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

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Abstract

Unprecedented sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of an oil spill in the Arctic. 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 in Canada's Northwest Passage. In this study, high Arctic beach sediments were used to characterize physicochemical variables in parallel with microbial analyses including abundances of culturable and unculturable microorganisms and community composition using 16S rRNA gene sequencing, to screen for potential hydrocarbon-degrading genera, and to evaluate the response of microbial communities to degrade fuel constituents, hexadecane and naphthalene, and a heavy marine fuel, Bunker C, with nutrient biostimulation. A common inorganic nitrogen, phosphorus and potassium fertilizer was used to prepare nutrient-amended and unamended hexadecane and naphthalene mineralization microcosms and Bunker C contaminated microcosms. Similarities in physicochemical variables and microbial abundances were observed between high Arctic beaches, but microbial community composition was distinct at each beach. Potential oil-degrading genera were detected at each beach, but in different proportions (0.01-0.20 of the total community). Overall, biodegradation of hexadecane, naphthalene and Bunker C was greater in nutrient-amended relative to unamended microcosms. Differences between microbial communities and abundance of key hydrocarbon biodegradation genes (*alkB, CYP153*, *phnAc* and *ncr*) were observed between nutrient-amended and unamended heavy marine fuel microcosms. *Pseudomonas* and *Rhodococcus* were significantly differentially abundant in heavy marine fuel relative to uncontaminated microcosms. This work suggests high Arctic beaches harbour oil degrading microbes and that nutrient biostimulation is a viable

bioremediation strategy. Moreover, the microbial insights in this work broaden the current understanding of shoreline biodegradation, particularly in the Canadian context. Ultimately, this study serves to proactively contribute to oil-spill bioremediation strategies in Canada's Northwest Passage.

Résumé

La perte sans précédent de glace de mer ouvre des routes de navigation dans le passage du Nord-Ouest du Canada, ce qui augmente le risque de déversement de pétrole dans l'Arctique. L'exploitation des capacités des micro-organismes naturels à dégrader le pétrole peut constituer une stratégie efficace de remise en état des côtes touchées. Cependant, il existe très peu de données sur la biodégradation et la biorestauration des hydrocarbures sur les plages du Haut-Arctique, en particulier dans le passage du Nord-Ouest du Canada. Dans cette étude, les sédiments des plages de l'Extrême-Arctique ont été utilisés pour caractériser les variables physicochimiques en parallèle avec les analyses microbiennes, notamment l'abondance des microorganismes cultivables et non cultivables et la composition des communautés à l'aide du séquençage des gènes de l'ARNr 16S, et pour évaluer la réponse de ces communautés microbiennes à la dégradation des constituants des carburants, l'hexadécane et le naphtalène, et d'un carburant marin lourd, le Bunker C, par biostimulation des nutriments. Un engrais inorganique commun à base d'azote, de phosphore et de potassium a été utilisé pour préparer des microcosmes de minéralisation de l'hexadécane et du naphtalène, modifiés ou non, et des microcosmes contaminés par le Bunker C. Des similitudes dans les variables physicochimiques et les abondances cellulaires ont été observées entre les plages du Haut-Arctique, mais la composition de la communauté microbienne était distincte sur chaque plage. Des genres susceptibles de dégrader le pétrole ont été détectés sur chaque plage, mais dans des proportions

différentes (0,01-0,20 de la communauté totale). Dans l'ensemble, la biodégradation de l'hexadécane, du naphtalène et du Bunker C'était plus importante dans les microcosmes modifiés par les nutriments que dans les microcosmes non modifiés. Des différences entre les communautés microbiennes et l'abondance des principaux gènes de biodégradation des hydrocarbures (*alkB*, *CYP153*, *phnAc* et *ncr*) ont été observées entre les microcosmes de combustibles marins lourds modifiés et non modifiés. *Pseudomonas* et *Rhodococcus* étaient significativement plus abondants dans les combustibles marins lourds que dans les microcosmes non contaminés. Ces travaux suggèrent que les plages du Haut-Arctique abritent des microbes dégradant le pétrole et que la biostimulation des nutriments est une stratégie de biorestauration viable. De plus, les connaissances microbiennes de ce travail élargissent la compréhension actuelle de la biodégradation du littoral, en particulier dans le contexte canadien. En fin de compte, cette étude contribue de manière proactive aux stratégies de biorestauration des marées noires dans le passage du Nord-Ouest du Canada.

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Contribution of Authors

Chapter 1: Literature Review

Madison Ellis, the MSc candidate, wrote the literature review with guidance and editing provided by Dr. Lyle G. Whyte.

Chapter 2: Hydrocarbon biodegradation potential of microbial communities from high Arctic beaches in Canada's Northwest Passage

This manuscript was made in collaboration with Dr. Ianina Altshuler, Dr. Mira Okschevsky and Dr. Lyle G. Whyte from McGill University and with Dr. Lars Schreiber and Dr. Charles W. Greer from National Research Council. Madison Ellis, the MSc candidate, conducted all laboratory work, data analysis and wrote the manuscript. Dr. Ianina Altshuler helped with experimental design and laboratory assistance. Dr. Lars Schreiber assisted with data analysis and provided manuscript guidance. Dr. Mira Okschevsky assisted with experimental design. Dr. Charles Greer assisted with sample collection and provided overall guidance. Dr. Lyle G. Whyte performed sample collection, provided overall guidance, and edited the manuscript.

Chapter 3: Discussion and Conclusion

Madison Ellis, the MSc candidate, wrote the discussion and conclusion with guidance and editing provided by Dr. Lyle G. Whyte.

Introduction

Unprecedented sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of an oil spill in the high Arctic (Overland and Wang 2013, Smith and Stephenson, 2013). 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 in Prince William Sound, Alaska, showed that 26 years later, an estimated 55,600 kg of sequestered oil persisted and notably, has remained unchanged from the 2001-2015 period (Short et al. 2004; Maselko et al. 2018). This persistence highlights not only the inherent challenges of oil spill clean-ups, which would be even more difficult in the Arctic's remote and harsh climate (Balaji et al. 2014), but also the need for improved long-term oil spill clean-up strategies. Naturally occurring microorganisms play an integral role in oil spill clean-up by metabolizing various oil compounds, known as hydrocarbon biodegradation (Atlas and Hazen 2011). Hydrocarbon biodegradation can be improved through various supplementary processes to circumvent environmental constraints, known as bioremediation strategies (Atlas and Cerniglia 1995). Oil-degrading microorganisms are found across 175 prokaryotic genera (Hazen et al. 2016) and, thus, are considered ubiquitous in the environment.

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 in Canada's Northwest Passage. Knowledge of hydrocarbon biodegradation on high Arctic shorelines to date is largely based on analyses from two large scale field experiments: the *Baffin Island Oil Spill* (BIOS) in the Canadian High Arctic in 1980 (Sergy and Blackall 1987) and the *Svalbard*

shoreline field trials in Spitsbergen, Norway in 1997 (Guénette et al. 2003). While these studies enhanced understanding of hydrocarbon biodegradation and bioremediation on high Arctic beaches, the characterization of microbial communities had limited resolution and was only conducted in Norway, highlighting the limited knowledge of hydrocarbon biodegradation on high Arctic beaches in Canada's Northwest Passage. In contrast to these studies, recent studies have used high throughput methodologies to advance the understanding of oil degrading microbial communities and function in other high Arctic environments including marine waters (Ribicic et al. 2018; Vergeynst et al. 2019; Krolicka et al. 2019), soils (Yergeau et al. 2012; Ferguson et al. 2020) and ice (Garneau et al. 2016; Vergeynst et al. 2019; Lofthus et al. 2020). The aim of this work is to expand the knowledge regarding hydrocarbon biodegradation and bioremediation on high Arctic shorelines, particularly in Canada's Northwest Passage by combining high throughput methodologies in parallel with characterization of oil biodegradation.

In this study, high Arctic beach sediments were used for three objectives: 1) to characterize the abundance and composition of naturally-occurring microbial communities on high Arctic beaches; 2) to explore whether hydrocarbon-degrading genera were present on high Arctic beaches; and 3) to evaluate the response of naturally occurring microbial communities to degrade fuel constituents, hexadecane and naphthalene, and a common marine heavy fuel oil, Bunker C, with nutrient biostimulation. The first goal was addressed by surveying 8 high Arctic beaches across four locations, including 7 beaches in 3 locations of the Northwest Passage, to assess physicochemical variables in parallel with microbial analyses including abundances of culturable and unculturable microorganisms and community composition using 16S rRNA gene sequencing. The second goal was address by screening sequence data of the microbial communities for the detection of known hydrocarbon-degrading genera. Finally, the third goal

was addressed using a common inorganic nitrogen, phosphorus and potassium fertilizer in nutrient-amended and unamended microcosms using fuel constituents, hexadecane and naphthalene, and the heavy marine fuel, Bunker C oil. Mineralization of hexadecane and naphthalene was assessed over 24 weeks using beach sediment from 3 beaches. Oil degradation in heavy marine fuel microcosms was determined using oxygen measurements and chemical analyses (alkane and aromatic fractions) in parallel with characterization of microbial community composition using 16S rRNA gene amplicon sequencing over 55 days using sediment from 1 beach. The abundance of key hydrocarbon biodegradation genes in nutrient-amended and unamended heavy marine fuel microcosms was assessed through metagenomic analysis. Given the ubiquity of hydrocarbon-degrading genera and the success of biostimulation in other studies, it is hypothesized that high Arctic beaches will harbor communities capable of degrading hydrocarbons that will be improved by nutrient biostimulation.

Chapter 1: Literature Review

1.1 Introduction

Unprecedented sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of oil spills in the High Arctic (Overland and Wang 2013; Smith and Stephenson 2013; Haas and Howell 2015). The Northwest Passage is a network of islands and gulfs in Canada's High Arctic (Fig. 1(i)). It is non-navigable most of the year due to near annual sea-ice cover. However, global warming is driving unprecedented sea-ice loss such that navigable and economically-favourable shipping routes in the Northwest Passage are projected by mid century (Fig. 1(ii); Smith and Stephenson 2013; Haas and Howell 2015). Ultimately, increased shipping traffic increases the risk of oil spills—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 in Prince William Sound, Alaska, showed that 26 years later, an estimated 55,600 kg of sequestered oil persisted and notably, has remained unchanged from the 2001-2015 period (Short et al. 2004; Maselko et al. 2018). This persistence highlights not only the inherent challenges of oil spill clean-up, which would be even more difficult in the High Arctic's remote and harsh climate (Balaji et al. 2014, Li et al. 2016), but also the need for improved long-term oil spill clean-up strategies.

Understanding the fate of oil released into the environment requires an understanding of its chemical composition. Hydrocarbons are compounds composed exclusively of hydrogen and carbon atoms and they constitute the dominant components of crude oil and processed petroleum products including gasoline, diesel and fuel oil (Abrajano Jr. et al. 2007). Hydrocarbons are

primarily grouped based on carbon-carbon bond arrangement: single (alkanes), double or triple (alkenes or olefins, and alkynes), or cyclic (aromatics) (Abrajano Jr. et al. 2007). Crude oil is composed primarily of alkane compounds in linear, branched or cyclic arrangements, and aromatic compounds (Abrajano Jr. et al. 2007). A minor component of crude oil is aromatics with two or more fused benzene rings known as polycyclic aromatic hydrocarbons (PAHs). Although PAHs are only a minor component of crude oil, they are particularly persistent and toxic in the environment (Cerniglia 1993; Abrajano Jr. et al. 2007) and are classified as chemicals of emerging Arctic concern (AMAP 2017). Their persistence in the environment is inversely related to their susceptibility to degradation, which for all hydrocarbons is related to their physical and chemical structure (Chandra et al. 2013).

When oil enters the marine environment, its chemical composition is immediately altered by several weathering processes including photooxidation and evaporation resulting in the loss of low molecular weight, volatile and water-soluble hydrocarbon molecules and leading to increased density and viscosity of residual oil (Fig. 1 (iii); Tarr et al. 2016; Wang et al. 2020). The remaining oil is further subject to natural attenuation processes—letting abiotic and biotic factors in the environment remove contamination (Tarr et al. 2016). In the marine system, natural attenuation is favoured by dissolution, emulsification and dispersion processes that favour physical-, chemical- and microbial- oil degradation (Alonso-Gutierrez et al. 2006; Tarr et al. 2016). However, physical processes may inhibit natural attenuation such as when oil mixes with sediment or other detritus forming aggregates that sink and may become trapped within marine sediments or that are deposited on shorelines (Warnock et al. 2015; Tarr et al. 2016). As oil is deposited on shorelines, in the form of liquid or aggregates, natural attenuation processes including evaporation, photo-oxidation and microbial biodegradation continue, however the

success of natural attenuation depends on several unique features of the shoreline environment including environmental conditions and geomorphic characteristics (Cravo-Laureau and Duran 2014; Wang et al. 2020). Of natural attenuation processes, this project is particularly interested in microbial-mediated oil degradation, known as hydrocarbon biodegradation.

Naturally occurring microorganisms play an integral role in oil spill clean-up by metabolizing various oil compounds (Atlas and Hazen 2011). When oil becomes available to microbial attack, biodegradation of most compounds occurs simultaneously, but at different rates (Atlas 1988). Rates are associated to chemical structure, whereby microorganisms readily utilize simple over complex hydrocarbons in oil mixtures (*i.e.* n-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes) (Perry 1984). What enables microorganisms to degrade hydrocarbons is the presence of a suite of genes that encode for multiple enzymes that enable metabolism of various hydrocarbons (Van Hamme et al. 2003). Oil-degrading microorganisms are found across 175 prokaryotic genera (Hazen et al. 2016) and, thus, are considered ubiquitous in the environment. Some marine microorganisms are described as obligate hydrocarbonoclastic bacteria (OHCB) (Yakimov et al. 2007), capable of degrading only hydrocarbons as their sole carbon source, though this phenomenon is contested (Radwan et al. 2019). Oil-degrading genera may be at low abundance levels in unpolluted environments, but shift to dominating abundance in the presence of oil (Atlas 1981). Prior pollution events may also enrich, or seed, hydrocarbon-degrading microorganisms through an autoinoculation effect, thus enhancing microbial degradation in response to future oil contamination in these environments (Valentine et al. 2012). Ultimately, while many microbes possessing abilities to degrade hydrocarbons are widespread in the environment, the success of degradation is often limited by environmental constraints.

Hydrocarbon biodegradation can be improved through various supplementary processes, known as bioremediation strategies, to circumvent environmental constraints (Atlas and Cerniglia 1995; Chaudhary and Kim 2019). These *in situ* strategies facilitate microbial oil metabolism by enhancing 1) the availability of limiting abiotic variables via biostimulation through supplying nutrient fertilizers or supplying oxygen through tilling (Leahy and Colwell 1990, Lindstrom et al. 1991), 2) oil bioavailability via chemical dispersants (Prince et al. 2016), and 3) the microbial system by inoculating with an established hydrocarbon-degrading consortia via bioaugmentation (Tyagi et al. 2011). Biostimulation with limiting nutrients is considered a favourable approach as it is cost-effective, improves degradation, and has minimal environmental impact (Macaulay and Rees 2014). In contrast, tilling, which helps to supplement limiting oxygen, is considered less favourable as it is destructive to the natural environment(Atlas and Bragg 2009). Dispersants, which enhance oil bioavailability by chemically breaking up oil into smaller droplets, are also considered a favourable approach, particularly in seawater and marine environments (Prince 1997; Tremblay et al. 2017, 2019; Ribicic et al. 2018b). Finally, bioaugmentation has been widely explored in laboratory testing, but is considered less favourable for *in situ* remediation as uncertainty remains about the efficacy over, and disturbance to, indigenous microorganisms (Van Hamme et al. 2003; Macaulay and Rees 2014).

Hydrocarbon biodegradation and bioremediation has been widely studied (Van Hamme et al. 2003; Varjani 2017); however, findings are difficult to generalize across systems, and many ecosystems, such as Arctic shorelines and beaches, remain understudied. While there is an understanding of many microbes and pathways involved in hydrocarbon degradation, and how they contribute to an array of effective bioremediation strategies, hydrocarbon biodegradation

depends on several factors that vary considerably across environments including microbial community composition, pollutant characteristics, concentration of oxygen and nutrients, and temperature (Varjani 2017). Overall, rates of hydrocarbon biodegradation are greater in warmer over colder temperatures, aerobic over anaerobic conditions, in low over high oil concentrations, and nutrient-rich (nitrogen, phosphorus and iron) over nutrient-limited environments (Atlas 1988). Ultimately, hydrocarbon biodegradation and bioremediation on high Arctic beaches in Canada's Northwest Passage requires consideration of factors affecting biodegradation on shorelines in cold environments and of methods to assess biodegradation and bioremediation.

