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HYDROCARBON BIODEGRADATION AND MICROBIAL COMMUNITY COMPOSITION IN FRESHWATER SYSTEMS AND ENRICHMENT CULTURES

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HYDROCARBON BIODEGRADATION AND MICROBIAL COMMUNITY
COMPOSITION IN FRESHWATER SYSTEMS AND ENRICHMENT CULTURES

By

Emily R. Byrne

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

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This thesis has been approved in partial fulfillment of the requirements for the Degree of
MASTER OF SCIENCE in Biological Sciences.

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Preface & Author Contribution Statement

The work presented in this document is original to the best of my knowledge. Chapter two of this document is currently in review at the Journal of Great Lakes Research as a completed manuscript titled “Seasonal Variation of Crude and Refined Oil Biodegradation Rates and Microbial Community Composition in Freshwater Systems.” Contributing authors include Kayley Roche, who assisted with data collection and writing the manuscript, Laura Schaerer, who assisted with data analysis, and Dr. Stephen Techtmann, who obtained funding for the project and served as the principal investigator. Chapter two of this document will be submitted soon to the Journal of Applied Microbiology. Other contributing authors include Simeon Schum, who assisted by generating GC/MS data for quantifying alkene breakdown, and Dr. Stephen Techtmann, who obtained funding for the project and served as the principal investigator.

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I would like to thank all members of the Techtmann lab for their encouragement and help in data analysis for these experiments. I would like to thank my advisor, Stephen Techtmann, for all of the guidance over the past three years and help in planning experiments. Finally, I would like to extend a thank you to my family and friends, in the Michigan Tech community and not, for all of the support over these past few years.

Abstract

In this study, we investigated if significant differences existed seasonally in the microbial response to oil in the Straits of Mackinac, and if crude (Bakken) and refined (non-highway diesel) oil exposure had impacts on microbial community composition and hydrocarbon biodegradation across seasons using a microcosm-level experiment. Ambient microbial communities differed between seasons, with significantly enriched microbial groups present between all sample types except for between fall 23 °C and fall 4 °C microcosms. We found significantly different microbial communities between control samples and oil-amended samples in every season, but no significant community differences between either oil type. We found Amplicon Sequence Variants (ASVs) from the bacterial family Solimonadaceae were significantly enriched in all oil-amended microcosms compared to the control microcosms across seasons. We assessed oil breakdown across seasons and oil types over the course of five weeks through measuring CO₂ production as a proxy variable for hydrocarbon metabolism using GC-FID. We observed a general trend of increasing respiration with oil amendment. No statistically significant differences in daily CO₂ production existed between oil types across seasons or within seasons across oil types. These findings suggest that microbial communities in the Straits of Mackinac shift over the course of seasons even without oil amendment, and that freshwater microbial communities are compositionally and metabolically responsive to the presence of varying oil types.

Given the responsiveness of microbial communities in the environment to both crude and refined oil in our microcosm-level study, we aimed to investigate the feasibility of using microbes from environmental inocula to establish laboratory cultures for breakdown of other hydrocarbon sources such as alkenes. Our study goal was to quantify alkene breakdown in laboratory cultures and monitor changes in the microbial communities to draw associations to which microorganisms may play a significant role in alkene breakdown. We monitored breakdown using various metrics including CO₂ production and found no significant distinctions in CO₂ production across nutrient levels or inocula types. After GC/MS quantification of residual compounds we observed extensive biodegradation of all quantified compounds in the majority of samples. Microbial community diversity analyses throughout our experiment found that cultures initialized with environmental inocula from varying starting microbial assemblages converged to display significant overlap of bacterial families. Significantly enriched families across all inocula types included ASVs from families Xanthomonadaceae, Nocardiaceae, and Beijerinckiaceae. Distinctly enriched ASVs overlapping across treatment types restrained divergence of the overall communities. Overall microbial communities across nutrient levels within inocula types in Caspian Sea sediment and farm compost were significantly different. These results ultimately suggest that the microorganisms necessary to achieve alkene breakdown may be present in a variety of environmental microbial assemblages, and that they are strongly selected for under optimized conditions such as nutrient amendment.

1 Oil Biodegradation Background

The oil industry is a multifaceted network that includes the processes of extracting and transporting oil. As the demand for oil increases, there is a push for increased means of oil transport. The Enbridge Line 5 pipeline that crosses the Straits of Mackinac serves as the primary means of transport for oil in the Great Lakes Region. The waters of the Great Lakes would be susceptible to an oil spill due to the presence of Line 5¹.

Oil in itself is a complex mixture of aliphatic and aromatic compounds of varying lengths and chemical constituents. These compounds are heavily composed of hydrocarbons, or compounds constituted of carbon and hydrogen bonds, with other constituents. Relative proportions of hydrocarbons to other chemical constituents in oil influence its behavior and degradation rate in an ecosystem. Crude oils typically consist of a broad range of aliphatic compounds, naphthenes, and aromatics. Asphaltenes and resins are also found in crude oils. Other possible constituents include compounds containing sulfur, nitrogen, or oxygen. Refined oils such as diesel tend to consist of lower proportions of aromatics. In the event of an oil spill, the chemical and physical properties of these compounds influence how they interact with an ecosystem and influence their remediation in aquatic systems.

1.1 Remediation Methods: Physical, Chemical, and Biological

Remediation of environmental pollutants, including oil, can be achieved in several ways. These are generally separated into physical, chemical, and biological methods. Physical methods encompass skimming, booms, and burning. It is important to consider the temporal aspect of a spill when choosing physical methods of remediation. Soon after a spill occurs, the oil can form a mousse-like substance on the surface when hit with waves and wind or can form clumps and sink in the water column. These processes lower the accessibility of the contaminant in the environment and thus lower remediation efficacy. Depending on the severity of the spill, physical methods of oil removal may not be feasible or cost-efficient.

Chemical means of oil remediation generally involve dispersants that increase the surface area of a spill to form droplets. This means of remediation is generally paired with bioremediation, or microbially-assisted remediation, due to the increase in availability from increased surface area. While dispersants have widespread use in marine environments, for freshwater bodies this method is generally not approved.

There are two primary approaches to addressing an oil spill with bioremediation methods. The first is bioaugmentation, in which the contaminated area is supplemented with oil-degrading microbes. The second is biostimulation, where environmental conditions are altered to create selective pressures on microbial communities. For instance, supplementing nutrients into water bodies to promote microbial growth for bioremediation of an oil spill may stimulate oil biodegradation. These disturbances combined may create a selective advantage for oil-degrading bacteria. In our study, we will investigate the efficiency of bioremediation on oil in the Great Lakes region by amending water samples with various oil types.

1.2 Constraining Environmental Factors for Bioremediation

Figure 1.1. A conceptual model of oil biodegradation from Hazen et al. 2016⁴.

Environmental conditions play a significant role in microbe-mediated remediation of oil. Temperature, ambient nutrients, oxygen availability, and salinity all contribute to

microbial community selection and activity (Figure 1.1). Oil biodegradation has been shown to be limited under anaerobic conditions in marine environments by some studies². However, hydrocarbons constitute a diverse class of compounds that may not all be biodegradable under the same set of conditions.

Oil spills supply a large flux of carbon sources into aquatic environments. Because of this sudden increase in the carbon supply, other nutrients required in smaller amounts by microorganisms such as nitrogen and phosphorus subsequently become the limiting factor⁵⁻⁶. Nutrient addition and stabilization are the most effective methods of stimulating bioremediation of oil spills. Utilizing nutrient addition as a strategy for stimulating bioremediation presents challenges in open bodies of water because it is difficult to maintain adequate nutrient concentrations that meet the area of the spill and the prevalent oil-degrading microbial communities. Once sufficient nutrient concentrations are achieved in oil-contaminated environments, the nutrients must dissolve into the aqueous phase to be made available for microbial communities⁷. In some environments, this makes bioremediation challenging to achieve. Oligotrophic environments, which are rich in oxygen and nutrient-poor produce challenges for achieving efficient oil biodegradation as a result.

For marine environments, the Redfield ratio is used to approximate the amounts of carbon, nitrogen, and phosphorous relative to one another. Carbon to nitrogen to phosphorous are estimated to be found in a 106:16:1 in marine phytoplankton. In freshwater environments, nutrient levels may vary more than marine environments. Anthropogenic disturbances such as the release of wastewater effluent or industrial waste may influence the flux of nutrients in watersheds. Nutrient levels induce selective pressure on the microbial communities and serve as limiting factors for microbial growth. Understanding the limiting in-situ factors on biodegradation is critical for making targeted ecosystem restoration decisions.

Current Gaps in Knowledge Regarding Freshwater Bioremediation of Oil

A significant amount of oil degradation studies were conducted in marine settings after the catastrophic Deepwater Horizon spill in 2010. Historically, oil biodegradation

has also been extensively studied in groundwater (Bemidji MN site). Currently, there is less information available about bioremediation in freshwater environments. Based on previous findings, we know that microbial community composition is subject to influence by *in-situ* environmental conditions and that distinct microbial communities arise in the presence of different oil types. However, quantifying the metabolic activity of these communities is crucial to assessing the extent of bioremediation and to determine optimal conditions for oil biodegradation. Currently, there are no studies published to our knowledge that characterize biodegradation rates of a diverse range of oil types in the Great Lakes region. Additionally, there is a lack of knowledge regarding seasonal shifts in microbial community composition in the Great Lakes arising from changing environmental conditions. Our study here aims to fill a critical need for determining biodegradation rates in the Great Lakes region using the ambient microbial communities present in water samples at various points during the year.

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2. Bioremediation of Oil in the Straits of Mackinac Freshwater System

The Great Lakes are the largest freshwater body on earth, accounting for one fifth of the global surface freshwater. As the Great Lakes are in proximity to numerous oil extraction sites and are a widespread avenue for the transport of oil, the risk of contamination is high. The waters of Lake Huron and Michigan in particular are susceptible to accidental hydrocarbon contamination from the Enbridge Line 5 pipeline that crosses the Straits of Mackinac¹. Physical methods of oil removal are time consuming, costly, and potentially ineffective in large bodies of water. Additionally, chemical dispersants, commonly used in marine environments, pose other hazardous risks for freshwater environments. Therefore, bioremediation has been increasingly considered an attractive remediation method for oil spills. Past research indicates that microbial hydrocarbon biodegradation plays a significant role in the elimination of oil contaminants in the environment²⁻⁵. Microbial communities are interacting assemblages that are responsive to environmental and anthropogenic pressures such as oil spills, nutrient runoff, and temperate climate shifts. An understanding of the selection pressures *in-situ* environmental factors such as nutrients and microbial community composition have on biodegradation rates in marine environments has been established⁶⁻⁸. However, a gap in knowledge exists surrounding the selection pressures of seasonal factors on freshwater microbial communities, as well as in studying hydrocarbon biodegradation rates in freshwater environments. Oil biodegradation is in part affected by the environmental conditions as well as the microbial community that is present at that time. Environmental conditions in the Great Lakes and associated estuaries exhibit dramatic differences between seasons. These alterations may involve both seasonal changes in temperature as well as ambient nutrient availability^{9,10}. Additionally, these changes in environmental conditions may also select for distinct microbial communities that may have different biodegradation capabilities. In the Straits of Mackinac in particular, it is unclear as to whether microbial community response will vary amongst exposure to an influx of diverse hydrocarbon sources transported by Line 5. Different oil types are known to

contain varying proportions of alkanes and aromatics and different size ranges within these broad hydrocarbon classes. These distinctions in the complexity and type of released hydrocarbons may result in a differential response to the released oil by microbial communities. There is a critical need to understand how microbial communities in the Straits of Mackinac respond to various oil types in the event of a spill. Additionally, developing a quantitative understanding of the various impacts environmental factors have on hydrocarbon bioremediation is essential for modeling potential oil spill implications, as well as to develop a broader understanding of how naturally fluctuating environmental factors may shape microbial community formation across seasons.

In this study, we compared the microbial communities and oil biodegradation rates in Straits of Mackinac water samples representing fall (fall water samples incubated at either 23°C or 4°C), spring, and summer starting microbial assemblages. The goal of our research was to quantify hydrocarbon biodegradation rates in the Straits of Mackinac by using CO₂ production as a measure of microbial respiration and presumed biodegradation of two oil types, as well as to characterize the microbial communities using 16S rRNA sequencing to further understand how community assemblage dynamics in the Straits of Mackinac are impacted with and without the presence of oil and by environmental factors such as temperature. Given the tendency for microbial communities to be dynamic in response to environmental selection pressures, we hypothesize that statistically significant changes in community composition will exist between oil-amended and control groups across seasons, with a group of significantly enriched ASVs (amplicon sequence variants) driving differences in community assemblages over time. We also hypothesize that oil biodegradation rates will vary significantly across seasons and oil types.

