

Microbial Dynamics in Aquatic Ecosystems: Insights from
Environmental Monitoring and Hydrocarbon-Impacted
Environments

by

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ABSTRACT

Microorganisms play a fundamental role in sustaining ecosystem functions and mediating the services that aquatic systems provide to human communities. However, microbial responses to anthropogenic disturbances can either enhance or compromise ecosystem services, depending on the environmental context. This thesis investigates how human activities drive microbial community shifts in Arctic marine and northern freshwater ecosystems, and how these shifts influence regulating, supporting, provisioning, and cultural ecosystem services.

Chapters 2–5 examine microbial responses to oil contamination in Arctic marine environments, with a focus on hydrocarbon degradation and Marine Oil Snow (MOS) formation. Through controlled microcosm and roller bottle experiments, gene metabarcoding, and chemical analysis of hydrocarbons, this research evaluates how microbial community composition changes in response to hydrocarbon exposure. Results demonstrate that indigenous bacterial communities in Hudson Bay have the capacity for oil biodegradation, representing a critical regulating ecosystem service in cold marine systems where abiotic weathering processes are limited. The reconstruction and annotation of a high-quality metagenome-assembled genome (MAG) of a novel *Paraperlucidibaca* sp. discovered from Hudson Bay upon oil spill experiments further revealed genetic pathways associated with hydrocarbon degradation and potential cold adaptation, highlighting its ecological role in Arctic oil biodegradation.

Chapter 6 shifts to freshwater systems in northern Manitoba, where microbial communities serve as indicators of lake ecosystem health. Using microbial DNA analysis, this work investigates how nutrient and organic matter loading influence microbial composition and water quality. In contrast to the beneficial microbial responses observed in oil-impacted marine systems, freshwater microbial shifts were associated with deteriorating water quality and increased risks to provisioning and cultural ecosystem services important to First Nations communities.

Together, this thesis demonstrates that microbial communities respond predictably to anthropogenic disturbances, but the consequences of these responses differ across ecosystems. By linking microbial functional potential and community dynamics to ecosystem service outcomes, this research advances our understanding of how human-driven environmental change reshapes microbial ecology and, ultimately, human well-being in northern aquatic environments.

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CHAPTER ONE: INTRODUCTION

1.0 Thesis overview

In this thesis, I will explore the diverse roles that microbes play in mediating ecosystem services and the benefits they provide to people. In Chapters 2-5, I investigate microbial communities that facilitate oil biodegradation in marine environments, contributing to water purification and other associated regulating and supporting ecosystem services that are vital to First Nations and Inuit communities in the Hudson Bay region. In Chapter 6, I investigate microbial communities that serve as indicators of lake ecosystem health, particularly in the context of deteriorating water quality and its impacts on provisioning and cultural ecosystem services that are important to First Nations communities in northern Manitoba. In both cases, the microbes under investigation are responding to human activities: oil spills stimulate the proliferation of hydrocarbon-degrading bacteria, while increased nutrient and sediment loading associated with the regulation of waters for hydroelectric power generation contribute to declining water quality in freshwater systems. However, the ecological and societal outcomes of these microbial responses differ substantially. Chapter 7 synthesizes the findings and provides an integrated assessment of how each research question is addressed, outlining future research directions for advancing the understanding of microbial functionality in sensitive aquatic ecosystems.

1.1 Background

1.1.1 Global environmental change and ecosystem deterioration

Ecosystem services are the benefits people obtain from nature (Balvanera et al., 2017), typically generated by complex natural cycles driven by energy inputs such as solar radiation or chemical energy (Daily et al., 1997). Microbial ecosystem services link ecosystem processes with societal benefits (Ducklow, 2008). Human survival is fundamentally dependent on the health of ecosystems and the

resources they provide by microbes (Depledge and Galloway, 2005). Nonetheless, human activity is considered to be the major driver of global environmental change (Vitousek et al., 1997), impacting the regulating, provisioning, and cultural services provided by ecosystems (Dodds et al., 2013). Provisioning services refer to tangible goods obtained from ecosystems (Shackleton, 2021), such as food and freshwater. Regulating services include processes that regulate environmental conditions, such as water purification and climate regulation (Mengist et al., 2020). Supporting services underpin ecosystem functioning through processes like primary production and nutrient cycling (Naeem, 2009). Cultural services encompass non-material benefits, including spiritual, cultural, and recreational values that influence the quality of life (Cheng et al., 2019).

Microbial processes contribute directly and indirectly to all four categories, highlighting their foundational role in sustaining ecosystem integrity and human well-being. Ecosystem services provided by microbes in Arctic environments include such processes as oil bioremediation (Scheibye et al., 2017; Gomes et al., 2022), fixation of atmospheric nitrogen (Urakawa et al., 2019), and fermentation of country foods (Campbell et al., 2022), primary production (Ardyna et al., 2020) and mediating numerous other biogeochemical cycles (Solan et al., 2020). Likewise, the recent rise in the frequency, extent, and duration of harmful algal blooms in aquatic ecosystems exemplifies the role that microbes can play in the degradation of ecosystem services when influenced by human activity (Vitousek et al., 1997). Despite their importance for food provision (Lu et al., 2016) and recreation (Hilderbrand et al., 2023), aquatic ecosystems are under unprecedented pressure from human activities, particularly pollution and climate change (Labbate et al., 2016).

1.1.2 Impacts of Arctic change

The rapid rise in greenhouse gas emissions has intensified global warming, with the Arctic undergoing the most pronounced temperature increases, a process referred to as Arctic amplification (Kayode-Edwards et al., 2024). Significant effectors in the Arctic are carbon dioxide, nitrogen oxides, and black carbon (Chen et al., 2024). Increased access to previously inaccessible Arctic waters is expected to raise geopolitical and economic tensions as new shipping routes and exploitation opportunities arise (Kayode-Edwards et al., 2024), especially considering that up to 20% of the world's currently undiscovered oil reserves are thought to be in the Arctic (Bird et al., 2008). The increasing interest in the Northwest Passage is fueled by the melting of Arctic sea ice and the discovery of new natural resources such as oil, natural gas, rare earth elements, and other valuable mineral deposits (Lu et al., 2014; Bernauer, 2020; Collins and Kumral, 2020), which has led to heightened activities in this region and thereby elevated the risk of oil spills occurring in the marine environment (Wright et al., 2022). Most importantly, due to its status as the only deep-water port in the Canadian Arctic, the Port of Churchill has become a focal point for strengthening trade with Europe and other international partners (Department of Finance Canada, 2025). In the meantime, Hudson Bay is experiencing progressively longer ice-free seasons (Stroeve et al., 2024), which are increasing shipping access and traffic in the region (Andrews et al., 2017). This expansion in maritime activity heightens the potential risk of oil spills and other associated environmental impacts. Indigenous communities are at the greatest risk, as traditional ways of life are threatened by these changes (Kayode-Edwards et al., 2024).

1.1.3 Microbial communities as drivers of ecosystem function

Microbial life was the first to emerge on Earth, yet only in recent decades have we begun to understand its true potential in sustaining ecosystem health (Tomasulo et al., 2024). Through their metabolic

activities, microorganisms transformed the early atmosphere and established biogeochemical cycles that continue to regulate Earth's systems today (Lyons et al., 2024). Because microbes drive nutrient cycling, primary production, and organic matter decomposition, they form the foundation of ecosystem functioning (Li et al., 2018) and, consequently, the ecosystem services upon which humans depend. Across aquatic environments, bacteria dominate in abundance and mediate a substantial fraction of annual primary production and carbon flux (Cole, 1999), directly influencing water quality and climate regulation. Their sensitivity to environmental disturbances further positions microbial communities as early indicators of ecosystem change, making them critical not only for maintaining, regulating, and supporting services but also for monitoring their stability (Ma et al., 2022). Although the relationship between microbial diversity and ecosystem function can vary depending on environmental context, it is clear that shifts in microbial community composition can significantly alter ecosystem processes and the services they provide (Ducklow, 2008). For example, proliferation of toxin-producing cyanobacteria in fresh waters alters water quality ecosystem services such as drinking water safety and recreation; in oil-contaminated marine environments, enrichment of hydrocarbon-degrading bacteria can enhance biodegradation rates, influencing the natural attenuation of pollutants and overall ecosystem recovery.

1.1.4 Microbial response to hydrocarbons

Biodegradation by naturally occurring microbial populations is one of the primary mechanisms by which oil is removed from marine systems (Bacosa et al., 2022). This microbial breakdown of hydrocarbons represents a critical regulating ecosystem service, as it contributes to water purification and reduces the persistence and toxicity of spilled oil. The efficiency of biodegradation depends on temperature and nutrient availability (Aislabie et al., 2006), and the composition and adaptive capacity of the microbial community (Bacosa et al., 2022). Bacteria are the dominant degraders in marine

environments, while fungi often play more substantial roles in freshwater and terrestrial systems (Leahy and Colwell, 1990).

In the Arctic, biodegradation is likely to play an even more crucial role, since abiotic weathering processes like dispersion and evaporation proceed more slowly in cold, ice-laden waters (Gomes et al., 2022). In those cases, biodegradation by indigenous bacteria can be enhanced by adding nutrients, also known as biostimulation (Xu and Obbard, 2003; Prince and Atlas, 2005; Warr et al., 2013) or by applying dispersants directly into the contaminated area (Merlin et al., 2021), reflecting deliberate human efforts to augment microbial ecosystem services. However, the use of dispersants remains controversial, following the 2010 Deepwater Horizon oil spill in the Gulf of Mexico, given their toxicity to marine species, including reef corals (Shafir et al., 2007; Wise and Wise, 2011).

Spilled oil may also undergo Marine Oil Snow Sedimentation and Flocculent Accumulation (MOSSFA), a process that influences its vertical transport and ultimate deposition (Daly et al., 2016). Following an oil spill, the oil composition changes through a process called weathering. The most soluble hydrocarbons, such as benzene and toluene, dissolve in the underlying water, while hydrocarbons containing up to 15 carbon atoms typically evaporate within the first day (Gregson et al., 2021). Few oils are dense enough to sink in seawater. However, sinking can occur when oil forms aggregates with minerals (known as oil-mineral aggregation) or when it becomes incorporated into aggregates of zooplankton feces or mucoid particles produced by phytoplankton and bacteria (Brakstad et al., 2018). Marine Oil Snow (MOS) can function as a localized hotspot for microbial oil biodegradation (Arnosti et al., 2016), further contributing to hydrocarbon removal. However, once MOS sinks to the seafloor, it may impact benthic organisms and sediment-associated food webs (Yang et al., 2016), illustrating how microbial processes simultaneously support carbon

cycling while potentially altering benthic ecosystem structure.

Together, these processes demonstrate that microbial responses to oil spills not only determine contaminant fate but also mediate regulating and supporting ecosystem services that directly influence environmental recovery and human well-being, particularly in vulnerable Arctic marine systems.

1.1.5 Freshwater microbial dynamics

While pristine Arctic ecosystems are increasingly threatened by the risk of oil spills associated with heightened maritime activity, boreal freshwater ecosystems are under pressure from anthropogenic disturbances such as water regulation and hydroelectric power development, which deliberately alter the natural flow of water and can disrupt ecosystem structure and function. These environmental changes may reshape microbial community composition, with cascading effects on key processes such as nutrient cycling, organic matter decomposition, and overall ecosystem services. Freshwater ecosystems maintain diverse and abundant microbial communities (Okafor, 2011) that are distinct from marine and terrestrial ecosystems (Logares et al., 2010) and tightly linked to surrounding catchments, making them particularly sensitive to environmental change. Because freshwater systems serve as major sources of drinking water, fisheries, and culturally significant landscapes, the structure and function of their microbial communities are critically important for Indigenous communities whose livelihoods, health, and cultural practices are closely connected to these waters.

In large freshwater systems regulated by damming, hydrological alterations can further modify microbial community composition and function, although these effects on prokaryotic communities are often overlooked (Ruiz-González et al., 2013). Such shifts may alter nutrient cycling, organic matter processing, and toxin production, ultimately influencing the ecosystem services upon which

human communities depend.

Human activities, including land-use change, urban and rural development, sewage inputs, and river regulation through the construction of dams and artificial channels, can substantially alter freshwater microbial communities (Rojas et al., 2022). Such disturbances often degrade water quality and compromise ecosystem health, thereby affecting provisioning services (e.g., safe drinking water and fisheries), regulating services (e.g., nutrient retention and water purification), and cultural services valued by local and Indigenous communities. This impact is further underscored by the presence of 39 active long-term boil water advisories impacting approximately 5756 households across Canada (Government of Canada, 2026).

1.1.6 Community co-developed environmental monitoring project at Split Lake

Tataskweyak Cree Nation is a remote community located on the north shore of Split Lake and has been under a boil water advisory since May 2017, which became long-term in May 2018. Hydrological changes in this region are largely linked to the Churchill River Diversion project, initiated in 1978 by Manitoba Hydro, which increased the flow of water in the Nelson River by approximately 32% through the Burntwood River system. This diversion altered the natural hydrology of the lower system, including Split Lake, causing additional water pressure on the lake and its surrounding environment. Concerns regarding water quality and the potential impacts on community health and livelihoods have been raised by members of the Tataskweyak Cree Nation (MBC News, 2026). In response, the community expressed interest in developing a multidisciplinary research initiative to assess the overall health of Split Lake. As a result, initial discussions began in early 2024 to design an effective long-term environmental monitoring framework for the lake in partnership with the Environmental Conservation Lab (ECL) at the University of Manitoba and Tataskweyak Environmental Monitoring

Agency (TEMA), a community-driven environmental monitoring initiative. In the summer of 2024, we embarked on the first long-term monitoring multi-disciplinary field campaign with multiple research groups at the University of Manitoba.



Figure 1. 1: Discussion sessions with community members and university partners on the progress of the first environmental monitoring campaign.



Figure 1. 2: Field sampling at Split Lake (left) and Assean Lake (right) in Summer 2024.

1.2. Research questions and objectives

Microbial communities act as the first responders to ecosystem disruption due to their rapid growth rates, metabolic versatility, and strong linkage to biogeochemical cycles. Environmental perturbations such as contamination, nutrient enrichment, or temperature change can trigger immediate shifts in microbial community composition and function, often preceding detectable responses in higher organisms. Through changes in abundance, gene expression, and metabolic activity, microbes provide early indicators of ecosystem stress while simultaneously driving initial recovery processes. This thesis aims to address the overarching research question: *How do anthropogenic activities influence microbial community shifts in aquatic ecosystems that affect ecosystem services?* This broader research question is addressed through a series of specific questions and hypotheses, each supported by defined objectives and corresponding methodological approaches, which are presented in the respective thesis chapters.

1.2.1 Research Question 1

Question 1: Can Marine Oil Snow formation be induced through biostimulation? We hypothesized that microbial community structure in microcosms would shift in response to different hydrocarbon sources, concentrations, and biostimulatory additives. These conditions were expected to influence both the early succession of oil-degrading microbes and the formation of MOS aggregates. In addition, dispersant addition was expected to increase the abundance of MOS aggregates.

Objective 1: Compare MOS formation in seawater exposed to different hydrocarbon sources (diesel vs. crude oil).

Objective 2: Compare early microbial community succession in seawater exposed to different hydrocarbons and concentration levels (0.1% (v/v) vs. 1% (v/v)).

Objective 3: Evaluate the effects of nutrients, dispersants, and their combination on early MOS formation in seawater exposed to diesel or crude oil.

1.2.2 Research Question 2

Question 2: Does Marine Oil Snow formation affect microbial community composition and hydrocarbon biodegradation in Hudson Bay seawater? We hypothesized that marine oil snow formation would increase the degradation rate of oil in microcosms seeded with Hudson Bay estuarine water. To test this hypothesis, the following objectives were formulated.

Objective 4: Test the effect of biostimulation (nutrients and alternative dispersants) on MOS formation in oil-in-seawater microcosm experiments.

Objective 5: Characterize microbial communities associated with oil-in-seawater microcosm experiments using molecular techniques (16S rRNA gene sequencing and metagenomics)

Objective 6: Quantify hydrocarbon biodegradation using Gas Chromatography – Mass Spectrometry (GC – MS) analysis and link it to microbial abundance and activity.

1.2.3 Research Question 3

Question 3: Do MOS-derived bacterial consortia produce biosurfactants? We hypothesized that oil-degrading bacteria in Hudson Bay encoded the potential to produce biosurfactants and bioemulsifiers, and that the yield of surfactants may vary with temperature and other environmental parameters.

Objective 7: Utilize qualitative and quantitative methods to evaluate potential biosurfactant-producing microbial consortia cultured directly from MOS aggregates.

Objective 8: Quantify the production of crude biosurfactants at environmentally relevant temperatures.

1.2.4 Research Question 4

Question 4: Do Arctic marine bacteria encode the potential for hydrocarbon degradation? We hypothesized that enrichment cultures fed with hydrocarbons as a sole carbon source would encode the potential to degrade alkanes and aromatics.

Objective 9: Reconstruct and annotate the complete genome of an oil-degrading bacterium enriched in Hudson Bay waters.

1.2.5 Research Question 5

Question 5: How does microbial community biogeography vary across Split Lake? We hypothesized that the microbial community composition of Split Lake varied spatially across the lake due to freshwater inputs from multiple river inflows influenced by hydroelectric power development.

Objective 10: Establish a baseline for microbial community composition of the surface waters of Split Lake, MB, using 16S rRNA metabarcoding.

Objective 11: Evaluate the environmental parameters affecting shifts in microbial community composition of Split Lake waters.

Objective 12: Assess the potential toxicological risk posed by microcystin in Split Lake.

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CHAPTER TWO: MARINE OIL SNOW FORMATION IN MICROCOSM INCUBATIONS OF ARTIFICIAL SEAWATER AT 4 °C.

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2.0. Abstract

An oil spill in the Arctic would have far-reaching impacts on the environment, the biota, and the Inuit communities that depend on this unique ecosystem for their livelihoods. Due to the rapid retreat of sea ice in the region, the Arctic is becoming more vulnerable to oil spills. Since microorganisms are the first responders to oil contamination, understanding their behavior is critical for designing bioremediation approaches tailored to Arctic conditions. In this study, we conducted microcosm experiments at 4 °C in the dark to simulate an Arctic oil spill, using crude oil and diesel as hydrocarbon sources. Biostimulation was tested with the addition of nutrients NaNO_3 and KH_2PO_4 , and soy lecithin–Tween 80 dissolved in ethanol as an alternative dispersant. Microcosms were placed on roller tubes at 3 rpm to mimic natural wave action and run with appropriate controls to assess microbial community shifts. Our objectives were to characterize the early microbial succession associated with oil degradation and marine oil snow (MOS) formation, and to determine how nutrients, dispersants, oil type (crude vs diesel), photochemical aging (fresh vs weathered), and oil concentration influence these processes.

We observed that the combined effect of nutrients and dispersant resulted in rapid MOS formation, occurring as early as 30 minutes after the initial setup. Microbial taxa exhibited varying tolerance to different concentrations of the same oil, and the dominant hydrocarbon degraders in fresh diesel treatments were distinctly different from those in weathered diesel. Furthermore, the nutrient–dispersant treatment promoted an earlier succession of oil-degrading taxa compared to nutrient-only or dispersant-only treatments. Together, these results highlight the importance of cold-adapted microbial communities in driving early oil degradation and MOS formation in Arctic waters, and they provide insights relevant to the development of Arctic-specific bioremediation strategies.

Key words: Arctic, oil spills, biostimulation, dispersants, MOS

2.1. Introduction

The Arctic is at risk from future oil spills due to increased vessel traffic elevated by the rapid retreat of ice cover (Kapsar et al., 2023). Northern communities rely heavily upon ships as a way of transporting goods to serve the region (Prowse et al., 2009; Pelletier and Guy, 2015), but the continued expansion of maritime activities in the Arctic is anticipated to raise the risk of oil spills (Afenyo et al., 2019), which pose acute and chronic stressors to the environment, wildlife, and Inuit communities living there (Wright et al., 2022a). The composition of spilled oil changes immediately after its release into water (Mariano et al., 2008). While spilled oil undergoes evaporation, photooxidation, and dispersion (Garrett et al., 1998), biological degradation is the primary process for removing oil from both surface and subsurface plumes (Scheibye et al., 2017). Breaking down hydrocarbons requires an appropriate consortium of degraders and conditions that support the growth and multiplication of these microorganisms (Ron and Rosenberg, 2014). Bioremediation in cold marine environments is considered a labor-effective technique since it does not produce harmful byproducts (Brakstad et al., 2017). However, the rate and extent of oil biodegradation are influenced by the availability of Nitrogen (N) and Phosphorus (P) in the marine environment (Hazen et al., 2016). Surface seawater typically contains low (nano or micromolar) concentrations of nitrogen and phosphorus, and hydrocarbons themselves are deficient in these essential nutrients (Atlas and Bartha, 1973). Therefore, the addition of N and P following an oil spill has been shown to support the native marine microbiota that contributes to oil biodegradation (Mirsaeidi, 2023). Furthermore, the rate of biodegradation depends on various factors such as temperature, oil composition, presence of electron acceptors, and an active microbial population (Rahsepar et al., 2016a). Nevertheless, biodegradation rates are often overestimated (Mariano et al., 2008), particularly due to oil being photo-oxidized and volatilized, and by ocean currents moving the oil to shore (Ron and Rosenberg, 2014).

Since different oils have distinct physical, chemical, and toxicological characteristics, they behave differently in the marine environment and can produce markedly different environmental impacts when spilled (Wright et al., 2022b). After an oil spill, skimmers are typically used to remove oil mechanically. However, harsh weather conditions can severely limit their effectiveness, and even the largest booms and skimmers may be unable to operate, e.g., in harsh Arctic conditions (Prince et al., 2017). Hence, responders use dispersants to reduce the impact of large oil slicks reaching shorelines and to promote biodegradation (Prince, 2015). Chemical dispersants consist of surfactants and solvents (Wise and Wise, 2011) that reduce the interfacial tension between oil and seawater, forming smaller oil droplets and making oil highly accessible for microbial degradation. (Lessard and DeMarco, 2000; Rahsepar et al., 2016a). Though the toxicity of dispersants varies depending on the dispersants and the species affected, oil concentration and type of oil dispersants may not pose a significant risk to aquatic species in field conditions (Bejarano, 2018). With advances in formulation chemistry, modern dispersants are far less toxic than earlier products (Kleindienst et al., 2015a), with food-grade and biological surfactants being promising eco-friendly dispersants, given their non-toxicity and biodegradability (Athas et al., 2014). Lecithin and Tween 80 are the most widely studied surfactants used as dispersants, owing to their enhanced emulsifying capacity (Jin et al., 2019). In this study, we used a lecithin–Tween 80 mixture at a 60:40 (w/w) ratio, dissolved in ethanol, as the dispersant. Lecithin is a mixture of phospholipids, glycolipids, triglycerides, and fatty acids, and Tween 80 (Polysorbate 80; $C_{64}H_{12}O_{26}$) is a complex nonionic surfactant and emulsifier; both are used widely in food production and industrial applications (Riehm et al., 2017). Dispersants promote the solubility of spilled oil by increasing the surface-to-volume ratio (Prince, 2015), making them accessible for microbes (Kleindienst et al., 2015); aggregation can also affect oil–microbe interactions, e.g. the

formation of Marine Oil Snow (MOS), a key process influencing the aggregation, sedimentation, and microbial degradation of oil in the water column (Passow, 2016a; Quigg et al., 2020; Gregson et al., 2021).

This microcosm study was designed as a pilot project to explore the impacts of fresh and weathered diesel and crude oil on MOS-associated biodegradation under controlled Arctic-like conditions. Experimental treatments incorporated varying hydrocarbon concentrations, nutrient amendments, and dispersant regimes to simulate realistic spill scenarios. We aimed to characterize the early microbial responders to oil contamination and evaluate their potential functional roles in degrading different hydrocarbon sources. These microbial dynamics provide insight into the mechanisms governing MOS formation and its role in oil aggregation, vertical transport, and biodegradation in cold marine environments.

2.2 Materials and Methods

2.2.1 Field sampling

Artificial seawater samples were collected into a large sterile cubitainer from the Sea-Ice Environment Research Facility (SERF) at the University of Manitoba on April 7th, 2025. The SERF pool (60 ft x 30 ft x 8 ft) had been filled with an artificial seawater mixture composed of a commercial brine slurry dissolved in groundwater to mimic seawater salt concentrations. The water sample was immediately stored at 4 °C in the cold room facility at the Centre for Earth Observation Science for 2 days until the microcosms were set up.

2.2.2 Microcosm setup and subsampling

The microcosms were established in 125 mL Pyrex glass bottles placed on tube rollers, following the method described by Gutierrez et al. (2013). Three sets of experiment groups were created with one replicate per sample, and each was monitored until the first signs of MOS were observed. The hydrocarbons used for this experiment included fresh Churchill diesel, UV-weathered Churchill diesel, and UV-weathered Tundra crude oil. Two concentrations were tested: 1% (v/v) and 0.1% (v/v).

Before the experiment, aliquots of Churchill diesel fuel and Tundra crude oil were exposed to UV weathering using UV-A (365 nm) and UV-B (302 nm) lamps for 5 days. A total of 24 microcosms were prepared, with each consisting of 98.9 mL of seawater amended with either UV-weathered Churchill diesel, UV-weathered crude oil, or fresh diesel at concentrations of 1% or 0.1%.

The experimental setup included three treatment types for each oil and concentration: (1) nutrients only, (2) dispersants only, and (3) a combination of nutrients and dispersants. For dispersants, soy lecithin and Tween 80 (60/40, w/w) dissolved in ethanol were added at a final concentration of 0.1% to the appropriate treatments. Two control treatments containing only seawater and UV-weathered diesel were prepared at 0.1% and 1%.

Microcosms that contained nutrients were amended with 7.785 mM NaNO₃ and 0.2 mM KH₂PO₄ to provide nutrient-replete conditions (1000x the natural environment) and satisfy nutrient requirements based on Redfield ratios. Microcosms were placed horizontally on tube rollers set at 3 rpm and maintained at 4 °C in the dark to simulate natural oceanic conditions. All treatments, except those amended with fresh diesel, were destructively sampled after one week, coinciding with the formation of a substantial amount of MOS in the majority of the treatments. Fresh diesel-amended treatments

were sampled after 3 weeks, once a comparable amount of MOS had developed. Observations were recorded at the time of sampling.

2.2.3 Genomic DNA extraction

Since the majority of MOS were at an early stage of development, they were not able to be separated reliably. Hence, the whole water sample was filtered onto a 0.22 µm Sterivex filter for genomic DNA extraction. The Qiagen DNeasy PowerSoil kit was used to extract DNA from the samples following the manufacturer's instructions, with slight modifications. For all extractions, the bead-beating tube containing the samples and CD1 solution were heated to 70 °C for 10 minutes to facilitate cell lysis. Additionally, an aliquot of elution buffer was heated to 50 °C before eluting the DNA in the final step. All extracted DNA products were quantified using a Qubit fluorometer and stored at -70 °C until library preparation and sequencing.

2.2.4 16S rRNA amplicon sequencing

The V4-V5 hypervariable regions of the 16S rRNA gene were amplified using the 515F (GTGYCAGCMGCCGCGGTAA) and 806 RB (GGACTACNVGGGTWTCTAAT) primer pair (CD Genomics, New York, USA). Amplicon libraries were sequenced using paired-end (2 x 250 bp) Illumina Novaseq (CD Genomics, New York, USA). Approximately 680k high-quality reads were obtained with a read depth ranging from 14,182 to 45,158 per sample. Sequencing reads were first quality-filtered to remove adapters and primer sequences using Cutadapt v5.2 (Martin, 2011). The resulting trimmed reads were processed with the DADA2 package in R (Callahan et al., 2016) to identify amplicon sequence variants (ASVs), followed by taxonomic classification using the SILVA v138.2 reference database (Quast et al., 2013). Code for the bioinformatics pipeline is available at

<https://github.com/rec3141/microscape>.

2.2.5 Statistical analysis and visualization

Microbial community composition was performed using R. All plots were generated in R (v4.3.1) using the ggplot2 package (Wickham, 2016).

2.3 Results

2.3.1 MOS development

The earliest development of MOS in roller incubations occurred within 30 minutes in the treatment containing 1% diesel fuel, supplemented with 0.1% dispersants and nutrients (Figure 2.1). In the weathered diesel treatments, MOS particles were suspended in the water column and were off-white in color, and were approximately 2 to 3 mm in size. However, after one week, they aggregated and became negatively buoyant and had settled at the bottom. The MOS produced in fresh diesel treatments differed significantly from that formed in weathered diesel treatments. The latter were noticeably whitish and had a mucous texture, remaining suspended throughout the water column, and small oil droplets were observed on the surface of the water. The shapes of those two categories varied, with some being linear and some being round. In weathered crude oil treatments, MOS particles were light brown in color, round in shape, and had negative buoyancy. The samples also contained linear structures that appeared to be an early stage of MOS development, which were neutrally buoyant and suspended at the surface (Table 2.1).

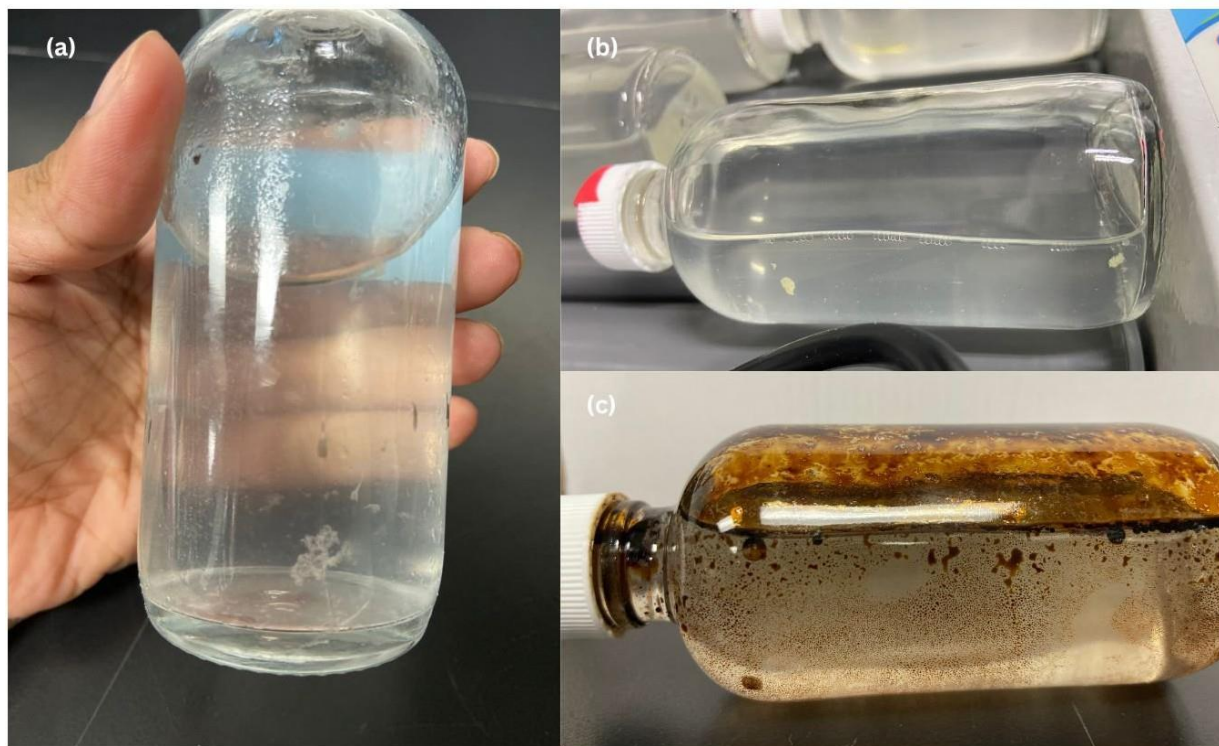


Figure 2. 1 : MOS aggregates formed in (a) fresh diesel (3 weeks old), (b) UV-weathered diesel (1week old), (c) UV-weathered crude oil treatments (1 week old).

