THE BIODEGRADATION OF OIL AND THE DISPERSANT COREXIT 9500 IN ARCTIC SEAWATER

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Abstract

As oil and gas production continues in the Arctic, oil exploration and shipping traffic have increased due to the decline of Arctic sea ice. This increased activity in the Arctic Ocean poses a risk to the environment through the potential release of oil from cargo ships, oil tankers, pipelines, and future oil exploration. Understanding the fate of oil is crucial to understanding the impacts of a spill on the marine ecosystem. Previous oil biodegradation studies have demonstrated the ability of Arctic and sub-Arctic microorganisms to biodegrade oil; however, the rate at which oil degrades and the identity of indigenous oil-degrading microorganisms and functional genes in Arctic seawater remain unknown. In addition to oil, it is also important to understand the fate and effects of chemicals potentially used in oil spill response. Corexit 9500 is a chemical dispersant that is pre-approved for use in sub-Arctic seawater and is likely the dispersant of choice for spill responders in Arctic offshore environments. Currently no literature exists concerning the biodegradation of Corexit 9500 in Arctic seawater.

Here we investigate the fate of oil, chemically dispersed oil, and the chemical dispersant, Corexit 9500, in laboratory mesocosms containing freshly collected Arctic surface seawater. The objectives of these experiments were to calculate the extent and rate of biodegradation (based on GC/MS & LC/MS/MS analysis) and to identify bacteria (determined using 16S rRNA gene sequencing) and genes (based on GeoChip 5.0 microarray) potentially involved in the biodegradation process. Indigenous microorganisms degraded both fresh and weathered oil, in both the presence and absence of Corexit 9500, with oil losses ranging from 36-41% within 28 days and 46-61% within 60 days. The biodegradation of the active components of Corexit 9500, which are dioctyl sodium sulfosuccinate (DOSS) and non-ionic surfactants, was also measured after 28 days. Biodegradation of DOSS was 77% in offshore seawater and 33% in nearshore seawater. Non-ionic surfactants were non-detectable after 28 days. Taxa known to include oil-degrading bacteria (e.g. *Oleispira*, *Polaribacter*, and *Colwellia*) and oil-biodegradation genes (e.g. *alkB*) increased in relative abundance in response to both oil and Corexit 9500. These results increase our understanding of oil and dispersant biodegradation in the Arctic and suggest that some bacteria may be capable of biodegrading both oil and Corexit 9500.

We also sought to understand baseline abundances of taxa known to include oil-degrading bacteria and functional genes involved in oil biodegradation in an offshore oil lease area. Aerobic oil-degradation genes (based on GeoChip 5.0 microarray) and taxa (determined using 16S rRNA gene sequencing) known to include oil-degrading bacteria were identified in seawater from the surface, middle, and bottom of the water column. Bacterial community structure differed significantly by depth (surface water vs. bottom water), while the relative abundance of major functional gene categories did not differ with depth. These findings support previous observations that two different water masses contribute to a stratified water column in the summer open-water season of the oil lease area, but indicate that potential function is fairly similar with depth. These results will contribute to understanding the potential for oil biodegradation throughout the Arctic water column and the fundamental microbial ecology of an offshore oil lease area.

Together, these mesocosm experiments and *in situ* studies address important data gaps concerning the fate of spilled oil and Corexit in Arctic seawater. These results provide novel insight into the ability of Arctic bacteria to biodegrade crude oil and Corexit 9500, and suggest similarities between Arctic and temperate deep-sea environments in regards to taxa and functional genes that respond to oil and Corexit.

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Chapter 1: General Introduction

Summer sea ice coverage in the Arctic has reached the lowest extent on record and continues to decline at a rate such that some models predict an ice-free Arctic by 2040 (Comiso et al., 2008; Holland et al., 2006). Areas that were once covered in ice year round are now accessible for shipping, tourism, and oil and gas exploration. This projected rise in activity increases the risk of oil spills and generates the need to advance environmental research to address data gaps regarding appropriate spill responses in Arctic marine environments.

Oil-degrading microorganisms have been discovered from pole to pole and are thought to be ubiquitous (Schneiker et al., 2006; Head et al., 2006; Yakimov et al., 2007). In a variety of environments, both terrestrial and marine, microorganisms have evolved over time to utilize petroleum hydrocarbons as a source of carbon and energy (Prince et al., 2010). Natural oil seeps have been discovered throughout the World's oceans, including in the Arctic (NRC, 2003), and these seeps continue to enrich oil-degrading microorganisms (Prince & Clark, 2004). With the advent of DNA sequencing technologies, indigenous oil-degrading bacteria in deep-sea and in temperate environments have been identified (Hazen et al., 2010; Chakraborty et al., 2012), but relatively little is known about the potential for Arctic marine bacteria to degrade oil.

When an oil spill necessitates a response, spill responders have three main options. Depending upon the conditions, these techniques are usually applied concurrently with no single response option applicable to all situations. With the aid of specifically engineered booms or herders, floating oil can be mechanically recovered (e.g. skimmers), or burnt (i.e. *in situ* burning) (Fingas, 2016). In addition, with the aid of aircraft or large boats affixed with sprayers, floating oil can also be dispersed into the water column with chemical dispersants. Corexit 9500 is the chemical dispersant most likely to be chosen for use in the Arctic Ocean due to its prior approval in subarctic Alaskan waters (ARRT, 2016) and reported effectiveness in temperate environments (Bejarano et al., 2013). When dispersants are applied to an oil spill, the concentrated oil slick is mixed into the water column as tiny droplets with the help of physical energy, such as wave action. The formation of these droplets is essential to the biodegradation process, as they increase the surface area available to oil-degrading microorganisms and can significantly increase oil biodegradation (Brakstad et al., 2015; Prince & Butler, 2014). Testing these response options in

environmentally relevant conditions and communicating these results to responders is critical to an efficient emergency response.

This dissertation addresses data gaps concerning the biodegradation of oil and Corexit 9500 in the Arctic Ocean. The fate and effects of chemically dispersed oil in the Arctic environment has recently been identified as a recommendation for future research by the National Academy of Sciences (NRC, 2014). The research herein reports rates at which whole oil and Corexit biodegrade in Arctic seawater and identifies bacteria and genes potentially involved in the biodegradation of oil and Corexit. Using a combination of laboratory mesocosms and *in situ* measurements; physical, chemical, and genetic analyses were conducted to understand the fate of oil and Corexit and the impact that these mixtures have on bacterial community structure and functional potential, as well as to survey an offshore oil lease area for the organisms and genes important to oil biodegradation. To my knowledge, these experiments are the first to use freshly collected Arctic seawater containing indigenous microorganisms to address these data gaps. Overall, these results indicate that significant oil and Corexit biodegradation can occur in the Arctic Ocean without adding large amounts of nutrients or microbial cultures.

This dissertation includes three research-based chapters. Chapter 2 describes the biodegradation of Alaska North Slope (ANS) crude oil by indigenous Arctic marine microorganisms in nearshore Arctic seawater in the presence and absence of added nutrients. At -1°C, primary biodegradation (total measureable and many individual hydrocarbons) and mineralization were measured in mesocosms that mimicked environmental conditions following a successful dispersion of a surface oil slick. Arctic microorganisms significantly degraded both fresh and weathered oil, in both the presence and absence of Corexit 9500. In addition, this study was the first to report the ability of indigenous Arctic marine microorganisms to mineralize Corexit 9500. This chapter provides novel insight into the extents of biodegradation at one of the lowest temperatures ever reported, -1˚C.

Chapter 3 details the biodegradation of ANS crude oil and Corexit 9500 in Arctic surface seawater collected from: (1) an offshore oil lease area (Burger; ~90 km from Wainwright, AK) and (2) a nearshore location (~1 km from Barrow, AK). This nearshore location is similar to the location where the seawater for Chapter 2 was collected. Mesocosm studies were conducted using freshly collected seawater spiked with either oil or Corexit to

determine rates of biodegradation and effects on natural Arctic bacteria. This chapter contains the first report of biodegradation rates of oil (total measurable hydrocarbons) and Corexit components (dioctyl sodium sulfosuccinate, DOSS, and the non-ionic surfactants) in Arctic seawater. Abundances of total prokaryotes, as well as bacterial community structure (using 16S rRNA gene sequencing) and functional genes known for oil-biodegradation (e.g. *alkB,* using the GeoChip 5.0 microarray) were compared between oil and Corexit incubations. In the natural seawater, both oil and Corexit enriched some of the same bacterial 'species' (97% similarity) and genes known to biodegrade oil, but overall Corexit was shown to enrich a greater abundance of prokaryotes compared to oil. These results suggest that some bacteria may be capable of biodegrading both oil and Corexit.

The final research chapter, Chapter 4, builds upon the mesocosm studies described in Chapters 2 and 3 by reporting *in situ* oil biodegradation potentials in the Arctic Ocean. In an offshore oil lease area, I characterized the bacterial community structure (using 16S rRNA gene sequencing) and detected the relative abundance of functional genes (using the GeoChip 5.0 microarray), including oil biodegradation and biogeochemical cycling (carbon, nitrogen, and phosphorus cycling) genes in surface, middle, and bottom seawater samples. These data were then correlated to physical and biogeochemical measurements within the oil lease area. Oil-degrading genes and taxa known to contain species able to biodegrade oil were located throughout the water column in relatively similar abundances. These findings support previous observations that two different water masses contribute to a stratified water column in the Chukchi Sea during the summer open-water season. The overall genetic potential for oil biodegradation or biogeochemical cycling was not affected by stratifications in temperature, water chemistry, and bacterial community structure. These baseline community trends may be useful to those assessing the effects of climate change and oil exploration on microbial communities in the Chukchi Sea.

The primary objectives of this dissertation were to (1) quantify the biodegradation of oil and the chemical dispersant Corexit 9500 by indigenous Arctic marine microorganisms, (2) characterize the effects of oil and Corexit on bacterial community structure, (3) determine the identity of biodegradation genes potentially utilized by indigenous Arctic marine bacteria when biodegrading oil or Corexit, and (4) contribute to an ecological baseline analysis of an offshore oil lease area by documenting bacterial community structure and functional potential to

biodegrade oil and cycle nutrients. Together, these results provide novel insight into the biodegradation of crude oil and Corexit 9500 by Arctic microorganisms and contribute to comprehensive oil spill research recommended by the National Academy of Sciences to assess oil spill response technologies in the Arctic marine environment.

References

ARRT. (2016). Alaska Federal/State Preparedness Plan for Response to Oil & Hazardous Substance Discharges/Releases (Unified Plan). Annex F, Appendix I. Alaska Regional Response Team Dispersant Use Plan for Alaska.

Bejarano AC, Levine E, Mearns AJ. (2013). Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. *Environ. Monitor. Assess.* 185:10281-10295.

Brakstad OG, Nordtug T, Throne-Holst M. (2015). Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Marine Poll. Bull.* 93:144-152.

Chakraborty R, Borglin SE, Dubinsky EA, Andersen GL, Hazen TC. (2012). Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. *Front. Microbiol.* 3:357.

Comiso JC, Parkinson CL, Gersten R, Stock L. (2008). Accelerated decline in the Arctic sea ice cover. *Geophys. Res. Lett.* 35:L01703.

Fingas M. (2016). *Oil spill science and technology*. Gulf professional publishing.

Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, et al. (2010). Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science*. 330:204-208.

Head IM, Jones DM, Röling WF. (2006). Marine microorganisms make a meal of oil. *Nature Rev. Microbiol.* 4:173-182.

Holland MM, Bitz CM, Tremblay B. (2006). Future abrupt reductions in the summer Arctic sea ice. *Geophys. Res. Lett.* 33:L23503.

National Research Council (NRC). *Oil in the Sea III: Inputs, Fates and Effects;* National Academy Press: Washington, DC, 2003.

National Research Council (NRC). *Responding to Oil Spills in the U.S. Arctic Marine Environment*; National Academies Press: Washington, DC, 2014.

Prince RC, Clark JR. (2004). Bioremediation of marine oil spills. In *Studies in Surface Science and Catalysis 151*; Vazques-Duhalt R, Quintero-Ramirez R, Eds.; Elsevier B.V.; pp 495-509.

Prince RC, Gramain A, McGenity TJ. (2010). Prokaryotic hydrocarbon degraders. In *Handbook of Hydrocarbon and Lipid Microbiology*; Timms KN, Ed.; Springer-Verlag: Heidelberg, Berlin; pp 1627-1692.

Prince RC, Butler JD. (2014) A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environ. Sci. Pollut. R.* 21:9506-9510.

Schneiker S, dos Santos VAM, Bartels D, Bekel T, Brecht M, Buhrmester J, et al. (2006). Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium *Alcanivorax borkumensis*. *Nature Biotechnol.* 24:997-1004.

Yakimov MM, Timmis KN, Golyshin PN. (2007). Obligate oil-degrading marine bacteria. *Curr. Opin. Biotech.* 18:257-266.

Chapter 2: Biodegradation of Dispersed Oil in Arctic Seawater at -1°C[1]

Abstract

As offshore oil and gas exploration expands in the Arctic, it is important to expand the scientific understanding of Arctic ecology and environmental impact to mitigate operational risks. Understanding the fate of oil in Arctic seawater is a key factor for consideration. Here we report the chemical loss due to the biodegradation of Alaska North Slope (ANS) crude oil that would occur in the water column following the successful dispersion of a surface oil slick. Primary biodegradation and mineralization were measured in mesocosms containing Arctic seawater collected from the Chukchi Sea, Alaska, incubated at -1°C. Indigenous microorganisms degraded both fresh and weathered oil, in both the presence and absence of Corexit 9500, with oil losses ranging from 46-61% and up to 11% mineralization over 60 days. When tested alone, 14% of 50 mg/L Corexit 9500 was mineralized within 60 days. Our study reveals that microorganisms indigenous to Arctic seawater are capable of performing extensive biodegradation of chemically and physically dispersed oil at an environmentally relevant temperature (-1˚C) without any additional nutrients.

[1] McFarlin KM, Prince RC, Perkins R, Leigh MB. (2014). Biodegradation of dispersed crude oil in Arctic seawater at -1˚C. PLoS ONE 9:e84297

Introduction

As the oil and gas industry continues offshore exploration in the Arctic, it is imperative to base design and operational plans on a deep scientific understanding of the Arctic ecology and the potential environmental impact in order to mitigate risks. The Arctic's fragile environment and sensitive ecology present unique challenges. Understanding the rate and extent of oil biodegradation in cold-water environments is a key factor for consideration. Biodegradation is generally believed to be the dominant process that removes petroleum compounds from the environment [1], but the process has not been thoroughly studied in the Arctic, and questions remain as to whether biodegradation is a significant process in cold conditions [2]. Microorganisms capable of using hydrocarbons as a source of carbon and energy are diverse and widespread [3], including in the Arctic [4] and other cold environments [5-8].

Dispersant application is a potential oil spill response option in Arctic marine environments. Corexit 9500 has been shown to be effective in dispersing Alaska North Slope (ANS) crude oil in a large outdoor wave tank at temperatures ranging from 0-3°C [9]. When Corexit 9500 was widely applied to the Deepwater Horizon spill, some research suggested that the dispersant was not toxic to the indigenous Gulf of Mexico microorganisms and that some bacterial species were capable of degrading various components of the dispersant [10]. However, little is known about the degradation of Corexit 9500 and dispersed oil in the Arctic marine environment.

When a dispersant is applied to an oil slick it reduces the interfacial surface tension between the water and the oil, allowing the oil to become mixed into the water column as tiny (1-70 μm) droplets [11] with mild wave action. The creation of small oil droplets increases the surface area available for microbial colonization [12] and can significantly increase biodegradation [11, 13-16]. The localized concentration of oil drastically decreases as it is chemically dispersed into the water column and has been reported to range from 1-15 mg/L beneath (1-5 m) oil slicks treated with dispersant within a few hours after the application [13, 17-19]. This study aimed to assess the biodegradation of oil at concentrations that are expected in the water column following successful dispersion in the Chukchi Sea.

Several laboratory studies have investigated sub-Arctic oil biodegradation in Alaska [20, 21], Canada [5], and Arctic and sub-Arctic Norway [6, 8, 22], but with diverse experimental methodologies, making direct comparisons of biodegradation data challenging. Previous studies

have used a range of oil loadings and in some cases the addition of enrichment cultures and/or high quantities of nutrient amendments. Most experiments have been conducted using high dispersed-oil concentrations (100-900 mg/L) [19, 21, 23], while few studies have focused on the low dispersed-oil concentrations (1-15 mg/L) that soon occur beneath a dispersed slick [13, 17-19]. Other studies have supplemented oil incubations with large quantities of nutrients, which accelerate biodegradation rates [24-26] but may create an experimental system that does not accurately represent environmental conditions.

This study measures the biodegradation of Alaska North Slope crude oil [27] that would occur in the water column following a successful application of Corexit 9500 to a surface oil slick. Arctic seawater with its indigenous microbial community was collected and experiments were conducted at the temperature of the water at the time of collection (-1°C) with natural or slightly enhanced nutrient levels. Biodegradation was measured using respirometry and gas chromatography-mass spectrometry (GC-MS) analysis.

Methods

Field studies did not involve endangered or protected species. Water collection did not require specific permission because the Chukchi Sea is not a federal or state protected area.

Seawater collection

Seawater (free of slush and ice) was collected from the eastern edge of the Chukchi Sea, approximately 2.5 km East of where the Chukchi Sea meets the Beaufort Sea. Samples were collected 1 km from Barrow, AK (N 71°21'43", W 156°40'13") from beneath 1 m of ice. Seawater was immediately transferred to the laboratory cold-room in clean Nalgene® rectangular carboys and aerated with aquarium air stones until test initiation. The test waters did not contain visible particulates and were not filtered. Measured seawater quality parameters at collection included pH (8.05), temperature (-1°C), dissolved oxygen (11.6 mg/L) and salinity (33 ppt). Nutrient levels (nitrate, nitrite, and ammonia) were below detection limits by simple colorimetric tests (DR/850, Hach, Colorado).

Experimental environmental parameters

All incubations were performed in a cold room, which was kept at -1°C, and under low light (Photosynthetically Active Radiation (PAR) of 1.83 µmol●s^{-1}●m^{-2}; LI-193SA Spherical Quantum Sensor, LI-COR, Nebraska).

Oil and dispersant

A single batch of Alaska North Slope (ANS) crude oil was collected from the Alyeska terminal in 2009. Some was artificially weathered by allowing a known weight of static fresh oil to evaporate (at room temperature) in a fume hood until it had lost 20% of its initial mass to approximate oil that might have been floating at sea for 12-24 hours [28]. The dispersant used in this study was Corexit 9500 [29]. Because of the small volumes involved, oil and dispersant were premixed before addition to the experimental chambers. The biodegradation of dispersed oil was tested at 1:20 and 1:15 dispersant to oil ratios (DORs) and the mineralization of Corexit 9500 alone was measured at 50 mg/L. The 1:20 DOR application rate is the target ratio in oil spill response, although ratios as high as 1:10 have been required with more emulsified and viscous heavy oils [19].

Biodegradation experiments

Low concentrations of oil were tested (2.5 mg/L and 15 mg/L) in order to assess the biodegradation of dispersed oil at concentrations that are expected to approach those found in the water column after successful dispersion. Two methods were used to quantify the biodegradation of oil. The first measured the primary biodegradation of the oil, i.e. the chemical disappearance of specific hydrocarbons, monitored with respect to a conserved internal marker within the oil (hopane) [30]. Primary biodegradation was measured in open top mesocosms (2.5 mg/L oil in 4 L of unamended seawater) and closed respirometer flasks (15 mg/L oil in 900 ml seawater + nutrients). Respirometer flasks contained their own oxygen generation system. The second method measured the mineralization of oil to CO_2 and H_2O with an electrolytic respirometer (Co-ordinated Environmental Service; Kent, England) and required the use of higher oil concentrations (15 mg/L oil in 900 ml seawater + nutrients) because of the detection limits of the instrument. Preliminary experiments indicated that the biodegradation of such concentrations (15 mg/L) would likely be limited by the availability of nitrogen and phosphorus in the seawater, so

low levels of nutrients (16 mg/L Bushnell Haas Broth [31]) were added to respirometer flasks, providing 75 μM phosphate, 49 μM nitrate and 76 μM ammonium. For comparison, Codispoti *et al.* [32] measured background nutrient levels adjacent to Point Barrow and reported 0.8 μM phosphate, 6 μM nitrate and 6 μM ammonium in summer surface seawater.

All treatments were continuously mixed (400 rpm) using Teflon coated stir-bars. Respirometer experiments were carried out in general agreement with the Organization for Economic Cooperation and Development 301F [33] guidelines for biodegradation testing, with the omission of a microbial inoculum. Mineralization was determined as a function of the sample's theoretical oxygen demand (ThOD): the amount of O_2 required to mineralize the sample. ThOD was calculated based upon analytical measurements of the substrate's elemental composition (QTI, Whitehouse, NJ): ANS crude oil contained 84.9% carbon, 12.0% hydrogen, and 0.39% nitrogen (ThOD = 3.32 mg O_2/mg oil), while oil plus Corexit 9500 (20:1) contained 83.8% carbon, 11.6% hydrogen, and 0.32% nitrogen (ThOD = 3.30 mg O_2/mg dispersed oil). Positive controls for the respirometry contained either sodium benzoate or peptone at 50 mg/L. Negative controls for the respirometry experiments contained seawater and 0.5% BH with no oil or dispersant addition. Materials necessary for sterile controls were unavailable in our remote Arctic research facility. The minimal respiration measured in the negative controls (3 replicates) was subtracted from all respirometer treatments.

Oil analyses

Petroleum hydrocarbons were extracted from all experimental incubations and analyzed with GC-MS as described by Douglas *et al.* [34]. Oil biodegradation was determined with respect to 17α(H),21β(H)-hopane as a conserved internal marker within the oil [30]. Each experimental container was extracted three times with methylene chloride. The combined extract was concentrated to a nominal concentration of approximately 10 mg/ml by evaporation to a small volume (but not to dryness), then dried of water and filtered by passage through a column of anhydrous sodium sulfate. All treatments were analyzed for total detectable hydrocarbons as well as individual aromatics and alkanes.

Statistical analyses

Levene's test for equality of variances and the t-test for equality of means were performed with a 95% confidence interval using a statistical package for social science, version 16 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The primary goal of this study was to quantify the chemical loss due to the biodegradation of dispersed oil at low temperature by indigenous microorganisms in Arctic seawater. Extensive biodegradation of ANS crude oil occurred at -1°C, both in the presence and absence of the dispersant Corexit 9500 (Figure 2-1). By the end of the incubation period (~60 days), 46-61% of total measurable oil was lost (Figure 2-2, Table 2-1). Percent losses were affected by prior weathering of oil and by the presence of Corexit 9500.

As reported by Venosa and Holder [21] at warmer temperatures, Corexit 9500 initially enhanced the rate of biodegradation, although differences between treatments with and without Corexit became smaller as time progressed (Figure 2-2). At the end of the experiments (56-63 days), the indigenous Arctic microbial community (in incubations containing 2.5 mg/L fresh and weathered oil), had biodegraded almost 100% of heptadecane, octadecane, and individual aromatics; including EPA listed priority pollutants (Figures 2-3 & 2-4) regardless of the presence of Corexit 9500. In addition, the complete loss of phenanthrene, C-1 phenanthrenes, dibenzothiophenes and C1-dibenzothiophenes was observed with or without Corexit in fresh oil incubations (Figure 2-4). The loss of C2-phenanthrenes was close to 90%, while almost 80% of C2-dibenzothiophenes was removed, as was approximately 60% of benz[a]anthracene (Figure 2-4). In addition, the indigenous microbial community was able to biodegrade the four-ringed PAH, chrysene (Figure 2-4). As expected, the degradation rates were lower for chrysene than for the lower molecular weight compounds (e.g., naphthalene, phenanthrene; Figures 2-3 & 2-4). The patterns of petroleum hydrocarbon biodegradation observed in these experiments were similar to those observed previously in both sub-Arctic and temperate conditions [34]; with the shorter, straight-chained alkanes more readily degraded than longer *n*-alkanes and branched alkanes, lower molecular weight PAHs more readily degraded than higher molecular weight PAHs, and parent PAHs degraded before their alkylated homologues.

It is not surprising that at such low oil concentrations only small differences in biodegradation were observed with the addition of Corexit 9500, although some have expressed concern that Corexit might be inhibitory [20, 35]. The desire to test equivalent oil loadings for both dispersant-treated and untreated mesocosms resulted in both treatments having very similar quantities of dispersed oil, as the small volumes of oil added to the mesocosms dispersed naturally without Corexit addition due to the physical mixing. In the environment, it is unlikely that a large oil slick would be physically dispersed to these low concentrations without dispersant application. The important finding of this work is that indigenous Arctic marine microorganisms are capable of extensive biodegradation of dispersed oil, regardless of whether physically or chemically dispersed. We note, though, that dispersants such as Corexit 9500 dramatically increase the amount of dispersed oil in the water column, and minimize surface slicks.

The oil losses detected in this study are primarily due to biodegradation, although volatilization may be responsible for a portion of the loss of low molecular weight compounds in fresh oil. The chromatograms shown in Figure 2-1 are normalized to an internal marker within the oil (17α(H),21β(H)-hopane) [30], so the losses represent the primary biodegradation of total extractable hydrocarbons rather than, for example, physical loss due to poor extraction. In addition to normalizing GC-MS data to conservative markers, such as hopane, reporting ratios of degradable hydrocarbons (alkanes or PAHs) to recalcitrant hydrocarbons (isoprenoids) has also been used as a measure of biodegradation [15, 36, 37]. Table 2-2 summarizes the C_{18} to phytane ratio at 10, 28 and 63 days in mesocosms containing 2.5 mg/L of oil with and without Corexit 9500. The ratio decreases with time, as expected during biodegradation.

