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Hydrocarbon biodegradation potential of microbial communities from high Arctic beaches in Canada's Northwest Passage

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ABSTRACT

Sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of an oil spill. Harnessing the capabilities of endemic microorganisms to degrade oil may be an effective remediation strategy for contaminated shorelines; however, limited data exists along Canada's Northwest Passage. In this study, hydrocarbon biodegradation potential of microbial communities from eight high Arctic beaches was assessed. Across high Arctic beaches, community composition was distinct, potential hydrocarbon-degrading genera were detected and microbial communities were able to degrade hydrocarbons (hexadecane, naphthalene, and alkanes) at low temperature (4 °C). Hexadecane and naphthalene biodegradation were stimulated by nutrients, but nutrients had little effect on Ultra Low Sulfur Fuel Oil biodegradation. Oiled microcosms showed a significant enrichment of *Pseudomonas* and *Rhodococcus*. Nutrient-amended microcosms showed increased abundances of key hydrocarbon biodegradation genes (*alk*B and *CYP153*). Ultimately, this work provides insight into hydrocarbon biodegradation on Arctic shorelines and oil-spill remediation in Canada's Northwest Passage.

1. Introduction

Unprecedented sea ice loss due to climatic warming is expanding the open-water season for Canada's Northwest Passage, resulting in increased marine traffic to service the growth of regional communities and industrial activities (Dawson et al., 2018; Overland and Wang, 2013; Smith and Stephenson, 2013). Increases in marine traffic inherently increases the risk of an oil spill in the Arctic. Oil spills are disastrous events with intertwined environmental, social, and economic impacts that require both short and long-term clean-up strategies. Follow-up studies from the 1989 Exxon Valdez oil spill, where 10.8 M gallons of crude oil spilled into Prince William Sound, Alaska, showed that 12 years later, an estimated 55,600 kg of sequestered oil persisted (Short et al., 2004) and notably, has remained largely unchanged from the 2001–2015 period (Lindeberg et al., 2018). This persistence highlights not only the inherent challenges of oil spill clean-ups, which are even more difficult in the Arctic's remote and harsh climate (Li et al., 2016), but also the need for improved long-term oil spill clean-up strategies. Harnessing the capabilities of naturally occurring microorganisms to degrade oil may be an effective remediation strategy for impacted shorelines. However, very limited data exists on hydrocarbon biodegradation and bioremediation on high Arctic beaches, particularly along Canada's Northwest Passage.

To date, knowledge of hydrocarbon biodegradation on high Arctic shorelines is based on analyses from three field projects: the *Baffin Island Oil Spill* (BIOS) in the Canadian high Arctic in 1980 (Sergy and Blackall, 1987), the *Svalbard shoreline field trials* on Spitsbergen, Norway in 1997 (Guénette et al., 2003) and two experimental spills in Svalbard in 2002 and 2003 (Røberg et al., 2007, 2011). Studies during BIOS focused mainly on weathering processes and the fate of oil on shorelines, and found that despite several abiotic degradative and dispersion processes contributing to oil reduction on shorelines, high Arctic beaches are still vulnerable to long-term oil persistence (Owens et al., 1987; Prince et al., 2002). Studies of the Svalbard field trials demonstrated that nutrient-fertilizers doubled the rate of biodegradation of an intermediate fuel oil (Sergy et al., 2003), increased microbial biomass as determined by

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phospholipid fatty acid analysis (Prince et al., 2003), and relative to unoiled sediment, resulted in an enrichment of Gammaproteobacteria, including the genera: *Pseudomonas*, *Cycloclasticus* and *Colwellia* (Grossman et al., 1999). Studies from the 2002 and 2003 experimental spills in Svalbard, where an oleophilic nutrient fertilizer was used on beach sediments contaminated with a light oil, reported an increased abundance of hydrocarbon-degraders (Røberg et al., 2007) and an altered community structure, resulting in an enrichment of Actinobacteria, Bacteroidetes and Gammaproteobacteria including: Pseudomonadaceae and *Shewanella* (Røberg et al., 2011).

In contrast to the early work conducted on high Arctic beaches, recent studies have used high-throughput methods to advance the understanding of oil degrading microbial communities in other high Arctic environments including marine waters (Krolicka et al., 2019; Ribicic et al., 2018; Vergeynst et al., 2019), soils (Ferguson et al., 2020; Yergeau et al., 2012) and ice (Garneau et al., 2016; Vergeynst et al., 2019; Lofthus et al., 2020). In the Canadian high Arctic, a survey of seawater and sea ice was conducted in the Northwest Passage, where taxonomic and functional differences were observed between microbial communities of seawater and sea-ice (Yergeau et al., 2017). Another study in the Northwest Passage examining hydrocarbon biodegradation by sub-ice and sea-ice microbial communities found communities degraded 94% and 48% of the oil, respectively (Garneau et al., 2016). The authors additionally found differences in microbial communities following hydrocarbon exposure, where Bacteroidetes (mainly *Polaribacter*) dominated sea-ice communities and Epsilonproteobacteria increased in sub-ice communities (Garneau et al., 2016). Relative to these other high Arctic environments, biodegradation on high Arctic shorelines is affected by many unique factors (Wang et al., 2020), particularly: limited ice-free days and sustained low temperatures even after thaw conditions (Owens and Harper, 1977; Prince et al., 2002), dynamic oil-sediment interactions influenced by tidal action (Gustitus and Clement, 2017; Owens et al., 1987), and limited concentrations of nutrients and oxygen that may vary with shoreline depth (Jansson, 1967; Lindeberg et al., 2018; Prince, 1993). Thus, the aim of this work was to broaden the body of knowledge of oil biodegradation on high Arctic shorelines, particularly along Canada's Northwest Passage.

In this study, the potential of hydrocarbon biodegradation of microbial communities of high Arctic beaches was assessed. First, the abundance and composition of endemic microbial communities and the physicochemical properties of the beach sediment were characterized. Secondly, mineralization assays were used to evaluate the ability of these microbial communities to degrade fuel constituents (hexadecane and naphthalene) with and without nutrient biostimulation using an inorganic N:P:K fertilizer. Lastly, microcosm experiments were set up to examine nutrient-amended biodegradation of a representative marine shipping fuel (Ultra Low Sulfur Fuel Oil (ULSFO)). Oxygen measurements and hydrocarbon analysis were conducted in parallel with microbial community characterization and metagenomic analysis of key hydrocarbon biodegradation genes. Here, the use of high-throughput methodologies in parallel with characterizing oil biodegradation contributes to the understanding of hydrocarbon biodegradation and bioremediation potential on high Arctic beaches, particularly along Canada's Northwest Passage. We hypothesize that oil-degrading communities from high Arctic tidal beaches will be a mixture of both marine and terrestrial bacteria and that nutrients will enhance hydrocarbon biodegradation. Ultimately, this study serves to inform for suitable oil-spill bioremediation strategies in the event of a spill in the extreme conditions of the Northwest Passage.

2. Materials and methods

2.1. Arctic beach sample sites

Beach sediment samples were collected in duplicate between July and August 2018 from eight high Arctic beaches across four locations in Nunavut, Canada: Alert, Cambridge Bay, Resolute, and Nanisivik (Fig. 1A, Supp. Table 1). Samples were immediately stored on site at −20 °C, transported to McGill University in coolers and stored at −20 °C until further analyses. Physicochemical parameters, microbial abundance, and microbial communities using 16S rRNA gene sequencing were analyzed from each sample. Beach sediment from Cambridge Bay, Nanisivik (East) and Resolute (Tank) was used in mineralization assays (Fig. 1B). Beach sediment from Resolute (Tank) was used in the Ultra Low Sulfur Fuel oil microcosms, where microbial communities using 16S rRNA gene sequencing, metagenomics, and total petroleum hydrocarbons (TPH) were analyzed (Fig. 1C).

2.2. Beach sediment physicochemical analyses and microbial abundance

Moisture content, organic matter, total nitrogen and phosphorus, salinity and dissolved oxygen were quantified from the sand fraction of the beach sediments. Moisture content was determined by heating samples to 105 °C for 24 h and calculating the difference in weight, expressed as a percentage, between the two steps. Organic matter was quantified following the Loss of Ignition (LOI) protocol (Schulte et al., 1991). Briefly, following the moisture content heating cycle, the samples were further heated at 360 °C for 4 h and the difference in weight between the two steps was calculated to obtain organic matter content, expressed as mg g^{-1}. Total nitrogen and phosphorus were quantified following the persulfate digestion protocol (Ebina et al., 1983). Salinity and dissolved oxygen were measured in situ at Resolute 1 year after sampling using a YSI probe (Xylem Inc.). Due to insufficient sample materials, physicochemical parameters were not obtained for Cambridge Bay.