2.1 Materials and Methods

Microcosm Assembly

The water samples used in this study were collected from the Straits of Mackinac in November of 2018, and in May and July of 2019. Samples were collected at the surface in a clean bucket adjacent to the Michigan Technological University monitoring buoy (Great Lakes Observing System Buoy 45175) (45.82526 N, 84.77217 W). Mean ambient temperatures of the water near each sampling period were as follows: 12.4 ± 1.9 °C during fall (late October 2018), 6.2 ± 1.4 °C during spring (May 2019), and 19.0 ± 1.1 °C during summer (July 2019). Water samples for microcosms were not pre-filtered to eliminate protists or other eukaryotic grazers. The microcosms were assembled in triplicate, as follows: each set contained 100 mL of water from the Straits and was enriched with 2.5 microliters of either refined oil (non-highway diesel) or crude oil (Bakken). These microcosms were prepared in 150 mL sealed serum bottles. All bottles were sealed with a butyl rubber stopper. A control set was also assembled, of which microcosms were not amended with oil. All bottles were incubated in the dark at 23 °C without shaking for five weeks. This procedure was repeated for each season shortly after initial water collection. Because winter water samples were not collected due to logistical difficulty caused by ice cover, microcosms with water collected in November were incubated at 4 °C to simulate the impact of lower temperatures on microbial community succession and biodegradation rates. One triplicate set of microcosms was used to determine biodegradation rates by measuring CO₂ produced once per week for the five-week experiment. A duplicate set of microcosms for microbial community analysis were sacrificially collected each week for five weeks. Sets of microcosm water were vacuum filtered on 0.2 µm pore size Sterlitech polyethersulfone filters and stored in -80 °C until processing. Half of the filter was used for DNA extraction, and the other half frozen at -80 °C as an archive. Sample DNA was extracted using a ZymoBIOMICS DNA microprep kit (Zymo Research Corporation, Irvine, CA) following manufacturers specifications

with the exception of homogenizing samples for 200 seconds at 5.5 m/s in a FastPrep 5G (MP Biomedicals). Extracted DNA was then prepared for high-throughput sequencing for construction of 16S rRNA gene libraries using a modified version of the Illumina 16S rRNA library preparation protocol. Briefly, the 16S rRNA gene was amplified using the 515YF and 926R primers¹¹. Sequencing adaptors and sequencing indices were added through a second eight-cycle PCR step. The libraries were purified, and concentrations of each library pool were determined using the PicoGreen dsDNA quantification assay (Thermo Scientific). Blank samples containing no added biomass were processed using the same protocol to monitor for possible contamination. The libraries were then pooled to give a single 4 nM pool for sequencing. Fall 23°C, fall 4°C, and summer samples were sequenced in one run using a v3 500-cycle kit. Spring samples were sequenced in a separate run using a v3 600-cycle Illumina kit. All sequencing was performed using Michigan Technological University's MiSeq instrument.

16S rRNA Reads Analysis

Using Rstudio¹², the package DADA2 (divisive amplicon denoising algorithm) was utilized to sort and trim sequencing reads¹³. Our criteria for trimming sequencing reads was to eliminate reads assigned a quality score less than 30. The forward reads from fall and summer samples sequences from the first run were trimmed at 280 cycles, and the reverse reads at 200 cycles. The spring samples sequenced in a separate run were trimmed at 280 cycles for forward and reverse reads. Both runs were denoised separately, forward and reverse reads were merged, and chimeras were identified. ASV tables from both runs were merged into one phyloseq object. A taxa table was constructed with the package "phyloseq"¹⁴ using taxonomical assignments from the SILVA v132 data set. Chloroplast and mitochondria sequences were filtered out of the dataset.

The "estimate_richness" function in phyloseq was used to determine alpha diversity, the number of ASVs and Shannon's index within the taxa table. Principal coordinate analysis (PCoA) was completed using the "phyloseq" package to visualize microbial community shifts across variables using the Bray-Curtis dissimilarity matrix

using a rarified taxa table normalized to a depth of 1000 reads. PERMANOVA analysis was used to determine if significant differences existed between microbial community composition using the *adonis2* package¹⁵. PERMANOVA was also used to determine the variation explained by the difference in microbial community structure between treatment types and seasons in the PCoA.

In order to test our hypotheses that significantly enriched ASVs would drive differences in community assembly across treatments, the package “DESeq2” was used to conduct differential abundance analyses between sample variables with an $\alpha = 0.01$. ASVs designated a log₂-fold difference >2 or <-2 were considered to be significantly enriched to a variable, whereas all other ASVs were considered non-significantly enriched.

Biodegradation Rate Determination

Carbon dioxide production as a measure of microbial respiration and hydrocarbon biodegradation was assessed in microcosm headspace samples over time using GC-FID (gas chromatography with flame ionization detection). A 1 mL sample was drawn from the headspace of each microcosm using a syringe and was replaced with 1 mL of room air. Once per week for five consecutive weeks, a 1 mL air sample was taken from the headspace of each microcosm and analyzed using GC-FID with a HayeSep D column to quantify carbon dioxide production. A linear calibration curve relating GC-FID CO₂ peak area values to CO₂ volume was created using known volumes of a standard mixture of gases with 1% CO₂, 1% CO, 1% H₂, 1% CH₄, 1% O₂, balanced with nitrogen. Percent CO₂ values were converted to total masses (mg) for determination of cumulative CO₂ production using the ideal gas law. Triplicate 1 mL ambient air samples were run to determine the approximate amount of CO₂ being injected back into the microcosms to allow for more accurate calculation of respiration rates. Daily CO₂ production rates were determined by subtracting starting CO₂ mass from the final CO₂ mass and dividing by the number of incubation days for each microcosm. To determine how much respiration was contributed to oil biodegradation, the mean control CO₂ production in milligrams from

the final time point was subtracted from Bakken and diesel-enriched samples before reporting the total output mass of CO₂ in a separate calculation.

To test our hypotheses that biodegradation rates varied significantly by season, we used the “pwr” package in R¹⁶ to determine if sample size between each season and oil type met the assumptions for an ANOVA test. We found that we did not have sufficient statistical power to do so with a significance level of 0.05 and power value of 0.8. Hence, we utilized the non-parametric Kruskal-Wallis test to assess for statistical differences in biodegradation amongst oil types and seasons in base R. The package “FSA” was used to conduct a post-hoc Dunn test to determine between which samples a significant difference occurred¹⁷.

2.2 Results

Alpha Diversity

To assess the impact of oil amendment on microbial diversity, we compared alpha diversity values represented as ASV richness between Bakken and diesel-enriched samples, as well as the control group (Fig A1). Across all sample types we observed 12525 distinct taxa. The highest median ASV richness (approximately 125 ASVs) was observed in spring diesel samples. The lowest median ASV richness (approximately 50 ASVs) was observed in spring control samples. Across fall 23°C samples, the control group contained the highest median ASV richness. Diesel-amended samples were observed to have the highest ASV richness in fall 4°C and spring samples. Bakken-amended samples were observed to have the highest ASV richness in the summer microcosms.

Seasonal Microbial Diversity

To visualize microbial diversity across all seasons and oil types, we used a taxa plot to visualize the spread of bacterial classes in our samples. We observed 14 total bacterial classes across all sample types. Gammaproteobacteria was highly abundant

across both oil treatments, and in the control treatment during the summer season (Fig. 2.1). The fall 23°C and fall 4°C samples appeared to display a trend of increasing abundance of Actinobacteria, whereas spring and summer samples displayed a general trend of increasing Bacteroidia relative to both fall sample types. All sample types contained abundant bacteria in the class Alphaproteobacteria, with the highest community proportion observed in spring control samples. Bakken and diesel-enriched samples did not appear to diverge significantly within each season regarding their overall bacterial taxa diversity (Fig. 2.1).

Figure 2.1. A taxa plot depicting microbial community composition in water samples collected from 2018-2019 from the Straits of Mackinac across seasons amended with one of two oil types: crude Bakken or non-highway diesel. Taxonomic assignments were based on sequencing of the V4-V5 16S rRNA gene. Trends of decreasing Actinobacteria from fall to summer were observed, and an increase of Gammaproteobacteria was observed over the course of sampling in summer samples relative to fall and spring.

We constructed a principal coordinate analysis (PCoA) plot to display trends in microbial community composition (Fig 2.2). Doing so allowed us to visualize if microbial community composition was distinct between seasons and treatments. Overall, seasonal microbial communities appeared to cluster apart from one another, except for both fall sample types, which were clustered together despite varying incubation temperatures. Closer clusters indicate increased similarity between 16S rRNA sequences in our samples. These results suggest that *in-situ* environmental conditions across seasons posed selection on the starting sample microbial assemblages used in this study, and that those distinctions are maintained despite incubations at temperatures not observed *in-situ*.

To test our hypothesis that microbial community composition varied significantly across seasons even without the presence of oil, we conducted a PERMANOVA test between the control groups in each season relative to one another (Table 1) and used the “adonis2” R package to determine how much of the variation was explained between samples in the PCoA plot. We observed statistically significant differences between the microbial communities of all seasons ($p\text{-value} < 0.001$), except in the case of fall 23°C and fall 4°C samples compared to one another ($p\text{-value} = 0.48$). Low amounts of variation were explained for each comparison. The highest amount of variation was explained for differences between fall 23°C and summer samples (19.78%). The lowest variation was explained by the PCoA for differences between fall 23 °C and fall 4°C samples (4.41%).

Figure 2.2. A Principal Coordinate Analysis (PCoA) plot depicting dissimilarities between the ASV composition of 16S rRNA sequences in water column samples enriched with Bakken crude oil, diesel, or control sets across seasons from the Straits of Mackinac. Distinct clustering indicates closer similarity between the ASVs present in samples. Clustering between seasons is observed for fall, spring, and summer. Fall 23 °C and fall 4°C samples appear to cluster despite varying incubation temperatures.

Table 1. A summary of PERMANOVA results depicting R^2 values between the microbial communities of the control group in each season. The F-model values are shown in parentheses for each comparison. The p-value depicting the probability of $>F$ for each comparison was <0.001 , except between control groups in fall 23°C and fall 4°C samples, which was 0.48. Statistically significant p-values indicate differences exist between the microbial communities.

	fall 23°C	fall 4°C	spring	summer
fall 23°C		0.04414 (0.97)	0.19451 (4.11)	0.19492 (6.30)
fall 4°C			0.17893 (4.36)	0.19785 (7.15)
spring				0.15698 (4.65)
summer				

In analyzing microbial community composition across seasons and treatments, we quantified the amount of significantly enriched ASVs present in certain sample types relative to others using differential abundance analysis (Table 3). We did not find any significantly enriched ASVs between fall 23°C and fall 4°C samples. We found significantly enriched ASVs in fall control samples compared to spring controls (27), in summer controls versus fall 23 °Ccontrols (66), in fall 23°C controls versus summer controls (5), in fall 4°C controls versus spring controls (20), in summer versus fall 4°C controls (102), in fall 4°C versus summer controls (2), and in summer controls relative to spring controls (57).

Oil-Enriched Microbial Communities

We conducted PERMANOVA comparisons between oil-enriched samples across seasons to test for significant differences (Table 2). Significant differences in microbial community dissimilarity existed between the control group and both oil types in all seasons (p-value <0.01), whereas no significant differences were found between samples enriched with Bakken or diesel when compared to each other in any season. Effect size tests conducted with the “adonis2” package revealed that the most variation was explained between spring control and diesel-enriched samples (18.58%) and the lowest amount of variation was explained between summer Bakken and diesel-enriched samples (3.16%) (Table 2).

Table 2. A summary of PERMANOVA results depicting R^2 values between the microbial communities of both oil types (Bakken crude and non-highway diesel) and the control group across seasons and incubation temperatures. The F-model values are shown in parentheses for each comparison. Comparisons with asterisk indicate the probability of generating a p-value $>F$ for each comparison was <0.01 .

fall 23°C	control	Bakken	diesel
control		0.07964 (1.73) *	0.07431 (1.20) *
Bakken			0.05341 (0.96)
diesel			
fall 4°C	control	Bakken	diesel
control		0.07879 (2.14) *	0.09447 (2.40) *
Bakken			0.03185 (0.79)
diesel			
spring	control	Bakken	diesel
control		0.1317 (2.43) *	0.18578 (3.88) *
Bakken			0.04597 (0.82)
diesel			
summer	control	Bakken	diesel
control		0.09179 (3.34) *	0.07456 (2.82) *
Bakken			0.03161 (0.11)
diesel			

In order to observe how microbial communities in treated and control samples responded over time, we plotted the second axis of the PCoA as a function of time (Fig A2). Fall 23°C samples appeared to begin clustered tightly together at week zero and become more distinctively clustered by treatment over time. Fall 4°C and spring samples appeared to diverge significantly two weeks after incubation. Summer samples appear to cluster according to treatment in week one, and cluster more closely together by the fifth week of the experiment.

