freshSW+oil were comparable at the start of the experiment at 4 °C, the concentrations of viable heterotrophic microbes (heterotrophic MPN) were lower in the melted ice than in freshSW treatments, suggesting an environmental stress imposed by the low temperature and high salinity conditions in the brine. Melting of ice allows for the degradation of dead cells and dissolved organic

matter (DOM) not metabolized in solid ice at -10 °C, in addition to selecting for oil degraders as the oil became bioavailable. The availability of easily available DOM was most likely main reason for the rapid increase in heterotrophic MPN concentrations in both MI and MI+oil during the first 15 days of incubations at 4 °C, favouring rapid biodegradation of organic debris instead of oil compounds. After 15 days, only 2% of the *n*-alkanes were biotransformed in MI+oil at 4 °C, compared to about 50% in freshSW+oil.

Several factors influence biodegradation of oil, and previous experiments have shown that degradation and microbial community dynamics are affected by temperature (Lofthus et al., 2018; Vergeynst et al., 2018), presence of frazil ice (Lofthus et al., 2020), oil chemistry (McKew et al., 2007; Ribicic et al., 2018c), the size of dispersed oil droplets (Brakstad et al., 2015a), and the location of the seawater source (Brakstad et al., 2018a; Ribicic et al., 2018d). Garneau et al. (2016) and Vergeynst et al. (2019) also revealed distinct differences between microbial communities which degraded oil in sub-ice seawater, communities in sea ice and communities from melted sea ice. Since temperature, oil composition, concentration and droplet size distribution were the same in both treatments (MI+oil and freshSW+oil) in our experiment at 4 °C, the differences in degradation rates were most likely caused by differences in the microbial communities in MI+oil and freshSW+oil.

Both treatments (MI+oil and freshSW+oil) shifted towards high relative abundances (80% at day 15) of Gammaproteobacteria, during the experimental period, suggesting the strong affiliation of this class with hydrocarbon degradation. Gammaproteobacteria are identified as the main players of oil degradation in cold marine environments, both in the presence and absence of ice (Brakstad et al., 2008; Garneau et al., 2016, Lofthus et al., 2018; Lofthus et al., 2020). However, the bacterial communities in fresh seawater with oil (freshSW+oil) responded faster to the presence of oil than the communities in melted ice (MI+oil), and the communities melted ice and fresh seawater were dominated by different OTUs.

The three most abundant OTUs in MI+oil during the period of most extensive degradation, *Piscirickettsiaceae* (OTU9), *Paraglaciecola* (OTU19) and *Sulfitobacter* (OTU21), all belongs to bacterial families with known oil degraders. Increased abundance of the genus *Paraglaciecola* has been associated with degradation of long chain *n*-alkanes in cold seawater (Bowman and Deming, 2014; Vergeynst et al., 2019), and the closely related genus *Glaciecola* was observed to be enriched in oil-contaminated sea ice (Brakstad et al., 2008). Biotransformation of PAHs could not be linked directly to increased abundances of any specific OTUs in MI+oil. However, members of the family *Piscirickettsiaceae* and the genus *Sulfitobacter* have been associated with degradation of PAHs in several biodegradation experiments (Brakstad et al., 2018a; Ribicic et al., 2018a; Ribicic et al., 2018b; Ribicic et al., 2018c), and these two taxa are most likely contributing to the degradation of PAHs in MI+oil. OTU9, OTU19, and OTU21 were also observed in MI but at lower abundances than in MI+oil. Although these taxa have been associated with oil degradation, it is also possible that they have enzymatic capacity to degrade hydrocarbons as well as heterotrophic degradation of organic compounds, therefore being present in both MI+oil and the controls without oil (MI). Other taxa present in MI+oil may not be related to oil degradation, but to degradation of other organic compounds, while at the same time surviving in the presence of oil. These taxa are likely to be present in both controls without oil (MI) and oil treated samples (MI+oil), causing the two treatments to change similarly over time, following the same trajectory in the PCoA plot, as well as showing the same initial decrease in richness and Shannon diversity.

In freshSW+oil *Oleispira* (OTU44), *Porticoccaceae* (OTU55) and *Cycloclasticus* (OTU92) were predominant. While *Oleispira* (OTU44) were emerging in MI+oil at day 30 at 4 °C, *Porticoccaceae* (OTU55) and *Cycloclasticus* (OTU92) were only observed in freshSW+oil, indicating that OTU55 and OTU92 may not survive freezing. Members of the genus *Oleispira* are considered to be obligate *n*-alkane degraders in cold seawater (Yakimov et al., 2003; Yakimov et al., 2007). The genus *Cycloclasticus* (belonging to the family *Piscirickettsiaceae*) is an obligate hydrocarbon degrader associated with the degradation of both mono- and polyaromatic hydrocarbons (Dyksterhouse et al., 1995; Yakimov et al., 2007; Wang et al., 2008; Ribicic et al., 2018b). *Cycloclasticus* has been identified as a PAH degrader in both Arctic and temperate seawater (Brakstad et al., 2015b; Ribicic et al., 2018d). Members of the family *Porticoccaceae* have also been associated with mono- and polyaromatic hydrocarbon degradation in cold seawater (Brakstad et al., 2018a; Ribicic et al., 2018b). This family also includes the obligate PAH-degrading species *Porticoccus hydrocarbonoclasticus* (Gutierrez et al., 2015).