Biodegradation of dilute oil is extensive in temperate [38, 39], tropical [40] and Arctic environments (this study), although the rates of biodegradation vary based on region. By the end of our incubations (~60 days), 46-61% of total GC-detectable oil had been lost (Figure 2-2). Thus the overall 'half-life' of crude oil in the Chukchi Sea at -1°C, when present at low concentrations (2.5-15 mg/L), was on the order of 60 days. Using the same methods as our study, Prince *et al.* [39] measured an 82-88% loss of weathered ANS crude oil without and with Corexit 9500 in incubations with New Jersey seawater at 8°C after 60 days. They also measured the percent loss of fresh oil and concluded that 81-82% of oil was lost after 41 days, which corresponds to an 11-14 day half-life with and without Corexit, respectively. Zahed *et al.* [40] also measured the half-life and percent loss of crude oil (100 mg/L) in incubations with

indigenous seawater enrichment cultures from Butterworth, Malaysia with and without Corexit 9500 at 28˚C. In tropical seawater and with a light crude oil, they reported a maximum loss of 67 and 64% of total petroleum hydrocarbons over 45-day incubations, with half-lives of 28 and 31 days, respectively. Hazen *et al.* [38] reported half-lives of soluble alkanes in MC252 oil dispersed with Corexit 9500 in the Deepwater Horizon subsurface (1099-1219 m) plume to range from 1.2-6.1 days at a temperature of 5˚C. Of course the half-life of petroleum does not predict the persistence of total oil, as the low molecular weight hydrocarbons are more readily biodegraded than the larger compounds. Half-life measurements are dependent on many factors, with oil concentration and composition being very important, making direct comparisons among different studies using different oil loadings and types challenging. However, all these studies show that indigenous microbial communities are able to degrade a substantial amount of petroleum in tropical, temperate and Arctic environments.

Crude oils are very dense sources of carbon and energy that provide no biologically available nitrogen or phosphorus, which are essential for microbial growth. If oil is stranded on shorelines, this limitation can be overcome by the application of fertilizers [41], even in the Arctic [42]. When successfully applied to an oil slick, dispersants dilute the oil so that the natural background levels of nutrients in the sea may support the microbial community during oil biodegradation [39]. In our 60-day experiments, 4 L mesocosms containing 2.5 mg/L oil and no added nutrients exhibited similar oil biodegradation extents as smaller (900 ml) respirometer flasks containing 15 mg/L oil and additional nutrients (16 mg/L Bushnell Haas medium). Because the aim of the study was to replicate environmental conditions as much as possible, higher concentrations of nutrients were not tested to evaluate whether additional nutrients might accelerate biodegradation.

In addition to measuring primary biodegradation (loss of individual chemicals) using GC/MS, we also measured mineralization (i.e. the complete respiration of substrate to CO_2 and H_2O) with respirometry. Although primary biodegradation was slightly enhanced by Corexit 9500 (Figure 2-2), no stimulatory effect of the dispersant was observed for mineralization (Figure 2-5). The indigenous microbial community mineralized 11% of weathered crude oil (15 mg/L) after 56 days in both physically and chemically dispersed treatments. Lindstrom and Braddock [20] compared the mineralization of oil with and without dispersant using a microbial enrichment culture from a sub-Arctic spill site, and similarly concluded that Corexit 9500 had

little effect on mineralization. Baelum *et al.* [15] also saw no significant difference in mineralization rates in incubations containing MC252 oil with and without Corexit 9500 at 5°C after 20 days, but reported a significant increase in primary biodegradation (60% vs. 25%) with the addition of Corexit.

There are several factors contributing to the fact that percent mineralization will likely always be lower than primary degradation. One is that oil-degrading bacteria utilize petroleum as a carbon source, integrating a portion of hydrocarbon metabolites directly into biomass [43-45]. Another is that GC analyses only measure a fraction of the hydrocarbons in most oils, while percent mineralization is based on total oil. The resin and polar fractions of the oil [46] are not significantly volatile, and do not enter the GC column, and nor do hydrocarbons with more than about 40 carbon atoms. All of these molecules are expected to degrade more slowly than hydrocarbons with <30 carbon atoms, although evidence is accumulating that resins and asphaltenes are at least partially degradable [47, 48]. Taking these two effects into consideration, our respirometry data are not inconsistent with our GC data.

Biodegradation of the dispersant alone was also examined using respirometry. Primary biodegradation of Corexit 9500 was not assessed, since solvent extraction and the GC/MS methods used in this study do not accurately measure all the components present in the dispersant. The concentration of Corexit 9500 (50 mg/L) in dispersant-only incubations was considerably higher than the concentration of Corexit in chemically dispersed oil incubations (0.75 mg/L) to enable detection of mineralization, and mineralization may have been nutrient limited due to the use of such a high concentration in our low-nutrient incubations. Nevertheless, approximately 14% of Corexit 9500 (50 mg/L) was mineralized by the indigenous Arctic marine microbial community within 60 days at -1˚C (Figure 2-5). Incubations with Corexit 9500 alone (50 mg/L) consumed more oxygen and at a much faster rate than treatments containing oil (15 mg/L oil + 0.75 mg/L Corexit). The rate of oxygen consumption in the Corexit 9500 treatment (50 mg/L) was the greatest over the first 10 days, while the treatments containing 15 mg/L weathered ANS and weathered ANS plus Corexit (15 mg/L oil + 0.75 mg/L Corexit) reached a maximum rate of oxygen consumption between day 12 and day 16, respectively. Almost no lag period was observed for the mineralization of Corexit 9500 alone (Figure 2-5), suggesting that the indigenous microbial community can readily initiate biodegradation of at least some components of the dispersant. The overall mineralization pattern of ANS and Corexit observed in

these experiments are similar to the results of Lindstrom and Braddock [20], who reported that Corexit 9500 was mineralized faster than fresh ANS crude, which in turn was mineralized faster than weathered ANS crude. Future studies using analytical methods capable of measuring chemical losses of dispersant components, such as LC-MS, would enable a more thorough understanding of Corexit 9500 biodegradation.

Conclusions

To our knowledge, this is the first study to measure the biodegradation of a crude oil, with and without a dispersant, at environmentally relevant concentrations [17] by an indigenous Arctic microbial community at sub-zero temperatures. Microorganisms indigenous to the Chukchi Sea were found to degrade both fresh and weathered crude oil in the presence and absence of Corexit 9500 at -1˚C, with oil losses ranging from 46-61% and up to 11% mineralization over 60 days. Weathered ANS dispersed with Corexit 9500 underwent a 57% loss in Arctic seawater after 60 days in our experiment, but experienced an 88% loss in New Jersey seawater in the same time [39]. These experiments suggest that in the Arctic, ANS crude oil degrades more slowly than oil in temperate regions, but that oil losses were still substantial even at -1˚C. There is evidence that Corexit 9500 initially stimulated oil biodegradation (Figures 2-1 & 2-2), but, as expected, its effects were minimal in longer-term incubations. We conclude that the biodegradation of oil in Arctic seawater is extensive at -1˚C, and that the biodegradation of dilute, dispersed oil is not inhibited by the presence of Corexit 9500. Although no microbial analyses are reported, it is apparent that the chemical loss of oil is indeed microbial. The respiration measured in all treatments could only be the result of indigenous microorganisms mineralizing oil and/or dispersant, since the minimal respiration measured in the background controls (seawater + nutrients) was subtracted from the respiration measured in the treatments (seawater + nutrients + oil and/or dispersant). Furthermore, the selective disappearance of some chemicals before others, whether referred to hopane, or for example the older C_{18} to phytane ratio, is a diagnostic for biodegradation [49]. Future work will focus on biodegradation rates in offshore Arctic oil lease areas and on the identification of microorganisms and genes active in biodegradation. Additional research in the Arctic is needed to address the behavior and biodegradation of oil spilled in ice-covered waters.

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References

1. National Research Council (2003) Oil in the sea III: inputs, fates and effects. National Academy Press: Washington DC.

2. Government Accountability Office (2012) Oil dispersants: Additional research needed, particularly on subsurface and Arctic applications. GAO-12-585 http://www.gao.gov/products/GAO-12-585. (accessed 3/26/2013)

3. Prince RC, Gramain A, McGenity TJ (2010) Prokaryotic hydrocarbon degraders. In: Timmis KN, editors. Handbook of Hydrocarbon and Lipid Microbiology: Springer-Verlag, Berlin, Heidelberg, pp. 1672-1692.

4. Mulkins-Phillips GJ, Stewart, JE (1974) Distribution of hydrocarbon-utilizing bacteria in Northwestern Atlantic waters and coastal sediments. Can J Microbiol 20: 955-962.

5. Siron R, Pelletier E, Brochu C (1995) Environmental factors influencing the biodegradation of petroleum hydrocarbons in cold seawater. Arch Environ Contam Toxicol 28: 406–416.

6. Deppe U, Richnow H, Michaelis W, Antranikian G (2005) Degradation of crude oil by an Arctic microbial consortium. Extremophiles 9: 461-470.

7. Gerdes B, Brinkmeyer R, Dieckmann G, Helmke E (2005) Influence of crude oil on changes of bacterial communities in Arctic sea-ice. FEMS Microbiol Ecol 53: 129-139.

8. Brakstad OG, Bonaunet K (2006) Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5˚C) and bacterial communities associated with degradation. Biodegradation 17: 71-82.

9. Belore RC, Trudel K, Mullin JV, Guarino A (2009) Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. Mar Pollut Bull 58: 118–128.

10. Chakraborty R, Borglin SE, Dubinsky EA, Andersen GL, Hazen TC (2012) Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. Front Microbiol 3: 357.

11. Lessard RR and G Demarco (2000) The significance of oil spill dispersants. Spill Sci Technol Bull 6: 59-68.

12. Ron EZ and E Rosenberg (2002) Biosurfactants and oil bioremediation. Curr Opin Biotechnol 13: 249-252.

13. Lunel T, Rusin J, Bailey N, Halliwell C, Davies L (1997) The net environmental benefit of a successful dispersant application at the *Sea Empress* incident. In: Proceedings of the 1997 International Oil Spill Conference. Fort Lauderdale, FL, American Petroleum Institute. pp. 185-194.

14. Swannell RPJ, Daniel F, Croft BC, Engelhardt MA, Wilson S, Mitchell DJ, Lunel T (1997) Influence of physical and chemical dispersion on the biodegradation of oil under simulated marine conditions. In: Proceedings of the 20th Arctic and Marine Oilspill Program Technical Seminar, Environment Canada, Vancouver, BC, Canada, 617-641.

15. Baelum J, Borglin S, Chakraborty R, Fortney JL, Lamendella R, Mason OU, Auer M, Zemla M, Bill M, Conrad ME, Malfatti SA, Tringe, SG, Holman H, Hazen TC, Jansson JK (2012) Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. Environ Microbiol 14: 2405-2416.

16. Prince RC and J Butler (2013) A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. Environ Sci Pollut Res http://dx.doi.org/10.1007/s11356-013-2053-7

17. Lee K, Nedwed T, Prince RC, Palandro D (2013) Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea. Mar Pollut Bull 73: 314-318.

18. Li Z, Lee K, King T, Boufadel MC, Venosa AD (2009) Evaluating crude oil chemical dispersion efficacy in a flow-through wave tank under regular non-breaking wave and breaking wave conditions. Mar Pollut Bull 58: 735–744.

19. National Research Council (2005) Oil spill dispersants: efficacy and effects. Washington DC: National Academy Press.

20. Lindstrom JE and JF Braddock (2002) Biodegradation of petroleum hydrocarbons at low temperatures in the presence of the dispersant Corexit 9500. Mar Pollut Bull 44: 739-747.

21. Venosa AD and EL Holder (2007) Biodegradability of dispersed crude oil at two temperatures. Mar Pollut Bull 54: 545-553.

22. Garrett RM, Rothenburger SJ, Prince RC (2003) Biodegradation of fuel oil under laboratory and Arctic marine conditions. Spill Sci Tech B 8: 297-302.

23. Davies L, Daniel F, Swannell R, Braddock J (2001) Biodegradability of chemically-dispersed oil. Report produced for the Minerals Management Service (MMS), Alaska Department of Environmental Conservation (ADEC) and the United States Coast Guard (USCG). http://dec.alaska.gov/spar/perp/r_d/aeat.pdf (accessed March 5, 2012).

24. Delille D, Basseres A, Dessommes A (1998) Effectiveness of bioremediation for oil-polluted Antarctic seawater. Polar Biol 19: 237–241.

25. Santas R, Korda A, Tenente A, Buchholz K, Santas PH (1999) Mesocosm assays of oil spill bioremediation with oleophilic fertilizers: Inipol, F1 or both? Mar Pollut Bull 38: 44-48.

26. Pelletier E, Delille D, Delille B (2004) Crude oil bioremediation in sub-Antarctic intertidal sediments: chemistry and toxicity of oiled residues. Mar Environ Res 57: 311–327.

27. ExxonMobil Corporation (2013) Alaska North Slope Crude Oil. http://www.exxonmobil.com/crudeoil/about_crudes_ans.aspx (accessed July 24, 2013)

28. Belore R (2005) The SL Ross oil spill fate and behavior model: SLROSM. www.slross.com/publications/SLR/Description_of_SLROSM.pdf (accessed May 7, 2012).

29. Nalco (2011) COREXIT Ingredients. http://www.nalcoesllc.com/nes/1602.htm > (accessed 10/29/2013).

30. Prince RC, Elmendorf DL, Lute JR, Hsu CS, Haith CE, et al. (1994) 17α(H),21β(H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. Environ. Sci. Technol. 28: 142-145.

31. Bushnell LD and HF Haas (1941) The utilization of certain hydrocarbons by microorganisms. J Bacteriol 41: 653-673.

32. Codispoti LA, Flagg C, Kelly V, Swift JH (2005) Hydrographic conditions during the 2002 SBI process experiments. Deep Sea Res Part II Top Stud Oceanogr 52: 3199-3226.

33. Organization for Economic Cooperation and Development (1992) Test No. 301: Ready Biodegradability; OECD guidelines for the testing of chemicals, Section 3. Paris: OECD Publishing. DOI: 10.1787/9789264070349-en.

34. Douglas GS, McCarthy KJ, Dahlen DT, Seavey JA, Steinhauer WG, et al. (1992) The use of hydrocarbon analyses for environmental assessment and remediation. J Soil Contam 1: 197–216.

35. Foght JM, Fedorak PM, Westlake DWS (1983) Effect of the dispersant Corexit 9527 on the microbial degradation of sulfur heterocycles in Prudhoe Bay oil. Can J Microbiol 29: 623-627.

36. Atlas RM, Boehm PD, Calder JA (1981) Chemical and biological weathering of oil from the Amoco Cadiz spillage, within the littoral zone. Estuar Coast Shelf Sci 12: 589-608.

37. Mearns AJ (1997) Cleaning oiled shores: putting bioremediation to the test. Spill Sci Technol B 4: 209-217.

38. Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, et al. (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330: 204-208.

39. Prince RC, McFarlin KM, Butler JD, Febbo EJ, Wang FCY, Nedwed TJ (2013) The primary biodegradation of dispersed crude oil in the sea. Chemosphere 90: 521-526.

40. Zahed MA, Aziz HA, Isa MH, Mohajeri L, Mohajeri S, Kutty SRM (2011) Kinetic modeling and half-life study on bioremediation of crude oil dispersed by Corexit 9500. J Hazard Mater 185: 1027-1031.

41. Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368: 413–418.

42. Prince RC, Bare RE, Garrett RM, Grossman MJ, Haith CE, et al. (2003) Bioremediation of stranded oil on an Arctic shoreline. Spill Sci Technol B 8: 303-312.

43. Davis JB (1964) Microbial incorporation of fatty acids from n-alkanes into glycerides and waxes. Appl Microbiol 12: 210-214.

44. Mazzella N, Molinet N, Syakti AD, Barriol A, Dodi A, et al. (2005) Effects of pure n-alkanes and crude oil on bacterial phospholipid classes and molecular species determined by electrospray ionization mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 822: 40–53.

45. Rontani JF (2010) Production of wax esters by bacteria. In: Timmis KN, editor. Handbook of Hydrocarbon and Lipid Microbiology. Heidelberg: Springer-Verlag, pp. 460-470.

46. Tissot BP and DH Welte (1984) Petroleum formation and occurrence. Berlin: Springer-Verlag.

47. Tavassoli T, Mousavi SM, Shojaosadati SA, Salehizadeh H (2012) Asphaltene biodegradation using microorganisms isolated from oil samples. Fuel 93: 142-148.

48. Uribe-Alvarez C, Ayala M, Perezgasga L, Naranjo L, Urbina H, Vazquez-Duhalt R (2011) First evidence of mineralization of petroleum asphaltenes by a strain of *Neosartorya fischeri*. Microb Biotechnol 4: 663-672.

49. National Research Council (1993) In situ bioremediation: when does it work? Washington DC: National Academy Press.

Figures

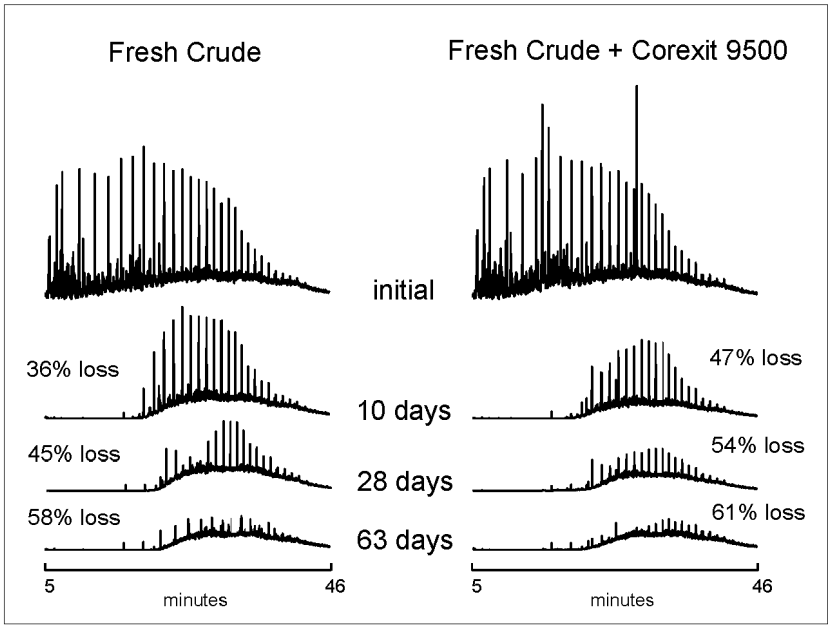

Figure 2-1. GC-MS total ion chromatograms. Open top mesocosm incubations contained an initial loading of 2.5 mg/L ANS crude oil without and with Corexit 9500 (1:15 DOR), and no added nutrients. Chromatograms show the pattern of biodegradation after 10 days, 28 days and 63 days.

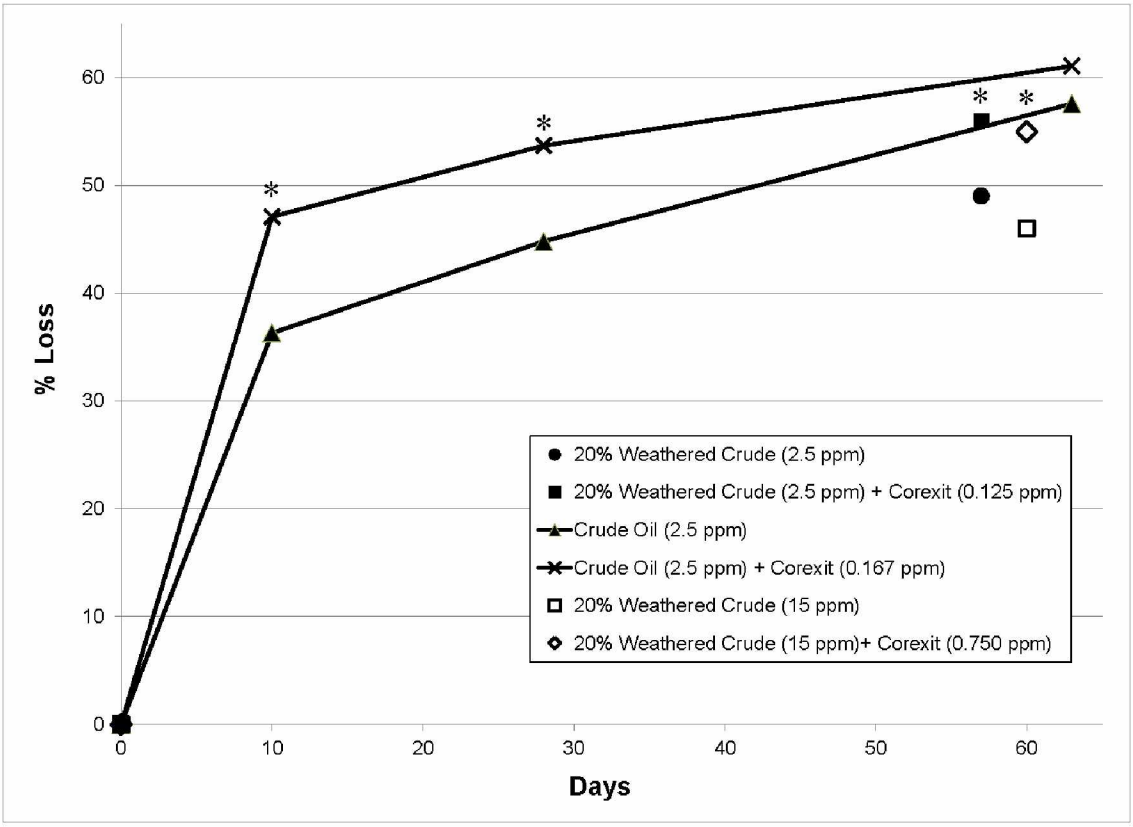

Figure 2-2. Primary biodegradation as measured by loss of total measurable petroleum hydrocarbons at -1°C. The higher concentrated (15 mg/L) weathered oil treatments contained a small amount of nutrient supplementation (16 mg/L Bushnell Haas), and were incubated in a sealed flask for parallel respirometry measurements (Figure 2-4). All other treatments had no added nutrients and were open to the atmosphere. Results are normalized to hopane. Standard deviations are reported in Table 2-1. *Denotes a significant difference between treatments with and without Corexit 9500 for each test ($p < 0.05$).

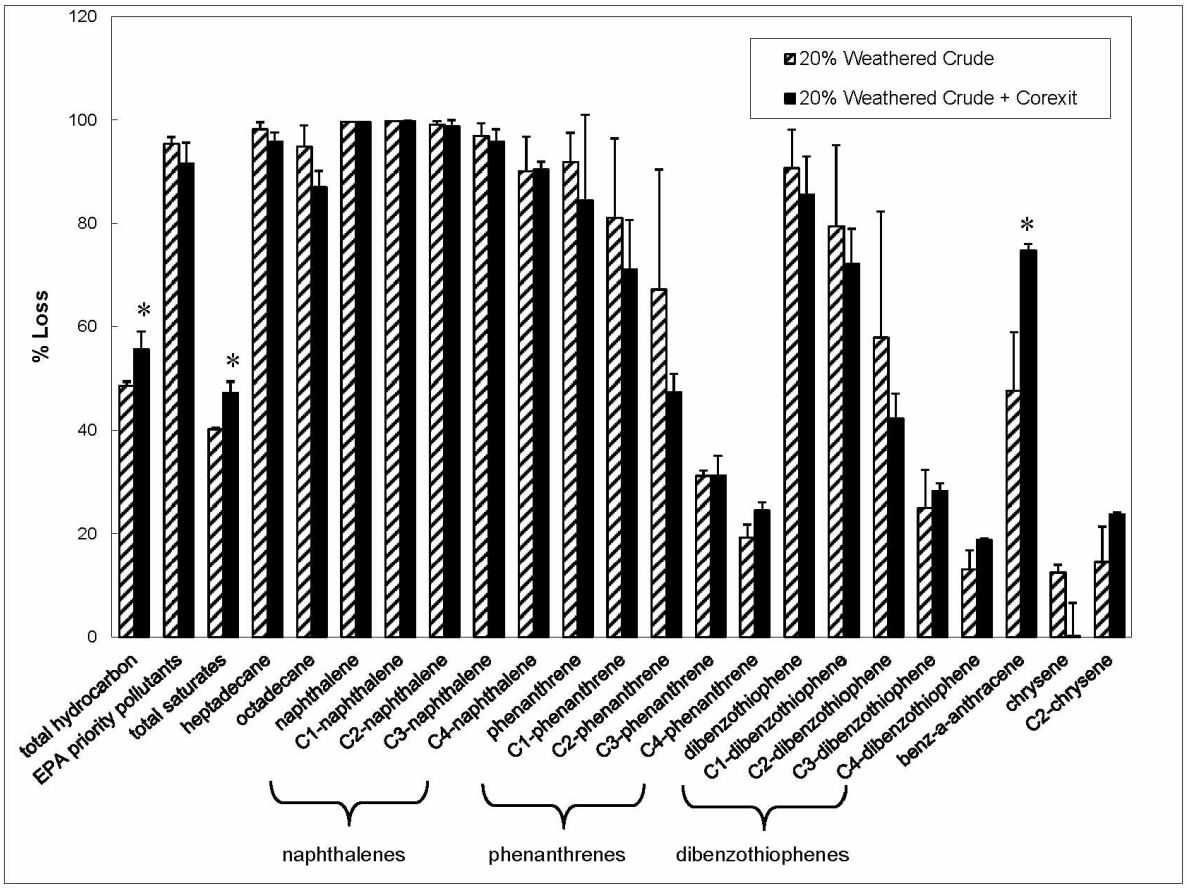

Figure 2-3. Percent loss of weathered oil after 56 days without nutrient addition at -1°C. Open top mesocosms contained an initial concentration of 2.5 mg/L of 20% weathered oil with and without the chemical dispersant Corexit 9500 at a 1:20 DOR. Standard errors are displayed. Results are normalized to hopane. *Denotes a significant difference between treatments with and without Corexit 9500 ($p < 0.05$).