Differential abundance analyses between 16S rRNA sequences across seasons and oil types were conducted using DESeq2 with an $\alpha = 0.01$. Complete results can be viewed in the electronic supplemental material. We found 1 significantly enriched ASV present in fall 23 °C control samples when compared to fall Bakken samples, and 0 ASVs enriched in fall 23 °C diesel samples relative to the control. In the fall 4°C group, we found 7 ASVs enriched in Bakken samples relative to the control, and 10 ASVs enriched in diesel samples relative to the control. No ASVs were enriched in spring Bakken samples relative to the control, and 41 ASVs were enriched in the spring diesel relative to the control. We found 14 ASVs enriched in summer Bakken samples relative to the control, and 1 ASV enriched in diesel samples. No significantly enriched ASVs were present between oil types within seasons except for 3 ASVs enriched in summer diesel samples relative to summer Bakken samples.

The taxonomy of significantly enriched ASVs within both oil types across seasons relative to their respective control group is displayed in Table 3. We observed seven bacterial families across all sample types. The spring oil-amended samples had three unique significantly enriched bacterial families: Rubinisphaeraceae, Sporichthyaceae and Chitinophagaceae. Fall 4°C held one unique enriched family (Moraxellaceae). Fall 4°C and spring shared ASVs in Burkholderiaceae, and spring and summer shared ASVs within Verrucomicrobiaceae. Bacteria from the families Burkholderiaceae and Solimonadaceae were enriched across all seasons, with a total of

56 significant ASVs observed across both groups. No significantly enriched ASVs were present in oil-amended samples relative to the control group in fall 23°C category.

Table 3. A summary depicting the taxonomy of significantly enriched bacterial ASVs within both oil types across seasons relative to their respective control group. The spring oil-amended samples had three unique significantly enriched bacterial families: Rubinisphaeraceae, Sporichthyaceae and Chitinophagaceae. Fall 4°C held one unique enriched family (Moraxellaceae). Fall 4°C and spring shared Burkholderiaceae, and spring and summer shared Verrucomicrobiaceae. Burkholderiaceae and Solimonadaceae were enriched across all seasons. No significantly enriched ASVs were present in oil-amended samples relative to the control group in fall 23°C category. The presence of significantly enriched ASVs in oil-amended samples relative to the control group may indicate the presence of robust microorganisms that would persist in the event of an oil spill.

Seasons Enriched In: (number of ASVs)	Phylum	Class	Family	Genus
spring (13)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Janthinobacterium
spring(9)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	NA
spring(7) , summer (3), fall 4°C (15)	Proteobacteria	Gammaproteobacteria	Solimonadaceae	Nevskia
spring(2) , fall 4°C (1)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Limnohabitans
summer (4)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Acidovorax
summer (1)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Tibeticola

summer (1)	Proteobacteria	Gammaproteobacteria	Burkholderiaceae	NA
fall 4°C (1)	Proteobacteria	Gammaproteobacteria	Moraxellaceae	Perluclidibacteria
spring (2), summer (1)	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiae	Prostheco bacter
spring (4)	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI clade
spring (2)	Bacteroidetes	Bacteroidia	Chitinophagaceae	Sediminibacterium
spring (1)	Planctomycetes	Planctomycetacia	Rubinisphaeraceae	NA

To visualize the response of potential key players for hydrocarbon biodegradation in the Straits, we plotted the relative abundance of the most significantly enriched ASV for each oil type in each season relative to the control group (Fig. 2.3). In spring diesel samples, we observed the greatest prevalence of ASV 4, with a peak relative abundance two weeks after oil amendment. ASV 4 was also observed in spring Bakken samples, with peak relative abundance three weeks after oil amendment. ASV 362 was most abundantly enriched in summer Bakken samples, with the greatest mean relative abundance observed five weeks after oil amendment. In summer samples, ASV 251 was most significantly enriched, with peak mean relative abundance four weeks after amendment in diesel samples and was also observed to reach peak relative abundance after two weeks in Bakken samples. ASV 21 was the most significantly enriched ASV for both Bakken and diesel samples in the fall 4°C group, both with peak relative abundance in week four.

Figure 2.3. Mean relative abundances of the most significantly enriched ASV for each oil type in each season relative to the control group plotted over time. ASV 4, enriched in spring diesel samples, belongs to the hgcl clade in the family Sporichthyaceae. ASV 362, enriched in summer Bakken samples, belongs to an unclassified genus in the Burkholderaceae family. ASV 251, enriched in summer diesel samples, belonged to the Prosthecobacter genus in the Verrucomicrobiaceae family. ASV 21, enriched in both fall 4°C Bakken and diesel samples, belonged to the Nevskia genus in the family Solimonadaceae.

Quantifying Biodegradation

Here we used CO₂ production as a proxy metric for assessing oil biodegradation. Weekly CO₂ production observations were calculated for each microcosm. After the course of five weeks, the lowest mass of CO₂ produced ($0.086 \text{ mg} \pm 0.003 \text{ mg}$) was observed in control fall 4°C samples (Fig. 2.4). The highest final mass of CO₂ produced at five weeks was observed in summer diesel-enriched samples ($0.348 \text{ mg} \pm 0.237 \text{ mg}$). To quantify differences in oil breakdown across seasons enriched with Bakken crude versus diesel, we performed a Kruskal-Wallis test. To identify between which samples a

significant difference occurred, we applied a Dunn *post hoc* test. We identified no statistically significant differences in daily CO₂ production between Bakken and diesel-enriched microcosms, or between oil-enriched samples and our control set (Table S1). Despite a lack of statistical significance, we observed a gradual trend of increasing carbon dioxide production in oil-enriched microcosms relative to the control set, indicating that the microbial communities were somewhat metabolically responsive to the addition of oil.

Figure 2.4. Mean CO₂ production values (mg) measured with GC-FID for each week over the course of five weeks in microcosm headspace for Straits water column samples enriched with 0.25 uL of crude Bakken, non-highway diesel, or a control group across seasons.

A Kruskal-Wallis test was applied to identify any significant differences in daily CO₂ production amongst seasons and oil types. There were no statistically significant differences between oil types within each season, or between oil types across each season (Table S1). In most cases the control microcosms showed low levels of CO₂ production

(ranging from 0.086 ± 0.003 mg after five weeks to 0.107 ± 0.095 mg after five weeks). Respiration levels were typically higher in the oil-amended microcosms. We also reported total CO₂ production with the mean total control CO₂ production subtracted from each season and oil type total (Fig. 2.5). We observed values ranging from 0.45 mg in summer diesel samples to -0.04 mg in spring Bakken samples. Since these masses are reported as values exceeding non-oil-enriched samples, it is likely that the CO₂ is more highly associated with hydrocarbon metabolism.

Figure 2.5. Mean mass of carbon dioxide produced in oil-enriched microcosms across seasons and incubation temperatures. Note: The mean total CO₂ production (mg) from control samples from each season was subtracted from these totals to observe respiration activity more likely associated with oil biodegradation.

2.3 Discussion

Given that fall 4°C CO₂ production decreased relative to fall 23°C despite consisting of the same starting environmental samples incubated at different temperatures (with no significant differences in microbial community composition), we infer that temperature in itself is an important controlling factor for rates of oil biodegradation. In the fall 23 °C control microcosms at 4°C, samples had exceptionally low respiration levels at nearly zero milligrams, which may indicate that microbial respiration is restrained in environments with lower temperatures. More work is needed to fully understand the impacts of temperature on biodegradation in the Great Lakes, as our experiment only accounts for differences observed by changing incubation temperature for fall samples. In the Straits of Mackinac environment, seasonally fluctuating temperature of the water column will likely play a significant role in oil biodegradation rates.

Furthermore, no significant shifts in microbial taxa amongst changing environmental conditions may imply that hydrocarbon-degrading communities in freshwater systems are robust. The lack of significantly differing microbial communities between Bakken and diesel-amended samples in seasons may suggest that microbial communities in the Straits of Mackinac are able to respond similarly to an influx of varying oil types in the event of an oil spill.

In general, microbial diversity in the marine environment tends to decrease after an oil spill when key species of hydrocarbon-degraders are selected for resulting in a bloom of oil-utilizing bacteria¹⁸⁻¹⁹. The addition of oil did not appear to result in decreased ASV richness relative to the control set (Fig A1), indicating that the addition of oil may not necessarily have strong selection pressure for certain taxa in this environment. It is also possible that other factors such as nutrient limitation and environmental constraints could control the extent of bacterial bloom in response to oil. Gammaproteobacteria are one of the primary classes of bacteria known to play a role in marine hydrocarbon biodegradation²⁰. Additionally, members of the Gammaproteobacteria class were shown to bloom in response to diluted bitumen

amendment to freshwater enriched oil-utilizing consortia²¹. Here we observe consistent phenomena, with Gammaproteobacteria being the most abundant class of bacteria across all oil-enriched microcosms (Fig. 2.1). Fall 23°C and fall 4°C samples displayed a trend of increasing Actinobacteria relative to spring and summer. Previous studies also note the ecological role of Actinobacteria in colder environments²².

We hypothesized that distinct seasonal shifts in the Straits microbiome would be present even without the presence of oil. In past studies, seasonal shifts between winter and summer seasons were seen in a permafrost thaw lake²³, and consistent seasonal diversity patterns in a eutrophic lake correlated with niche environmental conditions²⁴. In this freshwater system, we also observed distinct microbial communities across seasons in the Straits of Mackinac between control groups in fall 23°C, spring, and summer samples (Table 2.1). We observed seven bacterial families that were significantly enriched across seasonal oil-amended samples (Table 2.3). Families unique to one season (Moraxellaceae in the fall 4°C samples, or Sporichthyaceae, Chitinophagaceae, and Rubinisphaeraceae in the spring) may be indicative of selective pressures in changing environmental conditions. Conversely, bacterial families enriched in every season in oil-amended samples (Solimonadaceae) may indicate higher tolerance to varying seasonal conditions and carbon sources. Species within this family are known hydrocarbon-degrading organisms²⁵.

We also hypothesized that a group of significantly enriched ASVs would drive differences in microbial community assembly and respond to oil influxes over time across seasons. We plotted the relative abundance of the most significantly enriched ASVs for each oil type across each season (Fig. 2.3). The peak mean relative abundances for these ASVs ranged from two to five weeks after incubation. This may suggest that hydrocarbon-degrading members of the microbial community can thrive relatively soon after a potential spill. We observed that some of the most significantly enriched ASVs only showed significant increases in relative abundance in samples amended with one oil type, such as ASV 362 enriched in summer Bakken samples. This could indicate that some members of the microbial community are selected for more heavily with exposure

to certain oil types, despite no significant changes in the overall communities. Our results also indicate that certain ASVs such as ASV 21, belonging to the *Nevskia* genus within the Solimonadaceae family, may thrive in abundance to a range of oil types in the event of a spill. ASVs from bacterial families significantly enriched in oil-amended microcosms could potentially be key seasonal players in hydrocarbon biodegradation.

Although we did not take measurements to quantify certain environmental factors such as ambient nutrient levels, past studies have indicated that snowmelt in the spring season generally impacts freshwater systems by releasing an influx of nitrogen and phosphorus²⁶⁻²⁷. Influxes of these limiting nutrients may result in microbial community shifts and differences in growth rates. Additionally, nutrient limitation over time in freshwater environments may pose challenges for the biodegradation of more recalcitrant hydrocarbon compounds. In this experiment, nutrient limitation over the course of five weeks was not considered in microcosms. Microbial communities in the Straits of Mackinac may experience nutrient limitation that could impact biodegradation. In future experiments, monitoring microbes within the community across fluctuating environmental factors such as nutrient amendment could provide more insight to diverse microbial metabolism responses.

In the present study, CO₂ production was measured as a proxy for biodegradation. CO₂ production only measures hydrocarbons that are mineralized to CO₂ but does not account for oil that is assimilated into biomass or is transformed into hydrocarbon daughter products. Therefore, biodegradation rates calculated based on CO₂ could be underestimated without accounting for assimilatory metabolism. Further assessment to determine the extent that hydrocarbon compounds are assimilated into cell biomass in freshwater systems is necessary for accurately monitoring oil bioremediation. Furthermore, other factors that could potentially impact the microbial community include spring blooms of eukaryotic algae that are not captured by our 16S rRNA sequencing results. Additional work is needed to understand the broad diversity and interactions of microorganisms that play roles in freshwater hydrocarbon biodegradation across seasonal conditions.

2.4 Conclusions

In this study we observed significantly different microbial community composition between all seasonal samples except between fall 23°C and fall 4°C. In regard to oil-amended microbial community composition, significant differences in ASV composition were present in Bakken and diesel-enriched samples when each individually compared to control samples, but only summer samples possessed significantly enriched ASVs (3) in diesel-amended microcosms relative to Bakken. Bacteria from the family *Solimondaceae* were significantly enriched in samples amended with both oil types in every season. We observed gradual increasing trends of respiration in oil-enriched microcosms, indicating that the microbial community was marginally responsive to the addition of oil. Oil type held less influence over breakdown rates, with no statistically significant differences in oil breakdown occurring between control and diesel-enriched samples, as well as between diesel and Bakken-enriched samples. However, diesel-enriched microcosms displayed a consistent trend of increased respiration rate and extent of respiration relative to Bakken-enriched and control microcosms. Non-significant differences reflected in breakdown rates across oil types may suggest that microbial communities may have similar responses to a diverse range of hydrocarbon sources in the event of a spill. We observed no significant differences present between the oil breakdown rates for each season, which may indicate that microbially-mediated hydrocarbon degradation in freshwater systems does not necessarily require a specialized community. Further work is needed to determine which specific *in-situ* factors hold deterministic influence over community formation and response to oil spills in freshwater systems.