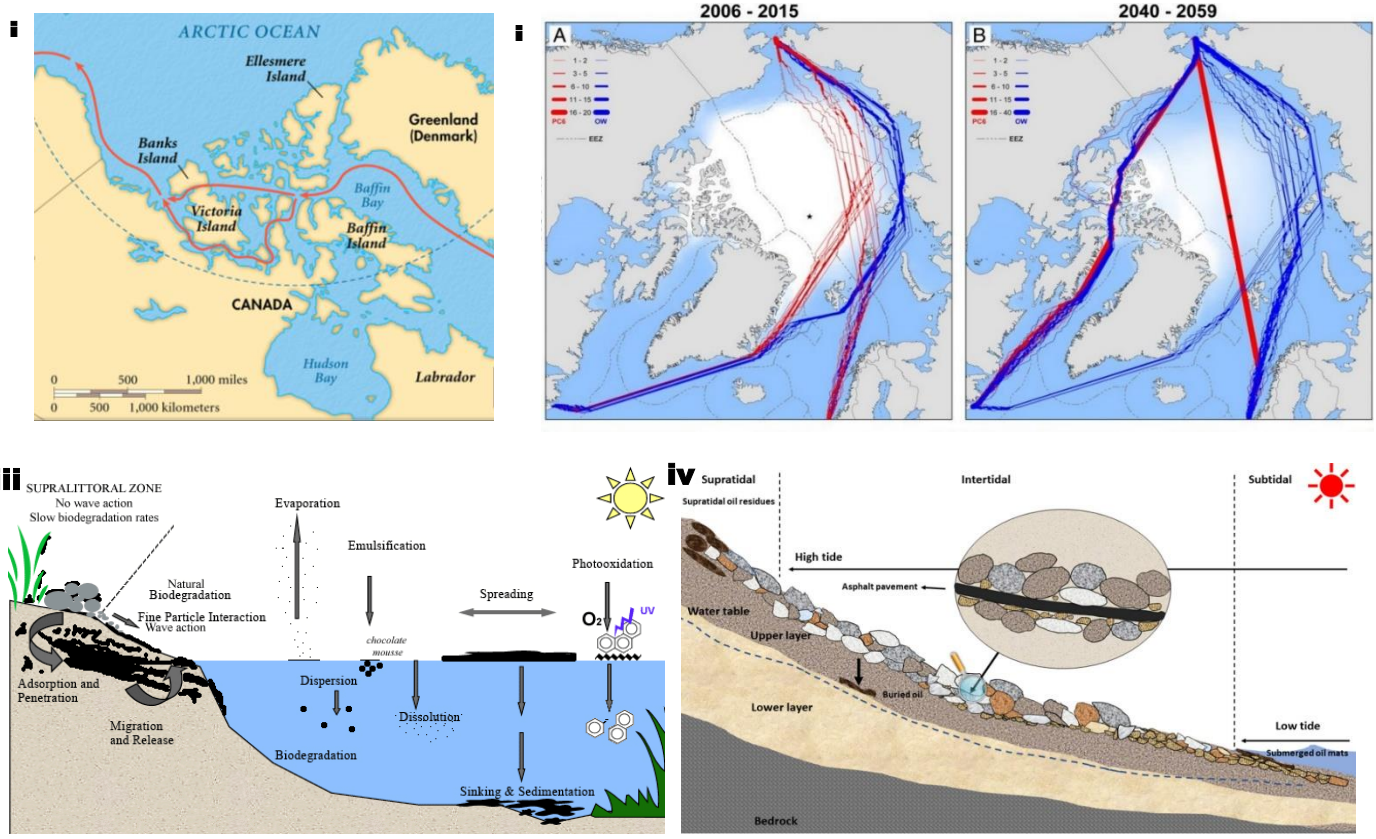

Figure 1. (i) Schematic representation of Canada's Northwest Passage (Encyclopædia Britannica); (ii) Model-derived projection of September navigation routes as per medium-low climate scenarios on sea ice concentration and thickness, highlighting increased shipping traffic (red/blue lines) through the Northwest Passage by mid century (Smith and Stephenson 2013); (iii) Overview of hydrocarbon biodegradation processes (Alonso-Gutierrez et al. 2006); and (iv) Overview of beach zones and

1.2 Factors affecting hydrocarbon biodegradation on shorelines in cold environments

1.2.1 Beach dynamics

When oil is transported to shorelines, it is distributed in different areas due to underlying beach sediment properties and hydraulics (tide, waves and groundwater flow) (Boufadel et al. 2019; Wang et al. 2020). Beach sediment can vary distinctly in its physical composition between rocky, coarse-grained and fine-grained sediment, that impact how oil behaves as surface or subsurface residue (Gundlach et al. 1985; Wang et al. 2020). Marine tar residues, which can be formed by oil-sediment interactions, may take the form of microscopic oil-particle aggregates with fine sediments interspersed, a physical form that may facilitate natural remediation; alternatively, marine tar residues may take the form of macroscopic oil-sediment aggregates or asphalt pavement with coarse sediments, which may be harmful to natural remediation (Gustitus and Clement 2017). Physical composition also affects oil penetration (vertical depth), with rocky shorelines more permeable than fine-grained sediment (Wang et al. 2020). Rates of hydrocarbon biodegradation are slower at greater depths where nutrient and oxygen levels are lower as observed in marine sediments where phenanthrene degradation was 2-3 times more rapid in surface sediments (0-3 cm) relative to deeper sediments (>6 cm) (Tang et al. 2006). Moreover, oil sequestered in subsurface sediments may remain more toxic and persistent in the long-term as observed with sequestered oil following *Exxon Valdez* over 20 years later (Maselko et al. 2018).

Beach hydraulics encompassing tides, waves and groundwater flow, play an important role in oil biodegradation on shorelines (Boufadel et al. 2019). Shorelines consist of beach sediment differentiated in many separate zones; however, zones of particular relevance for biodegradation include the intertidal zone (region between mean low tide and mean high tide)

and the supratidal zone (above the high tide level, but subject to occasional water action from storms) (Fig. 1(iv); Solo-Gabriele et al. 2016; Wang et al. 2020). Beach zones experience different environmental conditions, whereby beach sediment near and above the high tide experience extensive drying and highly variable temperature largely influenced by the atmosphere, whereas beach sediment near low tide experience extensive water saturation and more constant temperatures related to sea water temperatures (Pollock and Hummon 1971). These variations translate into different hydrocarbon biodegradation outcomes as demonstrated in model-simulations, whereby subsurface hydrocarbon biodegradation occurred at greater depths near the upper intertidal zone than the lower intertidal zone due to differential oxygen concentrations (Geng et al. 2015). However, tidal and wave action differentially affect the shoreline whereby the upper intertidal zone is likely to experience greater persistence of high oil concentration as observed in a field experiment on Baffin Island, in the Canadian high Arctic (Owens et al. 1987a) and following *Exxon Valdez* (Taylor and Reimer 2008).

Groundwater flow and leaching typically enhances oil biodegradation on shorelines. The presence of strong ground water flow may limit depth of oil penetration, particularly during low tides (Boufadel et al. 2019), as observed in an *Exxon Valdez* impacted gravel beach (Guo et al. 2010). One recent temperate shoreline study suggests supra-permafrost groundwater may also deliver large amounts of freshwater and dissolved organic carbon and nitrogen to coastal regions daily during late summer (Connolly et al. 2020). This indicates that groundwater leaching may not only limit oil penetration on shorelines, but also may facilitate microbial biodegradation through increasing the supply of nitrogen and phosphorus to microbes. Groundwater leaching was a contributing element to oil removal on Baffin Island beaches in the Canadian high Arctic through a washing effect (Owens et al. 1987a); however, the precise relationship between

groundwater nutrients and intertidal nutrients on Arctic beaches remains unexplored.

1.2.2 Physicochemical variables

Many physicochemical variables affect hydrocarbon biodegradation on shorelines in cold environments including nutrients, salinity, and pH. Given petroleum is carbon rich, microorganisms must obtain mineral nutrients including nitrogen, phosphorus and iron from the environment to effectively metabolize petroleum compounds (Atlas 1988). The importance of nitrogen and phosphorus on shorelines has been widely characterized as these nutrients are often limited both in surface and subsurface sediments (Prince 1993; Boufadel et al. 2010). In cold environments, freeze-thaw cycles further affect water availability in the sediments and thus, the distribution of inorganic nutrients (Chaudhary and Kim 2019). Both salinity and pH also effect cellular functions. Hydrocarbon biodegradation is considered to be optimal in a pH range of 6 to 9 (Das and Chandran 2011), and can be adjusted for with a buffering system (Chaudhary and Kim 2019). Greater biodegradation of crude oil has been observed in Arctic shoreline sediments where salinity was slightly higher (3.5% from 3.0%) (Sharma and Schiewer 2016).

The presence of oxygen is an important determinant to whether aerobic or anaerobic hydrocarbon biodegradation occurs. Low oxygen concentrations may limit aerobic hydrocarbon biodegradation, as oxygen is required both for the action of microbial attack via catabolic enzymes including monooxygenases, dioxygenases, dehydrogenases, hydrolases, and transferases, and as a terminal electron acceptor (Atlas and Cerniglia 1995; Van Hamme et al. 2003). In the presence of oxygen, aliphatic hydrocarbons are degraded via monoterminal-, biterminal- or subterminal- oxidation pathways and aromatic hydrocarbons are degraded via mono- and di- oxygenases pathways (Chaudhary and Kim 2019). While oxygen concentrations

may be less rate-limiting on shoreline surfaces, oxygen concentration within interstitial water varies with depth (Jansson 1967; Maselko et al. 2018). Following *Exxon Valdez,* both nutrients and dissolved oxygen were thought to be limited in subsurface sediments suggesting anaerobic degradation occurred (Boufadel et al. 2010, 2016). During anaerobic degradation, a comparatively slower degradation process, the rate-limiting factor is availability of suitable electron acceptors including nitrate, sulfate, ferrous iron and manganese (Tang et al. 2006; Chaudhary and Kim 2019).

1.2.3 Temperature

Temperature plays a critical role in determining hydrocarbon biodegradation potential on Arctic shorelines. The Arctic's climate is one of the world's harshest. Decreased hydrocarbon biodegradation rates are associated with lower temperatures as a consequence of decreased rates of enzymatic activity, known as the Q_{10} effect (Atlas 1991; Sharma and Schiewer 2016). However, cold-adapted microorganisms are able to function at lower temperatures including psychrotrophs that tolerate temperatures between 0-5°C, but grow optimally at >15°C, and psychrophiles that tolerate 0°C temperatures, but grow optimally between 0-15°C (Margesin and Schinner 1999). Hydrocarbon biodegradation has even been documented below 0°C (Rike et al. 2005). Cold-adapted microorganisms function at lower temperatures through various evolutionary modifications that enable cellular activity and protection including cold-active enzymes, cold- shock and acclimation proteins and the production of cryoprotective substances (Margesin et al. 2007; Giudice et al. 2010).

While cold-adapted microorganisms are widely distributed and can be active at low temperatures (Margesin and Schinner 2001), optimal activity still depends on thaw conditions

and oil bioavailability. Thawing of the nearshore intertidal environment is influenced first by surface snow and ice coverage, then by a subsurface frost-table which can only begin to thaw once the surface is exposed and seawater begins to infiltrate (Owens and Harper 1977). It was estimated that beaches on Baffin Island in the Canadian high Arctic experienced approximately 63 ice-free days a year for the 1981-2002 period (Prince et al. 2002a); however, this approximation is likely an underestimate for the current and future climates (Farquharson et al. 2018). Low temperatures not only affect microbial activity, but also affects oil properties. At lower temperatures, biodegradation is delayed due to increased oil viscosity and decreased rates of volatilization, solubility and dissolution (Atlas 1991; Ribicic et al. 2018a). A recent study using models to elucidate the relationship between oil properties and temperature in seawater proposed that with heavier oils at low temperatures, indeed, biological availability may be more rate-limiting than biological activity (Nordam et al. 2020).

1.3 Methods to assess biodegradation and bioremediation potential

1.3.1 Microcosm experiments

Assessing the potential for biodegradation and bioremediation on Arctic shorelines necessitates proactive approaches that emulate oil contamination. While oil spills, accidental or controlled, enable a large-scale assessment of observed biodegradation and potential remediation in actual conditions (Sergy and Blackall 1987; Pritchard et al. 1992; Atlas and Hazen 2011), they are nonetheless large-scale pollution events. Microcosm experiments enable exploration of ecological patterns with advantages of control, replication, post-hoc analysis and range of scale

though face criticisms for being contrived, simplistic and not truly representative of the macro-world (Jessup et al. 2004). In contrast to laboratory microcosms, *in situ* microcosms (used in the field), circumvent these criticisms (Kaster and Richnow 2010). Microcosms enable control over factors that influence biodegradation such as oil type, oil concentration, nutrients, oxygen, salinity, and temperature as well as the ability to examine different bioremediation strategies.

1.3.2 Quantifying oil biodegradation

Assessing biodegradation requires measures of oil loss or oil composition changes. One approach is to use isolated components of oil in experiments that utilize principles of mineralization *i.e.* oil biodegradation is derived from production of respired carbon dioxide or other gases (Phillips and Nickerson 2015; Pitombo et al. 2018). In hydrocarbon biodegradation studies, these respirometry assays typically utilize radiolabeled substrates and are commonly referred to as mineralization or mineralization assays (Steven et al. 2007; Aislabie et al. 2012). Another approach is to quantify from an oil mixture, the concentrations of total petroleum hydrocarbons, groupings such as aliphatics and aromatics or specific analytes through analytical chemistry methods such as gas chromatography mass spectrometry (Reddy and Quinn 1999; Prince and Douglas 2005). While both respirometry and analytical chemistry methods provide insight into the rate of biodegradation, respirometry can provide rapid insight into the biodegradation of a specific compound, whereas analytical methods provide insight into changes in chemical composition, encompassing information about specific compounds (Balba et al. 1998). While each of these methods for quantifying oil biodegradation has their own advantages and limitations, combining both methods provide a complimentary approach to assessing oil biodegradation.

1.3.3 Characterizing microbial communities and functional potential

Hydrocarbon biodegradation can be further elucidated through an understanding of microbial diversity and community function. Overall, microbial methods can be divided into culture-dependent and culture-independent approaches, each method with its own purpose and limitations (Van Hamme et al. 2003; Knight et al. 2018). As molecular technologies advanced, recent hydrocarbon biodegradation studies have focused on characterizing microbial diversity and potential hydrocarbon degraders through marker-gene sequencing (Bidja Abena et al. 2020) and on characterizing high resolution taxonomy and function through determining which genes are present in a community through metagenomics and through determining which genes are expressed in a community through metatranscriptomics (Narasimhan et al. 2016; Tremblay et al. 2019). These metrics provide insight into biodegradation and bioremediation potential as the sequence data from natural unpolluted environments can be mined for known hydrocarbon biodegradative microorganisms and key hydrocarbon biodegradative genes. This biomonitoring approach using biomarker taxa has been proposed in deep-water subarctic sediments (Gontikaki et al. 2018) and using biomarker genes has been proposed in Polar soils (Gittel et al. 2015).

While hydrocarbon-degrading microorganisms are ubiquitous as described previously, of particular interest on Arctic shorelines would be cold-adapted genera such as those previously described from Polar environments including: *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* (Table 1;Brakstad et al. 2017)). Of these genera, the majority are associated to the phylum Proteobacteria, a large phylum of

gram-negative organisms, encapsulating the alphaproteobacteria, gammapreoteobacteria and epsilonproteobacteria classes (Prince et al. 2018). Collectively the genera in this phylum are associated to degrading a broad range of hydrocarbon substrates including alkanes, phenanthrene, dibenzofuran, crude oil, gas oil, biphenyl and polycyclic aromatic hydrocarbons (Prince et al. 2018).

Metagenomic analysis enables the widespread screening for key alkane- and aromatic-degradation genes that have been experimentally characterized (Table 2) and enables the assembly of genomes to further elucidate relevant microbes and degradation genes. Aerobic alkane-degradation involves the genes *AlkB* and *CYP153* as alkane hydroxylases, which have been detected in 369 and 87 genomes, respectively, across 85 and 30 genera, respectively from terrestrial, freshwater and marine environments (Nie et al. 2014). Moreover, in extreme conditions, a thermophilic *Geobacillus* has demonstrated broad range alkane degradation via *ladA* genes rather than *alkB* genes (Boonmak et al. 2014) and homologous sequences have been described in cold-adapted genomes (Bowman and Deming 2014). Anaerobic alkane degradation has been demonstrated with *masD/AssA* genes from cold marine sediments (Gittel et al. 2015) and affiliated in the draft genome of an uncultivated Firmicute derived from single-cell sorting of methanogenic alkane-degrading cultures (Tan et al. 2014). Aerobic polycyclic aromatic hydrocarbon (PAH) degradation has been demonstrated with *phnAc* genes from Polar soils (Ding et al. 2010) and from coastal sub-Antarctic marine sediments (Lozada et al. 2008). Anaerobic PAH degradation has been demonstrated with *ncr* genes from groundwater and sediment samples (Morris et al. 2014).

Table 1. Taxonomy of Arctic and Antarctic oil-degrading bacteria (modified from Brakstad et al. 2017, source references therein).

Class	Family	Genus	Source[a]
Alphaproteobacteria	Rhodobacteraceae	*Loktanella*	Ar, SW
		Sulfitobacter	Ar, SW
	Sphingomonadaceae	*Sphingopyxis*	Ar, SW
		Sphingomonas	An, SW
Gammaproteobacteria	Alteromonadaceae	*Alteromonas*	SW
		Glaciecola	Ar, SI
		Marinobacter	An, Ar, SI, SW
	Colwelliaceae	*Colwellia*	An, Ar, S, SI, SW
		Thalassomonas	SW
	Moritellaceae	*Moritella*	Ar, S, SI, SW
	Pseudoalteromonadaceae	*Algicola*	Ar, SI
		Pseudoalteromonas	An, Ar, S, SI, SW
	Psychromonadaceae	*Psychromonas*	Ar, SW
	Shewanellaceae	*Shewanella*	An, Ar, S, SI, SW
	Alcanivoracaceae	*Alcanivorax*	Ar, S, SW
	Oceanospirillaceae	*Marinomonas*	An, Ar, S, SI, SW
		Oleispira	Ar, An, SI, SW
	Halomonadaceae	*Halomonas*	An, Ar, S, SI SW
	Moraxellaceae	*Psychrobacter*	Ar, SW
	Pseudomonadaceae	*Pseudomonas*	An, Ar, S, SI, SW
	Piscirickettsiaceae	*Cycloclasticus*	Ar, S, SW
Epsilonproteobacteria	Campylobacteraceae	*Arcobacter*	An, Ar, SW
Bacteroidetes	Cytophagales	*Cytophagia*	An, SW
Flavobacteriia	Flavobacteriaceae	*Ulvibacter*	Ar, SW
		Polaribacter	Ar, SI, SW
Actinobacteria	Nocardiaceae	*Rhodococcus*	An, SW
	Microbacteriaceae	*Agreia*	Ar, SI, SW
		Arthrobacter	An, SW

[a]*An* Antarctic, *Ar* Arctic, *S* sediment, *SI* sea ice, *SW* seawater

Table 2. Key hydrocarbon biodegradation genes.

Gene	Degradation	Method	Environment	References
alkB	Alkanes	Genome/metagenome	Terrestrial, freshwater, marine	Nie et al. 2014
CYP153	Alkanes	Functional assay	-	Van Beilen et al. 2006
		Genome/metagenome	Terrestrial, freshwater, marine	Nie et al. 2014
ladA	Alkanes	Genome	Petroleum reservoir	Boonmak et al. 2014
masD	Alkanes (anaerobic)	Genome	Oil sand tailing ponds	Tan et al. 2014
		Functional assay	Cold, marine sediments	Gittel et al. 2015
phnAc	PAHs	Functional assay	Cold, marine sediments	Lozada et al. 2008
			Polar soils	Ding et al. 2010
ncr	PAHs (anaerobic)	Functional assay	Groundwater, sediment	Morris et al. 2014

1.4 Hydrocarbon biodegradation and bioremediation studies on Arctic shorelines

1.4.1 Biostimulation as an approach to oil spill clean-up on Arctic shorelines

The most common approach for shoreline bioremediation is through biostimulation using nitrogen and phosphorus since these nutrients become limited once a concentrated supply of carbon-rich substrates is introduced into an environment following an oil spill and, importantly, have been shown to be effective for improving hydrocarbon biodegradation (Sergy et al. 2003; Prince 2005; Røberg et al. 2007; Prince et al. 2015). Two common approaches to nitrogen and phosphorus biostimulation are through the addition of 1) inorganic fertilizers and 2) slow release fertilizers, such as Inipol EAP 22. The Exxon Valdez clean-up was the first large-scale bioremediation effort that utilized the application of Inipol EAP 22 demonstrating *in situ* success (Swannell et al. 1996; Prince et al. 2015). Inorganic fertilizers supply nutrients immediately upon dissolution, whereas slow release fertilizers are coated such that nutrients are released more slowly over time as the coating breaks down. Inipol EAP 22 was a specialized oleophilic, slow release fertilizer designed to deliver nutrients, enhance oil bioavailability and be less susceptible to wash-out by wave action (Ladousse and Tramier 1991); however, this particular fertilizer is no longer manufactured, though other similar agents are available (United States Environmental Protection Agency 2020). While studies often highlight the potential advantages of slow-release fertilizers, related mainly to increased nutrient delivery efficiency and decreased cost due to fewer applications, slow-release fertilizers may be less effective than inorganic nutrient fertilizers at lower temperatures (<15° C) due to reduced coating permeability (Lee et al. 1993). Determining appropriate quantities and application rates with either fertilizer class is imperative to avoid potential adverse effects such as eutrophication (Macaulay and Rees 2014). Overall, it has been recommended use Redfield stoichiometry (a marine ratio of carbon, nitrogen and

phosphorus of 106:16:1, respectively) and nitrogen concentrations less than 2000 mg/kg (Filler et al. 2006), or concentrations that aim for ratios of carbon/nitrogen and carbon/phosphorous near 10:1 and 10:0.3, respectively (Oh et al. 2001).