Culturable cells were quantified using the spread plate method. In detail, 5 g of sediment was suspended in 15 mL artificial seawater (Sigma-Aldrich) and vortexed for 45 s. Dilutions were spread on plates containing 10% R2A (Difco) with artificial seawater agar medium. The agar plates were incubated at 10 °C for 4 weeks. Following incubation, colony-forming units (CFUs) were counted. Total microbial cells were quantified using fluorescence microscopy. Cells were fixed for 1 h at room temperature by suspending 0.5 g of sediment in 450 μL of formaldehyde solution (4%). The fixed cells were dislodged from the sediment by sonication for 30 s at 65 watts. The supernatant was collected and replaced with artificial seawater. After 3 sonication cycles, the pooled supernatant was stained with DAPI (NucBlue Fixed Cell Stain ReadyProbes reagent, Invitrogen) and spot dilutions were prepared on a glass slide. Once evaporated, all fluorescent cells were manually counted and averaged from three fields of view using fluorescent microscopy (Nikon Eclipse 80i) at 400× magnification.

2.3. Mineralization assays

To examine nutrient-enhanced biodegradation of hexadecane and naphthalene, ^{14}C-mineralization microcosms (as described by Steven et al., 2007) were prepared (Fig. 1B). Briefly, 5–10 g of sediment was added to 20 mL glass vials (Sigma-Aldrich). Radioactive hexadecane (American Radiolabeled Chemicals, ARC 0576-50 μCi), naphthalene (American Radiolabeled Chemicals, ARC 1260-50 μCi), or acetate (as a positive control; PerkinElmer, NEC553050UC) was added to a final activity of 100,000 disintegrations per minute (dpm). Microcosms were supplemented with non-radioactive substrates to a final concentration of 100 ppm for hexadecane and acetate and 10 ppm for naphthalene—reduced as naphthalene risks being toxic to microorganisms (Ahn et al., 1998). Inorganic nutrients were added to applicable microcosms using a 20:20:20 nitrogen (urea, nitrate, ammonia), phosphorus and potassium fertilizer (Master Plant-Prod Inc.) to a final concentration of 15 ppm to approximate Redfield stoichiometry (a marine ratio of carbon, nitrogen and phosphorus of 106:16:1, respectively) (Filler et al., 2006). Inorganic nutrients were used in for their accessibility at lower temperatures (<15 °C) (Lee et al., 1993) and

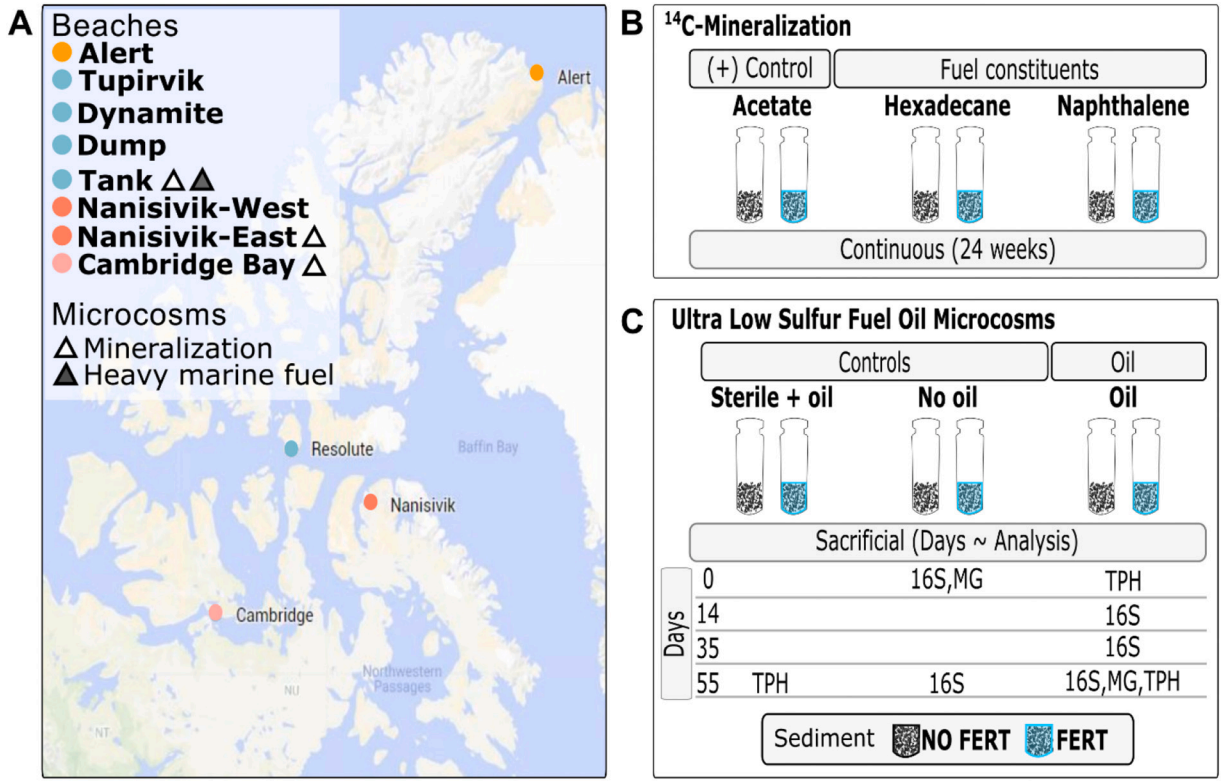

Fig. 1. Eight Arctic beach sample sites across four locations, collected between July and August 2018 (A). Overview of set-up for ^{14}C-mineralization assays (B) and ULSFO treated sediment microcosms (abbreviations: 16S = 16S rRNA community analysis, MG = Metagenome, TPH = Total Petroleum Hydrocarbons) (C).

Table 1
Physicochemical analyses and microbial abundances across high Arctic beaches.

Beach	Moisture (wt/wt%)	OM[A] (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	Salinity (psu)	DO[B] (mg L^{-1})	Culturable bacteria g^{-1}	Total bacteria g^{-1}	Potential HC genera[C]
Alert	7.450	3.04	0.34	0.853	–	–	3.8×10^6	3.3×10^6	0.087 ± 0.098
Tupirvik	6.044	9.51	0.25	0.122	1.80	12.56	4.6×10^5	1.2×10^7	0.057 ± 0.004
Dynamite	2.806	2.58	0.19	0.158	0.31	13.75	3.5×10^5	3.2×10^7	0.053 ± 0.003
Dump	1.777	3.79	0.16	0.645	6.00	11.45	3.1×10^5	1.6×10^6	0.132 ± 0.125
Tank	4.174	4.25	0.17	0.376	0.57	9.47	2.4×10^6	4.4×10^6	0.111 ± 0.012
Nanisivik - West	7.695	3.72	0.25	0.423	–	–	1.1×10^6	1.3×10^7	0.019 ± 0.002
Nanisivik - East	5.888	5.58	0.29	0.574	–	–	6.2×10^6	2.3×10^6	0.031 ± 0.007
Cambridge Bay	–	–	–	–	–	–	2.7×10^6	1.5×10^6	0.076 ± 0.001

[A] OM: Organic matter.
[B] DO: Dissolved oxygen.
[C] Potential HC genera expressed as mean sum of relative abundance of potential oil degrading genera screened for in 16S rRNA data of beach duplicates.

efficacy in in situ bioremediation (Boufadel et al., 2016). Set-up occurred in triplicate including negative controls (autoclaved sediment). Microcosms were incubated in the dark at 4 °C. Radioactivity was measured using a liquid scintillation counter (Perkin Elmer Tri-Carb Series) at set-up and after two, five, eight, twelve and twenty-four weeks of incubation. Concentration of mineralized ^{14}C that accumulated in between each sampling was reported as cumulative percent degradation.

2.4. Experimental microcosms

2.4.1. Set-up

To examine nutrient-enhanced biodegradation of a heavy marine fuel, microcosms were prepared using Ultra Low Sulfur Fuel Oil (ULSFO) (Shell Trading Canada, 002D4509) designed to comply with International Maritime Organization (IMO) regulations to reduce sulfur emissions from ships (Berger et al., 2017) (Fig. 1C). More information on the ULSFO used can be found from the Shell website (https://www.shell.com/business-customers/marine/fuel/marine-safety-data-sheets.html). Each microcosm consisted of a 20 mL glass vial (Sigma-Aldrich) containing 10 g of beach sediment from Resolute (Tank beach), 2 mL of filtered artificial seawater (to saturate the sediment), and an oxygen sensor spot (PreSens) placed into the upper headspace. Using a positive displacement pipette, ULSFO, heated to 75 °C, was added to a final concentration of 2000 ppm approximating residual shoreline oil concentrations following the Baffin Island Oil Spill (Prince et al., 2002). Nutrient-enhanced microcosms were supplemented with 15 ppm of inorganic nutrients as described in the *Mineralization assays* section. Microcosms were closed with a rubber stopper and incubated in the dark at 4 °C for 55 days. Microcosms were set up in triplicate with triplicate

negative controls (autoclaved sediment). Triplicate nutrient-amended and unamended ULSFO contaminated microcosms were sacrificed for microbial analyses at T0 and after 14, 35 and 55 days of incubation, and for hydrocarbon analyses at T0 and after 55 days of incubation. Unoiled control microcosms were sacrificed after 55 days incubation.