Table 2. 1: Experimental design for microcosm setup and visual observations of MOS formation

Time	Treatment Group	Oil type	Oil: water ratio (%)	MOS presence	Observations
3 WEEKS	SW + Oil + N ¹	Fresh Diesel	0.1	Y	Tiny MOS particles scattered in the water column
	SW + Oil + D ²	Fresh Diesel	0.1	N	Cloudy water, small oil droplets on the surface water
	SW + Oil + N+D ³	Fresh Diesel	0.1	Y	Mesh-textured MOS, neutral buoyancy
	SW + Oil + N	Fresh Diesel	1	N	Oil droplets on the surface water
	SW + Oil + D	Fresh Diesel	1	N	-
	SW + Oil + N + D	Fresh Diesel	1	N	-
1 WEEK	SW + Oil + N	Weathered Diesel	0.1	N	-
	SW + Oil + D	Weathered Diesel	0.1	N	-
	SW + Oil + N+D	Weathered Diesel	0.1	N	-
	SW + Oil + N	Weathered Diesel	1	N	-
	SW + Oil ⁴	Weathered Diesel	0.1	N	-
	SW + Oil	Weathered Diesel	0.1	N	-
	SW + Oil + D	Weathered Diesel	1	N	-
	SW + Oil + N + D	Weathered Diesel	1	Y	Off-white and dense MOS sunk in the bottom
1 WEEK	SW + Oil + N	Weathered Crude Oil	0.1	Y	Linear structured MOS, positively buoyant.
	SW + Oil + D	Weathered Crude Oil	0.1	N	-

¹ Nutrient alone treatment

² Dispersant alone treatment

³ Nutrient and dispersant treatment

⁴ Control treatment

	SW + Oil + N+D	Weathered Crude Oil	0.1	Y	A light brown MOS was suspended for a few minutes
	SW + Oil + N	Weathered Crude Oil	1	Y	Round-shaped MOS, positively buoyant
	SW + Oil + D	Weathered Crude Oil	1	N	-
	SW + Oil + N + D	Weathered Crude Oil	1	Y	A light brown MOS, negatively buoyant

2.3.2 Bacterial community response to hydrocarbon inputs and biostimulation

Sequences clustered at 100% similarity yielded 243 ASVs, representing 10 phyla, 13 classes, 44 orders, 74 families, and 100 genera. Microbial community composition shifted significantly from the unincubated seawater (pre-treatment) to the amended treatments, indicating strong selective effects of oil exposure and experimental manipulations (Figure 2.2).

Control treatments containing UV-weathered diesel at 0.1% and 1% displayed nearly identical community structures at the time of sampling. In both controls, *Salinibacterium* dominated the communities (37.2% and 32.1%, respectively), suggesting limited compositional differentiation in the absence of nutrient and dispersant amendments.

In contrast, the addition of nutrients and dispersants induced pronounced shifts in community structure, indicating strong selective enrichment of hydrocarbon-degrading taxa. Treatments amended with fresh diesel at 0.1% and 1% were dominated by distinct microbial taxa, reflecting concentration-dependent ecological selection. The 0.1% treatment was overwhelmingly dominated by *Colwellia* (89.5%), whereas the 1% treatment was dominated by *Marinomonas* (95.2%), suggesting that hydrocarbon concentration, in combination with nutrient and dispersant addition, exerted a powerful structuring effect on the microbial community.

Similarly, the nutrient-only treatments at 0.1% and 1% differed markedly in community composition, suggesting a concentration-dependent response. The 1% treatment was dominated by *Oleibacter* (62.0%), suggesting strong enrichment of hydrocarbon-associated taxa under higher substrate availability. In contrast, the 0.1% treatment exhibited a more diverse community structure, with *Salinibacterium* (27.3%) as the most abundant taxon, followed by *Marinobacter* (15.6%), *Shewanella*

(10.8%), and *Seohaecicola* (9.9%). These differences highlight the influence of hydrocarbon concentration on microbial selection even in the absence of dispersant addition.

Dispersant-only treatments exhibited broadly similar microbial community structures across the two diesel concentrations, although differences in relative abundances were evident. At 1%, *Marinomonas* was the most abundant taxon (38.0%), followed by *Shewanella* (22.2%), indicating strong enrichment of known copiotrophs at higher oil concentrations. In the 0.1% treatment, *Marinomonas* remained highly abundant (29.4%), but at a level comparable to *Colwellia* (26.8%), suggesting a more balanced community structure at the lower diesel concentration. Overall, these results suggest that while dispersant addition shaped community composition consistently across concentrations, the hydrocarbon level also influenced the relative dominance of key taxa.

Similar compositional divergences were observed in the UV-weathered diesel treatments. At the lower weathered diesel concentration (0.1% v/v), treatments amended with dispersants alone and with the combination of nutrients and dispersants were dominated by *Pseudoalteromonas*, with relative abundances of 63.3% and 67.1%, respectively. In contrast, the corresponding treatments at higher diesel concentration (1% v/v) exhibited distinct community structures. Notably, *Paraperlucidibaca* was most abundant in the nutrient-and dispersant-amended treatment at 1% weathered diesel (42.8%), suggesting a concentration-dependent shift in taxonomic dominance under UV-weathered oil exposure.

Clear divergence was also observed between fresh and UV-weathered diesel treatments, highlighting the influence of oil type on microbial assembly (Figure 3). Weathered crude oil treatments after one week exhibited distinct microbial community compositions compared to the pre-treatment (unincubated seawater), suggesting rapid shifts in response to crude oil exposure. Treatments amended with nutrients and dispersants at 0.1% and 1% weathered crude oil supported markedly different

microbial assemblages, suggesting concentration-dependent selection. At the lower crude oil concentration, *Colwellia* dominated the community (80.8% relative abundance), whereas at the higher concentration, *Pseudoalteromonas* was most abundant (66.2%). These results suggest that increasing hydrocarbon concentration under nutrient-and dispersant-amended conditions strongly influenced taxonomic dominance and community structure.

Dispersant-only treatments exhibited greater variability in microbial community composition between the two crude oil concentrations. At the lower crude oil concentration, communities were characterized by the presence of *Colwellia*, *Yoonia*, *Salinibacterium*, and *Pseudoalteromonas*. In contrast, the higher crude oil concentration was predominantly dominated by *Yoonia* and *Pseudoalteromonas*, indicating a concentration-dependent shift in community structure under dispersant exposure.

In contrast, nutrient-only treatments after one week at both crude oil concentrations supported more diverse microbial communities overall, while largely maintaining a community composition similar to that of the unincubated seawater. This suggests that nutrient addition alone exerted a relatively limited selective pressure compared to treatments amended with both nutrients and dispersants.

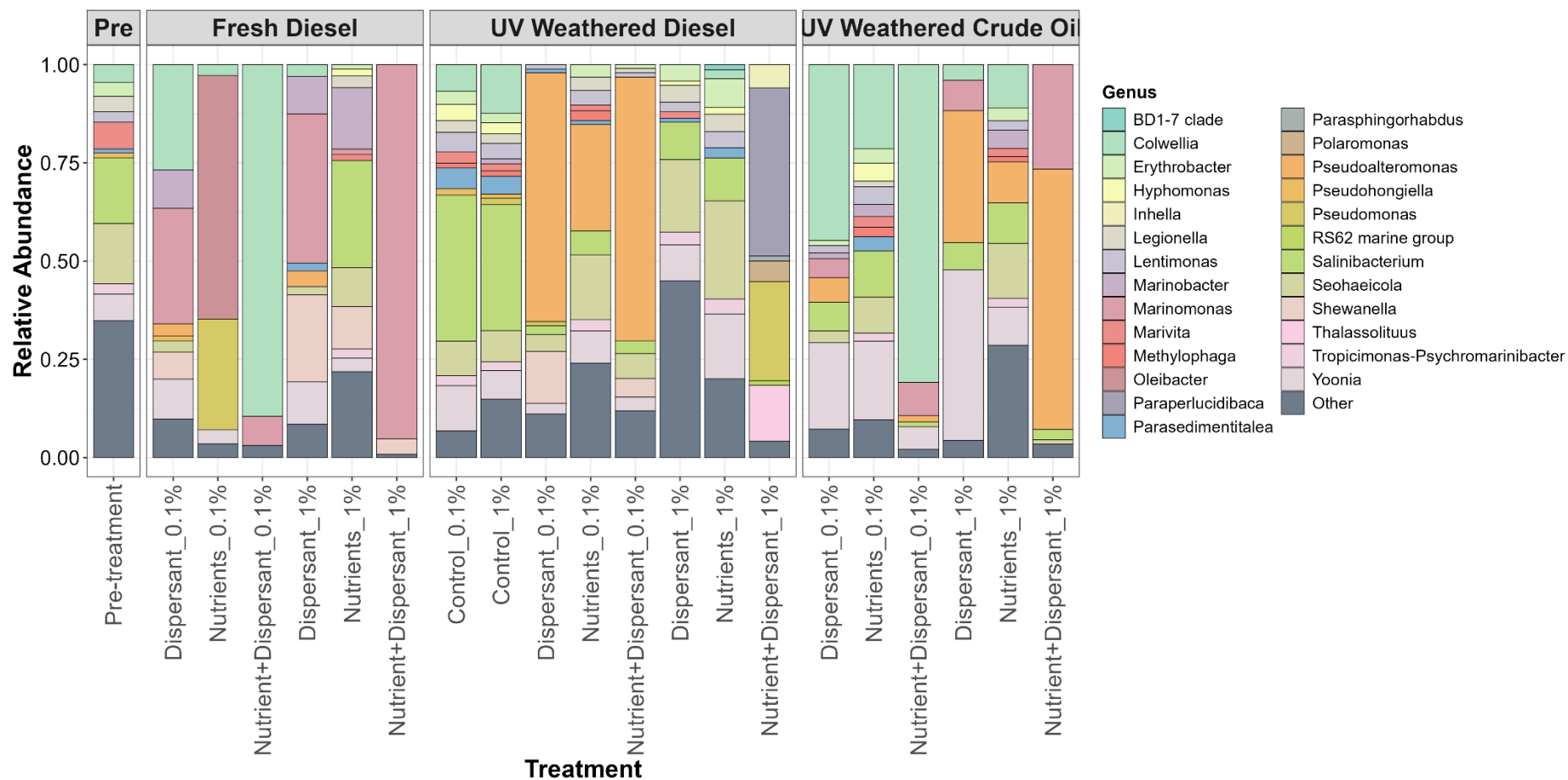


Figure 2. 2: Bacterial community composition at the genus level for the incubated seawater (pre- treatment), fresh diesel (after 3 weeks of incubation), control, UV-weathered diesel, and UV-weathered crude oil treatments (after 1 week of incubation). The hydrocarbon concentrations are shown as percentages (0.1% or 1%) with each treatment type. Each treatment had only one sample (n=1).

2.3.3 Bacterial community responses to hydrocarbon weathering and biostimulation

Specific bacterial taxa were associated with distinct hydrocarbon substrates, concentrations, and biostimulation regimes (Figure 2.3). In particular, *Marinobacter* and *Oleibacter* were detected in comparatively higher abundance in fresh diesel treatments, although their individual relative abundances varied across treatment types, suggesting differential affinity or tolerance to fresh diesel concentration and biostimulation conditions. Notably, *Oleibacter* exhibited the highest relative abundance in treatments containing the lower fresh diesel concentration amended with nutrients, indicating a potential preference for gaseous hydrocarbon availability under nutrient-enriched conditions.

In contrast, *Paraperlucidibaca* and *Erythrobacter* were detected at relatively higher abundances in weathered diesel treatments, suggesting substrate-specific selection or limited tolerance to fresh diesel and crude oil environments. *Pseudoalteromonas* dominated in weathered oil treatments (0.1% weathered diesel and 1% weathered crude oil), suggesting their preference for more complex or oxidized residuals and a later succession of this taxon in oil spills.

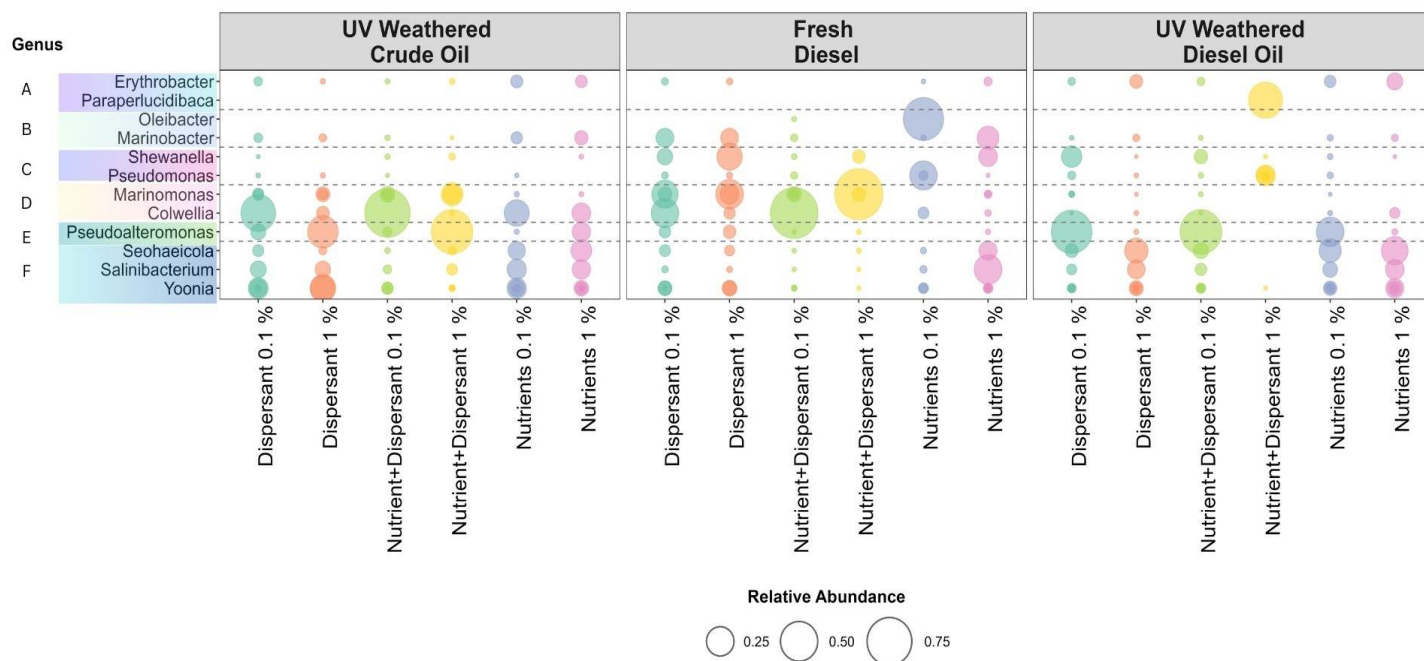


Figure 2. 3: Relative abundance of bacterial taxa across different hydrocarbon inputs, concentrations, and biostimulation conditions. Bubble size is proportional to the relative abundance (%). Taxa were considered prominent if their relative abundance reached $\geq 0.05\%$ in any treatment and were grouped according to their distribution patterns: (A) prominent only in UV-weathered diesel, (B) in fresh diesel, (C) in both fresh and UV-weathered diesel, (D) in both UV-weathered crude oil and fresh diesel, (E) in both UV-weathered diesel and UV-weathered crude oil, and (F) in all three treatments.

2.4 Discussion

Bacterial exudates play a significant role in the formation, modification, and degradation of Marine Oil Snow (Simon et al., 2002; Grossart et al., 2003). Many microbes produce high molecular weight bacterial exudates in response to oil, which aid them in attachment, and also solubilize and emulsify the oil compounds, thus increasing the bioavailability of oil (Head et al., 2006a; McGenity et al., 2012). While the majority of the MOS studies have investigated the environmental effects, such as phytoplankton biomass, presence of particulate matter, and presence of EPS (extracellular polymeric substances) producing bacteria, not many studies have focused on how weathered oil with nutrients

and dispersants would be involved in MOS formation.

The roller bottle incubations used in this study effectively simulated the formation of MOS under varying hydrocarbon input concentrations and different biostimulatory conditions. Neither of the control treatments resulted in the formation of MOS during the sampling period, whereas MOS formation was observed in 4/6 nutrient+dispersant treatments, 3/6 nutrient-only treatments, and 0/6 dispersant-only treatments.

In the present study, MOS formation was strongly influenced by the oil weathering state. Treatments containing fresh diesel required approximately three weeks for visible MOS formation, which formed in 2/6 treatments. In contrast, treatments with weathered diesel formed MOS more rarely (1/8 treatments), but they formed rapidly (within 30 minutes of incubation). Weathered crude oil produced MOS most frequently by far (4/6 treatments). These observations suggest that photochemically aged oil has an enhanced propensity to aggregate and form MOS. This finding is consistent with previous reports, including (Passow, 2016b), which demonstrated that photochemically aged oil promotes rapid MOS formation. The physical characteristics of MOS also differed markedly between treatments. Marine Oil Snow formed in weathered diesel treatments was denser and more compact, whereas MOS produced in fresh diesel treatments appeared loose and exhibited a more porous, mesh-like structure. These structural differences likely reflect alterations in oil chemistry following photo-oxidation, which may enhance aggregation potential and particle cohesion. Similar observations have been reported in microcosm experiments using photo-oxidized Macondo crude oil, where aggregates were smaller and more compact compared to those formed during the Deepwater Horizon oil spill (Passow, 2016b). Together, these findings suggest that weathering processes significantly influence not only the rate of MOS formation but also its physical structure and aggregation properties.

Though fundamentally limited in our interpretive power by the lack of replicates, the pronounced shifts in microbial community structure towards known oil-degrading taxa in nutrient and dispersant amendments do suggest a selective enrichment response to oil exposure. Similar restructuring of microbial assemblages after nutrient addition has been widely reported in oil-contaminated marine systems, where nitrogen and phosphorus limitation often constrain hydrocarbon biodegradation (Head et al., 2006b; McGenity et al., 2012). Nutrient enrichment can stimulate the rapid proliferation of obligate hydrocarbonoclastic bacteria, thereby accelerating biodegradation rates.

Dispersant addition has also been shown to alter microbial community composition by increasing hydrocarbon bioavailability through emulsification, although responses can vary depending on oil type and environmental conditions. For example, studies following the Deepwater Horizon oil spill documented enrichment of hydrocarbon-degrading taxa in response to dispersed oil fractions (Bælum et al., 2012). Our findings align with these observations, suggesting that the combined effect of nutrients and dispersants acts as a strong ecological filter, favoring taxa adapted to exploit dissolved and dispersed hydrocarbons.

Treatments containing the same hydrocarbon substrate but applied at different concentrations (0.1% and 1% v/v) under similar biostimulation regimes exhibited differences in microbial community composition. Without replicates, it is not possible to be certain whether these were stochastic batch effects or treatment effects, but the results suggest that oil concentration acted as a strong driver shaping microbial assemblages. Such concentration-dependent shifts likely reflect differences in bacterial tolerance thresholds, toxicity effects, and substrate availability. While lower concentrations may support a broader range of hydrocarbon-utilizing taxa, higher concentrations can impose physiological stress, selectively favoring taxa capable of tolerating elevated hydrocarbon exposure and associated

toxic intermediates, as outlined in previous research (Zahed et al., 2010; Vilcáez et al., 2013).

The pronounced enrichment of *Colwellia* in fresh diesel treatments is consistent with previous observations that this genus rapidly responds to increased availability of gaseous and simple aromatic hydrocarbons (Mason et al., 2014). Fresh diesel is characterized by a relatively high proportion of volatile and low-molecular-weight aromatic compounds (Miracolo et al., 2010), which likely provided readily bioavailable substrates that favored *Colwellia* proliferation. The elevated abundance of *Colwellia* in treatments containing 0.1% fresh diesel under nutrient and dispersant amendments further suggests that its growth was stimulated not only by substrate composition but also by enhanced hydrocarbon bioavailability and relieved nutrient limitation. Together, these findings support the view that *Colwellia* functions as an early responder to labile hydrocarbon inputs, particularly under conditions where dispersal and nutrient availability facilitate rapid substrate uptake.

Marinomonas exhibited significantly higher relative abundance in treatments containing 1% fresh diesel, including those amended with nutrients and dispersants, as well as dispersant-only treatments, whereas nutrient-only amendments did not stimulate its growth. This pattern suggests that *Marinomonas* respond primarily to elevated hydrocarbon availability rather than nutrient enrichment alone. Supporting this observation, (Zannotti et al., 2023) demonstrated that 1% diesel can sustain *Marinomonas* growth, with optimal biodegradation performance reported at 22 °C. Although our incubations were conducted under colder conditions, the enrichment observed at higher diesel concentrations indicates that *Marinomonas* can tolerate and exploit substantial hydrocarbon loads even outside its reported temperature optimum.

Furthermore, consistent with our findings, (Mugge et al., 2021) reported that *Marinomonas* proliferated in oil- and dispersant-treated surface seawater during a microcosm experiment evaluating biofilm

responses to oil contamination. The enrichment of *Marinomonas* in dispersant-amended treatments in our study further suggests that increased hydrocarbon dispersion and bioavailability may facilitate its proliferation. Collectively, these results indicate that *Marinomonas* may be particularly adapted to environments with high hydrocarbon concentrations and enhanced oil dispersion, reinforcing its potential role as a concentration-responsive hydrocarbon-associated taxon.

The enrichment of *Pseudoalteromonas* in weathered and dispersant-amended treatments is consistent with previous reports describing its role in hydrocarbon biodegradation (Yakimov et al., 2004; Brakstad and Bonaunet, 2006). In addition to its metabolic capacity to degrade oil components, *Pseudoalteromonas* is known to produce EPS in cold marine environments (Suja et al., 2017). EPS production can enhance cell aggregation and provide a structural matrix that facilitates MOS formation (Passow et al., 2012). Therefore, its dominance in weathered oil treatments in the present study may not only reflect substrate utilization but also a functional contribution to aggregate formation and stabilization. This dual role in hydrocarbon degradation and EPS-mediated aggregation highlights *Pseudoalteromonas* as a potentially important taxon linking microbial succession with MOS dynamics under cold, oil-impacted conditions.

Comparison among treatment groups revealed that nutrient-amended treatments, irrespective of hydrocarbon concentration, exhibited microbial community compositions broadly similar to oil-only controls. This suggests that nutrient addition alone did not substantially restructure the microbial assemblage within the timeframe of this study. In oligotrophic environments such as the Arctic, hydrocarbon degradation is often limited by low nutrient availability; however, the response to nutrient enrichment may depend on the concurrent availability and physical state of hydrocarbons. Previous studies have shown that dispersant application can markedly enhance biodegradation by reducing oil

droplet size and increasing the surface area-to-volume ratio, thereby improving microbial access to hydrocarbons (North et al., 2015). In contrast, nutrient addition without enhanced oil dispersion may not sufficiently alter substrate accessibility to drive pronounced community shifts.

Furthermore, significant restructuring of microbial communities under enhanced nutrient conditions during oil degradation in the Labrador Sea was reported to occur over approximately 28 days, often accompanied by accelerated hydrocarbon degradation (Murphy et al., 2021). In contrast, the present experiment was conducted over a one-week incubation period. It is therefore possible that this shorter timeframe was insufficient to capture more pronounced nutrient-driven community shifts, particularly in treatments where hydrocarbon bioavailability was not concurrently enhanced. These observations suggest that nutrient-mediated restructuring may require longer exposure periods to manifest fully under cold marine conditions.

Dispersant-only treatments, as well as combined dispersant and nutrient amendments, induced pronounced shifts in microbial community composition compared to oil-only controls within just one week, transitioning from a more generalist assemblage toward enrichment of specialized hydrocarbon-degrading taxa. This rapid restructuring suggests that dispersant-mediated increases in hydrocarbon bioavailability imposed strong selective pressure on the microbial community. The additional presence of nutrients may have further supported the proliferation of these specialized degraders by alleviating potential nutrient limitation. Together, these findings indicate that the combined application of dispersants and nutrients is likely to enhance the potential for hydrocarbon biodegradation within a short time.

The application of chemical dispersants as an oil remediation strategy remains controversial due to concerns regarding their potential long-term toxicity to marine fauna (Judson et al., 2010). Several

studies have reported that dispersant addition can stimulate the growth of hydrocarbon-degrading bacteria (Dubinsky et al., 2013) and enhance overall biodegradation rates (Prince et al., 2016; Brakstad et al., 2018), primarily by increasing oil dispersion and bioavailability. In contrast, other investigations have suggested that dispersants may not enhance biodegradation and, in some cases, may inhibit the growth of certain hydrocarbon-degrading taxa (Lindstrom and Braddock, 2002; Kleindienst et al., 2015a; Rahsepar et al., 2016b).

In the present study, well-known hydrocarbon degraders thrived in dispersant-only treatments, even at elevated oil concentrations 1%, suggesting that dispersant-mediated increases in substrate accessibility may have outweighed potential inhibitory effects under our experimental conditions. However, previous work has demonstrated that dispersants can differentially affect specific taxa. For example, (Kleindienst et al., 2015b) reported that dispersant application altered competitive dynamics among hydrocarbon degraders, potentially suppressing taxa such as *Marinobacter* while favoring dispersant- and hydrocarbon-degrading bacteria, including *Colwellia*. Although our results show enrichment of several hydrocarbon-associated taxa under dispersant exposure, the present findings are unable to disentangle whether these shifts were driven by hydrocarbon utilization, dispersant degradation, or competitive interactions between taxa.

Furthermore, the ecological impact of dispersants is likely influenced by their chemical composition, as different formulations can variably shape microbial community assembly (Thomas et al., 2021). Collectively, these contrasting findings highlight the complexity of dispersant-microbe interactions and underscore the need to evaluate dispersant efficacy and ecological consequences under environmentally relevant conditions.

Although the present study did not directly quantify hydrocarbon biodegradation rates, the observed community restructuring implies that nutrient and dispersant amendments can substantially alter microbial assembly processes. Given these pronounced compositional shifts, the role of nutrients and dispersants may be critical in shaping microbial responses and ultimately influencing the environmental fate of spilled oil.

2.5 Conclusion

Overall, this study highlights the importance of combined nutrient and dispersant application as a potential bioremediation strategy for oil spills, particularly in facilitating the early succession of oil-degrading bacteria. The food-grade dispersant used in this study may also have contributed to the selective enrichment of certain bacterial genera, potentially conferring a competitive advantage to taxa capable of utilizing either hydrocarbons or dispersant components.

Furthermore, oil weathering appeared to influence bacterial community composition, likely due to alterations in hydrocarbon chemistry and the relative availability of different compound classes. Although several taxa were shared between weathered diesel and crude oil treatments, genera such as *Paraperlucidibaca*, *Thalassolituus*, *Pseudomonas*, and *Shewanella* were absent in crude oil treatments, indicating that oil type and composition significantly shape microbial community structure. These differences underscore the role of substrate characteristics, including physicochemical properties and weathering state, in driving microbial succession.

This study is limited by its microcosm design, which does not fully replicate natural environmental conditions, including continuous nutrient flux, dynamic gas exchange, and ongoing oil weathering and evaporation processes. Additionally, each treatment combination was represented by a single biological replicate, which restricts the ability to quantify within-treatment variability and limits the statistical

applications for comparative analyses. Consequently, future research should investigate longer-term microbial succession under more environmentally realistic conditions and directly quantify biodegradation efficiencies across different stages of oil transformation to better evaluate the effectiveness of combined biostimulation strategies.

2.6 Data availability

All raw 16S rRNA gene sequence reads generated from this experiment have been deposited at NCBI's Sequence Read Archive (SRA) under the BioProject [PRJNA1176269](#) (accession numbers [SAMN54986490](#) to [SAMN54986481](#)).

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CHAPTER THREE: OIL BIODEGRADATION AND MARINE OIL SNOW FORMATION IN ARCTIC SEAWATER MICROCOSMS AT 4 °C.

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3.0 Abstract

Hudson Bay is a sub-Arctic inland sea characterized by seasonal sea-ice cover, which governs marine circulation, biological productivity, and ecosystem structure. In recent decades, the duration of sea-ice cover has declined, resulting in longer ice-free seasons that are expected to elevate shipping activity and environmental exposure to anthropogenic disturbances. As such, the potential for oil spill incidents associated with increased marine operations is expected to rise. To understand microbial biodegradation dynamics associated with Marine Oil Snow (MOS), seawater microcosms amended with diesel fuel were incubated at 4 °C for 6 weeks. We amended all our microcosms with UV-weathered Churchill diesel fuel at 1% (v/v). 16S rRNA sequencing of the incubations revealed a decline in overall microbial diversity and a dramatic increase in specialized oil-degrading taxa, with *Pseudomonas* and *Pseudoalteromonas* being the main taxa involved, as both effectively degrade hydrocarbons under ambient and stimulated conditions. Biostimulation with nutrients (NaNO₃, KH₂PO₄), and an alternative dispersant at 0.1% (v/v) resulted in a greater abundance of MOS aggregates and rapid enrichment of oil-degraders. Simple n-alkanes exhibited the highest biodegradation loss relative to complex Polycyclic Aromatic Hydrocarbons (PAHs); however, biostimulation did not substantially enhance their degradation. Additionally, UV weathering resulted in a 22% loss of PAHs, which is three times higher than the UV loss observed for n-alkanes. Biostimulated samples exhibited an elevated transfer of high molecular weight alkanes and PAHs from the oil into the aqueous phase, accompanied by a rapid enrichment of oil-degrading taxa.

Keywords: Arctic oil spills, biostimulation, dispersants, polycyclic aromatic hydrocarbons

3.1 Introduction

The Arctic is becoming more accessible as a result of the recent decreases in sea ice coverage brought about by climate change (Scheibye et al., 2017). In the last five decades, Arctic sea ice extent has decreased by 5 to 20% each decade, particularly during the summer months (Mudryk et al., 2021). The Canadian Arctic is a pivotal region for trans-Arctic shipping, with interest gaining in transit through the Northwest Passage (Mudryk et al., 2021). Between the years 2013 and 2023, the distance traveled by ships in the Arctic has doubled (PAME, 2024), with fishing and cargo shipments being the major drivers of the increased vessel traffic (Eguíluz et al., 2016; Pizzolato et al., 2016).