1.4.2 Existing Arctic hydrocarbon biodegradation and bioremediation studies

Knowledge of hydrocarbon biodegradation on high Arctic shorelines to date is largely based on analyses from two large scale field experiments: the *Baffin Island Oil Spill* (BIOS) in the Canadian High Arctic in 1980 (Sergy and Blackall 1987) and the *Svalbard shoreline field trials* in Spitsbergen, Norway in 1997 (Guénette et al. 2003). During BIOS, a medium gravity crude oil was released in the nearshore environment to evaluate the fate and effects of spilled oil washed onto the shoreline and in the shoreline environment to evaluate various clean-up techniques including burning, mixing, chemical surfactants, solidifying agents and flushing (Owens et al. 1987b) and the use of nutrients in the supralittoral shoreline (above high tide) (Eimjhellen et al. 1982). Findings from BIOS suggested that despite several weathering 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). During the Svalbard field trials, an intermediate fuel oil was applied to the upper intertidal and supratidal zone and various remediation strategies including bioremediation, mixing and sediment relocation were examined (Guénette et al. 2003). Findings from the Svalbard field trials demonstrated that the use of nutrient-fertilizers on Arctic shorelines doubled the rate of biodegradation of an intermediate fuel oil (Sergy et al. 2003), increased microbial biomass as determined by phospholipid fatty acid analysis (Prince et al. 2003) and altered relative abundance of microbial taxa characterized using 16S rRNA gene clone library sequencing (Grossman et al. 1999).

Other *in situ* high Arctic beach studies in Svalbard have explored the effects of using an oleophilic nutrient fertilizer on beach sediments contaminated with the light oil, kerosene, demonstrating increased abundance of hydrocarbon-degrading genera through enumeration estimated through most probable number cultivation (Røberg et al. 2007) and altered community structure as characterized through 16S rRNA gene-based fingerprinting and clone library analyses (Røberg et al. 2011). Both of the *in situ* studies conducted in Svalbard (Prince et al. 2003; Røberg et al. 2011) provided insight into potentially relevant hydrocarbon-degrading microorganisms on high Arctic beaches. Grossman et al. (1999) found Gammaproteobacteria and Bacteroidia were dominant groups, associated to sulfur-oxidizing bacteria, enteric bacteria and the genera: *Oceanospirillum, Legionella* and *Cytophaga.* Similarly, Røberg et al. (2007) also found Gammaproteobacteria and Bacteroidetes to be dominant groups along with Actinobacteria, and further determined Gammaproteobacteria were dominated by *Pseudoalteromonas*, Pseudomonadaceae and *Shewanella.*

Many recent studies have used high throughput methodologies to advance the understanding of oil degrading microbial communities and function in other high Arctic environments including marine waters (Ribicic et al. 2018; Vergeynst et al. 2019; Krolicka et al. 2019), soils (Yergeau et al. 2012; Ferguson et al. 2020) and ice (Garneau et al. 2016; Vergeynst et al. 2019; Lofthus et al. 2020). In Arctic seawater, 16S rRNA gene analysis and reconstructed genomes have enabled the description of microbial succession in hydrocarbon biodegradation and the identification of specific genera associated to alkane and aromatic degradation and associated as key-indicators of oil contamination (Ribicic et al. 2018; Vergeynst et al. 2019; Krolicka et al. 2019). In the Canadian high Arctic, a recent survey on the naturally occurring microbial communities and functional potential in seawater and sea ice was conducted in the

Northwest Passage, whereby taxonomic and functional differences were observed between microbial communities of seawater and sea-ice (Yergeau et al. 2017). Microcosm experiments examining hydrocarbon biodegradation by sub-ice and sea-ice microbial communities in the Northwest Passage found communities were able to degrade hydrocarbons to varying extents, 94% and 48% of initial hydrocarbons, respectively and found differences in microbial communities following hydrocarbon exposure, whereby Bacteroidetes (mainly *Polaribacter*) dominated sea-ice communities and Epsilonproteobacteria increased in sub-ice communities (Garneau et al. 2016).

1.4.3 Knowledge gap of hydrocarbon biodegradation and bioremediation on Arctic shorelines

While understanding of potential biostimulation remediation strategies and potential hydrocarbon-degrading communities has been explored on high Arctic shorelines (Owens et al. 1987b; Sergy and Blackall 1987; Guénette et al. 2003; Røberg et al. 2007, 2011), limited data exists on high Arctic beaches in Canada, particularly in the Northwest Passage. The early work on microbial characterization from high Arctic beaches in Svalbard provide limited resolution as compared to more recent high throughput methodologies used to enhance understanding of hydrocarbon-degrading microbial communities and function in other Arctic environments (Garneau et al. 2016; Ribicic et al. 2018b; Vergeynst et al. 2019; Krolicka et al. 2019; Lofthus et al. 2020; Ferguson et al. 2020). The aim of this work is to expand the knowledge regarding biostimulation-associated oil biodegradation on high Arctic shorelines, particularly in Canada's Northwest Passage by combining high throughput methodologies in parallel with characterization of oil biodegradation.

Connecting text

All research goals aimed at exploring hydrocarbon biodegradation and bioremediation potential on high Arctic beaches, particularly in the Northwest Passage, were addressed within one manuscript. This manuscript titled "Hydrocarbon biodegradation potential of microbial communities from high Arctic beaches in Canada's Northwest Passage", corresponding to *Chapter 2* of this thesis, will be submitted to ASM's journal of *Applied and Environmental Microbiology*.

Chapter 2: Hydrocarbon biodegradation potential of microbial communities from high Arctic beaches in Canada's Northwest Passage

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Abstract

Unprecedented sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of an oil spill in the Arctic. 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 in Canada's Northwest Passage. In this study, high Arctic beach sediments were used to characterize physicochemical variables in parallel with microbial analyses including abundances of culturable and unculturable microorganisms and community composition using 16S rRNA gene sequencing, to screen for potential hydrocarbon-degrading

genera, and to evaluate the response of microbial communities to degrade fuel constituents, hexadecane and naphthalene, and a heavy marine fuel, Bunker C, with nutrient biostimulation. Similarities in physicochemical variables and microbial abundances were observed between high Arctic beaches, but microbial community composition was distinct at each beach. Potential oil-degrading genera were detected at each beach, but in different proportions (0.01-0.20 of the total community). Overall, biodegradation of hexadecane, naphthalene and Bunker C was greater in nutrient-amended relative to unamended microcosms. Differences between microbial communities and abundance of key hydrocarbon biodegradation genes (*alkB*, *CYP153*, *phnAc* and *ncr*) were observed between nutrient-amended and unamended heavy marine fuel microcosms. *Pseudomonas* and *Rhodococcus* were significantly differentially abundant in heavy marine fuel relative to uncontaminated microcosms. This work suggests high Arctic beaches harbour oil degrading microbes and that nutrient biostimulation is a viable bioremediation strategy. Moreover, the microbial insights in this work broaden the current understanding of shoreline biodegradation, particularly in the Canadian context.

2.1 Introduction

Unprecedented sea ice loss is opening shipping routes in Canada's Northwest Passage, increasing the risk of an oil spill in the Arctic (Overland and Wang 2013, Smith and Stephenson, 2013). 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 in Prince William Sound, Alaska, showed that 26 years later, an estimated 55,600 kg of sequestered oil persisted and

notably, has remained unchanged from the 2001-2015 period (Short et al. 2004; Maselko et al. 2018). This persistence highlights not only the inherent challenges of oil spill clean-ups, which would be even more difficult in the Arctic's remote and harsh climate (Balaji et al. 2014), 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 in Canada's Northwest Passage.

Knowledge of hydrocarbon biodegradation on high Arctic shorelines to date is largely based on analyses from two large scale field experiments: the *Baffin Island Oil Spill* (BIOS) in the Canadian High Arctic in 1980 (Sergy and Blackall 1987) and the *Svalbard shoreline field trials* in Spitsbergen, Norway in 1997 (Guénette et al. 2003). 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 investigated nutrient biostimulation as a remediation strategy and its impact on microbial communities, demonstrating that the use of nutrient-fertilizers on Arctic shorelines doubled the rate of biodegradation of an intermediate fuel oil (Sergy et al. 2003), increased microbial biomass as determined by phospholipid fatty acid analysis (Prince et al. 2003) and altered relative abundance of microbial taxa characterized using 16S rRNA gene clone library sequencing (Grossman et al. 1999).

Other *in situ* high Arctic beach studies in Svalbard have explored the effects of using an oleophilic nutrient fertilizer on beach sediments contaminated with the light oil, kerosene, demonstrating increased abundance of hydrocarbon-degrading genera through enumeration

estimated through most probable number cultivation (Røberg et al. 2007) and altered community structure as characterized through 16S rRNA gene-based fingerprinting and clone library analyses (Røberg et al. 2011). Both of the *in situ* studies conducted in Svalbard (Prince et al. 2003; Røberg et al. 2011) provided insight into potentially relevant hydrocarbon-degrading microorganisms on high Arctic beaches. Grossman et al. (1999) found Gammaproteobacteria and Bacteroidia were dominant groups, associated to sulfur-oxidizing bacteria, enteric bacteria and the genera: *Oceanospirillum, Legionella* and *Cytophaga.* Similarly, Røberg et al. (2007) also found Gammaproteobacteria and Bacteroidetes to be dominant groups along with Actinobacteria, and further determined that Gammaproteobacteria were dominated by *Pseudoalteromonas*, Pseudomonadaceae and *Shewanella.*

In contrast to the early work conducted on high Arctic beaches, recent studies have used high throughput methodologies to advance the understanding of oil degrading microbial communities and function in other high Arctic environments including marine waters (Ribicic et al. 2018; Vergeynst et al. 2019; Krolicka et al. 2019), soils (Yergeau et al. 2012; Ferguson et al. 2020) and ice (Garneau et al. 2016; Vergeynst et al. 2019; Lofthus et al. 2020). In the Canadian high Arctic, a recent survey on the naturally occurring microbial communities and functional potential in seawater and sea ice was conducted in the Northwest Passage, whereby taxonomic and functional differences were observed between microbial communities of seawater and sea-ice. (Yergeau et al. 2017). Microcosm experiments examining hydrocarbon biodegradation by sub-ice and sea-ice microbial communities in the Northwest passage found communities were able to degrade 94% and 48% of initial oil introduced into microcosms, respectively (Garneau et al. 2016). The authors additionally found differences in microbial communities following hydrocarbon exposure, whereby Bacteroidetes (mainly Polaribacter) dominated sea-ice

communities and *Epsilonproteobacteria* increased in sub-ice communities (Garneau et al. 2016). While these studies enhance understanding of high Arctic hydrocarbon biodegradation, shoreline environments are affected by many unique factors, particularly, sediment properties and structure, tidal and wave action, and concentration of nutrients, oxygen and salinity (reviewed in Boufadel et al. 2019; Wang et al. 2020). Thus, the aim of this work was to broaden the current understanding of high Arctic shoreline biodegradation, particularly in Canada's Northwest Passage.

In this study, high Arctic beach sediments were used to characterize the abundance and composition of naturally-occurring microbial communities, to determine whether hydrocarbon-degrading genera were present and to evaluate the response of these microbial communities to degrade fuel constituents, hexadecane and naphthalene, and a heavy fuel oil used in marine transportation, Bunker C, with nutrient biostimulation. Using 8 high Arctic beaches across four locations, including 7 beaches in 3 locations of the Northwest Passage, we assessed physicochemical variables in parallel with microbial analyses including abundances of culturable and unculturable microorganisms and community composition using 16S rRNA gene sequencing. Sequence data of the microbial communities was screened for the detection of known hydrocarbon-degrading genera. To evaluate hydrocarbon biodegradation and bioremediation potential, a common inorganic nitrogen, phosphorus, and potassium fertilizer was used to prepare nutrient-amended and unamended microcosms using fuel constituents, hexadecane and naphthalene, and the heavy marine fuel, Bunker C oil. Mineralization of hexadecane and naphthalene was assessed over 24 weeks using Arctic beach sediments. Oil degradation in heavy marine fuel microcosms was determined using oxygen measurements and chemical analyses (alkane and aromatic fractions) in parallel with characterization of microbial

community composition using 16S rRNA gene amplicon sequencing over 55 days. The abundance of key hydrocarbon biodegradation genes in nutrient-amended and unamended heavy marine fuel microcosms was assessed through metagenomic analysis. In this study, the use of high throughput methodologies in parallel with characterizing oil biodegradation enhances understanding of the potential for hydrocarbon biodegradation and bioremediation on high Arctic beaches, particularly in Canada's Northwest Passage. Ultimately, this study serves to proactively contribute to oil-spill bioremediation strategies.

2.2 Materials and Methods

2.2.1 Arctic beach sample sites

Beach sediment samples were collected in duplicate between July-August 2018 from eight Canadian high Arctic beaches (Fig. 2). Beach sediment samples were collected from four locations in Nunavut, Canada: Alert (Ellesmere Island), Cambridge Bay (Victoria Island), Resolute (Cornwallis Island), and Nanisivik (Baffin Island). Beach sediment samples from the latter three locations are located on the Northwest Passage. One beach sample was collected at both Alert and Cambridge Bay, two beach samples were collected at Nanisivik (East, West) and four beach samples were collected at Resolute (Tupirvik, Dynamite, Tank and Dump). All samples were immediately stored on site at -20°C, transported to McGill University in coolers and stored at -20°C until further analyses.

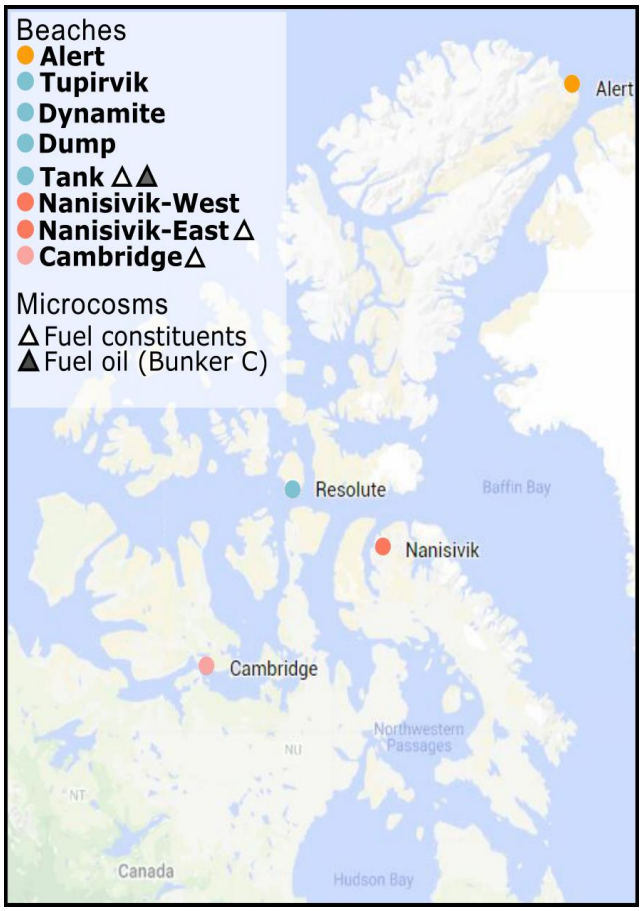

Figure 2. Arctic beach sample sites across four locations, collected between July-August 2018

2.2.2 Beach sediment physicochemical analyses

Moisture content, organic matter, total nitrogen and phosphorus, salinity and dissolved oxygen were quantified in each sample. Within the sand fraction of the samples, moisture content was determined by heating samples to 105^{o}C for 24 hours and calculating the difference in weight, expressed as a percentage, between the two steps. Organic matter within the sand fraction of the samples was quantified following the Loss of Ignition (LOI) protocol as described in Schulte et al. 1991. Briefly, following the moisture content heating cycle, the samples were further burned at 360^{o}C for 4 hours and the difference in weight between the two steps was calculated to obtain organic matter content, expressed as mg g^{-}. Total nitrogen and phosphorus

within the sand fraction of the samples were quantified following the persulfate digestion protocol as described in Ebina et al. 1983. Salinity and dissolved oxygen were measured *in situ* in Resolute the following year from the interstitial pore water using a YSI probe (Xylem Inc.). Physicochemical analyses from Cambridge Bay were not conducted due to limited sediment materials.

2.2.3 Microbial abundance

To characterize microbial abundance in the samples, culturable cells were enumerated using the spread plate method and total cells were enumerated using fluorescent staining and microscopy. To quantify culturable microorganisms, 5g of sediment was vortexed with 15mL of 4.0% artificial seawater (Sigma-Aldrich S9883) for 45 seconds. Dilutions were spread on 3 agar media in triplicate: marine broth (Difco 2216), R2A (Teknova R0005) with 4.0% artificial, and 10% R2A with 4% artificial seawater. Culturable hydrocarbon degraders were screened for by inoculating a mineral salt (Margesin and Schinner 1997) agarose media spiked with 100 µL of marine diesel. All plates were incubated at 10°C for four weeks. Following incubation, colony forming units (CFUs) were enumerated. To quantify total cells, 0.5g of sediment was fixed in 450 µL of 4% formaldehyde for 1 hour and then sonicated for 30 seconds at low power using a probe sonicator. The supernatant was collected after each sonication and replaced with artificial seawater. After 3 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 400X magnification.

2.2.4 Community composition across beaches

To characterize microbial communities across all beaches, DNA from the beach sediments was extracted in triplicate using the DNeasy PowerLyzer PowerSoil Kit (Qiagen) as per protocol, with the following modification: the final elution was completed with 50µL of nuclease-free water. Downstream DNA processing is described under the *Library preparation, sequencing, and bioinformatics* section below.

2.2.5 Mineralization assays

Experimental set-up. To examine nutrient-amended biodegradation of hexadecane and naphthalene, ^{14}C-mineralization microcosms using sediment from three beaches were prepared essentially as described in Steven et al. 2007 (Fig. 3). Briefly, 5-10g of sediment from Resolute (Tank), Cambridge Bay and Nanisivik (East) were added to 20 mL glass vials (Sigma-Aldrich). Into appropriate microcosms, either radioactive hexadecane (American Radiolabeled Chemicals, ARC 0576-50 µCi), naphthalene (American Radiolabeled Chemicals, ARC 1260-50 µCi), or acetic acid (PerkinElmer, NEC553050UC), as a positive control, 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 100ppm for hexadecane and acetic acid and 10ppm for naphthalene—lowered as naphthalene risks being toxic to microbes (Ahn et al. 1998)). Nutrient-amended microcosms were supplemented with 15 ppm of inorganic nutrients, to approximate Redfield stoichiometry (a marine ratio of carbon, nitrogen and phosphorus of 106:16:1, respectively) (Filler et al. 2006). Inorganic nutrients, supplied using a 20:20:20 nitrogen (urea, nitrate, ammonia), phosphorus and potassium fertilizer (Master Plant-Prod Inc., 10529), were selected in place of organic nutrients for their accessibility and favourability at lower

temperatures (<15° C) (Lee et al. 1993). Set-up occurred in triplicate including negative controls with sediment autoclaved twice with 24 hours between cycles. Microcosms were incubated in the dark at 4°C. Biodegradation of each substrate was determined by measuring radioactivity using a liquid scintillation counter (Perkin Elmer Tri-Carb Series) at set-up and at two, five, eight, twelve and twenty-four weeks. Concentration of mineralized ^{14}C that accumulated in between each sampling time point were reported as cumulative percent degradation over time. Statistical significance between nutrient-amended and unamended microcosms was assessed using a one-tail, paired Student's t-test in Excel.