2.4.2. Oxygen measurements and hydrocarbon analyses within microcosms

In between sampling events, headspace oxygen was measured intermittingly as a proxy for biodegradation under the assumption that depletion of headspace oxygen corresponded to increased microbial respiration (Brown et al., 2018). Using the OXY-4 mini sensing system and software (PreSens), headspace oxygen measurements were taken in triplicate at 4 °C for each replicate microcosm. Microcosms were aerated intermittently by opening and closing the rubber stopper to ensure microbial processes were aerobic. Oxygen measurements were converted from air saturation percent to micromole of oxygen based on estimated headspace volume. Decreased headspace oxygen was reported as cumulative consumption, determined from oxygen concentrations that accumulated in between each sampling time point. Sacrificed experimental microcosms were sent to the University of Manitoba to quantify aliphatic and aromatic hydrocarbons using a LECO Pegasus® gas chromatography-high resolution time of flight mass spectrometry system and an Agilent 7010B triple quadrupole gas chromatography mass spectrometry system (Saltymakova et al., 2020). To determine hydrocarbon biodegradation, analyte concentrations were normalized to the internal marker 17α(H),21β(H)-hopane (Prince and Douglas, 2005).

2.5. DNA extraction, library preparation and sequencing

Microbial communities from beach sediments and experimental microcosms were characterized by 16S rRNA gene amplicon analysis. DNA was extracted from the beach sediments in triplicate using the DNeasy PowerLyzer PowerSoil Kit (Qiagen) as per protocol except DNA was eluted with 50 μL of nuclease-free water. DNA was extracted from experimental microcosm sediment using the DNeasy PowerMax Soil Kit (Qiagen) as per protocol except DNA was eluted with 3 mL of nuclease-free water. For both the beach sediments and experimental microcosms, the 16S rRNA gene libraries were prepared following Illumina's 16S Metagenomic Sequencing Library Preparation protocol, except for three modifications. First, 2× HotStarTaq Plus Master Mix (Qiagen) was used in PCR steps. Second, the ratio of amplicon PCR reagents were adjusted as follows: 7.5uL nuclease-free water, 1.5 μL 10 μM forward primer, 1.5 μL 10 μM reverse primer, 12.5 μL HotStarTaq Plus and 2 μL genomic DNA. Third, primers from the earth microbiome project were used (515F-Y (5′-GTGYCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYMTTTRAGTTT)) (Parada et al., 2016). Amplicons from the beach sediments were indexed, pooled and sequenced using the 600-cycle MiSeq Reagent Kit v3 on an Illumina MiSeq platform in house. Amplicons from experimental microcosms were indexed, pooled and sequenced using the MiSeq Reagent Kit v2 nano configuration on an Illumina MiSeq platform at the UBC Sequencing Centre.

Metagenomic libraries from T0 and triplicate samples from 55-day nutrient-amended and unamended microcosms were prepared using the Nextera XT Library Prep kit as per protocol (#15031942V04). Briefly, extracted DNA was tagmented, amplified and cleaned with a Sera-Mag Select bead clean-up kit (Cytiva). Normalization between libraries was performed by pooling equimolar amounts of libraries after a quantification using an Agilent High Sensitivity DNA kit on a 2100 Bioanalyzer (Agilent Technologies). The library pool was purified using a Nucleic Acid Purification PCR and DNA clean-up kit (Monarch). Denaturation and dilution of the library pool was prepared following protocol A of Illumina's Denature and Dilute Libraries Guide (15,039,740 v10). The final library was sequenced using a 600-cycle MiSeq Reagent kit v3 on an Illumina MiSeq platform. The 16S rRNA gene sequencing data from the beach sediments and the 16S rRNA gene and metagenomic sequencing data from the experimental microcosms are available at NCBI under the BioProject PRJNA735418.

2.6. Bioinformatics

The 16S rRNA sequencing output was processed following the 'Bioconductor Workflow' (Callahan et al., 2016a). Briefly, raw sequencing reads were imported into R (version 4.0.2; The R Foundation for Statistical Computing) and processed using the R package "dada2" v. 1.16.0 (Callahan et al., 2016b) to generate amplicon sequence variants (ASVs) with assigned taxonomy from the SILVA database v. 132 (Callahan, 2018). ASV counts, taxonomy and sample metadata were combined and manipulated using the R package "phyloseq" v. 1.32.0 (McMurdie and Holmes, 2013), whereby uncharacterized taxa, taxa prevalent in less than three samples and taxa present in less than 5% of total samples were removed.

Microbial communities from the beach sediments were screened for the prevalence of potential hydrocarbon-degrading genera previously detected in polar environments: *Loktanella, Sulfitobacter*, *Sphingopyxis*, *Sphingomonas*, *Alteromonas*, *Glaciecola*, *Marinobacter*, *Colwellia*, *Thalassomonas*, *Moritella, Algicola*, *Pseudoalteromonas*, *Psychromonas*, *Shewanella*, *Alcanivorax*, *Marinomonas*, *Oleispira*, *Halomonas*, *Psychrobacter*, *Pseudomonas*, *Cycloclasticus*, *Arcobacter*, *Cytophagia*, *Ulvibacter*, *Polaribacter*, *Rhodococcus*, *Agreia* and *Arthrobacter* (Brakstad et al., 2017). The mean sum and standard deviation of these genera was determined through averaging the sum of each beach replicate. Alpha diversity metrics within the experimental microcosms were determined using the indices "Chao1" (Chao, 1984) for community richness and "Shannon-Weiner" (Shannon, 1948) for diversity using the R package "phyloseq" (McMurdie and Holmes, 2013). Obtained Shannon diversity indices were converted to "estimated number of species" (Jost, 2006). Beta diversity among microbial communities of the beach sediments and experimental microcosms were assessed under the principles of compositional data (Gloor et al., 2017). Dissimilarities between communities were evaluated using the proportionality metric phiT (φ) (Lovell et al., 2015) using the R package "propr" v. 4.2.6, which implements a centered log-ratio transformation (Quinn et al., 2017). Obtained dissimilarities were explored using nonmetric multidimensional scaling (NMDS) ordination using the R packages "vegan" v. 2.5.6 (Oksanen et al., 2013), "EnvStats" v. 2.4.0 (Millard, 2013) and "ellipse" v. 0.4.2 (Murdoch and Chow, 2020).

The experimental microcosms metagenomic data was analyzed by screening the assembled metagenomic data for functional genes of interest. Raw reads were controlled for quality in several steps: 1) excess barcodes generated during sequencing were discarded 2) remaining reads were quality checked via FastQC (Andrews, 2010) and trimmed using "trimmomatic" (Bolger et al., 2014) 3) synthetic artifacts were discarded using "bbmap" (Bushnell, 2014) 4) resulting reads were error-corrected and subsequently assembled using "metaSPAdes" (Nurk et al., 2017). The assembled contigs were annotated using "MetaErg," which included a taxonomic classification of genes (Dong and Strous, 2019). Protein coding sequences derived during contig annotation were used as a reference to map error-corrected reads from originating metagenomic sample using "bbmap" (Bushnell, 2014). Mapped reads obtained using "bbmap" (Bushnell, 2014) were compiled into a gene × read count matrix.

Hidden Markov Models (HMM) were generated to screen for the presence of hydrocarbon biodegradation genes with demonstrated biological function including: aerobic alkane-degradation genes, *alk*B encoding an alkane monooxygenase (Nie et al., 2014), *CYP153* (Nie et al., 2014; Van Beilen et al., 2006) and *ladA*; an anaerobic alkane-degradtion gene, *masD/AssA* (Gittel et al., 2015); an aerobic polycyclic aromatic hydrocarbon (PAH)-degradation gene, *phnAc* (Ding et al., 2010; Lozada et al., 2008); and an anaerobic PAH-degradation gene, *ncr* (Morris et al., 2014). Amino acid sequences of these genes were compiled and a multiple sequence alignment was generated for each gene using MAFFT (Katoh and Standley, 2013) with the *E*-INS-I

alignment. The multiple sequence alignment was used to create an HMM using "hmmbuild" on Hmmer (http://hmmer.org/). The produced HMM for each hydrocarbon biodegradation gene was used to identify homolog amino acid sequences (potential degradation genes) within the annotated protein sequences using "hmmsearch" on Hmmer (http://hmmer.org/). Potential gene hits that had an e-value of $<1 \times 10^{-10}$ and a domain score below the lowest domain score of reference sequences were filtered. The resulting gene hits were imported into R using the R package "rhmmer" v. 0.1.0 (Arendsee, 2017) and gene abundance across samples was calculated as counts per million (CPM).