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3. Impacts of Nutrients on Alkene Biodegradation Rates and Microbial Community Composition in Enriched Consortia from Natural Inocula

Introduction

Oil contamination in the environment provides an ample carbon source for microbial communities in a wide range of environments. Bacteria have been known to metabolize hydrocarbon compounds in both marine and freshwater aquatic systems, as well as in sediments¹⁻⁴. Over 175 bacterial genera have been classified as oil-degraders in a variety of conditions⁵, and therefore are widespread across environments. This ubiquitous nature of these bacteria in the environment is likely attributed to the presence of both aerobic and anaerobic oil-utilizing metabolisms in microbial communities^{6,7}. Hydrocarbons are also naturally produced by other bacteria and algae and may serve as a natural substrate for hydrocarbon metabolizing bacteria⁸⁻⁹. In the event of an oil spill, oil-degrading microbial taxa rapidly bloom to outcompete other microbes, dominating as much as 90% of the community within 72 hours¹⁰. Hydrocarbon contamination in the environment typically consists of a mixture of a broad range of compounds including alkanes, alkenes, and aromatics of varying sizes and complexities. The biodegradation of alkanes is well-characterized in aquatic systems and soils both in regards to rate determination and associated microbial composition¹¹⁻¹⁵. However, the community-wide roles of microorganisms in the biodegradation of alkene hydrocarbon compounds is less understood. Biodegradation of alkene compounds in particular holds significance for not only the role of alkenes as environmental contaminants, but may also be analogous to the processing of solid industrial waste.

The use of polyethylene plastics in industrial applications has surged by over 600% since the 1970's¹⁶. Globally, over 350 million tons of plastic is produced annually, and the majority of plastics are not recycled but accumulate in landfills or aquatic environments^{17,18}. In 2015 most manufactured plastic and plastic waste produced consisted of polyolefin plastics such as low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polypropylene (PP)¹⁷.

Figure 3.1. A graphic depicting global plastic production from (Geyer, Jambeck, and Law 2017).

High-density polyethylene in particular is a popular plastic that is sturdy and used commonly in piping and water bottles. The structural complexity of polyethylene plastics makes them recalcitrant environmental pollutants¹⁹. Therefore, the development of alternative means of plastic degradation would fill a critical need. Polyethylene plastics are polymers of straight-chain alkene compounds, which are general components of oil mixtures. Given the ubiquity of oil-degrading microorganisms in the environment, the use of environmental inocula may serve as a starting consortium for the breakdown of polyethylene plastic monomers. Gaining further understanding of microbial community response to influxes of alkene compounds as their primary carbon source in a variety of environments may indicate the feasibility of late-stage plastic biodegradation.

Apart from community composition alone, biodegradation rates and extent in the environment are generally dependent upon *in-situ* conditions such as temperature and ambient nutrients^{20,21}. Under suboptimal conditions in the environment, biodegradation of hydrocarbons is still often achievable. However, developing enrichment cultures of oil-degrading microbes operating under optimal conditions in a laboratory setting may hold increased potential for enhanced biodegradation. Additionally, the use of enriched microbial consortia allows for creation of a simplified community to study the process of biodegradation and allow for targeted questions related to oil-degrading microorganisms²²⁻²⁴. In this experiment, we aim to demonstrate that multiple environmental inocula sources with differing initial microbial communities are capable of alkene biodegradation in controlled laboratory conditions under three nutrient treatments. Doing so may also provide more insight as to under which conditions biodegradation of alkenes is most efficient.

We hypothesize that biodegradation extent and rates will vary with alkene chain length, with the catabolism of longer chain-length carbon sources possibly requiring longer lag phases in cultures. We also hypothesize that significantly different microbial communities will be selected for across inocula sources within enrichment cultures, and that distinctive community formation will drive differences in alkene biodegradation response. Being that *in-situ* microbial community dynamics are generally responsive to shifts in temperature, nutrients, pH, and other environmental factors, we anticipate that altering nutrient concentrations under controlled laboratory conditions will significantly influence microbial community composition and alkene breakdown rates in our cultures.

3.1 Materials & Methods

Sample Collection

Caspian Sea sediment cores from the southern basin were sampled at 39.745 59 °N, 50.480 600 °E²⁵. Iron-rich sediment was collected from a stream in Michigamme, Michigan. Compost samples were collected from a farm in Calumet, Michigan. Environmental inocula samples were collected in sterile containers and stored at 4°C until use in cultures.

Enrichment Cultures

Cultures under each inocula type and nutrient treatment were assembled in triplicate. Bushnell Haas media was used in this study due to its lack of carbon source (0.2 g MgSO₄, 0.02 g CaCl₂, 1 g KH₂PO₄, 1 g (NH₄)₃PO₄, 1 g KNO₃, 0.05 g FeCl₃). In each replicate, one gram of environmental inocula was combined with 100 mL of either low-nutrients (0.25 times concentrated Bushnell-Haas media), standard Bushnell Haas media, or high-nutrients (2 times concentrated Bushnell-Haas media). Nitrogen and phosphorous levels present in each treatment condition as ammonium phosphate and potassium nitrate were as follows: 0.46 g/L N and 0.77 g/L K (high), 0.23 g/L N and 0.38 g/L K (standard), and 0.05 g/L N and 0.09 g/L K (low). As a carbon source, 0.5 mL of a 1:1:1:1 by volume mixture of 1-hexene, 1-decene, 1-hexadecene, and and-1-eicosene was added. These were chosen to represent a range of carbon lengths of olefin compounds. Cultures were inoculated in 250 mL flasks and placed on a stir plate with Teflon coated stir bars at 200 rotations per minute at room temperature (23°C). After one week, 7 mL of each culture was transferred to a new flask with 93 mL of fresh Bushnell Haas media and 0.5 mL of the same alkene mixture. Cultures were transferred twice before a final transfer to begin growth measurements. In the final transfer, 7 ml were transferred into fresh medium with the appropriate nutrient concentration. A control set of flasks was also assembled with uninoculated Bushnell Haas medium.

Biodegradation Rate Determination

To assess culture response to alkenes, carbon dioxide production rates were measured as a proxy for alkene biodegradation using GC with an -FID with attached methanizer. Each day for five days, a 1 mL sample from the headspace of each flask was analyzed with GC-FID with a methanizer using the HayeSep D column. After sampling, each flask was opened daily for one minute to allow for gas exchange and allow for calculation of daily CO₂ production. We did not account for CO₂ reentering the flasks during that period in our calculations of respiration rates. We used a linear calibration line of best-fit relating GC-FID CO₂ peak area values to estimated gaseous volume with known volumes of a standard mixture of gases with 1% CO₂, 1% CO, 1% H₂, 1% CH₄, 1% O₂, balanced with nitrogen. CO₂ values were converted to mass (mg) for determination of CO₂ production using the ideal gas law, and scaled to represent the entire headspace of each flask.

Since these cultures were grown using batch culture, we observed typical growth phases. To determine the rates of CO₂ production, linear regression lines were calculated for CO₂ produced between time points representative of the log phases of microbial growth determined using OD600 measurements. The slope of these regression lines were used as the rate constant for CO₂ produced during the exponential CO₂ production. In order to test our hypothesis that microbial communities from different inocula sources and nutrient levels will result in varying biodegradation rates, the slopes of the regression lines for each treatment were compared using the nonparametric Kruskal-Wallis test since sample data did not meet the assumptions for an ANOVA. To identify between which sample types a significant difference occurred, the “Dunn” package in R was used to perform a post hoc test.

Quantifying Residual Alkene Compounds

From each culture after five days, 70 mL were subsampled and frozen in amber glass bottles until hydrocarbon extraction. For extractions, 70 mL of dichloromethane (DCM) was used to rinse residual hydrocarbon compounds in the bottles and then added as a solvent to each sample in separatory funnels. Funnels were shaken vigorously for one minute, and left to settle into nonpolar and polar fractions for approximately one hour. The miscible fraction containing non-polar compounds was poured into amber glass vials through Wattman glass fiber filter for each sample to remove residual particles and biomass that would inhibit GC/MS analysis. Filters were rinsed with 5 mL of DCM to remove any residual alkene compounds. The solvent fraction was blown down with nitrogen gas to a volume < 2 mL. All extractions were stored at 4 °C until analysis with GC/MS

Quantification of Alkenes With GC/MS

The volume of all extracts was measured for subsequent quantification of alkene mass. Hydrocarbon extracts were diluted to 1% of their initial concentration in DCM. 10 μ L of 1,3,5-trichlorobenzene was added to each diluted sample as an internal standard to correct for injection error by the autosampler. Samples were analyzed using GC/Ion Trap mass spectrometer. The GC was heated at 65 °C, held for 3 minutes, then heated from 65°C-240°C, ramping at 30°C until reaching a temperature of 240 °C and held for one minute using the TG-5MS column from ThermoScientific. The mass spectrometer ion source temperature was heated to 275 °C. A full scan was conducted with a m/z ratio of 20-300, with a max ion time of 75 μ S. An autosampler was used for injections and was rinsed three times with 3 μ L of DCM between each run. Injection volumes of 1 μ L per sample were used for analysis. Each sample was run in a series of five technical replicates. Calibration curves were created for each target compound to relate peak intensity to analyte concentration.

To determine if there were statistical differences in alkene concentrations between sample types and nutrient sources, we started by using the “pwr” package in R to determine if sample sizes of each inocula source and nutrient treatment met the assumptions for statistical comparison with ANOVA. We found that with the number of technical replicates we did have sufficient statistical power to do so with an alpha value of 0.05 and power value of 0.8. Hence, we utilized the ANOVA test in base R to assess for statistical differences in alkene concentrations amongst sample types. Pairwise comparisons between sample types were done using the post hoc Tukey HSD function.

Culture Growth and Biomass Quantification

In order to quantify growth partitioned to biomass production in our cultures, we quantified protein content in the culture at the initial and final timepoints. A detergent solution was made using 1 g of sodium dodecyl sulfate, 5 ml of Tris (pH = 8), and 9.5 mL of sterilized water. 75 μ L of the detergent solution was combined with 75 μ L of culture subsample from each time point for a total volume of 150 μ L in a 96-well plate and heated in a thermocycler at 100 °C for ten minutes. The Pierce bicinchoninic acid assay protein kit produced by Thermo Fisher Scientific was used. 200 μ L of the BCA working reagents was mixed with 10 μ L of boiled sample in a separate 96-well plate. The plate incubated at 37 °C for 30 minutes before absorbance values were measured using a spectrophotometer at 562 nm for each well. A standard curve was created with various concentrations of an Albumin protein stock standard solution to correlate absorbance with protein concentration.

From each culture, 30 mL were vacuum filtered on 0.03µm Sterlitech polyethersulfone filters and stored at -80 °C. Sample DNA was extracted from the entire filter using a ZymoBIOMICS DNA microprep kit (Zymo Research Corporation, Irvine, CA) following manufacturers specifications with the exception of homogenizing samples for 200 seconds at 5.5 m/s using a FastPrep 5G (MP Biomedicals). Extracted DNA was then prepared for high-throughput sequencing for construction of V4-V5 16S rRNA gene libraries using a modified version of the Illumina 16S rRNA library preparation protocol. Briefly, the V4-V5 hypervariable regions of the 16S rRNA gene were amplified using the 515YF and 926R primers²⁶ with 25 cycles. After PCR purification using Axyprep magnetic beads (Corning), sequencing indexing barcodes were added through a second 8-cycle PCR step. In order to account for possible contamination of our reagents at each step, blank wells containing no added DNA were run. The barcoded amplicons were purified using the Axyprep magnetic bead PCR clean up kit. The picogreen dsDNA quantification assay (Thermo Scientific) was used to determine the concentrations of each library pool. The libraries were then pooled to give a single 4 nM pool for sequencing. The pool was sequenced using a v3 600-cycle Illumina kit using Michigan Technological University's MiSeq instrument.

Figure 3.2. A more detailed graphic depicting our experimental design for 16S rRNA sequencing and hydrocarbon extraction subsampling.

All sequencing data was analyzed in R. We used the package DADA2 (divisive amplicon denoising algorithm) to process and trim sequencing reads²⁷. Reads falling below a quality score of 30 were trimmed. The forward reads and reverse reads were trimmed at 250 cycles. Reads were denoised, forward and reverse reads were merged, and chimeras were identified. For the ASV table, samples were rarified to a depth of 1000 reads. A taxa table was constructed with the package “phyloseq”, using taxonomical assignments from the SILVA v132 data set for DADA2. Sequences from mitochondria and chloroplasts were filtered out of the dataset. To determine alpha diversity on the non-rarified taxa table, the “estimate_richness” function in phyloseq was used.