2.2.6 Heavy marine fuel microcosms

Experimental set-up. To examine nutrient-enhanced biodegradation of a heavy marine fuel, sacrificial microcosms were prepared using Bunker C fuel oil (Tesoro Refining & Marketing Co.) (Fig. 3). Each microcosm contained 10g of beach sediment from Resolute (Tank beach) plus 2 mL of filtered 4% artificial seawater (that saturated the sediment) in a 20 mL glass vials (Sigma-Aldrich). Using a positive displacement pipette, Bunker C oil, heated to 75°C, was added into the sediment to a final concentration of 2000ppm. Bunker C oil concentration was based on residual oil on shorelines following the Baffin Island Oil Spill (Prince et al. 2002b). Nutrients-enhanced microcosms were supplemented with 15ppm of inorganic nutrients as described in the *Mineralization assays* section. An oxygen sensor spot (PreSens) was placed into the upper headspace of each microcosm. 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 as described in the *Mineralization assays* section. Triplicate nutrient-amended and unamended heavy marine fuel microcosms were sacrificed for microbial analyses at set-up and at 14-, 35- and 55-days incubation and for hydrocarbon analyses at set-up and at 14- and 55-

days incubation. In addition, triplicate nutrient-amended and unamended no oil control microcosms were sacrificed at 55 days incubation.

Oxygen monitoring. In between sampling, 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 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 theoretical headspace volume. Decreased headspace oxygen was reported as cumulative consumption, determined from oxygen concentrations that accumulated in between each sampling time point. Statistical differences between nutrient-amended and unamended treatments were calculated using a one-tail Student T-test in Excel.

Microbial analyses of heavy marine fuel microcosms. To characterize microbial communities and functional potential within sacrificed heavy marine fuel microcosms, DNA was extracted from 8g of sediment using the DNeasy PowerMax Soil Kit (Qiagen) as per protocol, with the following modification: the final elution was completed in 3 mL of nuclease-free water. Downstream DNA processing is described in the *Library preparation, sequencing and bioinformatics* section below.

Hydrocarbon analyses of heavy marine fuel microcosms. To characterize hydrocarbon biodegradation, sacrificed heavy marine fuel 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 as described (Saltymakova et al. 2020). However, due to Covid-19 they are still being processed. To determine hydrocarbon biodegradation, all analytical hydrocarbon data will be normalized to the internal marker 17α(H),21β(H)-hopane (Prince and Douglas 2005).

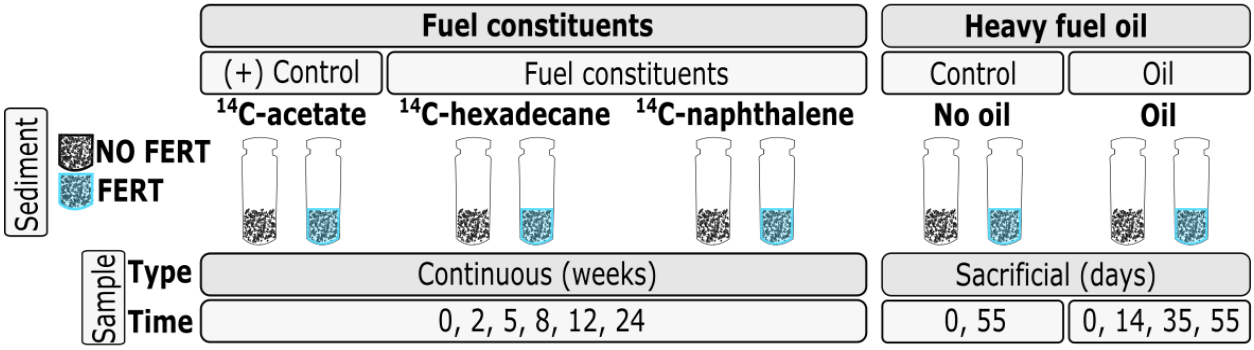

Figure 3. Experimental overview of mineralization assays and heavy marine fuel microcosms.

2.2.7 Library preparation, sequencing, and bioinformatics

Library preparation and sequencing. To characterize microbial communities within the collected arctic beach sediments and heavy marine fuel microcosms, 16S rRNA gene libraries were prepared following Illumina's 16S Metagenomic Sequencing Library Preparation protocol, except for three modifications. First, during amplicon and index PCR steps, 2x HotStarTaq Plus Master Mix (Qiagen) was used. Second, the ratio of amplicon PCR reagents were adjusted as follows: 7.5uL Nuclease-free water, 1.5uL 10uM forward primer, 1.5uL 10uM reverse primer, 12.5uL HotStarTaq Plus and 2uL genomic DNA to a total volume of 25uL per reaction. Third, during amplicon PCR, primers from the earth microbiome project, 515F-Y (5′-GTGYCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYMTTTRAGTTT) (Parada et al.

2015), were used. Beach sediment amplicons were indexed, pooled and sequenced using the 600-cycle MiSeq Reagent Kit v3 on an Illumina MiSeq platform in house. Heavy marine fuel microcosm amplicons were indexed, pooled and sequenced using the MiSeq Reagent Kit v2 nano configuration on an Illumina MiSeq platform at the UBC Sequencing Centre.

To characterize functional potential of microbial communities in the heavy marine fuel microcosms, metagenomic libraries from set-up (0 days incubation) and triplicate samples from nutrient-amended and unamended microcosms after 55 days incubation were prepared using the Nextera XT Library Prep kit as per protocol (#15031942 V04). Briefly, extracted DNA was tagmented, amplified and cleaned with a Sera-Mag Select bead clean-up kit (Cytiva). Normalization was performed by pooling equimolar amounts of libraries after quantification using an Agilent High Sensitivity DNA kit on the 2100 Bioanalyzer (Agilent Technologies) as per the manufacturers instruction. The pooled library was further purified using a Nucleic Acid Purification PCR and DNA clean-up kit (Monarch) as per protocol. Denaturation and dilution of the pooled library was prepared following protocol A of Illumina's Denature and Dilute Libraries Guide (15039740 v10). The final library was sequenced using a 600-cycle MiSeq Reagent kit v3 on an Illumina MiSeq platform.

Bioinformatics. Raw 16S rRNA sequencing output from both the beach sediments and the heavy marine fuel microcosms was processed following the 'Bioconductor Workflow' (Callahan et al. 2016b). Briefly, raw sequencing reads were processed using the R package "dada2" (Callahan et al. 2016a) to generate amplicon sequence variants (ASVs) with assigned taxonomy from the SILVA database (Callahan 2018). ASV counts, taxonomy and sample metadata were combined and manipulated using the R package "phyloseq" (McMurdie and Holmes 2013), whereby uncharacterized phyla, phyla prevalent in two samples or less and taxa

present in less than 5% of total samples were removed.

Relative abundance of dominant genera from both the beach sediments and heavy marine fuel microcosms were visualized using the R package "ggplot2" (Wickham H 2016). Alpha diversity metrics within heavy marine fuel 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). For better interpretation, obtained Shannon diversity indices were converted to "estimated number of species" (Jost 2006). Alpha diversity metrics were visualized using the R package "ggplot2" (Wickham H 2016). Statistical significance of community richness and diversity between nutrient-amended and unamended heavy fuel oil microcosms was assessed using a two-tailed, paired Student's t-test in Excel.

Relationships between samples were assessed under the principles of compositional data (Gloor et al. 2017). Dissimilarities between communities was assessed using the proportionality metric phiT as calculated using the R package "propr" (Lovell et al. 2015) and nonmetric multidimensional scaling (NMDS) ordination using the R package "vegan" (Oksanen et al. 2013), and visualized using the R package "ggplot2" (Wickham H 2016). Sequence data from beach sediments were screened for the prevalence of potential hydrocarbon-degrading genera previously characterized in polar environments including: *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* (reviewed in Brakstad et al. 2017). For heavy marine fuel microcosms, statistical

differences between differentially abundant genera was assessed using "aldex2" (Fernandes et al. 2013).

Metagenomic reads were analyzed in an assembly-based functional gene annotation approach. 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 the stand-alone automated metagenomic pipeline "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 derived using "bbmap" (Bushnell 2014) were compiled into a read count matrix.

Hidden Markov Models (HMM) were generated using hydrocarbon biodegradation genes including: *alkB*, *CYP153*, *ladA*, *masD*, *ncr*, and *phnAc*. Amino acid sequences from 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 algorithm. 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 $<1x10^{-10}$ and a sequence score below the lowest sequence score of the reference group were filtered (Fig. S1). Gene hits output was read into RStudio using the R package "rhmmer" (Arendsee 2017) and

gene abundance across samples was reported as counts per million.

2.3 Results

2.3.1 Beach sediment physicochemical analyses

Physicochemical analyses across beaches showed low nutrient availability with limited variation across beaches (Table 3). Across beaches, moisture content ranged from 1.7% - 7.7%, organic matter (OM) ranged from 2.58 mg g^- to 9.51 mg g^-, total nitrogen ranged from 0.19 mg g^- – 0.34 mg g^- and total phosphorus ranged from 0.122 mg g^- – 0.853 mg g^- Additionally, in the Resolute samples, interstitial water measurements showed a range for salinity of 0.31 psu – 6 psu, the largest variation observed, and for dissolved oxygen (DO) of 9.47 mg L^- -13.75 mg L^-.

2.3.2 Beach sediment microbial abundance

Across all beaches, similar orders of magnitudes for both culturable and total cells were observed (Fig. 3). Culturable cells were in highest abundance on the 10% R2A + seawater agar media (Table 3), ranging from 1.11 x 10^5 – 3.75 x 10^6 colony forming units (CFUs) g^- with a median across beaches of 8.63 x 10^5 CFUs g^-; followed by R2A and seawater agar media ranging from 2.49 x 10^5 – 2.8 x 10^6 CFUs g^- with a median across beaches of 3.96 x 10^5 CFUs g^-; and then, marine broth agar media, ranging from 1.24 x 10^5 – 2.50 x 10^6 CFUs g^- with a median across beaches of 3.75 x 10^5 CFUs g^-. Total cells ranged from 1.51 x 10^6 – 3.16 x 10^7 cells g^- with a median across beaches of 3.84 x 10^6 cells g^-. Culturable hydrocarbon degraders were not detected after 4 weeks of incubation at 10°C.

Table 3. Physicochemical analyses and microbial abundances across high Arctic beaches.

Beach	Moisture (wt/wt %)	OM (mg g^{-})	Total N (mg g^{-})	Total P (mg g^{-})	Salinity (psu)	DO (mg L^{-})	Culturable bacteria g^{-}	Total bacteria g^{-}
Alert	7.450	3.04	0.34	0.853	-	-	3.8×10^{6}	3.3×10^{6}
Tupirvik	6.044	9.51	0.25	0.122	1.80	12.56	4.6×10^{5}	1.2×10^{7}
Dynamite	2.806	2.58	0.19	0.158	0.31	13.75	3.5×10^{5}	3.2×10^{7}
Dump	1.777	3.79	0.16	0.645	6.00	11.45	3.1×10^{5}	1.6×10^{6}
Tank	4.174	4.25	0.17	0.376	0.57	9.47	2.4×10^{6}	4.4×10^{6}
Nanisivik - West	7.695	3.72	0.25	0.423	-	-	1.1×10^{6}	1.3×10^{7}
Nanisivik - East	5.888	5.58	0.29	0.574	-	-	6.2×10^{6}	2.3×10^{6}
Cambridge Bay	-	-	-	-	-	-	2.7×10^{6}	1.5×10^{6}

Figure 4. Abundances of colony forming units (CFUs g^{-} sediment) enumerated from Marine Broth, R2A and seawater and 10% R2A and seawater; and of total cell counts (cells g^{-} sediment) enumerated from DAPI staining and fluorescent microscopy across eight Arctic beaches.

2.3.3 16S rRNA community composition across collected Arctic beach sediments

Across beaches, communities were dominated at phyla-level by: Proteobacteria, Bacteroidetes and Actinobacteria (Fig. 4A) and at class-level: Bacteroidia, Gammaproteobacteria and Alphaproteobacteria (Fig. 4B). Microbial communities differentiated at the order and family classification level, with the most abundant groups as less representative of all beach communities, including: Flavobacteriales, Cellvibrionales, Microtrichales (Fig. 4C); and *Flavobacteriaceae*, *Psychromonadaceae* and *Porticoccaceae* (Fig 4D). Across beaches, the most abundant genera included: *Gillisia*, *Illumatobacter*, *Granulosicoccus*, *Maritimimonas* and *Sphingorhabdus* (Fig. 5A). However, these genera were more representative of Alert, Nanisivik and Resolute beaches and largely absent in Cambridge Bay beaches. The most abundant genera of Cambridge Bay beaches included: *Gillisia*, *Ellin6067*, *Zeaxanthinibacter*, *Woeseia* and *UTBCD1* (Fig. S2). Across beaches, NMDS ordination of the proportionality metric phiT showed differentiation between microbial communities. Four distinct clusters were observed representative of each beach location, with Cambridge Bay samples clustering furthest from the remaining locations; however, clear clusters between Nanisivik, Resolute and Alert beaches were also still observed (Fig. 5C). While some overlap between clusters was observed between Resolute beaches (Dynamite, Tupirvik and Dump) and between Nanisivik beaches (East and West), PERMANOVA analysis still supported that dissimilarities between microbial communities was explained 24% by beach (p-value: 0.0002).

Of the 28 potential hydrocarbon-degrading genera that were screened for, 22 genera were detected across beaches including: *Loktanella, Sulfitobacter, Sphingopyxis, Sphingomonas, Glaciecola, Marinobacter, Colwellia, Moritella, Pseudoalteromonas, Psychromonas, Shewanella, Alcanivorax, Marinomonas, Oleispira, Halomonas, Psychrobacter, Pseudomonas,*

Cycloclasticus, *Ulvibacter*, *Polaribacter*, *Rhodococcus*, and *Arthrobacter* (Fig. 5B). The total relative proportion of detected genera ranged from 0.01 to 0.2 across beach samples. The total relative proportion of detected genera was greatest in 1 replicate at Dump beach, Resolute (<0.2), driven by *Psychromonas* accounting for near half of the total proportion (Fig. 5b). Following Dump beach, total relative proportion of detected genera was greatest in Tank beach, Resolute (<0.12), followed by Alert (<0.09) and Cambridge Bay beaches (<0.08) (Fig. 5B). The total relative proportion of detected genera at Nanisivik beaches (East and West) and remaining Resolute beaches (Dynamite and Tupirvik) was near or below 0.05.

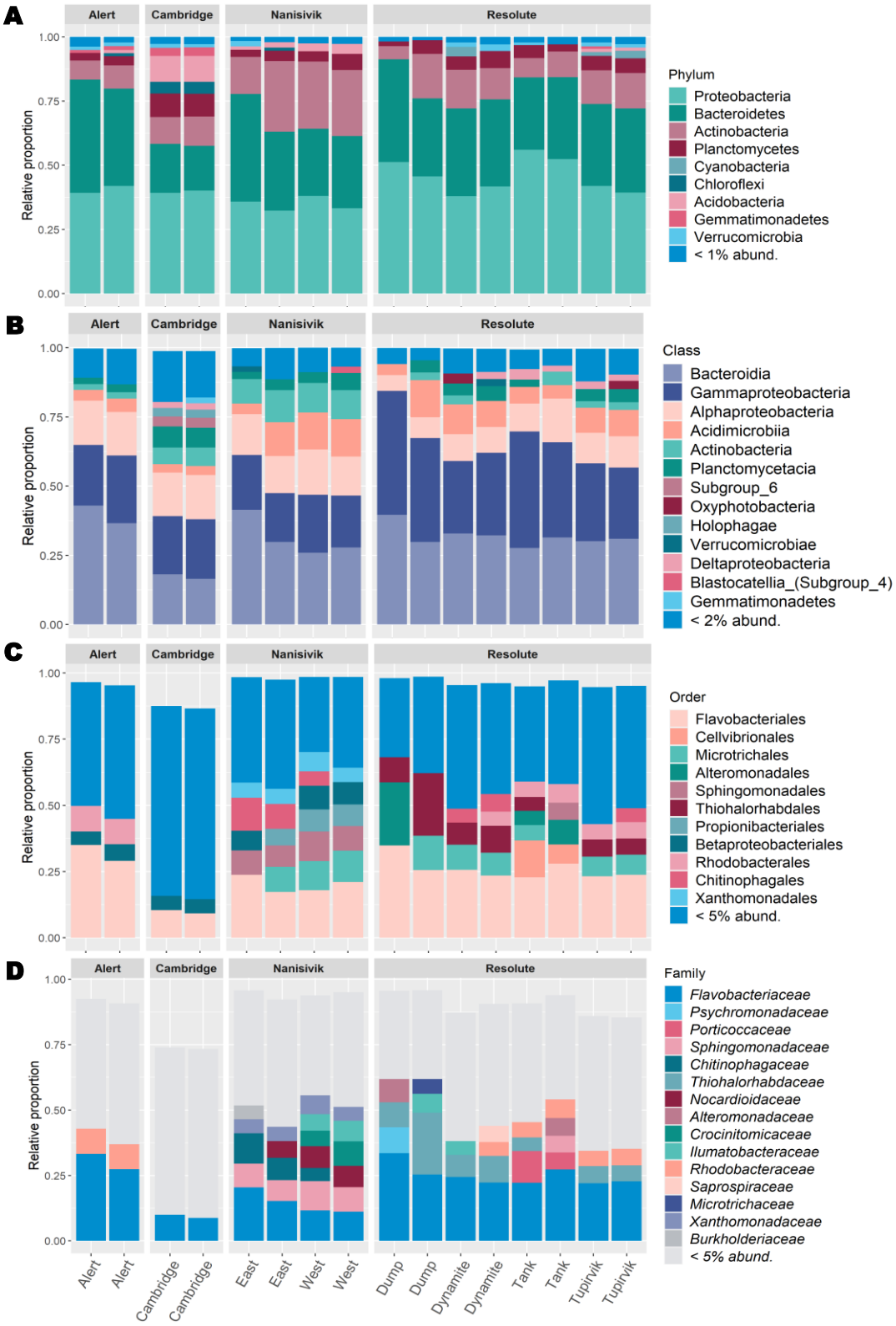

Figure 5. Relative abundance across replicate beach sediment samples at phyla-level (A), class-level (B), order-level (C) and family-level (D).