Hudson Bay experiences longer ice-free seasons than the high Arctic (Stroeve et al., 2024), facilitating increased shipping access and traffic in the region (Andrews et al., 2017). Sea ice extent in Hudson Bay in the Spring of 2024 reached a new record low for the month, falling below the median from 1979 to 2023 (Soriot et al., 2025), raising alarms regarding the potential oil spill risks associated with shipping. The port of Churchill is the only deep-water port in the Canadian Arctic, and that provides access to North America via the Hudson Bay railway (Andrews et al., 2018). Despite its closure in 2016, the 2025 federal budget shed light on developing transformative energy and trade corridors by expanding the export capacity of the Port of Churchill to strengthen trade with Europe and other partners, and improve connectivity between Churchill, Manitoba, and the rest of Canada (Department of Finance Canada, 2025). In addition, the sea ice extent in Hudson Bay in the Spring of 2024 reached a new record low for the month, falling below the median from 1979 to 2023 (Soriot et al., 2025), which alarms the potential oil spill risks associated with shipping. Shipping in Hudson Bay and Hudson Strait generated by the mining industry is expected to grow quite rapidly in the coming years (Andrews et al., 2017), while the majority of the shipping traffic in Hudson Bay appears to be concentrated along the corridor across the Bay to Churchill (Judson, 2010). Though shipping can improve the livelihoods of

communities by delivering essential supplies, it also carries risks that can affect the marine environment, including oil spills, the introduction of invasive species, and disturbances to the migration routes of Arctic mammals. Ultimately, these economic activities most affect the Inuit communities in the regions that rely on harvesting country foods from the natural environment for subsistence (Olsen et al., 2019; Dawson et al., 2020; Lasserre, 2022; Adeniran-Obey and Osagie, 2024).

In aerobic marine waters, the majority of hydrocarbons are degraded by microbes that use oil as their source of energy and carbon, with a half-life of days to months (Hazen et al., 2016). Nonetheless, rates of biodegradation depend on the oil composition, state, and concentration (Leahy and Colwell, 1990). Even though psychrophilic (cold-loving) oil degraders adapt to low temperatures with specialized enzymatic pathways (Ribicic et al., 2018), removal of oil from biodegradation alone is challenging due to freezing temperatures that slow down the recovery (Garneau et al., 2016; Stern et al., 2023), thus the impact on marine life, communities, and socioeconomic conditions can be long-lasting (Bi et al., 2025).

Dispersed oil undergoes a range of physical, chemical, and biological processes, such as evaporation, dissolution, photochemical oxidation, biodegradation, and sorption onto particulate matter in seawater (Zhao et al., 2016). Under certain conditions, interactions between dispersed oil, organic matter, minerals, and microorganisms promote the formation of Marine Oil Snow (MOS) that eventually sinks to the sea floor (Gregson et al., 2021; Ross et al., 2021). MOS may develop as pre-formed Marine Snow (MS) interacting with oil, or oil acting as an agent for microbial biofilm growth and floc formation (Gregson et al., 2021), and host a distinct bacterial community different from the free-living community in the surrounding seawater (Arnosti et al., 2016). These aggregates transfer oil and EPS-derived dissolved organic carbon (DOC) into the water column and contribute to the carbon flux through oil degradation (Ziervogel et al., 2012). The formation and aging of MOS foster the

establishment of specialized microbial communities that enhance the degradation of oil and oil-derived residues (Arnosti et al., 2016). As aggregates descend, microbial degradation preferentially removes labile hydrocarbons, enriching the residue in less bioavailable compounds (Gregson et al., 2021).

Sinking MOS aggregates deliver oil and oil residues to deeper marine environments, where distinct microbial communities further transform less bioavailable hydrocarbons (Quigg et al., 2020). MOS can potentially impact the benthic organisms through direct toxicity and reduced oxygen availability due to microbial respiration (Yang et al., 2016; van Eenennaam et al., 2018). Therefore, understanding the rate at which hydrocarbon composition changes during transit through the water column and subsequent sedimentation is critical for evaluating potential risks to benthic communities (Atlas and Hazen, 2011).

The Arctic region is expected to be particularly sensitive to MOS formation due to unique environmental conditions, including low temperatures and the presence of sea ice, which together result in slower biodegradation rates (Murk et al., 2020). Diesel fuel is a complex mixture of hydrocarbons that includes both aliphatic compounds, such as alkanes, and aromatic compounds, including polycyclic aromatic hydrocarbons (PAHs; Liang et al., 2005). Aliphatic hydrocarbons constitute roughly 64% of diesel fuel, with approximately 35% including both monoaromatic compounds and PAHs. Olefinic hydrocarbons typically represent a minor fraction, comprising approximately 1–2% of the total composition (Gad, 2014).

Both diesel and crude oil contain hundreds of PAHs plus alkylated PAH compounds that can be more persistent than their parent compounds (Latimer and Zheng, 2003) and exhibit varying degrees of hydrophobicity depending on the number of aromatic rings (Sakellari et al., 2021). The United States Environmental Protection Agency (USEPA) has listed 16 PAHs as priority pollutants given their potential toxicity including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene,

anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[ghi]perylene (USEPA, 2014). Generally, PAHs have greater resistance to degradation than other compound groups such as alkanes (Friedman et al., 2014). PAHs associated with oil spills are expected to be fully bioavailable to marine fauna (Yunker et al., 2015), with some being carcinogenic and mutagenic (Latimer and Zheng, 2003).

Despite extensive research on hydrocarbon biodegradation, uncertainties remain regarding how nutrient enrichment and dispersant applications influence the fate of different hydrocarbon classes and associated microbial communities. In particular, the combined effects of biostimulation on the degradation of n-alkanes and polycyclic aromatic hydrocarbons (PAHs), as well as on the selective enrichment of hydrocarbon-degrading microorganisms, are not fully understood under environmentally relevant conditions. In addition, oil–microbe interactions can promote the formation of MOS, which plays a critical role in the aggregation, vertical transport, and microbial processing of oil in the water column. However, the extent to which nutrient and dispersant additions modulate MOS formation and its associated microbial consortia, and how these processes influence hydrocarbon biodegradation and partitioning, remains poorly understood.

In previous work, we characterized short-term microbial responses to differential oil exposure and biostimulation within artificial seawater microcosms over one week, capturing early community shifts but not longer-term succession or sustained hydrocarbon transformation processes. In contrast, the present manuscript extends this work by examining microbial community dynamics and biodegradation patterns over six weeks under more Arctic-relevant conditions. This longer experimental duration enables evaluation of temporal restructuring, treatment persistence, and the

sustained enrichment of hydrocarbon-degrading taxa, providing insight into longer-term ecosystem responses to oil contamination. This study aimed (1) to elucidate the processes underlying MOS formation in the Canadian Arctic, (2) to characterize microbial community shifts in a low-temperature seawater microcosm, and (3) to evaluate hydrocarbon compositional changes and phase partitioning under biostimulation.

3.2 Materials and methods

3.2.1 Field sampling

Sea water samples were collected using a peristaltic pump into a large sterile cubitainer from the Control pool at the Ocean Sea Ice Mesocosm (OSIM) facility of the Churchill Marine Observatory (CMO) on 21st April 2025 (Figure 1). The water had been previously pumped into the mesocosm pools from the Churchill estuary on October 16, 2024; the salinity was 26.4 PSU.

Upon returning to the University of Manitoba, the water sample was stored at 4 °C in the cold room facility at the Centre for Earth Observation Science for 2 weeks until the microcosms were set up.

3.2.2 Microcosm setup and subsampling

The microcosms were established in 125 mL Pyrex glass bottles on three tube rollers, as previously described by Gutierrez et al. (2013). Two types of treatment groups were established, and each treatment was run in duplicate for a period of six weeks (Figure 3.1). Aliquots of Churchill diesel fuel were UV-weathered using UV-A (365 nm) and UV-B (302 nm) lamps for 5 days before the experiment. A total of 16 microcosms were set up, along with six additional samples for chemical analysis. Eight microcosms consisted of 98.9 mL of seawater amended with UV-weathered Churchill diesel fuel at a ratio of 1% (v/v), 0.1% (v/v) alternative dispersants (soy lecithin and Tween 80 dissolved in ethanol

(Athas et al., 2014) at a 60/40 [w/w] ratio), and nutrients (7.785 mM NaNO₃ and 0.2 mM KH₂PO₄) to satisfy the Redfield ratios. The other eight microcosms consisted of 99 mL of seawater and 1% (v/v) UV-weathered Churchill diesel fuel. All the microcosms were placed on the tube rollers horizontally, and the speed was set at 3 rpm at 4°C to mimic the natural environmental conditions of the ocean. The treatments and controls were sampled at four time points over 6 weeks for microbial analysis: T0 at Day 0, T1 at 1 week, T2 at 2.5 weeks, T3 at 4 weeks, and T5 at 6 weeks. Observations were recorded at each sampling point.

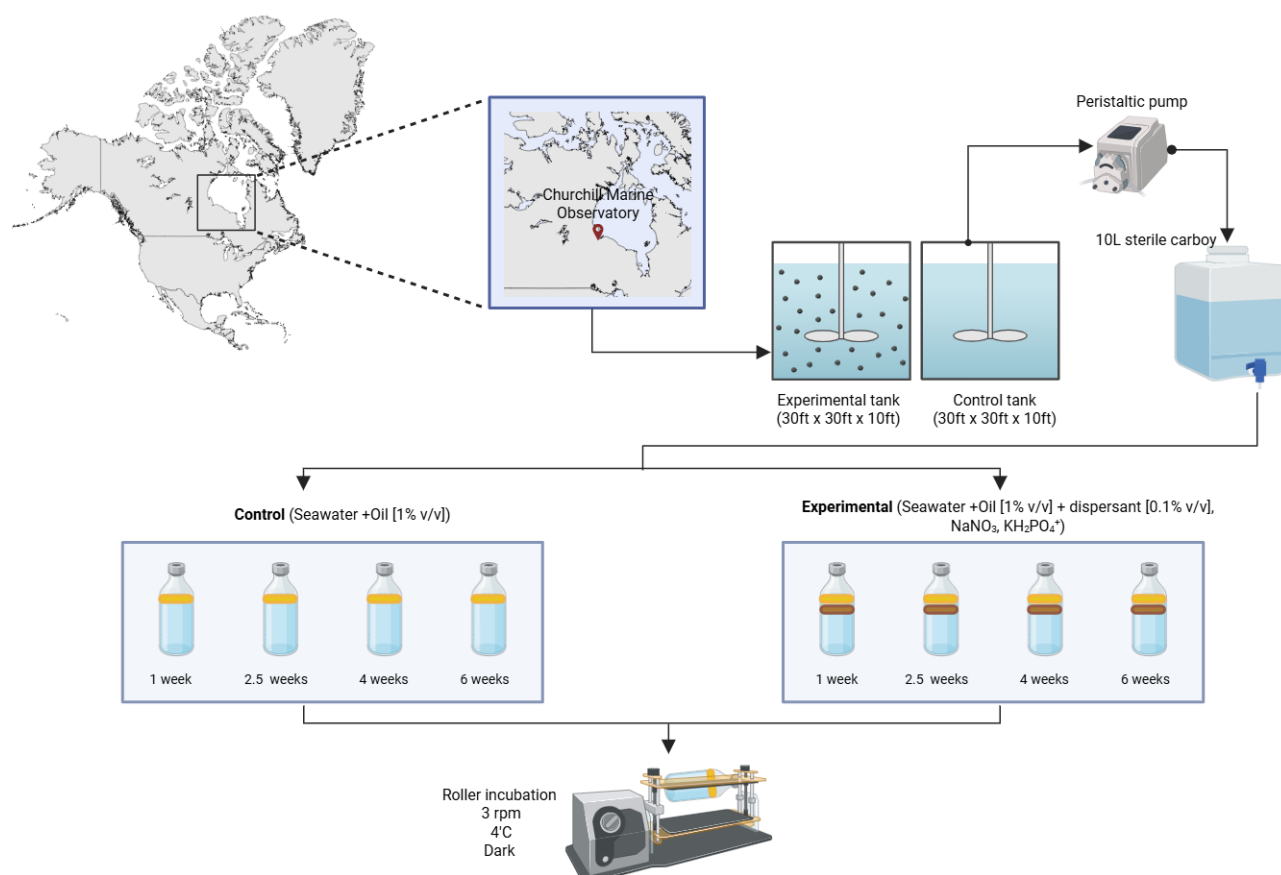


Figure 3. 1: Water sample collection from CMO and roller incubation setup at 3 rpm rotation speed and at 4 °C in the dark. Created with [BioRender.com](https://www.biorender.com/).

3.2.3 Genomic DNA extraction

The majority of the MOS aggregates were too fragile to withdraw reliably (Figure 3.2). Hence, the whole sample was filtered onto a 0.22 µm Sterivex filter for genomic DNA extraction. The Qiagen DNeasy PowerSoil kit was used to extract DNA from the samples following the manufacturer's instructions, with slight modifications. For all extractions, the bead-beating tubes containing the samples and CD1 solution were heated to 70 °C for 10 minutes to facilitate cell lysis (Murphy et al., 2021). Additionally, an aliquot of elution buffer was heated to 50 °C before eluting the DNA in the final step. All extracted DNA products were quantified using a Qubit fluorometer and stored at -70 °C until the sequencing was performed.

3.2.4 16S rRNA amplicon sequencing

Paired-end (2 x 250) amplicon sequencing across the V4-V5 hypervariable region of the 16S rRNA gene was carried out on the Illumina Novaseq platform using the 515F (GTGYCAGCMGCCGCGGTAA) and 806 RB (GGACTACNVGGGTWTCTAAT) primer pair (CD Genomics, New York, USA). Approximately 605k high-quality reads were obtained with a read depth of 19,197 to 58,613 per sample. Raw sequencing reads were quality-controlled by removing adapters and primer sequences using Cutadapt v5.2 (Martin, 2011). Trimmed reads were then processed using the DADA2 (Callahan et al., 2016) package in R to infer amplicon sequence variants (ASVs), and taxonomic assignment was performed using the SILVA v138.2 reference database (Quast et al., 2013). Code for the bioinformatics pipeline is available at <https://github.com/rec3141/microscape>.

3.2.5 Hydrocarbon analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

Hydrocarbons were extracted from the microcosms after 6 weeks. To account for the hydrocarbon fractions that were transported from the oil phase to the aqueous phase, each microcosm group was analyzed in three sub-sections: oil phase (consisting of two replicates), aqueous phase (consisting of two replicates), and whole sample (with no replicates) for both control and biostimulated treatments. The oil phase was decanted and measured volumetrically, and a homogenized aliquot (20 μ L) was diluted to 2%, 1%, and 0.1% with isooctane before chemical analysis. For the aqueous phase, each microcosm was extracted three times with hexane and dichloromethane (DCM) as solvents. Extracts were filtered through sodium sulphate to remove any residual water. The extracts were then evaporated on a BUCHI Rotary Evaporator with a 30 °C water bath, a starting pressure of 450 mbar, and a final reduced pressure of 150 mbar, until the extracts reached approximately 1 mL (in isooctane). For the whole sample, extraction and reduction proceeded identically to the aqueous phase but were instead reduced to a 10 mL hexane stock before further diluting by 5% and 3% with isooctane. For each final extract, a 2- fluorobiphenyl (2-FB) internal standard was added and the final sample volume was adjusted to 1 mL (by mass) in a GC vial. All chemicals used were Fisher Chemical Optima grade.

An Agilent 7010B Triple Quadrupole GC-MS (QQQ-MS) was employed for the analysis of aromatics and alkanes using multiple reaction monitoring (MRM). The system was equipped with an Rxi-PAH column (60 m $\times$ 250 μ m $\times$ 0.1 μ m). For aromatics, the oven temperature program was held at 40 °C for 1.5 minutes, then ramped to 140 °C at 30 °C/min. It was subsequently increased to 250 °C at 3 °C/min, followed by a third ramp to 320 °C at 2 °C/min, where it was held for 20 minutes. For alkanes, the oven temperature program was held at 30 °C for 2 minutes, then ramped to 120 °C at 15 °C/min. It was subsequently increased to 250 °C at 3 °C/min, followed by a third ramp to 320 °C at 2 °C/min,

where it was held for 30 minutes. An electron ionization of 70 eV was used. The injection volume of each sample was 1 μ L, which was carried by helium gas at a flow rate of 1.1 mL/min through the GC column. Nitrogen was used as the collision gas at a flow rate of 1.5 mL/min and an ion gain of 5. 2-FB was measured in each sample to correct for instrument response.

Quantification of aromatics was carried out using a PAH calibration mixture composed of the following standards: Polycyclic Aromatic Hydrocarbons Mix (CV Calibration Mix #5, Restek) and PAH/Dibenzothiophenes Mixture (Chiron). All aromatic calibration curves consisted of at least 9 points and had R^2 values ≥ 0.95 . Quantification of alkanes was carried out using an n-alkane calibration mix consisting of C8–C40 n-Alkanes (Sigma-Aldrich). All alkane calibration curves consisted of at least 8 points and had R^2 values ≥ 0.98 . A minimum signal-to-noise ratio of 5 was achieved for all compounds. A full list of the compounds analyzed, and their MRM quantifier settings, is included in the supplementary.

3.2.6 Diagnostic ratios for determining biodegradation

The extent of n-alkane and PAH biodegradation was determined from the abundance of heptadecane relative to pristane (n- C17/ pristane; Johnston et al., 2007) and (2-methylnaphthalene/ 1-methylnaphthalene; Murphy et al., 2021) in fresh diesel, UV-weathered diesel, biostimulated, and oil-only control treatments.

3.2.7 Statistical analysis

Microbial community analysis was performed using R. All plots were generated in R (v4.3.1) using the ggplot2 package (Wickham, 2016). Statistical significance across the treatment groups was analyzed using two-tailed t-tests ($\alpha = 0.05$).

3.3 Results

3.3.1 MOS development

The earliest time for MOS development in the roller incubations was less than 24 hours. Initially, MOS particles were suspended in the water column and were off-white in color and approximately 2 to 3mm in size (Figure 3.2). However, over time, they aggregated and sank; the majority of MOS particles seen after 2.5 weeks were settled at the bottom, and only a very few tiny particles could be seen scattered in the water column. The MOS settled at the bottom had a mucus texture rather than being dense. The shapes varied from linear to round. Notably, MOS were observed only in the biostimulated (nutrient and dispersant added) treatments. No control treatment had MOS or even visible signs of MOS formation during the 6 weeks. Instead, the control treatment had the oil layer visible on the top of the water column when the bottle was inverted. Since the MOS particles were extremely fragile, they disintegrated quickly when inverting the bottles for sampling or photography.

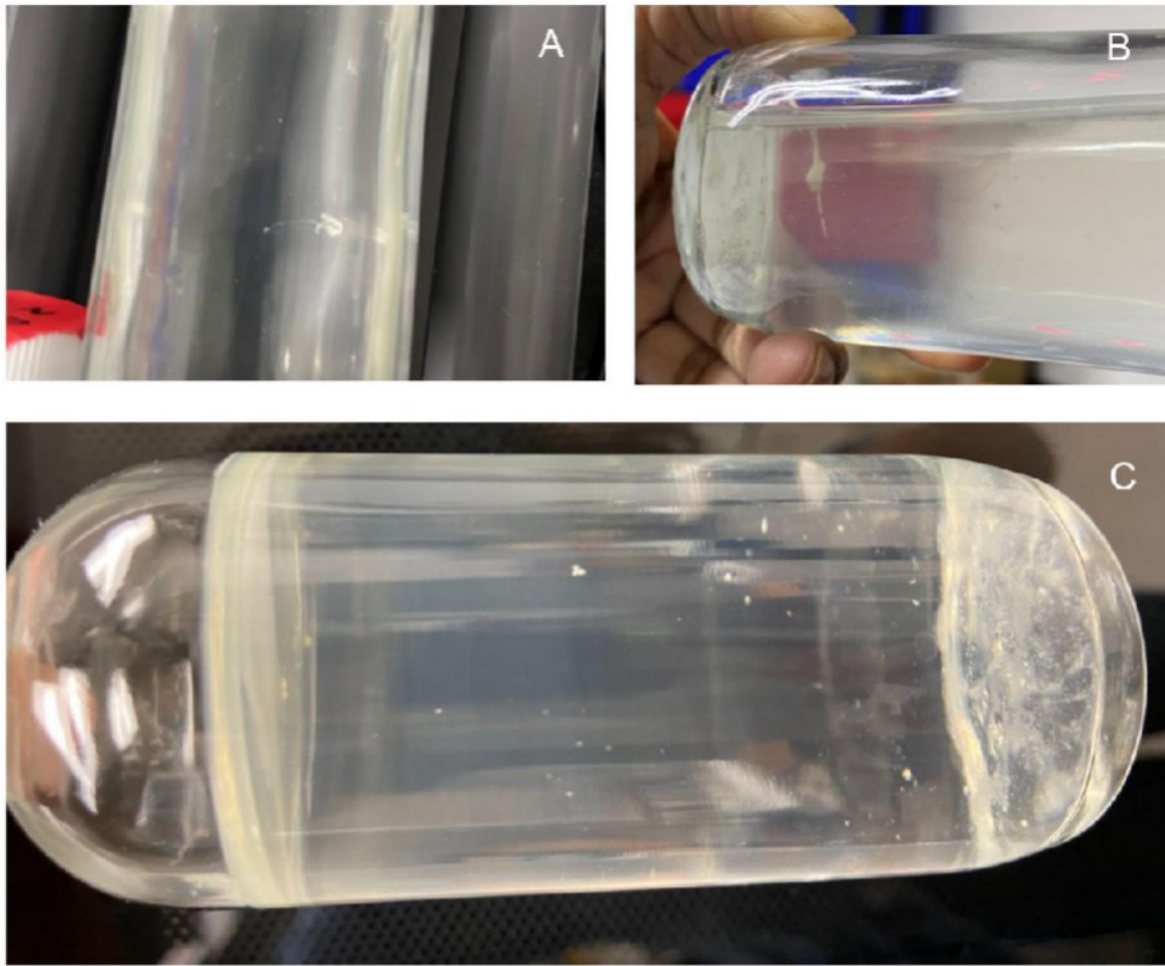


Figure 3. 2: MOS formation in the biostimulated treatments (A) A MOS formed in a biostimulated treatment group within a day. (B) the same MOS after 1 week of roller incubation (C). The Mucous MOS settled at the bottom of the bottle.

3.3.2 Microbial community composition between treatment groups

Following quality filtering and denoising, amplicon sequence variants (ASVs) were inferred, resulting in 243 ASVs spanning 10 phyla, 13 classes, 44 orders, 74 families, and 100 genera. A pronounced dominance of *Pseudoalteromonas* and *Pseudomonas* was observed at 2.5 weeks in the biostimulated treatments, with *Pseudoalteromonas* accounting for 82.5% of the total microbial abundance (Figure 3.3). However, the relative abundance of *Pseudoalteromonas* declined markedly over time, decreasing

from 46.9% at four weeks to 11.2% at six weeks. In contrast, *Pseudomonas* exhibited a strong increase, rising from 52.2% at four weeks to 85.4% at six weeks.

The addition of nutrients and dispersants rapidly altered microbial community composition, compared to oil-only controls, favoring the proliferation of a limited number of oil-degrading taxa under these enriched conditions. In the control treatments, the relative abundances of *Pseudomonas* and *Pseudoalteromonas* also increased over time; however, a more diverse assemblage of oil-degrading taxa was observed compared to the biostimulated treatments. *Colwellia* was present in the initial seawater at 13.6% and increased to 26.1% after one week before subsequently declining. During the first 2.5 weeks of control treatments, *Sulfitobacter* was the dominant taxon, accounting for 37.7% of the community. As *Pseudomonas* populations increased, the relative abundance of *Sulfitobacter* declined. By four weeks, both *Pseudoalteromonas* and *Pseudomonas* had emerged as dominant taxa in control treatments. Nevertheless, *Pseudomonas* appeared to outcompete *Pseudoalteromonas*, ultimately becoming the predominant taxon after six weeks.

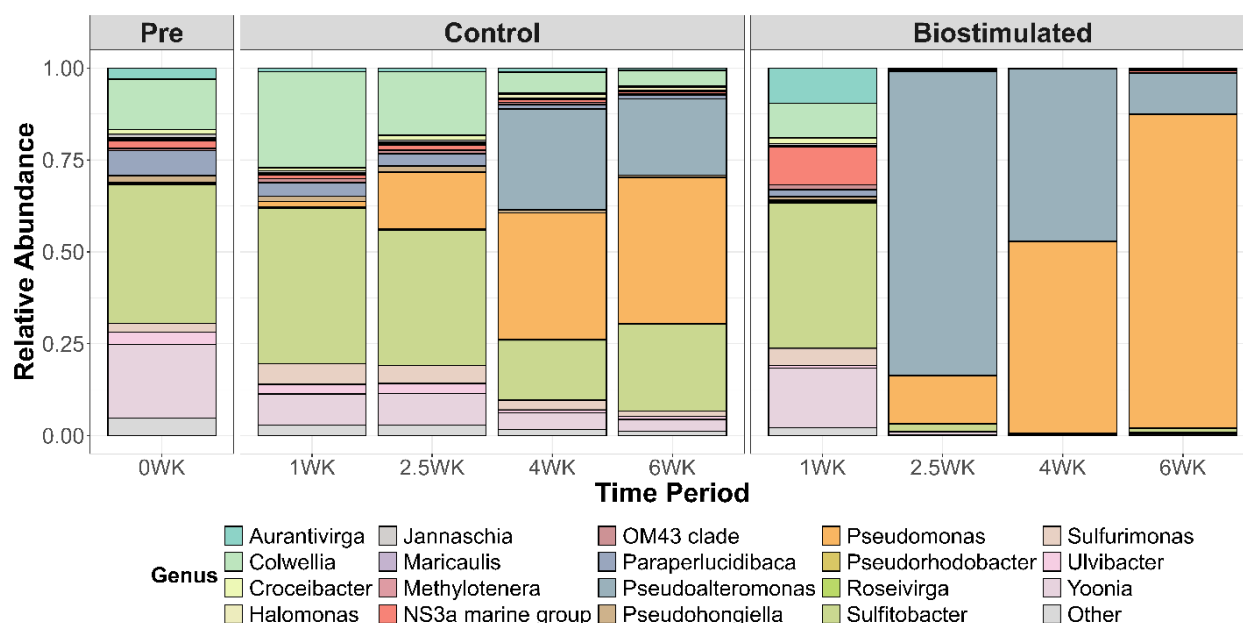


Figure 3. 3: The mean genus-level composition of the microbial taxa present in the treatment groups (n=2 per treatment). The taxa with relative abundance less than 0.001 were grouped as “other”.

3.3.3 Biodegradation of hydrocarbon compounds

Compared to PAHs, n-alkanes showed a more pronounced loss in both control and biostimulated treatments. Nonetheless, biostimulation had a similar impact on the overall biodegradation of both n-alkanes (C₁₀-C₂₃) and PAHs as revealed by gas chromatography-mass spectrometry (GC-MS; Figure 3.4). This is evident by the nearly similar ratio of n- C₁₇/pristane (0.83) and 2MN/1MN (1.39) in both treatments (Figure 3.S1). Notably, the above ratios were lower than those of the n- C₁₇/pristane and 2MN/1MN ratios for fresh diesel (1.36 and 1.54) and UV-weathered diesel (1.42 and 1.53), suggesting active biodegradation processes in both treatments. Reduced n- C₁₇/pristane and 2MN/1MN diagnostic ratios in control and biostimulated treatments relative to fresh and UV-weathered diesel, along with similar ratios between fresh and UV-weathered diesel, indicate comparable biodegradation patterns, as UV exposure primarily drives physicochemical weathering rather than biodegradation. A significant loss of polycyclic aromatic hydrocarbons (PAHs) was observed during the UV weathering process

compared to the n-alkanes ($p = 0.028$; Figure 3.S2). PAHs displayed compound-specific responses to UV-driven weathering, with the highest loss (68.2%) observed for pyrene ($C_{16}H_{10}$). Despite this, UV exposure did not produce a systematic or consistent alteration pattern among PAHs.

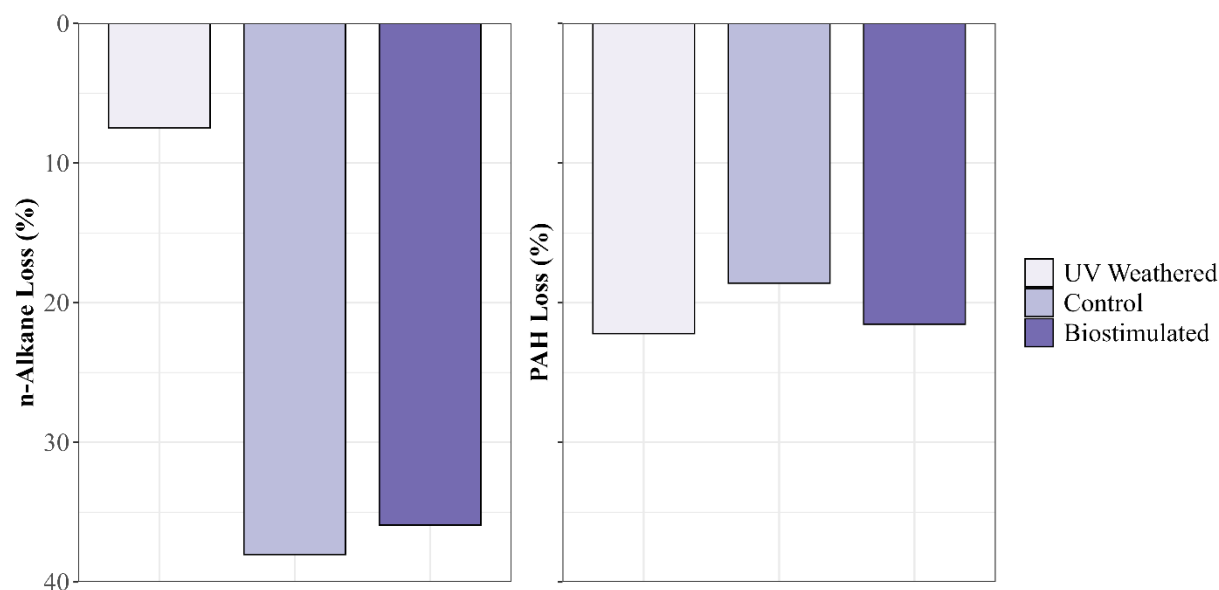


Figure 3. 4: Total loss (%) of n-alkanes (a) and PAHs (b) due to UV weathering and biodegradation after 6 weeks of incubation ($n=1$).

3.3.4 Partitioning of hydrocarbon compounds

Higher oil-to-aqueous ratios for alkanes in the control treatments reflect the absence of enhanced partitioning processes (e.g., dispersant-mediated dispersion or incorporation into marine oil snow), resulting in a greater proportion of oil remaining in the bulk phase (Figure 3.5 & Figure 3.S3). Within the controls, low-molecular-weight (LMW) alkanes exhibited higher ratios compared to high-molecular-weight (HMW) alkanes, likely due to the greater susceptibility of LMW compounds to biodegradation in the aqueous phase. A similar trend was observed for the biostimulated treatments in which LMW alkanes exhibited higher ratios compared to HMW, albeit less pronounced. These patterns

are likely driven by enhanced partitioning of alkanes into the aqueous phase in the presence of dispersants and MOS aggregates. Among the partitioned n-alkanes, LMW compounds were preferentially biodegraded, whereas HMW n-alkanes exhibited higher retention in the aqueous phase through partitioning relative to their biodegradation rates.