2.7. Data visualization and statistical analyses

All plots and statistical analyses were completed in R. Plots were generated using the R packages "ggplot2" v. 3.3.2 (Wickham, 2016), "ggpubr" v. 0.4.0 (Kassambara, 2020a), "forcats" v. 0.5.0 (Wickham, 2020), "unikn" v. 0.3.0 (Neth and Gradwohl, 2020), "polychrome" v.1.2.6 (Coombes et al., 2019) and "dplyr" v. 1.0.2 (Wickham et al., 2020). Significant differences between nutrient treatments and controls within ^{14}C mineralization microcosms, and oxygen consumption, total petroleum hydrocarbon (TPH) concentration and hydrocarbon gene abundances within experimental microcosms were tested using a one-tailed *t-test* in the R package "RStatix" v. 0.6.0 (Kassambara, 2020b). Within experimental microcosms, TPH differences between time points and significance of community richness and diversity between nutrient treatments and controls were assessed using a two-tailed *t-test* with the same R package. Within experimental microcosms, differences between differentially abundant genera of microbial communities was assessed using "aldex2" v. 1.20.0 (Fernandes et al., 2013). Significant effects of community dissimilarity were tested by multivariate permutational ANOVA (PERMANOVA) using the 'adonis' function in the R package "vegan" v. 2.5.6 (Oksanen et al., 2013), where multivariate homogeneity of group dispersion was evaluated with the 'betadisper' function.

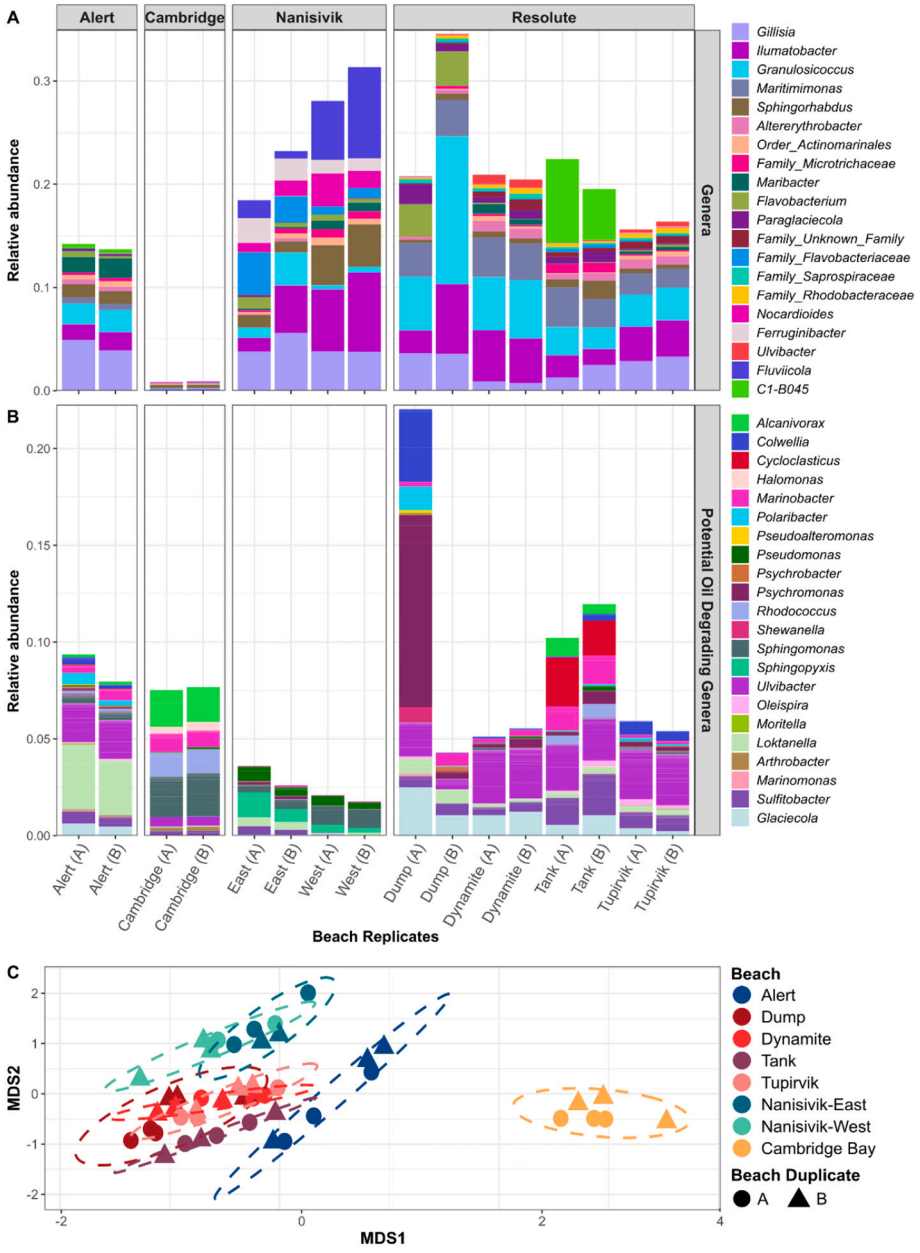

Fig. 2. Relative abundance across replicate beach samples of the top 20 most abundant genera (mean across all samples) (A), and potential hydrocarbon-degrading genera compiled from the literature (B). Dissimilarities between microbial communities across beaches represented by the proportionality metric phiT (Stress value: 0.154), where PERMANOVA analysis supported that dissimilarities between microbial communities was explained 41.1% by beach biological replicate (p-value: 0.0002) (C).

3. Results

Physicochemical analyses across beaches showed low nutrient availability (Table 1). Across beaches, moisture content ranged from 1.7–7.7%, organic matter (OM) ranged from 2.58–9.51 mg g^{-1}, total nitrogen ranged from 0.19–0.34 mg g^{-1} and total phosphorus ranged from 0.122–0.853 mg g^{-1} (Table 1). Additionally, in the Resolute samples, interstitial water measurements showed a range for salinity of 0.31–6 psu, the largest variation observed, and for dissolved oxygen (DO) of 9.47–13.75 mg L^{-1} (Table 1). Across all beaches, similar counts for both culturable and total cells were observed (Table 1). Culturable cells ranged from 1.11 × 10^5–3.75 × 10^6 colony forming units (CFUs) g^{-1} with a median across beaches of 8.63 × 10^5 CFUs g^{-1}. Total cells ranged from 1.51 × 10^6–3.16 × 10^7 cells g^{-1} with a median across beaches of 3.84 × 10^6 cells g^{-1} (Table 1).

3.1. 16S rRNA community composition across high Arctic beach sediments

Across beaches, microbial communities were dominated by similar classes (Bacteroidia, Gammaproteobacteria and Alphaproteobacteria (Fig. S1B), but differentiated at the family-level, where the shared groups (*Flavobacteriaceae*, *Psychromonadaceae* and *Porticoccaceae*) represented less than 50% of the beach communities (Fig. S1D). Across Alert, Nanisivik and Resolute beaches, *Gillisia*, *Illumatobacter* and *Granulosicoccus* genera were dominant (Fig. 2A), whereas Cambridge Bay beaches were dominated by *Gillisia*, *Algoriphagus* and *Zeaxanthinibacter* (Fig. S2). Across beaches, an analysis of beta diversity using nonmetric multidimensional scaling (NMDS) ordination of the proportionality metric phiT showed microbial communities in four distinct clusters representative of each beach location, with Cambridge Bay samples clustering furthest from the remaining locations (Fig. 2C). PERMANOVA analysis supported that dissimilarities between microbial communities was explained 41.1% by beach biological duplicate (*p*-value: 0.0002). Across beaches, 22 of the 28 potential hydrocarbon-degrading genera previously detected in polar environments (Brakstad et al., 2017) were observed (Fig. 2B). Potential oil degrading genera were most abundant at two Resolute beaches (Dump and Tank) and least abundant at Nanisivik beaches (East and West) (Fig. 2B and Table 1).

3.2. Mineralization assays

Across representative beaches along the Northwest Passage, mineralization of hexadecane was generally greater than naphthalene and overall, nutrients improved mineralization (Fig. 3). Nutrients enhanced hexadecane mineralization at each beach: Cambridge Bay (20.5 ± 10.6% vs. 1.0 ± 0.4%; *p*-value: 0.04), Nanisivik (10.9 ± 6.7% vs. 4.3 ± 0.7%; NS), and Resolute (10.8 ± 13.1% vs. 4.5 ± 0.7%; NS). Nutrients enhanced naphthalene mineralization for Cambridge Bay (8.0 ± 2.9% vs. 3.8 ± 1.3%; NS) and Nanisivik (1.1 ± 0.6% vs. 0.7 ± 0.04%, NS); however, mineralization was greater in unamended microcosms for Resolute (1.2 ± 0.5% vs. 1.0 ± 0.3%; NS). Nutrients improved mineralization of acetate, used as a positive control, for Cambridge Bay (19.9 ± 3.6% vs. 9.8 ± 4.7%; *p*-value: 0.02) and Nanisivik (31.3 ± 4.0% vs. 30.1 ± 3.5%; NS); however, mineralization was greater in unamended microcosms for Resolute (37.4 ± 15.8% vs.