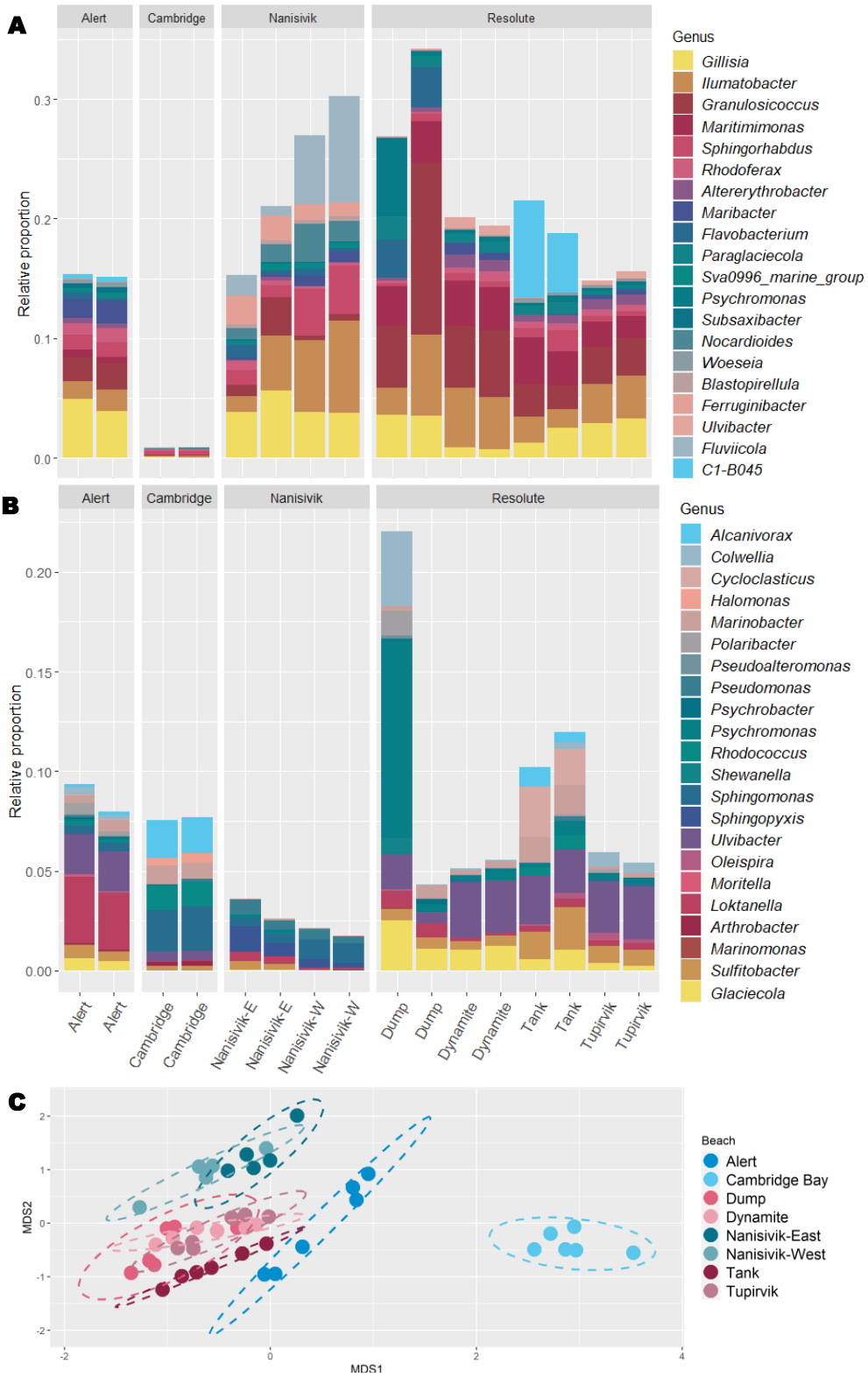

Figure 6. Relative proportion across replicate beach samples of the top 20 most abundant genera (A), and potential hydrocarbon-degrading genera compiled from literature (B). Dissimilarities between microbial communities across beaches represented by the proportionality metric phiT (Stress value: 0.154) (C)

2.3.4 Mineralization assays

Mineralization of hexadecane and naphthalene was observed in each of the 3 Northwest Passage beaches used in the mineralization microcosms (Fig. 7). Cumulative mineralization of hexadecane was generally greater than naphthalene and for both compounds, cumulative mineralization was greater in nutrient-amended relative to unamended microcosms (Fig. 7). For hexadecane, cumulative mineralization was greater in nutrient-amended relative to unamended microcosms for 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%; not significant), and Resolute (10.8 ± 13.1% vs. 4.5 ± 0.7%; not significant). For naphthalene, cumulative mineralization was greater in nutrient-amended relative to unamended microcosms for Cambridge Bay (8.0 ± 2.9% vs. 3.8 ± 1.3%; not significant) and Nanisivik (1.1 ± 0.6% vs. 0.7 ± 0.04%, not significant); however, cumulative mineralization was greater in unamended relative to nutrient-amended microcosms for Resolute (1.2 ± 0.5% vs. 1.0 ± 0.3%; not significant). Overall, for all three beaches collectively per constituent, cumulative hexadecane mineralization was significantly greater in nutrient-amended microcosms relative to unamended microcosms (14.11 ± 4.55% SEM vs. 3.23 ±1.59% SEM; p-value: 0.008); cumulative naphthalene mineralization was also greater in nutrient-amended microcosms relative to unamended microcosms (3.39 ±3.27% SEM vs. 1.92 ± 1.33%), but not significantly. Overall, for both hexadecane and naphthalene, mineralization occurred more rapidly within the first 8 weeks of incubation relative to the last 16 weeks of incubation (Fig. 7). Cumulative mineralization of acetate, used as a positive control, was observed across all beaches with little differentiation between nutrient-amended and unamended microcosms (Fig. S3). **C**umulative mineralization in sterile negative controls was below 0.5% for acetate and hexadecane and below 1.0% for naphthalene (Fig. 7 and Fig. S3).

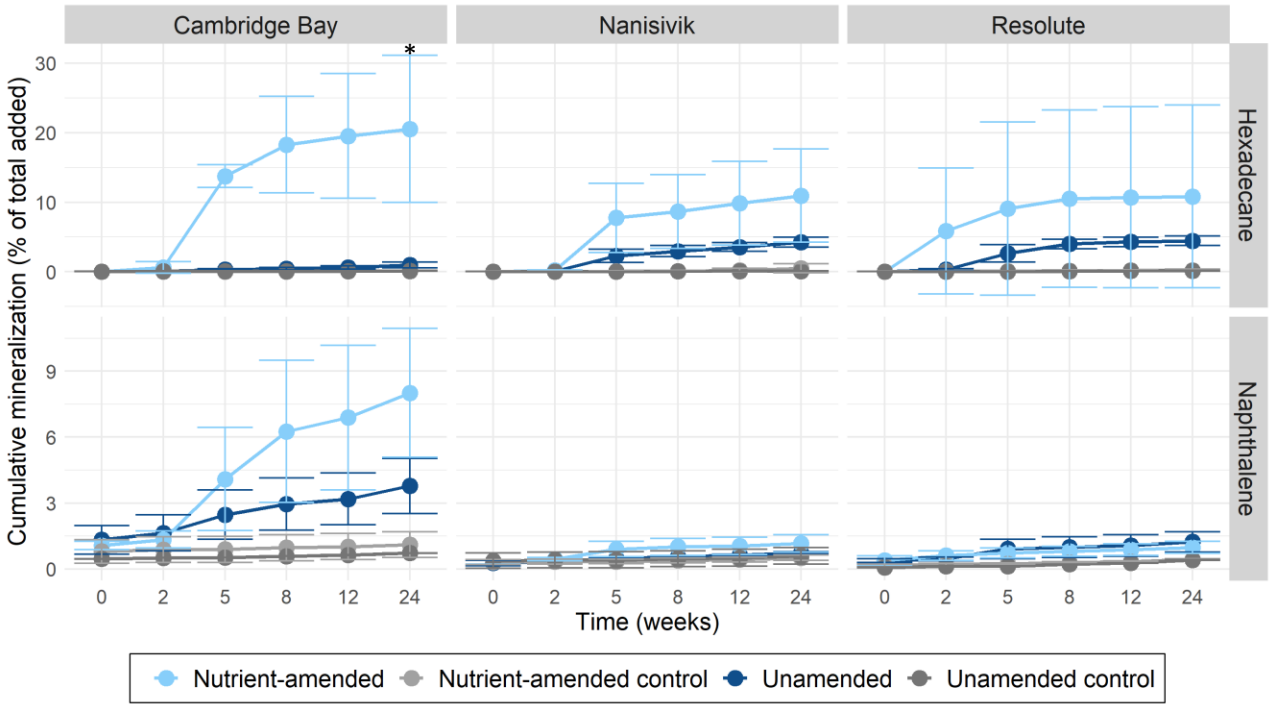

Figure 7. Mean 24-week cumulative hexadecane mineralization (%) in nutrient-amended relative to unamended microcosms incubated at 4°C from Cambridge Bay (20.5 ± 10.6% vs. 1.0 ± 0.4%; p-value: 0.04), Nanisivik (10.9 ± 6.7% vs. 4.3 ± 0.7%; NS), and Resolute (10.8 ± 13.1% vs. 4.5 ± 0.7%; NS). Mean 24-week cumulative naphthalene mineralization (%) in nutrient-amended relative to unamended microcosms from Cambridge Bay (8.0 ± 2.9% vs. 3.8 ± 1.3%; NS), Nanisivik (1.1 ± 0.6% vs. 0.7 ± 0.04%; NS), and Resolute (1.0 ± 0.3% vs. 1.2 ± 0.5%; NS). Error bars representing standard deviation of the mean from triplicate microcosms.

2.3.5 Heavy marine fuel microcosms

Oxygen monitoring of marine heavy fuel microcosms

Oxygen measured during the 55-day incubation period of the heavy marine fuel microcosms showed a steady depletion of headspace oxygen, a proxy for microbial respiration, over the course of the experiment and greater oxygen depletion in nutrient-amended microcosms relative to unamended microcosms (Fig. 8). Cumulative depletion of headspace oxygen at 55 days was significantly higher in the nutrient-amended relative to unamended fuel contaminated

microcosms (Fig. S4; 132.6 ± 16.4 µmol vs 104.1 ± 10.0 µmol; p-value: 0.0007) as well as for the nutrient-amended relative to unamended uncontaminated control microcosms (Fig. S4; 105.7 ± 12.5 µmol vs. 65.2 ± 28.2 µmol; p-value: 0.0264). Negative control microcosms, with autoclaved sediment and fuel contamination, showed some depletion of headspace oxygen in both nutrient-amended and unamended microcosms (Fig. 8 and Fig. S4; 47.7 ± 3.6 µmol; 42.8 ± 2.0 µmol, respectively) (Fig. 8 and Fig. S4). Cumulative mean depletion of headspace oxygen at 55-days was significantly greater in the nutrient-amended fuel contaminated microcosms relative to the nutrient-amended uncontaminated control microcosms (p-value: 0.0149), in the unamended fuel contaminated microcosms relative to the unamended uncontaminated control microcosms (p-value: 0.0077), and collectively, in the fuel contaminated microcosms over the uncontaminated control microcosms (Fig. S4; p value:0.0054).

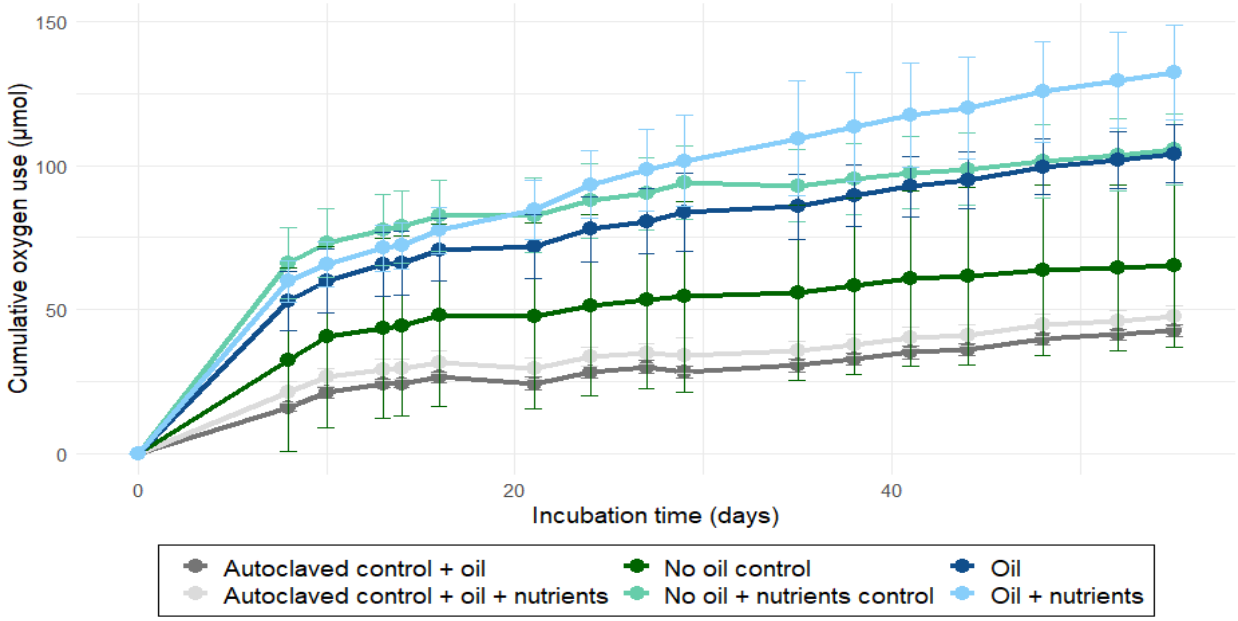

Figure 8. Mean cumulative loss of headspace oxygen (µmol) over 55 days at 4°C for nutrient-amended and unamended marine fuel oil microcosms (n=6), no oil controls (n=3) and autoclaved oil controls (n=3). Error bars representing standard deviation of the mean of microcosms

16S rRNA community composition of marine heavy fuel microcosms

Through 16S rRNA gene sequencing, community composition was characterized in nutrient-amended and unamended fuel contaminated microcosms at 14-, 35- and 55-day incubations. Overall, similarities in relative abundances between nutrient-amended and unamended replicate microcosms were observed at all taxonomic levels (Fig. 9). Shifts in community composition from the T0 sediment were observed in both the 55-day fuel contaminated and uncontaminated control microcosms (Fig. 9A). In the 55-day fuel contaminated relative to uncontaminated microcosms, a larger dominance in abundance was observed for Proteobacteria at the phylum-level (Fig. 9A), Gammaproteobacteria and Actinobacteria at the class-level (Fig. 9B), Pseudomonadales and Sphingomonadales at the order-level (Fig. 9C), and *Pseudomonadaceae* and *Sphingomonadaceae* at the family-level (Fig. 9D). The most abundant genera in fuel contaminated microcosms, included: *Gillisia, Granulosicoccus, Pseudomonas, Planococcus, Flavobacterium, Psychrobacter* and *Rhodococcus*, which have all been associated to hydrocarbon biodegradation (Fig. 10A). Interestingly, many of the genera within the uncontaminated 0-day microcosms have also been associated to hydrocarbon biodegradation, including: *Gillisia, Granulosicoccus, Cycloclsaticus, C1-B045, Sulfitobacter, Maritimimonas, Alterytrhobacter* and *Maribacter*. Of those genera, *Cycloclsaticus, C1-B045, Sulfitobacter, Maritimimonas, Alterytrhobacter*, and *Maribacter* were not detected in any later time point. There were no genera that were significantly differentially abundant between nutrient-amended and unamended fuel contaminated microcosms at any time point. However, in both the 55-day and 35-day microcosms, *Rhodococcus* was only detected in nutrient-amended fuel contaminated microcosms relative to unamended microcosms (Fig. 10A). Collectively in the 55-day microcosms both *Pseudomonas* and *Rhodococcus* were significantly

differentially abundant (p-values*:* 0.0027, 0.0141, respectively) in the fuel contaminated relative to uncontaminated microcosms.

While there were no clear genera differences between nutrient-amended and unamended fuel contaminated microcosms at any time point, at the ASV-level, NMDS ordination of the proportionality metric phiT provided better differentiation between microbial communities (Fig. 10B). Nutrient-amended and unamended fuel contaminated microcosms were represented in two distinct clusters in the 14-day and 55-day microcosms, with some overlap in the 35-day microcosms (Fig. 10B). However, this nutrient effect was not found to be significant by PERMANOVA analysis (P-values: 0.2, 0.1, 0.4, respectively). In the 55-day microcosms, when including both fuel contaminated and uncontaminated control microcosms, four distinct clusters formed differentiating fuel contamination from uncontaminated microcosms, and nutrient-amended from unamended microcosms (Fig. 10B). This observation was further supported by PERMANOVA analysis that showed dissimilarities between communities was explained 22.5% by oil (p-value: 0.0020) and 16.9% by nutrients (p-value: 0.0134). Ordination patterns were more difficult to distinguish when considering all incubation microcosms (Fig. 11); however, PERMANOVA analysis showed dissimilarities between communities was explained 20.2% by incubation time (P-value: 0.0004), 10.5% by oil (p-value: 0.0008) and 6.96% by nutrients (p-value: 0.0178).

Species richness and diversity within microcosms both increased over time (Fig. 12). After 55 days of incubation, species richness increased from a mean of 126 ± 22 species to 348 ± 44 species in fuel contaminated microcosms and to 271 ± 23 species in unoiled control microcosms (Fig. 12A). Similarly, after 55 days of incubation, effective number of species increased from a mean of 102 ± 17 species to 280 ± 33 species in fuel contaminated microcosms

and to 223 ± 15 in unoiled control microcosms (Fig. 12B). Differences between nutrient-amended and unamended fuel-contaminated microcosms were not significant at any time point for community richness, nor for effective number of species. However, in 55-day fuel contaminated relative to uncontaminated microcosms, both community richness and effective number of species were significantly greater (p-values: 0.0064, 0.014, respectively) (Fig. 12).