Consistent with these patterns, PAHs in the control treatments exhibited higher oil-to-aqueous ratios compared to the biostimulated treatments. Within the controls, HMW PAHs displayed comparatively lower ratios. Biostimulated treatments showed markedly reduced ratios relative to controls, reflecting enhanced biodegradation facilitated by increased partitioning of PAHs into the aqueous phase through MOS sorption and dispersant action, in which HMW PAHs in biostimulated treatments were retained in the water column to a greater extent than they were biodegraded, as indicated by the declining oil-to-aqueous ratios (Figure 3.6 & Figure 3.S4).

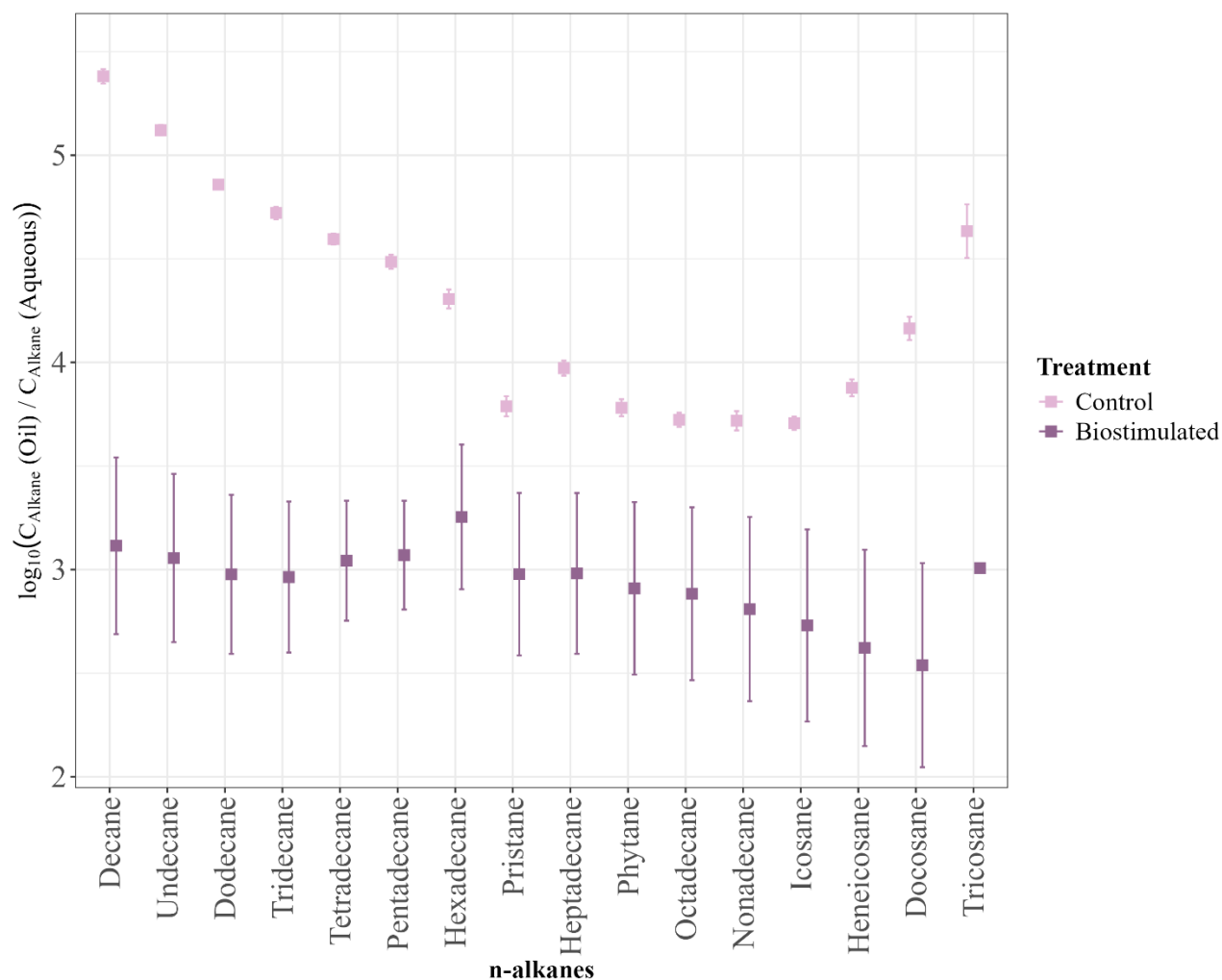


Figure 3. 5: Partitioning of individual n-alkanes from diesel fuel between the oil and aqueous phases in control and biostimulated treatments after six weeks of incubation. Compounds are arranged in order of increasing molecular weight. The ratio of n-alkane concentrations in oil versus aqueous phases is shown on a $\log_{10}$ scale. Error bars represent standard deviations across replicates, and concentrations were normalized to total sample volume for direct comparison of partitioning behavior across treatments (n=2).

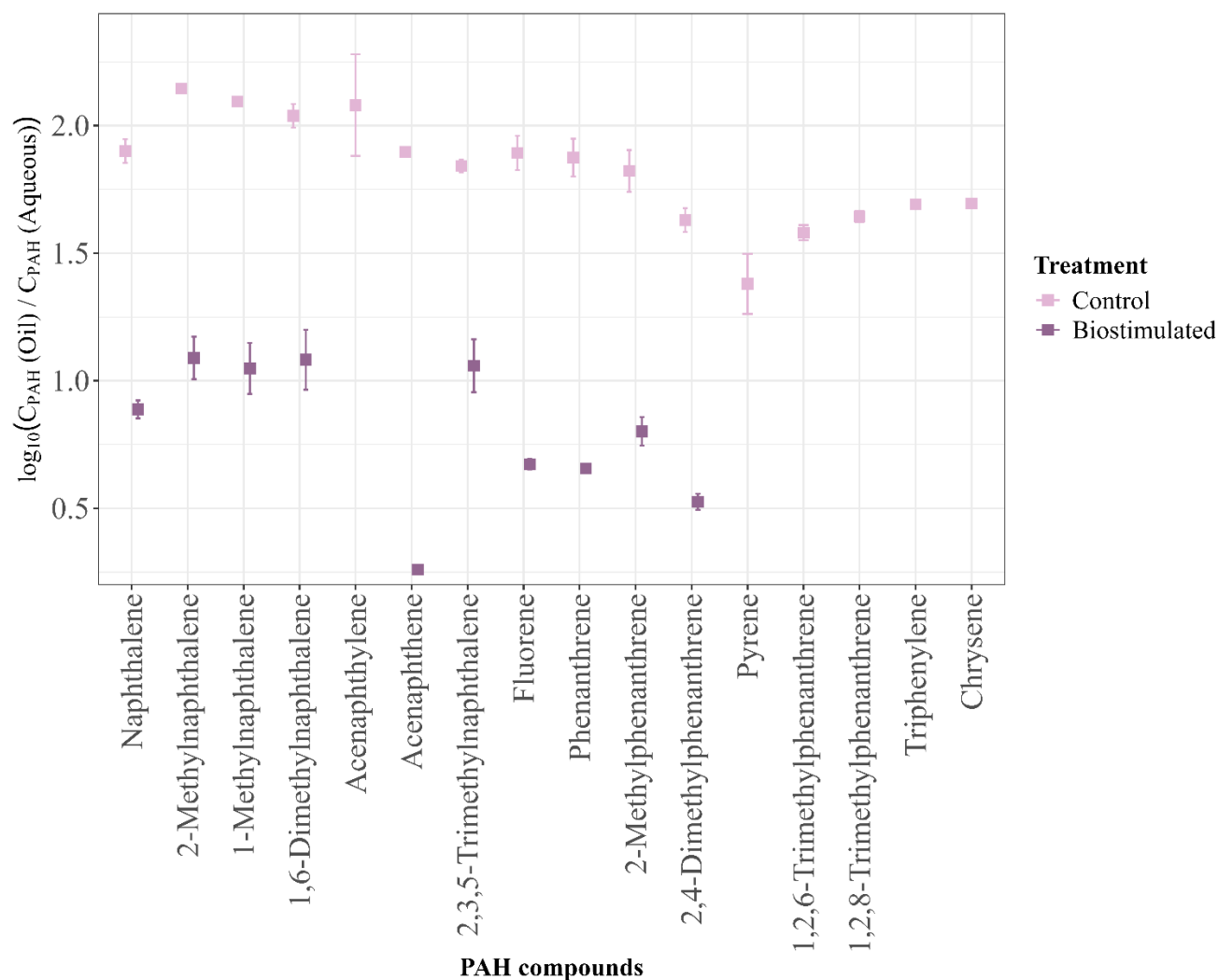


Figure 3. 6: Partitioning of individual PAHs from diesel fuel between the oil and aqueous phases in control and biostimulated treatments after six weeks of incubation. Compounds are arranged in order of increasing molecular weight. The ratio of PAH concentrations in oil versus aqueous phases is shown on a log₁₀ scale. Error bars represent standard deviations across replicates, and concentrations were normalized to total sample volume for direct comparison of partitioning behavior across treatments (n=2).

3.4 Discussion

3.4.1 Biostimulation influences the early succession of oil-degrading taxa

To our knowledge, this is the first study conducted on MOS formation from Canadian Arctic waters. Our roller bottle incubations indicate that MOS formation is independent of climate, as it has also been observed in other studies conducted in temperate and the Northeast Atlantic oceans (Passow et al., 2012; Fu et al., 2014; Passow, 2016; Suja et al., 2017). MOS formation was observed exclusively in biostimulated treatments containing nutrients and dispersants, where no aggregation occurred in oil-in-seawater controls. This suggests that nutrient enrichment and dispersant application facilitate oil-particle interactions and EPS production, which are central to MOS formation. Similar mechanisms have been reported following the Deepwater Horizon (DWH) spill, where microbial EPS production promoted rapid formation of oil-rich marine snow (Passow et al., 2012). This confirms that MOS formation is not an inherent property of oil alone, but a biologically mediated process sensitive to spill response strategies. Microorganisms play a vital role in the natural attenuation of oil spills, and bioremediation serves as a cost-effective approach in a wide range of ecosystems, including the Arctic (Aislabie et al., 2006; Góngora et al., 2022). In natural marine environments, biodegradation of petroleum hydrocarbons involves a succession of microbial consortia (Peeb et al., 2022). Following an oil spill, groups of hydrocarbon degraders, typically found in low relative abundance (<1%) in seawater, increase their numbers and become dominant as a response to the spillage (Neethu et al., 2019). We observed that oil-degrading bacteria proliferated even in control treatments upon the addition of oil, although the onset of colonization was delayed. Also, MOS formation was accompanied by shifts in microbial community composition relative to controls. These changes suggest the selective enrichment of hydrocarbon-associated and EPS-producing bacteria within MOS aggregates. We posit that the

dramatic increase in *Pseudoalteromonas* population within 2.5 weeks of incubation was due to EPS production, which concurrently caused MOS formation. Similar observations have been reported from the oil-in-seawater microcosms conducted using North Atlantic seawater, where an increase in the abundance of *Pseudoalteromonas* in MOS particles was observed after 2.5 weeks (Suja et al., 2017). Species of *Pseudoalteromonas* are members of the “generalist” hydrocarbon degraders in the marine environments (Gutierrez et al., 2013) with the ability to produce EPS (Suja et al., 2017). These bacteria significantly contribute to the ocean's pool of Dissolved Organic Matter (DOM) during spill events. They enhance oil aggregation, dissolution, bioavailability, and the eventual degradation of hydrocarbons by indigenous bacterial communities (Gutierrez et al., 2013). In general, EPS provides a matrix for MOS formation (Passow, 2016), and nutrients-enriched treatments yield higher concentrations of EPS, highlighting the supportive role of nutrient enrichment for EPS-producing bacteria (Suja et al., 2017).

Colwellia populations did not increase in abundance in biostimulated treatments, despite being relatively abundant in control treatments within 2.5 weeks. One possible explanation is *Colwellia*'s preference for degrading gaseous and simple hydrocarbon aromatics (Mason et al., 2014) that are commonly present in fresh petroleum products (Reddy et al., 2012). As oil undergoes weathering, these volatile and gaseous components are progressively lost through evaporation, dissolution, and biodegradation (Tarr et al., 2016). The depletion of these preferred substrates provides a plausible explanation for the decline in *Colwellia* relative abundance observed after approximately 2.5 weeks, even in oil-amended control treatments. Consistent with its predominantly copiotrophic lifestyle, *Colwellia* is well adapted to rapidly exploit transient pulses of labile organic carbon during early stages of oil exposure, but becomes less competitive as these substrates are depleted and community composition shifts during later stages of oil weathering (Labbate et al., 2016; Roller et al., 2016).

Further, these findings suggest that *Colwellia* dominance is not universal following oil exposure, but instead depends on environmental context, spill response strategies, and microbial niche availability.

The dominance of *Pseudomonas* and *Pseudoalteromonas* was observed exclusively in biostimulated treatments, in which, after approximately 2.5 weeks, *Pseudomonas* populations increased markedly, coinciding with a decline in relative abundance of *Pseudoalteromonas* and other community members. Nutrient enrichment likely favored fast-growing, copiotrophic taxa with high metabolic flexibility, enabling *Pseudomonas* to outcompete other community members, as previously observed (Suja et al., 2017; Murphy et al., 2021). Although *Pseudoalteromonas* persisted, its relative decline suggests competitive exclusion as *Pseudomonas* populations expanded under nutrient-replete conditions. In contrast, control treatments lacking nutrient and dispersant amendments maintained a more diverse community structure and did not converge toward dominance by a limited number of taxa. *Pseudomonas* is a paradigmatic bacterial genus associated with both alkane and aromatic degradation (Mason et al., 2012; Pasumarthi et al., 2013; Nogales et al., 2017), whereas *Pseudoalteromonas* has been reported to persist in environments enriched in aromatic hydrocarbons (Dubinsky et al., 2013). Although direct interactions between these taxa were not examined in this study, the observed shift toward *Pseudomonas* dominance under nutrient-rich, biostimulated conditions is consistent with competitive dynamics favoring metabolically versatile alkane-degrading taxa. This pattern occurred despite the continued presence of both n-alkanes and PAHs in the system, suggesting that nutrient availability and substrate accessibility, rather than hydrocarbon presence alone, played a key role in structuring the microbial community.

Sulfitobacter is a common and often dominant member of surface-water microbial communities in Hudson Bay and other polar marine systems, where it participates in the transformation of inorganic

sulfur compounds (Buchan et al., 2005). Consistent with this, *Sulfitobacter* was the most abundant taxon in the initial seawater before oil addition. In oil-only control treatments, *Sulfitobacter* relative abundance gradually declined over the six-week incubation period following diesel addition, while its relative abundance rapidly declined in biostimulated treatments, with almost no detection after 1 week. This rapid decline reflects strong environmental selectivity under nutrient and dispersant-replenished conditions, in which *Sulfitobacter* may be at a competitive disadvantage.

Our results show that microbial community composition shifted rapidly within 1 to 2.5 weeks in response to nutrient and dispersant amendments, indicating that biostimulated conditions strongly and selectively favored hydrocarbon-degrading taxa. This rapid restructuring suggests that nutrient and dispersant additions accelerate microbial succession by enhancing hydrocarbon bioavailability and promoting the growth of metabolically versatile putative oil degraders. The Hudson Bay surface-water microbial community appears to include populations capable of responding to hydrocarbon inputs, with *Pseudomonas* and *Pseudoalteromonas* emerging as dominant taxa under both ambient and biostimulated conditions. Together, these findings indicate that while a diverse background community is present under natural conditions, biostimulation can rapidly select for a narrow subset of highly competitive hydrocarbon-degrading bacteria.

3.4.2 UV exposure causes greater loss of PAHs than of n-alkanes

UV-weathered Churchill diesel was used to simulate conditions conducive to MOS formation. (Passow, 2016) Photochemically aged oil has been shown to promote marine snow formation, though the resulting aggregates are typically smaller and more compact than those formed with fresh oil (Passow, 2016). In our roller incubations, the MOS observed was relatively compact, which is consistent with these previous observations. This may be related to the higher viscosity and altered

interfacial properties of irradiated diesel, which has lost volatile compounds and contains more surface-active photooxidation products (Janus et al., 2019). PAHs are generally more susceptible to photooxidation than n-alkanes (Bacosa et al., 2015), which can alter their environmental fate following an oil spill. Consistent with this, our UV-weathering experiments showed a greater loss of PAHs (22.2%) compared to n-alkanes (3.3%). Among the PAHs, pyrene, a high molecular weight compound, experienced the highest loss (68.2%) under UV exposure, reflecting its susceptibility to photooxidation. While photooxidation reduced PAH abundance, UV irradiation can also generate more reactive or toxic intermediates through photo-activation and photo-modification (Ekunwe et al., 2005), which may influence microbial degradation pathways and the potential toxicity of oil-contaminated waters. These changes in hydrocarbon composition could affect MOS formation and microbial community dynamics, as altered PAH and alkane profiles modify the chemical environment available for microbial colonization and hydrocarbon degradation.

3.4.3 MOS influences elevated partitioning of high molecular weight hydrocarbons

The observation of higher oil-to-aqueous ratios for n-alkanes in the control treatments suggests that, in the absence of dispersants or MOS formation, these compounds largely remain in the bulk oil phase. This is consistent with the hydrophobic nature of n-alkanes (Turner et al., 2014; Desmond et al., 2021), which limits their spontaneous partitioning into the aqueous phase. In biostimulated treatments, overall oil-to-water ratios were markedly lower, consistent with dispersant-mediated solubilization or incorporation into marine oil snow, followed by microbial processing of hydrocarbons. Therefore, the resulting higher oil:water ratios from lower aqueous concentrations in control treatments indicate limited natural dispersion and a reduced immediate substrate availability for hydrocarbonoclastic microbes, which may slow the early-stage biodegradation of these compounds compared to treatments with enhanced partitioning mechanisms.

LMW n-alkanes exhibited higher oil-to-water ratios compared to HMW n-alkanes in both the treatments, which may reflect the relatively higher bioavailability and greater susceptibility of LMW compounds to microbial uptake and biodegradation. These observations highlight how both molecular weight and treatment regime influence hydrocarbon fate, with LMW compounds more readily mobilized and degraded in the aqueous phase than HMW homologues, as described by previous research (Singh et al., 2012).

Overall, lower oil-to-water ratios observed for PAHs compared to n-alkanes likely reflect their relatively higher aqueous solubility (Desmond et al., 2022) and structural characteristics (Zhang et al., 2015) that promote partial partitioning into the water phase. In contrast, the more hydrophobic n-alkanes remain largely in the bulk oil phase, leading to higher oil-to-water ratios in the absence of dispersants or MOS formation.

Biostimulated treatments exhibited markedly lower oil-to-water ratios for PAHs compared to controls, reflecting enhanced mobilization and biodegradation facilitated by dispersant action and incorporation into marine oil snow. LMW PAHs were more readily partitioned and degraded, whereas HMW PAHs were retained more in the water column, likely via MOS sorption, rather than being biodegraded, a pattern attributed to their lower aqueous solubility and greater incorporation into the organic fraction, which limits microbial access (Achten et al., 2011; Frapiccini and Marini, 2015). This interpretation is consistent with previous observations showing that dispersant addition promotes the incorporation and enrichment of HMW oil compounds within MOS (Wirth et al., 2018).

Phenanthrene and its alkylated homologues (1-methylphenanthrene and 2-methylphenanthrene) are frequently among the most abundant PAH compounds detected in petroleum-contaminated marine

environments (Wang et al., 2011), making their transport behavior particularly relevant for assessing oil spill impacts. Moreover, surveys of monomethylated phenanthrene isomers have shown that these compounds can be two- to five-fold more toxic than parent phenanthrene (Lee and McCain, 2015), highlighting their ecological significance. The enhanced partitioning of these compounds under biostimulated conditions, therefore, suggests that MOS-mediated transport may increase the mobility and potential bioavailability of toxic PAHs to pelagic organisms.

Overall, n-alkanes exhibited a higher percentage of degradation than PAHs, consistent with their susceptibility to biodegradation (Prince et al., 2016; Stout and Wang, 2016). Nonetheless, alkane and PAH losses did not differ substantially between control and biostimulated treatments, suggesting that alkane and PAH biodegradation were not strongly limited by nutrient or dispersant availability under the conditions tested. This indicates that the microbial community sampled from Hudson Bay surface waters possessed an inherent capacity to efficiently degrade alkanes and PAHs, even in the absence of external stimulation. While biostimulation did not significantly increase total alkane and PAH degradation, it altered hydrocarbon partitioning dynamics, with increased transfer of HMW compounds into the aqueous phase through sorption via MOS formation. The absence of independent nutrient-only and dispersant-only treatments prevents differentiation between physicochemical dispersion effects and biologically mediated aggregation processes such as MOS formation. It is therefore unclear whether enhanced partitioning resulted primarily from dispersant-induced emulsification or microbially driven EPS production and aggregate formation. Future studies incorporating factorial treatment designs and direct quantification of MOS would help disentangle these mechanisms.

3.5 Conclusion

Findings of this study indicate that biostimulation through nutrient and dispersant addition substantially alters the fate and transport of diesel-derived hydrocarbons in Arctic marine surface waters by promoting marine oil snow formation and restructuring microbial communities with hydrocarbon-degrading taxa such as *Pseudomonas* and *Pseudoalteromonas* becoming dominant within weeks. Enhanced transfer of high-molecular-weight alkanes and selected PAHs into the aqueous phase under stimulated conditions suggests that MOS potentially acts as an effective vector for redistributing hydrophobic hydrocarbons within the water column. Although the biostimulation can accelerate the microbial responses to oil contamination, it may modify the hydrocarbon transport pathways in ways that influence ecological risk. Understanding these trade-offs is critical for informing oil spill response strategies in cold and sensitive marine environments such as the Arctic.

3.6 Data availability

All raw 16S rRNA gene sequence reads generated from this experiment have been deposited at NCBI's Sequence Read Archive (SRA) under the BioProject [PRJNA1176269](#) (accession numbers [SAMN54986455](#) to [SAMN54986446](#)).

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Appendix 3A – Additional figures

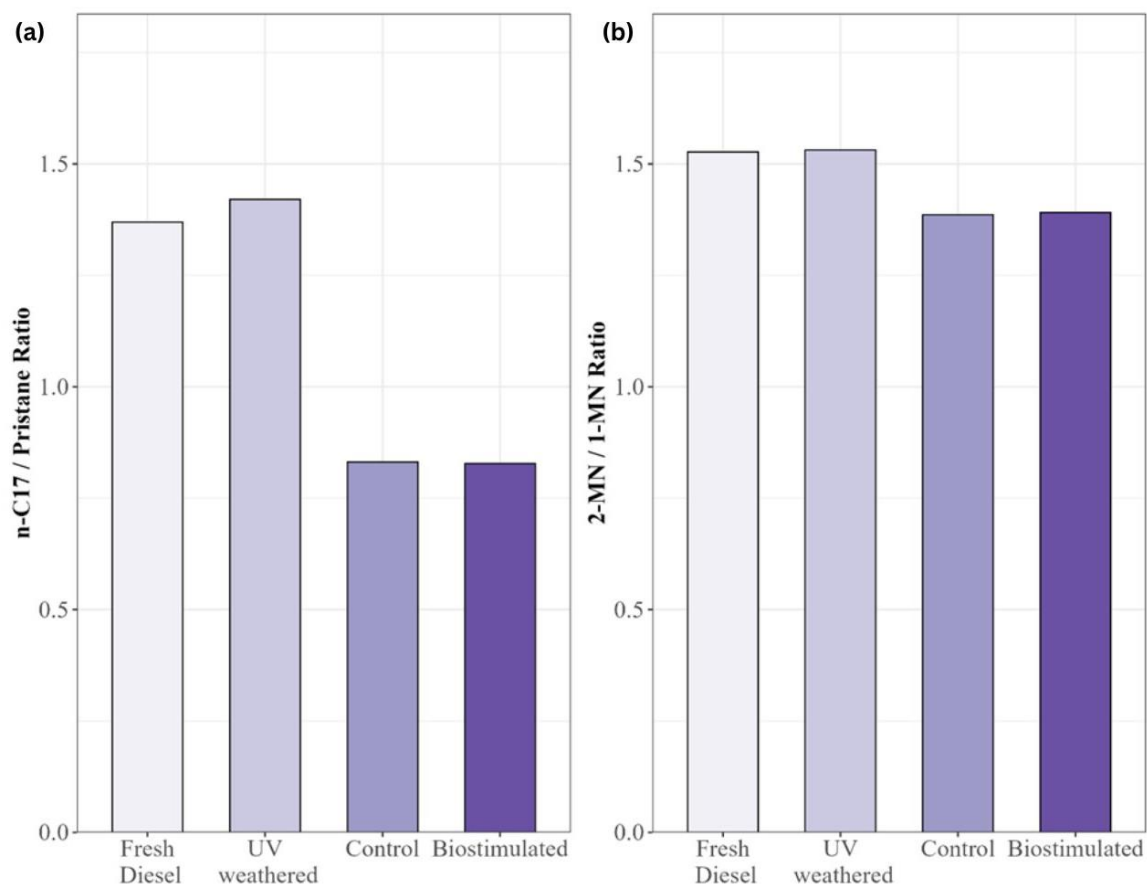


Figure 3.S 1: Diagnostic ratios for the biodegradation of n-alkanes (a), PAHs (b) after 6 weeks of incubation.

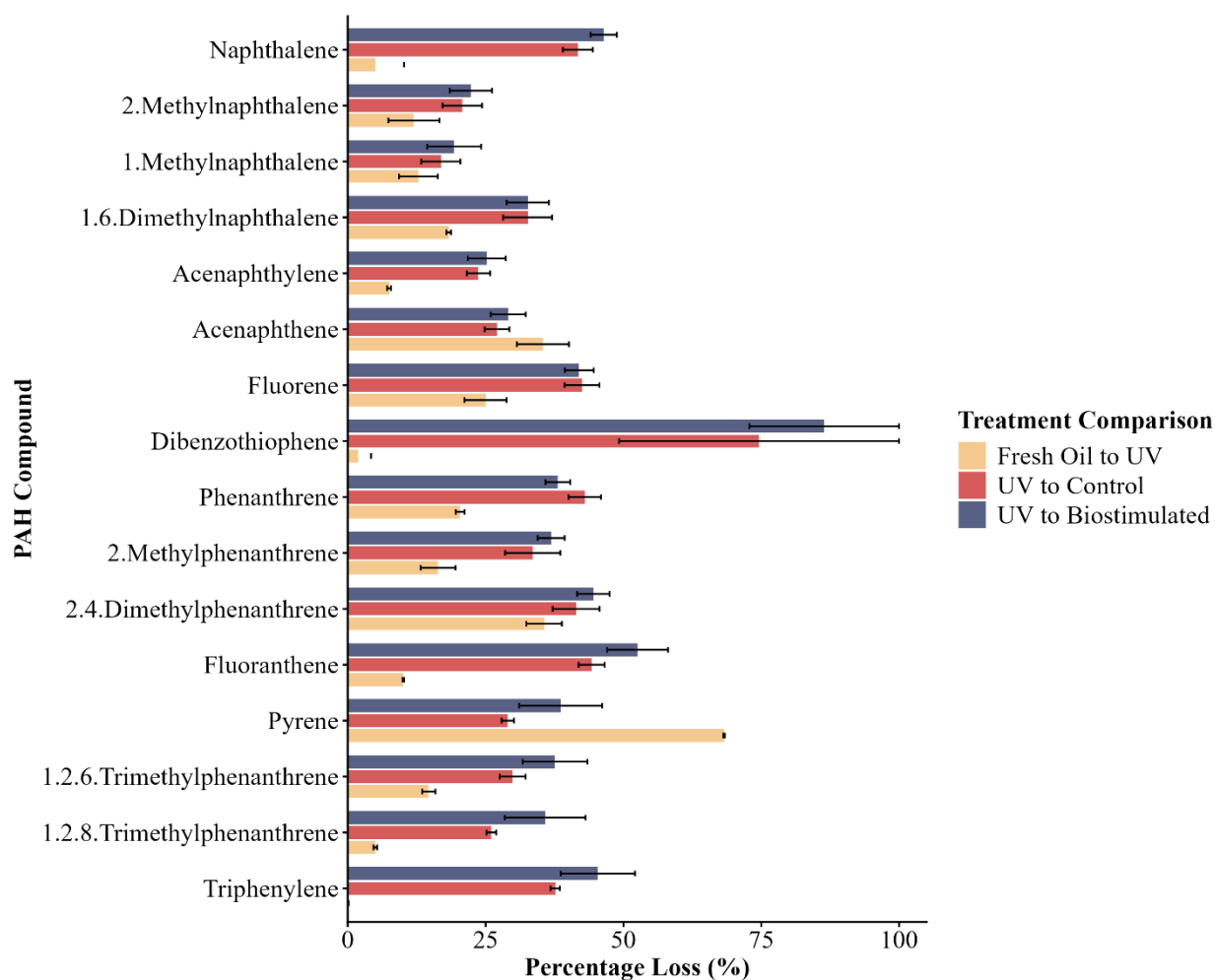


Figure 3.S 2: Percentage loss of individual PAH compounds during the 6-week incubations. Bars represent the mean percentage loss (%) for each compound. Error bars indicate the standard error of the mean (SE). Compounds are arranged in order of increasing molecular weight. The data are based on the bulk oil phase.

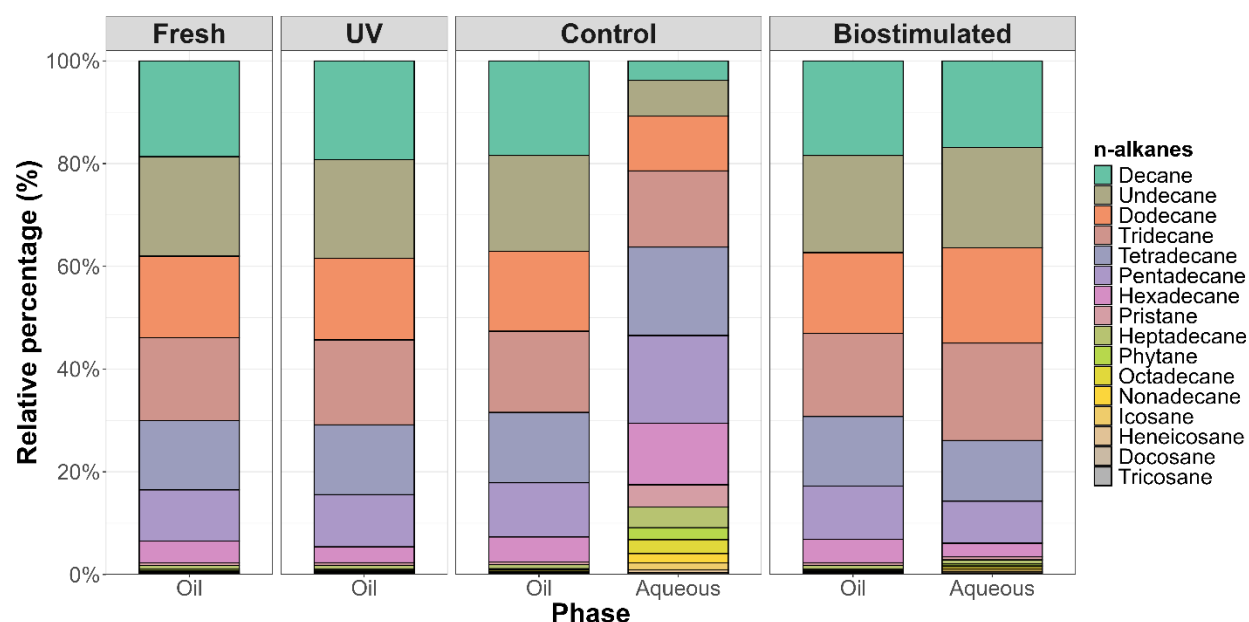


Figure 3.S 3: Relative concentration (%) of individual n-alkanes in fresh and UV-weathered diesel, and in the control and biostimulated treatments after incubation. For control and biostimulated treatments, PAH distributions are shown separately for the oil and aqueous phases. Values represent the percentage contribution of each compound to the total measured PAH concentration of each phase within each sample.