All statistical analyses were carried out using R. We used the “pwr” package in R to determine if sample size between each inocula source and nutrient level met the assumptions for statistical comparison with ANOVA and found that we did not have sufficient statistical power to do so with a power value of 0.8 and a significance level of 0.05. As a result, we utilized the non-parametric Kruskal-Wallis test to assess for statistical differences in microbial communities amongst inocula types and nutrient levels. Pairwise comparisons between sample types were done using the Dunn function in the package “FSA”²⁸. Principal coordinate analysis (PCoA) was completed using the “phyloseq” package to visualize microbial community shifts across treatments using the Bray-Curtis dissimilarity matrix. A PERMANOVA test was used to determine the extent of variation explained by the difference in microbial community structure between treatment types in the PCoA. The Adonis2 function was used to determine the percentage of variation that could be explained by the PCoA within the package “vegan”²⁹.

Differential Abundance Analysis

In order to determine if significantly enriched ASVs were present between inocula sources and nutrient levels in the 16S rRNA reads, the package “DESeq2”³⁰ was used to conduct differential abundance analyses between sample variables with an alpha = 0.01. ASVs present with a log2 fold value >2 or <-2 were considered to represent significant differences in abundance.

3.2 Results

Biodegradation Rates: CO₂ Production

Figure 3.3. Total CO₂ production over the course of five days across inocula types and nutrient levels (high, standard, and low) in laboratory cultures indicating metabolic response of model alkene compounds. Alkene cultures were amended at day 0 with 0.5 mL of 1:1:1:1 1-hexene, 1-decene, 1-hexadecene, 1-eicosene mix. CO₂ values were obtained using GC-FID with 1 mL subsamples of flask headspace air analyzed. Each replicate across treatments was opened daily for 1 minute to allow gas exchange with ambient room air and to prevent anoxic conditions from occurring within the cultures.

Here we used CO₂ production as an indirect means of quantifying alkene biodegradation. For each sample, CO₂ production was calculated over the course of five days following inoculation. Values of CO₂ produced ranged from 13.74 mg in standard-nutrient compost cultures to 0.97 mg in standard-nutrient Caspian sediment cultures (Figure 3.3). In order to identify statistically significant differences in total mass of CO₂ produced across inocula types and nutrient levels, a Kruskal-Wallis test was used. A non-significant p-value was obtained (0.1712). To verify that no significant differences were present between any sample types, a Dunn *post-hoc* function was used. We found no significant differences present between any samples.

Slopes of Linear Regressions for CO₂ Production

Table 3.1. A summary table depicting the slopes of linear regression equations for mg of CO₂ produced per day during the exponential phase of microbial growth. R² values detailing the amount of variation explained by the trendline drawn are in parentheses.

Nutrient Level	Inocula Source		
	Farm Compost	Iron-Rich Sediment	Caspian Sediment
High	2.31 (0.913)	2.99 (0.999)	1.71 (0.986)
Standard	3.17 (0.975)	4.59 (1)	0.92 (0.994)
Low	3.31 (0.919)	2.55 (0.864)	1.59 (0.961)

In order to test our hypothesis that biodegradation rates would differ significantly between different environmental inocula sources or nutrient levels, we compared the linear regression slopes using the nonparametric Kruskal-Wallis test. This test revealed a p-value of 0.4335, indicating that no statistically significant differences existed between the slopes of CO₂ production in the log phase between any combination of inocula sources or nutrient levels in our samples.

In order to differentiate how much CO₂ produced was attributed more likely to alkene biodegradation versus other background processes, we calculated CO₂ production taking into account mean control production (Figure 3.4).

Figure 3.4. Total net CO₂ production after subtraction of the mean CO₂ production in mg from a control set of replicates containing no alkene input. Representing net CO₂ allows for more direct means of quantifying respiration attributable to alkene biodegradation.

Quantification of Residual Alkenes With GC/MS

Figure 3.5. End mass (g) of 1-decene, 1-hexadecene, and 1-eicosene in laboratory cultures consisting of various inocula sources after the course of five days. These values were obtained using GC/MS following extraction of hydrocarbons from culture subsamples in dichloromethane.

We quantified residual alkene concentrations in our cultures in order to determine how much of our initial alkene input was degraded over the course of five days (Figure 3.5). We found masses (g) for 1-decene ranging from 0.04 g in low nutrient Caspian Sea sediment cultures to 0.0007 g in high nutrient compost cultures. For 1-hexadecene, we found masses ranging from 0.21 g in low nutrient Caspian Sea sediment cultures to 0.006 g in high-nutrient compost samples. Masses for 1-eicosene ranged from 0.22 g in low nutrient Caspian Sea sediment samples to 0.008 g in standard nutrient compost samples. We were unable to quantify the hexene that was added to the cultures.

To determine if significant differences existed in mass values for each alkene compound across nutrient levels and inocula sources, we conducted an ANOVA test, which revealed a p-value of <0.001 . To determine between which specific sample types significant differences existed, we conducted a Tukey HSD post hoc test. For 1-decene quantification, our results revealed significant differences between high and low nutrient levels in Caspian Sea samples (Table 3.1). For 1-hexadecene, significant differences existed between high and low and standard and low nutrient levels in Caspian Sea sediment samples (Table 3.2). For 1-eicosene, the similar differences were observed (Table 3).

Table 3.2. A summary of Tukey HSD post hoc results depicting adjusted p-values for comparisons between the residual masses of 1-decene across nutrient treatments within Caspian Sea Sediment, iron-rich sediment, and farm compost inocula cultures. Bolded values indicate significant differences (p-value <0.05).

<i>Caspian Sediment</i>	High	Standard	Low
High		0.98	0.03
Standard			0.19
Low			
<i>Iron-Rich Sediment</i>	High	Standard	Low
High		0.86	0.99
Standard			0.97
Low			
<i>Farm Compost</i>	High	Standard	Low
High		0.97	0.98
Standard			0.99
Low			

Table 3.3. A summary of Tukey post hoc results depicting adjusted p-values for comparisons between the residual masses of 1-hexadecene across nutrient treatments within Caspian Sea Sediment, iron-rich sediment, and farm compost inocula cultures. Bolded values indicate significant differences (p-value <0.05).

<i>Caspian Sediment</i>	High	Standard	Low
High		1.00	<0.01
Standard			<0.01
Low			
<i>Iron-Rich Sediment</i>	High	Standard	Low
High		0.22	0.64
Standard			0.99
Low			
<i>Farm Compost</i>	High	Standard	Low
High		0.99	0.99
Standard			0.99
Low			

Table 3.4. A summary of Tukey post hoc results depicting adjusted p-values for comparisons between the residual masses of 1-eicosene across nutrient treatments within Caspian Sea Sediment, iron-rich sediment, and farm compost inocula cultures. Bolded values indicate significant differences (p-value <0.05).

<i>Caspian Sediment</i>	High	Standard	Low
High		0.99	<0.01
Standard			<0.01
Low			
<i>Iron-Rich Sediment</i>	High	Standard	Low
High		0.05	0.76
Standard			0.87
Low			
<i>Farm Compost</i>	High	Standard	Low
High		0.81	0.98
Standard			0.99
Low			

We used the quantification of alkenes to determine the percent mass of degradation across all treatment types and compounds (Figure 3.6). We observed mean biodegradation extents of approximately 90% and above in all sample types. Lower extents of biodegradation ranging from 85 to 95% were observed for 1-eicosene amendment. Biodegradation of shorter chain-length compounds such as 1-decene or 1-hexadecene was more thoroughly achieved at 95% degradation or above.

Figure 3.6. Percent biodegradation values of 1-decene, 1-hexadecene, and 1-eicosene in laboratory cultures consisting of various inocula sources after the course of five days. These values were obtained using GC/MS following extraction of hydrocarbons from culture subsamples in dichloromethane. Cultures were enriched on these compounds for two transfers over the course of two weeks preceding this monitoring period.

We quantified protein content in our cultures in order to estimate how much of our initial alkene input was being allocated towards assimilatory metabolism in cells. We found protein concentrations ranging from 1.49 mg/mL in high-nutrient iron-rich sediment cultures after five days to near zero mg/mL in low-nutrient compost samples at the initial time point of zero days following inoculation (Figure 3.7).

Protein Quantification

Figure 3.7. Estimated protein content values within laboratory cultures consisting of various environmental inocula sources after the course of five days. These values were obtained using a ThermoFisher protein extraction kit and creating a calibration curve using Albumin protein standards.

Mass Balance

To attempt to follow the flow of carbon from alkenes through this system we attempted a rough mass balance based on the data that was measured.

Table 3.4. Mass balances for enrichment cultures from several inocula sources grown on liquid alkene. The mean mass (+/- SD) of estimated protein content, headspace CO₂ production, and remaining residual alkenes (C10, C16, and C20) were accounted for. Note: This table and calculations do not take into account total biomass production, for which protein production is approximately a 50% underestimation of. They also do not account for the remaining alkenes that may have been present in the initial mixture, or possible daughter productions formed during metabolism processes.

Inocula Group	Initial input of alkene mixture (g)	Mean Mass Attributed to Protein Production (g)	Mean Mass Attributed to CO ₂ Production (g)	Mean Mass Leftover of Alkenes Combined(GC/MS) (g)	Mass Unaccounted For (g)
Farm Compost-High	0.374	0.03+/- 0.019	0.007+/- 0.006	0.06+/-0.012	0.277
Farm Compost-Standard	0.374	0.02+/- 0.028	0.01 +/- 0.003	0.03+/- 0.009	0.314
Farm Compost-Low	0.374	0.0003 +/- 0.005	0.01 +/- 0.001	0.04+/- 0.007	0.321
Iron-Rich High	0.374	0.09+/- 0.051	0.009 +/- 0.002	0.08+/- 0.016	0.195
Iron-Rich Standard	0.374	0.02+/- 0.003	0.01 +/- 0.0005	0.03+/- 0.004	0.314
Iron-Rich Low	0.374	0.01+/- 0.003	0.01 +/- 0.001	0.05+/- 0.008	0.304
Caspian Sea High	0.374	0.02+/- 0.040	0.005 +/- 0.004	0.13+/- 0.022	0.219
Caspian Sea Standard	0.374	0.0004+/- 0.0006	0.002 +/- 0.0006	0.13+/- 0.021	0.238
Caspian Sea Sediment-Low	0.374	0.004+/- 0.015	0.007 +/- 0.002	0.27+/- 0.054	0.093

Microbial Diversity In Raw Inocula Sources

In 16S rRNA sequences for raw inocula without alkene amendment, we detected 26,069 ASVs across 13 bacterial phyla (Figure 3.8). We observed phylum-level microbial diversity across all inocula sources and nutrient levels using a taxa plot. The most prevalent phylum across the microbial communities in all inocula sources was Proteobacteria, with the highest number of taxa observed in iron-rich sediment inocula. The second most prevalent bacterial phylum observed was Bacteroida, from which taxa were observed in iron-rich sediment and Caspian Sea sediment. Compost samples contained less microbial diversity, containing bacteria only represented in Proteobacteria.

Figure 3.8. A taxa plot depicting microbial community composition in raw inocula samples not amended with alkene compounds. Taxonomic assignments were based on sequencing of the V4-V5 16S rRNA gene. Across all samples, 13 phyla were observed. Trends of high abundances of Proteobacteria across all inocula types were observed, as well as increased Bacteroidota in iron-rich sediment samples and Caspian Sea sediment samples.

Microbial Diversity of Alkene-Amended Cultures: Alpha Diversity

To assess for differences in selection between alkene-enriched microbial communities, we compared ASV richness values between compost, Caspian sediment, and iron-rich sediments samples. Alkene-amended cultures with compost inocula appeared to have the highest alpha diversity overall, and standard and high nutrient Caspian sediment cultures having the lowest diversity across all seasons and treatments (Figure 3.9).

Figure 3.9. Alpha diversity represented as ASV richness across Caspian Sea sediment, farm compost, and iron-rich sediment. The highest diversity was present in compost samples, with the high nutrient treatment group presenting the largest observed ASV richness. Conversely, the standard nutrient iron-rich sediment group contained the lowest observed ASV richness.

To assess microbial diversity across all inocula sources and nutrient levels, we used a taxa plot to visualize the spread of bacterial classes in our samples. We observed taxa within four phylum (Proteobacteria, Firmicutes, Actinobacteria, and Bacteriodetes) and nine total bacterial families across all sample types. Xanthomonadaceae was highly abundant across all sample types, with the most abundant portion of the community present in low nutrient compost samples at approximately 90% of the total community composition (Figure 3.10). Standard nutrient samples across all inocula types contained increased Nocardiaceae (15-20%) relative to high and low nutrient samples. Compost samples of all nutrient levels displayed a gradual trend of to cultures with sediment from the Caspian Sea and from iron-rich sediments.