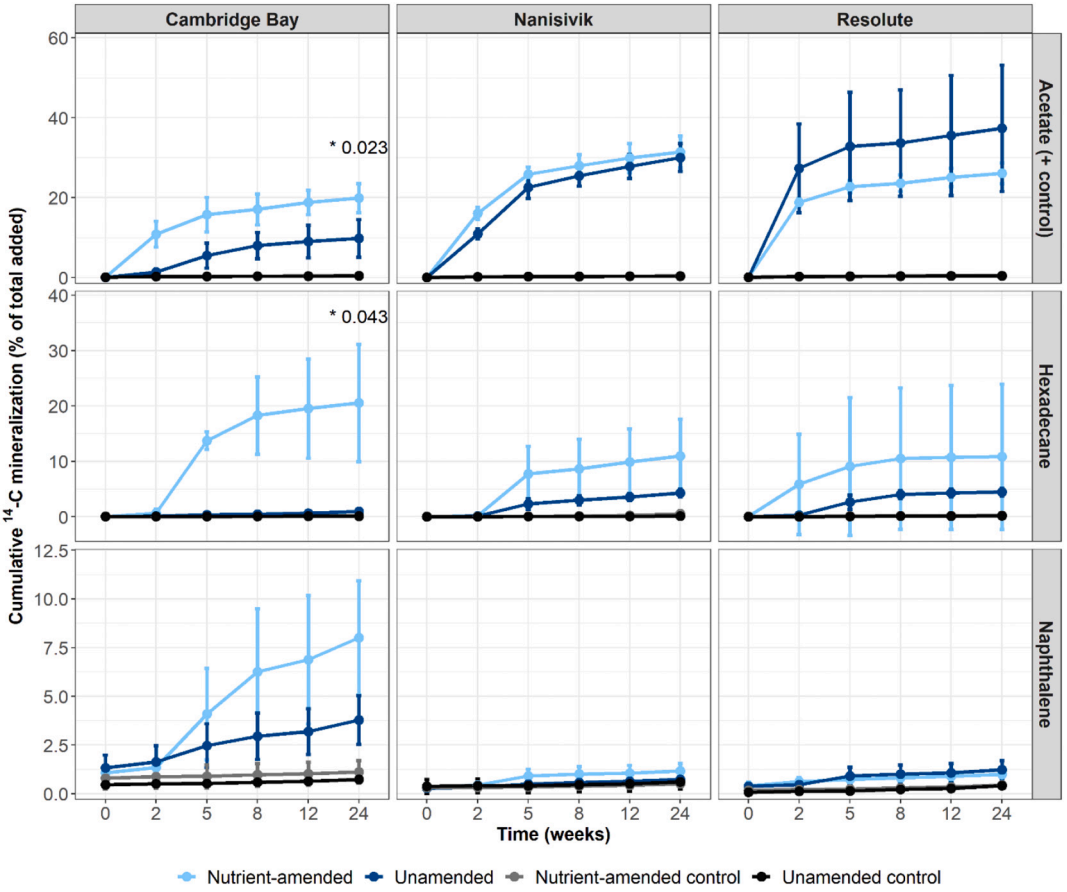

Fig. 3. Mean cumulative mineralization (% of total added) of ^{14}C-acetate (positive control), hexadecane and naphthalene incubated at 4 °C in nutrient-amended and unamended microcosms and control microcosms using beach sediment from three locations: Cambridge Bay, Nanisivik and Resolute. Error bars representing standard deviation of the mean from triplicate microcosms. Significance between unamended and nutrient-amended microcosms at 24 weeks determined using a one-tailed *t*-test.

26.1 ± 2.6%; NS). Mineralization was not observed in any of the sterile negative controls, indicating that mineralization was microbially driven in the microcosms (Fig. S3).

3.3. Experimental microcosms

3.3.1. ULSFO degradation in microcosms by oxygen estimates and hydrocarbon analyses

In Ultra Low Sulfur Fuel Oil (ULSFO) microcosms, oxygen depletion (a proxy for microbial respiration) was observed (Fig. 4A) and petroleomic analyses showed alkanes being primarily degraded over polycyclic aromatic hydrocarbons (PAHs) (Fig. 4B). Nutrients significantly enhanced depletion of headspace oxygen in microcosms (132.6 ± 14.6 μmol vs 104.1 ± 10.0 μmol; *p*-value: 0.002). For nutrient treatments, mean cumulative oxygen depletion was significantly higher in oiled microcosms relative to unoiled controls (105 ± 12.5 μmol; p-value: 0.018) and sterile controls (47.7 ± 3.6 μmol; p-value: <0.001). For unamended treatments, mean cumulative depletion of headspace oxygen was greater relative to unoiled controls (65.2 ± 28.2 μmol; NS) and was significantly greater relative to sterile controls (42.8 ± 2.0 μmol; p-value: <0.001). Alkane concentration decreased, but not significantly from 64.6 ± 25.0 ng g^{-1} to 39.7 ± 6.49 ng g^{-1} in unamended microcosms and to 39.9 ± 5.70 ng g^{-1} in nutrient-amended microcosms. Alkane concentration of biotic microcosms was lower, but not significantly, than the sterile controls (nutrient-amended: 60.5 ± 19.1 ng g^{-1}; unamended: 51.3 ± 27.4 ng g^{-1}). PAH concentration decreased, but not significantly, from 21.1 ± 8.08 ng g^{-1} to 13.3 ± 11.1 ng g^{-1} in unamended microcosms; however, PAH concentration increased slightly to 23.2 ± 3.94 ng g^{-1} in nutrient-amended microcosms. PAH concentration of biotic microcosms was lower, but not significantly, than the sterile controls (nutrient-amended: 25.8 ± 11.0 ng g^{-1}; unamended: 20.3 ± 13.0 ng g^{-1}).

3.3.2. 16S rRNA community composition of oiled-sediment microcosms

Similarities in relative abundances at all taxonomic levels were observed across microcosms (Fig. S4). No genera were significantly differentially abundant between nutrient treatments of oiled microcosms; however, *Rhodococcus* was only detected in nutrient-amended oiled microcosms after 55 days (Fig. 5A). Oiled microcosms, relative to unoiled controls, had higher abundances of *Pseudomonadaceae* and *Sphingomonadaceae* (Fig. S4). The most abundant genera in oiled microcosms: *Gillisia*, *Granulosicoccus*, *Pseudomonas*, *Planococcus*, *Flavobacterium*, *Psychrobacter* and *Rhodococcus* (Fig. 5A), have all previously been associated with hydrocarbon biodegradation (Chaudhary et al., 2019; Deppe et al., 2005; Liang et al., 2021; Margesin et al., 2003; Rizzo et al., 2019; Yang et al., 2018). Interestingly, many genera within the unoiled microcosms at T0: *Gillisia*, *Granulosicoccus*, *Cycloclsaticus*, *C1-B045*, *Sulfitobacter*, *Alterythrobacter* and *Maribacter* (Fig. 5A), have also previously been implicated in hydrocarbon biodegradation (Bagi et al., 2014; Dunlevy et al., 2013; Gontikaki et al., 2018; Kasai et al., 2002; Liang et al., 2021; Peng et al., 2020; Rizzo et al., 2019). Apart from *Gillisia*, none of these genera were later detected in the unoiled microcosms. Both *Pseudomonas* and *Rhodococcus* were significantly differentially abundant (*p*-values: 0.0027, 0.0141, respectively) in the 55-day oiled relative to unoiled microcosms.

Alpha diversity was influenced by the addition of oil rather than nutrients (Fig. 5B), whereas beta diversity was influenced by the addition of both (Fig. 5C). Both community richness and effective number of species were significantly greater (*p*-values: 0.004, 0.007, respectively) in nutrient-amended oiled microcosms relative to unoiled controls, whereas these metrics were not significantly different between unamended oiled microcosms and unoiled controls (Fig. 5B). Nutrients did not enhance either community richness or effective number of species, where species were greater in unamended oiled microcosms (360 ± 73 vs. 337 ± 12 species; NS and 283 ± 56 vs. 278 ± 13 species; NS,