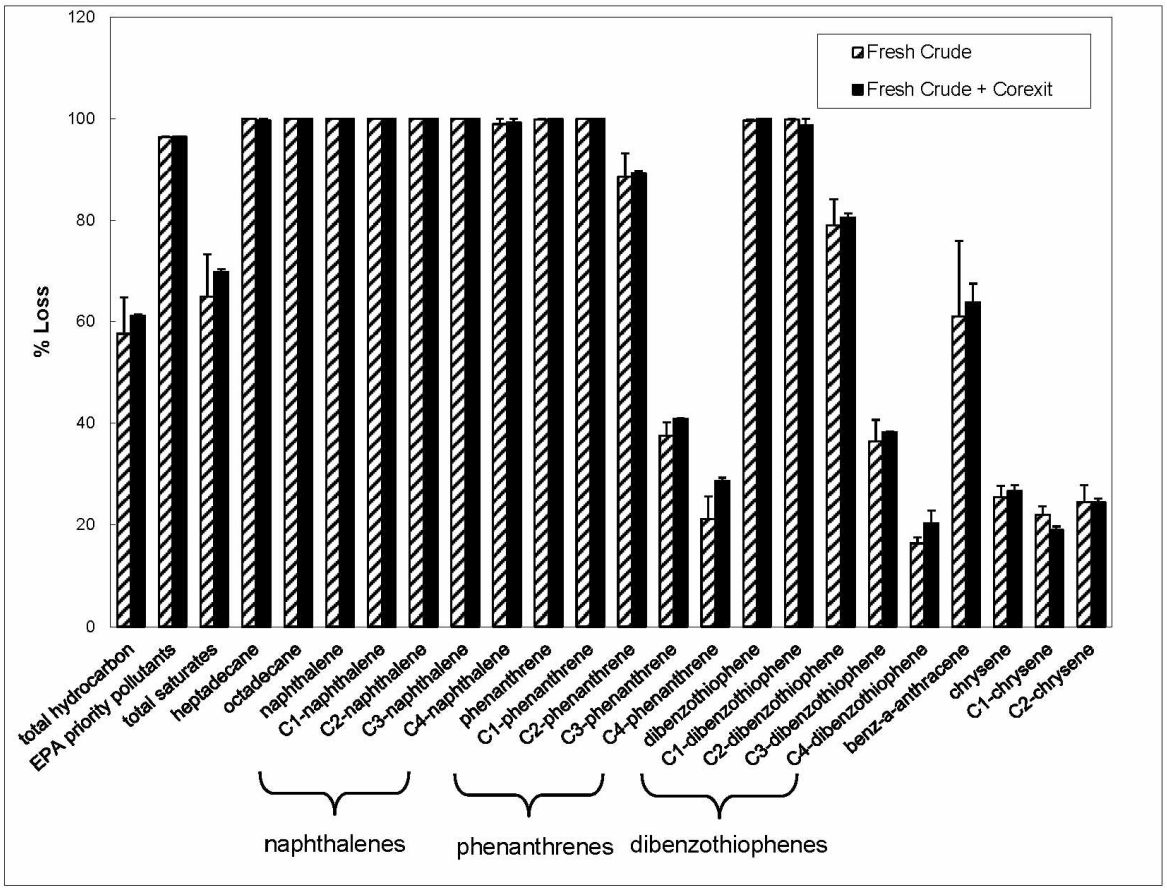

Figure 2-4. Percent loss of fresh oil after 63 days without nutrient addition at -1°C. Open top mesocosms contained an initial concentration of 2.5 mg/L of fresh oil with and without the chemical dispersant Corexit 9500 at a 1:15 DOR. Standard errors are displayed. Results are normalized to hopane. No significant differences ($p < 0.05$) were observed.

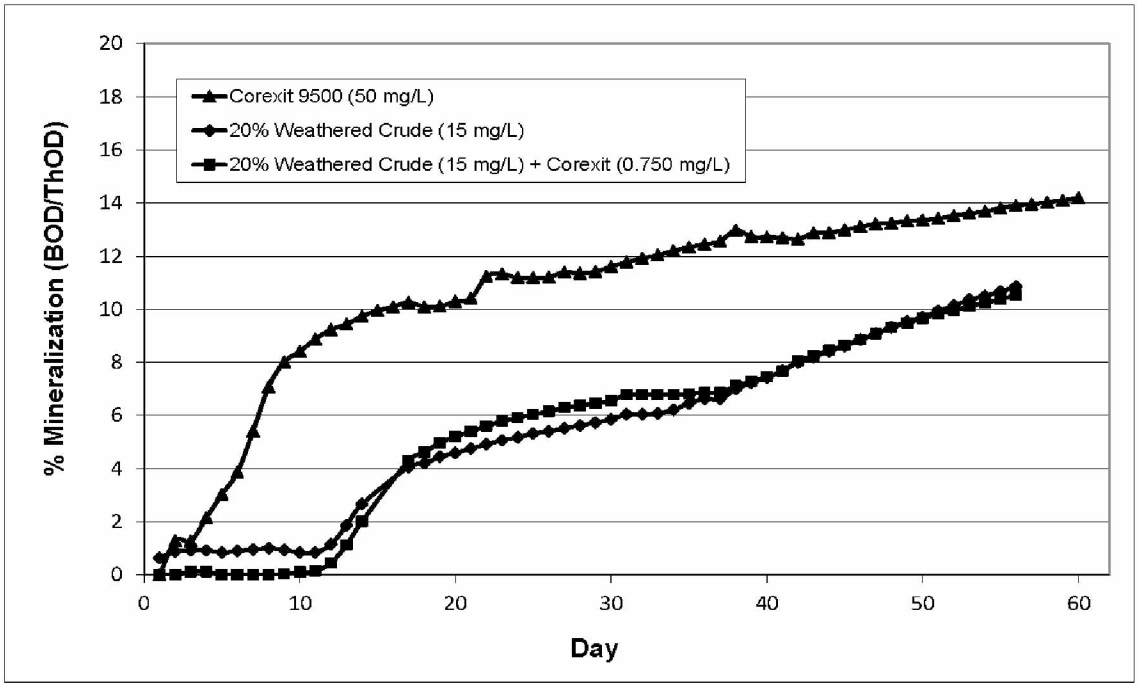

Figure 2-5. Percent mineralization at -1˚C. Sealed respirometer experiments contained Corexit 9500 (50 mg/L) alone or 20% weathered ANS crude (15 mg/L) with and without Corexit 9500 (1:20 DOR). All treatments contained seawater and 16 mg/L of Bushnell Haas.

Tables

Table 2-1. Mineralization and primary biodegradation of Corexit 9500 and ANS crude oil as determined by respirometry and GC-MS analysis. All treatments were incubated at -1°C.

Test ID	Treatments	Bushnell Haas (mg/L)	Dispersant Concentration (mg/L)	Oil Concentration (mg/L)	% Loss of Detectable Hydrocarbons	% Mineralized
Respirometer Flasks (900 ml seawater)						
R2	Corexit Only	0.016	50.0	na	nm	14
R1	20% Weathered	0.016	na	15	46 ± 5.1	11
	20% Weathered + Corexit (1:20 DOR)	0.016	0.750	15	55 ± 1.5*	11
Mesocosms (4 L seawater)						
M2	Fresh Oil	na	na	2.5	58 ± 10	nm
	Fresh Oil + Corexit (1:15 DOR)	na	0.167	2.5	61 ± 0.45	nm
M1	20% Weathered	na	na	2.5	48 ± 1.5	nm
	20% Weathered + Corexit (1:20 DOR)	na	0.125	2.5	57 ± 4.2*	nm

*Denotes significant difference ($p < 0.05$) between treatments in each test, na: not applicable, nm: not measured. Standard deviations are presented for primary biodegradation.

Table 2-2. Biodegradation index (C18 / Phytane) of mesocosms containing 2.5 mg/L fresh ANS crude oil without nutrient addition incubated at -1° with and without Corexit 9500 (DOR 1:15) analyzed at 10, 28 and 63 days.

	C18 / Phytane	
	Crude Oil	**Crude Oil + Corexit 9500**
Initial Oil	1.8 ± 0.03	1.8 ± 0.03
10-day	1.7 ± 0.04	0.97 ± 0.13
28-day	0.098 ± 0.1	0.082 ± 0.02
63-day	0.009 ± 0.004	0.003 ± 0.0001

Chapter 3: Biodegradation of Crude Oil and Corexit 9500 in Arctic seawater[1]

Abstract

The need to understand the biodegradation of oil and chemical dispersants in Arctic marine environments is increasing alongside growth in oil exploration and marine transport. The rates at which oil and Corexit 9500 biodegrade in Arctic seawater and the microorganisms and genes involved have yet to be identified. We conducted incubation experiments with Arctic seawater to determine the chemical loss of crude oil and Corexit 9500 over time, as well as associated shifts in bacterial community structure (16S rRNA genes) and abundance of biodegradation genes (GeoChip 5.0 microarray). In separate incubations, the indigenous microbial community biodegraded 36% (k = 0.010 day^{-1}) and 41% (k = 0.014 day^{-1}) of crude oil within 28 days, and biodegraded 77% and 33% (k = 0.015 day^{-1}) of dioctyl sodium sulfosuccinate (DOSS) within 28 days. Non-ionic surfactants were non-detectable at 28 days. More microorganisms grew in response to Corexit than oil alone within 28 days. Taxa known to include oil-degrading bacteria (e.g. *Oleispira*, *Polaribacter*, and *Colwellia*) and oil-biodegradation genes (e.g. *alkB*) increased in relative abundance in response to both oil and Corexit 9500. These results increase our understanding of oil and dispersant biodegradation in the Arctic and suggest that some bacteria may be capable of biodegrading both oil and Corexit 9500.

[1] McFarlin KM, Perkins MJ, Field JA, Leigh MB. (2017). Biodegradation of crude oil and Corexit 9500 in Arctic seawater. (prepared for submission: *Environmental Science & Technology*)

Introduction

As Arctic sea ice cover retreats due to climate change (Comiso et al., 2008), oil exploration and shipping traffic have increased (NRC, 2014) and so has the probability of oil spills (BOEM, 2015). One oil spill response option is the use of dispersants. Dispersants have been applied to oil spills worldwide (Chapman et al., 2007), with substantial applications in the Gulf of Mexico (Deepwater Horizon oil spill, > 43,000 barrels; National Commission, 2010) and England (Sea Empress incident, > 3,000 barrels; Lunel et al., 1997). When applied to a surface oil slick, surfactant compounds within dispersants reduce the surface tension between water and oil (Rouse et al., 1994), allowing the oil to become mixed into the water column as tiny droplets (Camilli et al., 2010; Brakstad et al., 2014). The creation of these droplets can significantly increase oil biodegradation (Brakstad et al., 2015; Brakstad et al., 2014; Prince & Butler, 2014); however, the use of dispersants adds more chemicals to the environment and negative impacts of prior Corexit formulations (i.e. 9527) have been reported (Bruheim et al., 1999).

If an oil spill occurs in the Arctic, the use of dispersants is a key oil spill response option. Corexit 9500 will likely be the dispersant of choice due to its efficacy (Belore et al., 2009; SL Ross, 2007), relatively low toxicity (US EPA, 2010), reported effectiveness during the Deepwater Horizon (DWH) oil spill (Bejarano et al., 2013), and prior approval in subarctic Alaskan waters (ARRT, 2004). The primary components of Corexit 9500 consist of the ionic surfactant DOSS (bis-(2-ethylhexyl) sulfosuccinate; 18% w/w; Place et al., 2016), the nonionic surfactants Span 80, Tween 80, and Tween 85 (27% w/w; Place et al., 2016), and the carrier solvents dipropylene glycol butyl ether and petroleum distillates (~55 % w/w) (NRC, 2005; Parker et al., 2014; Ramirez et al., 2013).

Currently no published reports exist for the primary biodegradation of Corexit 9500 (hereafter referred to as Corexit) in Arctic seawater. The mineralization of Corexit 9500 (based on respirometry) and the extent of crude oil biodegradation in Arctic seawater have been reported (McFarlin et al., 2014), although the rates at which Corexit and oil degrade in Arctic environments are still unknown (NRC, 2014). Corexit biodegradation studies have been conducted with Gulf of Mexico seawater (Campo et al., 2013; Kleindienst et al., 2015; Seidel et al. 2016) at low temperatures (5 and 8°C), but Corexit-degrading functional genes remain unknown and the metabolic functions of these deep-sea microbial communities may not be representative of Arctic communities. Rates of oil biodegradation are thought to be slower in

Arctic than temperate regions (Margesin et al., 2003; Michaud et al., 2004) due to the influence of temperature on metabolic processes (Mihelcic, 1999); however, some microorganisms are adapted to low temperatures (Feller, 2003), which may explain why some have reported similar oil biodegradation rates in cold and temperate environments (Braddock and McCarthy, 1996; Gibb et al., 2001; Margesin and Schinner, 1997).

Although previous studies have demonstrated the ability of indigenous Arctic microorganisms to biodegrade oil (Atlas et al., 1978; Garneau et al., 2016; McFarlin et al., 2014), data gaps remain regarding the taxonomic identity of Arctic oil-degraders in seawater. Experiments with indigenous microbial communities have been conducted with Arctic sea ice (Brakstad et al., 2008; Garneau et al., 2016; Gerdes et al., 2005), but what has been reported for seawater relies primarily on culture-based methods that may be taxonomically and functionally unrepresentative of the sampled environment. Such studies have, however, indicated that a diverse community of bacteria is associated with oil biodegradation in Arctic seawater and sea ice (Deppe et al., 2005). In culture-based studies, the majority of Arctic and Antarctic bacteria associated with oil in seawater have been classified as Gammaproteobacteria within the genera *Marinobacter*, *Pseudoalteromonas*, *Colwellia*, *Oleispira*, and *Psychrobacter* (Deppe et al., 2005; Yakimov et al., 2003, 2007). Some of these taxa, including *Colwellia*, were also enriched by chemically dispersed oil in a deep-water plume during the DWH oil spill (Redmond & Valentine, 2012; Dubinsky et al., 2013). The increased abundance of *Colwellia*, a known oil-degrading taxon (Dubinsky et al., 2013), in experimental incubations containing Corexit 9500-only and chemically dispersed oil suggests that members within this genus may be able to degrade Corexit in addition to oil (Brakstad et al., 2015; Kleindienst et al., 2015).

It is likely that microorganisms capable of degrading oil are also capable of degrading some components of dispersants due to some similarities in chemical composition. For example, bacterial *alkB* genes encode enzymes responsible for attacking alkane hydrocarbons (Vomberg & Klinner, 2000). Crude oil generally consists of hydrocarbons classified as alkanes (linear and branched; 33% by wt.), cycloalkanes (32%), and aromatics (35%) (Tissot & Welte, 1984). The petroleum distillate (CAS# 64742-47-8) fraction of Corexit 9500 also consists of hydrocarbons (C_9 - C_{16}), including C_{10} naphthenes (ringed paraffins), and *iso*- and *n*-paraffins (Petroleum Distillates, SDS). In addition to the hydrocarbons present in the carrier solvent fraction of Corexit, hydrocarbon side chains are also present in DOSS (Seidel et al., 2016), and the nonionic

surfactants (Corexit 9500A SDS; Word et al., 2015). Since hydrocarbons are found in both oil and Corexit 9500, bacteria may use similar metabolic pathways when biodegrading these compounds.

Here we combine chemical analyses with microbial analyses to characterize the biodegradation of oil and Corexit, separately, in Arctic seawater. To our knowledge, we are the first to report biodegradation rates of oil and Corexit (DOSS) in Arctic seawater, and the identity of oil-degradation genes (e.g. *alkB*) potentially associated with the biodegradation of Corexit and oil in Arctic seawater. We found that some of the same taxa and oil-biodegradation genes increased in relative abundance in response to both oil and Corexit, suggesting that some organisms may be capable of biodegrading components of both and that Corexit is not inhibitory to these oil-degrading bacteria.

Materials and Methods

Seawater collection

Three separate batches of seawater were collected, two (September and October, 2013) from the Burger lease area, ~90 km from Wainwright, Alaska (71.1050° N, -162.2668° W), and one with nearshore seawater collected ~1 km from Barrow, Alaska (71.3647° N, -156.5241° W) in August 2014. Surface seawater temperature was 2°C upon collection. Samples were held at 2°C onboard the vessel and transported in coolers with icepacks to the University of Alaska Fairbanks within 24 hours. Upon receipt, seawater was placed in a cold room (2°C), aerated, and amended with a nutrient medium (Bushnell & Haas, 1941). These nutrients provided 75 µM phosphate, 49 µM nitrate, and 76 µM ammonium, and are within an order of magnitude of background nutrient concentrations reported for Arctic seawater (Codispoti et al., 2005). After 24 hours of aeration (approximately 48 hours from the time of collection), biodegradation experiments were initiated.

Oil and Corexit 9500

Alaska North Slope (ANS) crude oil was obtained from the Alyeska pipeline terminal in Valdez, Alaska (summer, 2009). The oil was not deliberately weathered prior to addition. Corexit 9500 was obtained from Nalco Inc. (Naperville Township, IL), during the summer of

2009. Positive displacement pipettes were used to accurately add defined volumes of oil or Corexit.

Mesocosms

Incubations were conducted in 1-L small-mouth glass bottles, with caps tilted to allow air exchange. Each contained 800 mL of Arctic surface seawater, a small amount of nutrients (16 mg/L Bushnell Haas), and ANS crude oil (15 mg/L) or Corexit 9500 (15 mg/L). We also incubated seawater and nutrients in the absence of oil or Corexit as a negative control to reveal incubation-associated microbial community shifts. Three replicates were included for each time point, except for the October offshore incubation in which the microbial analyses contained 2-3 replicates due to the limited quantity of collected seawater.

Incubations were constantly stirred using magnetic stir bars (Teflon coated; no vortex) at 2°C in a cold room with an 8-hour/day light cycle (8.88 μmol s^{-1} m^{-2}; LI-COR light meter, LI-250, Quantum Sensor, Lincoln, NE). After the bottles for microbial analyses were filtered (0.2 μm), the filters were frozen (-80°C) until DNA extraction. Separate bottles for chemical analyses were also frozen (-20°C) until extraction.

Experimental design

Two offshore incubations (September and October 2013) and one nearshore incubation (August 2014) were conducted with the collected seawater. The offshore September experiment incubated oil, seawater, and nutrients for 0, 5, 10, and 28 days and incubations were only analyzed for the chemical loss of oil. The October offshore incubation experiment incubated oil, seawater, and nutrients for 0, 5, 10, and 28 days and also included a Corexit-only treatment at day 0 and day 28; chemical losses of oil and Corexit were analyzed. In addition, the offshore October experiment included separate incubations for microbial analyses of mesocosms containing oil or Corexit. The nearshore August 2014 incubation focused exclusively on Corexit; incubating Corexit, seawater and nutrients for 0, 10, 28, and 60 days, and included incubations for chemical loss and microbial analyses.

We are not reporting the data for day 60 of the Corexit-only incubation due to bottle effects on the microbial community. Bacterial abundance decreased by half between day 28 and day 60, which may be due to nutrient depletion and other bottle effects that would not be

expected to occur in open water. For these volumes of Arctic seawater (800 mL) and quantities of Corexit (15 mg/L), we do not recommend incubating experimental flasks longer than 28 days. Incubations containing oil (15 mg/L) were incubated for a maximum of 28 days.

Chemical analysis of crude oil

The chemical loss of ANS crude oil was determined in both biotic and abiotic control incubations. The entire contents of each flask were extracted and analyzed for total petroleum hydrocarbons (TPH) by gas chromatography-flame ionization detector (GC-FID), and individual alkanes and aromatics by GC/MS-selected ion monitoring (SIM) (B&B Laboratories, College Station, TX). We normalized concentrations to 17α(H), 21β(H)-hopane as a conserved internal marker (Prince *et al.*, 1994) and determined biodegradation as percent loss relative to time zero. Abiotic losses were measured in killed (autoclaved seawater) controls and were subtracted from all time points to calculate biodegradation. Using first-order kinetics (Mihelcic, 1999), we used the rate law for a first order reaction ($[C] = [C_0]e^{-kt}$) to calculate the rate constant (k) for the biodegradation of total measureable hydrocarbons (Brakstad et al., 2008; Venosa & Holder, 2007; Stewart et al., 1993).

Chemical analysis of Corexit 9500

At each time point, incubation bottles were immediately diluted to 75% with isopropanol (seawater:isopropanol, 75:25), and stored at -20°C. Aliquots (50 mL) were shipped overnight on dry ice to Oregon State University and stored at -20°C until analysis. Aliquots were diluted (0 - 100 fold) with a combination of Instant Ocean (Spectrum Brands, Blaksburg) and isopropanol (75:25), and quantitative analysis of surfactant components was performed as described by Place et al. (2016), with minor modifications. Mass spectrometric detection was performed with a Waters Micromass Quattro Mass Spectrometer. Details regarding chromatographic separation, calibration, and QA/QC are provided in the Supplementary Information (SI). To calculate the rate constant (k) for DOSS, we used the concentration at day 28 as the final concentration in the rate law for a first order reaction ($[C] = [C_0]e^{-kt}$).

Quantitative real-time PCR

Quantitative real-time polymerase chain reaction (q-PCR) targeting 16S rRNA genes was used to quantify prokaryotic populations in treatments containing oil, Corexit, and no carbon amendment (i.e. biotic control). Details of the qPCR analysis are located in the SI. Results are reported as copies of 16S rRNA gene sequences per 800 mL of seawater.

Microbial community analysis

We sequenced 16S rRNA genes to determine the taxonomic identity and relative abundance of bacteria in the October offshore and August nearshore incubations. We extracted total DNA from filters containing microorganisms from each treatment flask (Miller et al., 1999). The DNA extract was sequenced on Illumina's MiSeq platform using indexed primers (F515/R806) that targeted the V4 region (Caporaso et al., 2012). Details are reported in the SI. We then analyzed DNA sequences with mothur open source software (Schloss et al., 2009) following the online standard operating procedure (Schloss et al., 2011) and determined the taxonomic identity of bacteria using the Ribosomal Database Project (Wang et al., 2007). Operational taxonomic units (OTUs) were defined at 97% similarity. After the removal of singletons, relative abundances were normalized to total abundance per sample.

Functional gene analysis

The GeoChip 5.0_108K (Glomics Inc., Norman, OK), a functional gene microarray, was used to determine the presence and relative abundance of petroleum degradation genes in the October offshore incubation. Using the same DNA as the microbial community analysis, Glomics, Inc. conducted the amplification, labeling, hybridization, and data preprocessing (Van Nostrand et al., 2016). GeoChips were imaged (NimbleGen MS 200 microarray scanner; Roche NimbleGen Inc., Madison, WI) and the data were extracted using the Agilent Feature Extraction program. Extracted data were then loaded onto the GeoChip data analysis pipeline (ieg.ou.edu/microarray/) where singletons were removed. Prior to statistical analyses, all signals were transferred into relative abundances.

Statistical analyses

Nonmetric multidimensional scaling (NMS), cluster analysis, indicator species analysis (ISA), and multi-response permutation procedures (MRPP) were all conducted with PC-ORD V6 (McCune & Mefford, 2011). Methodological details concerning NMS, cluster analysis, and ISA are included in the SI.

Statistical differences were calculated using MRPP, a nonparametric procedure for testing differences among groups (Mielke, 1984; Mielke & Berry, 2001). We found the data to be non-normal using the Shapiro-Wilk test ($W = 0.837$, $p = 0.053$). Each MRPP was conducted using a Bray-Curtis distance measure. We compared the percent loss of oil and Corexit among their respective time points and reported the p-value.

Results

Biodegradation of crude oil

The indigenous microorganisms biodegraded 16 ± 4% (mean ± SD) of ANS crude oil within the first 5 days in both offshore incubations (Table 3-1). The extent of oil biodegradation at 28 days was similar between September and October, with extents ranging from 36 ± 6% to 41 ± 0.0% (Table 3-1; MRPP, $p > 0.05$). The rate constants (k) for the September and October offshore oil biodegradation experiments were 0.010 day^{-1} and 0.014 day^{-1}, respectively.

Biodegradation of Corexit 9500

The concentration of DOSS significantly decreased between day 0 and 28 in the offshore (by 77 ± 0.5%) and nearshore (by 33 ± 7%) incubations, while the abiotic controls showed no significant difference in DOSS ($p > 0.05$; Table 3-2). The nearshore incubation included an earlier time point and indicated that the majority of DOSS was biodegraded within the first 10 days (23 ± 9% loss; $p > 0.05$; Table 3-2). Since the offshore incubation included two time points (day 0 and 28) and the nearshore incubation included three time points (day 0, 10, and 28), we used the nearshore incubation to calculate the rate constant (k) for DOSS, which was 0.015 day^{-1}.

With respect to the non-ionic surfactants, the communities substantially biodegraded Span 80, Tween 80, and Tween 85. Within 28 days, microbes in offshore and nearshore seawater

biodegraded Span 80, Tween 80, and Tween 85 to concentrations below their limits of detection, 5.5 µg/L, 15 µg/L, and 6.5 µg/L, respectively (Table 3-2). In the nearshore incubation at day 10, Tween 80 and Tween 85 were both below detection limits, while Span 80 was still detectable at day 10 (75% loss), but by day 28, Span 80 was also below its detection limit (5.5 µg/L; Table 3-2). Out of all surfactants, Tweens were the only ones that exhibited any abiotic loss, where significant loss occurred at day 10 (82% loss) and at day 28 (87% loss; Table 3-2) in the offshore incubation.

It should be noted that these percent loss calculations (for oil and Corexit) are based upon mean values. Furthermore, when calculating biodegradation we subtracted mean abiotic loss at each time point and therefore assumed that compounds lost through abiotic means were never available for biodegradation. Therefore, the rates and extents reported here are likely conservative.

Prokaryotic population size

Over the course of the offshore and nearshore incubations, we quantified prokaryotic 16S rRNA gene copies using qPCR to determine if population growth occurred concurrently with the biodegradation of oil or Corexit. Total 16S rRNA genes increased in abundance in response to the presence of oil or Corexit within 28 days (Figure S3-1). At 28 days, 69% more 16S rRNA genes were present in offshore seawater with Corexit than with oil (1.1×10^{10} vs. 3.5×10^{9}, Figure S3-1); however, the Corexit treatment was more variable than the oil treatment. In the seawater-only treatment (i.e. no oil or no Corexit addition), prokaryotes significantly increased between day 0 (3.3×10^{9} gene copies) and day 10 (9.2×10^{9} gene copies) in nearshore seawater, but by day 28, only 2% of the initial abundance remained (7.0×10^{7} gene copies; Figure S3-1). In both offshore and nearshore incubations, 16S rRNA gene abundance decreased in the seawater-only treatments between day 10 and 28.