Figure 3.10. A taxa plot depicting microbial diversity across inocula sources and nutrient levels for cultures amended with four alkene compounds (C6, C10, C16, C20).

PCoA (Beta Diversity)

Figure 3.11. A PCoA plot depicting diversity between samples for bacterial 16S rRNA sequences across three nutrient levels and inocula types amended with a mixture of alkene compounds. Closer clustering of samples indicates increased similarity.

We conducted principal component analysis to visualize shifts in microbial community composition across treatments with the Bray-Curtis dissimilarity matrix. We observed distinct clustering of samples based on 16S rRNA sequences within each inocula type (Figure 3.11). Sample clustering by nutrient level was less distinct but may indicate gradual trends in microbial community composition shifts when nutrient contents are enhanced. Overall, the first two dimensions of the PCoA plot explained 23.3% of the variation. We used a PERMANOVA test to determine if statistically significant microbial community composition shifts were present across treatments. PERMANOVA was also used to determine the variation explained by the difference in microbial community structure between inocula types and nutrient levels in the PCoA. We used the `adonis2` program as part of the `vegan` package in R to perform the PERMANOVA test in order to determine between which sample types significant differences were present. We observed significant differences between the microbial communities of each inocula type when collapsing nutrient treatments together (Table 3.5).

PERMANOVA Comparison of 16S ASVs Across Inocula Type

Table 3.5. A summary of PERMANOVA results depicting R^2 values between the microbial communities across Caspian sediment, iron-rich sediment, and compost. The F-model values are shown in parentheses for each comparison. Bolded values indicate the p-value depicting the probability of $>F$ for each comparison was <0.001 .

	Caspian Sea Sediment	Iron-Rich Sediment	Farm Compost
Caspian Sea Sediment		0.15052 (4.6069)	0.1588 (5.6635)
Iron-Rich Sediment			0.17444 (5.0711)
Farm Compost			

PERMANOVA Comparison of 16S Microbial Communities Across Nutrient Treatments Within Each Inocula Group

Table 3.6. A summary of PERMANOVA results depicting R^2 values between the microbial communities across nutrient treatments within Caspian Sea Sediment, iron-rich sediment, and farm compost inocula cultures. The F-model values are shown in parentheses for each comparison. Bolded values indicate the probability of $>F$ for each comparison was <0.01 .

<i>Caspian Sediment</i>	High	Standard	Low
High		0.37573 (5.4169)	0.36331 (5.7063)
Standard			0.2724 (3.3694)
Low			
<i>Iron-Rich Sediment</i>	High	Standard	Low
High		0.27787 (1.5392)	0.13407 (0.92899)
Standard			0.19977 (1.4978)
Low			

<i>Farm Compost</i>	High	Standard	Low
High		0.35346 (5.4669)	0.3504 (3.7758)
Standard			0.37827 (4.259)
Low			

Differential Abundance Analysis

To begin to understand which bacteria were enriched in different treatments, we performed differential abundance analysis using DESeq2. We found significantly enriched bacterial ASVs between nutrient groups in every inocula type (Table 3.7). All significantly enriched ASVs belonged to one of three bacterial families:

Xanthomonadaceae, *Beijerinckiaceae*, or *Nocardiaceae*. All of the enriched ASVs from the *Xanthomonadaceae* were classified as members of *Stenotrophomonas* genus.

Similarly, the significantly enriched ASVs classified as *Beijerinckiaceae* were members of the *Methylobacterium* f. The *Gordonia* and *Rhodococcus* genera were the only taxonomic assignments represented across enriched ASVs within *Nocardiaceae*.

Table 3.7. A summary depicting the number of significantly enriched ASVs within inocula types across nutrient levels. All significantly enriched ASVs belonged to one of three bacterial families: Xanthomonadaceae, Beijerinckiaceae, or Nocardiaceae.

Inocula Source	Nutrient Level Comparison	Number of ASVs Enriched in First Group
Caspian Sea	High vs. Standard	8
	Standard vs. High	6
	Standard vs. Low	3
	Low vs. Standard	2
	High vs. Low	3
	Low vs. High	8
Iron-rich sediment	Standard vs. High	3
	High vs. Low	4
	Low vs. High	2
Farm Compost	High vs. Standard	2
	Standard vs. High	15
	Low vs. Standard	3
	High vs. Low	3

3.3 Discussion

We observed no significant differences in either total CO₂ production or CO₂ production rates in culture headspace across all treatment types after the course of five days. Given that CO₂ production did not display significant differences under varying nutrient levels despite consisting of the same starting environmental inocula (with significant differences in microbial community composition), we infer that nutrient availability may not be the most critical controlling factor for alkene biodegradation rates in a culture setting. However, it is possible that even our lowest nutrient amendment (0.25 g/L of nitrogen and phosphorous) is substantially more nutrients than what the microbial communities would likely be exposed to in an uncontrolled *in-situ* environment. Because of this, even the lowest nutrient levels in this experiment may represent conditions where nutrients are not limiting biodegradation rates. More work is needed to fully understand the impacts of nutrients on biodegradation of alkene compounds in environmental settings and enrichment cultures, as our experiment only accounts for the impacts of nutrients biodegradation of four model compounds (C6, C10, C16, C20).

We observed on average between 90 and 100% biodegradation of our chosen alkenes over the course of five days (Figure 3.6) along with significant selection pressure within the microbial community (Figure 3.10). Our results align with previous work characterizing the selection of microbial communities contributing to alkane breakdown³¹. Specialized communities were also selected for in our consortias for alkene breakdown. Longer carbon chains and increasing complex chemical structures, such as branching, will likely play a significant role in the alkene biodegradation rates of recalcitrant pollutants. Previous work has suggested that biodegradation is most rapid for alkanes, followed by alkenes, then branched compounds, and then aromatics³²⁻³⁴. Gaining further insight to the roles other environmental factors such as temperature play in biodegradation would be beneficial for upscaling microbial-aided alkene biodegradation.

Another consideration for our study results is the partitioning of the alkene carbon sources into dissimilatory and assimilatory metabolism sources. In regard to protein production in cultures, low nutrient samples across all inocula sources presented distinctly lower protein concentration relative to high nutrient samples. However, when observing CO₂ production over the same length of time, significant differences are not observed between high and low nutrient samples. This suggests that metrics of dissimilatory metabolism such as CO₂ production may not be as heavily impacted as cell growth metrics by nutrient content in cultures. Furthermore, more than one metabolism monitoring technique may be required to discern which treatment conditions are most efficient for optimizing alkene breakdown. Bacterial growth efficiency is defined as the quantity of biomass synthesized per unit of substrate assimilated³⁵. In natural settings this can be measured as the ratio of bacterial production to bacterial respiration. Since bacterial production is biomass production and respiration can be measured as CO₂ production, it's possible to consider these results from the perspective of bacterial growth efficiency. Previous studies have shown that increased supply of nutrients results in increased bacterial growth efficiency³⁶⁻³⁷. High nutrient enrichments showed a trend of increased protein production after the course of five days relative to low nutrient enrichments. Our results also show that low nutrient enrichments displayed trends of

increased total CO₂ produced relative to high nutrient enrichments. This may indicate that high nutrient enrichments possess higher growth efficiency ratios relative to low nutrient enrichments. In general, microbial diversity in the environment tends to be higher than microbial diversity in these laboratory consortia, since a large percentage of environmental microbes resist culturing³⁸. The selection for an alkene-degrading consortium resulted in decreased bacterial diversity relative to the raw inocula (Figure 3.8), indicating that the addition of alkenes holds strong selection pressure for certain taxa in many environments. Also, other factors such as nutrient limitation and environmental constraints such as temperature could control the extent of bacterial selection in response to alkenes in the environment. In natural systems nutrient limitation often is one of the strongest constraints on oil biodegradation. In our system, we assumed that the high nutrient levels in the cultures would allow for the cultures to not reach nutrient limitation by the end of the experiment. However, our optical density and CO₂ production indicate that the enriched cultures had reached stationary phase by the end of the five day experiment.

Furthermore, we were able to enrich taxa from similar bacterial families despite the fact that we used different nutrient levels and started with inocula with distinct community composition. This finding suggests that alkene-degrading taxa in environmental systems are found to be ubiquitous in many locations. Bacteria from three families were identified as being significantly enriched across all nutrient levels and inocula types: *Xanthomonadaceae*, *Beijerinckiaceae*, and *Nocardiaceae*. ASVs within *Xanthomonadaceae* were significantly enriched in every inocula and nutrient level apart from low-nutrient iron sediment samples. ASVs within *Beijerinckiaceae* was significantly enriched in standard and low-nutrient Caspian samples, in all nutrient levels of iron-rich sediment samples, and in standard nutrient samples from compost inocula. ASVs from *Nocardiaceae* were enriched in high-nutrient Caspian samples, standard nutrient iron-rich samples, and in high and standard-nutrient compost samples. All three enriched families of bacteria have been previously observed to play a role in hydrocarbon biodegradation³⁹⁻⁴¹. Microbes from *Xanthomonadaceae* in particular were previously

observed to be enriched in ethylbenzene-enriched soil microcosms⁴². The widespread presence of enriched ASVs from each of these families within varying inocula types may indicate that the bloom of bacteria after exposure to alkenes may not directly be impacted by the starting microbial community. Additionally, a lack of significant presence of Nocardiaceae in low-nutrient samples may indicate that other bacterial families are more strongly selected for and outcompeting them in lower nutrient conditions.

Another limitation of our study was that we only examined bacterial diversity. It is possible that fungi are present in our cultures and contribute to alkene biodegradation across nutrient levels. Previous work indicates that fungi are important key players for the biodegradation of other recalcitrant compounds such as lignin⁴³, and therefore may be important to consider in the context of our environmental consortium. Future work that focuses on sequencing of universal regions of fungal genomes such as inter-transcribed spacers (ITS) regions would provide more insight to the fungal community dynamics contributing to alkene breakdown.

We hypothesized that significantly different microbial communities will be selected for across inocula sources within enrichment cultures, and that distinctive community formation will drive differences in alkene biodegradation response. In our cultures, we observed convergence of microbial communities across treatment types and inocula sources, despite beginning with three distinct microbial communities from different environments. Only three families of bacteria across two phyla were found to have taxa significantly enriched despite more than 35 bacterial phyla represented in the initial community sequences from raw inocula. Although contradictory to our initial hypothesis, selection of a specialized microbial community may suggest that cultures from varying environmental inocula are adaptable to alkene sources of varying chain lengths, such as those in this study.

3.4 Conclusions

Our study goal was to quantify alkene breakdown in laboratory cultures and monitor changes in the microbial communities to draw associations to which microorganisms may play a significant role in alkene breakdown. We monitored breakdown using various metrics including CO₂ production and GC/MS and found no significant distinctions in CO₂ production across nutrient levels or inocula types. We observed high extents of biodegradation in all sample types, with the majority of samples achieving between 90 to 100% biodegradation of all quantified compounds. Microbial community diversity analyses throughout our experiment found that cultures initialized with environmental inocula from varying starting microbial assemblages converged to display significant overlap of bacterial families. Significantly enriched families across all inocula types included ASVs from families *Xanthomonadaceae*, *Nocardiaceae*, and *Beijerinckiaceae*. Distinctly enriched ASVs overlapping across treatment types restrained divergence of the overall communities. Overall microbial communities across nutrient levels within Caspian Sea sediment and farm compost cultures were significantly different. These results ultimately suggest that the microorganisms necessary to achieve alkene breakdown may be present in a variety of environmental microbial assemblages, and that they are strongly selected for under optimized conditions such as nutrient amendment.

Chapter Two Supplementary Material

Table A2.1. *Post-hoc* Dunn function p-values for statistical comparisons of daily CO₂ production (mg/day) made between oil-enriched microcosms amended with varying oil types (Bakken crude, non-highway diesel, and a control group) within seasons.

fall 23°C	Bakken	diesel	control
Bakken		0.5605649	0.6504271
diesel			0.7095834
control			
fall 4°C	Bakken	diesel	control
Bakken		0.32587057	0.85337210
diesel			0.44688337
control			
spring	Bakken	diesel	control
Bakken		0.32835689	0.84847461
diesel			0.25463754
control			
summer	Bakken	diesel	control
Bakken		0.32744443	0.62825028
diesel			0.66873104
control			

Table A2.2. *Post-hoc* Dunn function p-values for statistical comparisons of daily CO₂ production (mg/day) made between oil-enriched microcosms amended with varying oil types (Bakken crude, non-highway diesel, and a control group) across seasons.

Bakken	fall 23°C	fall 4°C	spring	summer
fall 23°C		0.22929796	0.64435926	0.25848476
fall 4°C			0.43472189	0.86390142
spring				0.53188245
summer				
diesel	fall 23°C	fall 4°C	spring	summer
fall 23°C		0.75151417	0.73558874	0.76725690
fall 4°C			0.57563240	0.94330521
spring				0.56425839
summer				
control	fall 23°C	fall 4°C	spring	summer
fall 23°C		0.92972949	0.72612846	0.65862569
fall 4°C			0.67359446	0.62437317
spring				0.91497188
summer				

Figure A2.1. Observed ASVs richness from 16S rRNA sequences across seasons and oil types in the Straits of Mackinac. Across all sample types we observed 12525 distinct taxa. The highest median ASV richness (approximately 125 ASVs) was observed in spring diesel samples. The lowest median ASV richness (approximately 50 ASVs) was observed in spring control samples.