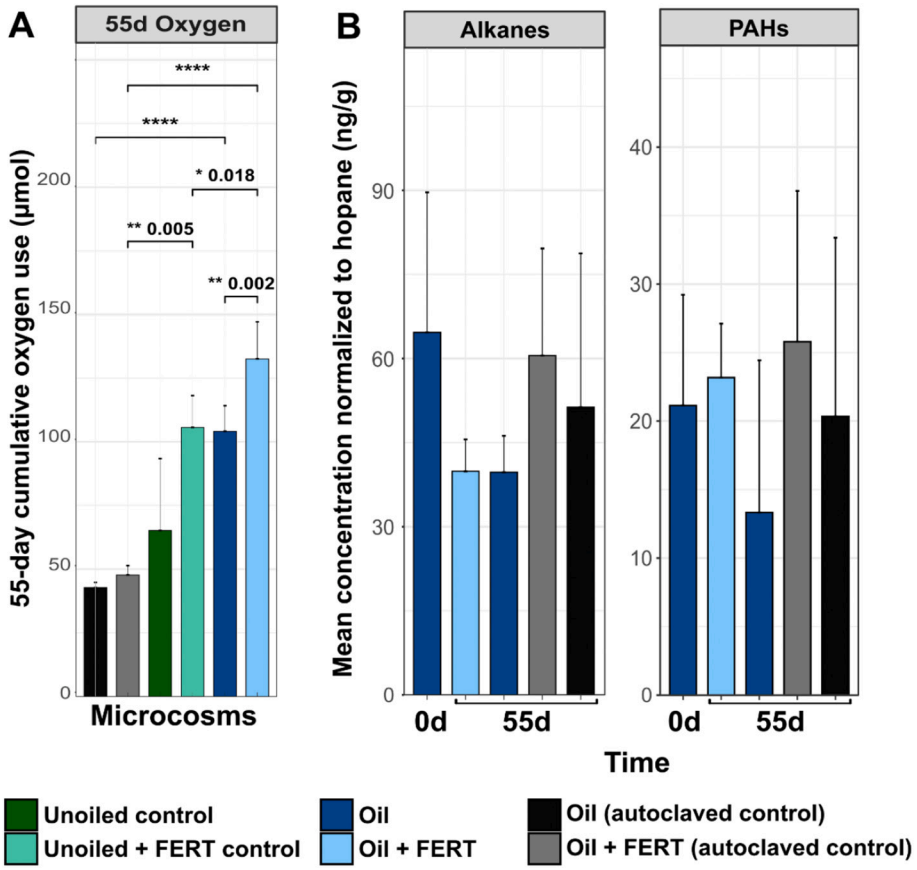

Fig. 4. Cumulative 55-day headspace oxygen depletion (μmol) incubated at 4 °C for nutrient-amended and unamended experimental microcosms (n = 6), unoiled controls (n = 3) and autoclaved sediment controls with ULSFO (n = 3), error bars representing standard deviation of the mean of microcosms and significance determined through a one tailed *t*-test (A). Concentration of hydrocarbons across nutrient-amended and unamended experimental microcosms and sterile control microcosms incubated at 4 °C, error bars representing standard deviation of the mean of microcosms.

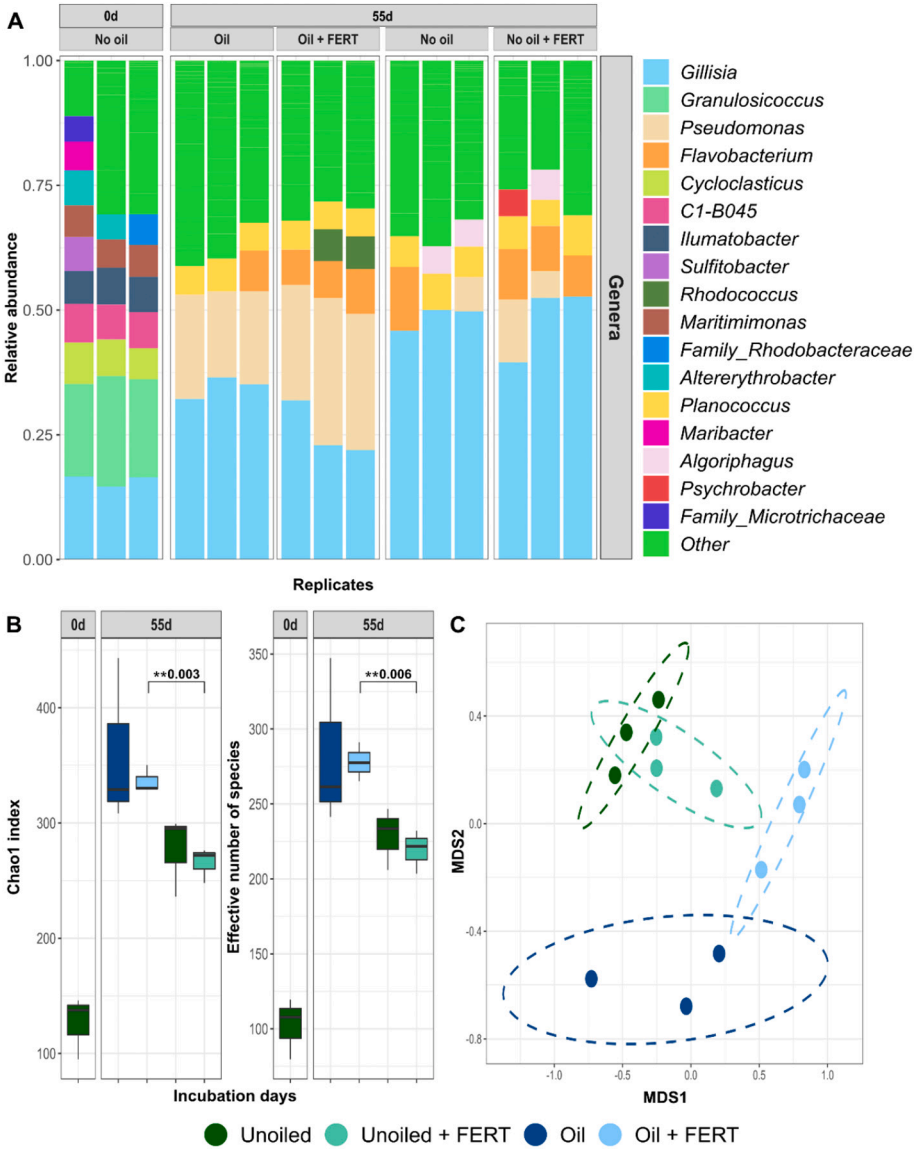

Fig. 5. Relative abundance of the top genera (A), development of species richness based on the Chao1 index and diversity quantified using Shannon-Wiener index expressed as effective number of species across microbial communities (B) and nonmetric multidimensional scaling (nMDS) ordination of the proportionality metric phiT between microbial communities (stress: 0.07) (C) across nutrient-amended and unamended ULSFO treated and unoiled control microcosms incubated at 4 °C.

respectively), but nonetheless increased from T0 (126 ± 27 and 102 ± 20 species, respectively) (Fig. 5B). NMDS ordination of the proportionality metric phiT showed four distinct clusters differentiating oiled and nutrient treated microcosms (Fig. 5C). This observation was supported by PERMANOVA analysis that showed dissimilarities between communities could be explained 22.5% by oil addition (*p*-value: 0.0020) and 16.9% by nutrient addition (p-value: 0.0134). Distinct clusters were also observed between nutrient treatments at 14 and 35 days of incubation, but this nutrient effect was not found to be significant (Fig. S5C). Ordination patterns were less distinct when considering all microcosms (Fig. S5C); however, PERMANOVA analysis showed that dissimilarities between communities could be explained 20.3% by incubation time (*P*-value: 0.0002), 10.5% by oil addition (p-value: 0.0008) and 7.00% by nutrient addition (p-value: 0.0196).

3.3.3. *Metagenomic analyses of oiled-sediment microcosms*

The hydrocarbon biodegradation genes *alk*B, *CYP153* and *phnAc* were detected in the experimental microcosms, while *ladA*, *ncr* and *masD* genes were not and overall, nutrients appeared to stimulate higher abundances of *alk*B and *CYP153* genes (Fig. 6). The abundance of *alk*B genes was ~2-fold greater in nutrient-amended microcosms (239 ± 70 vs. 133 ± 37 CPM; NS), increasing from T0 (14 CPM) (Fig. 6). Within oiled microcosms, *alk*B genes were primarily associated to the genera *Pseudomonas, Rhodococcus* and *Sphingorhabdus* (Fig. 6). The abundance of *CYP153* genes was ~2-fold greater at T0 (192 CPM) relative to oiled microcosms; however, these genes were primarily associated to the genera *Cycloclasticus* and *50–400-T64* (a Gammaproteobacteria*)*, which were not observed in the oiled microcosms (Fig. 6). The abundance of *CYP153* genes was ~2-fold greater in nutrient-amended microcosms (99 ± 43 vs. 54 ± 23 CPM; NS). In oiled microcosms, *CYP153* genes were associated to the genera *Williamsia*, *Rhodococcus* and *Alterythrobacter* (Fig. 6). Abundance of *phnAc* genes was greater at T0 (77 CPM) relative to oiled microcosms; however, as with *CYP153*, these genes were associated to the genus *Cycloclasticus*, which was not observed in oiled microcosms (Fig. 6). Abundance of *phnAc* genes was low in unamended and nutrient-amended oiled microcosms (8 ± 12 CPM; <1 CPM, respectively). The *phnAc* genes were associated to the genus *Sphingobium* (Fig. 6).