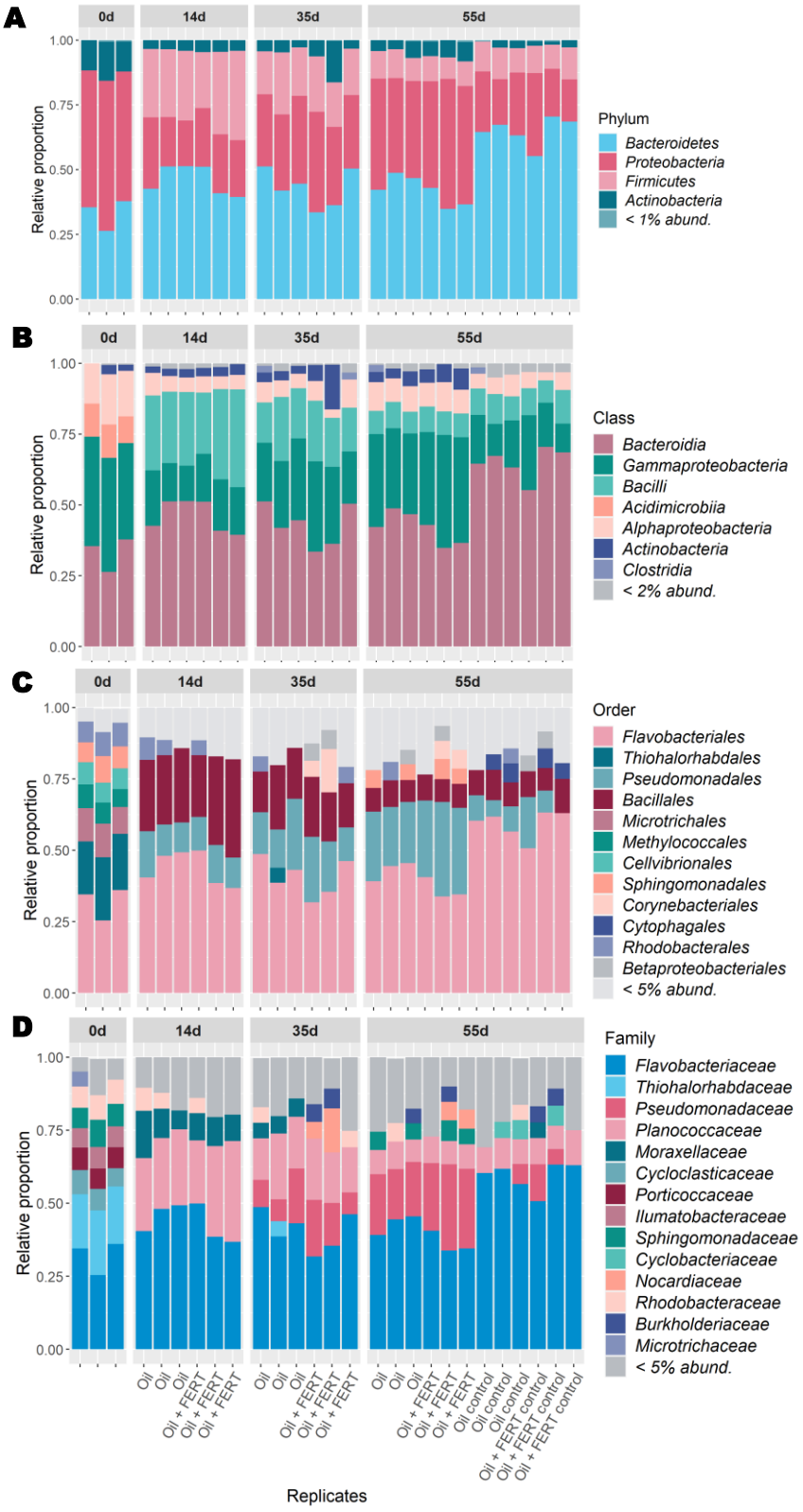

Figure 9. Relative abundance across replicate heavy marine fuel microcosms at phyla-level (A), class-level (B), order-level (C) and family-level (D).

Figure 10. Relative abundance of genera per replicate across all treatments (A). Nonmetric multidimensional scaling (nMDS) ordination of the proportionality metric phiT between microbial communities differentiating nutrient-amended and unamended oil microcosms at 14d (stress: 0.03), 35d (stress: 0) and 55d (stress: 0) and differentiating nutrient-amended and unamended oil and no oil control microcosms at 55d (stress: 0.07) (B).

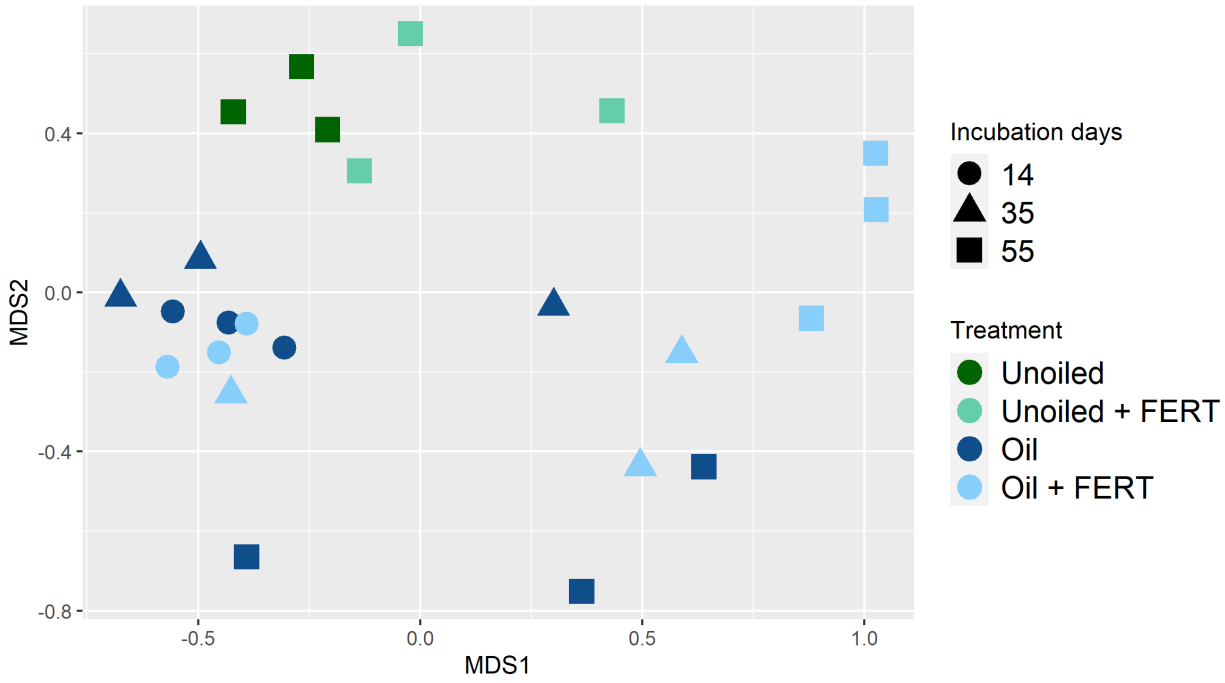

Figure 12. Non-metric multidimensional scaling ordination of proportionality metric phiT distances of microbial communities from 14-, 35- and 55- day nutrient-amended and unamended heavy marine fuel and no oil control microcosms (stress: 0.145).

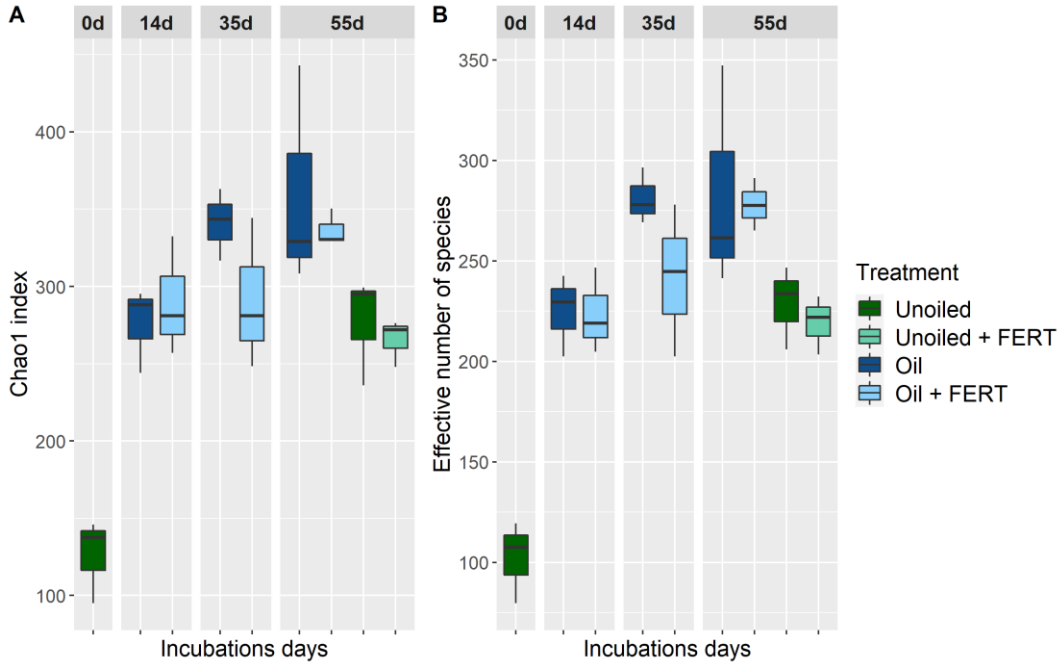

Figure 11. Development of species richness based on the Chao1 index (A) and diversity quantified using Shannon-Wiener index expressed as effective number of species (B) across microbial communities from 14-, 35- and 55- day nutrient-amended and unamended heavy marine fuel and 0- and 55- day no oil control microcosms.

Metagenomic analyses of marine heavy fuel microcosms

Metagenomic analyses were conducted from 7 microcosms: 1 microcosm for the 0-day uncontaminated beach sediment, 3 replicates for the 55-day unamended fuel contaminated microcosms and 3 replicates for the 55-day nutrient-amended fuel contaminated microcosms. These samples were screened for the presence of hydrocarbon biodegradation genes that have demonstrated true biological function, including: aerobic alkane-degrading genes, *alkB* (Nie et al. 2014), *CYP153* (Van Beilen et al. 2006; Nie et al. 2014) and *ladA* (Boonmak et al. 2014); an anaerobic alkane-degrading gene, *masD/AssA* (Tan et al. 2014; Gittel et al. 2015)*;* an aerobic polycyclic aromatic hydrocarbon (PAH)-degrading gene, *phnAc* (Lozada et al. 2008; Ding et al. 2010); and an anaerobic PAH-degrading gene, *ncr* (Morris et al. 2014). Of these genes that were screened for, *alkB, CYP153, phnAc* and *ncr* were detected in the 55-day nutrient-amended and unamended fuel contaminated microcosms (Fig. 13 and 14) and overall, in higher abundance in the former; however, these differences in abundance were not significant. Genes *ladA* and *masD* were not detected in any of the heavy marine fuel microcosms (Fig. S1).

Alkane-degrading genes

For *alkB,* gene abundance was ~ 2-fold greater in the 55-day nutrient-amended relative to unamended fuel contaminated microcosms (262.0 ±80.2 vs. 140.7 ±36.0) and gene abundance in the 55-day fuel contaminated microcosms was substantially higher than the 0-day microcosm (20.6) (Fig. 12A). Within the fuel contaminated microcosms, *alkB* genes were associated to *Rhodococcus*, *Sphingorhabdus*, *Pseudomonas*, *Psychrobacter*, *Sulfitobacter*, *Marinobacter*, *Congregibacter*, *Nocardiodes*, and *Aquabacterium* (Fig. 13A). For *CYP153*, gene abundance was ~ 2-fold greater in the 0-day microcosm (191.6) relative to the 55-day fuel contaminated

microcosms; however, these genes were mainly associated to *Cycloclasticus* and *50-400-T64*, which were not observed in the later 55-day fuel contaminated microcosms (Fig. 13B). In the 55-day fuel contaminated microcosms, gene abundance ~ 2-fold greater in the nutrient-amended relative to unamended microcosms (98.9 ±42.7 vs. 53.2 ±22.9). In the 55-day fuel contaminated microcosms, *CYP153* genes were associated to *Williamsia*, *Rhodococcus*, *Alterythrobacter*, *Polaromonas*, *Ilumatobacter*, *Sphingorhabdus, UBA5078* and *Blastomonas* (Fig. 13B).

PAH-degrading genes

For *phnAc*, gene abundance was ~ 4-fold greater in the 0-day microcosm (410.9) relative to the 55-day fuel contaminated microcosms; however, as with *CYP153*, these genes were associated to *Cycloclasticus*, which was not observed in the later 55-day microcosms (Fig. 14A). In the 55-day fuel contaminated microcosms, *phnAc* gene abundance was similar in both nutrient-amended and unamended microcosms (78.8 ±10.9 vs. 82.0 ±9.4). In the 55-day fuel contaminated microcosms, *phnAc* genes were associated to *Sphingopyxis*, *Sphingorhabdus*, *Hyphomonas*, *Pseudomonas, Alicyclobacillus* and *Sphingobium* (Fig. 14A). For *ncr*, gene abundance was greater in the 55-day nutrient-amended relative to unamended fuel contaminated microcosms (513.9 ±20.9 vs. 442.6 ±48.8) and gene abundance in both fuel contaminated microcosms was substantially greater than in the 0-day microcosm (281.9) (Fig. 14B). As with *CYP153* and *phnAc*, the majority of *ncr* genes in the 0-day microcosm were associated to *Cycloclasticus* and *50-400-T64,* which were not observed in the later 55-day microcosms. In the 55-day fuel contaminated microcosms, *ncr* genes were associated to 27 genera, the most abundant including: *Ramlibacter*, *Rhodococcus, Pseudomonas*, *Anderseniella* and *Novosphingobium* (Fig. 14B).

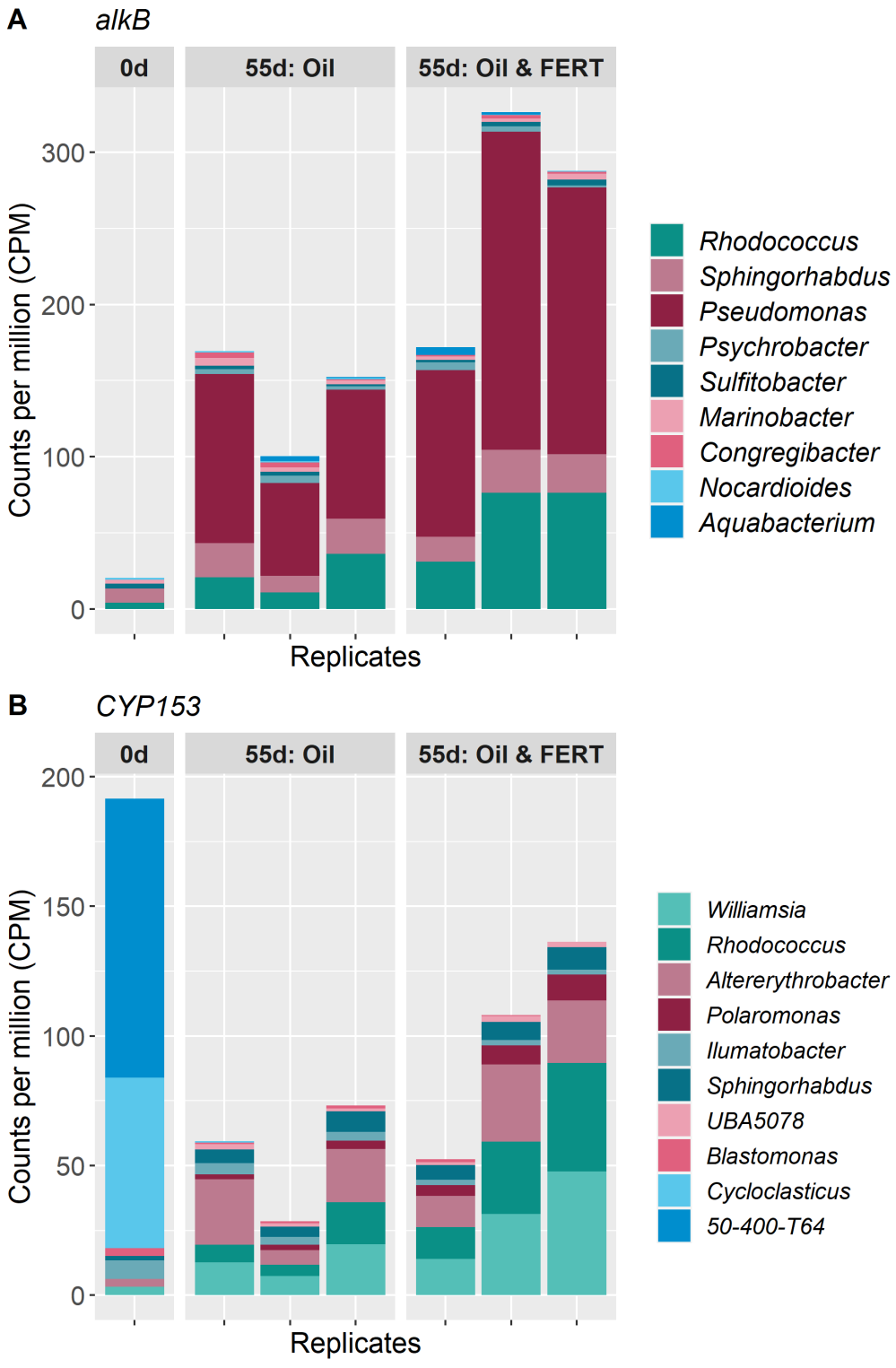

Figure 13. Abundance (counts per million) of alkane hydrocarbon biodegradation genes: *alkB* (A) and *CYP153* (B) in a 0-day uncontaminated microcosm and in 55-day heavy marine fuel contaminated triplicate unamended microcosms (Oil) and triplicate nutrient-amended microcosms (Oil & FERT). Genes are colored by contig taxonomy at genus classification.

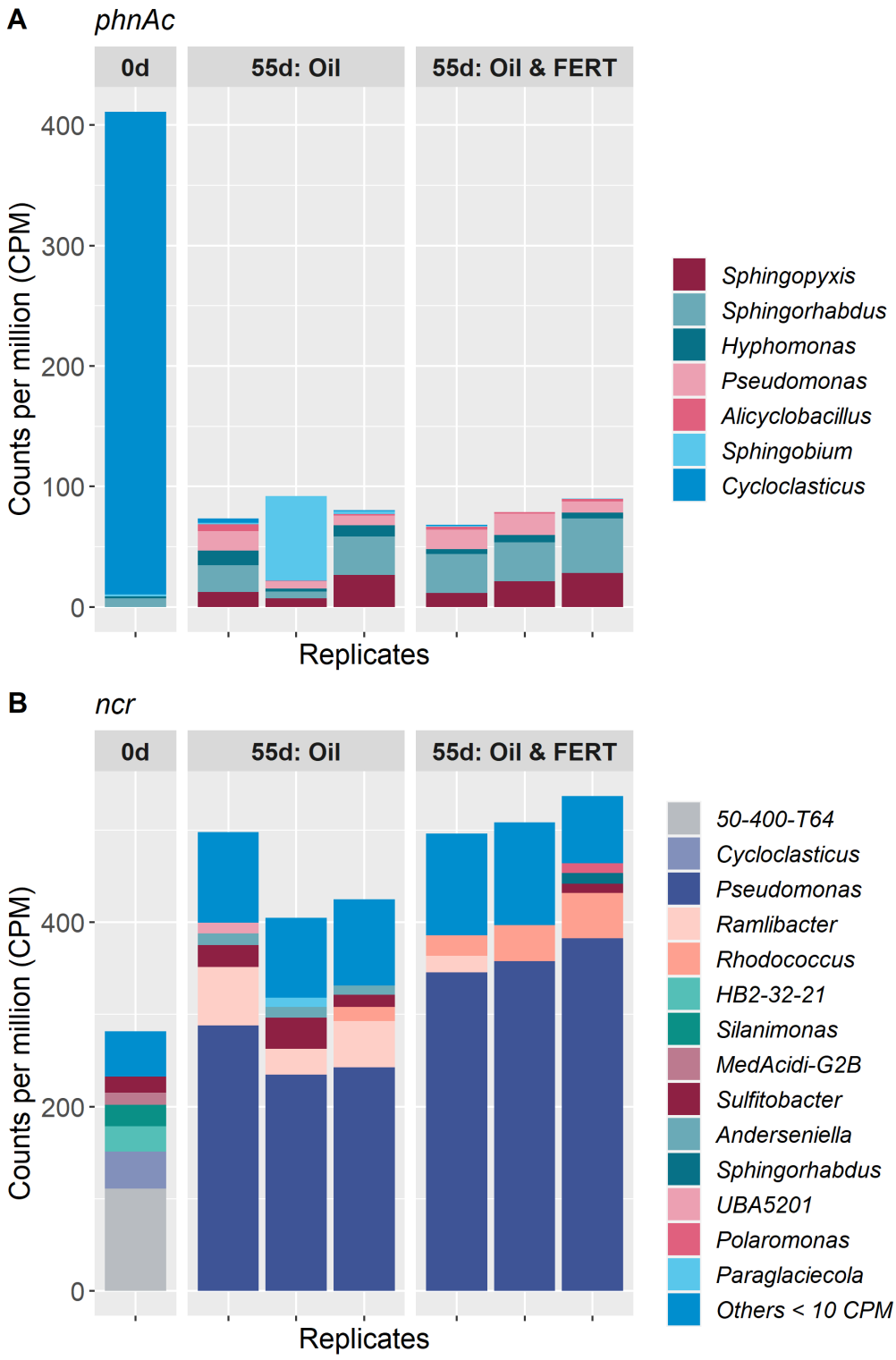

Figure 14. Abundance (counts per million) of aromatic hydrocarbon biodegradation genes: phnAc (A) and ncr (B) in a 0-day uncontaminated microcosm and in 55-day heavy marine fuel contaminated triplicate unamended microcosms (Oil) and triplicate nutrient-amended microcosms (Oil & FERT). Genes are colored by contig taxonomy at genus classification.