Microbial community analysis

The response of indigenous microbial communities to ANS crude oil and Corexit 9500 was monitored over the course of the incubations in order to identify potential oil- and Corexit-degrading bacteria in Arctic seawater. The 16S rRNA gene sequencing results produced an average of 23,836 different OTUs. In the offshore incubation, the NMS ordination and the

dendrogram both illustrated a strong separation in bacterial community structure between oil and Corexit incubations at day 28 (Figures S3-2 & S3-3). In the offshore incubation, only two replicate mesocosms were incubated for the majority of treatments analyzed for microbial analyses due to limited seawater availability; however, these duplicates showed consistent grouping in our dendrogram and our NMS ordination (Figures S3-2 & S3-3). In the nearshore incubation, bacterial sequences from replicate incubations were grouped together and the biotic controls (seawater in the absence of Corexit) grouped separately from the Corexit treatments (Figure S3-4).

MRPP was conducted to determine if microbial community shifts over the course of Corexit incubations were statistically significant. Each treatment contained a significantly different microbial community at each time point (Figure S3-4; MRPP, p-value < 0.05). In nearshore seawater, the biotic control at day 10 contained a similar abundance of prokaryotes as the Corexit treatment at day 10, but the structure of the microbial communities was drastically different (Figure 3-1; MRPP, p-value < 0.05). The microbial communities in all incubations with Corexit were significantly different from communities without Corexit (i.e. the biotic controls; MRPP, p-value < 0.05).

Taxa that responded to oil or Corexit

In the offshore incubation, *Oleispira* (Figures S3-5 & S3-8a), *Colwellia* (Figures S3-5 & S3-7a), *Lutibacter* (Figure S3-6a), and an unclassified member of Flavobacteriaceae (OTU83; Figure S3-6a) increased in relative abundance in response to both oil and Corexit. One *Oleispira* OTU (OTU8) increased in relative abundance in response to oil at day 5 by 18% and day 10 by 14% (based on only 1 replicate in the latter case), and in response to Corexit at day 28 compared to the biotic controls ($n = 2$; Figure S3-8a). In addition, one *Colwellia* OTU (OTU12) increased in response to oil at day 10 (by 16%), oil at day 28 (by 8%), and Corexit at day 28 (by 22%) compared to the biotic control (Figure S3-7a). Overall, the relative abundance of *Colwellia* increased in response to oil within 10 days and increased in response to Corexit between 10 and 28 days in the offshore incubation; however, more *Colwellia* grew in response to Corexit than oil (relative abundance, Figure S3-7a). Three *Colwellia* OTUs (OTUs 12, 19, and 21) were identified as indicator species for the presence of Corexit at day 28, with indicator values and p-values of IV = 88, $p = 0.013$; IV = 67, $p = 0.012$; and IV = 56, $p = 0.013$, respectively.

In the offshore incubation, one unique *Colwellia* (OTU19; Figure S3-7a), and an unclassified Oceanospirillaceae (OTU246; Figure S3-8a) increased in response to Corexit, but not oil. In addition, an individual unclassified Flavobacteriaceae (OTU83) was also identified as an indicator species for the presence of Corexit at day 28 (IV = 88; $p = 0.013$). In response to oil-only, *Sulfitobacter* (Figure S3-9), an unclassified Flavobacteriaceae (OTU50; Figures S3-6a & S3-10), and an unclassified Rhodobacteraceae (OTU4; Figures S3-9 & S3-10) increased in relative abundance compared to the biotic controls within 28 days. Furthermore, ISA concluded that an individual *Sulfitobacter* (OTU72) was an indicator species of the presence of oil at day 28 (IV = 88; $p = 0.013$).

In the nearshore experiment, members of Oceanospirillaceae, Flavobacteriaceae, and *Colwellia* also increased in response to Corexit compared to the biotic controls. *Polaribacter*, a member of Flavobacteriaceae, increased in relative abundance in response to Corexit at day 10 (by 34%; Figures 3-1 & S3-6b), while Colwelliaceae (Figure S3-7b) and Oceanospirillaceae (Figure S3-8b) increased in response to Corexit at day 28.

Functional gene analysis

We used GeoChip analyses to detect and compare abundances of genes that encode microbial enzymes used in biodegradation to identify metabolic processes potentially utilized by oil- and Corexit-degrading microorganisms in offshore surface seawater. At 28 days, the normalized intensities of total petroleum degradation genes (*alkB*, *apc*, *bbs*, *catB*, *ebdA*, *edbABC*, *hbh*, *pchCF*, *tomoABE*, *tamA*, *tutFDG*, *xylM*, *catA*, multi-ring-1,2-dixoygenase, one-ring-1,2-dioxygenase, *nagG*, one-ring-2,3-dioxygenase) in the biotic control grouped separately from treatments amended with oil or Corexit in the NMS ordination (Figure S3-11). In regards to total petroleum degradation genes, the NMS ordination did not indicate a strong separation between oil and Corexit incubations (Figure S3-11); however, the relative abundance of several individual genes did shift over the course of incubation with oil or Corexit (Figures 3-2 & S3-12). At day 28, *alkB* (alkane monooxygenase), *nagG* (salicylate 5-hydroxylase), and *pchCF* (p-hydroxybenzaldehyde dehydrogenase) genes showed the greatest differences in abundance compared to the unamended control (Figure 3-2). In seawater without oil, the richness of *alkB* decreased from 67 different genes to 32 within the first 28 days. In the presence of oil and dispersant, the richness of *alkB* dramatically increased from the unamended control at day 28,

with an increase of 52 different *alkB* genes in the oil treatment and 112 *alkB* genes in the Corexit treatment.

When comparing oil incubations ($n = 2$) to Corexit incubations ($n = 3$), the relative abundance of *catA* (catechol dioxygenase), *catB* (muconate cycloisomerase), and to a smaller extent *one-ring-2,3-diox* (aromatic-ring-2,3-dioxygenase) increased in response to oil, but not in response to Corexit at day 28 compared to the unamended control (Figure S3-12). Oil and Corexit had no effect on *one-ring-1,2-diox* (aromatic-ring-1,2-dioxygenase; Figure S3-12). At day 28, *nagG* and *alkB* were the only genes that had intensities in Corexit and oil treatments above those measured at day 0 (Figure 3-2).

Discussion

We report here that ANS crude oil and surfactant components of Corexit 9500 can undergo significant biodegradation (i.e. 36-41% loss of oil; 33-77% loss of DOSS; ~100% loss of Span and Tweens) within 28 days in Arctic surface seawater at 2°C (Tables 3-1 & 3-2). Corexit likely enriched a different microbial community than crude oil in offshore surface seawater at 28 days (Figures S3-2 & S3-3); however, throughout the incubation a subset of taxa (*Colwellia*, *Oleispira*, *Lutibacter*, and an unclassified Flavobacteriaceae spp. OTU83; Figures S3-5, S3-6a, S3-7a, & S3-8a) and functional genes associated with oil biodegradation (*alkB*, *nagG*, and *pchCF*; Figure 3-2) increased in response to both oil and Corexit. These results suggest that some oil-degrading bacteria may also have the potential to biodegrade components in Corexit.

Whole ANS crude oil degraded at a rate of 0.010 day^{-1} and 0.014 day^{-1} in offshore Arctic seawater collected in September and October, respectively. While there is a lack of literature reporting whole oil biodegradation rates in Arctic marine environments (NRC, 2014), these rate constants are aligned with others who reported volumetric degradation rates of crude oil in seawater (without added nutrients) of 0.011 gC/m^3*d (18°C, Atlas & Bartha, 1973) and 0.015 gC/m^3*d (1°C, Laake et al., 1984), in temperate and sub-Arctic seawater, respectively (Stewart et al., 1993). While oil biodegradation rates are dependent upon many factors, especially concentration (Prince et al., 2017), these results support previous reports that cold-adapted bacteria have similar metabolic rates to warm-adapted bacteria in their respective environments (Arnosti et al., 1998; Robador et al., 2009). The influence of temperature on oil

biodegradation rates has been extensively studied (Atlas & Bartha 1972; Atlas, 1981; Brakstad et al., 2008); however, recent research suggests that physico-chemical properties of oil at low temperature more likely limit oil biodegradation than metabolism (Bagi et al., 2013). Nonetheless, the rate of abiotic factors such as evaporation and diffusion increase with increasing temperature (Honrath & Mihelcic, 1999), which can result in more oil loss in temperate (Prince et al., 2013) vs. Arctic environments (McFarlin et al., 2014).

The surfactant constituents of Corexit were significantly biodegraded (33-77% loss of DOSS, ~100% loss of non-ionics) within 28 days in both offshore and nearshore incubations. Complete consumption of Tweens was observed within 10 days (Table 3-2), which was also observed by Kleindienst et al. (2015) in a laboratory microcosm study using only Corexit 9500 and Gulf of Mexico deep seawater at 8°C. Degradation rates of DOSS have yet to be reported in other cold environments; however, the rapid consumption of DOSS (k = 0.015 day^{-1}) observed in our study (2°C) is in contrast to Kleindienst et al. (2015) who reported an 8% loss of DOSS over 28 days in their Corexit-only incubation (8°C). These differences are likely a function of different methodologies, with the most notable difference in regards to the management of seawater. While our incubations were performed in bottles open to the atmosphere on stir plates, Kleindienst et al. (2015) performed theirs in closed bottles on a roller table. In addition, the seawater used in our incubations was stored for < 2 days at 2°C prior to the start of the experiment (2°C), while the seawater used by Kleindienst et al. (2015) was stored for >1 month at varying temperatures (4-8°C). This prolonged storage (> 1-month) was likely responsible for the difference in microbial community structure between *in situ* samples and incubation samples at day 0 (Kleindienst et al., 2015) and may have impacted the indigenous community's ability to biodegrade Corexit. In addition to our study, Campo et al. (2013) also published rates of DOSS biodegradation in the absence of oil, with extents > 99% after 8 days and a first-order rate constant of 0.30 day^{-1} in incubations at 25°C. Here we report a substantially lower rate of DOSS biodegradation in Arctic seawater at 2°C, with a first-order rate constant of 0.015 day^{-1}. These data may simply suggest that DOSS biodegrades slower at 2°C than 25°C (Campo et al., 2013); however, the extent of DOSS biodegradation was highly variable between our offshore and nearshore incubations (33 ± 7% loss vs. 77 ± 0.5% loss; Table 3-2) at the same temperature (2°C), suggesting that other variables (e.g. microbial community structure) may have a stronger impact on the extent of DOSS biodegradation than temperature.

Differences between microbial community structures in offshore and nearshore incubations may have contributed to the variability we observed in DOSS biodegradation, as dominant community members in both incubations were drastically different (i.e. *Colwellia* vs. *Polaribacter*; Figures 3-1 & S3-5). These community differences are likely due to different water masses flowing through the two sampling locations. The offshore location is characterized by Bering Sea water flowing north from the Bering Strait, and the nearshore location is characterized by coastal water that flows northward via the Alaskan Coastal Current (Day et al., 2013). Greater DOSS biodegradation occurred in offshore (77% loss) than nearshore (33% loss) incubations in 28 days, which also correlated with a greater response of *Colwellia* (42% and 4% of the community at 28 days, respectively) in offshore seawater. *Colwellia* was present at 3% (mean relative abundance) at day 0 in the offshore incubation, but only made up 0.2% of the total community in the nearshore incubation. While *Colwellia* was the genus that increased in abundance most in the offshore incubations at 28 days, in the nearshore incubations, *Polaribacter* showed the greatest change, which occurred at day 10 when it increased by 30% within the first 10 days. Within our dataset, psychrotrophs *Colwellia* and *Polaribacter* (Deming & Junge, 2005; Moyer & Morita, 2007) are likely the most influential in the biodegradation of Corexit compounds in Arctic seawater (Figures 3-1 & S3-5).

Colwellia may have a greater role in degrading Corexit than crude oil in Arctic marine environments. At day 28, *Colwellia* spp. (OTUs 12, 19 and 21) were indicators of the presence of Corexit (ISA), and incubations containing Corexit had a higher relative abundance of *Colwellia* and total prokaryotes than incubations containing oil (Figures 3-1 & S3-7a). *Colwellia* spp. are known for their psychrophilic members isolated from deep sea and polar marine ice (Deming & Junge, 2005), and have been associated with the biodegradation of oil in Antarctic seawater cultures (Yakimov et al., 2003), Arctic marine ice (Brakstad et al., 2008), and sub-Arctic seawater (Brakstad & Bonaunet, 2006). To our knowledge, this is the first report of *Colwellia* growing in response to oil in Arctic seawater. *Colwellia* spp. were identified as dominant members in a deep-water dispersed plume during the DWH oil spill and in enrichment incubations containing chemically dispersed oil in water from the Gulf of Mexico (Baelum et al., 2012; Chakraborty et al., 2012; Kleindienst et al., 2015; Mason et al., 2014; Redmond & Valentine, 2012). *Colwellia* spp. have also been shown to incorporate ^{13}C from ethane, propane, and benzene at 6°C in stable isotope experiments (Redmond & Valentine, 2012) and grow on

MC252 oil as the sole carbon source at 5°C (Dubinsky et al., 2013). Different *Colwellia* strains have genetic potentials to biodegrade a variety of hydrocarbons (gaseous, aromatics, n-alkanes, and cycloalkanes; Techtmann et al., 2016), which may be due to their acquisition of different degradative pathways through horizontal gene transfer (Collins & Deming, 2013). The increased relative abundance of *Colwellia* in our incubations with Corexit (Figure S3-7) together with the increase in total prokaryotic abundance (Figure S3-1) supports prior reports of their rapid response to labile carbon substrates in Arctic environments (Collins & Deming, 2013).

The relative abundance of *Polaribacter* coincided with the biodegradation of nonionic surfactant components of Corexit at day 10 (Figure 3-1; Table 3-2) and may indicate growth on these components. *Polaribacter* spp. have also been found to increase in abundance in response to oil in sub-Antarctic seawater cultures (Prabagaran et al., 2007) and mesocosms consisting of Arctic sea ice (Garneau et al., 2016). *Polaribacter* spp. were also suggested to play a role in the degradation of complex organic matter in the deep-sea decaying microbial bloom in the aftermath of the DWH oil spill (Dubinsky et al., 2013); however, to our knowledge, we are the first to report the association of *Polaribacter* with Corexit in Arctic seawater (Figure S3-6b).

Oleispira, another genus known to contain oil-degraders (Yakimov et al., 2007), also increased in relative abundance in our offshore incubation in response to Corexit at day 28 (Figure S3-8a), and increased in response to oil at day 5 and 10 (Figure S3-5). *Oleispira* spp. were shown to play an important role in the degradation of dispersed Macondo oil in the deep-sea oil plume in the Gulf of Mexico (Hazen et al., 2010); and have also been associated with enrichment cultures containing crude oil and Antarctic seawater (Yakimov et al., 2003; Prabagaran et al., 2007), and in clone libraries from Arctic sea ice incubated with crude oil (Brakstad et al., 2008).

Some microorganisms use dispersants as growth substrates (Chakraborty et al., 2012). As was observed by Lindstrom & Braddock (2002) and Kleindienst et al. (2015), Corexit also enriched a higher abundance of microorganisms than oil in our seawater incubations (Figure S3-1). When oil is chemically dispersed, laboratory studies have shown that oil-degrading microorganisms rapidly colonize dispersed oil droplets (Macnaughton et al., 2003), and may preferentially degrade some dispersant compounds over oil compounds (Foght & Westlake, 1982; Bunch et al., 1983; Foght et al., 1983). Even at subzero temperatures (-1°C), McFarlin et al. (2014) reported that indigenous Arctic marine microorganisms continued to mineralize more

Corexit 9500 than ANS crude oil (20% weathered) throughout a 60-day respirometer experiment. Corexit contains more water-soluble components than oil (Corexit 9500A SDS), thus making the mixture more bioavailable to bacteria as a food source. In addition to its physical effects (i.e. movement of oil into the water column; Prince & Butler, 2014; Brakstad et al., 2015), Corexit also impacts the Arctic marine microbial community by increasing the abundance of microorganisms (Figure S3-1), while enriching oil-degradation genes (Figures 3-2 & S3-12), and taxa known to include oil-degrading bacteria (Figures 3-1 & S3-5).

Together, these results suggest that known oil-degrading taxa also have the ability to biodegrade components in Corexit 9500. Microorganisms may use some of the same metabolic pathways to biodegrade Corexit as they do oil, as known oil-degradation genes, most notably *alkB* and *nagG*, increased in abundance in both oil and Corexit incubations (Figure 3-2). The *nagG* gene encodes salicylate-5-hydroxylase, an enzyme that converts salicylic acid to gentisic acid, which is ultimately degraded to pyruvic and fumaric acid (Fuenmayor et al., 1998). Alkane monooxygenases (*alkB*) hydroxylate alkanes to alcohols (Rojo, 2009), and are the most common alkane hydroxylating enzymes found in bacteria (Smits et al., 1999, 2002). The abundance of *alkB* at day 28 in incubations with Corexit coincides with the abundance of *Colwellia* (Figures 3-1 & S3-7), a taxon that has shown a preference for Corexit (Chakraborty et al., 2012; Dubinsky et al., 2013; Kleindienst et al., 2015), and may indicate their use of these genes for biodegrading alkanes in the petroleum distillate fraction of Corexit or the hydrocarbon side chains of the surfactants. In a functional gene survey (GeoChip 4.0) during the DWH oil spill, *alkB* was also significantly higher in dispersed plume samples compared to non-plume samples (Lu et al., 2012). These results suggest that *alkB* may also play a role in the biodegradation of Corexit in surface waters of the Arctic Ocean.

Correlating observed losses of Corexit and oil with increases in abundance of prokaryotic cells and of specific taxa and functional genes allowed us to suggest which microorganisms are degrading oil and Corexit compounds, as well as which relevant biodegradation genes they possess and may be involved. Our results support prior research indicating that significant oil and Corexit biodegradation can occur in the marine environment without adding large amounts of nutrients or cultures of oil-degrading microorganisms (Mearns, 1997; Head et al., 2006). Including the biodegradation rates reported here in future oil spill

trajectory models may improve the accuracy of predicted fates of spilled oil and Corexit 9500 in near shore and offshore Arctic environments.

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Conflict of interest

The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH or other research funders.

References

Apprill, A.; McNally, S.; Parsons, R.; Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **2015**, *75*, 129-137.

Arnosti, C.; Jorgensen, B.B.; Sagemann, J.;Thamdrup, B. Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. *Mar. Ecol. Prog. Ser.* **1998**, *165*, 59-70.

ARRT (Alaska Regional Response Team); *Dispersant Approval for Selendang Ayu*; 2004; https://dec.alaska.gov/spar/ppr/response/sum_fy05/041207201/041207201_index.htm

ARRT (Alaska Regional Response Team); *Alaska Federal/State Preparedness Plan for Response to Oil & Hazardous Substance Discharges/Releases (Unified Plan)*; Annex F, Appendix I; Dispersant Use Plan for Alaska; 2016.

Atlas, R.M.; Bartha, R. Biodegradation of petroleum in seawater at low temperatures. *Can. J. Microbiol.* **1972**, *18*, 1851-1855.

Atlas, R.M.; Bartha, R. Stimulated biodegradation of oil slicks using oleophilic fertilizers. *Environ. Sci. Technol.* **1973**, *7*, 538-541.

Atlas, R.M.; Horowitz, A.; Busdosh, M. Prudhoe crude oil in arctic marine ice, water and sediment ecosystems: degradation and interactions with microbial and benthic communities. *J. Fish. Res. Board Can.* **1978**, *35*, 585-590.

Atlas, R.M. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **1981**, *45*, 180-209.

Baelum, J.; Borglin, S.; Chakraborty, R.; Fortney, J.L.; Lamendella, R.; Mason, O.U.; Auer, M.; et al. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environ. Microbiol.* **2012**, *14*, 2405-2416.

Bagi, A.; Pampanin, D.M.; Brakstad, O.G.; Kommedal, R. Estimation of hydrocarbon biodegradation rates in marine environments: A critical review of the Q 10 approach. *Marine Environ. Res.* **2013**, *89*, 83-90.

Bejarano, A.C.; Levine, E.; Mearns, A.J. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. *Environ. Monitor. Assess.* **2013**, *185*, 10281-10295.

Belore, R.C.; Trudel, K.; Mullin, J.V.; Guarino, A. Large-scale cold water dispersant effectiveness experiments with Alaskan crude oils and Corexit 9500 and 9527 dispersants. *Marine Poll. Bull.* **2009**, *58*, 118-128.

Braddock, J.F.; McCarthy, K.A. Hydrologic and microbial factors affecting persistence and migration of petroleum hydrocarbons spilled in a continuous-permafrost region. *Envir. Sci. Technol.* **1996**, *30*, 2626-2633.

Brakstad, O.G.; Bonaunet, K. Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5˚C) and bacterial communities associated with degradation. *Biodegradation*, **2006**, *17*, 71-82.

Brakstad, O.G.; Nonstad, I.; Faksness, L.G.; Brandvik, P.J. Responses of microbial communities in Arctic sea ice after contamination by crude petroleum oil. *Microb. Ecol.* **2008**, *55*, 540-552.

Brakstad, O.G.; Daling, P.S.; Faksness, L.G.; Almås, I.K.; Vang, S.H.; Syslak, L.; Leirvik, F. Depletion and biodegradation of hydrocarbons in dispersions and emulsions of the Macondo 252 oil generated in an oil-on-seawater mesocosm flume basin. *Marine Poll. Bull.* **2014**, *84*, 125-134.

Brakstad, O.G.; Nordtug, T.; Throne-Holst, M. Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Marine Poll. Bull.* **2015**, *93*, 144-152.

Bruheim, P.; Bredholt, H.; Eimhjellen, K. Effects of surfactant mixtures, including Corexit 9527, on bacterial oxidation of acetate and alkanes in crude oil. *Appl. Environ. Microbiol.* **1999**, *65*, 1658-1661.

Bunch, J.N.; Bedard, C.; Cartier, T. *Abundance and activity of heterotrophic marine bacteria in selected bays at Cape Hatt, N.W.T.: Effects of oil spills, 1981*; Canadian Manuscript Report of Fisheries and Aquatic Sciences, No.1708, Baffin Island Oil Spill (BIOS) project; Canadian Fisheries and Aquatic Sciences: Sainte-Anne-de-Bellevue, PQ, Canada, 1983; pp 96.

Bureau of Ocean Energy Management (BOEM), *Supplement to the Oil-Spill Risk Analysis: Chukchi Sea Planning Area, OCS Lease Sale 193*; U.S. Department of the Interior: Herndon, VA, 2015; http://www.boem.gov/Environmental-Stewardship/Environmental-Assessment/Oil-Spill-Modeling/Oil-Spill-Risk-Analysis-Reports.aspx

Bushnell, L. D.; Haas, H.F. The utilization of certain hydrocarbons by microorganisms. *J. Bacteriol.* **1941**, *41*, 653-673.

Camilli, R.; Reddy, C.M.; Yoerger, D.R.; Van Mooy, B.A.; Jakuba, M.V.; Kinsey, J.C.; McIntyre, C.P.; Sylva, S.P.; Maloney, J.V. Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science*, **2010**, *330*, 201-204.

Campo, P.; Venosa, A.D.; Suidan, M.T. Biodegradability of Corexit 9500 and dispersed South Louisiana crude oil at 5 and 25C. *Environ. Sci. Technol.* **2013**, *47*, 1960-1967.

Caporaso, J.; Gregory, C.L.; Lauber, W.A.; Walters, D.B.L.; Huntley, J.; Fierer, N.; Owens, S.M.; et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **2012**, *6*, 1621-1624.

Chakraborty, R.; Borglin, S.E.; Dubinsky, E. A.; Andersen, G. L.; Hazen, T. C. Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. *Front. Microbiol.* **2012**, *3*, 357.

Chapman, H.; Purnell, K.; Law, R.J.; Kirby, M.F. The use of chemical dispersants to combat oil spills at sea: A review of practice and research needs in Europe. *Mar. Pollut. Bull.* **2007**, *54*, 827–838.

Codispoti, L.A.; Flagg, C.; Kelly, V.; Swift, J.H. Hydrographic conditions during the 2002 SBI process experiments: Deep Sea Research Part II: Topical Studies. *Oceanography*, **2005**, *52*, 3199-3226.

Collins, R.E.; Deming, J.W. An inter-order horizontal gene transfer event enables the catabolism of compatible solutes by Colwellia psychrerythraea 34H. *Extremophiles*, **2013**, *17*, 601-610.

Comiso, J.C.; Parkinson, C.L.; Gersten, R.; Stock, L. Accelerated decline in the Arctic sea ice cover. *Geophys. Res. Lett.* **2008**, *35*:L01703.

Corexit 9500A Safety Data Sheet; COREXIT ® EC9500A; Nalco Company, Naperville, IL, USA; https://dec.alaska.gov/spar/ppr/docs/dispersant_MSDS/Corexit%209500A%20MSDS.pdf.

Deming, J.W.; Junge, K. *Bergey's Manual of Systematic Bacteriology* (Bergey's Manual Trust, East Lansing, MI) Vol. 2, 2005.

Deppe, U.; Richnow, H.H.; Michaelis, W; Antranikian, G. Degradation of crude oil by an arctic microbial consortium. *Extremophiles*, **2005**, *9*, 461-470.

Dubinsky, E.A.; Conrad, M.E.; Chakraborty, R.; Bill, M.; Borglin, S.E.; Hollibaugh, J.T.; et al. Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environ. Sci. Technol.* **2013**, *47*, 10860-10867.

Feller, G. Molecular adaptations to cold in psychrophilic enzymes. *Cell. Mol. Life Sci.* **2003**, *60*, 648-662.

Foght, J.M.; Westlake, D.W.S. Effect of the dispersant Corexit 9527 on the microbial degradation of Prudhoe Bay oil. *Can. J. Microbiol.* **1982**, *28*, 117-122.

Foght, J.M.; Fedorak, P.M.; Westlake D.W.S. Effect of the dispersant Corexit 9527 on the microbial degradation of sulfur heterocycles in Prudhoe Bay oil. *Can. J. Microbiol.* **1983**, 29, 623-627.

Fuenmayor, S.L.; Wild, M.; Boyes, A.L.; Williams, P.A. A gene cluster encoding steps in conversion of naphthalene to gentisate in Pseudomonas sp. strain U2. *J. Bacteriol.* **1998**, *180*, 2522-2530.