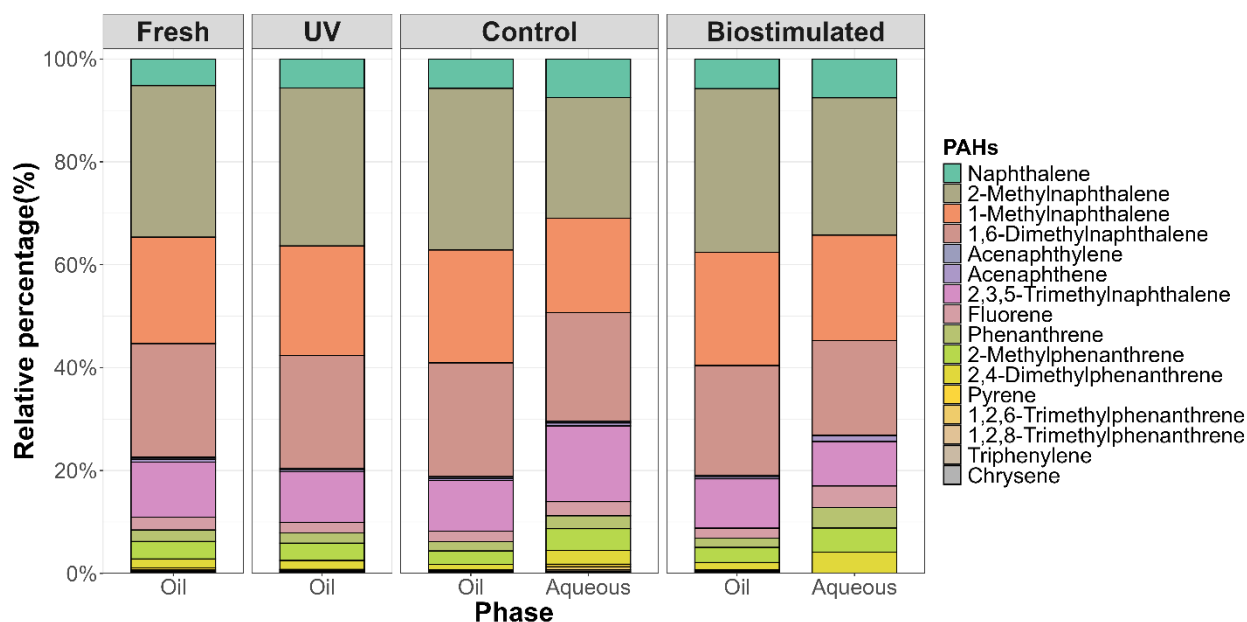


Figure 3.S 4: Relative concentration (%) of individual PAHs in fresh and UV-weathered diesel, and in the control and biostimulated treatments after incubation. For control and biostimulated treatments,

PAH distributions are shown separately for the oil and aqueous phases. Values represent the percentage contribution of each compound to the total measured PAH concentration of each phase within each sample.

Appendix 3B – GC-MS instrumental parameters

Table 3.S. 1: Table of identified compounds and their multiple reaction monitoring mass spectrometry method quantifier settings

Investigated Compounds	Standard Quantifiers	Precursor Ion	Product Ion	Dwell	CE (eV)
Naphthalene	Naphthalene	128	128	54.7	5
2-Methylnaphthene	2-Methylnaphthene	142	142	54.8	5
1-Methylnaphthene	1-Methylnaphthene	142	142	54.8	5
1,6-Dimethylnaphthene	1,6-Dimethylnaphthene	156	156	110.3	5
Acenaphthylene	Acenaphthylene	152	152	32.5	5
Acenaphthene	Acenaphthene	154	154	32.5	5
2,3,5-Trimethylnaphthalene	2,3,5-Trimethylnaphthalene	170	170	110.4	5
Fluorene	Fluorene	166	165	40.9	26
Dibenzothiophene	Dibenzothiophene	184	184	110.3	5
Phenanthrene	Phenanthrene	178	178	40.9	5
Anthracene	Anthracene	178	178	40.9	5
2-Methyldibenzothiophene	2-Methyldibenzothiophene	198	198	54.8	5
2-Methylphenanthrene	2-Methylphenanthrene	192	192	54.8	5
2,8-Dimethyldibenzothiophene	2,8-Dimethyldibenzothiophene	212	212	110.4	5
2,4-Dimethylphenanthrene	2,4-Dimethylphenanthrene	206	206	54.8	5
2,4,7-Trimethyldibenzothiophene	2,4,7-Trimethyldibenzothiophene	226	226	54.8	5
Fluoranthene	Fluoranthene	202	202	54.8	5
Pyrene	Pyrene	202	202	41	5
1,2,6-Trimethylphenanthrene	1,2,6-Trimethylphenanthrene	220	220	36.3	5
1,2,8-Trimethylphenanthrene	1,2,8-Trimethylphenanthrene	220	220	36.3	5
1-Methylfluoranthene	1-Methylfluoranthene	216	216	36.3	10
Benzo[e]phenanthrene	Benzo[e]phenanthrene	228	228	110.4	5
Benz[a]anthracene	Benz[a]anthracene	228	228	36.2	14
Triphenylene	Triphenylene	228	228	46.8	5
Chrysene	Chrysene	228	228	46.8	5
1-Methylchrysene	1-Methylchrysene	242	242	54.8	5
6-Ethylchrysene	6-Ethylchrysene	256	241	54.8	18
Benzo[b]fluoranthene	Benzo[b]fluoranthene	252	250	21.4	50
Benzo[k]fluoranthene	Benzo[k]fluoranthene	252	252	2.14	14
Benzo[j]fluoranthene	Benzo[j]fluoranthene	252	252	21.4	14
Benzo[e]pyrene	Benzo[e]pyrene	252	252	36.2	14
Benzo[a]pyrene	Benzo[a]pyrene	252	252	36.2	14
Perylene	Perylene	252	252	54.7	14

Indeno[1,2,3-c,d]pyrene	Indeno[1,2,3-c,d]pyrene	276	276	32.5	14
Dibenz[a,h]anthracene	Dibenz[a,h]anthracene	278	278	32.5	14
Benzo[g,h,i]perylene	Benzo[g,h,i]perylene	276	276	54.8	14
Decane	Decane	57	57	110.2	0
Undecane	Undecane	57	57	110.2	0
Dodecane	Dodecane	57	57	54.7	0
Tridecane	Tridecane	57	57	110.2	0
Tetradecane	Tetradecane	57	57	100.2	0
Pentadecane	Pentadecane	57	57	110.2	0
Hexadecane	Hexadecane	57	57	36.2	0
Pristane	Pristane	57	57	54.8	0
Heptadecane	Heptadecane	57	57	54.8	0
Phytane	Phytane	57	57	54.8	0
Octadecane	Octadecane	57	57	54.8	0
Nonadecane	Nonadecane	57	57	110.2	0
Eicosane	Eicosane	57	57	54.7	0
Heneicosane	Heneicosane	57	57	110.2	0
Docosane	Docosane	57	57	110.2	0
Tricosane	Tricosane	57	57	110.2	0
Tetracosane	Tetracosane	71	71	40.8	0
Pentacosane	Pentacosane	57	57	110.2	0
Hexacosane	Hexacosane	57	57	110.2	0
Heptacosane	Heptacosane	57	57	110.2	0
Octacosane	Octacosane	57	57	54.7	0
Nonacosane	Nonacosane	57	57	110.2	0
triacontane	triacontane	57	57	110.2	0
Hentriacontane	Hentriacontane	57	57	54.7	0
Dotriacontane	Dotriacontane	57	57	110.2	0
Tritriacontane	Tritriacontane	57	57	110.2	0
Tetratriacontane	Tetratriacontane	57	57	110.2	0
Pentatriacontane	Pentatriacontane	57	57	54.7	0
Hexatriacontane	Hexatriacontane	57	57	110.2	0
Heptatriacontane	Heptatriacontane	57	57	110.2	0
Octatriacontane	Octatriacontane	57	57	110.2	0
Nonatriacontane	Nonatriacontane	57	57	110.2	0
Tetracontane	Tetracontane	57	57	110.2	0

CHAPTER FOUR: PUTATIVE BIOSURFACTANTS FROM POLAR MICROBIAL CONSORTIA

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R. Eric Collins - Conceptualization, Methodology, Writing – Review & Editing, Validation, Supervision, Funding acquisition, Project Administration, Supervision

4.0 Abstract

Biosurfactants (BS) are amphiphilic microbial metabolites that are biodegradable, low in toxicity, and environmentally compatible, making them attractive for applications in food, pharmaceutical, and bioremediation industries. This study investigated the phylogenetic composition and biosurfactant-producing potential of marine oil snow (MOS)-associated microbial consortia from Southern Hudson Bay. MOS aggregates were cultivated in mineral Bushnell–Haas (BH) medium supplemented with 3% (w/v) NaCl, using motor oil as the sole hydrocarbon source. Enrichment cultures capable of growth in BH medium were subsequently evaluated for biosurfactant production using qualitative and quantitative assays at 4 °C and 20 °C. All cultures were maintained as enrichment consortia throughout the study.

A total of eight enrichment cultures were identified as biosurfactant-producing consortia. 16S rRNA gene sequencing revealed that *Pseudoalteromonas* dominated all consortia, while *Paraglaciicola*, *Marinomonas*, and other taxa were present at varying relative abundances. Although none of the consortia exhibited high emulsification activity ($E_{24} > 40\%$), biosurfactant yields were significantly higher at 4 °C. Additionally, three consortia tested positive for rhamnolipid-type biosurfactant production.

Keywords: biosurfactants, microbial consortia, rhamnolipids

4.1 Introduction

Biosurfactants (BS) can reduce fuel-water interfacial tension to form emulsions that facilitate the assimilation of hydrocarbons by microorganisms (Ruiz et al., 2021). Chemical and microbial surfactants are amphiphilic compounds, meaning that they contain both hydrophobic and hydrophilic groups. The hydrophilic groups consist of polysaccharides, peptides, or proteins, while the hydrophobic groups are made up of hydroxylated fatty acids or fatty alcohols (Lang, 2002). Bioemulsifiers (BE) are high-weight molecules such as lipoproteins, lipopolysaccharides, proteins, and exopolysaccharides (EPS; Collins and Margesin, 2019). Both BS and BE are bioproducts produced by microorganisms that are less toxic than their synthetic counterparts (Banat et al., 2014). The physical and chemical properties of biosurfactants make them suitable for various applications, including bioremediation of contaminated sites, microbial enhanced oil recovery, and use in the pharmaceutical, cosmetic, and food industries (de Lemos et al., 2023). Using biosurfactants as a potential oil remediation method has gained a lot of interest due to their effectiveness, environmental safety, and sustainability. Even though short-chain hydrocarbons such as alkanes and alkenes are readily biodegradable by many bacteria, long-chain petroleum hydrocarbons' biodegradation takes a long time. Hence, the removal of those complex hydrocarbons from oil spills is challenging (Kubota et al., 2008).

The amphiphilic properties of biosurfactants provide a significant advantage by allowing them to spontaneously separate into two immiscible phases. This occurs as they reduce surface tension and improve the solubility of hydrophobic compounds (Abdoli et al., 2025). Biosurfactant activity plays a role in biofilm formation and also in biofilm detachment (Ward, 2010). Biofilms are microbial cells that are irreversibly attached to a substratum or an interface embedded in a matrix of extracellular polymeric substances produced by them (Dheilly et al., 2010).

Production of biosurfactants occurs predominantly on hydrophobic substrates such as petroleum hydrocarbons (Ward, 2010). Recently, 55 biosurfactant producers were identified and characterized in North Atlantic Canada. Those belonged to 8 genera, including *Alcanivorax*, *Exiguobacterium*, *Halomonas*, *Rhodococcus*, *Bacillus*, *Acinetobacter*, *Pseudomonas*, and *Streptomyces* (Cai et al., 2014). Biosurfactant production, mediated by the RhIA enzyme, plays a role in the maintenance of the channels within the biofilm and occurs in microcolonies early in biofilm development (Ward, 2010).

Among biosurfactants, glycolipids and glycopeptides with lower molecular weights are generally more effective in reducing surface and interfacial tensions, whereas high molecular weight compounds such as amphipathic polysaccharides, lipopolysaccharides, and lipoproteins serve as stabilizers for oil-in-water mixtures (Lin, 1996). Microbes access hydrocarbon either via direct interaction with the particulate or liquid droplet hydrophobic substance and interaction with pseudo-solubilized oil or molecules in the form of a surfactant-generated oil-in-water / water-in- oil emulsions, or the surfactant secreted by microbes promotes emulsion formation and facilitates cellular uptake of the hydrophobic substrate (Ward, 2010).

Generally, both monocultures and mixed microbial cultures can be applied in bioremediation; however, higher hydrocarbon degradation rates are often achieved using enrichment consortia isolated from the contaminated environment targeted for restoration (Juhasz et al., 2000). Nonetheless, these naturally occurring surface-active microbial metabolites provide environmentally friendly alternatives for their synthetic counterparts (Abdoli et al., 2025). Hence, bioremediation using biosurfactant-producing oil-degrading autochthonous bacteria serves as a promising green clean-up alternative (Lee et al., 2018).

This study aims to improve understanding of biosurfactant production by cold-adapted marine microbial consortia, which may play a key role in enhancing hydrocarbon bioavailability and influencing oil fate in Arctic environments. Despite the ecological importance of biosurfactants in cold-oil interactions, their functional performance under polar conditions remains insufficiently characterized, particularly in enrichment cultures representing natural microbial communities.

4.2 Methodology

4.2.1 Field sampling at the Churchill Marine Observatory (CMO)

The Churchill Marine Observatory is a multidisciplinary research facility designed specifically for studying oil spills. It features two mesocosm outdoor pools that allow for controlled and experimental investigations in both freshwater and seawater environments contaminated with hydrocarbons. As part of a collaborative research conducted by the Centre for Earth Observation Science (CEOS) at the University of Manitoba aiming at investigating the oil behavior in sea ice and the microbial response to such contamination, a total of 114 L of Churchill diesel oil was added to the experimental pool, on December 3rd, 2024 followed by systematic sampling of both the sea ice and seawater over the next two months. Approximately 55 days after the oil contamination, light brown aggregates were observed on the surface of the experimental pool, which is referred to as Marine Oil Snow (MOS; Figure 4.1).



Figure 4. 1: MOS aggregates observed on January 27, 2025, at the surface of the experimental pool in the Ocean–Sea Ice Mesocosm (OSIM) facility at the Churchill Marine Observatory, ~ 55 days after the addition of Churchill diesel fuel.

4.2.2 Bacterial cultivation

The MOS aggregates observed on January 27, 2025 at the surface of the experimental pool in the Ocean—Sea Ice Mesocosm (OSIM) facility at the Churchill Marine Observatory were collected in aseptic amber glass jars and cultured on Bushnell-Hass (BH) Agar (plus 3% w/v NaCl) with motor oil spotted on a sterile filter paper and placed on the lid to use as the sole carbon source for bacterial growth (Malavenda et al., 2015). Cultures were maintained at 4 °C for a month in the dark. Several of bacterial enrichment cultures were obtained by the streak plate method and labeled alphanumerically, with subcultures denoted by numerals (CMO_MOS_A, CMO_MOS_Q1, CMO_MOS_Q2, CMO_MOS_Q3, CMO_MOS_Q4, CMO_MOS_R, CMO_MOS_S, CMO_MOS_T).

4.2.3 Bacterial growth assessment

Bacterial cultures that could grow with motor oil as the added hydrocarbon substrate were transferred to BH mineral medium (plus 3% w/v NaCl) and allowed to grow aerobically for a month at 4 °C. The

composition of the BH medium (g L^{-1}) used in this study was: MgSO_4 (0.20), CaCl_2 (0.02), KH_2PO_4 (1.00), K_2HPO_4 (1.00), NH_4NO_3 (1.00), and FeCl_3 (0.05). The pH was adjusted to 7.0, and the medium was autoclaved. Bacterial growth was compared against the respective negative controls, which had no added hydrocarbon substrate.

4.2.4 Screening of biosurfactant producers

Bacterial cultures that grew in the presence of hydrocarbons were screened for their ability to produce surface-active compounds such as biosurfactants (BS) and bioemulsions (BE). From each culture, an aliquot of 100 μL was transferred to an Erlenmeyer flask containing 100 mL of BH mineral medium (plus 3% w/v NaCl) and 2% (v/v) motor oil and grown under shaking at 110 rpm for 1 week at 20 °C and 4 °C (Malavenda et al., 2015).

4.2.4.1 Emulsification index test (E24)

The ability of the bacterial cultures to form stable emulsions was assessed using the E_{24} test. After 1 week of growing bacterial consortia under shaking, the medium was centrifuged at 4200 rpm for 40 minutes to obtain the cell-free supernatant. A small volume (2 mL) of each supernatant was added to a flat-bottom glass vial that contained an equal volume of kerosene as the hydrocarbon source. The solution was vigorously vortexed for 1 minute and allowed to stand for 24 hours. The total height of the mixture and the height of the emulsion layer were noted (Malavenda et al., 2015). The following equation was used to calculate the emulsification index of the bacterial isolates, where $h_{emulsion}$ and h_{total} are the height of the emulsion layer and the total mixture, respectively. The test was done in triplicate.

$$E24 = \frac{h_{emulsion}}{h_{total}} \times 100$$

4.2.4.2 Blue agar plate method

The blue agar plate method is a semi-quantitative assay for the detection of glycolipid biosurfactants or other anionic surfactants such as rhamnolipids (Walter et al., 2013). The blue agar plates were prepared with BH media that were supplemented with peptone 2% (w/v) and cetyltrimethylammonium bromide (CTAB: 0.5 mg/mL)-methylene blue (MB: 0.2 mg/mL) and 3.5% (w/v) NaCl. Inoculum from enrichment cultures was spot inoculated in duplicate onto wells prepared on blue agar plates and incubated at 20 °C for one week. Appearance of dark blue halos around the spotted colonies was considered an indication of surfactant production (Satpute et al., 2008).

4.2.4.3 Oil displacement method

This method is used to determine the ability of biosurfactants to reduce surface tension and displace oil (Degang et al., 2023). A total of 40 mL of distilled water was added to a Petri dish, followed by the addition of 100 µL of crude oil to form a thin layer on the surface. Then, 10 µL of cell-free supernatant from each liquid enrichment culture was carefully placed onto the oil layer. Formation of a clear zone was considered an indication of biosurfactant production. The diameter of the resulting clear zone was measured to assess biosurfactant activity (Trudgeon et al., 2020). Clear zone diameters were determined by measuring two perpendicular diameters across each zone after 2 minutes and calculating the average. Tween 80 was used as the positive control.

4.2.4.4 Extraction of crude biosurfactants

Putative biosurfactants produced by each bacterial enrichment culture were enriched by acid precipitation. While this simplified procedure enriches for amphiphilic molecules like biosurfactants, it may also co-extract other cell components. A volume of 6N HCl was added to the cell-free

supernatant until pH 2 was achieved, and samples were stored at 4 °C overnight for precipitation. The solution was then centrifuged at 4200 rpm for 1 hour to collect the precipitate, resuspended in distilled water, and neutralized. The crude precipitates were then subjected to a methanol-chloroform extraction (1:1), followed by rotary evaporation at 40 °C. The pellet was collected in pre-combusted (450 °C for 5 h) glass vials, which were dried in a drying oven at 80 °C for 72 h (Phulpoto et al., 2020).

As residual oil did not fully evaporate during oven drying, the dried material was subsequently dissolved in chloroform and filtered onto pre-combusted, pre-weighed GF/F filters. Filters were then dried at 80 °C for an additional 72 h, after which the dry weight of the crude biosurfactant was determined gravimetrically using the following formulae:

$$\text{Dry weight of extract} = \text{Oven dried vial weight} - \text{clean vial weight} \quad (1)$$

$$\text{Dry weight of extract} = \text{Oven dried filter weight} - \text{clean filter weight} \quad (2)$$

$$\text{Crude extract yield} = \text{Eq. (1)} + \text{Eq. (2)}$$

4.2.4.5 Bacterial phylogenetic characterization

Genomic DNA of the enrichment cultures was extracted using the PowerSoil DNA isolation kit (Qiagen, CA, USA) following the manufacturer's instructions. The V4-V5 hypervariable regions of the 16S rRNA gene were amplified using the 515F (GTGYCAGCMGCCGCGGTAA) and 806 RB (GGACTACNVGGGTWTCTAAT) primer pair (CD Genomics, New York, USA) and were sequenced using paired-end (2 x 250 bp) Illumina Novaseq (CD Genomics, New York, USA). Quality filtering of the reads was performed using the DADA2 package in R (Callahan et al., 2016), resulting in 250,208 reads with an average depth of 54, 292, and 17,010 per sample. Sequences were aligned using the SILVA v138.2 reference database for taxonomic classification (Quast et al., 2013).

4.3 Results

4.3.1 Qualitative assays of the biosurfactants

4.3.1.1 Foaming

Surfactants can foam, characterized by bubbles visible through the liquid and on the gas-liquid interface (Satpute et al., 2010). All eight cultures exhibited visible foaming for approximately 2 hours after incubation on a shaker at 110 rpm, at both 20 °C and 4 °C (Figure 4.2). This observation suggests biosurfactant production by the bacterial enrichment cultures.



Figure 4. 2: Foaming properties observed in liquid enrichment cultures at 4 °C. The culture consisted of 100 mL of BH mineral media, 2 mL of motor oil, and 100 μ L of bacterial inoculum.

4.3.1.2 E₂₄ test

The ability of bacteria to produce stable emulsions was analyzed using the E₂₄ test. The emulsification index (E₂₄ %) indicates the strength of the biosurfactant. The samples exhibited varying emulsion indices when produced at different temperatures, with all samples tested at a neutral pH of 6-8. All

samples, except for A, demonstrated stable emulsions at 4 °C (Figure 4.3 & Figure 4. S3). At 20 °C, only three samples (CMO_MOS_R, CMO_MOS_Q1, and CMO_MOS_S) showed stable emulsions that were 16.1%, 3.2%, and 6.4%, respectively. CMO_MOS_R demonstrated notably higher emulsion stability at 20 °C (16.1%). None of the above bacterial consortia can be considered efficient bioemulsifiers under the above environmental conditions since the E₂₄ values were relatively low.

4.3.1.3 Oil displacement method

R demonstrated the highest biosurfactant activity (57.5 cm) by pushing the oil layer to the edges, while CMO_MOS_T, CMO_MOS_Q1, and CMO_MOS_Q3 exhibited moderate biosurfactant activities. However, the activity varied at different temperatures (Figures 4.3 & 4.4).

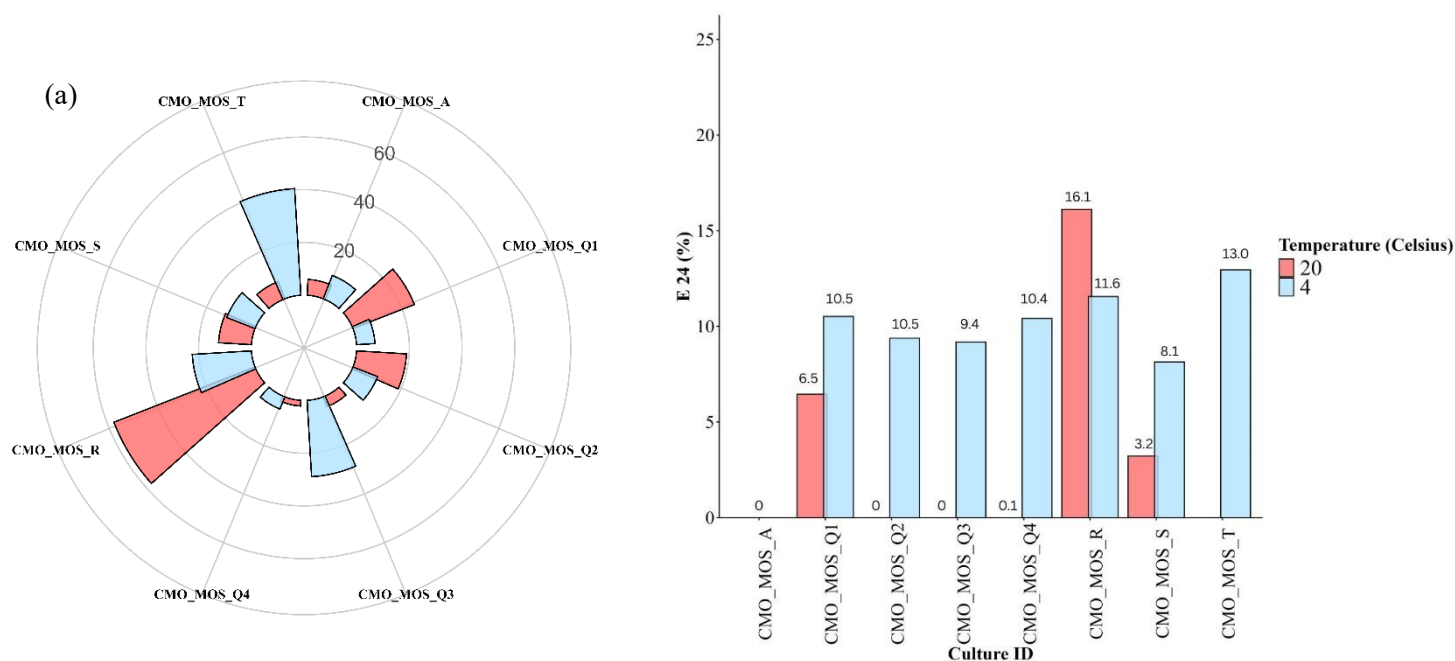


Figure 4. 3: Oil displacement assay of the biosurfactants at 20 °C (red) and 4 °C (blue) (a) E₂₄ % of the biosurfactants at 20 °C (red) and 4 °C (blue) (b)

4.3.1.4 Blue agar test

Enrichment cultures CMO_MOS_A, CMO_MOS_R, and CMO_MOS_S showed positive results

in the blue agar assay, indicating potential rhamnolipid production (Figure 4.5).

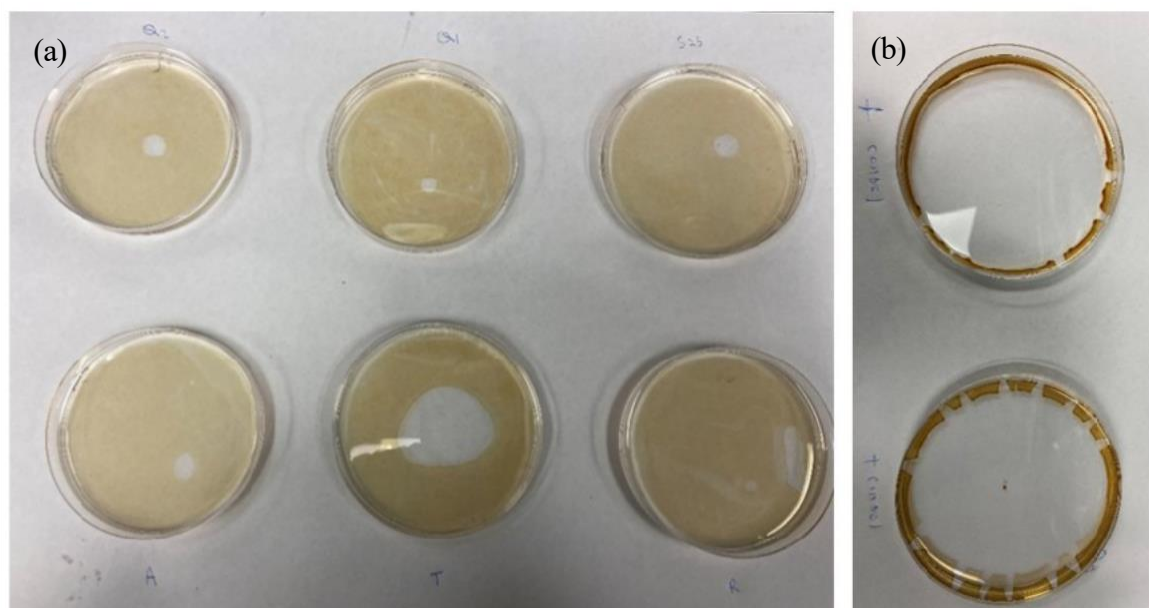


Figure 4. 4: Oil displacement assay of biosurfactants tested on distilled water with crude oil on the top at 4 °C. The samples are arranged from left to right as follows: CMO_MOS_Q2, CMO_MOS_Q1, CMO_MOS_S (top row), CMO_MOS_A, CMO_MOS_T, and CMO_MOS_R (bottom row) (a), while Tween 80 serves as the positive control (b).

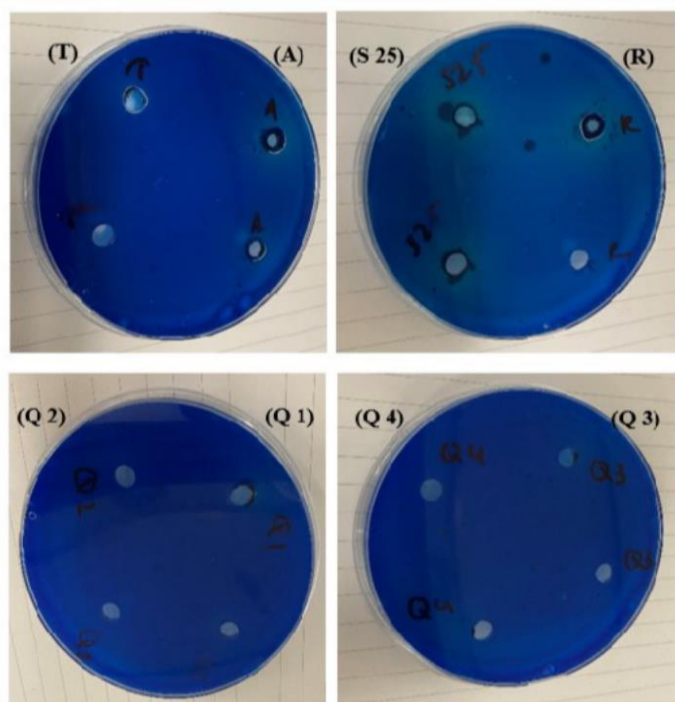


Figure 4. 5: Blue agar test for biosurfactants tested at room temperature (20 °C), showing blue halo zones around CMO_MOS_A, CMO_MOS_S, and CMO_MOS_R inoculated spots.