2.4 Discussion

Arctic sea ice loss is driving the opening of the Northwest Passage, which may increase shipping traffic and the risk of an oil spill in the Arctic. 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 in Canada's Northwest Passage. The aim of this work was to explore whether hydrocarbon-degrading genera were present on high Arctic beaches, particularly beaches of the Northwest Passage, and to evaluate the response of naturally occurring high Arctic beach microbial communities to degrade hydrocarbons with nutrient biostimulation as a remediation strategy.

2.4.1 Abundance and composition of high Arctic beach microbial communities

Arctic beaches were characterized by similarities in sediment chemistry and microbial abundances and by differences in microbial community composition. Within the Arctic beach sediments, the low amounts of total nitrogen (<0.3 mg g^{-}), total phosphorus (<0.9 mg g^{-}), organic matter (<9 mg g^{-}) and moisture content (<7.7 %) were similar in value and range to Antarctic coastal soils (Aislabie et al. 2012) and Arctic soils (Mohn and Stewart 2000). Microbial abundances ($10^5 – 10^7$ cells g^{-}) were similar to what was characterized on Canadian high Arctic shorelines during the *Baffin Island Oil Spill Project* (Eimjhellen et al. 1982) and were also similar in range to hydrocarbon-contaminated soils in the Canadian high Arctic (Whyte et al. 2001) and coastal soils in Antarctica (Aislabie et al. 2012). To our knowledge, this is the first description of microbial community composition of Canadian high Arctic beaches, including 7 beaches across 3 locations in the Northwest Passage.

Dominant microbial phyla across beaches including Proteobacteria and Bacteroidetes, followed by Actinobacteria, were similar to those detected in a survey of microbial communities in sea-ice and seawater in the Northwest Passage near Resolute, where Bacteroidetes and Gammaproteobacteria dominated sea-ice samples and Actinobacteria, Deltaproteobacteria and Planctomycetes 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 lower resolution 16S rRNA gene clones, where Gammaproteobacteria and Bacteroidia were dominant (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 and/or marine environments (Asker et al. 2007; Matsumoto et al. 2009; Park et al. 2009; Jogler et al. 2013; Roh et al. 2013; Du et al. 2016; Kang et al. 2018), but differed from findings reported by Grossman et al. (1999) where 16S rRNA gene clones from a Norwegian beach were most related to sulfur-oxidizing bacteria, enteric bacteria and the genera: *Oceanospirillum*, *Legionella*, and *Cytophaga.* Of the most abundant genera, *Gillisia*, *Maritimimonas*, and *Zeaxanthinibacter* are all classified within the order Flavobacteriales, which was also a dominant order in microbial communities of seawater in the Northwest Passage based on classification of the top 15 most abundant OTUs (Yergeau et al. 2017); however, none of the other dominant OTUs observed in seawater or sub-ice communities in Yergeau et al. (2017) were present in high abundances in the high Arctic beach sediments reported here. Overall, ordination at the amplicon sequence variant (ASV) level showed distinct community composition between beaches. Such differentiation is congruent with the literature on the heterogeneity of beaches, whereby bacterial abundance may

differ at a millimetre scale (Seymour et al. 2000). Distinct community composition may have implications on the universality of a bioremediation approach.

Despite distinct microbial community composition at each beach, cumulative mineralization of hexadecane and naphthalene was observed from each of the 3 Northwest Passage beaches used in mineralization microcosms. Moreover, mineralization of these compounds at each beach was improved by nutrient-biostimulation. These mineralization results suggest pristine high Arctic beaches along the Northwest Passage harbour microbes capable of degrading hydrocarbons. This was further supported by the detection of potential cold-adapted hydrocarbon-degrading genera in the 16S rRNA community data. Of the beaches used in the mineralization microcosms, sediment from Resolute had the highest total proportion of potential hydrocarbon-degrading genera (<10%), followed by Cambridge Bay (<7.5%) then Nanisivik (<2.5%). While hydrocarbon-degrading genera may be present in up to 10% of a microbial community (Atlas 1981) and have been detected by hydrocarbon-degrader enumeration methods in pristine high Arctic beach sediments previously (Røberg et al. 2007), the proportion of genera detected is surprisingly high. Screening of potential oil degrading genera was conducted using a selected 28 hydrocarbon degraders previously characterized in cold environments (Brakstad et al. 2017) and thus, may be an underestimate of the true hydrocarbon-degrading community. One possibility for the high total proportion of hydrocarbon-degrading genera may be a result of minor pollution events enriching microorganisms (Valentine et al. 2012). For Cambridge Bay, the beach sediment was sampled adjacent to a pier, increasing the likeliness that the shorelines experienced minor pollution. Another possibility is that these microbes are being enriched by undiscovered oil seeps as the High Arctic is thought to harbour 13% of the world's undiscovered oil (Gautier et al. 2009). Oil seeps have been previously characterized in Baffin Bay at Scott Inlet

and west Lancaster Sound (Blasco et al. 2010), which is over 500km away from the nearest beach sample collected in this study. In Alaska, 29 oil seep areas have been identified (Becker and Manen 1988).

The detection of hydrocarbon-degrading genera within naturally occurring communities of high Arctic beaches, including beaches in the Northwest Passage, may provide insight as to which shorelines may be vulnerable to oil spills. This biomonitoring approach using the identification of biomarker taxa has been explored in deep-water subarctic sediments (Gontikaki et al. 2018). While hexadecane and naphthalene mineralization were observed at each beach, the extent of mineralization was quite variable between beaches. Similar results have been characterized in 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). In the presented 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 extent of hydrocarbon biodegradation. These results highlight that the identification of specific taxa, rather than a broad list of potential degraders, may better resolve predicting potential response to oil degradation on Arctic shorelines. Greater organic matter content may also positively impact extent of hydrocarbon biodegradation (Chen et al. 2020). Both the Resolute and Nanisivik beaches used in the mineralization experiments had similar levels of organic matter content (3.79 mg g^{-} and 3.72 mg g^{-}, respectively) which may explain their similar extent of hexadecane and naphthalene degradation, and while physio-chemical analyses were not collected for Cambridge Bay, it is possible its lower latitudinal position relative to Resolute and Nanisivik may correlate

with a higher organic matter content (Paré and Bedard-Haughn 2013), and ultimately, greater extent of hydrocarbon biodegradation.

2.4.2 Biostimulation as a viable remediation strategy on Arctic shorelines

Influence of nutrients on fuel constituent and heavy marine fuel degradation

The addition of a common inorganic nitrogen, phosphorus and potassium fertilizer improved the biodegradation of fuel constituents, hexadecane and naphthalene, and increased depletion of headspace oxygen—a proxy for aerobic biodegradation—of the heavy marine fuel, Bunker C oil. This finding suggests that nutrient biostimulation is a viable remediation strategy for oil spill remediation on high Arctic beaches of the Northwest Passage. This finding also corroborates the positive impact of supplying nutrients as characterized previously on Arctic shorelines (Prince et al. 2003; Røberg et al. 2011), including in the Canadian context (Eimjhellen et al. 1982). Increased nutrient-stimulated hexadecane and naphthalene mineralization has also been reported using hydrocarbon contaminated soils from the Canadian High Arctic, with the application of a similar 20:20:20 fertilizer incubated at a similar temperature (5°C) (Whyte et al. 2001). It is not surprising the addition of nutrients had a positive impact since total nitrogen and phosphorus across beaches were in very low concentrations, which did not limit the mineralization of acetic acid, confirming that rather the supply of carbon-rich substrates (hexadecane, naphthalene and Bunker C oil) induce conditions whereby these nutrients becoming limiting. Determining an appropriate concentration of nutrients to supply is essential to avoid potential adverse effects such as eutrophication (Macaulay and Rees 2014). Here, hydrocarbon biodegradation improved not only when nutrients were supplied in a 100:15 C/N ratio in the mineralization assays—a recommended C:N nutrient stimulation ratio (Oh et al.

2001) that approximates Redfield stoichiometry (Filler et al. 2006)—but also, when supplied in a 2000:15 C/N ratio in the heavy marine fuel microcosms. Similar results on temperate shorelines have reported lower nutrient concentration being suitable to improve rates of oil biodegradation (Lee et al. 1993; Röling et al. 2002), but that nutrient concentration was a factor affecting microbial community composition (Röling et al. 2002).

Degradation of hexadecane and naphthalene

Across mineralization microcosms, the extent of hexadecane degradation in nutrient-amended microcosms ranged from 10.8%-20.5% and in unamended microcosms ranged from 1.0%-4.5%, whereas the extent of naphthalene degradation in nutrient-amended microcosms ranged 1.0-10.8% and in unamended microcosms ranged from 1.1-3.8%. Greater hexadecane relative to naphthalene degradation is expected as alkanes are favoured over polycyclic aromatic hydrocarbons (Perry 1984). Most studies of similar environments have been conducted with contaminated rather than uncontaminated sediments, though one study using uncontaminated Antarctic desert soils as a control found hexadecane and naphthalene degradation to be less than 5% degraded after incubating at 8°C for 90 days, even with the addition of nitrogen (Aislabie et al. 1998).

The extent of hexadecane degradation in unamended microcosms was similar to other studies where hexadecane was less than 5% degraded in unamended microcosms using hydrocarbon contaminated Arctic soils incubated at 5°C (Whyte et al. 2001; Børresen and Rike 2007), but was substantially lower than other studies where hexadecane was ~40-60% degraded using unamended hydrocarbon contaminated Arctic soils incubated at 7°C (Mohn and Stewart 2000) and was ~1-45% degraded using unamended hydrocarbon contaminated Antarctic coastal

soils incubated at 15°C (Aislabie et al. 2012). The extent of hexadecane degradation in nutrient-amended microcosms was slightly lower than what has been reported in other similar environments, where hexadecane was ~18-30% degraded in nutrient-amended microcosms using hydrocarbon contaminated high Arctic soils incubated at 5°C (Whyte et al. 2001) and was ~25-60% degraded in nutrient-amended microcosms using hydrocarbon contaminated Antarctic coastal soils incubated at 15°C (Aislabie et al. 2012).

The extent of naphthalene degradation in unamended microcosms was generally lower than what has been observed in other studies, where naphthalene was ~2.5-20% degraded in unamended microcosms using hydrocarbon contaminated high Arctic soils incubated at 5°C (Whyte et al. 2001), was ~8% degraded in unamended microcosms using uncontaminated temperate intertidal mudflat sediments at 10°C (Bauer and Capone 1985) and where phenanthrene, another aromatic compound, was 40-60% degraded using in unamended microcosms using hydrocarbon-contaminated Arctic soils incubated at 7°C (Mohn and Stewart 2000). The extent of naphthalene degradation in nutrient-amended microcosms was substantially lower than the reported ~25-50% of naphthalene degraded in nutrient-amended microcosms using hydrocarbon contaminated high Arctic soils incubated at 5°C (Whyte et al. 2001).

The comparison in extent of degradation to other studies suggest that low incubation temperature and unacclimated sediment (i.e. no prior exposure to oil contamination) likely influenced the total extent of hexadecane and naphthalene mineralization observed in the microcosms using sediment from uncontaminated high Arctic beaches in the Northwest Passage. Whyte et al. (2001) also reported higher mineralization in hexadecane and naphthalene microcosms incubated at 23°C rather than 5°C. Further insight on the chemical analysis of alkane and aromatic fractions in the heavy marine fuel microcosms (*data pending*), may improve

understanding of extent of biodegradation in high Arctic beach sediments at low temperature.

Influence of nutrients and heavy marine fuel on microbial community structure and function

Not only do the mineralization assays and heavy marine fuel microcosms corroborate previous work on Arctic shorelines suggesting biostimulation may be an effective oil spill strategy, but they further expand on the knowledge of high Arctic shorelines by providing insight into the effect of nutrients and oil on community structure and functional potential. Overall, the addition of nutrients impacted community structure as observed in the ordination of microbial communities in two distinct clusters at 14-, 35- and 55- days and increased abundance of key hydrocarbon biodegradation genes at 55 days, particularly for alkane-degrading genes: *alkB* and *CYP153.* Differences between microbial communities were observed on Arctic shorelines previously, where the addition of an oleophilic fertilizer had a strong effect in shaping community structure such that microbial communities from fertilizer alone and fertilizer-amended kerosene treatments were more similar and were distinct from microbial communities from kerosene alone treatments (Røberg et al. 2011). In contrast, in the presented study, the addition of inorganic nutrients and oil resulted in four distinct groupings between treatment combinations suggesting the influence of both nutrients and oil in shaping community structure. Shifts in community composition, that were observed from the 0-day sediment to the 55-day fuel contaminated and uncontaminated sediment, have also been described in hydrocarbon contaminated and uncontaminated microcosms over 15 days using sea-ice and sub-ice samples from the Northwest Passage (Garneau et al. 2016).

While there were no significant differences between genera, community richness or effective number of species between nutrient-amended and unamended treatments, the

significant differential abundance of *Pseudomonas* and *Rhodococcus* and significantly greater species richness and effective number of species in the 55-day oil microcosms relative to unoiled control microcosms suggest these two genera may be being selected for to degrade heavy marine fuel as part of a richer and more diverse community. Both genera are well characterized with oil degradation (Astashkina et al. 2015), including in polar environments (Chong et al. 2018; Rizzo et al. 2019) and the dominance of *Pseudomonas* and *Rhodococcus* in 16S rRNA gene clone libraries has been previously reported in oiled arctic shoreline sediments in Norway (Grossman et al. 1999). Moreover, in the 55-day fuel contaminated microcosms, *Pseudomonas* was associated to *alkB*, *ncr* and *phnAc* genes, while *Rhodococcus* was associated to *alkB*, *ncr* and *CYP153* genes. The 16S rRNA community data in conjunction with the metagenomic data provide evidence on the potential importance of these genera in oil degradation on high arctic beaches in the Northwest Passage. Increased abundance of *alkB* and *CYP* genes and the active expression of hydrocarbon biodegradation genes of *Pseudomonas* and *Rhodococcus*, have also been described in diesel-contaminated biopile remediation soils in the Canadian high Arctic (Yergeau et al. 2012). Species richness and effective number of species was significantly greater in fuel contaminated relative to uncontaminated microcosms suggesting a diverse consortia may be degrading hydrocarbons as seen in cold environments where aromatic degradation involves a more diverse community and alkane degradation involves a more specialized community (Brakstad et al. 2017). However, decreases in community richness and diversity following exposure to oil have also been described in other cold environments (Yang et al. 2016; Schreiber et al. 2019).

The taxonomic classification of hydrocarbon biodegradation genes observed in the 55-day fuel contaminated microcosms further suggest several other alkane-degrading and aromatic-

degrading genera. Many of the dominating associated genera have been described previously in oil-contaminated cold environments suggesting these genera may be important for cold-adapted biodegradation, including alkane-degrading genera: *Sphingorhabdus*, *Sulfitobacter*, *Psychrobacter*, *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 aromatic-degrading genera: *Novosphingobium*, *Sphingobium*, *Alicyclobacillus* from permafrost (Yang et al. 2014), and *Hyphomonas* and *Sphingopyxis* from arctic seawater (Crisafi et al. 2016). Grossman et al. (1999) detected genera found exclusively in biostimulated-oiled shoreline sediments in Norway, but these genera were not detected in the heavy marine fuel microcosms apart from *Cycloclasticus* in the 0-day sediment. Similarly, the dominant genera found in oil and nitrogen-stimulated oil microcosms using temperatures shorelines including *Alcanivorax/Fundibacter* and *Erythrobacter* were not detected in the heavy marine fuel microcosms, however some of the minor clones they detected including *Planococcus* in unamended oil sediments and *Sphingomonas*, *Roseobacter*, and *Nocardiodes* in biostimulated-oil sediments (Röling et al. 2002) were detected in community 16S rRNA gene sequencing data of heavy marine fuel microcosms or in the taxonomic classification of the metagenomic sequencing data in the 55-day heavy marine fuel microcosms. In contrast to the presented study, genera from oil contaminated microcosms using sea-ice and sub-ice samples of the Northwest Passage including *Moritella*, *Alcanivorax*, *Sulfitobacter*, and *Oleispira* (Garneau et al. 2016), were not detected as abundant in the marine heavy fuel 16S rRNA communities, nor in the taxonomic classification of the metagenomic sequencing data in the 55-day heavy marine fuel microcosms, except for *Sulfitobacter*.