Garneau, M.È.; Michel, C.; Meisterhans, G.; Fortin, N.; King, T.L.; Greer, C.W.; Lee, K. Hydrocarbon biodegradation by Arctic sea-ice and sub-ice microbial communities during microcosm experiments, Northwest Passage (Nunavut, Canada). *FEMS Microbiol. Ecol.* **2016**, p.fiw130.

Gerdes, B.; Brinkmeyer, R.; Dieckmann, G.; Helmke, E. Influence of crude oil on changes of bacterial communities in Arctic sea-ice. *FEMS Microbiol. Ecol.* **2005**, *53*, 129-139.

Gibb, A.; Chu, A.; Wong, R.C.K.; Goodman, R.H. Bioremediation kinetics of crude oil at 5C. *J. Environ. Eng.* **2001**, *127*, 818-824.

Hazen, T.C.; Dubinsky, E.A.; DeSantis, T.Z.; Andersen, G.L.; Piceno, Y.M.; Singh, N.; Jansson, J.K.; Probst, A.; Borglin, S.E.; Fortney, J.L.; Stringfellow, W.T. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science*, **2010**, *330*, 204-208.

Head, I.M.; Jones, D.M.; Röling, W.F. Marine microorganisms make a meal of oil. *Nature Rev. Microbiol.* **2006**, *4*, 173-182.

Honrath, R.E.; Mihelcic, J.R. Physical Processes. In *Fundamentals of Environmental Engineering*; Anderson, W., Eds.; John Wiley & Sons, Inc.: New York 1999; pp 180.

Kleindienst, S.; Seidel, M.; Ziervogel, K.; Grim, S.; Loftis, K.; Harrison, S.; Malkin, S.Y.; Perkins, M.J.; Field, J.; Sogin, M.L.; Dittmar, T. Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *PNAS*. **2015**, *112*, 14900-14905.

Laake, M.; Tjessem, K.; Rein, K. Fate of a tritiated Ekofisk crude oil in a controlled ecosystem experiment with North Sea plankton. *Environ. Sci. Technol.* **1984**, *18*, 641-647.

Landes, K. Mother Nature as an oil polluter. *Am. Assoc. Pet. Geol. Bull.* **1973**, *57*, 637-641.

Lu, Z.; Deng, Y.; Van Nostrand, J.D.; He, Z.; Voordeckers, J.; Zhou, A.; Lee, Y.J.; Mason, O.U.; Dubinsky, E.A.; Chavarria, K.L; Tom, L.M. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. *ISME J.* **2012**, *6*, 451-460.

Lunel, T.; Rusin, J.; Bailey, N.; Halliwell, C.; Davies, L. The net environmental benefit of a successful dispersant operation at the Sea Empress incident. *IOSC*, **1997**, *1*, 185-194.

Macnaughton, S.J.; Swannell, R.; Daniel, F.; Bristow, L. Biodegradation of dispersed Forties crude and Alaskan North Slope oils in microcosms under simulated marine conditions. *Spill Sci. Technol. B.* **2003**, *8*, 179-186.

Margesin, R.; Schinner, F. Efficiency of indigenous and inoculated cold-adapted soil microorganisms for biodegradation of diesel oil in alpine soils. *Appl. Envir. Microbiol.* **1997**, *63*, 2660-2664.

Margesin, R.; Gander, S.; Zacke, G.; Gounot, A.M.; Schinner, F. Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts. *Extremophiles.* **2003**, *7*, 451-458.

Mason, O.U.; Han, J.; Woyke, T.; Jansson, J.K. Single-cell genomics reveals features of a Colwellia species that was dominant during the Deepwater Horizon oil spill. *Front. Microbiol.* **2014**, *5*, 29.

McCune, B.; Mefford, M.J. *PC-ORD v. 6.255 beta. MjM Software Design*. Gleneden Beach, Oregon, 2011.

McFarlin, K.M.; Prince, R.C.; Perkins, R; Leigh, M.B. Biodegradation of dispersed oil in arctic seawater at -1C. *PloS One*, **2014**, *9*, p.e84297.

Mearns, A.J., Cleaning oiled shores: putting bioremediation to the test. *Spill Sci. Technol. Bull.* **1997**, *4*, 209-217.

Michaud, L.; Giudice, A.L.; Saitta, M.; De Domenico, M.; Bruni, V. The biodegradation efficiency on diesel oil by two psychotrophic Antarctic marine bacteria during a two-month-long experiment. *Mar. Pollut. Bull.* **2004**, *49*, 405-409.

Mielke, PW. Meteorological Applications of Permutation Techniques Based on Distance Functions. In *Handbook of Statistics, Vol. 4: Nonparametric Methods*; Krishnaiah, P.R.; Sen, P.K., Eds.; Elsevier North-Holland: Amsterdam 1984; pp 813-830.

Mielke, P.W.; Berry, K.Y. *Permutation Methods: A Distance Function Approach*. Springer Series in Statistics: New York 2001.

Mihelcic, J.R. Chemistry. In *Fundamentals of Environmental Engineering*; Anderson, W., Eds.; John Wiley & Sons, Inc.: New York 1999; pp 49-55.

Miller, D.N.; Bryant, J.E.; Madsen, E.L.; Ghiorse, W.C. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *App. Env. Micro.* **1999**, *65*, 4715-4724.

Moyer, C.L.; Morita, R.Y. Psychrophiles and psychrotrophs. *eLS*. **2007**, DOI: 10.1002/9780470015902.a0000402.pub2

National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2010. http://www.iadc.org/archived-2014-osc-report/response/response-actions-dispersants.html. Date accessed: 8.18.2016

National Research Council (NRC). *Oil Spill Dispersants: Efficacy and Effects*; National Academy Press: Washington DC, 2005.

National Research Council (NRC). *Responding to Oil Spills in the U.S. Arctic Marine Environment*; National Academies Press: Washington, DC, 2014.

Parker, A.M.; Ferrer, I.; Thurman, E.M.; Rosario-Ortiz, F.L.; Linden, K.G. Determination of COREXIT components used in the Deepwater Horizon cleanup by liquid chromatography-ion trap mass spectrometry. *Anal. Methods*, **2014**, *6*, 5498-5502.

Petroleum Distillates SDS; REACH Registration No. 01-2119484819-18-0010; Shell Trading Rotterdam B.V.; Netherlands, 2012.

Place, B.J.; Perkins, M.J.; Sinclair, E.; Barsamian, A.L.; Blakemore, P.R.; Field, J.A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep-Sea Res. Pt. II.* **2016**, *129*, 276-281.

Prabagaran, S.R.; Manorama, R.; Delille, D.; Shivaji, S. Predominance of Roseobacter, Sulfitobacter, Glaciecola and Psychrobacter in seawater collected off Ushuaia, Argentina, Sub-Antarctica. *FEMS Microbiol. Ecol.* **2007**, *59*, 342-355.

Prince, R.C.; Elmendorf, D.L.; Lute, J.R.; Hsu, C.S.; Haith, C.E.; et al. 17a(H),21b(H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environ. Sci. Technol.* **1994**, *28*, 142-145.

Prince, R.C.; McFarlin, K.M.; Butler, J.D;, Febbo, E.J.; Wang, F.C. Nedwed, T.J. The primary biodegradation of dispersed crude oil in the sea. *Chemosphere*, **2013**, *90*, 521-526.

Prince, R.C.; Butler, J.D. A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environ. Sci. Pollut. Res. Int.* **2014**, *21*, 9506-9510.

Prince, R.C.; Butler, J.D.; Redman, A.D. The rate of crude oil biodegradation in the sea. *Environ. Sci. Technol.* **2017**, *51*, 1278-1284.

Ramirez, C.E.; Batchu, S.R.; Gardinali, P.R. High sensitivity liquid chromatography tandem mass spectrometric methods for the analysis of dioctyl sulfosuccinate in different stages of an oil spill response monitoring effort. *Anal. Bioanal. Chem.* **2013**, *405*, 4167-4175.

Redmond, M.C.; Valentine, D.L. Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. *PNAS*, **2012**, *109*, 20292-20297.

Robador, A.; Brüchert, V.; Jørgensen, B.B. The impact of temperature change on the activity and community composition of sulfate□reducing bacteria in arctic versus temperate marine sediments. *Environ. Microbiol.* **2009,** *11*, 1692-1703.

Rojo, F. Degradation of alkanes by bacteria. *Environ. Microbiol.* **2009**, *11*, 2477-2490.

Rouse, J.D.; Sabatini, D.A.; Suflita, J.M.; Harwell, J.H. Influence of surfactants on microbial degradation of organic compounds. *Crit. Rev. Environ. Sci. Technol.* **1994**, *24*, 325-370.

Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, EB; et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *App Env Microbiol.* **2009**, *75*, 7537-7541.

Schloss, P.D.; Gevers, D.; Westcott, S.L. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PloS One*, **2011**, 6:e27310.

Seidel, M.; Kleindienst, S.; Dittmar, T.; Joye, S.B.; Medeiros, P.M. Biodegradation of crude oil and dispersants in deep seawater from the Gulf of Mexico: Insights from ultra-high resolution mass spectrometry. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2016**, *129*, 108-118.

SL Ross. *Corexit 9500 Dispersant Effectiveness Testing In Cold Water On Four Alaskan Crude Oils.* Report to U.S. Department of the Interior, Mineral Management Service, 2007.

Smits, T.H.M.; Rothlisberger, M.; Witholt, B.; van Beilen, J.B. Molecular screening for alkane hydroxylase genes in Gram-negative and Gram-positive strains. *Environ. Microbiol.* **1999**, *1*, 307-317.

Smits, T.H.M.; Balada, S.B.; Witholt, B.; van Beilen, J.B. Functional analysis of alkane hydroxylases from Gram-negative and Gram-positive bacteria. *J. Bacteriol.* **2002**, *184*, 1733-1742.

Stewart, P.S.; Tedaldi, D.J.; Lewis, A.R.; Goldman, E. Biodegradation rates of crude oil in seawater. *Water Environ. Res.* **1993**, *65*, 845-848.

Techtmann, S.M.; Fitzgerald, K.S.; Stelling, S.C.; Joyner, D.C.; Uttukar, S.M.; Harris, A.P.; Alshibli, N.K.; Brown, S.D.; Hazen, T.C. *Colwellia psychrerythraea* strains from distant deep sea basins show adaptation to local conditions. *Front. Environ. Sci.* **2016**, *4*, 33.

Tissot, B.P.; Welte, D.H. Composition of crude oils. In *Petroleum Formation and Occurrence*. Springer-Verlag: Berlin 1984; pp 384.

US EPA. Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species. U.S. Environmental Protection Agency, Office of Research and Development, 2010; https://archive.epa.gov/bpspill/web/html/dispersants-testing.html

Van Nostrand, J.D.; Yin, H.; He, Z.; Zhou, J. Hybridization of Environmental Microbial Community Nucleic Acids by GeoChip. In *Methods in Molecular Biology - Microbial Environmental Genomics*; Martin, F.M., Uroz, S., Eds.; Springer Press, 2016.

Venosa, A.D; Holder, E.L. Biodegradability of dispersed crude oil at two different temperatures. *Mar. Pollut. Bull.* **2007**, *54*, 545-553.

Vomberg, A.; Klinner, U., Distribution of *alkB* genes within n□alkane□degrading bacteria. *J. Appl. Microbiol.* **2000**, *89*, 339-348.

Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261-5267.

Word, J.Q.; Clark, J.R.; Word, L.S. Comparison of the acute toxicity of Corexit 9500 and household cleaning products. *Hum. Ecol. Risk Assess.* **2015**, *21*, 707-725.

Yakimov, M.M.; Giuliano, L.; Gentile, G.; Crisafi, E.; Chernikova, T.N.; Abraham, W.R.; Lünsdorf, H.; Timmis, K.N.; Golyshin, P.N. *Oleispira antarctica* gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 779-785.

Yakimov, M.M.; Timmis, K.N.; Golyshin, P.N. Obligate oil-degrading marine bacteria. *Curr. Opin. Biotechnol.* **2007**, *18*, 257-266.

Figures

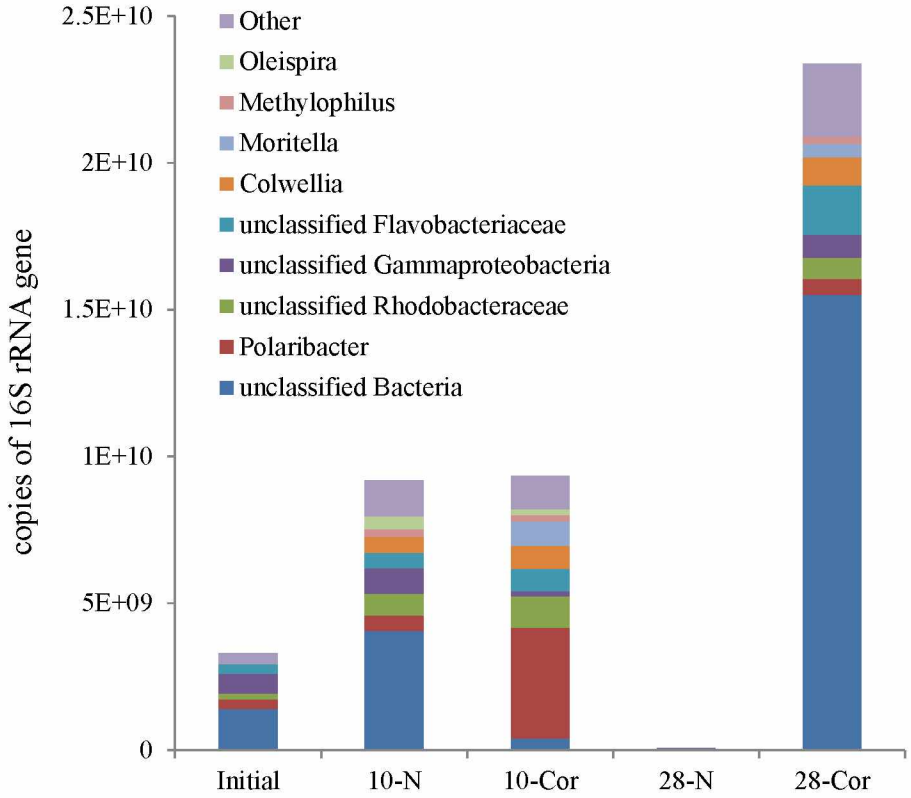

Figure 3-1. Mean relative abundance of bacterial sequences as a portion of mean prokaryotic abundance in the nearshore Corexit-only experiment. Bottles contained surface seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N, biotic control) or Corexit 9500 (Cor; 15 mg/L), and were incubated at 2°C for 0, 10, and 28 days ($n = 3$).

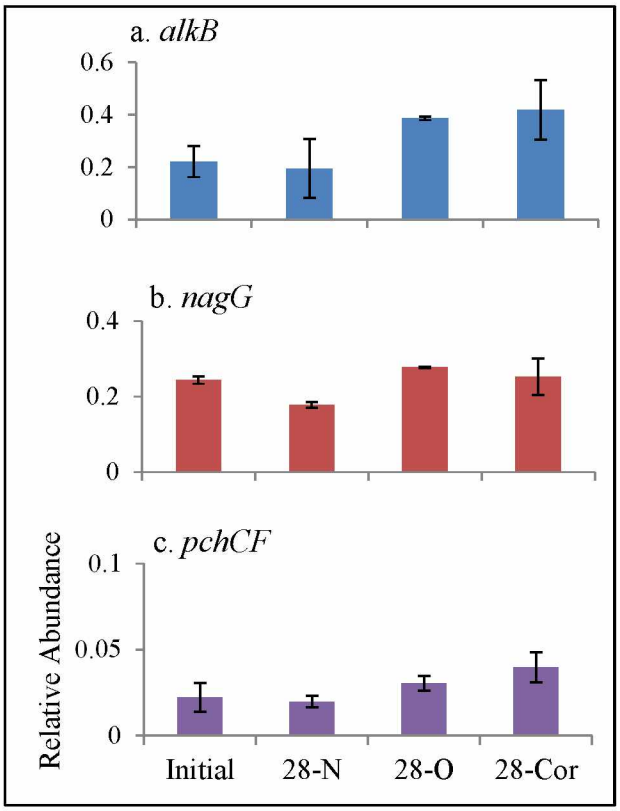

Figure 3-2. Relative abundance of *alkB*, *nagG*, and *pchCF* genes in offshore experiment at day 0 and 28. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) and were incubated at 2°C. Error bars are standard deviations.

Tables

Table 3-1. Mean percent loss of total measureable hydrocarbons in Arctic surface seawater. All incubations contained whole ANS crude oil ($n = 3$). Different letters correspond to significant differences among time points (MRPP, $p < 0.05$). Errors are standard deviation. nm: not measured.

Location	Citation	Oil (mg/L)	Temp (°C)	Nutrients (mg/L)	Percent Loss			
					Day 5	Day 10	Day 28	Day 63
Offshore (Sept.)	This study	15	2	16	16 ± 4.2^{a}	29 ± 5.5^{b}	36 ± 6.2^{bc}	nm
Offshore (Oct.)	This study	15	2	16	16 ± 4.6^{a}	28 ± 3.1^{b}	41 ± 0.0^{c}	nm
Nearshore (Feb.)	McFarlin et al., 2014*	2.5	-1	0	nm	36 ± 3.0	45 ± 3.6	58 ± 10

* Percent losses includes abiotic losses

Table 3-2. Mean concentration of Corexit 9500 surfactant components in offshore and nearshore seawater (n = 3). Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and Corexit (15 mg/L) and were incubated at 2°C. Abiotic controls are designated with an 'A' after the time point. Different letters correspond to significant differences among time points within each surfactant ($p < 0.05$; MRPP). Error bars represent standard deviation. LOD: limit of detection.

Offshore		DOSS (µg/L)	Span 80 (µg/L)	Tweens (µg/L)
	Day 0	2875 ± 697^{a}	244 ± 30^{e}	2006 ± 21^{q}
	Day 28A	2269 ± 397^{a}	132 ± 91^{efg}	1228 ± 568^{q}
	Day 28	71 ± 15^{d}	<LOD	<LOD
Nearshore				
	Day 0	2256 ± 211^{a}	179 ± 23^{efg}	2242 ± 110^{r}
	Day 10A	2188 ± 123^{a}	150 ± 10^{f}	409 ± 33^{s}
	Day 10	1659 ± 196^{b}	44 ± 2^{h}	<LOD
	Day 28A	2209 ± 219^{a}	177 ± 8^{g}	298 ± 41^{t}
	Day 28	1464 ± 166^{c}	<LOD	<LOD

Supplementary Information

Methods

Chemical analysis of Corexit 9500

Mass spectrometric detection was performed with a Waters Micromass Quattro Mass Spectrometer as described previously (Place et al., 2016). We used an Agilent Proshell 120 EC-C18 guard column (4.6 mm ID x 5 mm length, with 2.7-μm particles) to accommodate high backpressure. A 50 mm Targa C18 analytical column (2.1 mm ID x 50 mm, with 5-mm particles; Higgins Analytical, Inc., Mountain View, CA) was used for chromatographic separations. The 50-mm column allowed for the flow rate to be increased to 1 mL/min during sample loading and washing non-volatile salts from the column (first 5.6 minutes) without degrading peak shape or percent recovery of analytes. The gradient was further modified such that the 97.5% acetonitrile was held for 3 min before returning to 5% acetonitrile for 6 min. The flow rate was 1 mL/min for the first 6 min, 0.5 mL/min for the next 5 min, and 1.0 mL/min for the last 6 minutes. The timing of the main-pass by-pass valve switching and divert valve switching, as described by Place et al. (2016), was adjusted to reflect changes in the flow rate and gradient.

Calibration curves consisted of at least 5 calibration standards and required a correlation coefficient of 0.99 or greater for use in analysis. All calibration curves were weighted (factor of 1/x), and standards with calculated concentrations above 20% of intended concentrations were removed from the calibration curve calculation. Calibration curves spanned from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ): for DOSS (0.2-25 μg/L), Span 80 (60-300 μg/L), Tween 80 (60-300 μg/L), and Tween 85 (60-300 μg/L). Each calibration standard was spiked to give a final concentration of 500 ng/L ^{13}C4-DOSS. Blank and check standards, as described by Place et al. (2016), were used for quality control purposes and consisted of at least 20% of the total samples run in any given sequence. Check standards for DOSS fell within 20% of the spiked concentration and the non-ionic Corexit surfactants fell within 35% of the spiked concentration. All blanks fell below the limit of detection.

Quantitative real-time PCR

Briefly, a synthetic double-stranded DNA molecule of 482 bp was synthesized (IDT, Coralville, Iowa) and re-suspended to known molarity. PCR oligonucleotide primers were also synthesized complementary to the 5' and 3' region of the synthesized gBlock fragment, and designed based on known prokaryotic 16S rRNA gene sequences. The primers were GTGCCAGCMGCCGCGGTAA ("515F Original", Caporaso et al., 2010; Walters et al., 2016) and GGACTACNVGGGTWTCTAAT ("806R Modified", Apprill et al., 2015; Walters et al., 2016). qPCR reactions were conducted in replicates with standard curves according to manufacturer recommendations using Power SYBR qPCR master mix (Life Technologies, Carlsbad, CA) and run on an Applied Biosystems 7900HT Sequence Detection System. A regression line, created from standards, was used to quantify abundance.

Microbial community analysis

PCR products were normalized and pooled using an Invitrogen SequalPrep DNA Normalization plate (Thermo Fisher Scientific, Waltham, MA). The pooled libraries were quality-controlled and quantified prior to loading on an Illumina MiSeq v2 flow cell and sequenced in a 2 x 250 bp format with a standard v2 500 cycle reagent cartridge. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v1.8.4.

Statistical analyses

We used NMS ordination plots to illustrate differences in bacterial community structure and the abundance of petroleum biodegradation genes in treatments containing ANS crude oil, Corexit 9500, and no carbon amendments. The dimensionality of the data within each NMS was determined with a Bray-Curtis distance measure in autopilot mode using 100 runs with real data and random starting configurations (Mather, 1976; Kruskal, 1964). After the NMS was created, a Monte Carlo test with 249 randomized runs was conducted to evaluate whether the NMS was extracting stronger axes than expected by chance. The stability of each solution was determined by plotting stress versus iteration (McCune & Mefford, 2011).

Cluster analysis objectively identifies groups that are most similar and builds groups within groups to show differences. We used hierarchical clustering to determine similarities

among treatments containing no amendments, ANS crude oil, and Corexit. Hierarchical clustering was performed with a Bray-Curtis distance measure and similarities were illustrated in a dendrogram. The dendrogram was created with a group average linkage method and was not scaled or pruned.

ISA is a statistical calculation that indicates which species are responsible for observed differences. ISA was used to identify taxa that were associated with either ANS crude oil or Corexit in seawater incubations. This statistical analysis revealed organisms that responded to oil or dispersant by calculating the proportional abundance and consistency of a particular species in a treatment relative to that species in all other treatments (McCune & Grace, 2002). We conducted an ISA using the default Dufrêne & Legendre (1997) analysis. Results are reported as an indicator value (IV) for each species and the statistical significance of each IV was evaluated by a Monte Carlo method. Indicator values range from zero (no indication) to 100 (perfect indication). Species that had statistically significant p-values ($p < 0.05$) are reported with their IVs for oil and dispersant treatments at day 28.

References

Apprill, A.; McNally, S.; Parsons, R.; Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **2015**, *75*, 129-137.

Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, **2010**, *7*, 335-336.

Dufrêne, M.; Legendre, P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **1997**, *67*, 345-366.

Kruskal, J.B. Nonmetric multidimensional scaling: a numerical method. *Psychometrika*, **1964**, *29*, 115-129.

Mather PM. Computational Methods of Multivariate Analysis in Physical Geography. Wiley & Sons: London, 1976.

McCune, B.; Grace, J.B. Analysis of Ecological Communities. MjM Software Design. Gleneden Beach, Oregon, USA, 2002.

McCune, B.; Mefford, M.J. *PC-ORD v. 6.255 beta. MjM Software Design*. Gleneden Beach, Oregon, 2011.

Place, B.J.; Perkins, M.J.; Sinclair, E.; Barsamian, A.L.; Blakemore, P.R.; Field, J.A. Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep-Sea Res. Pt. II.* **2016**, *129*, 276-281.

Walters, W.; Hyde, E.R.; Berg-Lyons, D.; Ackermann, G.; Humphrey, G.; Parada, A.; Gilbert, J.A.; Jansson, J.K.; Caporaso, J.G.; Fuhrman, J.A.; Apprill, A. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems*. **2016**, *1*, e00009-15.

Figures

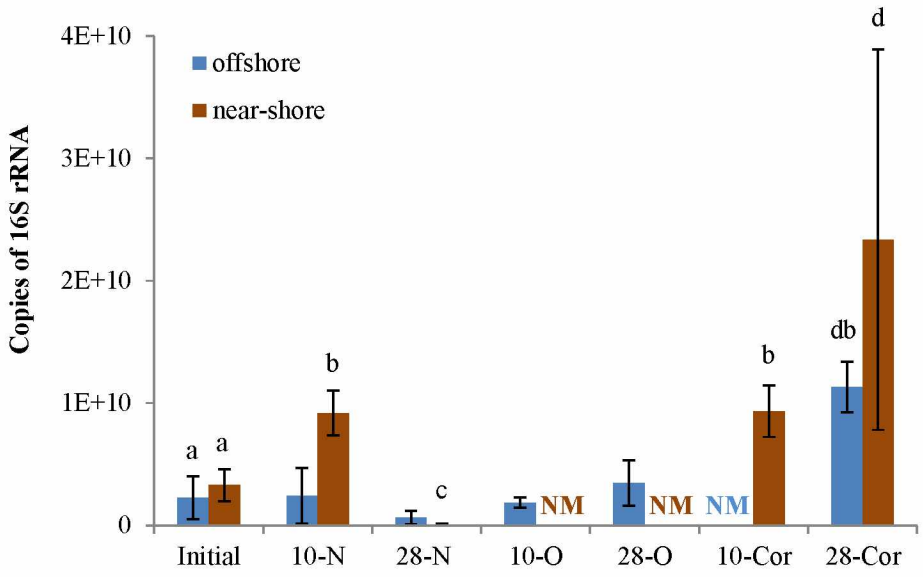

Figure S3-1. Mean abundance of prokaryotes in offshore and nearshore experiments at 2°C. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L). Different letters correspond to significant differences among treatments containing three replicates (MRPP, $p < 0.05$). NM: not measured.