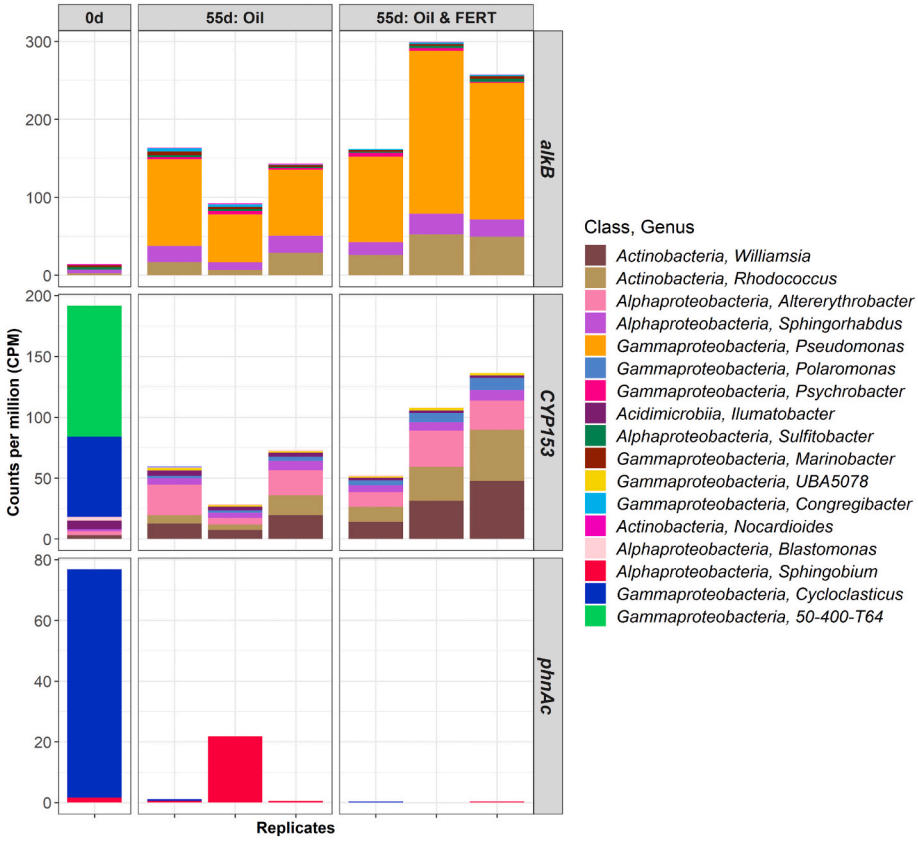

Fig. 6. Abundance (counts per million) of alkane biodegradation genes: alkB and CYP153 and aromatic hydrocarbon biodegradation genes: phnAc in a 0-day uncontaminated microcosm and in 55-day ULSFO contaminated triplicate unamended microcosms (Oil) and triplicate nutrient-amended microcosms (Oil & FERT). Genes are colored by contig taxonomy at the genus level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Characterizing high Arctic beaches and evaluating hydrocarbon biodegradation potential

Artic beaches were characterized by similarities in sediment chemistry and microbial abundances and by differences in microbial community composition. The Arctic beach sediments of this study featured low amounts of total nitrogen (<0.3 mg g^{-1}), total phosphorus (<0.9 mg g^{-1}), organic matter (<9 mg g^{-1}) and moisture content (<7.7%), which were similar to previously described Antarctic coastal soils (Aislabie et al., 2012) and Arctic soils (Mohn and Stewart, 2000). Dominant microbial phyla across beaches including: Proteobacteria, Bacteroidetes and Actinobacteria, were consistent with a previous study in the Northwest Passage, where Bacteroidetes and Gammaproteobacteria dominated sea-ice samples and Actinobacteria dominated seawater samples (Yergeau et al., 2017). Dominant microbial classes across beaches including Bacteroidia, Gammaproteobacteria and Bacilli were similar to those detected previously from a Norwegian beach using 16S rRNA gene clones (Grossman et al., 1999). In this study, the most dominant genera, *Gillisia*, *Illumatobacter*, *Granulosicoccus*, *Sphingorhabdus, Maritimimonas, Woeseia,* and *Zeaxanthinibacter,* have been detected in numerous other cold or marine environments (Asker et al., 2007; Du et al., 2016; Jogler et al., 2013; Kang et al., 2018; Matsumoto et al., 2009; Park et al., 2009; Roh et al., 2013). Our beta diversity analysis suggested distinct community composition between beaches, where clustering was largely explained by the beach duplicate collected. Such differentiation is congruent with the literature on the heterogeneity of beaches, where bacterial abundance may differ at a millimetre scale (Seymour et al., 2000). Overall, microbial communities clustered by geographic location with the sediments from Cambridge Bay as farthest from Resolute, Nanisivik and Alert (Fig. 2C).

Despite unique microbial community composition at each beach, mineralization of hexadecane and naphthalene was observed demonstrating pristine high Arctic beaches along the Northwest Passage harbour microbes capable of degrading hydrocarbons at low temperature (4 °C). Of the beaches used in the mineralization microcosms, sediment from Resolute had the highest abundance of potential hydrocarbon-degrading genera (Tank, 0.111 ± 0.012), followed by Cambridge Bay (0.076 ± 0.001), then Nanisivik East (0.031 ± 0.007). Screening for potential oil degraders was conducted using a set of genera previously implicated with hydrocarbon biodegradation in cold environments (Brakstad et al., 2017) and thus, may be an underestimate of the true hydrocarbon-degrading community. Nonetheless, the abundance reported is congruent with the observation that hydrocarbon-degrading genera may be present in up to 10% of a microbial community (Atlas, 1981) and the detection of oil degraders by enumeration in pristine high Arctic beach sediments (Røberg et al., 2007). The differences between the total abundance of potential hydrocarbon degrading genera across beaches may be explained by microbe enrichment from minor pollution events (Valentine et al., 2012) or from undiscovered oil seeps (Gautier et al., 2009). Oil seeps have been detected in areas of Baffin Bay (Blasco et al., 2010; Levy and Lee, 1988), which is over 500 km away from the nearest beach sample collected in this study.

While hexadecane and naphthalene mineralization were observed at each beach, the extent of mineralization was variable between beaches. In this study, Cambridge Bay showed the greatest extent of biodegradation relative to Nanisivik and Resolute. However, Resolute had the highest total proportion of potential hydrocarbon-degrading genera. Thus, a higher proportion of total hydrocarbon-degrading genera was not a reliable indicator of the extent of hydrocarbon biodegradation. Similar results have been obtained for pristine sub-Antarctic intertidal sediments where despite similarities in most probable number of hydrocarbon-degrading microorganisms initially, strong differences in extent of oil degradation were observed (Delille and Delille, 2000). Higher organic matter content may also positively impact the extent of

hydrocarbon biodegradation (Chen et al., 2020). Both the Resolute and Nanisivik beaches used in the mineralization experiments had similar organic matter contents (3.79 mg g^{-1} and 3.72 mg g^{-1}, respectively) which may explain their similar extent of hexadecane and naphthalene degradation. While physio-chemical analyses were not collected for Cambridge Bay, it is possible its lower latitude relative to Resolute and Nanisivik may correlate with a higher organic matter content (Paré and Bedard-Haughn, 2013), and ultimately, a greater extent of hydrocarbon biodegradation.

4.2. Influence of nutrients on oil biodegradation

Overall, the addition of a common inorganic N:P:K fertilizer had variable results on the biodegradation of hydrocarbons. Nutrients improved hexadecane and naphthalene biodegradation for Cambridge Bay and Nanisivik and increased depletion of headspace oxygen (a proxy for aerobic biodegradation) in Ultra Low Sulfur Fuel Oil (ULSFO) microcosms. However, nutrients had little effect on hexadecane and naphthalene biodegradation for Resolute samples and on alkane and polycyclic aromatic hydrocarbon (PAH) biodegradation in ULSFO microcosms. Nutrient enhanced hexadecane and naphthalene mineralization has been reported using hydrocarbon contaminated soils from the Canadian high Arctic, after the application of a similar 20:20:20 fertilizer and incubated at a similar temperature (5 °C) (Whyte et al., 2001). It is not surprising that nutrients had some positive impact on degradation since total nitrogen and phosphorus were in very low concentrations across the beaches. In fact, the lack of a measurable nutrient effect on alkane and PAH degradation was surprising, since previous studies on Arctic shorelines have successfully demonstrated enhanced nutrient biodegradation (Prince et al., 2003; Røberg et al., 2011; Eimjhellen et al., 1982). While nutrient concentration should not be too high to avoid adverse effects such as eutrophication (Macaulay and Rees, 2014), it is likely that the high concentration of ULSFO used (2000 ppm) rendered the minor nutrient addition (15 ppm) negligible.