Figure A2.2. A PCoA plot with the second axis replaced with time depicting the temporal shifts of the microbial communities in the Straits of Mackinac across season and oil type over the course of five weeks. Fall 23°C samples appeared to begin clustered tightly together at week zero and become more distinctively clustered by treatment over time. Fall 4°C and spring samples appeared to diverge significantly two weeks after incubation. Summer samples appear to cluster according to treatment in week one, and cluster more closely together by the fifth week of the experiment.

Table A2.3. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 23 °C control samples or spring control samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Family	Genus	Season
2.670447	1.13E-07	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
1.976717	0.000136	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	Non-significant
2.03851	0.000442	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.186288	0.000182	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.200825	0.001429	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.295819	0.008015	Proteobacteria	Alphaproteobacteria	Clade_III	NA	fall
1.574895	0.009249	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	Non-significant
2.355614	0.000136	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.203595	0.000136	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.095258	0.00165	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.159559	0.00122	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall

2.167423	0.00044 2	Proteobacteria	Alphaproteobacteria	Clade_III	NA	fall
2.1184	0.00034 2	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.179407	0.00062 3	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.402147	9.76E- 05	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.305514	0.00101 1	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.176077	0.00060 6	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.024024	0.00359 2	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.133445	0.00147 7	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.121421	0.00867 2	Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	Sphingobium	fall
2.080572	0.00142 9	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.151928	0.00118 6	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.212852	0.00011 6	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall
2.049392	0.00557 4	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	fall

1.895923	0.008015	Actinobacteria	Actinobacteria	Sporichthyaceae	hgcI_clade	Non-significant
2.177993	0.000116	Proteobacteria	Gammaproteobacteria	Methylophilaceae	Candidatus_Methylopumilus	fall
1.979687	0.003236	Actinobacteria	Actinobacteria	Sporichthyaceae	Candidatus_Planktophila	Non-significant
2.214967	8.32E-05	Proteobacteria	Gammaproteobacteria	Methylophilaceae	Candidatus_Methylopumilus	fall
1.959955	0.006166	Actinobacteria	Actinobacteria	Sporichthyaceae	Candidatus_Planktophila	Non-significant
2.11587	0.003236	Verrucomicrobia	Verrucomicrobiae	Chthoniobacteraceae	Chthoniobacter	fall
1.910933	0.009068	Proteobacteria	Gammaproteobacteria	Methylophilaceae	Candidatus_Methylopumilus	Non-significant
2.461266	2.73E-05	Verrucomicrobia	Verrucomicrobiae	Pedosphaeraceae	NA	fall
-2.29028	0.001215	Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	Novosphingobium	spring

Table A2.4. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 23 °C control samples or summer control samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Season
-3.42716	9.35E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-1.79653	0.005959	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Non-significant
-3.32466	9.35E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.83587	4.64E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.34158	0.000114	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	summer
-3.31197	8.58E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.09421	0.005664	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-3.23199	2E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.59396	5.14E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.23689	2.52E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.13237	0.005674	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-1.96585	0.003639	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant
-3.18767	2.22E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-3.10305	8.58E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.10185	9.35E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.96539	0.001514	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant
-3.10541	7.35E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.27816	0.00466	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-3.16025	9.48E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.11663	9.48E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.99531	1.29E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.13856	0.006983	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-1.84924	0.005674	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Non-significant
-3.00944	1.54E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.01423	8.58E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-2.93897	1.35E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.9916	8.58E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.07829	0.005171	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	summer
-3.05796	7.98E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.96958	1.54E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.76829	2.93E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.92651	1.54E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.03138	0.003639	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-2.75226	7.77E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.78343	0.007713	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Non-significant
-2.8405	0.000472	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.96654	3.56E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-2.94508	8.58E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.81297	9.48E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.88556	0.00768	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant
-2.601	1.69E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.82798	8.19E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.77197	5.92E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.80414	3.19E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.75134	0.000117	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.83444	2.45E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.84745	6.58E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.73725	9.36E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.01963	0.005959	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraeae	NA	summer

-1.79124	0.002537	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
2.076176	0.000394	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	fall
-2.61747	0.000864	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.81045	0.000864	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
1.962775	0.009391	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	Non-significant
-2.44624	0.003685	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.30619	2.97E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.35138	0.005959	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
2.054318	0.000394	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	fall
-2.51899	0.005024	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.82276	0.005639	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.57526	0.005171	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant

-1.93948	0.000562	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.03613	0.000356	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.00736	0.005674	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.09651	0.004464	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.13546	0.000102	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.86404	0.005664	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
2.051758	3.41E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopusillus	fall
-2.28317	0.004464	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.04934	0.000562	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.17437	0.004978	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.13853	0.008827	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.20237	0.000217	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer

-1.94803	0.009509	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	Non-significant
-2.2679	0.002024	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	summer
1.482384	0.007653	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	Candidatus_Planktophilus	Non-significant
2.162163	7.71E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylophilus	fall
-1.72271	0.006034	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.03385	0.000582	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.40796	0.008543	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.94503	0.002549	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.9615	0.003734	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	Non-significant
-2.23089	0.006034	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	summer
-2.08925	0.00466	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	summer
-2.06611	0.006983	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraaceae	NA	summer

-2.0575	0.00197	Planctomyces	Planctomycetia	Planctomycetales	Rubinisphaeraeae	NA	summer
-2.01587	0.009546	Planctomyces	Planctomycetia	Planctomycetales	Rubinisphaeraeae	NA	summer
1.689136	0.004554	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopusillus	Non-significant
1.942045	0.003014	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter	Non-significant
1.740144	0.006034	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopusillus	Non-significant
-1.78086	0.007029	Planctomyces	Planctomycetia	Planctomycetales	Rubinisphaeraeae	NA	Non-significant
-2.16046	0.003019	Planctomyces	Planctomycetia	Planctomycetales	Rubinisphaeraeae	NA	summer
2.239734	1.52E-05	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	fall
-1.80525	0.003753	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
1.806426	0.008401	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.86896	0.008743	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant
-1.86607	0.007029	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant

-1.72632	0.009 864	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.99446	0.004 139	Armatimonadetes	Armatimonadida	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.97323	0.001 817	Armatimonadetes	Armatimonadida	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
1.721421	0.009 864	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylophilus	Non-significant
-1.96477	0.004 139	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
1.956147	0.002 65	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.75099	0.009 864	Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	Hirschia	Non-significant
-2.00865	0.001 63	Armatimonadetes	Armatimonadida	Armatimonadales	Armatimonadaceae	Armatimonas	summer
-1.64445	0.006 983	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.91153	0.005 893	Armatimonadetes	Armatimonadida	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.99035	0.004 139	Armatimonadetes	Armatimonadida	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.70063	0.006 983	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant

Table A2.5. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 4 °C control samples or spring control samples generated in DESeq2

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Season
2.907376	3.91E-12	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
1.945056	0.000216	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	Non-significant
2.092967	0.004428	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
2.378857	5.39E-05	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
2.156556	5.33E-05	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
2.2024	0.000871	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
2.317885	0.000576	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter
2.395074	5.81E-05	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	winter

2.156002	0.0008 95	Proteobacteri a	Alphaproteobacte ria	SAR11_clade	Clade_III	NA	winter
2.16383	0.0013 32	Proteobacteri a	Alphaproteobacte ria	SAR11_clade	Clade_III	NA	winter
2.035849	0.0054 77	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.034177	0.0048 2	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.057811	0.0046 52	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.057918	0.0007 75	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.134959	0.0049 11	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.228911	0.0008 95	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.2824	0.0006 45	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	winter
2.019875	0.0032 85	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Methylophilacea e	Candidatus_ Methylopumil us	winter
2.202815	0.0007 07	Verrucomicr obia	Verrucomicrobia e	Pedosphaerales	Pedosphaeracea e	NA	winter
2.009872	0.0094 16	Verrucomicr obia	Verrucomicrobia e	Pedosphaerales	Pedosphaeracea e	NA	winter

-2.40838	0.001332	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	spring
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Table A2.6. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 4 °C control samples or summer control samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Season
-3.41803	8.42E-11	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.48489	1.89E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.44328	8.42E-11	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.29838	9.82E-10	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.5986	0.005268	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Non-significant
-3.29517	7.11E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.20837	0.000474	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-3.09447	2.67E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.23174	2.89E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-3.35311	5.78E-10	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.24502	0.000967	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-2.8771	1.07E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.21929	2.89E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.22663	8.42E-11	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.10174	0.00503	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-3.20742	4.16E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.18341	0.003776	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	summer
-2.39081	0.000766	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-3.01429	3.81E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.9715	3.82E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.11005	8.2E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.24425	0.001314	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer

-1.98543	0.000889	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Non-significant
-3.12395	1.14E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.12947	2.89E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.92363	1.65E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.10884	3.28E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.19528	0.000474	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	summer
-3.17842	2.61E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.29063	0.002498	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	summer
-3.08853	1.14E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.1581	0.000766	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-2.63348	1.93E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.95999	0.002087	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Non-significant
-2.06182	0.004539	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer

-3.04416	1.14E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.14756	0.000635	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	NA	summer
-2.86123	3.72E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.04878	0.000945	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Limnohabitans	summer
-2.94328	4.43E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-3.08208	3.97E-08	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-3.06384	2.79E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.93733	4.44E-09	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.0017	0.001959	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	NA	summer
-2.47608	2.5E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.93745	4.16E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	Janthinobacterium	summer
-2.1637	0.003049	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.14587	0.006165	Proteobacteria	Gammaproteobacteria	Betaproteobacterales	Burkholderiaceae	NA	summer

-2.88196	2.51E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.91967	2.37E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.85941	6.75E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.94751	1.71E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.96146	1E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.26198	9.33E-06	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.85273	1.65E-07	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.92448	0.000248	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
1.872723	0.002087	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	Non-significant
-2.06789	0.005848	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	summer
-2.72875	0.000967	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.72073	9.11E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.35259	0.000259	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer

-2.51194	1.65E-07	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
1.8661	0.002204	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	Non-significant
-2.72738	0.000482	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.43636	6.63E-07	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.24039	2.33E-06	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.02265	0.002421	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.0988	0.000189	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.49593	0.000766	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.94183	0.001073	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.91499	9.44E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.21643	1.08E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.46003	0.000889	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.90375	0.002793	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant

-2.16493	2.02E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.26018	0.000391	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.01636	0.001939	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.26772	3.75E-06	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.99056	0.00104	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.96863	0.000372	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
1.948505	0.000286	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus	Non-significant
-2.40707	0.000766	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.02901	3.47E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.17389	4.24E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.79682	0.00425	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.1666	0.000976	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.28642	0.000108	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer

-2.55264	0.001744	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.37351	0.0031	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.32602	1.24E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.53662	0.00104	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.52773	0.000242	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.45572	0.000387	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.70024	0.0074	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant
-1.78723	0.000451	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.84682	0.001083	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.6268	0.003074	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.70967	0.009979	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.16636	3.98E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.65762	0.006717	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant

-2.02504	5.34E-05	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
2.185683	5.32E-05	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	winter
-1.80638	0.006165	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.07019	0.00034	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-1.82582	0.002419	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant
-2.35276	0.001232	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
1.829913	0.00425	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-2.22244	0.000293	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.70278	0.004539	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.99287	0.000286	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
2.071284	0.002023	Bacteroidetes	Bacteroidia	Sphingobacteriales	env.OPS_17	NA	winter
-2.48364	0.001237	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.7806	0.003016	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant

-2.27378	0.00222	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.27243	9.16E-06	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
1.636788	0.003666	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus	Non-significant
-2.19712	0.002408	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
1.690428	0.005896	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	Candidatus_Methylopumilus	Non-significant
1.358878	0.006271	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	Non-significant
-2.15743	0.002632	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.69154	0.002498	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-2.04849	0.000937	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.2816	0.000203	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.67058	0.007947	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.03846	0.00208	Planctomyces	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.76687	0.001877	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant

-1.76014	0.005268	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.00882	0.000889	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
-1.97962	0.000437	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.54834	0.003666	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.77679	0.0015	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant
-1.60135	0.005363	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-2.0586	0.000847	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
1.851656	0.001744	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.84979	0.00257	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant
-1.89759	0.002858	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.71198	0.002809	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.71621	0.003666	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-2.11235	0.000766	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	summer