4.3.1.5 Acid extraction yield

A Mann–Whitney U test revealed a significant difference between yields of crude extractions obtained at 4 °C and 20 °C ($W = 56$, $p = 0.014$), indicating a consistent directional trend with decreasing temperature. The magnitude of yield varied considerably among samples, with some exhibiting substantial increases at 20 °C (CMO_MOS_Q1; Figure 4.6 & Figure 4.S1). These patterns suggest strain-specific and potentially cold-adaptive production of putative biosurfactants.

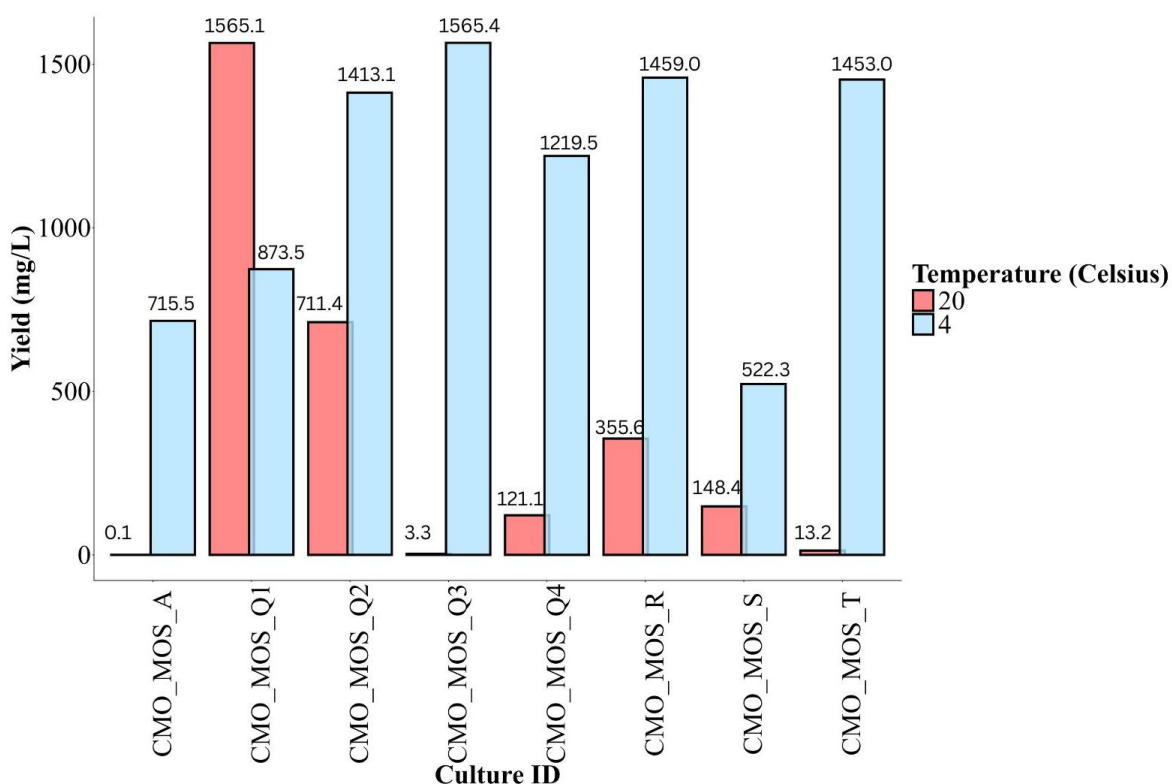


Figure 4. 6: Acid extract yield from bacterial enrichment cultures at two temperatures, 20 °C (red bars), 4 °C (blue bars)

4.3.1.6 Phylogeny of surfactant producers

Across all enrichments (CMO_MOS_A, CMO_MOS_Q1–Q4, CMO_MOS_R, CMO_MOS_T, and CMO_MOS_S), *Pseudalteromonas* accounted for the majority of the total relative abundance, ranging from 73% to 98% (Figure 4. 7) with the highest relative abundances observed in cultures CMO_MOS_S (98%) and CMO_MOS_T (93%), indicating consistent enrichment of this genus under cold, motor oil-based growth conditions.

In addition to *Pseudalteromonas*, several other known surfactant-producing marine genera were detected at lower relative abundances. *Paraglacliecola* was the most prominent secondary contributor, particularly in CMO_MOS_Q1 (17%), CMO_MOS_R (10%), and CMO_MOS_Q2 (8%). *Marinomonas* was also present in multiple enrichments, with its highest relative abundance observed in CMO_MOS_Q3 (12%). Other genera, including *Shewanella*, *Paraperlucidibaca*, *Halomonas*, *Psychrobacter*, and *Sulfitobacter*, were detected sporadically and generally comprised less than 5% of the community in each enrichment culture.

Community composition varied among enrichments in terms of relative contributions of secondary taxa; however, overall community diversity was low, particularly in cultures CMO_MOS_T and CMO_MOS_S, which exhibited near-exclusive dominance by *Pseudalteromonas*. These patterns indicate strong and consistent selection for a limited subset of surfactant-producing bacteria under cold, exogenous carbon enrichment conditions.

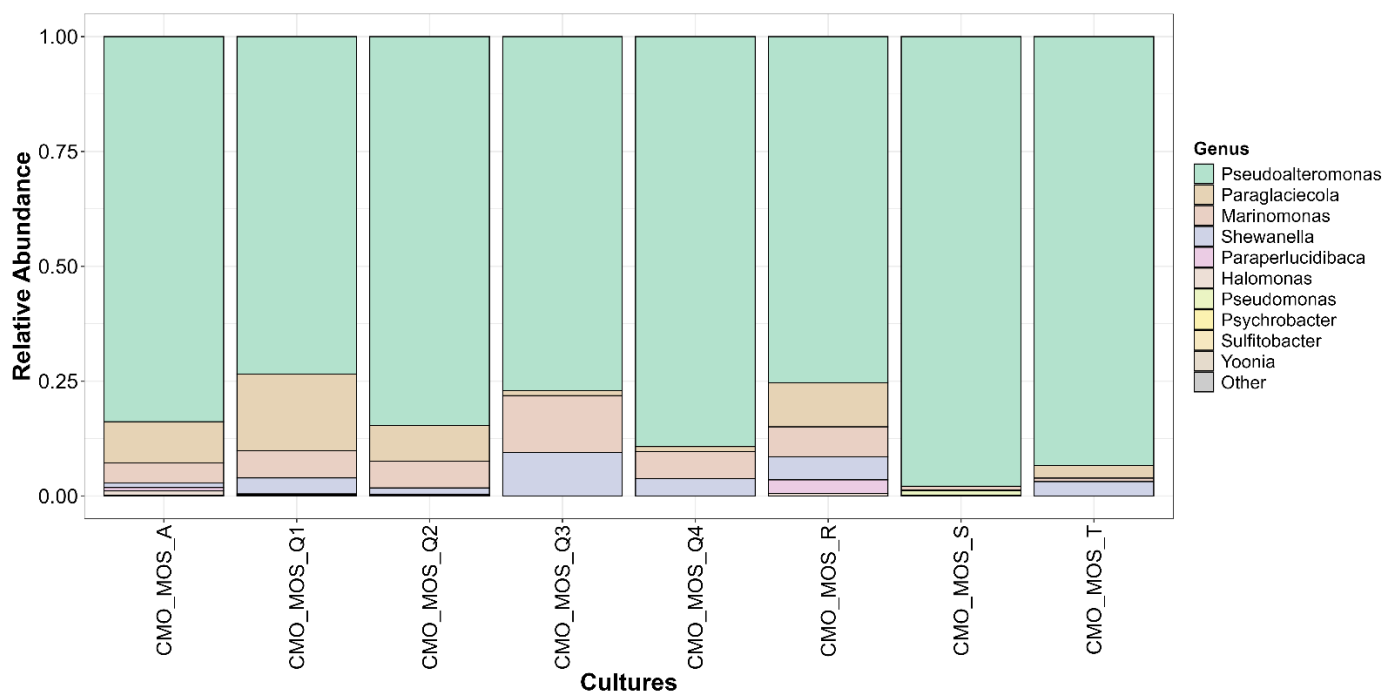


Figure 4. 7: Bacterial community composition of enrichment cultures (CMO_MOS_A, CMO_MOS_Q1–Q4, CMO_MOS_R, CMO_MOS_S, and CMO_MOS_T) incubated at 4 °C with motor oil as the sole source of carbon.

4.4 Discussion

A total of eight bacterial enrichment cultures were screened for the ability to produce biosurfactants on Bushnell Hass media in the presence of motor oil as the hydrocarbon source. Across all enrichment treatments (CMO_MOS_A, CMO_MOS_Q1–Q4, CMO_MOS_R, CMO_MOS_S, and CMO_MOS_T), *Pseudoalteromonas* dominated the microbial community, representing 73–98% of the total relative abundance. The highest proportions observed in cultures CMO_MOS_S (98%) and CMO_MOS_T (93%) indicate strong and consistent selection of this genus under cold, motor oil-amended conditions. This pattern suggests that the enrichment strategy imposed significant selective pressure favoring taxa capable of surviving and proliferating under hydrocarbon stress at low temperatures. Members of this genus are members of the ‘generalist’ hydrocarbon-degrading community and are known for their metabolic versatility (Gutierrez et al., 2013), surface-associated

lifestyles (Thomas et al., 2008), and potential production of extracellular compounds (Suja et al., 2017) that may facilitate hydrocarbon interaction and emulsification. The dominance of *Pseudoalteromonas* therefore indicates its potential ecological role in biosurfactant-associated processes and hydrocarbon adaptation under cold conditions.

Although biosurfactants produced at 4 °C demonstrated measurable emulsification activity, none reached the $E_{24} \geq 40\%$ benchmark commonly used to classify strong bioemulsifiers. These results indicate that cold-temperature conditions supported biosurfactant production to some extent, but the emulsifying performance remained moderate.

The methylene blue (MB), cetyltrimethylammonium bromide (CTAB) agar plate assay (Pinzon and Ju, 2009) was used for rapid screening of rhamnolipid-producing cultures. Positive halo formation was observed for enrichment cultures CMO_MOS_A, CMO_MOS_R, and CMO_MOS_S, indicating production of anionic surfactant(s), possibly including rhamnolipids.

The oil displacement assay performed on enrichment cultures revealed clear temperature- dependent differences in biosurfactant activity. The highest oil displacement was observed for R produced at 20 °C (57.5 mm), followed by CMO_MOS_T produced at 4 °C (40.5 mm). Although CMO_MOS_T was overwhelmingly dominated by *Pseudoalteromonas* (93%), enrichment culture CMO_MOS_R exhibited a more diverse community structure, comprising *Pseudoalteromonas* (75%), *Paraglaciecola* (10%), *Marinomonas* (7%), and *Shewanella* (5%). The superior oil displacement observed in CMO_MOS_R, produced at 20 °C, may reflect enhanced microbial metabolic activity at this temperature, which can promote synergistic interactions such as complementary metabolic pathways, cooperative EPS production, and cross-feeding mechanisms that improve hydrocarbon solubilization.

Although all enrichment cultures exhibited broadly similar microbial community compositions, their relative abundances differed, and their functional responses to biosurfactant screening assays varied considerably. Some cultures showed higher oil displacement and greater biosurfactant yield, whereas others did not, despite taxonomic similarity. This suggests that functional performance is not determined solely by bacterial community, but rather by the abundance of key taxa that influence the potential synergistic interactions within the consortium.

Importantly, biosurfactant production can vary even among strains within the same species, meaning that genetically similar isolates may still exhibit markedly different levels of biosurfactant activity (Suthar and Nerurkar, 2016; Aboelkhair et al., 2022). Therefore, even minor shifts in strain-level composition undetectable at higher taxonomic resolution may explain the observed differences in oil displacement and yield among our enrichment cultures. These findings highlight the importance of considering intraspecific diversity and functional heterogeneity when evaluating biosurfactant-mediated oil degradation processes.

Although both CMO_MOS_T and CMO_MOS_R exhibited higher biosurfactant yields at 4 °C, their oil displacement activity did not consistently correspond to yield values. For example, enrichment culture CMO_MOS_R produced a higher acid extract yield at 4 °C (1459 mg L⁻¹) compared to 20 °C (355.5 mg L⁻¹); however, oil displacement was greater at 20 °C (57.5 mm) than at 4 °C (22.5 mm). Similar temperature-dependent discrepancies between acid extract yield and oil displacement activity were observed in enrichment cultures CMO_MOS_Q4 and CMO_MOS_Q2. These results indicate that acid extract yield alone does not necessarily predict their performance, suggesting that differences in chemical composition, structural properties, or community interactions may influence functional effectiveness. Thus, temperature may affect not only production levels but also the physicochemical

characteristics and emulsification efficiency of the biosurfactants produced, as demonstrated in previous studies (Ortiz et al., 2021).

4.5 Conclusion

This study evaluated the biosurfactant-producing potential of eight bacterial enrichments derived from marine oil snow collected at the Ocean Sea Ice Mesocosm (OSIM) facility at the Churchill Marine Observatory. Biosurfactant production varied significantly with incubation temperature, with enhanced activity observed at lower temperatures. Given that these bacterial enrichments originated from polar environments, their metabolic activity is potentially adapted to low temperature conditions. Among the enrichments, CMO_MOS_R exhibited the highest emulsification and oil displacement activity, along with a comparatively high acid extract at 4°C. Enrichment CMO_MOS_R also tested positive in the blue agar assay, suggesting the possible production of a rhamnolipid-type biosurfactant. Collectively, these findings highlight the potential of cold-adapted marine bacterial communities associated with marine oil snow to produce biosurfactants under low-temperature conditions. With further characterization and optimization, biosurfactants derived from such strains may represent promising candidates for future bioremediation applications in cold marine environments.

4.6 Data availability

All raw 16S rRNA gene sequence reads generated from this experiment have been deposited at NCBI's Sequence Read Archive (SRA) under the BioProject [PRJNA1176269](#) (accession numbers [SAMN54986504](#) to [SAMN54986511](#)).

4.7 Acknowledgements

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Appendix 4A – Data tables

Table 4.S. 1: Summary of the biosurfactant screening results for samples (CMO_MOS_A-CMO_MOS_T) at 20 °C and 4 °C.

Sample ID	E24 (%)		Oil Displacement (mm)		Blue Agar	Crude extract yield (mg/L)	
	20 °C	4 °C	20 °C	4 °C	20 °C	20 °C	4 °C
CMO_MOS_A	0.0	0.0	6.0	9.5	+	0.1	715.5
CMO_MOS_Q1	6.5	10.5	25.0	7.0	-	1565.1	873.5
CMO_MOS_Q2	0.0	9.3	19.0	10.0	-	711.4	1413.1
CMO_MOS_Q3	0.1	9.2	4.0	29.0	-	3.3	1565.4
CMO_MOS_Q4	0.0	10.4	2.2	5.0	-	121.1	1219.5
CMO_MOS_R	16.1	11.6	57.5	22.5	+	355.6	1459.0
CMO_MOS_S	3.2	8.1	12.5	11.5	+	148.4	522.3
CMO_MOS_T	0.0	13.0	7.0	40.5	-	13.2	1453.0

Appendix 4B - Additional figures

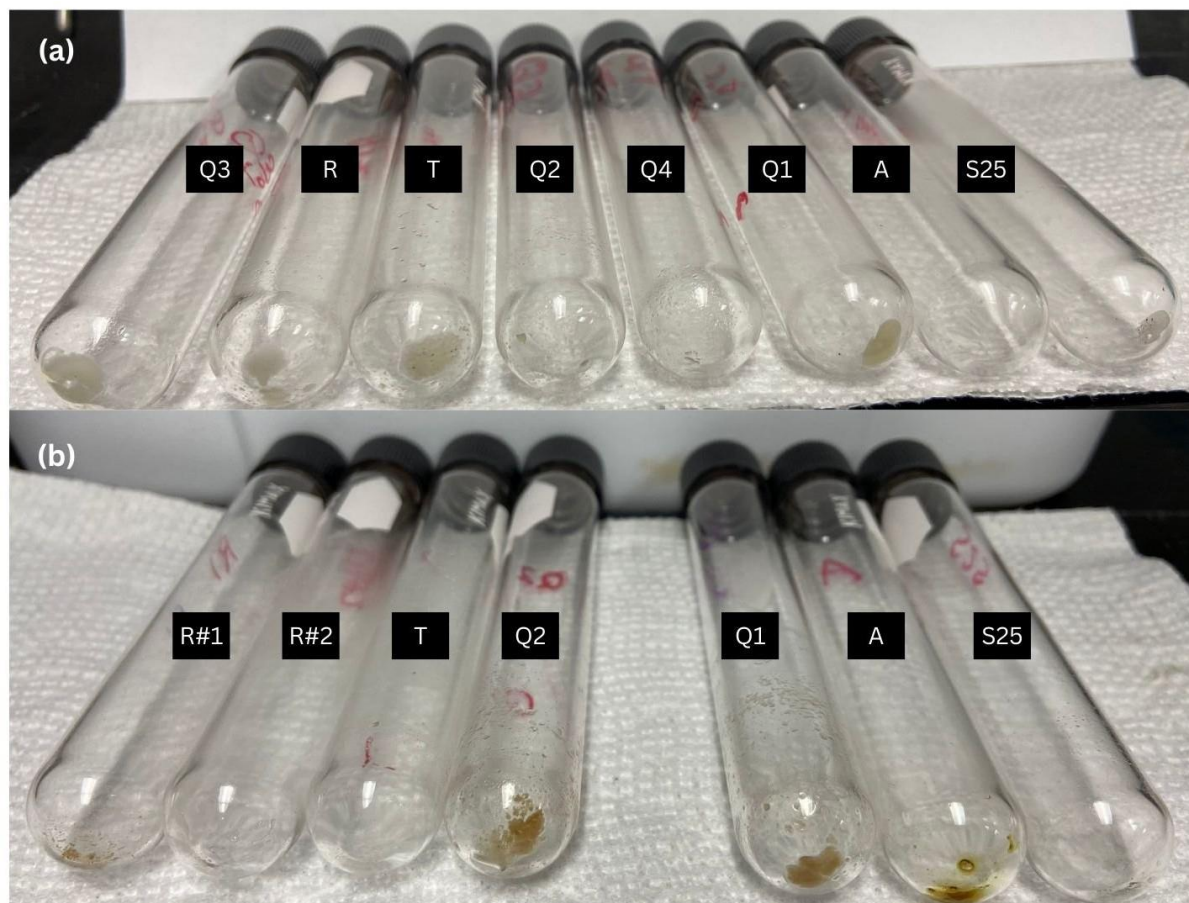


Figure 4.S. 1: Extracted biosurfactant products obtained from different enrichment cultures incubated at (a) 4 °C and (b) 20 °C. Cultures are arranged from highest to lowest biosurfactant yield based on production at 4 °C. Missing vials in panel (b) indicate samples that were entirely filtered onto pre-combusted GF/F filters for dry weight determination.

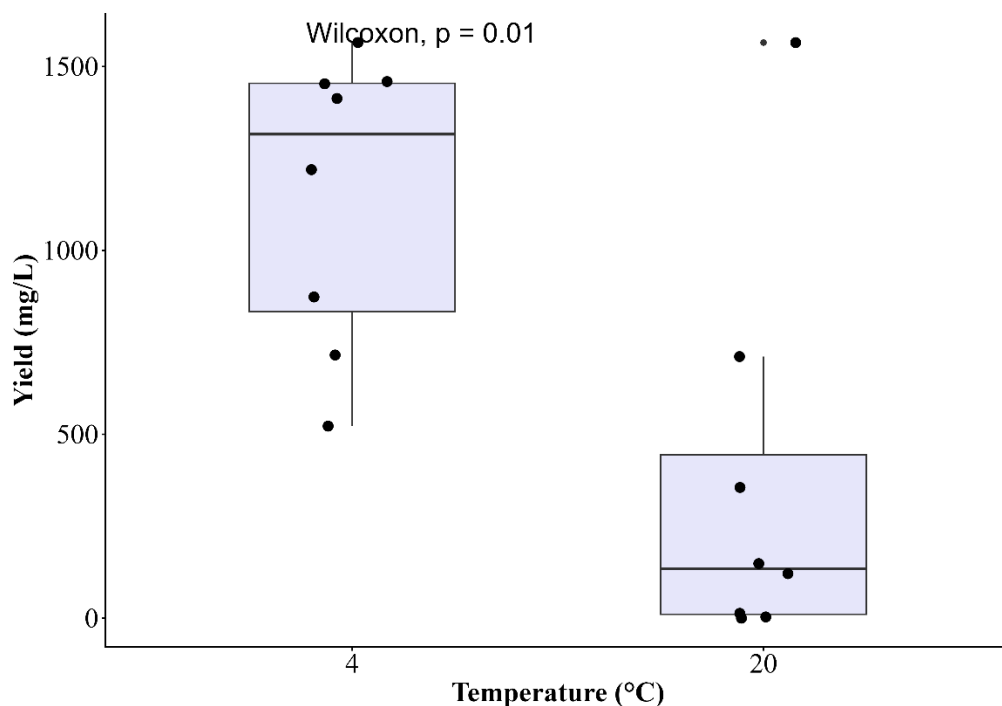


Figure 4.S. 2: Biosurfactant yield produced at 4 °C and 20 °C by enrichment cultures. Boxes indicate the interquartile range and median, while points represent individual yield measurements. Yield differed significantly between temperatures based on a Mann-Whitney U test ($p = 0.014$).



Figure 4.S. 3: Emulsification index ($E_{24\%}$) test of cell-free supernatants from enrichment cultures CMO_MOS_T and CMO_MOS_R grown at 4 °C, and CMO_MOS_Q4 grown at 20 °C, demonstrating their ability to form stable emulsions. Visible emulsification was observed in cultures CMO_MOS_T and CMO_MOS_R, whereas CMO_MOS_Q4 showed no detectable emulsification after 24 h.

CHAPTER FIVE: DRAFT GENOME OF *PARAPERLUCIDIBACA* SP. CMO1 ISOLATED FROM MARINE OIL SNOW IN THE CHURCHILL MARINE OBSERVATORY, MB, CANADA

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5.0. Genome Announcement

An oil-degrading enrichment culture was inoculated from Marine Oil Snow (MOS) collected during an oil spill experiment at the Churchill Marine Observatory in winter 2024. The complete metagenome-assembled genome for *Paraperlucidibaca* sp. CMO1 was reconstructed, exhibiting the genetic potential to degrade hydrocarbons, including alkanes and aromatic compounds, as well as the ability to produce surfactants.

Key words: hydrocarbons, *Paraperlucidibaca*, surfactants

Paraperlucidibaca is a genus of Gram-negative, rod-shaped bacteria belonging to the class *Gammaproteobacteria* (Oh et al., 2011). *Paraperlucidibaca* was first proposed in 2011 based on the notable differences in phenotypic properties with its closest member, *Perlucidibaca* (Oh et al., 2011). *Paraperlucidibaca* is a well-known hydrocarbon degrader in oil-contaminated seawater, exhibiting a significant enrichment in the presence of nutrients (Murphy et al., 2021). Here, we report the complete metagenome-assembled genome of *Paraperlucidibaca* sp. CMO1, isolated from the Ocean-Sea Ice Mesocosm (OSIM) facility at the Churchill Marine Observatory, Manitoba, Canada, during a winter 2024 oil-spill experiment.

A total of 114 L of Churchill Diesel oil was added to the experimental pool at the OSIM on December 3rd, 2024. Light brown flocs were observed floating on the surface of the pool 55 days after oil was added, which we refer to as Marine Oil Snow (MOS). The MOS particles were collected in aseptic glass jars and grown on Bushnell-Hass agar (plus 3% w/v NaCl) plates with motor oil spotted on the lid to use as the sole carbon source for bacterial growth (Malavenda et al., 2015). Cultures were maintained at 4°C, and subcultures were obtained through streak-planting, but pure cultures were not obtained after multiple streaking, so analysis proceeded with the mixed culture. Genomic DNA extraction of the enrichment culture was performed using the DNeasy PowerSoil kit (Qiagen). Multiplexed DNA libraries were prepared using the Rapid PCR Barcoding Kit 24 v14 (SQK-RPB114.24) and loaded onto a FLO-MIN114 flowcell and sequenced on the Oxford Nanopore Technology, GridION device according to the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). Twelve enrichment cultures were co-assembled using Flye 2.9.4-b1799 in metagenomics mode with a minimum overlap of 1000 bp. The co-assembly was polished in Flye using the built-in polisher, and a single circular contig containing the *Paraperlucidibaca* genome was reconstructed from the resulting assembly graph using Bandage v0.8.1 (Wick et al., 2015). Genome

annotation was performed using RASTtk via the BV-BRC annotation service.

The complete genome is 2,526,375 bp in length, with a G+C content of 52.39% and 2,577 predicted protein-coding sequences, 45 tRNAs, and 4 rRNA genes (Table 1). Average Nucleotide Identity (ANI) of 98.37% with uncultured *Paraperlucidibaca* sp. (GCA_963974805.1) and 90.09% with type strain *Paraperlucidibaca baekdonensis* DSM 26022.

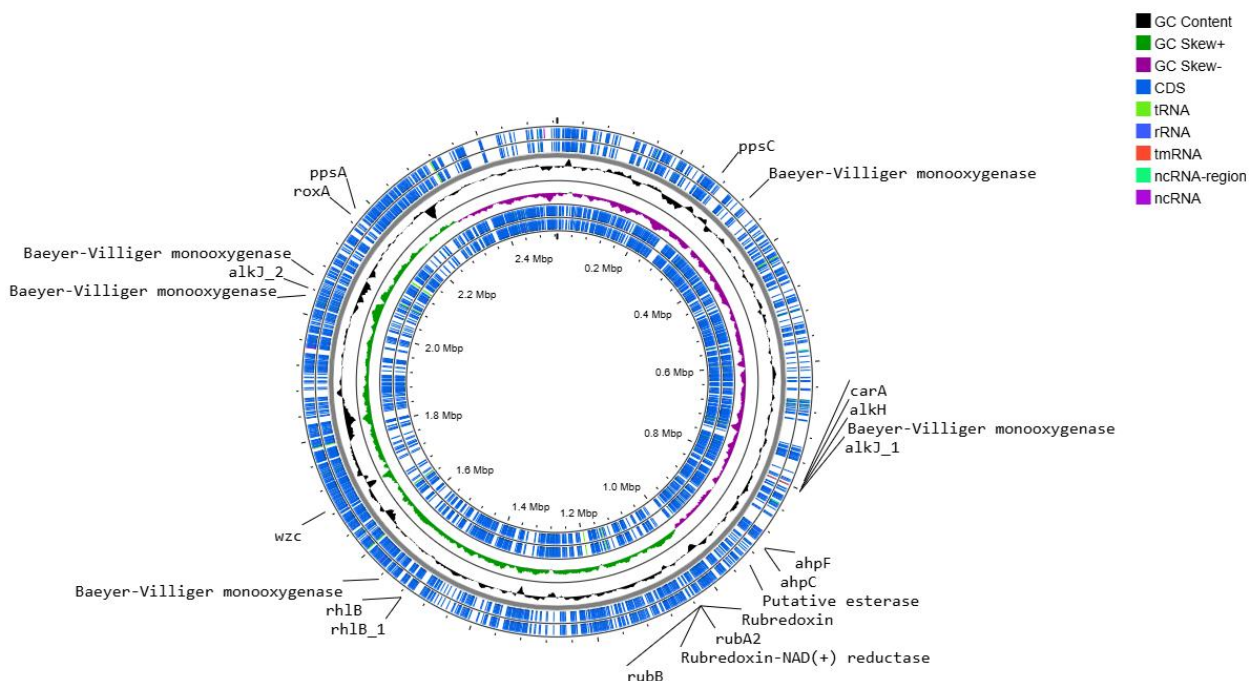


Figure 5. 1: Genome circular diagram of *Paraperlucidibaca* sp. CMO1 generated by Proksee (<https://proksee.ca/tools/prokka>)

Genes associated with hydrocarbon degradation were identified by querying the genome against the Hydrocarbon Aerobic Degradation Enzymes and Genes (HADEG; Rojas-Vargas et al., 2023) and Calgary approach to ANnotating HYDrocarbon degradation genes (CANT-HYD; Khot et al., 2022) databases. A noise cutoff was applied to CANT-HYD annotations to retain high-confidence gene hits. Functional gene annotation using HADEG was performed with thresholds set to –identity = 50 and –conn = 0.3, yielding a 94.68% true-positive rate and a 20.80% false-negative rate. Manual annotation using Prokka and Bakta revealed that the genome also encodes rubber oxygenase (*roxA*; Cui et al., 2023), as well as genes associated with biosurfactant production, including rhamnolipids (*rhIB*; Kumar et al., 2024), emulsan (*wzc*; Nayariseri and Singh, 2023), and plipastatin (*ppsC*; Wang et al., 2024).

Table 5. 1: Genes related to hydrocarbon biodegradation identified in the metagenome-assembled genome based on BV-BRC annotation and matches to curated hydrocarbon-degradation databases (HADEG and CANT-HYD). Genes retained following CANT-HYD noise cutoff filtering are indicated with an asterisk (*).