From day 30, members of the families *Rhodobacteraceae* and *Flavobacteriaceae* increased in abundances, both in MI+oil and freshSW+oil. The late increases in abundance of *Rhodobacteraceae* are in agreement with results from other biodegradation experiments in cold water (Dubinsky et al., 2013; Brakstad et al., 2018a; Ribicic et al., 2018d). It has been proposed that *Rhodobacteraceae* may be involved in the degradation of recalcitrant hydrocarbons, both larger PAHs and branched alkanes (Ribicic et al., 2018a). *Flavobacteriaceae* has also been observed to increase in abundance in response to crude oil in cold seawater (Brakstad et al., 2018a; McFarlin et al., 2018), being linked to the degradation of PAHs, both naphthalenes (Bagi et al., 2014) and 4-6 ring PAHs (Ribicic et al., 2018a).

The results of this study are in agreement with the results of Garneau et al. (2016), studying the degradation capacity of microbial communities in sub-sea ice seawater and melted sea ice. Different from our study, Garneau et al. (2016) introduced oil to their samples after melting. Garneau et al. (2016) found oil degradation to be more extensive in sub-ice seawater than in melted sea ice. Further, they observed that Bray-Curtis similarities of the microbial communities in sub-ice seawater and melted sea ice were significantly different, while oil treatments and no oil controls in melted sea ice were very similar at the end of the experiment. The same patterns were observed in our study, between fresh seawater and melted ice (exposed to oil prior to melting) taken from the same seawater source. Comparison of these two studies, suggests that as long as oil is not bioavailable in ice to select for a oil degrading community, the bacterial community in melted ice will have reduced potential to degrade oil compared to non-frozen seawater, due to the extreme environmental conditions in the sea ice (low temperatures and high salinity). It is therefore worth to mention that in our study the transition period between impermeable winter ice, to ice more similar to that of spring melt, were not simulated. Spring melt in the Arctic has been shown to select for a sea ice community totally different from source seawaters and the mid-winter ice (Collins et al., 2010). Since our studies showed that microbial communities with abilities to biodegrade oil compounds may survive several months in solid sea ice, transition periods during spring melt, may stimulate the sea ice communities to start oil compound degradation while still trapped inside the ice matrix, as temperature increases, and oil becomes more bioavailable. This is also supported by observations by Brakstad et al. (2008) in oil-contaminated sea ice in Svalbard, where communities from both oil-contaminated and clean ice were similar during the first month of exposure to oil, in March, followed by a change in communities in both clean and polluted ice in April and June, that were different from one another.

5. Conclusion

Results from this study showed that both the total amount of oil and target oil compounds were biotransformed by the microbial communities in melted sea ice. However, oil released from melted sea ice was degraded at a slower rate compared to oil incubated in fresh seawater, at the same temperature (4 °C), by a different microbial community. *n*-Alkanes were biotransformed to the same extent in melted sea ice as in fresh seawater after 64 days, whereas the total amount of oil and PAHs were degraded to a lesser extent. These data showed that microbial communities incubated for 200 days in solid sea ice with oil were able to biodegrade oil compounds in melted ice, but were not better adapted to hydrocarbon degradation at 4 °C than communities in fresh seawater not previously exposed to oil.

During 200 days of incubation in oil polluted solid sea ice at -10 °C, no oil was degraded and there was no detectable stimulation of oil degrading microorganisms. It is therefore reasonable to assume that the bioavailability of weathered oil frozen into solid ice is extremely low, since temperatures are far below the pour point of the oil, even though dissolved oil compoundsare present in liquid brine.

In conclusion, dispersed weathered oil frozen into ice will persist, until ice melts during the Arctic summer. Melted ice contains bacterial communities with the capacity to degrade hydrocarbons. However, degradation rates of oil compounds caused by the melted ice communities are slower than for communities from seawater not previously frozen.

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CRediT authorship contribution statement

Synnøve Lofthus: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Ingrid Bakke:** Methodology, Resources, Writing – review & editing, Supervision. **Charles W. Greer:** Writing – review & editing. **Odd Gunnar Brakstad:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112823.

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