-2.0961	0.000249	Armatimonadetes	Armatimonadiales	Armatimonadales	Armatimonadaceae	Armatimonas	summer
-2.01833	0.005964	Armatimonadetes	Armatimonadiales	Armatimonadales	Armatimonadaceae	Armatimonas	summer
-1.63611	0.007307	Verrucomicrobia	Verrucomicrobiales	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.61003	0.008405	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.88693	0.006271	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	NA	Non-significant
-1.84468	0.007049	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	NA	Non-significant
1.810534	0.001155	Verrucomicrobia	Verrucomicrobiales	Chthoniobacteriales	Chthoniobacteraceae	Chthoniobacter	Non-significant
-1.86477	0.002548	Proteobacteria	Alphaproteobacteria	Caulobacteriales	Hyphomonadaceae	Hirschia	Non-significant
-1.79899	0.008417	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-2.13273	0.000222	Armatimonadetes	Armatimonadiales	Armatimonadales	Armatimonadaceae	Armatimonas	summer
-1.91892	0.000635	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-1.89493	0.00503	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant
-2.03298	0.000672	Verrucomicrobia	Verrucomicrobiales	Pedosphaerales	Pedosphaeraceae	NA	summer

-1.75059	0.00425	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	NA	Non-significant
-1.90386	0.001811	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.93433	0.004624	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.72446	0.009683	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.97488	0.003419	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.99802	0.007049	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-2.06053	0.001237	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
-1.86813	0.003776	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.79125	0.007694	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Methylophilaceae	NA	Non-significant
-1.97825	0.003419	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-2.11251	0.000766	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	summer
-1.85917	0.006562	Armatimonadetes	Armatimonadadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.89705	0.005401	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant

-1.66563	0.008417	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.96573	0.003049	Armatimonadetes	Armatimonadiales	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.96344	0.000847	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.74657	0.007635	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
1.880059	0.002774	Planctomycetes	Planctomycetacia	Planctomycetales	Schlesneriaceae	Schlesneria	Non-significant
-1.72761	0.003776	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant

Table A2.7. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in spring control samples or summer control samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Season
-2.55513	6.3E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.88764	0.004877	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Non-significant
-2.05519	0.001012	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.23196	0.005299	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcl_clade	summer

-2.8119	1.79E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.159	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.74293	1.43E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-3.09781	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.56788	5.61E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.17384	0.004666	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.2232	0.001012	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.44608	5.1E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.27993	0.008141	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.65771	9.49E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.61959	7.7E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.12751	0.002199	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.51391	0.000105	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-2.87099	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-1.75018	0.007562	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Non-significant
-2.84953	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.91675	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.47799	0.00011	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.63017	3.47E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.78362	1.79E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.06225	0.007133	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.67299	0.001883	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.80725	9.78E-06	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.26708	0.004718	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	summer
-2.05235	0.007707	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	summer
-2.68106	1.18E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-1.95123	0.00139	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Non-significant
-2.67092	0.000431	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.6558	9.52E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.19204	0.00895	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	summer
-2.59337	0.000612	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.68419	8.4E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.69969	6.3E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.06833	0.007707	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	summer
-2.43892	0.00774	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.01138	0.00103	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.59156	8.4E-05	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer
-2.33954	0.004877	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.45178	0.003103	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	summer

-2.34819	0.004572	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.26189	8.62E-05	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.5876	0.007707	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.015	0.000727	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.25506	0.007133	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.99544	0.000431	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.16631	0.007853	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	summer
-2.45909	0.003643	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.84083	0.005579	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.79593	0.0033	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.14588	0.000714	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
-1.74552	0.004572	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.16059	0.005672	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer

-2.08887	0.00883	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.92205	0.002726	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-2.27879	0.00274	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
-2.07127	0.001281	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	summer
-2.25864	0.004877	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-2.1867	0.007028	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.79015	0.003354	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Non-significant
-1.90083	0.003103	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	Non-significant
-2.15336	0.000394	Bacteroidetes	Bacteroidia	Cytophagales	Cyclobacteriaceae	Algoriphagus	summer
-2.0612	0.004711	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	summer
-1.8041	0.00774	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.93594	0.008055	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Non-significant
-2.1154	0.001883	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	summer

-1.67597	0.009661	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.77499	0.008788	Bacteroidetes	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Non-significant
-1.76134	0.009286	Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Pseudarcicella	Non-significant
-1.89062	0.0033	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
-1.83664	0.006185	Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
-1.87623	0.005672	Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	Armatimonas	Non-significant
1.995387	0.004877	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	Non-significant

Table A2.8. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 23°C control samples or fall 23 °C Bakken samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil Type
2.705214	0.000206	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Control
-1.95644	0.003327	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant

Table A2.9. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in spring control samples or spring diesel samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil Type
2.772816	0.000915	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	Diesel
2.349721	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.340454	0.004346	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.750541	0.001115	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Diesel
2.146937	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.776372	0.000915	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.169033	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.022789	0.008169	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Diesel
2.170822	0.008421	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.669104	0.005182	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Diesel
2.633506	0.00173	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.27088	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Diesel

2.420886	0.0077 26	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel
2.501453	0.0041 64	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	NA	Diesel
2.340281	0.0057 08	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel
2.176752	0.0057 08	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	Diesel
2.651692	0.0057 08	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel
2.384254	0.0077 26	Proteobacteria	Gammaproteobact eria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.449366	0.0041 64	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel
2.186852	0.0058 63	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	NA	Diesel
2.591682	0.0057 08	Actinobacteri a	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	Diesel
2.338117	0.0057 08	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel
2.58928	0.0058 63	Proteobacteria	Gammaproteobact eria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.410121	0.0081 43	Proteobacteria	Gammaproteobact eria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.30115	0.0056 79	Proteobacteria	Gammaproteobact eria	Betaproteobacteri ales	Burkholderiaceae	Janthinobacteri um	Diesel

2.255356	0.007892	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Diesel
2.345943	0.00585	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	Diesel
2.222771	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.217016	0.006792	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.123197	0.005708	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.331681	0.005708	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Janthinobacterium	Diesel
2.307061	0.005863	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade	Diesel
2.188599	0.007726	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.282703	0.006035	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Diesel
2.331096	0.005719	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Diesel
2.174731	0.009286	Proteobacteria	Alphaproteobacteria	SAR11_clade	Clade_III	NA	Diesel
2.258327	0.006035	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prostheco bacter	Diesel
2.187275	0.008555	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prostheco bacter	Diesel

2.07751	0.009162	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.087527	0.005863	Planctomycetes	Planctomycetacia	Planctomycetales	Rubinisphaeraceae	NA	Diesel
2.201557	0.00585	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	Diesel

Table A2.10. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in summer control samples or summer Bakken samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil_Type
1.846314	0.000709	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.72136	0.001601	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.932397	0.001601	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.735284	0.004789	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.721381	0.002816	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.892517	0.004196	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
2.117758	0.000348	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken

1.793758	0.003668	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.604865	0.00403	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.625186	0.007224	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.965674	0.001601	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.649297	0.004789	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
2.066637	0.001601	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
1.89741	0.006434	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.685345	0.006448	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.701687	0.002488	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.77111	0.005444	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
2.030501	0.003107	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
1.831416	0.002816	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant
1.789402	0.004789	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Non-significant

1.743951	0.0029 94	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
1.737243	0.0066 14	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
1.616912	0.0066 14	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
1.849342	0.0054 44	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
1.860956	0.0047 89	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
1.824067	0.0041 96	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
2.079344	0.0041 96	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	Acidovorax	Bakken
1.796701	0.0067 55	Proteobacteri a	Gammaproteobac teria	Salinisphaerales	Solimonadaceae	Nevskia	Non- significant
2.294786	0.0007 95	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	Acidovorax	Bakken
2.302535	0.0016 01	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	NA	Bakken
2.382969	0.0007 95	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	NA	Bakken
2.115385	0.0016 11	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	NA	Bakken
2.205236	0.0016 01	Proteobacteri a	Gammaproteobac teria	Betaproteobacter iales	Burkholderiaceae	NA	Bakken

1.986399	0.003107	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant
-1.66691	0.006434	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA	Non-significant
2.218171	0.001601	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	Bakken
2.089997	0.004789	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Acidovorax	Bakken
2.060291	0.002488	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Bakken
1.946895	0.007403	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant
2.006083	0.005577	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Tibeticola	Bakken
1.563374	0.004789	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sandarakinorhabdus	Non-significant
2.058631	0.004789	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Bakken
1.900415	0.007359	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	NA	Non-significant

Table A2.11. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in summer control samples or summer diesel samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil Type
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2.111955	0.005125	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prostheco bacter	Diesel
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Table A2.12. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 4°C control samples or fall 4°C Bakken samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil Type
2.655699	0.000829	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.357644	0.001689	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.294325	0.001208	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.486884	0.003788	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.283318	0.000829	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.088641	0.007433	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Bakken
2.03728	0.003788	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Limnohabitans	Bakken

Table A2.13. Analysis of 16S rRNA sequence reads results depicting ASVs significantly enriched in fall 4°C control samples or fall 4°C diesel samples generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Oil Type
2.746002	2.73E-05	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.133235	0.001999	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.327433	0.001354	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.111817	0.004222	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.395301	0.004222	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.20704	0.004222	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.469144	0.000594	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.327444	0.008313	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.387221	0.004222	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	Nevskia	Diesel
2.447345	0.001999	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Perlucidibactera	Diesel

Chapter Three Supplementary Material:

Table A3.1. Analysis of 16S rRNA sequence reads results depicting 14 ASVs significantly enriched in Caspian Sea sediment high or standard nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
13.90682	6.03E-30	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
13.21194	9.15E-27	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-10.21	1.77E-09	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Standard
-20.4841	2.75E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Standard
-4.0501	0.000944	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Standard
8.98777	3.02E-06	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-7.55318	0.000547	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Standard
-6.70887	1.82E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
8.380128	9.11E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
9.558014	5.28E-07	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
9.174595	1.3E-06	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
8.138125	0.000582	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	High

8.910586	3.09E-06	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	High
-7.16248	0.000582	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard

Table A3.2. Analysis of 16S rRNA sequence reads results depicting 4 ASVs significantly enriched in Caspian Sea sediment low or standard nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
-13.0831	1.58E-24	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
-12.391	2.58E-14	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
21.09336	3.66E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Stan.
21.07873	3.66E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Stan.
20.1038	8.63E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Stan.

Table A3.3. Analysis of 16S rRNA sequence reads results depicting 14 ASVs significantly enriched in Caspian Sea sediment high or low nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
12.26574	0.009732	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
20.37867	1.63E-06	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
-10.5575	3.48E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-9.18194	0.000192	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-9.64558	6.57E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	High
23.13342	1.14E-07	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Low
22.21212	2.65E-07	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
23.11717	1.14E-07	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Low
20.88584	9.08E-07	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
21.71275	3.72E-07	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	Low
22.11557	2.65E-07	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
21.61741	3.72E-07	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Low

Table A3.4. Analysis of 16S rRNA sequence reads results depicting 3 ASVs significantly enriched in iron-rich sediment standard or high nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
23.32299	1.82E-07	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Standard
23.24545	1.82E-07	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
20.80418	4.63E-06	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Standard

Table A3.5. Analysis of 16S rRNA sequence reads results depicting 7 ASVs significantly enriched in iron-rich sediment high or low nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
-21.6206	2.24E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
24.56694	1.64E-06	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
-23.1711	5.82E-06	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-20.9506	3.46E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
23.30494	4.33E-06	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Low

-22.1268	1.55E-05	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	High
-21.4262	2.34E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	High

Table A3.6. Analysis of 16S rRNA sequence reads results depicting 17 ASVs significantly enriched in farm compost high or standard nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrients
8.767105	0.001428	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard
8.224972	0.002518	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard
10.99216	1.07E-12	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard
8.973592	0.001086	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
7.885399	0.003052	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard
-5.14697	0.003119	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	High
8.697218	0.001155	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard
10.24821	1.5E-08	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadales	Stenotrophomonas	Standard

9.483126	0.000176	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
9.204119	7.73E-05	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
9.004795	0.001907	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
9.048224	0.001688	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	Standard
8.757543	0.000349	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Gordonia	Standard
8.893572	0.003052	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Standard
8.826653	0.000349	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	Standard
8.416042	0.001209	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Standard
-21.4619	3.98E-11	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	High

Table A3.7. Analysis of 16S rRNA sequence reads results depicting 3 ASVs significantly enriched in farm compost standard or low nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrient
10.4994	0.003009	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
9.738499	0.0022	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low

9.367789	0.0022	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Low
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Table A3.8. Analysis of 16S rRNA sequence reads results depicting 3 ASVs significantly enriched in farm compost standard or low nutrient enrichments generated in DESeq2.

log2FoldChange	padj	Phylum	Class	Order	Family	Genus	Nutrient
-22.8475	0.001799	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	High
-21.0854	0.001799	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	High
-19.8798	0.002633	Actinobacteria	Actinobacteria	Corynebacteriales	Nocardiaceae	Rhodococcus	High

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The contents of chapter two in this document are currently in review at the Journal of Great Lakes Research.

The contents of chapter three in this document will soon be submitted to the Journal of Applied Microbiology for publication.