Target name	Gene name	Description	CANT-HYD E- value	Availability in CANT-HYD	HADEG E- value	Availability in HADEG	BLAST hit E- value
fig 2708021.11.peg.115	<i>est</i>	Arylesterase precursor	-	-	7.80e-57	√	W6R2Y2 est 1.25e-75
fig 2708021.11.peg.360	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	3.3e-43	√	-	-	A5H9N6 almA A5H9N8 almA 1.29e-37
fig 2708021.11.peg.812	<i>alkH_ald</i>	Aldehyde dehydrogenase	-	-	8.27e-137	√	P12693 alkH_ald 1.71e-175
fig 2708021.11.peg.816	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	6e-44	√	-	-	G1C7M4 almA 1.91e-36
fig 2708021.11.peg.915	<i>ahpF</i>	Alkyl hydroperoxide reductase protein F	-	-	2.68e-203	√	Q9I6Z2 ahpF 0.0
fig 2708021.11.peg.916	<i>ahpC</i>	Alkyl hydroperoxide reductase protein C	-	-	2.77e-71	√	Q02UU0 ahpC 9.78e-91
fig 2708021.11.peg.1041	<i>alkG_rubA2_rdx</i>	Rubredoxin	-	-	1.14e-26	√	Q9HTK8 alkG_rubA2_rdx 3.1e-29
fig 2708021.11.peg.1042	<i>alkT_rubB_rdxR</i>	Rubredoxin-NAD (+) reductase	-	-	9.70e-120	√	Q0VTB0 alkT_rubB_rdxR 3.15e-154
fig 2708021.11.peg.1585	<i>BVMO</i>	Cyclohexanone monooxygenase	-	-	5.07e-144	√	Q9I3H5 BVMO 9e-180
fig 2708021.11.peg.1585	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	5.2e-45	√	-	-	K0CFH5 almA E3UUD9 almA 4e-36
fig 2708021.11.peg.2019	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	1.9e-238	√	6.06e-171	√	A5H9N6 almA A5H9N8 almA A5H9P0 almA E3UUD9 almA G1C7M4 almA GOM1 almA

							K0CFH5 almA Q6F7T9 almA 0.0
fig 2708021.11.peg.2076	<i>AlmA_GroupI</i> ,	Flavin-binding alkane	1.4e-40	√	-	-	A5H9N6 almA
	<i>AlmA_GroupIII</i>	monooxygenase					4.81e-35
fig 2708021.11.peg.2080	<i>carAc</i>	Ferredoxin, 2Fe-2S	-	-	2.62e-23	√	D5IGG4 carAc 1.97e-30
fig 2708021.11.peg.2081	<i>CYP153*</i>	Alkane-oxidizing cytochrome P450	8.5e-201	√	1.68e-189	√	L7QFU8 CYP153 0.0
fig 2708021.11.peg.2086	<i>CYP153*</i>	Alkane-oxidizing cytochrome P450	3.8e-201	√	1.75e-245	√	L7QFU8 CYP153 0.0
fig 2708021.11.peg.2086	<i>CYP450</i>	Putative cytochrome P450 hydroxylase	-	-	1.75e-245	√	Q6RCE3 CYP450 D0Q1H3 CYP450 0.0
fig 2708021.11.peg.2109	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	1.2e-52	√	-	-	K0CFH5 almA 1.87e-44
fig 2708021.11.peg.2119	<i>carAc</i>	Ferredoxin, 2Fe-2S	-	-	2.85e-24	√	D5IGG4 carAc 3.06e-32
fig 2708021.11.peg.2120	<i>CYP153*</i>	Alkane-oxidizing cytochrome P450	1.3e-201	√	8.40e-190	√	L7QFU8 CYP153 D0Q1H3 CYP450 Q6RCE3 CYP450 0.0
fig 2708021.11.peg.2168	<i>AlmA_GroupI</i> , <i>AlmA_GroupIII</i>	Flavin-binding alkane monooxygenase	2.3e-213	√	-	-	A5H9N6 almA A5H9N8 almA A5H9P0 almA E3UUD9 almA G1C7M4 almA GOM1 almA K0CFH5 almA Q6F7T9 almA 0.0
fig 2708021.11.peg.2316	<i>AlkB*</i>	Alkane hydrolase	4.9e-208	√	6.17e-142	√	C0LMW6 alkB 2.01e-141

Table 5. 2: Annotation statistics of *Paraperlucidibaca* sp.

Features	<i>Paraperlucidibaca</i> genome	Community co-assembly	Original reads
Genome length (bp)	2526375	45525428	897915959
No. of contigs/reads	1	2864	479225
No. of coding DNA sequences	2577	-	-
N50	1	223	60354
L50 (bp)	2526375	33629	4344
GC content (%)	52.39	46.48	44.13
tRNA	45	-	-
rRNA	1	-	-
Proteins with functional assignments	1640	-	-
Proteins with EC number assignments	715	-	-
Proteins with Pathway assignments	530	-	-
Proteins with GO assignments	606	-	-
Proteins with Subsystem assignments	826	-	-

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CHAPTER SIX: FACTORS AFFECTING MICROBIAL COMMUNITY COMPOSITION IN SPLIT LAKE, MB, CANADA.

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6.0 Abstract

Split Lake is a large freshwater lake in Northern Manitoba, Canada, that receives major inflows from the Nelson River, Burntwood River, and Grassy River. In 1976, during the Churchill River Diversion (CRD) project, most of the water in the Churchill River was diverted into the Nelson River via the Rat River, and the Burntwood River, creating a large inflow pressure in the lower Nelson area, including Split Lake. Decades after its implementation, the Churchill River Diversion still influences ecological and environmental processes in the area. In this study, metabarcoding of the 16S rRNA gene was employed to characterize the microbial community structure of the lake. Environmental factors (temperature, pH, turbidity, dissolved oxygen) and cyanotoxin levels were also assessed.

The objectives of this study were to establish a baseline for microbiota across the lake and examine how environmental variables affect microbial population dynamics. The study was conducted in the summer of 2024, encompassing 14 sites on Split Lake and 2 sites on Assean Lake, an adjacent waterbody with minimal influence from hydroelectric infrastructure. Split Lake was found to harbor six distinct microbial clusters, with turbidity and dissolved oxygen levels correlating with different aquatic regimes within the lake. The predominant taxa in Split Lake included *Actinobacteria* hgcI clade and *Cyanobium*. Microcystin concentrations measured throughout the study were always below the Maximum Acceptable Concentration (MAC) set for Canadian federal drinking water standards.

Keywords: aquatic microbial ecology, metabarcoding, microcystin, freshwater lake ecosystem, community-based monitoring.

6.1 Introduction

Split Lake is a shallow, seasonally ice-covered riverine lake that spans approximately 46 km of the Nelson River in northern Manitoba, Canada. Tataskweyak is a remote community of about 2,300 residents located on the north shore of Split Lake. In May 2017, a boil water advisory was issued for the Tataskweyak Cree Public Water System (#6602), becoming a long-term advisory in May 2018 (Indigenous Service Canada 2025). Since 1978, Manitoba Hydro has operated the Churchill River Diversion (CRD) water management project, which redirects 75% of the Churchill River drainage system into the Burntwood River and then into the Nelson River at Split Lake (Duncan & Williamson, 1988). The CRD diversion increased flow in the Nelson River by approximately 32% (Wiens, 1994), resulting in extensive shoreline erosion of clay and silt sediments (Manitoba Hydro, 2015). In response to these impacts, researchers from the University of Manitoba and Land Guardians from the Tataskweyak Environmental Monitoring Agency co-developed this Community-Led Monitoring project with the goal of understanding changes in water quality observed by community members and to monitor for future harms to the aquatic ecosystems. The specific objectives of this study were to establish a baseline for microbial community structure in Split Lake, identify environmental drivers of microbial community structure, and explore harmful algal bloom risks in the lake.

Freshwater microorganisms play a crucial role in biogeochemical cycles and influence water quality in lakes (Newton et al., 2011). They also act as primary sources of food and nutrients for higher trophic level organisms (Islam et al., 2019). Their absence distorts the food chain, disrupting ecosystem functioning and ultimately affecting the balance of biotic and abiotic systems in lakes (Joshi, 2016). Microorganisms in aquatic environments are highly sensitive to environmental changes and human-induced stressors (Islam et al., 2019). Anthropogenic activities such as agriculture, fishing, and tourism affect the structure and function of the microbial community, which leads to pathogenic

bacteria being introduced into the lakes (Islam et al., 2019). Bacteria are more sensitive to anthropogenic changes than fungi (Luo et al., 2022) and are therefore widely used as indicators in assessments of water quality worldwide (Dufour, 1984; Gronewold et al., 2008; Méndez et al., 2004; Wen et al., 2020). Freshwater bacterial community data provide valuable insights into the effects of land use on stream ecosystems and can serve as a complementary tool for screening stream catchment attributes (Hermans et al., 2024). Human activities have profoundly altered natural river connectivity by disrupting longitudinal and lateral linkages through structures such as dams and levees, and by indirectly modifying hydrological, thermal, and sediment regimes that shape riverine ecosystem processes (Nilsson & Berggren, 2000). Damming is the most significant human-induced disturbance to rivers, with about 70% of the world's rivers being dammed (Grill et al., 2019). Studies in dam-impacted reservoirs have documented pronounced longitudinal shifts in bacterioplankton community structure, which are ultimately linked to increased water residence time within impoundments (Mašín et al., 2003).

Freshwater cyanobacterial harmful algal blooms represent a significant risk to human health and aquatic ecosystems, with evidence indicating that their occurrence and intensity are increasing worldwide (Bramburger et al., 2023). Nowicka-Krawczyk et al. 2022 showed that the emergence of Cyanobacterial harmful algal blooms (CyanoHAB) is linked with the mismanaged use of water bodies and land by humans (Nowicka-Krawczyk et al., 2022). Such changes in aquatic communities can further drive eutrophication processes by affecting trophic interactions, changing nutrient ratios, and promoting the movement of nutrients from benthic layers to the overlying water column (Schindler, 2006). In addition, CyanoHAB pose serious socioeconomic and ecological costs that impact the water quality, habitats, food webs, and tourism.

These bloom-forming genera can produce cyanotoxins (microcystin, cylindrospermopsin, nodularin) that can cause acute or chronic health effects for mammals and other organisms (Carmichael & Boyer, 2016; Metcalf & Codd, 2012). Cyanobacteria are widely recognized as key indicators of eutrophication in freshwater systems, as their blooms frequently occur in waters affected by excessive nutrient loading (Thajuddin & Dhanasekaran, 2016). As such, human-driven changes in water physicochemical properties can lead to localized sorting of aquatic bacterial communities (Chen et al., 2018), which in turn results in significant spatiotemporal variability in bacteria-mediated processes within aquatic ecosystems (Islam et al., 2019).

Using bacterial 16S rRNA gene metabarcoding, this study aimed to (1) establish a baseline characterization of the microbial community structure in the surface waters of Split Lake, northern Manitoba, a freshwater system influenced by hydroelectric development; (2) examine spatial variability in microbial community composition across the lake; (3) evaluate relationships between microbial community patterns and key physicochemical water characteristics; and (4) assess the potential of microbial metabarcoding data as an indicator of ecosystem disturbance associated with hydroelectric regulation.

6.2 Materials and methods

6.2.1 Site description and sampling

Surface water samples were collected by a small boat from 14 sites across Split Lake (56.2°N, 96.1°W) and 2 sites from Assean Lake (56.2°N, 96.5°W) from August 13 – 16, 2024 (Figure 1). At each site, approximately 1 L of water was filtered through 0.22 µm Sterivex filters and stored frozen until DNA extraction. Sample temperature and pH were determined using a digital pH meter, and dissolved oxygen (DO) was determined using the CHEMets Dissolved Oxygen visual water testing kit. Turbidity

was measured using the Palintest® Turbimeter Plus instrument. Secchi disc depth measurements were also taken at each site to assess water clarity.



Figure 6. 1: True color satellite image of the Split Lake region, Manitoba, taken on August 5, 2024, by the Sentinel-2 mission. Suspended sedimentary material (clay and silt) discolors the water, producing lighter shades. Water samples were collected by a small boat from August 13-16, 2024; pins are colored by hierarchical clustering of microbial community composition. Local rivers (blue waves) and Manitoba Hydro structures (red squares) are shown for context.

6.2.2 DNA extraction, amplicon library preparation, and processing

Qiagen DNeasy PowerSoil kits were used to extract DNA from Sterivex filters following the manufacturer's protocols. Extracted DNA was quantified using a Qubit fluorometer and stored at -70 °C until sequencing. The V4-V5 hypervariable regions of the 16S rRNA gene were amplified using the 515F (GTGYCAGCMGCCGCGGTAA) and 806 RB (GGACTACNVGGGTWTCTAAT) primer pair. Amplicon libraries were sequenced using paired-end (2 x 250 bp) Illumina Sequencing by CD Genomics (New York, USA). A total of 40k high-quality reads were obtained with a sequence depth range of 29,686 to 73, 860 reads per sample. Raw sequencing reads were quality-controlled by

removing adapters and primer sequences using Cutadapt (Martin, 2011). Trimmed reads were then processed using the DADA2 (Callahan et al., 2016) package in R to infer amplicon sequence variants (ASVs), and taxonomic assignment was performed using the SILVA v138.2 reference database (Quast et al., 2013). Code for the bioinformatics pipeline is available at <https://github.com/rec3141/microscape>.

6.2.3 Statistical analysis

Statistical analyses and visualizations were performed in R. To explore the correlations between microbial community composition and environmental variables, Spearman correlation analysis was performed. The corrplot and pheatmap packages in R were used to produce heatmaps and correlation analysis visualizations, with p-values adjusted for multiple comparisons using the Benjamini–Hochberg method. Redundancy analysis (RDA) was performed to explore relationships between bacterial community composition and environmental factors using the vegan package (Oksanen et al., 2025)

6.2.4 Water chemistry analysis

The American Society Testing Materials method D6919-03 (ASTM, 2021) was followed for major cations (Mg^{2+} , Ca^{2+} , Na^{+}), and EPA method 300.0 (Pfaff, 1996) was used for anions (F^{-} , Cl^{-} , SO_4^{2-}). Concentrations were quantified using the Dionex 5000+ ion chromatography instrument at the Centre for Earth Observation Science, UCTEL, University of Manitoba. Standard reagents and consumables were sourced from Thermo Fisher Scientific.

6.2.5 Cyanotoxin analysis

The ABRAXIS® Microcystins Strip Test for Source Drinking Water was used to qualitatively screen microcystins in lake water samples following the manufacturer's instructions. The sensitivity of the test kit is 1 mg/L or higher. All samples and reagents were allowed to reach room temperature before testing.

6.3 Results

6.3.1 Environmental parameters

Water temperature of the 16 sampling sites ranged between 20.6 °C and 23.2 °C. Measurements of pH ranged from 7.2 to 8.0 and fell within federal guidelines (pH 7.0 - 8.7) for freshwater. The minimum federal guideline for dissolved oxygen in warm freshwater ecosystems is above 5.5 mg/L (Canadian Council of Ministers of the Environment 1999), which was met by all except one site (Maskwa Bay). The highest turbidity was observed in Burntwood River (65.00 NTU), while the lowest was observed in Assean Lake – west (2.51 NTU).

Table 6. 1: Metadata of sampling sites in Split Lake during the August 13th – 16th, 2024 sampling expedition.

Date	Site Name	Site ID	Longitude (deg E)	Latitude (deg N)	Temperature (°C)	pH	Dissolved Oxygen (ppm)	Turbidity (NTU)	Secchi depth_(m)	Cluster color
13/08/2024	York Landing Channel	YLC	-96.2434	56.1472	23.2	8.0	8.5	5.78	1.50	Red
15/08/2024	Aiken River	AK	-96.0824	56.0764	23.1	7.2	7.0	9.09	0.98	
14/08/2024	Odei River	OR	-96.9186	56.0905	21.1	7.8	7.5	34.70	0.47	
16/08/2024	Assean Lake (east)	ALe	-96.5961	56.1851	20.8	7.2	7.0	4.57	2.20	Yellow
16/08/2024	Assean Lake (west)	ALw	-96.4192	56.2330	21.6	7.5	5.5	2.51	2.97	
13/08/2024	Nelson Burntwood	NB	-96.4879	56.1171	20.1	7.5	8.5	52.90	0.30	Green
14/08/2024	Burntwood River	BR	-96.8768	56.0895	20.8	7.8	8.0	65.00	0.33	
14/08/2024	Old Fish Camp Narrow	OFCN	-96.2446	56.1565	21.6	7.2	8.0	40.10	0.39	
16/08/2024	Split Clark Narrow	SCN	-95.9498	56.2848	21.3	7.8	6.0	14.50	0.90	Cyan
16/08/2024	Clark Lake	CL	-95.9198	56.2799	21.2	7.5	8.0	12.70	1.10	
13/08/2024	Split Lake Treatment Plant	SLTP	-96.1045	56.2409	21.2	7.5	10.0	19.10	NA	
16/08/2024	Maskwa Bay	MB	-95.9155	56.2890	20.8	7.5	5.0	13.60	1.08	

13/08/2024	Kelsey Dam	KD	-96.5290	56.0442	21.0	7.6	6.0	7.25	1.65	Purple
15/08/2024	Hole in the Rock Bay	HRB	-96.4686	56.1148	21.1	7.5	6.0	7.94	1.58	
13/08/2024	Grassy River	GR	-96.5618	56.0424	20.6	7.8	6.0	6.47	1.70	
15/08/2024	Josey Spence Island	JSI	missing	missing	21.6	7.5	7.0	36.60	0.42	Magenta

6.3.2 Water chemistry in the lake

Concentrations of major cations (Mg^{2+} , Ca^{2+} , Na^+ , K^+) and anions (F^- , Cl^- , NO_3^- , SO_4^{2-}) were determined from each water sample (Supplementary table S.1). Phosphates were not detected at any of the sampling sites. Sulfate concentrations were relatively high at Kelsey Dam (33.32 ppm) and Hole in the Rock Bay (28.66 ppm) sites, both located along the upper Nelson River watershed. Comparable sulfate levels were also observed at the York Landing Channel (28.65 ppm). In contrast, the lowest sulfate level was observed at Josey Spence Island (0.44 ppm); however, all measured ion concentrations remained below applicable federal guideline limits (Canadian Council of Ministers of the Environment, 2007).

6.3.3 Cyanotoxin concentration analysis

Microcystin concentrations measured across the sampling sites were consistently low. At York Landing Channel, Old Fish Camp Narrow, Nelson Burntwood, Kelsey Dam, Burntwood River, Assean Lake – west, Clark Lake, Hole in the Rock Bay, and Maskwa Bay, microcystin was not detected (0 $\mu\text{g/L}$). Several sites recorded concentrations of 0.5 $\mu\text{g/L}$, including Odei River, Assean Lake – east, Josey Spence Island, Split Clark Narrow, Split Lake Treatment Plant, Grassy River, and Aiekn River. Importantly, none of the sites exceeded the maximum acceptable concentration established in the Canadian Water Quality Guidelines for drinking water.

6.3.4 Taxonomic abundance profiles

All quality-controlled sequences across 16 samples were resolved into 842 ASVs. Bray-Curtis dissimilarities among samples were calculated from fourth root transformed relative abundances and hierarchically clustered using the Ward.D algorithm in R using `vegan::hclust()`.

The sample dendrogram was cut into six clusters containing distinct community assemblages, including in waters strongly influenced by the Burntwood River watershed (green), upper Nelson River (purple), and Assean Lake (yellow), while samples from waters mostly influenced by the Odei River and Aiken River clustered together (red). Waters from the lower Nelson River watershed region of the Split Lake, where inflowing river waters become well-mixed, were clustered with each other (cyan). Another site, “Josey Spence Island” (location information missing), clustered separately from all other samples (magenta; Figure 6.2).

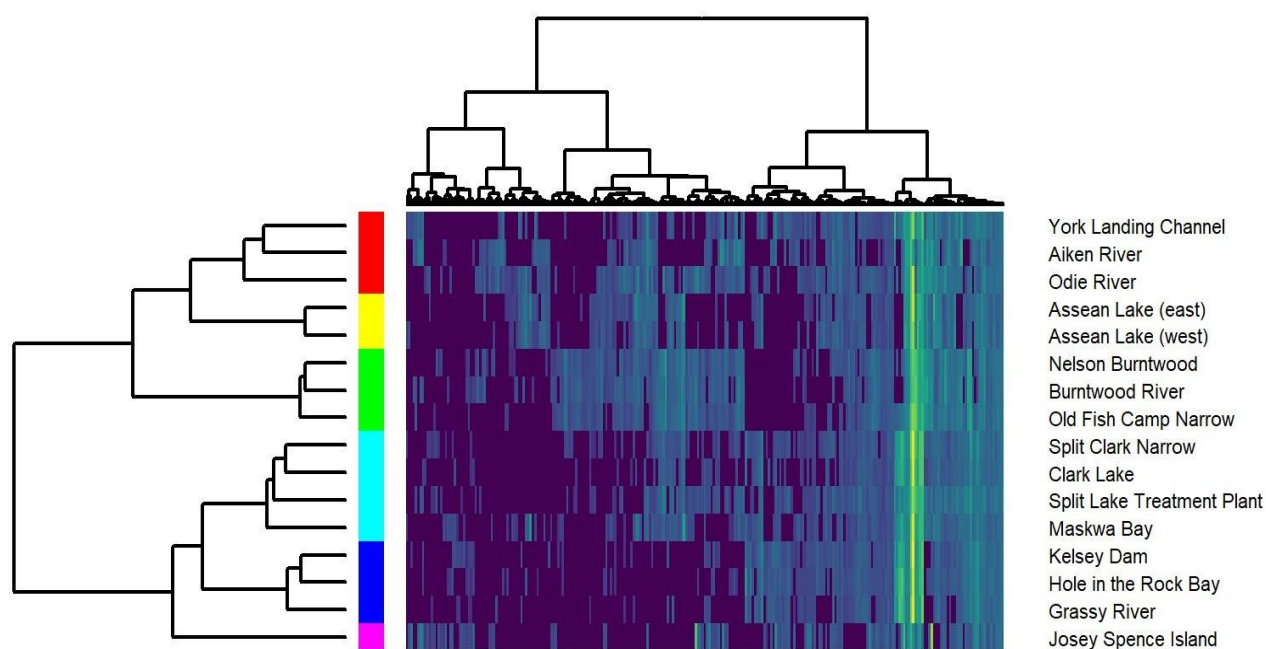


Figure 6. 2: Hierarchical clustering of microbial community composition. Heatmap shows the relative abundance of ASVs (columns) across sites (rows), with darker colors indicating lower relative abundance and brighter colors indicating higher relative abundance. Dendrograms display Ward clustering of Bray-Curtis dissimilarity among taxa (top) and sampling sites (left). The colored bar to the left of the heatmap indicates community clusters.

6.3.5 Microbial community composition

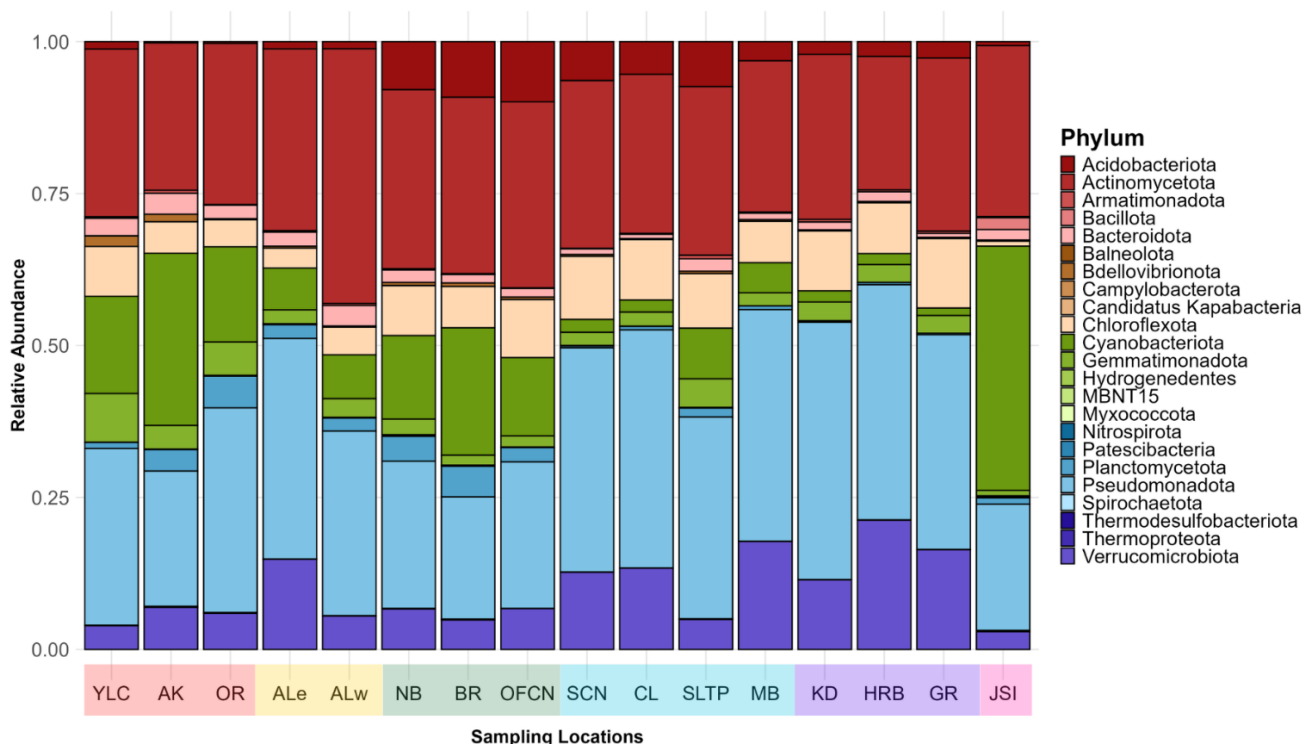


Figure 6. 3: Relative abundance of bacterial phyla across sampling sites. Each bar represents the normalized relative abundance at a given station; YLC – York Landing Channel, AK – Aiken River, OR – Odei River, ALe – Assean Lake east, ALw – Assean Lake west, NB – Nelson Burntwood, BR – Burntwood River, OFCN – Old Fish Camp Narrow, SCN – Split Clark Narrow, CL – Clark Lake, SLTP – Split Lake Treatment Plant, MB – Maskwa Bay, KD – Kelsey Dam, HRB – Hole in the Rock Bay, GR – Grassy River, JSI – Josey Spence Island.

ASVs were taxonomically classified to 23 phyla, 42 classes, 81 orders, 113 families, and 176 genera.

Pseudomonadota was the most abundant phylum observed, accounting for 31.6% of the total sequences (Figure 6.3). The other dominant phyla were Actinomycetota (28.2%), Cyanobacteriota (11.5%), Verrucomicrobiota (9.75%), Chloroflexota (7.30%), and Acidobacteriota (3.8%). Josey Spence Island (JSI) had a distinct profile dominated by Cyanobacteriota (40.2%) and comparatively lower abundance of Verrucomicrobiota (2.9%) and Bacteroidota (1.7%). Nelson Burntwood, Burntwood River, Old Fish Camp Narrow, Split Clark Narrow, Clark Lake, and Split Lake Treatment

Plant had a comparatively higher abundance of Acidobacteriota.

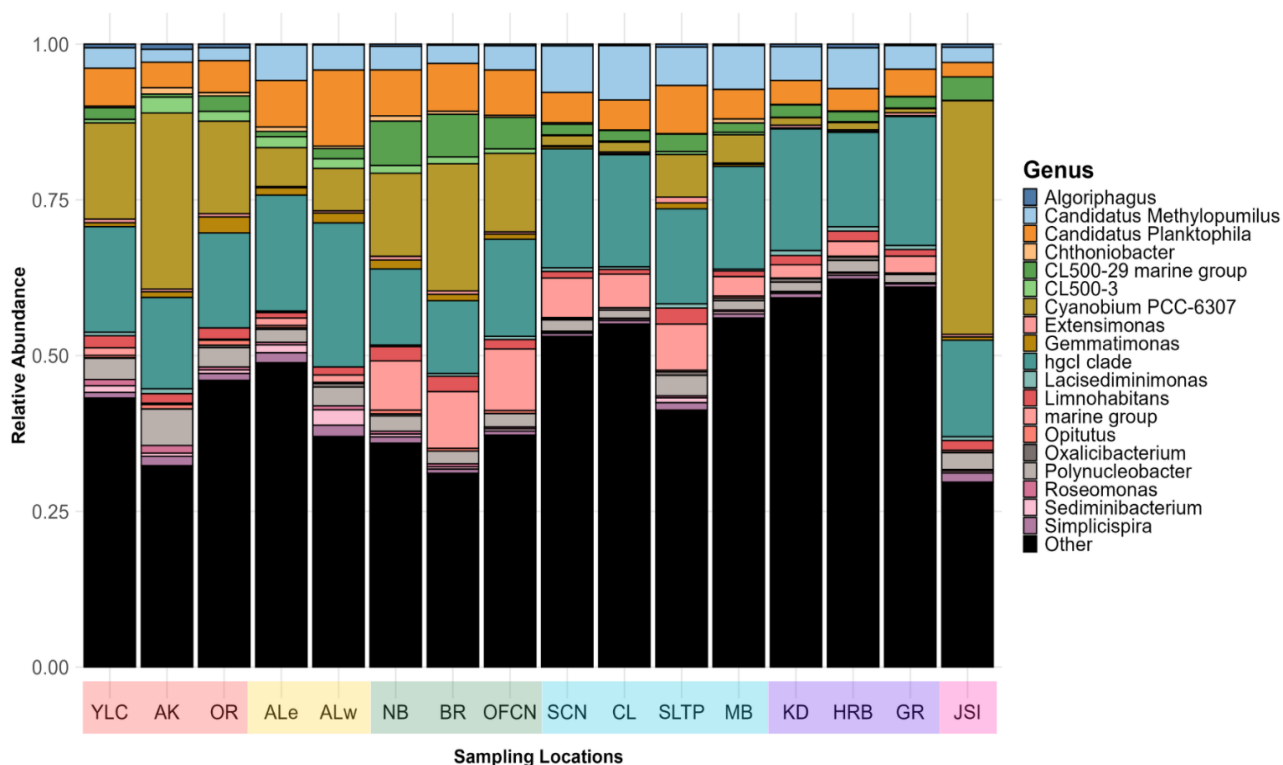


Figure 6. 4: Relative abundance of bacterial genera across sampling sites. The top 20 most abundant genera are shown individually, while all other less abundant genera, including "Incertae Sedis," are grouped as "Other" (black). Each bar represents the normalized relative abundance at a given station; YLC – York Landing Channel, AK – Aiken River, OR – Odei River, ALe – Assean Lake east, ALw – Assean Lake west, NB – Nelson Burntwood, BR – Burntwood River, OFCN – Old Fish Camp Narrow, SCN – Split Clark Narrow, CL – Clark Lake, SLTP – Split Lake Treatment Plant, MB – Maskwa Bay, KD – Kelsey Dam, HRB – Hole in the Rock Bay, GR – Grassy River, JSI – Josey Spence Island.

Among all samples, unclassified taxa (incertae sedis) made up the plurality of the relative abundance at the genus level (41.3%); hence clustered together with less abundant genera and were grouped as “other” (Figure 6.4). The hgcI clade accounted for 16.7% of the community, followed by *Cyanobium* with 10.8%. *Cyanobium* was the most abundant bacterial genus (37.5%) in Josey Spence Island (JSI), suggesting site-specific enrichment, perhaps due to differences in nutrient availability and local hydrodynamics or temporal bloom conditions. Nevertheless, *Cyanobium* abundance was significantly

low in Split Clark Narrow, Clark Lake, Kelsey Dam, Hole in the Rock Bay, and Grassy River locations. The CL500-29marine group was found in comparatively high relative abundance at Nelson Burntwood, Burntwood River, Old Fish Camp Narrow, Split Clark Narrow, Clark Lake, and Split Lake Treatment Plant locations. Additionally, the relative abundance of *Polynucleobacter* was comparatively higher in the Aiken River.