Overall, it is likely that low incubation temperature and unacclimated sediment (i.e., no prior exposure to oil contamination) limited hexadecane and naphthalene mineralization. Unamended hexadecane degradation was similar to studies using hydrocarbon contaminated Arctic soils incubated at 5 °C (Børresen and Rike, 2007; Whyte et al., 2001), but was substantially lower than studies using hydrocarbon contaminated polar soils incubated at warmer temperatures (Mohn and Stewart, 2000; Aislabie et al., 2012). Nutrient enhanced hexadecane degradation was slightly lower than what has been reported in other nutrient treatments of similar environments, where hexadecane degraded ~18–30% in hydrocarbon contaminated high Arctic soils incubated at 5 °C (Whyte et al., 2001) and ~ 25–60% in hydrocarbon contaminated Antarctic coastal soils incubated at 15 °C (Aislabie et al., 2012). Unamended naphthalene degradation was generally lower than what has been observed in other studies, where naphthalene was ~2.5–20% degraded using hydrocarbon contaminated high Arctic soils incubated at 5 °C (Whyte et al., 2001) and where phenanthrene was 40–60% degraded using hydrocarbon-contaminated Arctic soils incubated at 7 °C (Mohn and Stewart, 2000). Nutrient enhanced naphthalene degradation was substantially lower than microcosms using hydrocarbon contaminated high Arctic soils incubated at 5 °C, where naphthalene degraded ~25–50% (Whyte et al., 2001). Whyte et al. (2001) also reported higher mineralization in hexadecane and naphthalene microcosms incubated at 23 °C rather than 5 °C.

Alongside the limitations of hexadecane and naphthalene mineralization, degradation of ULSFO was also likely limited by too high an oil concentration and too short an incubation period. In ULSFO-contaminated sediments, greater biodegradation of alkanes over aromatics was observed; however, overall degradation of these compounds was relatively low in comparison to previous Arctic biodegradation studies (Garneau et al., 2016; Lofthus et al., 2020; Sanscartier et al., 2009). Lofthus et al. (2020), who examined biodegradation with similar environmental parameters to the present study (i.e., unacclimated samples and a − 2 °C incubation temperature), but with a much lower crude oil concentration (2-3 ppm), reported slow biodegradation rates of alkanes and aromatics that were insignificant until after 55 days of incubation.

4.3. Influence of nutrients and ULSFO on microbial community structure and abundance of hydrocarbon biodegradation genes

Microbial analyses showed that the addition of both nutrients and oil altered community structure, resulting in an enrichment of *Pseudomonas* and *Rhodococcus*. Four distinct clusters were observed in the ordination of microbial communities from experimental microcosms suggesting the influence of both nutrients and oil in shaping community structure. Nutrients influencing microbial communities has been previously observed on Arctic shorelines, where an oleophilic fertilizer had a strong effect on community structure (Røberg et al., 2011). The significant differential abundance of *Pseudomonas* and *Rhodococcus* in oiled microcosms suggest these two genera may be being selected for to degrade oil at low temperature (4 °C). *Pseudomonas* has been detected previously within 16S rRNA gene clone libraries from oiled high Arctic shoreline sediments (Grossman et al., 1999); however, to our knowledge, this is the first detection of *Rhodococcus* from oiled high Arctic shoreline sediments. Nonetheless, both genera are well known oil degraders (Astashkina et al., 2015), and have been detected in other hydrocarbon-contaminated Polar environments including seawater (Brakstad and Bonaunet, 2006; Crisafi et al., 2016), ice (Gerdes et al., 2005) and soil (Rizzo et al., 2019; Ruberto et al., 2005; Whyte et al., 1997). In conjunction with the alpha diversity metrics, *Pseudomonas* and *Rhodococcus* are likely part of a diverse consortia that may be degrading hydrocarbons on high Arctic beaches.

Metagenomic analyses showed that the addition of nutrients and oil altered abundance of hydrocarbon biodegradation genes and suggested several genera that may degrade oil at low temperatures that have not been previously identified on High Arctic shorelines. Nutrients favoured higher abundances of the key genes for hydrocarbon degradation *alk*B and *CYP15*3. Moreover, *alk*B genes were associated with *Pseudomonas* and *Rhodococcus*, and *CYP153* genes were associated with *Rhodococcus*—supporting the importance of these genera in oil degradation on high Arctic shorelines. An increased abundance of *alk*B and *CYP153* genes and the active expression of hydrocarbon biodegradation genes of *Pseudomonas* and *Rhodococcus*, have also been described in diesel-contaminated soil biopiles in the Canadian high Arctic (Yergeau et al., 2012). The taxonomic classification of hydrocarbon biodegradation genes observed in ULSFO microcosms suggest several potential low-temperature alkane-degrading and aromatic-degrading genera that have not been previously detected on high Arctic shorelines. Many of the genera have been detected previously in oil-contaminated cold environments, including the alkane-degrading genera: *Sphingorhabdus*, *Sulfitobacter, Psychrobacter* and *Marinobacter* from polar and subpolar seawater (Crisafi et al., 2016; Prabagaran et al., 2007; Lofthus et al., 2020) and *Nocardiodes* from Antarctic sediments (Deng et al., 2015); and the aromatic-degrading genera: *Novosphingobium*, *Sphingobium* and *Alicyclobacillus* from permafrost (Yang et al., 2014), and *Hyphomonas* and *Sphingopyxis* from arctic seawater (Crisafi et al., 2016). Dominant genera within oil contaminated sea- and sub-ice microcosms from the Northwest Passage (*Moritella*, *Alcanivorax*, *Sulfitobacter*, and *Oleispira*) (Garneau et al., 2016), were not detected as abundant in the presented experimental microcosms (except for *Sulfitobacter*). This suggests Northwest Passage beaches may have a unique hydrocarbon-degrading consortia relative to other Northwest Passage environments, comprised predominantly with genera found in high Arctic soils (Yergeau et al., 2012) and polar and sub-polar seawater (Crisafi et al., 2016; Prabagaran et al., 2007; Lofthus et al., 2020), with hydrocarbon biodegradation response most similar to contaminated high Arctic soils (Whyte et al., 2001; Yergeau et al., 2012).

5. Conclusions

In this work, we characterized microbial communities of high Arctic beaches, assessed their potential to degrade hydrocarbons and tested biostimulation as a remediation strategy in the scenario of an oil spill. Across high Arctic beaches, community composition was distinct, potential hydrocarbon-degrading genera were detected at each beach, and microbial communities were able to degrade hydrocarbons (hexadecane, naphthalene, and alkanes) at low ambient temperature (4 °C) in lab microcosm assays. Nutrients improved the biodegradation of hexadecane and naphthalene, altered microbial community composition, and resulted in higher abundances of *alk*B and *CYP153* genes involved in alkane degradation. However, nutrients did not have a significant effect on the biodegradation of Ultra Low Sulfur Fuel Oil (ULSFO), alpha diversity metrics of microbial communities or the abundance of the *phnAc* gene involved in PAH degradation. As discussed, overall hydrocarbon biodegradation may have been limited by low incubation temperature, unacclimated sediment, concentration of inorganic nutrients supplied, concentration of ULSFO, and length of incubation. Future studies that address these limitations, while maintaining realistic High Arctic conditions, would further improve understanding of oil bioremediation in such extreme environments.

The use of high-throughput methodologies in parallel with characterizing oil biodegradation in this study has enhanced understanding of oil bioremediation and biodegradation on high Arctic shorelines. To date, knowledge of oil bioremediation and biodegradation on high Arctic shorelines is comprised mainly from three projects (Guénette et al., 2003; Røberg et al., 2007; Sergy and Blackall, 1987), where only two studies characterized microbial communities (Grossman et al., 1999; Røberg et al., 2011). In the present study, microbial analyses from the low temperature ULSFO microcosms suggested *Pseudomonas*, previously detected on high Arctic shorelines, and *Rhodococcus*, not previously detected on high Arctic shorelines, as important oil degrading genera. Metagenomic analyses further implicated several potential oil-degrading genera that have not previously been detected on high Arctic shorelines (*Nocardiodes, Sphingorhabdus*, *Sulfitobacter*, *Psychrobacter*, *Marinobacter*, *Novosphingobium*, *Sphingobium*, *Alicyclobacillus*, *Hyphomonas* and *Sphingopyxi*). Ultimately, this work enhances understanding of oil biodegradation and bioremediation on high Arctic shorelines in the event of a future spill in the extreme conditions of Canada's Northwest Passage.

CRediT authorship contribution statement

Madison Ellis: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft, Visualization. **Ianina Altshuler:** Conceptualization, Methodology, Writing – review & editing. **Lars Schreiber:** Methodology, Software, Writing – review & editing. **Ya-Jou Chen:** Writing – review & editing. **Mira Okshevsky:** Conceptualization, Methodology, Project administration. **Kenneth Lee:** Funding acquisition, Writing – review & editing. **Charles W. Greer:** Supervision, Resources, Funding acquisition, Writing – review & editing. **Lyle G. Whyte:** Supervision, Conceptualization, Resources, Funding acquisition, Writing – review & editing.

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

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