2.4.3 Limitations and challenges

While this work corroborates early studies on biostimulation-associated oil biodegradation on Arctic shorelines and further broadens the knowledge of naturally-occurring microbial communities on high Arctic beaches in the Northwest Passage and the response to oil and nutrients, limitations in identifying key biomarker taxa and the development of the hydrocarbon degrading microbial consortia and challenges for translating these laboratory findings to *in situ* applications remain. As discussed earlier, the identification of key biomarker taxa within naturally occurring communities may provide insight into the predictability of how microbial communities respond to oil exposure. While the mineralization assays do not enable further microbial characterizations, the heavy marine fuel microcosms could have provided further insight. However, uncontaminated control microcosms were only sacrificed at 55-days. Thus, it could not be determined whether differentially abundant genera observed in the 14- and 35-day microcosms were being selected for oil degradation. Such insight may have given indication of hydrocarbon biodegradation succession as seen in other cold environments (Ribicic et al. 2018b; Vergeynst et al. 2018) and ultimately, may have helped to identify biomarker taxa within the naturally-occurring microbial communities of high Arctic beach sediments in the Northwest Passage. In heavy marine fuel microcosms, certain genera associated with hydrocarbon biodegradation were detected exclusively within the uncontaminated 0-day sediment including *Cycloclasticus* (Kasai et al. 2002), *C1-B045* (Peng et al. 2020) and *Alterythrobacter* (Dunlevy et al. 2013). Thus, the detection of these genera within uncontaminated beach sediment may not correlate with their relative importance to oil biodegradation. Further studies that elucidate succession of biodegradation on high Arctic shorelines may provide further insight into potential biomarker taxa. While the 55-day fuel

contaminated relative to uncontaminated microcosms suggested an increase in species richness and estimated number of species, without absolute abundance metrics and without uncontaminated microcosms in earlier stages of degradation (14 and 35 days), it is difficult to corroborate this observation which would further help elucidate the development of the hydrocarbon degrading consortia.

The lack of predictability may be a result of the microcosm environment. Variation between microcosm replicates was particularly apparent in nutrient-amended hexadecane mineralization assays, where at least a 10% difference in extent of mineralization was observed between replicates. Variation between abundances of genera and hydrocarbon biodegradation genes, particularly alkane degrading genes, were observed in heavy marine fuel microcosms replicates. Variation between community composition has been observed in replicate microcosms using temperate shoreline sediments (Röling et al. 2002) and between community composition and extent of degradation in dilbit-amended seawater microcosms (Schreiber et al. 2019). Variability not only causes challenges in interpreting laboratory results, but also poses challenges for extrapolating results to a field setting. Inherently, the use of microcosms make such extrapolation difficult since many environmental factors are not accounted for and as such, laboratory data may be an overestimate of field data (Diplock et al. 2009; Horel et al. 2015). While the mineralization assays and heavy marine fuel microcosms presented in this study provide insight into oil biodegradation and biostimulation on high Arctic beaches, follow-up microcosm studies that account for beach heterogeneity (via larger sized microcosms), and beach structure and hydrodynamics particularly tides (Guo et al. 2010; Cravo-Laureau and Duran 2014), may better extrapolate findings to *in situ* application whilst corroborating the findings presented here.

2.5 Conclusions

In this work, potential hydrocarbon-degrading genera were detected within the naturally occurring microbial communities of high Arctic beaches in the Northwest Passage. Moreover, these naturally occurring microbial communities demonstrate an ability to degrade hexadecane, naphthalene and a common marine heavy fuel oil, Bunker C, which is further enhanced with the addition of inorganic nutrients suggesting biostimulation as a viable bioremediation strategy on beaches of the Northwest Passage. Overall extent of hydrocarbon biodegradation was variable between high Arctic beaches and was likely limited due to very low nitrogen and phosphorus concentration, low incubation temperature and the prior uncontaminated nature of the beach sediments. The addition of nutrients not only improved extent of hydrocarbon biodegradation, but further altered microbial community composition and the abundance of key hydrocarbon biodegradation genes within heavy marine fuel microcosms. Clear differentiation between microbial communities in unamended relative to nutrient-amended fuel contaminated microcosms was observed, though specific differences in genera between treatments were not detected. The addition of nutrients favoured higher abundance of key hydrocarbon biodegradation genes, particularly alkane-degrading genes, in fuel contaminated microcosms after 55 days. Across high Arctic beaches, community composition was distinct, but potential hydrocarbon-degrading genera were detected at each beach. Characterization of microbial communities from heavy marine fuel microcosms suggested *Pseudomonas* and *Rhodococcus* may be important oil degrading genera on high Arctic beaches. Within the heavy marine fuel microcosms, 16S rRNA microbial communities and the taxonomy of hydrocarbon biodegradation genes further suggested potential cold-adapted oil degrading genera. Ultimately, this work provides insight into biostimulated-oil biodegradation on Arctic shorelines and

contributes to planning oil-spill remediation strategies in Canada's Northwest Passage.

2.6 Conflict of Interest

The authors declare no conflict of interest.

2.7 Acknowledgements

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2.8 Supplementary Materials

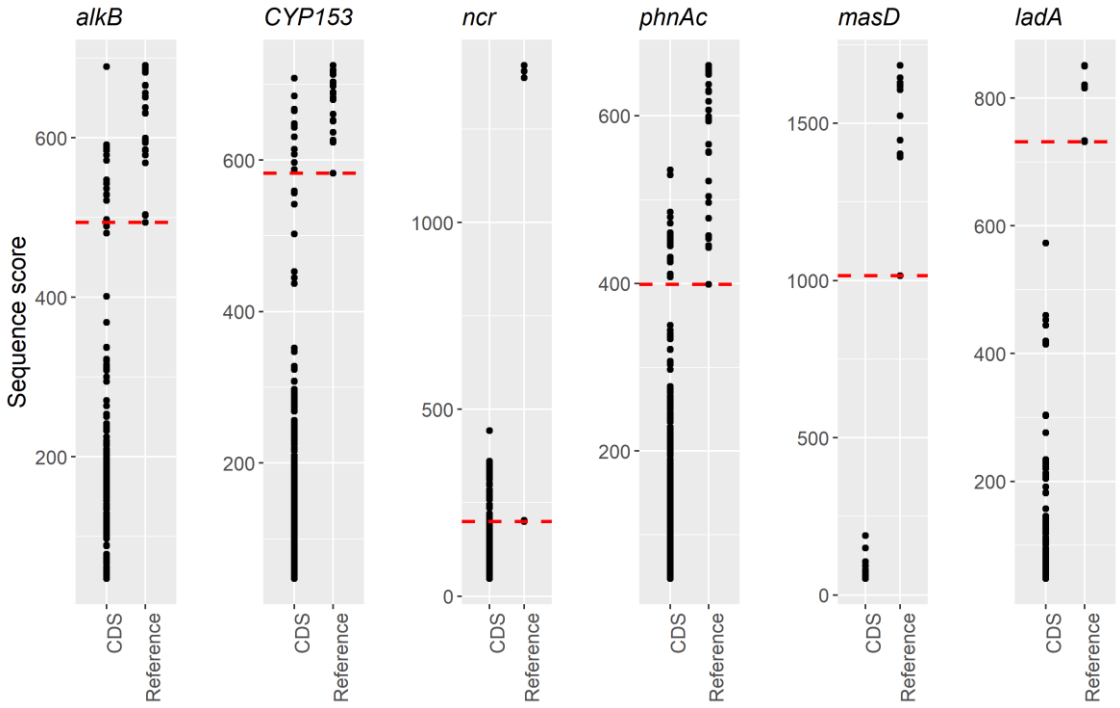

Figure S1. Graphical representation of coding sequences (CDS) against reference-group Hidden Markov Models. Coding sequences above the sequence score threshold (dotted red line) were considered in analysis as homologous genes, for each representative gene.

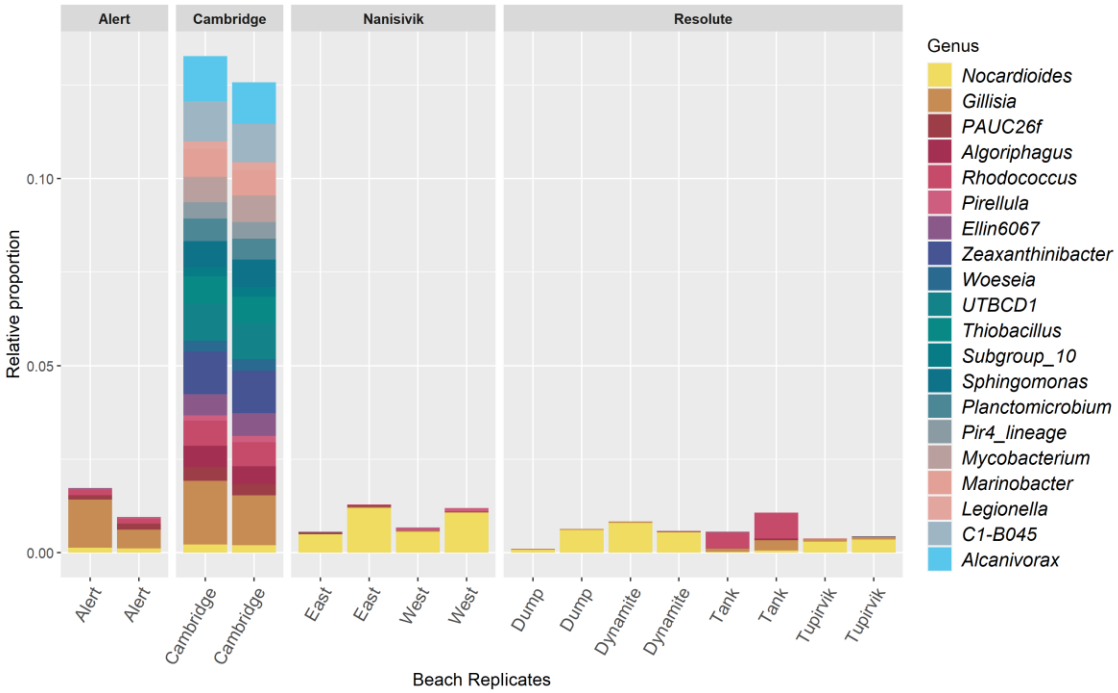

Figure S2. Relative abundances of the top 20 genera from Cambridge Bay beaches across all replicate beach samples.

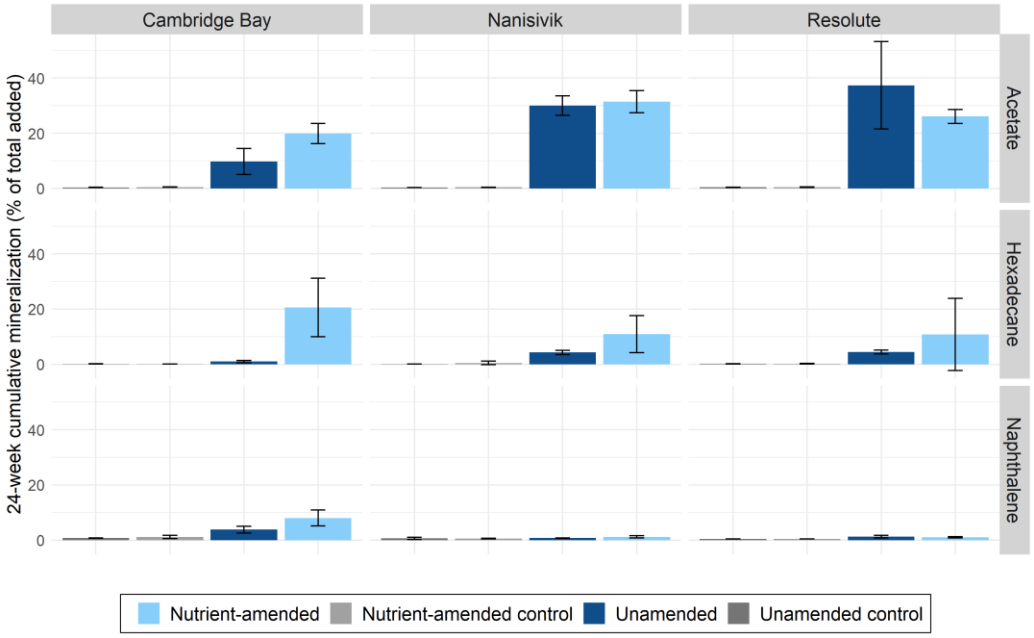

Figure S4. Mean 24-week cumulative mineralization (%) of ^{14}C- acetate (positive control) (A), hexadecane (B) and naphthalene (C) incubated at 4°C in nutrient-amended and unamended microcosms using beach sediment from Cambridge Bay, Nanisivik and Resolute beaches. Error bars representing standard deviation of the mean from triplicate microcosms.

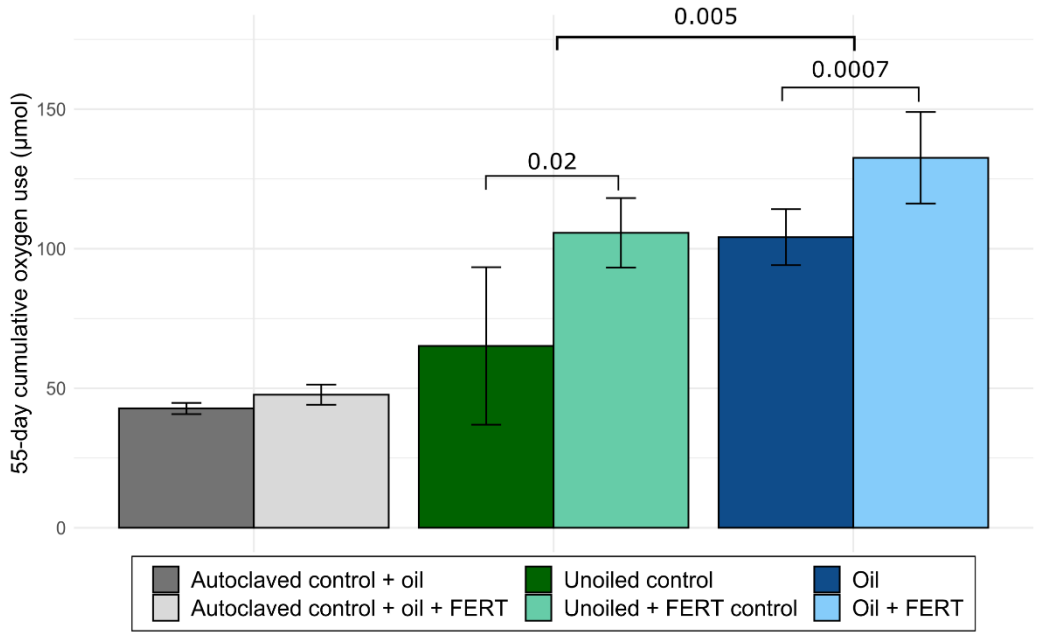

Figure S3. Mean 55-day cumulative headspace oxygen depletion (µmol) incubated at 4°C for nutrient-amended and unamended heavy marine fuel microcosms (n=6), no oil controls (n=3) and autoclaved sediment controls with heavy marine fuel (n=3). Error bars representing standard deviation of the mean of microcosms. Significance determined through one way, Student's T-Test.

Chapter 3: Discussion and Conclusions

Understanding the ability of naturally occurring microorganisms on Arctic shorelines to degrade oil can proactively inform oil spill bioremediation strategies. The aim of this work was to enhance understanding of hydrocarbon biodegradation and bioremediation on high Arctic beaches, particularly along Canada's Northwest Passage, through characterizing physicochemical variables and abundance and composition of naturally occurring microbial communities of high Arctic beaches, through screening these naturally occurring communities for potential hydrocarbon-degrading genera, and finally, through evaluating the response of these naturally occurring high Arctic beach microbial communities to degrade hydrocarbons with nutrient biostimulation, using inorganic nutrients, as a remediation strategy.

In this study, physicochemical properties and microbial abundances and composition were characterized in beach sediments from 8 high Arctic beaches, including 7 beaches from 3 locations in the Northwest Passage. Using sediment from 3 beaches, 1 representative of each Northwest Passage location, the potential to degrade hexadecane and naphthalene, common fuel compounds, was evaluated in nutrient-amended and unamended microcosms. Using sediment from 1 beach, the potential to degrade a marine shipping heavy fuel oil, Bunker C, was evaluated in nutrient-amended and unamended microcosms. Community composition and metagenomic analyses were conducted on heavy marine fuel microcosms to explore the effects of oil and nutrients on community structure and function and to elucidate potentially relevant hydrocarbon-degrading microorganisms on high Arctic beaches.

Similarities in physio-chemical variables and cellular abundances were observed between high Arctic beaches, but microbial community composition was distinct at each beach. However,

regardless of the initial microbial community composition at each beach, potential hydrocarbon-degrading genera were detected across beaches. The naturally occurring microbial communities were capable of degrading hexadecane, naphthalene and a heavy marine fuel, which was further enhanced with the addition of inorganic nutrients, suggesting biostimulation as a viable bioremediation strategy on beaches of the Northwest Passage. These findings are in accordance with the low abundance, yet ubiquitous nature of oil degrading microorganisms and the positive impact of biostimulation on oil degradation. Total extent of hexadecane and naphthalene biodegradation was variable between beaches and was likely limited due to limiting nitrogen and phosphorus concentration even after nutrient addition, low incubation temperature and the prior uncontaminated nature of the beach sediments. Heavy marine fuel microcosms showed that the addition of nutrients altered microbial community structure and function, differentiating microbial communities at the ASV-level in nutrient-amended relative to unamended microcosms and favouring higher abundance of hydrocarbon biodegradative genes, particularly alkane-degrading genes, in nutrient-amended microcosms. Collectively, between fuel contaminated and uncontaminated microcosms, *Pseudomonas* and *Rhodococcus* were significantly differentially abundant in the former, suggesting their importance in shoreline hydrocarbon biodegradation.

While this study improves the understanding of hydrocarbon biodegradation on high Arctic beaches in the Northwest Passage, particularly in the Canadian context, future research can further enhance this understanding. In this study, the addition of inorganic nutrients was examined; while inorganic nutrient fertilizers may offer advantages in low temperatures (Lee et al. 1993), they may also be subject to higher rates of washing out in comparison to oleophilic nutrient fertilizers which adhere to oil surfaces (Ladousse and Tramier 1991; Wrenn et al. 1997; Røberg et al. 2011; Prince et al. 2015). In this study the potential for hydrocarbon biodegradation

was evaluated using hexadecane, naphthalene, and a heavy marine fuel oil in microcosm experiments. Future research could evaluate the potential of biodegradation of other common fuel types, as this would lend to recommendations on whether some fuel types are more or less susceptible to oil degradation, given success of oil biodegradation inherently depends on the chemical mixture (Mishra and Kumar 2015; Varjani 2017). Exploring the potential for biodegradation in larger microcosms that account for beach heterogeneity (via larger sized microcosms), and beach structure and hydrodynamics particularly tides (Guo et al. 2010; Cravo-Laureau and Duran 2014; Wang et al. 2020), may better extrapolate findings to *in situ* application and further corroborate the findings presented here. *In situ* shoreline studies, such as the *Baffin Island Oil Spill* (BIOS) project conducted in the Canadian high Arctic in 1980 (Sergy and Blackall 1987) and the *Svalbard shoreline field trials* in Spitsbergen, Norway in 1997 (Guénette et al. 2003), in conjunction with high throughput methodologies would better enhance hydrocarbon biodegradation understanding on high Arctic beaches in the Northwest Passage.

Nonetheless, this work corroborates early studies of hydrocarbon biodegradation on high Arctic beaches and further demonstrates that hydrocarbon-degrading genera are present on high Arctic beaches of the Northwest Passage and the naturally occurring microbial communities can degrade hydrocarbons which is improved by addition of inorganic nutrients, suggesting nutrient biostimulation as a viable remediation strategy on high Arctic beaches. Moreover, the microbial analyses presented broaden the understanding of hydrocarbon-degrading communities on high Arctic shorelines, particularly in the Canadian context. Ultimately, this study serves to proactively contribute to oil-spill bioremediation strategies in Canada's Northwest Passage.

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