6.3.6 Correlation between microbial community and environmental factors

Redundancy analysis (RDA) was performed to evaluate the relationship between bacterial community composition at the ASV level and environmental variables (Figure 6.5). The first two canonical axes (RDA1 and RDA2) explained the majority of the constrained variation in community composition. Environmental vectors indicated that turbidity and dissolved oxygen were positively associated with RDA1, whereas major ions such as NO_3^- and Ca^{2+} were aligned primarily along the negative direction of RDA2. However, sample clustering was weak, and most ASVs were distributed near the ordination centroid, suggesting limited structuring of the community by the measured environmental variables. The overall RDA model was not statistically significant based on permutation testing ($F = 1.63$, $p = 0.057$, $N = 999$), indicating that the measured environmental variables explained only a limited proportion of the variation in community composition.

To further assess potential associations between bacterial phyla and environmental variables, Spearman rank correlations were calculated and visualized as a heatmap (Figure 6.S1). Consistent with the RDA results, no statistically significant correlations were detected after Benjamini–Hochberg false discovery rate correction (adjusted $p < 0.05$). Together, these analyses indicate that the measured environmental variables did not exert strong or consistent control on bacterial community composition across samples.

6.3.7 Correlation among microbial phyla

Notably, Pseudomonadota abundance was strongly and negatively correlated with Cyanobacteriota ($R = -0.87, p = 0.002$), as were the Cyanobacteriota and Verrucomicrobiota ($R = -0.80, p = 0.015$). Strong positive correlations were observed between Cyanobacteriota and Planctomycetota ($R = +0.76, p = 0.029$), Myxococcota and Planctomycetota ($R = +0.78, p = 0.018$), and Pseudomonadota and Verrucomicrobiota ($R = +0.74, p = 0.036$; Figure 6.6).

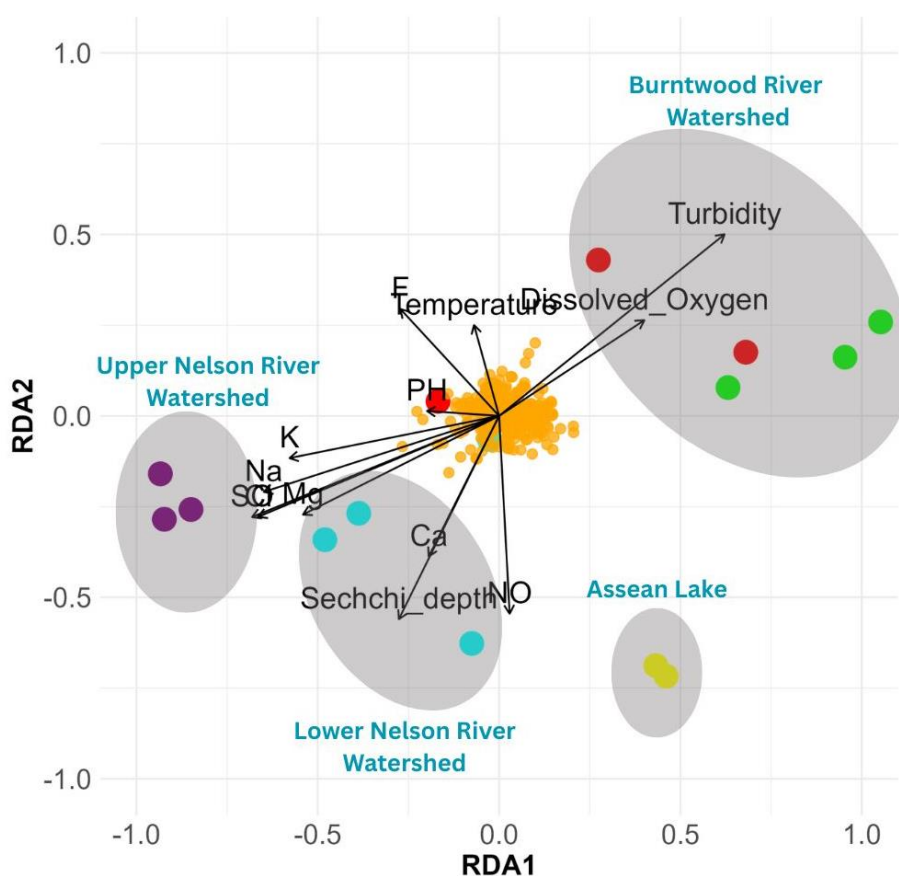


Figure 6. 5: Redundancy analysis (RDA) biplot showing the relationship between ASV community composition (orange dots) and sampling locations (colored dots denote the different community clusters). ASV composition was constrained by environmental parameters, with arrows representing the direction and relative strength of environmental gradients. The watersheds are shown in shaded ellipses. The overall model was not statistically significant based on permutation testing ($F = 1.63, p = 0.057, N = 999$).

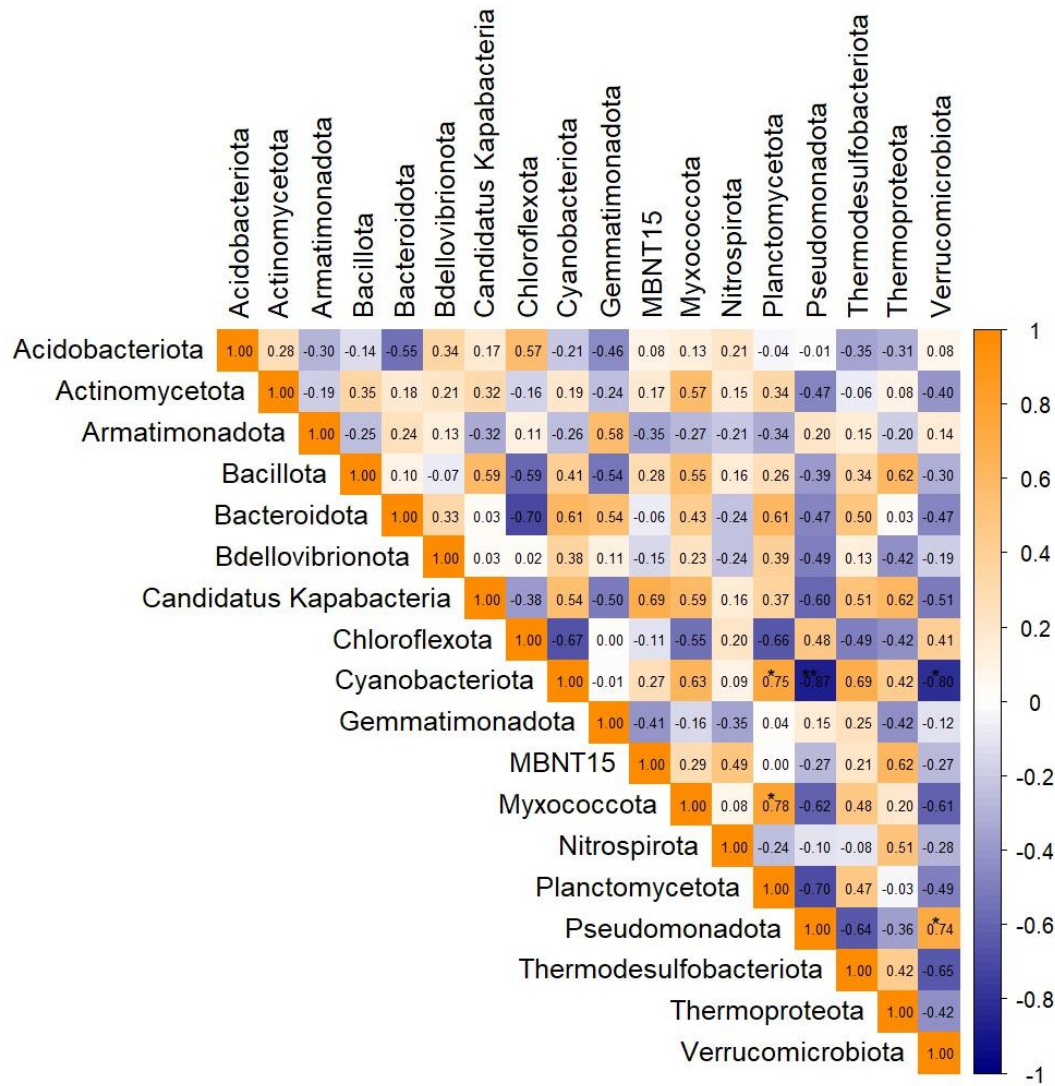


Figure 6. 6: Spearman correlation matrix showing pairwise relationships between bacterial phyla observed in the samples. The color gradient represents the strength and direction of the correlation, with orange indicating positive correlations and blue indicating negative correlations. Values in each cell represent the Spearman correlation coefficient (ρ). P-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Asterisks denote significance thresholds (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

6.4 Discussion

This study establishes a baseline for microbial community composition in Split Lake, Manitoba, providing a foundation for future investigations across other freshwater lakes in the region. Environmental explanatory variables (including surface water chemistry), dependent on geography, were evaluated as potential drivers of microbial community structure. Freshwater ecosystems host diverse microbial communities that play key roles in nutrient cycling, water quality, and ecosystem functioning. In this study, we used 16S rRNA metabarcoding to characterize microbial community composition across Split Lake in August 2024. We evaluated the relationships between community structure and measured environmental variables using correlation-based statistical approaches. Previous research from Lake Winnipeg, located several hundred kilometers upstream of Split Lake, has shown that bacterial community composition across the lake was relatively stable, with only moderate seasonal variability (Kuzyk et al., 2022). In contrast, microbial communities in Split Lake exhibited pronounced spatial heterogeneity across sampling sites. For example, the Josey Spence Island (JSI) site supported a distinctly different microbial community structure compared to all other sites. This spatial variability likely reflects the stronger influence of localized river inflows, hydrological regulation, and physicochemical gradients within Split Lake, which may override the homogenizing effects observed in larger, more hydrodynamically stable systems such as Lake Winnipeg.

JSI exhibited the highest relative abundance of *Cyanobium*, coinciding with very low sulfate concentrations (0.44 ppm), chloride concentrations (0.24 ppm), and moderately elevated turbidity (36.6 NTU). A similar combination of sulfate, chloride, and turbidity was observed at the Odei River site; however, these sites did not cluster together based on the microbial community composition.

Given that other measured environmental variables were largely similar between these sites, the

contrasting microbial assemblages likely reflect differences in hydrological connectivity, water residence time, or biological interactions rather than direct chemical control, as outlined in previous studies on freshwater lakes' microbial community dynamics (Kent et al., 2007; Sadeghi et al., 2021, 2024).

Across all sampling sites, Actinobacteria represented the most abundant taxa in Split Lake and its associated inflows. This dominance is consistent with the observations from Lake Winnipeg (Kuzyk et al., 2022), other Canadian freshwater lakes (Luo et al., 2022; Sharma et al., 2009), and all around the world (Allgaier & Grossart, 2006; Newton et al., 2007), where Actinobacteria are frequently among the most prevalent taxa due to their small cell size, high surface area-to-volume ratio, and efficient utilization of low molecular weight dissolved organic carbon (Yaradoddi & Kontro, 2021). Notably, Actinobacteria remained abundant despite pronounced spatial heterogeneity in turbidity, sulfate concentrations, and hydrological influence, suggesting that this group occupies a broadly resilient ecological niche across the Split Lake system. A similar spatial pattern was observed for *Candidatus* Planktophila, a dominant lineage within the Actinobacteriota, which was consistently abundant across all sampling sites. *Candidatus* Planktophila is a ubiquitous freshwater taxon known for its adaptation to oligotrophic conditions, allowing it to persist across a wide range of physicochemical environments (Amadei Martínez et al., 2025).

Hierarchical clustering analysis revealed spatial structuring of microbial communities across Split Lake, identifying six distinct microbial clusters among the 16 sampling sites. These clusters were particularly associated with river inflow regions, suggesting that tributary inputs play a predominant role in shaping microbial community composition within the lake. For example, sites influenced by the upper Nelson River watershed were grouped into different microbial clusters than those associated

with the Burntwood River watershed, despite both rivers discharging into Split Lake.

This differentiation likely reflects contrasts in upstream watershed characteristics, major ion and organic matter inputs, and physicochemical conditions associated with each river system. In particular, sulfate concentrations were relatively high in the upper Nelson River watershed (14.46 to 33.32 ppm), compared to the Burntwood River watershed (1.35 to 2.55 ppm), providing a potential geochemical driver for the distinct microbial communities associated with each river system. The increased relative abundance of Verrucomicrobiota and reduced relative abundance in Cyanobacteriota further distinguish these sites from the Burntwood River and are consistent with previous studies on Verrucomicrobiota that describe their metabolic capacity in organic matter degradation, including complex sulfated polysaccharides (SPs; Sichert et al., 2020; Tan et al., 2025). Spearman correlation revealed several strong negative associations among dominant bacterial phyla, including Cyanobacteria and Verrucomicrobiota, and Cyanobacteriota and Pseudomonadota. These inverse relationships likely reflect differences in ecological strategies and environmental preferences rather than direct biotic interactions. Many of the negatively associated phyla were also linked to contrasting physicochemical conditions, such as turbidity and dissolved oxygen level, underscoring the relevance of environmental filtering on community structure in Split Lake.

Burntwood River inflows were characterized by higher turbidity levels, and the sampling sites within the lake exhibiting similar turbidity levels to those of the Burntwood River supported microbial assemblages that clustered together and were distinct from communities associated with Nelson River watersheds, Aiken River inflows, and Assean Lake. Elevated turbidity can reduce light penetration and increase particulate matter availability, thereby favoring heterotrophic and particle-associated bacteria while constraining phototrophic taxa (McGowan et al., 2018; Pick & Caron, 1987). These

findings indicate that physical drivers such as turbidity act alongside chemical gradients to independently structure microbial communities across Split Lake. The green microbial cluster was characterized by a relatively high abundance of the CL500-29 marine group and was primarily associated with Burntwood River inflows and the lower Nelson River watershed, which exhibited elevated turbidity. The CL500-29 clade has been reported in freshwater and brackish systems, where the organic matter is rich (Guo et al., 2023; B. Wang et al., 2024; Q. Wang et al., 2024). The higher turbidity observed in the Burntwood River watershed likely reflects increased suspended particles and organic matter, which may create favorable niches for the CL500-29. In contrast, lower-turbidity inflows and adjacent unregulated waters did not support similar assemblages, underscoring the importance of physical forcing in shaping microbial distributions.

Assean Lake, an adjacent and unregulated freshwater system, lacking direct hydroelectric power influence, exhibited distinct physicochemical and microbial characteristics compared to Split Lake and its inflows. The markedly low turbidity in Assean Lake likely promotes greater light penetration and reduced particle-associated disturbance, conditions that favor free-living, oligotrophic bacterial lineages. The comparatively high relative abundance of the hgcI clade (Actinobacteria) under these conditions is consistent with studies describing this group as adapted to low-nutrient, low-turbidity freshwater environments (Neuenschwander et al., 2018; Yaradoddi & Kontro, 2021).

Prairie lakes, including Lake Winnipeg, are well-documented hotspots for cyanobacterial blooms and elevated microcystin concentrations compared to many other Canadian freshwater systems (McKindles et al., 2019; Onana et al., 2025). In contrast, measured microcystin concentrations in Split Lake remained below maximum acceptable guideline values during the study period. This difference likely reflects the distinct hydrological and physicochemical characteristics of Split Lake, including

strong riverine influence, higher turbidity, and reduced water residence times, which can limit bloom development and toxin accumulation. Additionally, although cyanobacterial taxa were present across sites, toxin-producing lineages did not dominate the microbial community, further supporting the observed low microcystin levels.

6.5 Limitations and summary

The present study used only high-throughput 16S rRNA gene sequencing data to explore the microbial community composition in Split Lake. However, an in-depth analysis would be required to understand the community dynamics and functional roles. For instance, these data only cover August 2024; hence, more sampling is required to know how the microbial composition changes between seasons and across years. The sampling primarily focused on the river inflows to Split Lake, with limited data available from the midstream region of the lake. In addition, the potential contribution of particle-associated bacteria cannot be excluded since only surface water samples were collected. Year-round long-term investigations would facilitate identifying the microbial community composition trends and potential ecosystem disruptions, including bloom-forming events, sediment loading, zebra mussel infestation, and contaminant accumulations. Furthermore, metagenomic-level investigations may provide insights into microbial community dynamics, their functional roles, and the underlying genetic mechanisms.

6.6 Conclusion

This study characterized the bacterial community structure of Split Lake, Manitoba, during the summer of 2024 and demonstrated that spatial variability in microbial composition is strongly influenced by environmental and hydrological gradients within the system. Although Actinobacteria, particularly the *hgcI* clade, and *Cyanobium* dominated most sampling sites, the JSI site exhibited a distinctly different microbial profile, with an unusually high relative abundance of *Cyanobium*

compared to all other sites. Hierarchical clustering further revealed six distinct microbial assemblages across Split Lake, highlighting pronounced spatial heterogeneity in community structure. This spatial distribution is likely driven by the unique environmental and hydrological influences in different parts of the lake since Split Lake receives inflows from major rivers, including Burntwood River, Nelson River, Grassy River, and potentially others, each contributing varying concentrations of major ions, sediment loads, and temperature regimes, and shaping the localized environmental conditions for selected microbiota.

Additionally, physical parameters such as flow rate, flow direction, sediment deposition, and light penetration may support niche partitioning among microbial taxa. Therefore, this hydrological complexity, coupled with environmental variables, likely contributes to the spatial diversity of microbiota in Split Lake.

6.7 Data availability

All raw 16S rRNA gene sequence reads generated from this experiment have been deposited at NCBI's Sequence Read Archive (SRA) under the BioProject [PRJNA1416953](#) (accession numbers [SAMN54985744](#) to [SAMN54985759](#)).

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Appendix 6A

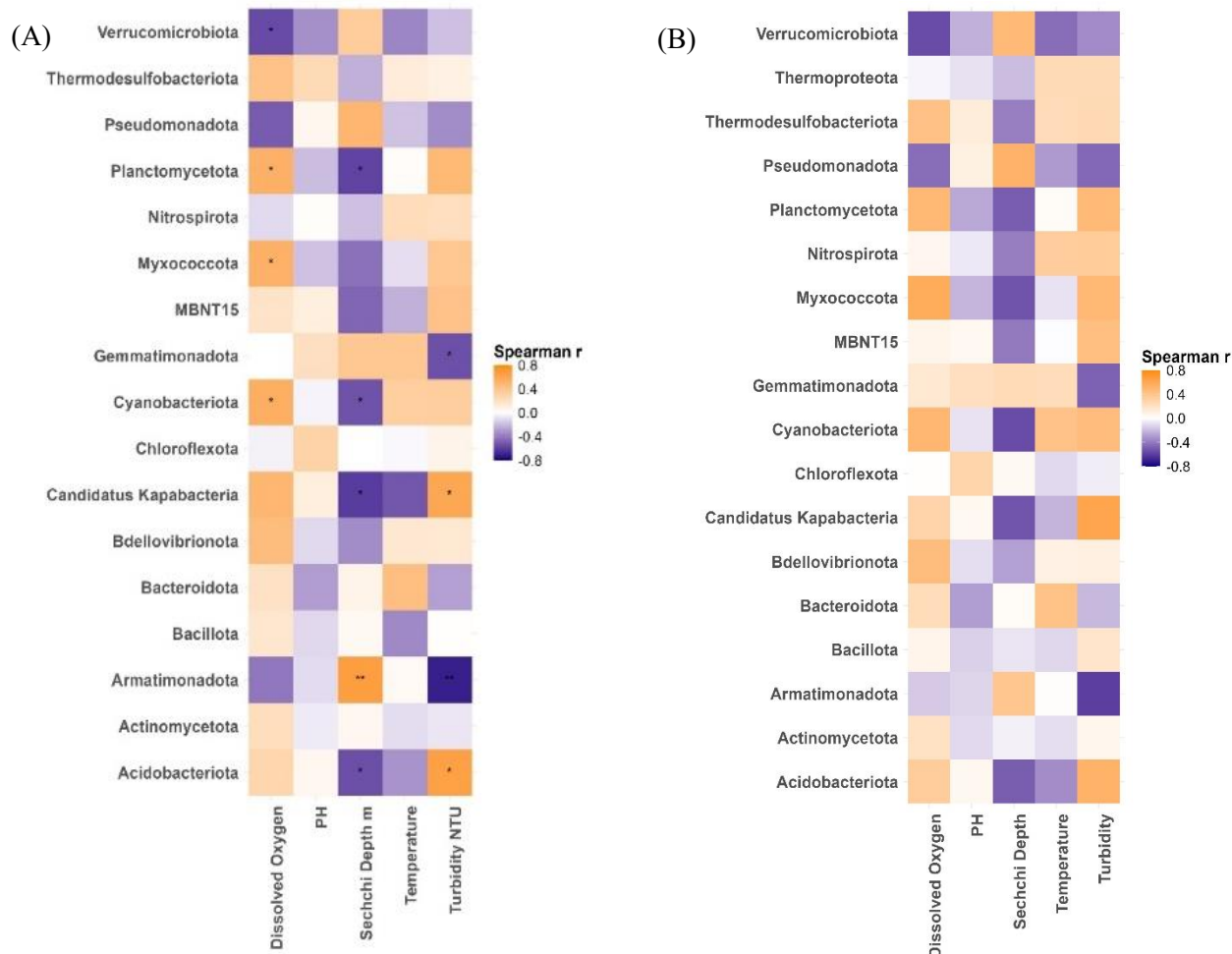


Figure 6.S 1: Pairwise Spearman correlations between bacterial phyla and environmental variables. (A) Raw p-values were used to determine statistical significance. (B) P-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Correlation strength is indicated by color intensity (Spearman's r). Asterisks denote significance thresholds (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Appendix 6B

Table 6.S. 1: Concentrations of the major cations and anions of the water samples (values are given in ppm)

Site ID	Mg ²⁺	Ca ²⁺	Na ⁺	K ⁺	F ⁻	Cl ⁻	SO ₄ ²⁻	NO ₃ ⁻	Cluster color
YLC	11.40	20.31	13.48	1.96	0.11	11.73	28.65	0.34	Red
AK	6.26	18.35	4.72	0.40	0.11	3.82	9.51	0.53	
OR	2.56	14.77	0.74	<DL	0.09	0.24	0.45	<DL	
ALe	5.46	22.12	1.97	0.2	<DL	0.81	1.22	0.58	Yellow
ALw	4.56	20.26	1.62	0.08	<DL	0.67	1.10	0.56	
NB	4.38	15.93	2.96	0.66	0.11	0.93	2.55	<DL	Green
BR	1.58	8.11	1.02	<DL	0.10	0.38	1.35	0.39	
OFCN	5.19	16.46	4.35	0.86	0.10	0.38	1.35	0.38	
SCN	9.38	18.88	10.61	1.69	0.11	8.97	21.93	0.45	Cyan
CL	1.79	10.95	1.80	<DL	0.09	1.34	4.83	<DL	
SLTP	7.79	17.62	8.10	1.34	0.11	5.98	15.06	0.36	
MB	9.40	23.25	9.84	1.52	0.12	7.93	19.27	0.42	
KD	12.25	20.84	15.41	2.25	0.11	13.52	33.32	0.38	Purple
HRB	10.74	20.60	13.12	1.84	0.11	11.39	28.66	0.37	
GR	5.23	12.53	6.28	0.66	0.10	5.35	14.46	0.33	
JSI	3.79	14.70	2.84	0.62	0.09	0.24	0.44	<DL	Magenta

CHAPTER SEVEN: THESIS CONCLUSIONS

7.1 Summary of research findings and their implications

The research presented in this thesis advances our understanding of the fundamental link between ecosystem services and microbial communities. Despite their central ecological roles, microbial contributions to ecosystem adaptation and resilience are often overlooked. As primary responders to environmental disturbances, microorganisms play a critical role in regulating biogeochemical processes, mediating contaminant transformation, and supporting overall ecosystem recovery. Therefore, understanding their adaptability, functional capacity, and metabolic traits is essential for developing effective environmental monitoring and protection strategies.

This study highlights the potential of microbial DNA as a sensitive and reliable proxy for assessing ecosystem health and detecting environmental disruptions, particularly oil contamination. In an increasingly industrialized and resource-driven world, anthropogenic activities continue to exert significant pressure on natural systems, leading to more frequent and pronounced environmental pollution. Within this context, incorporating microbial indicators into environmental assessment frameworks is not only timely but necessary for improving our ability to predict, monitor, and mitigate ecosystem disturbances.

The primary research question addressed in Chapter 2 of this thesis was to determine which biostimulation conditions promote the early enrichment of oil-degrading taxa in seawater under Arctic-relevant conditions. The results clearly demonstrated that the combined application of nutrients and alternative dispersants enhanced the rapid activation and enrichment of specialized hydrocarbon-degrading microorganisms compared to other treatments. This combined biostimulation approach accelerated the rapid MOS formation and early establishment of key oil-degrading taxa, which could potentially support more efficient biodegradation under cold conditions.

Nitrogen-based fertilizers, in particular, have previously been shown to enhance oil degradation rates without causing measurable toxicity (Prince et al., 2003). Further, Lewis and Prince, 2018 argued that dispersants should play a critical role in contingency planning for accidental oil spills in the Arctic Ocean, as they not only enhance the rapid biodegradation of oil but also reduce surface oil slicks. Building on this perspective, the findings of this study provide empirical support for an integrated response strategy. Specifically, our results demonstrate that dispersant–nutrient biostimulation can promote rapid MOS formation and the enrichment of specialized oil-degrading taxa under Arctic-relevant conditions, ultimately influencing the environmental fate of spilled oil through MOS sorption and redistribution within the water column. Overall, these findings strongly support the strategic use of dispersant–nutrient biostimulation approaches in future Arctic oil spill response frameworks.

In addition, oil biodegradation is strongly influenced by substrate type and chemical composition, as evidenced by the distinct bacterial communities observed in response to fresh diesel, UV-weathered diesel, and UV-weathered crude oil. Photooxidation enhances oil dissolution (Snyder et al., 2021) and chemically transforms petroleum hydrocarbons (Bacosa et al., 2015), thereby modifying their bioavailability and toxicity. Consequently, microbial succession during oil degradation is closely linked to photooxidative processes, as shifts in oil chemistry select for microorganisms adapted to distinct hydrocarbon substrates at different stages of degradation.

To further examine the implications of MOS formation, Chapter 3 evaluated overall biodegradation losses and oil partitioning under Arctic-relevant conditions. Although biostimulation did not result in statistically significant biodegradation losses within the six-week incubation period, the observed partitioning of HMW hydrocarbons into the water column suggests that vertical transport processes were active during biodegradation. These results indicate that MOS-mediated vertical transport of

hydrocarbons must be considered in future Arctic oil spill risk assessment frameworks, as the potential impacts on benthic ecosystems cannot be overlooked when evaluating overall spill consequences. Importantly, the findings also demonstrate that Hudson Bay microbial communities possess an inherent capacity to degrade hydrocarbons even in the absence of biostimulation under cold conditions.

Although biodegradation is widely recognized and discussed in the context of oil spills, the underlying functional mechanisms by which microorganisms mediate hydrocarbon degradation remain poorly understood. In particular, biodegradation is often closely associated with biosurfactant production, which enhances hydrocarbon solubility and facilitates microbial access to hydrophobic substrates present in oil. Growing public concern over environmental protection, along with increasingly stringent regulations, especially in Arctic and sub-Arctic regions, has prompted the exploration of more sustainable oil remediation alternatives, such as biosurfactants (Farooq et al., 2024).

In Chapter 4, eight bacterial consortia originating from Marine Oil Snow collected from the 2024 Churchill Marine Observatory (CMO) oil spill experiment were identified as capable of producing biosurfactants, as confirmed through multiple qualitative and quantitative assays.

Although the widespread industrial application of biosurfactants remains constrained by high production costs (Onaizi et al., 2012), these findings provide a foundational basis for the development of environmentally sustainable, biologically driven spill remediation strategies tailored to Arctic oil spill scenarios.

Building upon the identification of biosurfactant-producing consortia within MOS aggregates, metagenomic analysis provided deeper insight into the functional mechanisms underlying this biodegradation potential as outlined in Chapter 5. Specifically, the metagenome of a previously

uncultured, cold-adapted oil-degrading bacterial species, *Paraperlucidibaca* sp. CMO1 revealed its genetic potential in the degradation of both alkanes and aromatic hydrocarbons. Notably, this species also possessed genes associated with surfactant production, highlighting its dual metabolic potential for hydrocarbon degradation and enhanced oil bioavailability within MOS aggregates.

Northern ecosystems, both marine and freshwater, are increasingly shaped by anthropogenic pressures, whether through expanding Arctic maritime activities or large-scale hydrological modifications in boreal watersheds. Hydroelectric development has significantly altered the hydrology, structure, and ecological functioning of many Canadian northern freshwater lakes (Bradford, 2022; Brown et al., 2024). The Churchill River Diversion (CRD), initiated in 1976, caused a seven-fold increase in sediment load in the Burntwood River, contributing to elevated turbidity and enhanced particulate inputs into downstream systems, including Split Lake (Goharrokhi et al., 2022), ultimately affecting the lake's water quality over time. We infer that the varying turbidity levels observed across the lake influenced nutrient availability and oxygen dynamics, thereby reshaping microbial community structure as reflected in the distinct microbial clusters identified, particularly at the major inflow regions. This mosaic of inflowing water masses creates strong spatial gradients within the lake, contributing to physicochemical stratification and localized environmental niches as discussed in Chapter 6.

These findings underscore that hydrological regulation not only reshapes the physical landscape of boreal lakes but also exerts spatial patterns on microbial community structure that are associated with gradients in water chemistry that differ among river inflows. Understanding these microbial responses is therefore critical for assessing long-term ecosystem health in hydroelectrically modified northern freshwater systems.

7.2 Recommendations for future work

Given the microcosm scale of this experiment, several limitations must be acknowledged. These controlled systems do not fully incorporate natural environmental processes such as continuous gas exchange, progressive oil weathering from evaporation and photooxidation, nutrient dynamics, and their cumulative ecological impacts. Therefore, future studies should evaluate the above objectives at larger, more environmentally representative scales where natural conditions are maintained. In particular, investigating individual MOS aggregates for their microbial community composition and HMW hydrocarbons sorption into MOS would provide deeper insight into oil fate.

For strengthened Arctic oil spill preparedness, the contribution of MOS to carbon flux through vertical transport and the chemical alteration of oil during its descent through the water column requires further investigation. Understanding these processes is critical for accurately assessing benthic exposure risks and long-term ecosystem consequences.

As biosurfactants continue to emerge globally as a greener alternative for oil spill remediation (Nikolova and Gutierrez, 2021), the Arctic spill response strategies should consider their potential as safer and more sustainable options. Even though the large-scale production of biosurfactants remains economically challenging, a comprehensive techno-economic and environmental assessment will be essential to determine the practicality and long-term benefits of implementing biosurfactant-based technologies in real-world spill scenarios. In addition to that, individual metabolic capacities of the identified surfactant producers and their biosurfactant production efficiencies under Arctic conditions should be further studied.

Finally, this work highlights the importance of incorporating microbial indicators into freshwater ecosystem monitoring, particularly in systems experiencing significant anthropogenic pressure from

large-scale hydrological modifications. Long-term monitoring of such impacted lakes is essential to evaluate changes in water quality and shifts in microbial community composition over time. Furthermore, incorporating comprehensive microbial surveillance programs within major environmental infrastructure projects would enable more effective impact assessment and support the advancement of sustainable energy development while safeguarding ecosystem health.

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