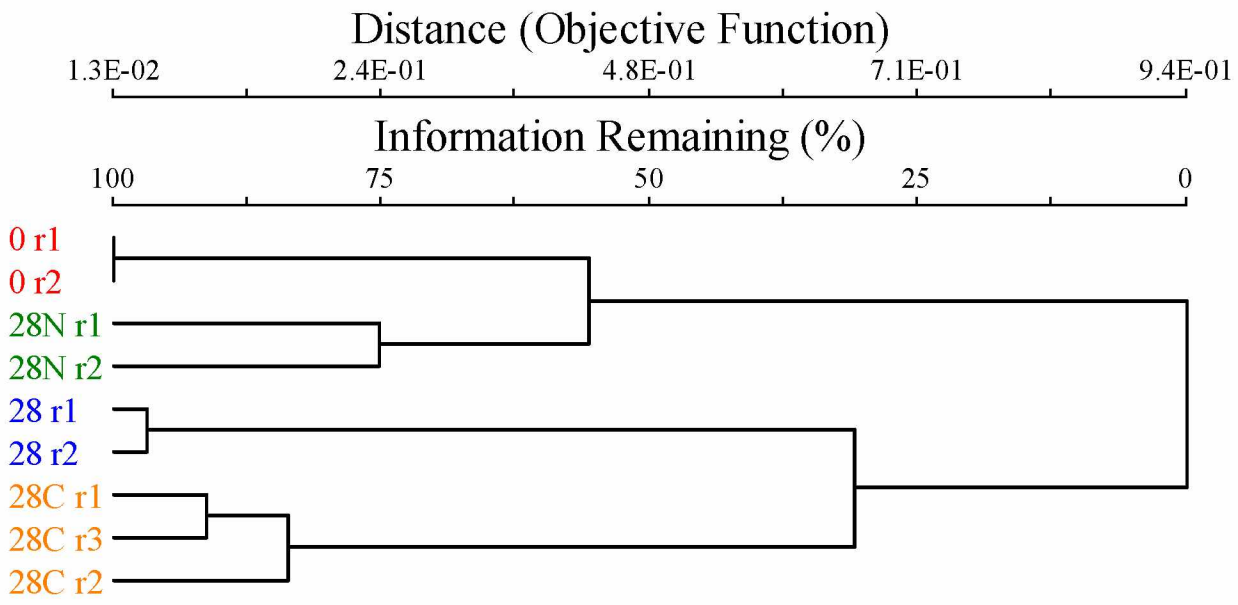

Figure S3-2. Dendrogram of bacterial sequences (16S rRNA genes) in offshore experiment at day 0 and 28. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no-amendment (N; biotic control), oil (15 mg/L), or Corexit 9500 (C; 15 mg/L), and were incubated at 2°C.

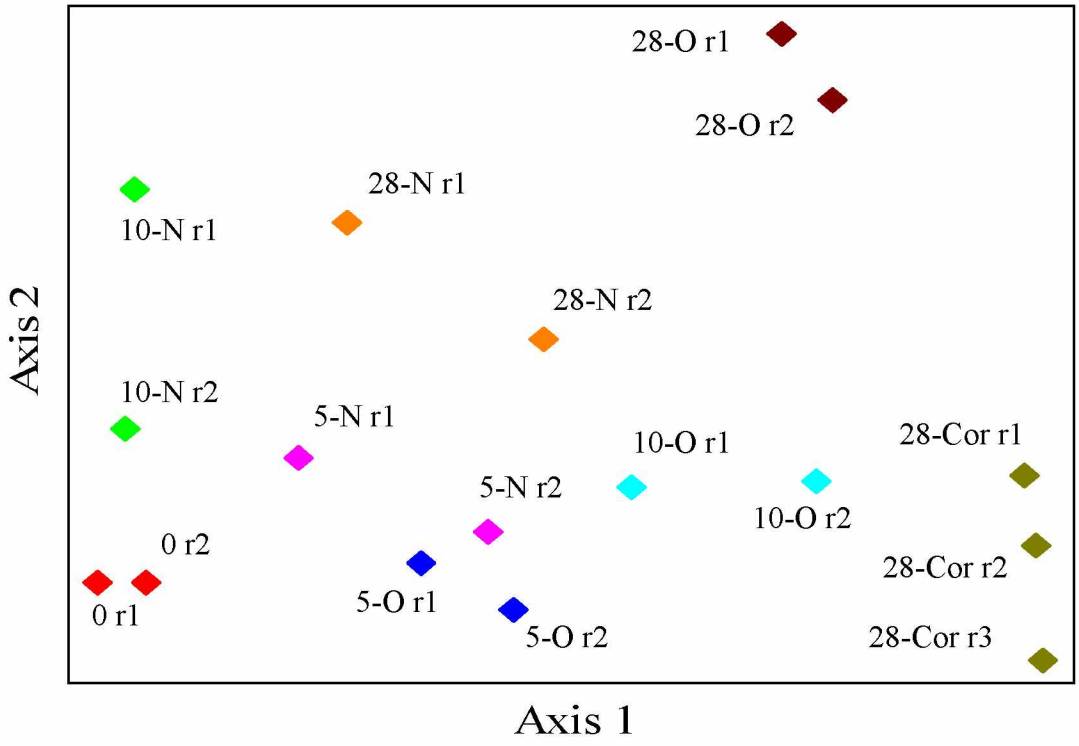

Figure S3-3. NMS ordination of bacterial sequences in the offshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L), and were incubated for 0, 5, 10, and 28 days at 2°C.

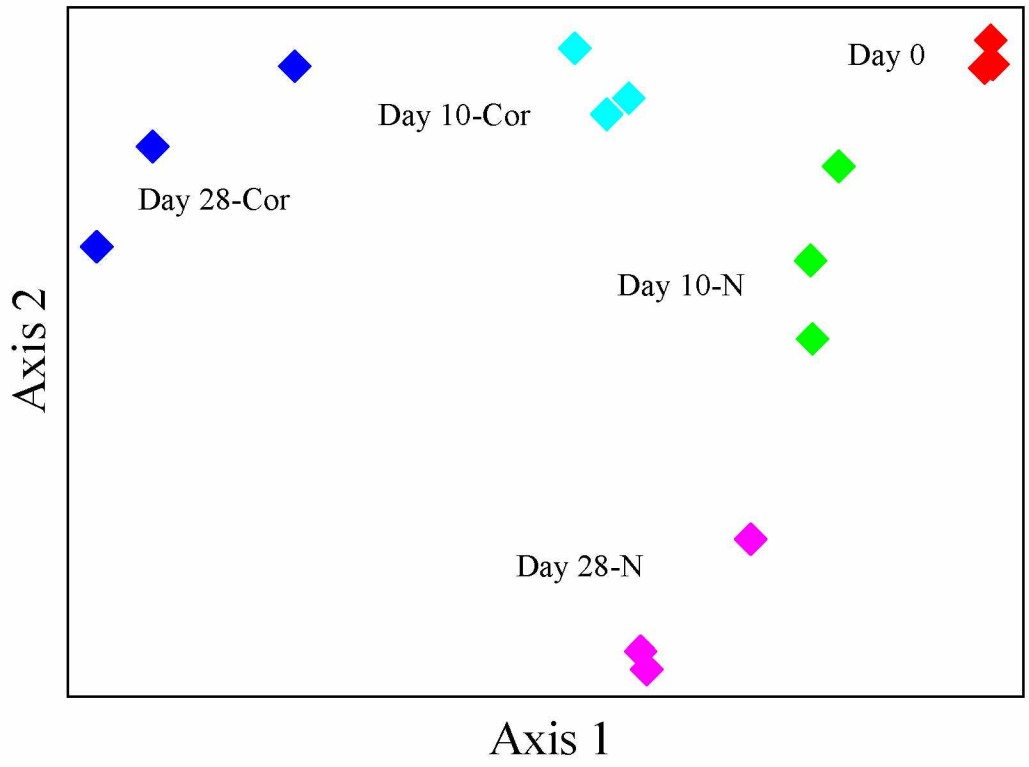

Figure S3-4. NMS ordination of bacterial sequences in the nearshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), or Corexit 9500 (Cor; 15 mg/L), and were incubated for 0, 10, and 28 days at 2°C ($n = 3$).

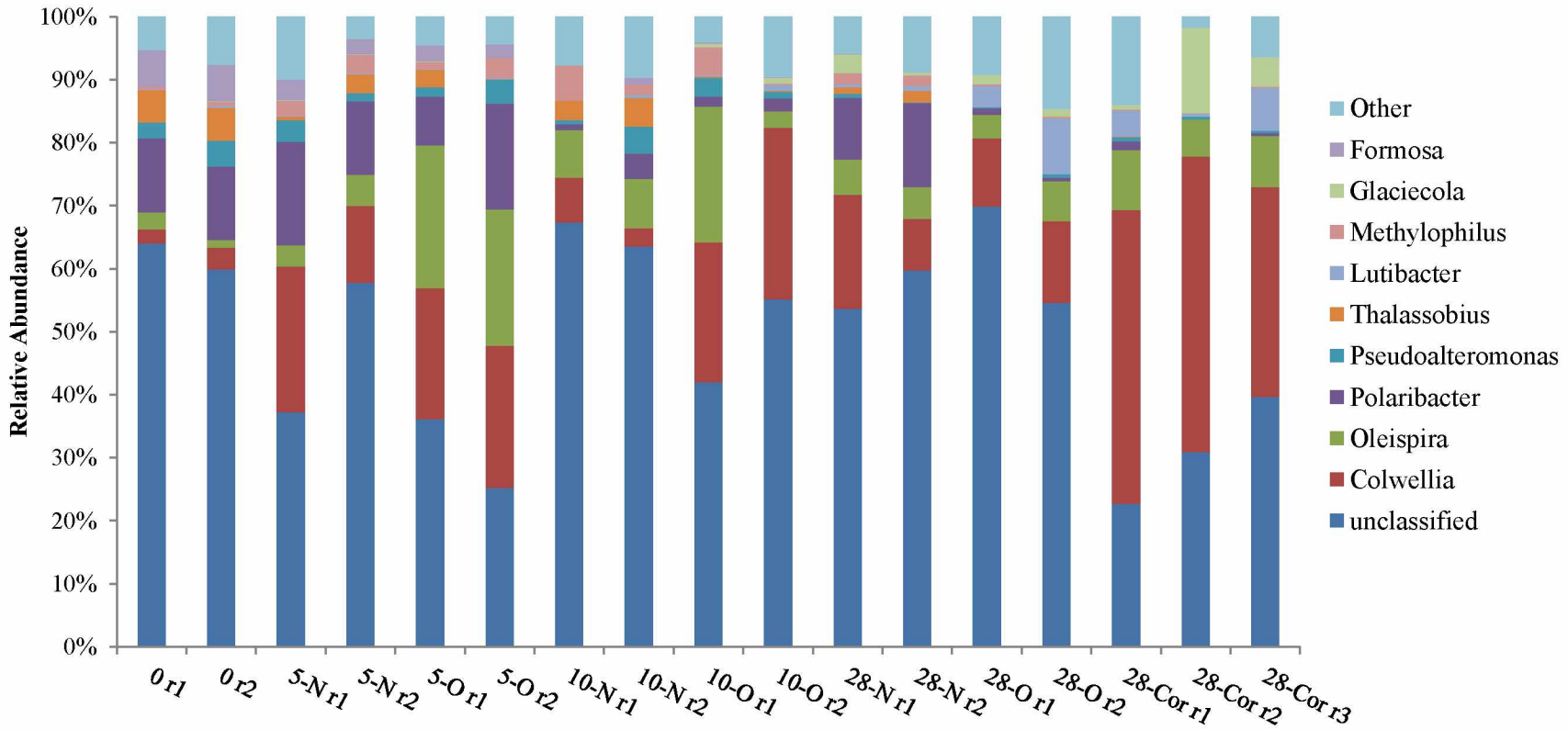

Figure S3-5. Relative abundance of bacterial genera in the offshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L), and were incubated for 0, 5, 10, and 28 days at 2°C. The Corexit treatment (28C, $n = 3$) was only incubated for 28 days in the offshore experiment.

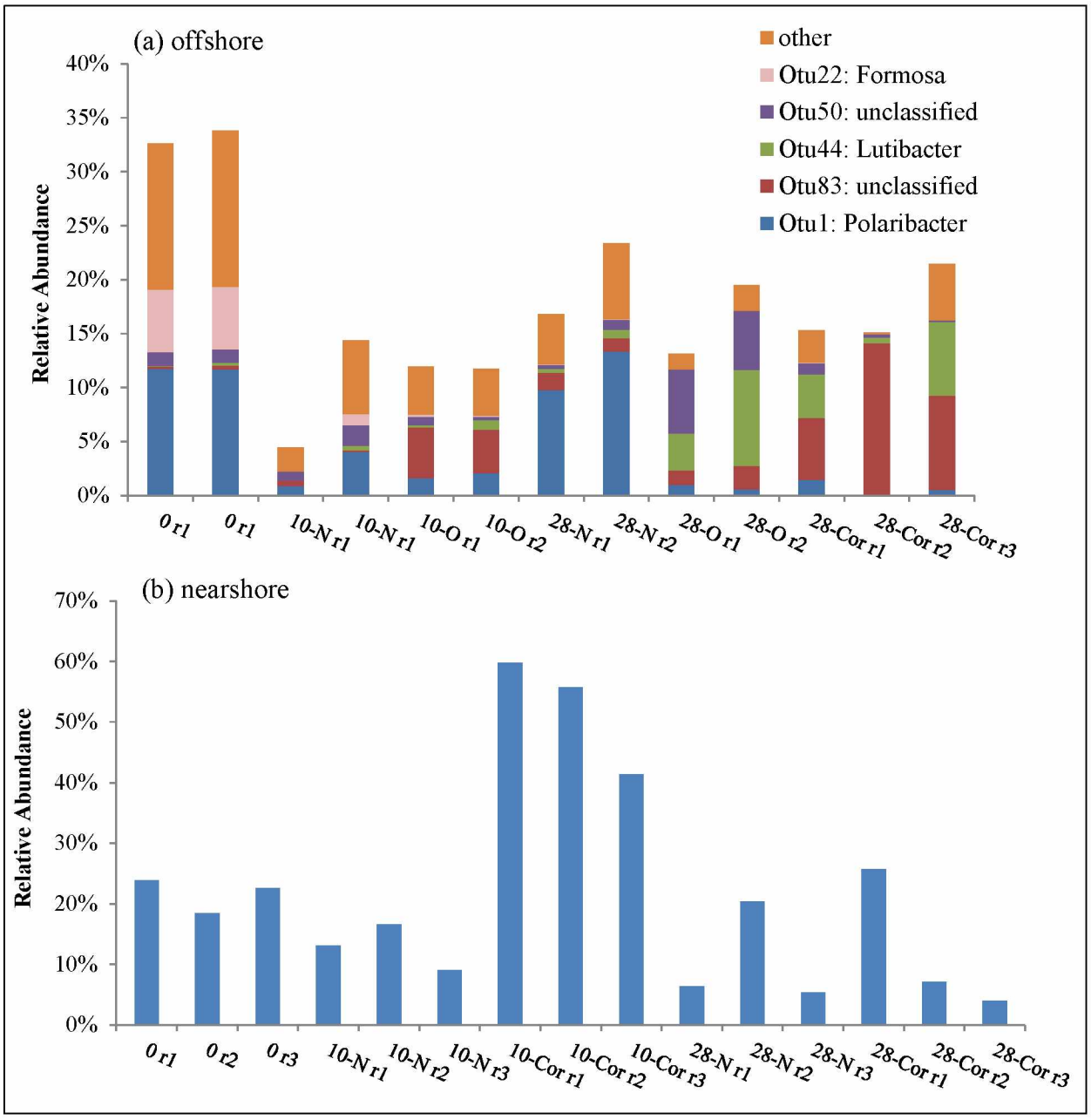

Figure S3-6. Relative abundance of bacterial sequences classified in the Flavobacteriaceae family in the (a) offshore experiment and (b) nearshore experiment at day 0, 10, and 28. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) and were incubated at 2°C. Individual OTUs (sequences) are identified in the offshore experiment to provide a specific comparison between oiled and Corexit treatments.

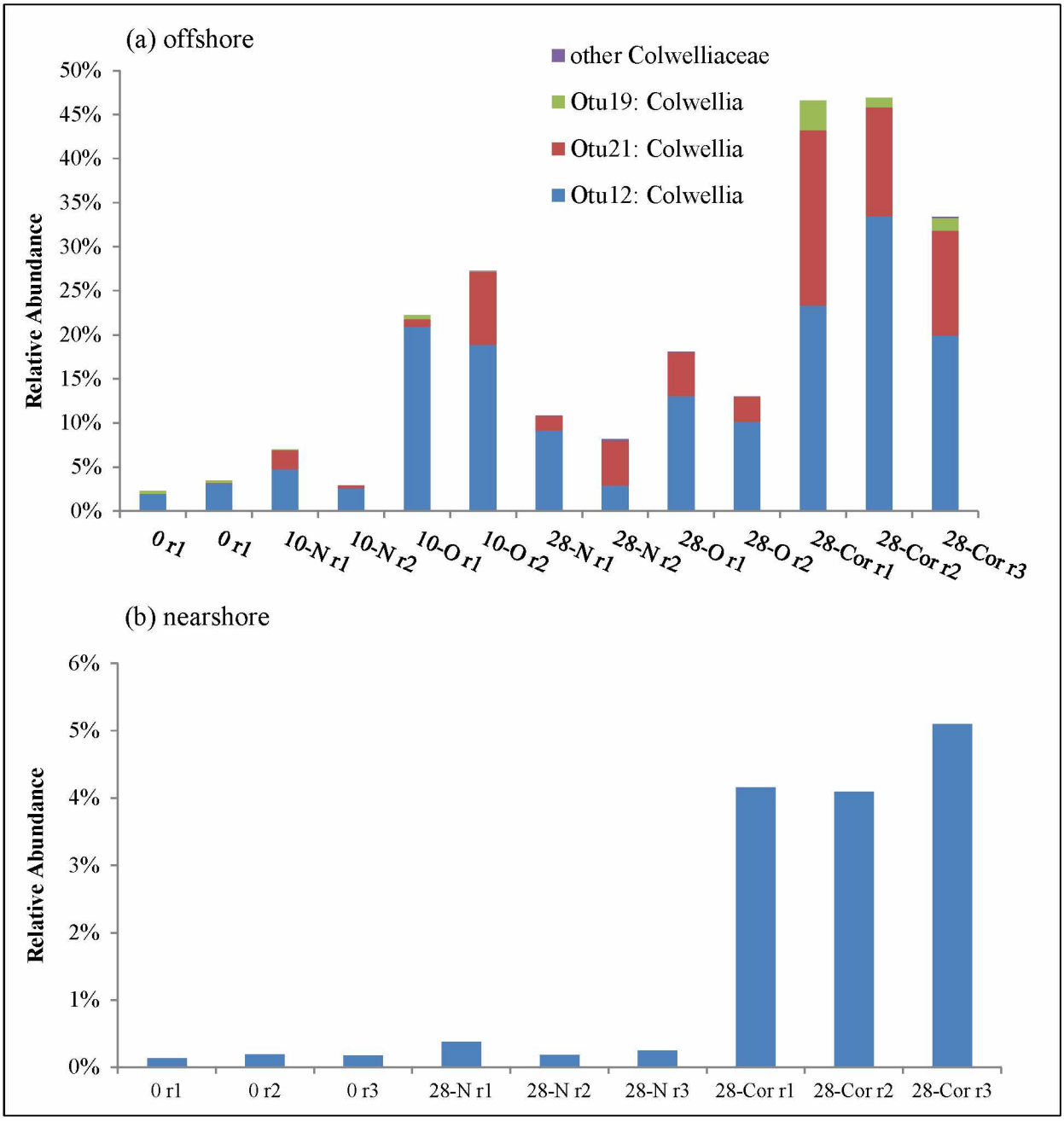

Figure S3-7. Relative abundance of bacterial sequences classified in the Colwelliaceae family in the (a) offshore experiment and (b) nearshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas) and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) and were incubated at 2°C. Individual OTUs (sequences) are identified in the October offshore experiment to provide a specific comparison between oiled and Corexit treatments.

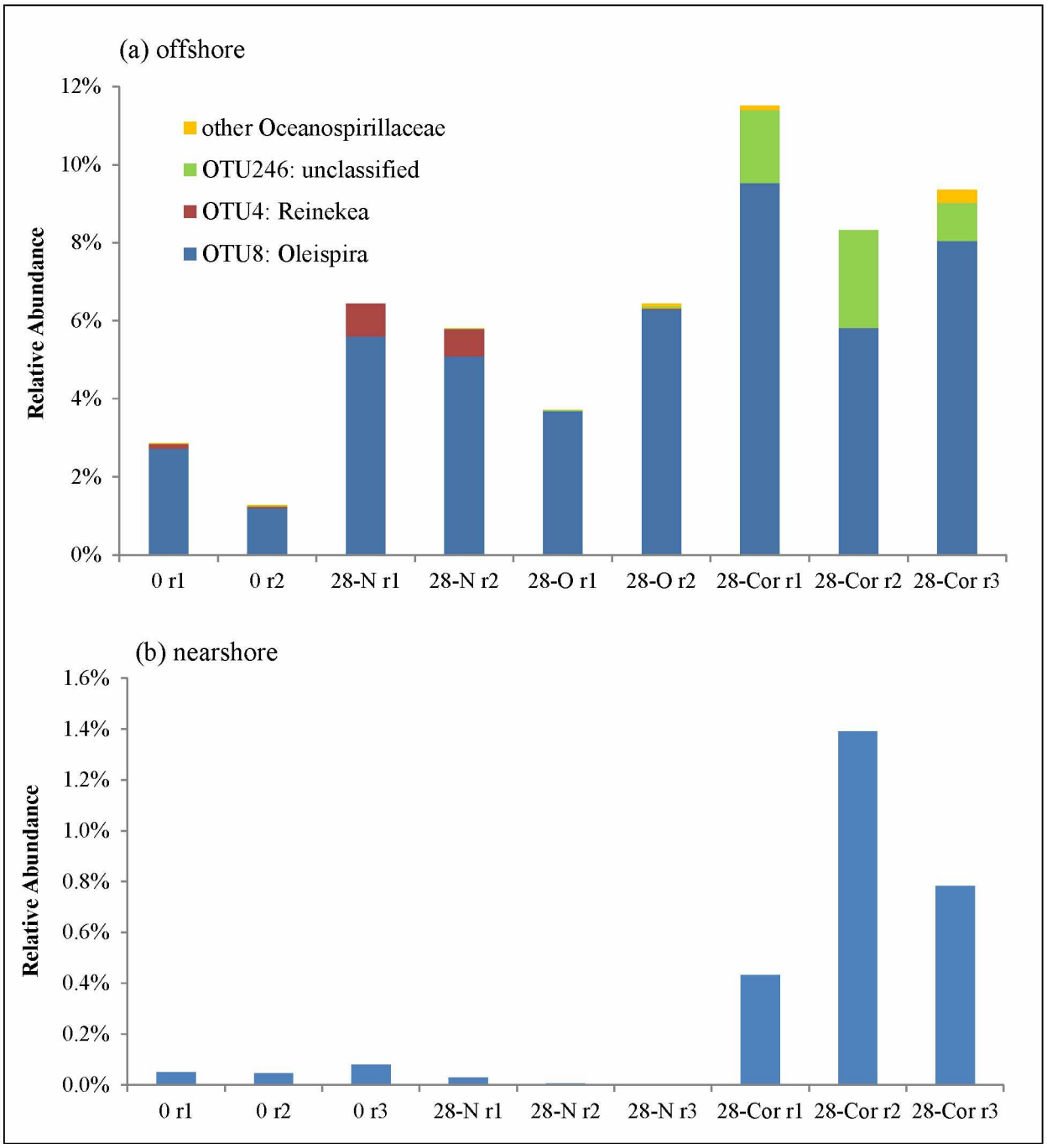

Figure S3-8. Relative abundance of bacterial sequences classified in the Oceanospirillaceae family at day 0 and 28 in the (a) offshore experiment and (b) nearshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) and were incubated at 2°C. Individual OTUs (sequences) are identified in the October offshore experiment to provide a specific comparison between oiled and Corexit treatments.

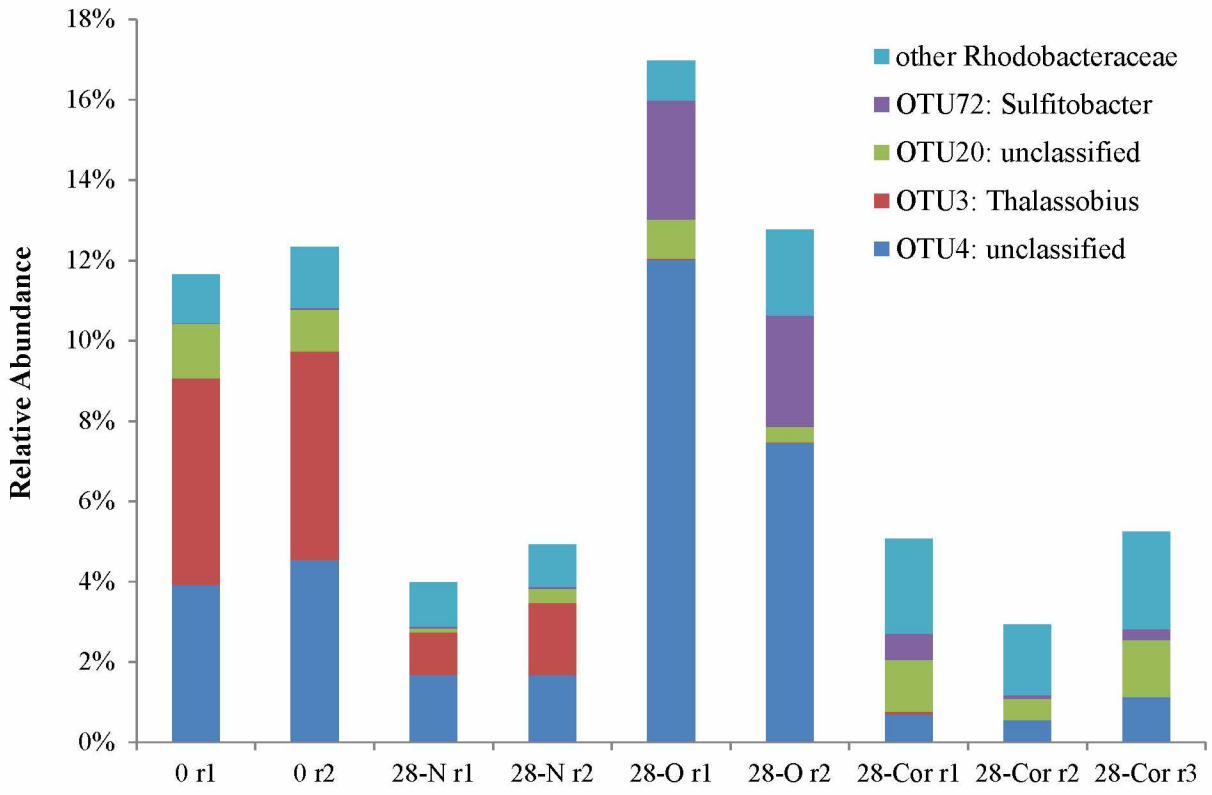

Figure S3-9. Relative abundance of sequences classified in the Rhodobacteraceae family at day 0 and 28 in incubations containing offshore seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) at 2°C. Individual OTUs (sequences) are identified to provide a specific comparison between oiled and Corexit treatments.

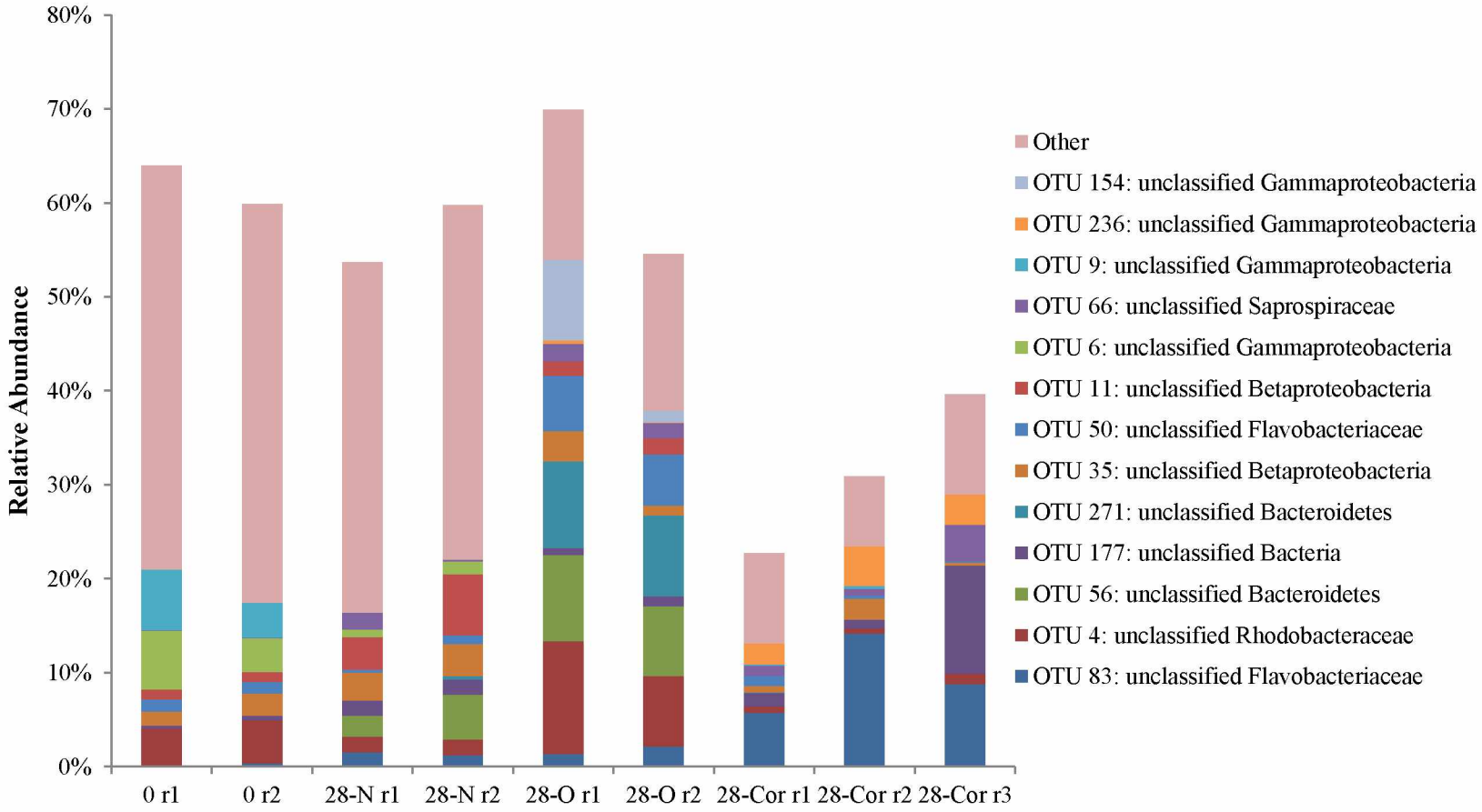

Figure S3-10. Unclassified sequences in the offshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) and were incubated at 2°C. Sequences with abundances greater than 2% of the community in day 0 and day 28 are identified.

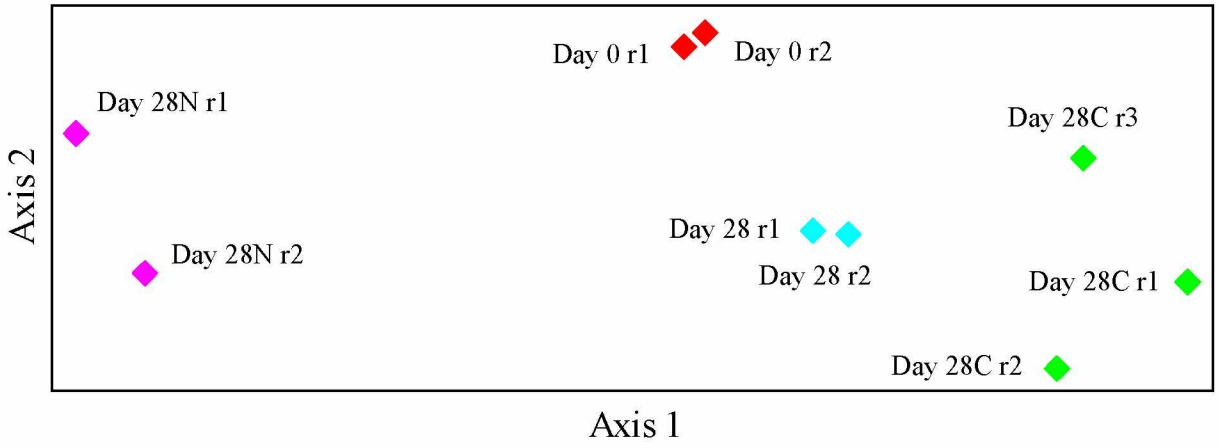

Figure S3-11. NMS of petroleum degradation genes in offshore experiment. Bottles contained offshore seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (15 mg/L), or Corexit 9500 (C; 15 mg/L), and were incubated at 2°C. Day 0 and 28 time points are shown.

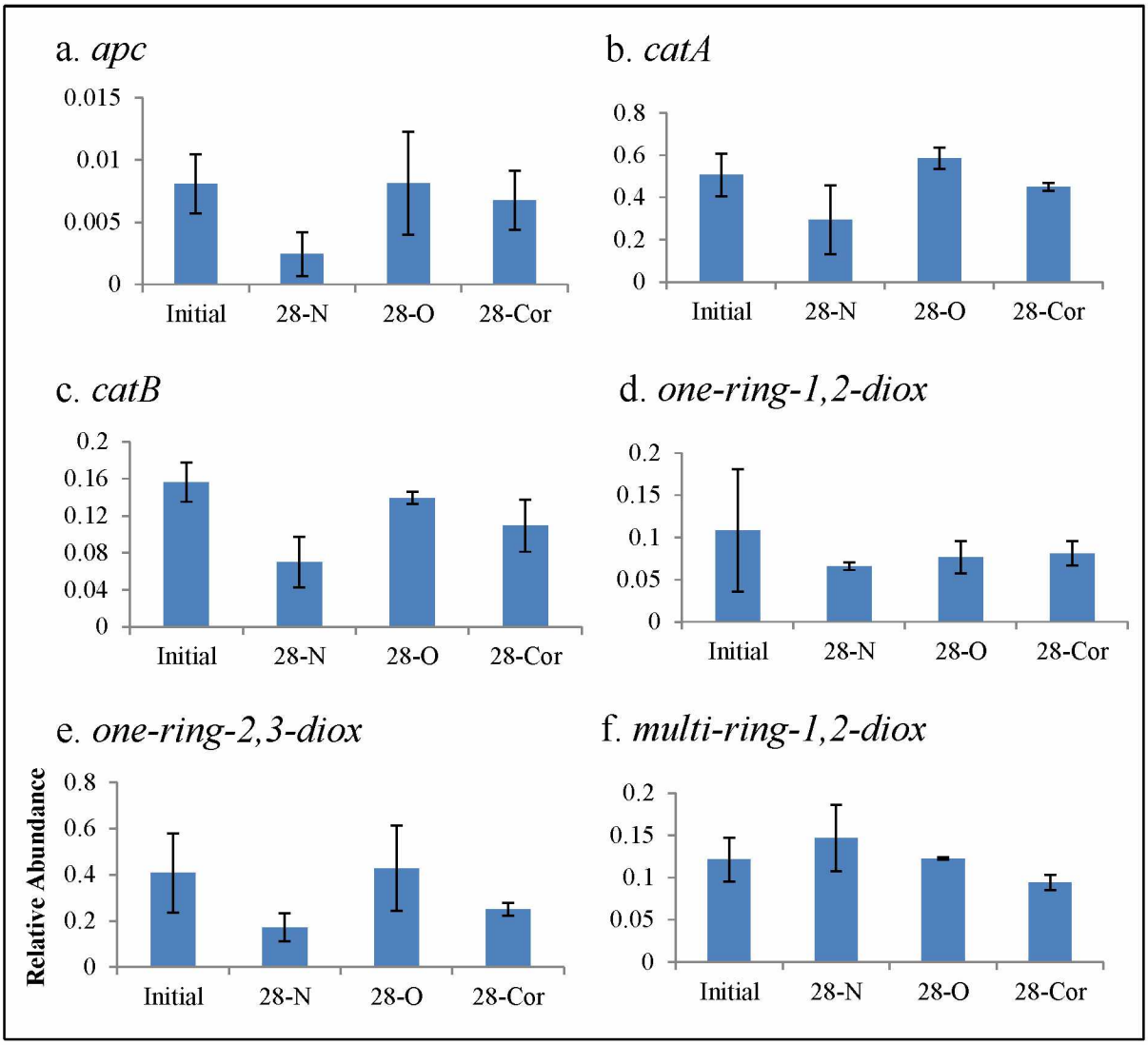

Figure S3-12. Mean relative abundance of petroleum degradation genes in incubations containing seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L) at day 0 and 28. Incubations were conducted at 2°C. Error bars represent standard deviation. Due to water availability, the Corexit treatment is the only treatment with three replicates; all other incubations have two replicates. apc (encodes acetophenone carboxylase); catA (encodes catechol 1,2-dioxygenase); catB (encodes muconate cycloisomerase); diox: dioxygenase.

Chapter 4: Bacterial Community Structure and Functional Potential in the Northeastern Chukchi Sea[1]

Abstract

We performed a molecular microbial ecological analysis in the northeastern Chukchi Sea in order to characterize bacterial community structure and genetic potential for biogeochemical cycling and oil biodegradation in a region targeted for oil and gas exploration (Burger lease area). Samples were collected from the surface, middle (20 m), and bottom (2-3 m above seafloor) of the water column during the open-water season of August and September 2012 at 17 different locations. We determined bacterial community structure with 16S rRNA gene sequencing and detected functional genes, including an array of oil biodegradation and biogeochemical cycling (carbon, nitrogen, and phosphorus cycling) genes, using the GeoChip 5.0 microarray, and then correlated molecular data to contextual physical and biogeochemical factors. Bacterial community structure differed significantly by depth (surface water vs. bottom water) and between sampling dates (August vs. September). While the relative abundance of major functional gene categories did not differ with depth, the abundance of individual functional genes for carbon cycling, nitrogen cycling, organic contaminant remediation, phosphorus cycling, sulfur cycling, virulence, and viruses differed between surface and bottom seawater samples. Aerobic oil-degradation genes and taxa known to include oil-degrading bacteria were found at all three depths. These findings support previous observations that two different water masses contribute to a stratified water column in the summer open-water season of the Burger lease area, but indicate that potential function is fairly similar with depth despite differences in temperature, water chemistry, bacterial community structure, and individual functional gene alleles.

[1] McFarlin KM, Questel JM, Hopcroft RR, Leigh MB. (2017). Bacterial Community Structure and Functional Potential in the Northeastern Chukchi Sea. *Cont. Shelf Res.* 136: 20-28.

Introduction

The potential for oil to be released into the environment is a prominent concern as marine traffic and offshore oil exploration activities continue to expand in the Arctic Ocean. The Burger prospect within the Chukchi Sea Lease Area 193 (herein after referred to as Burger) is located approximately 90 km offshore from Wainwright, Alaska, USA, and is a likely target for future development (Shell Gulf of Mexico Inc., 2015; Figure 4-1). It is increasingly important to understand the ecology of this region as it responds both to a changing climate and potential oil and gas development. Microorganisms are critical to ecological function, thus baseline characterizations are important to understanding biogeochemical cycling, predicting the impacts of disturbance, aiding in predictions of oil biodegradation potential, and assessing recovery.

The structure and biogeochemistry of the Arctic marine ecosystem is defined by the presence of sea ice, inputs from its surrounding water masses, and associated stratification (Michel et al., 2012). The Chukchi Sea, a shallow sea (~50 m deep) located in the western Arctic Ocean, is linked to the Pacific Ocean by a northward flow through the Bering Strait. The long duration of summer sunlight and the Bering Strait's influx of heat, nutrients, carbon, and organisms drive the seasonally high productivity and strong benthic-pelagic coupling that characterize this region (Dunton et al., 2005; Grebmeier & Maslowski, 2014). This region is experiencing the effects of climate change (Grebmeier et al., 2006) as increasing seawater temperatures are leading to declines in sea ice (Comiso et al., 2008) and the subsequent increase of fresh water inputs into surface waters (Kwok & Cunningham, 2010; Michel et al., 2012; Serreze et al., 2007).

During the summer open-water season, salinity and temperature gradients associated with sea ice melt and dense winter water stratify the Burger water column (Weingartner et al., 2013a, 2013b). Water from the Bering Sea is thought to first displace melt water present in the upper portion of the Burger water column and later the colder winter water occupying the lower portion (Weingartner et al., 2013a, 2013b). The co-occurrence of these different water masses typically yields a continuously stratified water column with salinity and temperature gradients creating a thick (~5 m) pycnocline at a depth of approximately 15 m (Day et al., 2013; Weingartner et al., 2013a, 2013b). An important aspect of the benthic ecology of Burger is that the cold winter water generally persists in the benthic system throughout the summer open-water season (Day et al., 2013; Blanchard et al., *submitted*). The persistence of the winter water in

Burger's benthic environment has been shown to vary seasonally and interannually and to affect many trophic levels (Day et al., 2013). These different water masses lead to differences in nutrient concentrations and zooplankton communities throughout the water column in the Chukchi Sea (Day et al., 2013; Blanchard et al., *submitted*; Questel et al., 2013) and may also contribute to shaping the biodiversity and function of microbial communities important to biogeochemical cycling and the biodegradation of contaminants.

The Chukchi Sea has been the subject of extensive ecological and oceanographic studies (Gradinger, 2009; Hopcroft et al., 2010; Mathis et al., 2007; Weingartner et al., 2013a, 2013b), including assessments of oil biodegradation in near shore environments (McFarlin et al., 2014), yet to date very little is known about the microbial ecology of this region. A number of studies have investigated Arctic marine bacteria (Ferrari & Hollibaugh, 1999; Gomez-Pereira et al., 2010; Kellogg & Deming, 2009; Kirchman et al., 2010; Malmstrom et al., 2007; Monier et al., 2014; Pedrós-Alió et al., 2015; Pommier et al., 2007); however, neither microbial community structure nor functional genetic potential (including oil-biodegradation genes and important biogeochemical processes) have yet been characterized in the Chukchi Sea. Even though no active oil seeps are known to exist in Burger (NRC, 2003), oil-degrading microorganisms are considered to be ubiquitous and are detectable in both polluted and unpolluted environments (Schneiker et al., 2006; Head et al., 2006; Yakimov et al., 2007; Kostka et al., 2011). Yet, little is known about the distribution of particular microbial taxa and genes associated with oil biodegradation in the Arctic marine environment, including along depth gradients within the water column, which is relevant to the fate of oil in the event of contamination.

Our objective was to obtain a detailed molecular analysis of the *in situ* structure of the bacterial community and its functional potential with regard to oil biodegradation and the cycling of carbon, nitrogen, phosphorus, and sulfur throughout the Burger water column. We also assessed the relationship between environmental factors (temperature, salinity, and nutrient concentrations) and both the structure and potential function of the microbial community to help identify important drivers of microbial community structure and the potential of specific biodegradation processes. We hypothesize that bacterial communities will differ between the distinctive upper and lower water masses, yet these genetically diverse communities will have similarly broad genetic potentials to cycle nutrients and degrade oil. These results may assist in developing an *in situ* baseline data set to assess ecosystem responses to environmental

disturbances, while also providing insight into the potential for indigenous marine bacteria to degrade oil in a sensitive offshore Arctic environment.

Materials and Methods

Study area

The Burger prospect within Lease Area 193 was the focus of this study. Lease Area 193, located in the Chukchi Sea (Arctic Ocean), contains an estimated 4.3 billion barrels of crude oil and gas (BOEM, 2015). Burger covers roughly 3,000 km^2 of ocean with an average depth of ~40 m and has been the focus of extensive ecological studies for over 6 years (Blanchard et al., *submitted*).

Hanna Shoal (~26 m in depth) borders Burger to the north (Figure 4-1). The Hanna Shoal and its surrounding oil lease areas are some of the most comprehensively characterized sites in the Arctic Ocean. Measurements of oceanographic parameters defining the physical (Weingartner et al., 2005; Weingartner et al., 2013a, 2013b), chemical (Mathis & Questel, 2013), and biological components (Blanchard et al., 2013a, 2013b; Questel et al., 2013) from the surface to the seafloor have been reported (Weingartner et al., 2013b). These detailed datasets offer an excellent opportunity to relate bacterial community structure and biogeochemical cycling to *in situ* conditions.

Sample collection

The Chukchi Sea is typically ice-free from July through November (Weingartner et al., 2005). During the open water season of 2012, we coordinated with the Chukchi Sea Environmental Studies Program (CSESP) operated by Olgoonik Fairweather LLC to obtain seawater samples from 17 different stations in Burger (Figure 4-1). Due to weather and logistics, 11 of the 17 stations were sampled once and 6 stations twice, resulting in a total of 23 samples per water column depth. At each station, seawater samples were collected from the surface, the middle of the water column (20 m), and 2-3 m above the seafloor on two separate research cruises from August 12-18 and from September 20-22. Seawater was collected using an SBE25 CTD with SBE55 rosette sampler (Questel et al., 2013; Weingartner et al., 2013b) and 1 L samples were immediately filtered onto 0.2-µm sterile Supor filters (Pall Corporation, Port

Washington, NY). Filters were frozen (-20°C) and shipped to the University of Alaska Fairbanks, where they were stored frozen (-80°C) until DNA extraction.

Samples for physical, environmental and biogeochemical analyses were collected from the same rosette casts as the samples for microbial analyses. Environmental measurements consisted of temperature, salinity, chlorophyll-*a*, pheophytin, and macronutrients at fixed depths within the Burger water column. Macronutrients included phosphate, silicate, nitrate, nitrite, ammonium, and dissolved inorganic nitrogen (DIN). Detailed methodology for the collection and analysis of environmental samples is described in Questel et al. (2013).

Microbial community analysis

Bacterial community DNA was extracted from each 1-L sample (23 stations, 3 depths at each station) using the methods of Miller et al. (1999). The DNA extracts were sequenced with 454-Pyrosequencing on the GS FLX Titanium platform using F563-577 and R907-924 primers to target the V4-V5 region on the 16S rRNA gene (http://pyro.cme.msu.edu/pyro/help.jsp). We then analyzed DNA sequences with mothur open source software (Schloss et al., 2009) following the online standard operating procedure (Schloss et al., 2011). The sequence length (350 bp) allowed us to determine the taxonomic identity of bacteria present (down to the genus level when possible) by comparing 16S rRNA gene sequences to a publicly available online database (Ribosomal Database Project; Wang et al., 2007). Operational taxonomic units (OTUs) were clustered at 97% similarity and relative abundances were normalized to total abundance per sample. Singletons were removed across water column locations (surface, middle, and bottom) and only sequences > 0.01% of the total relative abundance were included in statistical analyses.

Functional gene analysis

We determined the presence and relative abundance of functional genes using the GeoChip functional gene microarray (GeoChip 5.0; Glomics Inc., Norman, OK). The GeoChip 5.0 contains 167,044 probes covering ~1,500 functional genes involved in microbial carbon, nitrogen, sulfur, and phosphorus cycling; organic contaminant remediation, energy metabolism, antibiotic resistance, metal resistance/reduction, stress response, virulence, and viruses (Zhou et al., 2015). We analyzed functional genes in surface, middle, and bottom water samples from three stations (BF04, BF07, and BF15; Figure 4-1) ($n = 3$ for each sampling station).

Amplification, labeling, hybridization, and data preprocessing were conducted by the Institute for Environmental Genomics at the University of Oklahoma. Whole-genome amplification was conducted on 50 ng of DNA (Wu et al., 2006). Labeling, hybridization, and washing followed published protocols (Van Nostrand et al., 2016). GeoChips were imaged as a Multi-TIFF using the NimbleGen MS 200 microarray scanner (Roche, Basel, Switzerland). The data were then extracted using the Agilent Feature Extraction program (Santa Clara, CA). Preprocessed signal intensities were initially normalized and filtered as previously described (Van Nostrand et al., 2016). Singletons were removed prior to statistical analyses and all reported abundances are relative, calculated by dividing the signal intensity of each gene by the mean signal intensity of the sample. In Figures depicting the most abundant genes, cutoffs were determined to display the 5 - 7 most abundant genes for the purpose of readability and focus.

Statistical analyses

We used PC-ORD V6 (McCune & Mefford, 2011) for Nonmetric Multidimensional Scaling (NMDS) and Multi-Response Permutation Procedures (MRPP) analyses using a Bray-Curtis distance measure. Additionally, variations in the bacterial diversity of water column locations were compared by calculating Chao1 and ACE richness estimates. Differences in environmental variables, bacterial community structure and functional potential (p-values ≤ 0.05) among the three water column depths were calculated using MRPP, a nonparametric procedure for testing differences among groups (Mielke, 1984; Mielke & Berry, 2001). We compared surface, middle and bottom samples with MRPP and reported the p-value and the test statistic with a description of the effect size independent of the sample size.

NMDS ordination plots illustrated differences in community structure and functional gene abundance. We assessed the dimensionality of the data in autopilot-mode using 100 runs with real data and random starting configurations (Kruskal, 1964; Mather, 1976). A Monte Carlo test with 249 randomized runs was conducted to evaluate whether NMDS was extracting stronger axes than expected by chance. We also assessed the stability of each solution by plotting stress (the departure from monotonicity) versus iteration and confirming that all curves stabilized at a relatively low level (McCune & Grace, 2002).

We overlaid environmental vectors and OTUs on NMDS ordinations of individual functional genes to independently assess the importance of these data on ordination gradients.

The direction of a vector shows its relative association with the two axes and its length is proportional to the magnitude of the association (McCune & Grace, 2002). We compared measured environmental variables (concentrations of phosphate, silicate, nitrate, nitrite, ammonium, DIN, chlorophyll-*a*, pheophytin, temperature, and salinity) to NMDS ordinations of functional genes in each category to determine which environmental factors were potentially driving the distribution of functional genes in surface, middle, and bottom samples. Relationships between functional gene intensity and either environmental variables or OTUs were assessed by calculating pairwise Pearson's correlation coefficients (r). Squared correlation coefficients are also provided to report the proportion of variation that is explained by the variable.

Results

Bacterial community structure

Seawater throughout Burger was dominated by members of the phylum Proteobacteria (Table S4-1). Bacteria belonging to the Bacteroidetes and Verrucomicrobia phyla, as well as many genera of unclassifiable bacteria, were also relatively abundant at the three depths sampled (Table S4-1). The majority of sequences in our samples were members of the classes Alphaproteobacteria and Gammaproteobacteria (24%; Figure 4-2A). Sequences of Gammaproteobacteria were most abundant in bottom samples (31% of total community), while sequences of Alphaproteobacteria were most abundant in surface samples (23% of total community; Figure 4-2A). At the order level, unclassified Gammaproteobacteria dominated the water column (Surface: 15±4%, Middle: 17±05%; Bottom: 24±5%), while the most abundant classified bacteria were Flavobacteriales (S: 19±3%; M: 15±2%; B: 10±2%), Rhodobacterales (S: 12±1%; M: 8±0%; B: 8±0%), Puniceicoccales (S:11±1%; M: 14±2%; B: 7±1%), SAR11 (S: 9±1%; M: 11±1%; B: 8±1%), and Alteromonadales (S: 4±1%; M: 3±1%; B: 4±1%) (Figure 4-2B). At the OTU level, in surface and middle samples, unclassified Puniceicoccaceae (OTU7) had the highest relative abundance, followed by SAR11 (OTU2; Table S4-1). In bottom samples, unclassified Gammaproteobacteria (OTU6) was more relatively abundant than SAR11 (OTU2; Table S4-1).

In both August and September, microbial communities in surface water samples differed from communities in bottom seawater samples, with p-values < 0.001 ($n = 23$; Table 4-

1). When we compared the microbial communities in August to the communities in September, the microbial community in surface ($p = 0.03$) and bottom ($p = 0.04$) seawater samples differed between the two months. The community shift observed in surface and bottom seawater samples may be due to the presence of low-abundance bacteria, as bacteria with relatively high abundances (>1.0%) remained more stable between August and September (Table 4-1).

When we combined August and September samples and grouped them by their water column location, samples from the surface and middle of the water column had similar alpha diversity and richness, and both indices differed from bottom samples (Table S4-2). Furthermore, in looking at our NMDS ordination, samples from surface and middle locations did not form discrete groups, but were separated in ordination space from bottom samples (Figure 4-3). Our middle samples (20 m) were sometimes obtained above the pycnocline and thus exhibited similarities to surface samples (Figure 4-3). When the pycnocline was above our 20-m fixed sampling depth, samples grouped with bottom samples (Figure 4-3). When August and September 16S rRNA sequences were analyzed together, middle samples that were obtained above the pycnocline were significantly different than middle samples that were obtained below the pycnocline ($p < 0.001$; Figure 4-3). In August, bacterial sequences in middle samples obtained above and below the pycnocline were similar to sequences obtained at the pycnocline ($p = 0.22$, $p = 0.53$, respectively); and were similar to surface and bottom samples, respectively ($p = 0.15$, $p = 0.43$). September samples were more variable as middle samples obtained above and below the pycnocline were different from surface and bottom samples, respectively ($p = 0.03$, $p = 0.04$). While the bacterial community structure in middle samples obtained outside the pycnocline did not follow the same trends in August and September, these data support differences in community structure between August and September in surface and bottom samples (Table 4-1).

Polaribacter, Pseudoalteromonas, *Psychrobacter,* and unclassified Flavobacteriaceae were located at all three depths. Sequences associated with *Polaribacter* (OTU1), *Pseudoaltermonas,* and unclassified Flavobacteriaceae (OTUs 36, 98, and 17) had slight differences in relative abundance with respect to depth (Table S4-1). *Psychrobacter* (OTU144) was also present in low abundance at the three depths sampled, but there was significantly more *Psychrobacter* in surface than middle and bottom samples (Table S4-1).

Functional gene distribution

GeoChip data revealed a strong stratification in the relative abundance of individual functional genes between surface and bottom samples (Table 4-2); however, when the abundance of individual genes in each functional category were averaged together, oil-degradation genes as well as carbon-, nitrogen-, phosphorus-, and sulfur-cycling genes were present in similar abundances at the three depths (Table 4-2). Individual genes involved in carbon cycling, carbon (non-petroleum) degradation, organic remediation, nitrogen cycling, phosphorus cycling, sulfur cycling, secondary metabolism, virulence, and viral processes were present in different relative abundances between surface and bottom samples ($p < 0.05$; Tables 4-2 & S4-3). Conversely, individual genes involved in carbon fixation were well mixed among the three depths sampled and exhibited no significant difference with depth (Tables 4-2 & S4-3).

The overall profile of oil degradation gene categories did not significantly differ in the three depths sampled, although individual degradation genes within several categories did vary with depth (Table 4-2). The most abundant petroleum-degradation genes (in terms of relative abundance) included alkane monooxygenase (*alkB)*, muconate cycloisomerase (*catB),* and p-hydroxybenzoate hydroxylase (*hbn)* (Table 4-3). The *alkB* genes are associated with the aerobic biodegradation of alkanes, while the *cat*B and *hbn* genes are associated with the aerobic biodegradation of BTEX (benzene, toluene, ethylbenzene, and xylenes) and related aromatics. Significant differences among water column depths were noted in two individual oil-degradation genes: *alkB* (GenBank accession #: 54018095) and *hbn* (GenBank accession #: 21106432) (Table 4-3). When biodegradation gene profiles were subjected to NMDS, vector analysis revealed correlative associations between *alkB*_54018095 and biodegradation genes in surface samples ($r^2 > 0.80$). In regards to specific bacteria, the distribution of petroleum biodegradation genes in surface samples was mostly correlated to *Octadecabacter* (OTU82; $r = 0.81$) and an unclassified bacterium (OTU435; $r = 0.82$); while the distribution in bottom samples was mostly correlated to *Formosa* (OTU22; $r = -0.78$) and an unclassified member of Betaproteobacteria (OTU47; $r = -0.76$) (Figure S4-1). *Octadecabacter* spp. are known to dominate Arctic sea ice communities (Brinkmeyer et al., 2003), and *Formosa* belongs to the Flavobacteriaceae family, which has been shown to increase in response to oil during the Deepwater Horizon oil spill (Dubinsky et al., 2013).

The separation of carbon (non-petroleum) degradation gene profiles between surface and bottom samples in the NMDS ordination was highly correlated with the relative abundance of amylase, cellobiase, and malate synthase (enzymes that break down starch, cellulose, and acetate, respectively), with Pearson's correlation coefficients (r) ranging from 0.92-0.94 in the negative direction (Figure S4-2). To a lesser extent, rhamnogalacturonan lyase (*rgl*; degrades cellulose, hemicellulose, and pectin; r = -0.88) and phenol oxidase (degrades lignin; r = -0.85) also influenced the distribution of carbon-degradation genes in the ordination (Figure S4-2). The carbon cycling category includes genetic sequences for RubisCO, a protein involved in autotrophic fixation and considered to be the most abundant protein in the world (Ellis, 1979). Surprisingly, the relative abundance of RubisCO genes was higher in bottom samples than surface or middle samples (Figure S4-3) and was not correlated with the concentration of chlorophyll-*a* in the water column; however, RubisCO genes were correlated with pheophytin (r = 0.81), an accessory pigment involved in photosynthesis. The most abundant RubisCO gene in bottom samples (RLP_91798420) was similar to the RubisCO-like gene found in the halotolerant bacterium *Chromohalobacter salexigens* (Figures S4-3 & S4-4).

The overall genetic potential to perform nitrogen cycling was similar in surface, middle, and bottom samples (Figure S4-5), while phosphorus-cycling genes were more variable among the three depths (Figure S4-6). The most abundant nitrogen-cycling genes included genes for ammonification (*gdh* and *ureC*), denitrification (*nosZ*, *nirK*, and *nirS*), and nitrification (*amoA*; Figure S4-5). The most abundant individual phosphorus cycling genes found in surface, middle, and bottom samples included genes for storage (*ppk* and *ppk2*; Rao & Kornberg, 1996), utilization (*ppx*; Akiyama et al., 1993), and mineralization (*phytase*; Menezes-Blackburn et al., 2013) (Figure S4-6). The *ppx* gene for phosphorus utilization was the most relatively abundant phosphorus cycling gene located in the water column and had a higher relative abundance in samples from the middle of the water column than bottom samples (Figure S4-6).

Environmental factors influencing gene distribution

At all sampling stations, temperature decreased between middle (August: 0.83°C ± 0.54; September: 1.79°C ± 0.49; average ± standard error) and bottom samples (August: -0.04°C ± 0.45; September: -0.61°C ± 0.25), and chlorophyll-*a* concentrations were highest in samples from the middle of the water column (Tables S4-4 & S4-5). In addition, concentrations of

phosphate, nitrate, ammonium, and pheophytin significantly increased with depth at every station (Table 4-4). Nitrate concentrations varied throughout the three depths, while similar concentrations of phosphate, nitrite, ammonium, and DIN were found in surface and middle samples with significantly higher concentrations in bottom samples (Table 4-4).

Temperature and phosphate appeared to have the greatest influence on the microbial community structure among the three depths (Figure 4-3, Table S4-6). The separation of bacteria in surface and bottom samples was most strongly correlated with temperature ($r = 0.77$). Out of all measured nutrients, phosphate ($r = -0.72$) statistically contributed the most to differences in the microbial community structure (Figure 4-3, Table S4-6).

In bottom samples, the separation of carbon (non-petroleum) degradation genes towards the bottom of axis 2 (Figure S4-2) was highly correlated with salinity ($r = -0.77$), nitrate ($r = -0.79$), ammonium ($r = -0.83$), phosphate ($r = -0.76$), and temperature ($r = 0.74$). Ammonium was the only environmental variable that was correlated with the distribution of petroleum degradation genes, with an $r = 0.64$. The complete environmental data for all water column samples, which correspond to the stations in Figure 4-1, are shown in Tables S4-4 & S4-5.

Discussion

Salinity and temperature densities brought on by seasonal ice melt stratify the Burger water column with respect to nutrient concentrations and zooplankton communities (Day et al., 2013; Questel et al., 2013). Stratification was apparent in our environmental data (Table 4-4), and was also reflected in the microbial community structure (Figure 4-3; Table 4-1), although minimal stratification was seen in the distribution of taxa known to include oil degraders or bacteria reportedly associated with the presence of oil (Table S4-1). Individual functional genes for biogeochemical cycling were stratified in the water column, with surface samples containing a different distribution of individual functional genes than bottom samples. The difference in microbial community structure and biogeochemical potential in surface and bottom samples is consistent with the existence of different water masses overlying each other at Burger.

Even though microbial communities and individual genetic potentials were stratified between surface and bottom seawater samples, the overall potential for biogeochemical cycling was similar at all depths (Table 4-2). This reveals that similar genetic potentials to perform biogeochemical cycling existed in surface, midwater, and bottom samples independent of

specific genetic sequences and potentially stratified water masses. As biogeochemical cycling is a function of the entire bacterial community and not of individual members (Follows et al., 2007), we hypothesize that changes in stratification will not affect the genetic potential for cycling nutrients in Burger. Furthermore, the genetic potential to biodegrade oil was similar among the three depths (Table 4-3), even though multiple data sets supported a stratified water column, so the microbial community is likely capable of degrading petroleum compounds in the presence or absence of stratified water masses. Yet, actual function (e.g. rates of biodegradation or nutrient cycling) is expected to vary among water column locations in response to environmental factors even when microbial functional genetic potential may not.

Bacterial community structure

Although SAR11 have previously been found to be the most abundant bacteria in ocean surface waters (Carlson et al., 2009; Morris et al., 2002; Rappé & Giovannoni, 2003) our Arctic survey data did not show this at the order or OTU level (Figure 4-2B; Table S4-1). It is possible that our primers (563F and 924R) were biased against the SAR11 clade, which could generate artifactual low relative abundance results. Apprill et al. (2015) found that bacteria belonging to the SAR11 clade were unrepresented when using 16S rRNA primers 515F and 806R. At the order level, our results indicate that Flavobacteriales and unclassified Gammaproteobacteria were more abundant than the SAR11 clade at all three depths (Figure 4-2B). Rhodobacterales were also more abundant than SAR11 in surface water samples while Puniceicoccales were more abundant than SAR11 in samples from the surface and middle of the water column (Figure 4-2B). This may be the first report of a high relative abundance of Puniceicoccales in pelagic Arctic Ocean samples, although the phylum Verrucomicrobia, to which this order belongs, has been reported to be abundant both in Arctic and temperate marine environments. Verrucomicrobia were found to be abundant (1.5% of total sequences) in surface waters of the western Arctic Ocean (Kirchman et al., 2010) and have been shown to dominate surface samples in temperate oceans (as Verrucomicrobiales; DeLong et al., 2006). Even though SAR11 did not dominate the middle of the water column in our samples, OTUs belonging to the SAR11 clade were found to be more abundant in our midwater samples, compared to surface and bottom samples. Comparably dominant members (e.g. SAR11, Rhodobacterales, and Flavobacteriales) were also detected in a recent global ocean survey conducted by Zinger et al. (2011) and in

Arctic Ocean surface samples (Kirchman et al., 2010; Malmstrom et al., 2007; Pommier et al., 2007). Similar to Malmstrom et al. (2007), these results indicate that the Arctic Ocean is dominated by a few bacterial groups, although our results suggest that spatial and temporal variation exists among the dominant members.

The presence of *Pseudoalteromonas*, *Polaribacter*, *Psychrobacter*, and unclassified Flavobacteriaceae at all three depths suggests that oil biodegradation potential may exist throughout the water column, as members of these taxa have been associated with the presence of oil in Arctic and Antarctic marine environments (Table S4-1; Brakstad et al., 2008; Deppe et al., 2005; Dubinsky et al., 2013; Gerdes et al., 2005; Lin et al., 2009; Prince et al., 2010; Yakimov et al., 2004); however, because bacteria within a genus often vary widely in their functional genetic capacity, it is important not to presume that all members of these genera are oil degraders. Members of the genera *Pseudoalteromonas* and *Polaribacter* have been associated with the presence of oil in Arctic marine ice (Brakstad et al., 2008; Gerdes et al., 2005). Additionally, *Pseudoalteromonas* spp. have also been associated with the biodegradation of oil in Antarctic and Arctic seawater (Deppe et al., 2005; Yakimov et al., 2004). *Pseudoalteromonas* and *Polaribacter* are known to contain oil-degrading species (Lin et. al., 2009; Wang et al., 2014). Unclassified Flavobacteriaceae spp., which were also quite abundant throughout the water column, and some *Flavobacteria* spp. have been associated with the biodegradation of oil in Arctic seawater and marine ice (Table S4-1; Gerdes et al., 2005). Related bacteria were also discovered during the Deepwater Horizon oil spill, as *Polaribacter*, *Pseudoalteromonas*, and sequences belonging to the family Flavobacteriaceae were all enriched in the subsurface plume of dispersed oil and gas (Dubinsky et al., 2013). In our study, taxa known to include oil-degrading microorganisms existed throughout the water column independent of potentially stratified water masses; however their presence only suggests the potential for oil biodegradation and functional genetic data are a stronger indication of actual oil degradation potential than taxonomic affiliations.

Oil-degrading bacteria as well as oil-degradation genes are thought to be ubiquitous and have been found in pristine environments with no known exposure to petroleum compounds (Head et al., 2006; Kostka et al., 2011; Schneiker et al., 2006; Yakimov et al., 2007). For this reason, it was not surprising to find taxa related to oil-degrading bacteria and oil-degradation genes in our Chukchi Sea study area. Since water from the Bering Sea is thought to displace

water masses in the Burger water column (Weingartner et al., 2013a, 2013b), it is possible that microorganisms were exposed to oil compounds prior to reaching the Burger water column, which would also contribute to the presence of oil-degradation genes.

Functional gene distribution

Similar to the microbial sequence data, the distribution of individual functional genes suggests that two different water masses exist within the Burger water column. While the relative abundance of major functional gene categories was similar at all depths, the abundance of individual functional genes for carbon cycling, nitrogen cycling, organic remediation, phosphorus cycling, sulfur cycling, virulence, and viruses differed between surface and bottom samples.

When examining the individual genes involved in carbon cycling, we were surprised by the relatively high presence of RubisCO genes in bottom seawater samples compared to surface and middle samples (Figure S4-3). The most abundant RubisCO gene in bottom samples was similar to the RubisCO-like gene in *Chromohalobacter salexigens* (RLP_91798420; Figure S4-4), a heterotrophic bacterium known to contain the RubisCO-like protein (RLP; form IV-non phototrophic bacteria; Ashida et al., 2008; Csonka et al., 2005). Over the past 10 years, genome sequencing has led to the discovery of RubisCO-like sequences in microorganisms that do not use CO_2 as a carbon source (Tabita, 1999; Tabita et al., 2007). RLPs do not cluster with genuine RubisCO sequences in phylogenetic trees and have yet to demonstrate the ability to catalyze ribulose-1,5-bisphosphate (RuBP)-dependent CO_2 fixation (Tabita et al., 2007). Although, RLP_91798420 increased in relative abundance with depth, the bottom of the water column contained other RubisCO genes, with similar mean signal intensities to surface and middle samples (Figure S4-4). The presence of *bona fide* RubisCO genes in bottom samples may indicate the presence of phototrophs in the bottom layer, however gene presence does not confirm function and only a small percent of surface light reaches the bottom of the turbid water column (Brown et al., 2015). Additionally, even though samples were filtered (0.2 μm) prior to DNA extraction, it is also possible that the filter may have captured extracellular DNA sorbed to particulates (Nielsen et al., 2006) or intracellular DNA from intact, yet nonviable phototrophs. The similar relative abundance of *bona fide* RubisCO genes in surface, middle, and bottom samples may also indicate the presence of chemolithotrophs in the bottom layer.

Out of the potential inorganic electron donors (e.g., hydrogen, sulfide, sulfur, ammonium, nitrite, and ferrous iron), sulfide oxidation may be contributing the most to chemolithotrophic reactions in Burger bottom samples. Individual nitrogen-, phosphorus-, and sulfur-cycling genes were different between surface and bottom samples and had relatively high abundances at all three depths (Table 4-2). When looking at the most abundant individual genes, *soxY*, a gene encoding a protein involved in sulfur oxidation, was the only abundant gene involved in oxidation-reduction reactions that was significantly more abundant in bottom samples (Figure S4-7). It should also be noted that some chemolithotrophs also grow as mixotrophs using organic compounds for a carbon source and thus do not utilize RubisCO (Ehrlich, 1978; Kelly & Wood, 2013). Since Burger is known for its rich benthic-pelagic coupling that contributes to high organic inputs into the benthic environment (Blanchard et al., *submitted*), the presence of mixotrophic bacteria obtaining energy from the oxidation of inorganic compounds in bottom seawater samples is likely.

Conclusions

Microbial community sampling along defined depths allowed us to identify trends in community structure and genetic potential throughout the water column. We found that broad functional groups related to biogeochemical cycling and oil degradation were similarly abundant throughout the Burger water column even when concentrations of available nutrients varied by orders of magnitude and microbial communities differed (Table 4-4). This finding suggests that varying bacterial communities have similar genetic potentials to biodegrade oil and cycle nutrients in this region. It is important to note that a similar magnitude of functional genetic potential does not necessarily translate into predictions of similar process rates, since environmental conditions (e.g. oxygen and nutrient availability) generally control microbial functions in the environment. Nonetheless, identifying baseline distributions of microbial communities and their functional genes provides significant insight into the potential for microbial processes to occur. These baseline community trends will be useful to researchers in assessing both the long-term effects of climate change as well as oil and gas development on microbial communities in the Chukchi Sea. These data may also be relevant for future Environmental Impact Statements. The pervasiveness of oil-degrading bacteria and functional genes may assist modelers in determining the fate and effects of oil in this biologically

productive environment, although further work is required to quantify biodegradation rates and to elucidate the scales of seasonal and interannual variability such as those observed in other ecosystem components (e.g. Day et al., 2013, Blanchard et al., *submitted*).

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Conflict of interest

The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIH or other research funders.

References

Akiyama M, Crooke E, Kornberg A. (1993). An exopolyphosphatase of *Escherichia coli*. The enzyme and its ppx gene in a polyphosphate operon. *J. Biol. Chem.* 268:633-639.

Apprill A, McNally S, Parsons R, Webe L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75:129-137.

Ashida H, Saito Y, Nakano T, de Marsac NT, Sekowska A, Danchin A, Yokota A. (2008). RuBisCO-like proteins as the enolase enzyme in the methionine salvage pathway: functional and evolutionary relationships between RuBisCO-like proteins and photosynthetic RuBisCO. *J. Exp. Bot.* 59:543-1554.

Blanchard AL, Parris CL, Knowlton AL, Wade NR. (2013a). Benthic ecology of the northeastern Chukchi Sea. Part I. Environmental characteristics and macrofaunal community structure, 2008–2010. *Cont. Shelf. Res.* 67:52-66.

Blanchard AL, Parris CL, Knowlton AL, Wade NR. (2013b). Benthic ecology of the northeastern Chukchi Sea. Part II. Spatial variation of megafaunal community structure, 2009–2010. *Cont. Shelf. Res.* 67:67-76.

Blanchard AL, Gall AE, Day RH, Hopcroft RR, Weingartner TJ, Lisanne AM, Aerts LAM, Delarue J, Dobbins EL, Questel JM, Wisdom SS. (*submitted*) Detecting change in an arctic ecosystem: can we separate a signal from natural variabililty? *Prog. Oceanogr.*

BOEM. (2015). Supplement to the Oil-Spill Risk Analysis: Chukchi Sea Planning Area, OCS Lease Sale 193. http://www.boem.gov/Oil-Spill-Risk-OCS-LEASE-SALE-193/ (accessed 4.6.15).

Brakstad OG, Nonstad I, Fasness LG, Brandvik PJ. (2008). Responses of microbial communities in Arctic sea ice after contamination by crude petroleum oil. *Microb. Ecol.* 55:540-552.

Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E. (2003). Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl. Environ. Microbiol.* 69:6610-6619.

Brown ZW, Lowry KE, Palmer MA, van Dijken GL, Mills MM, Pickart RS, Arrigo KR. (2015). Characterizing the subsurface chlorophyll a maximum in the Chukchi Sea and Canada Basin. *Deep Sea Res. II.* 118:88-140.

Carlson CA, Morris R, Parsons R, Treusch AH, Giovannoni SJ, Vergin K. (2009). Seasonal dynamics of SAR11 populations in the euphotic and mesopelagic zones of the northwestern Sargasso Sea. *ISME J.* 3:283-295.

Comiso JC, Parkinson CL, Gersten R, Stock L. (2008). Accelerated decline in the Arctic sea ice cover. *Geophys. Res. Lett.* 35:L01703.

Csonka LN, O'Connor K, Larimer F, Richardson P, Lapidus A, Ewing AD, Goodner BW, Oren A. (2005). What we can deduce about metabolism in the moderate halophile *Chromohalobacter salexigens* from its genomic sequence. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds). Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. Springer: Netherlands, pp 267-285.

Day RH, Weingartner TJ, Hopcroft RR, Aerts LAM, Blanchard AL, Gall AE, Gallaway BJ, Hannay DE, Holladay BA, Mathis JT, Norcross BL, Questel JM, Wisdom SS. (2013). The offshore northeastern Chukchi Sea, Alaska: a complex high-latitude ecosystem. *Cont. Shelf. Res.* 67:147-165.

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, *et al.* (2006). Community genomics among stratified microbial assemblages in the ocean's interior. *Science.* 311:496-503.

Deppe U, Richnow H, Michaelis W, Antranikian G. (2005). Degradation of crude oil by an arctic microbial consortium. *Extremophiles.* 9:461-470.

Dubinsky EA, Conrad ME, Chakraborty R, Bill M, Borglin SE, Hollibaugh JT, *et al.* (2013). Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environ. Sci. Technol.* 47:10860-10867.

Dunton KH, Goodall JL, Schonberg SV, Grebmeier JM, Maidment DR. (2005). Multi-decadal synthesis of benthic–pelagic coupling in the western arctic: role of cross-shelf advective processes. *Deep Sea Res. II.* 52:3462-3477.

Ehrlich HL. (1978). Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. *Geomicrobiol. J.* 1:65-83.

Ellis RJ. (1979). Most abundant protein in the world. *Trends Biochem. Sci.* 4:241-244.

Ferrari VC, Hollibaugh JT. (1999). Distribution of microbial assemblages in the Central Arctic Ocean Basin studied by PCR/DGGE: analysis of a large data set. *Hydrobiologia.* 401:55-68.

Follows MJ, Dutkiewicz S, Grant S, Chisholm SW. (2007). Emergent biogeography of microbial communities in a model ocean. *Science.* 315:1843–46.

Gerdes B, Brinkmeyer R, Dieckmann G, Helmke E. (2005). Influence of crude oil on changes of bacterial communities in Arctic sea-ice. *FEMS Microbiol. Ecol.* 53:129-139.

Gomez-Pereira PR, Fuchs BM, Alonso C, Oliver MJ, van Beusekom JE, Amann R. (2010). Distinct flavobacterial communities in contrasting water masses of the North Atlantic Ocean. *ISME J.* 4:472-487.

Gradinger R. (2009). Sea-ice algae: Major contributors to primary production and algal biomass in the Chukchi and Beaufort Seas during May/June 2002. *Deep Sea Res. II.* 56:1201-1212.

Grebmeier JM, Overland JE, Moore SE, Farley EV, Carmack EC, Cooper LW, Frey KE, Helle JH, McLaughlin FA, McNutt SL. (2006). A major ecosystem shift in the northern Bering Sea. *Science* 311:1461-1464.

Grebmeier JM, Maslowski W. (2014). The Pacific Arctic Region: Ecosystem Status and Trends in a Rapidly Changing Environment. Springer.

Head IM, Jones DM, Röling WF. (2006). Marine microorganisms make a meal of oil. *Nature Rev. Microbiol.* 4:173-182.

Hopcroft RR, Kosobokova KN, Pinchuk AI. (2010). Zooplankton community patterns in the Chukchi Sea during summer 2004. *Deep Sea Res. II.* 57:27-39.

Kellogg CTE, Deming JW. (2009). Comparison of free-living, suspended particle, and aggregate-associated bacterial and archaeal communities in the Laptev Sea. *Aquat. Microb. Ecol.* 57:1-18.

Kelly DP, Wood AP. (2013). The Chemolithotrophic Prokaryotes. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed). The Prokaryotes. Springer Berlin Heidelberg, pp 275-136.

Kirchman DL, Cottrell MT, Lovejoy C. (2010). The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. *Environ. Microbiol.* 12: 1132-1143.

Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M. (2011). Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Appl. Environ. Microbiol.* 77:7962-7974.

Kruskal JB. (1964). Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115-129.

Kwok R, Cunningham GF. (2010). Contribution of melt in the Beaufort Sea to the decline in Arctic multiyear sea ice coverage: 1993-2009. *Geophys. Res. Lett.* 37:1-5.

Lin X, Yang B, Shen J, Du N. (2009). Biodegradation of crude oil by an arctic psychrotrophic bacterium *Pseudoalteromomas* sp. P29. *Curr. Microbiol.* 59:341-345.

Malmstrom RR, Straza TR, Cottrell MT, Kirchman DL. (2007). Diversity, abundance, and biomass production of bacterial groups in the western Arctic Ocean. *Aquat. Microb. Ecol.* 47:45-55.

Mather PM. (1976). Computational Methods of Multivariate Analysis in Physical Geography. Wiley & Sons: London.

Mathis JT, Pickart RS, Hansell DA, Kadko D, Bates NR. (2007). Eddy transport of organic carbon and nutrients from the Chukchi Shelf: Impact on the upper halocline of the western Arctic Ocean. *J. Geophys. Res.* 112:*C05011, doi:*10.1029/2006JC003899.

Mathis JT, Questel JM. (2013). Assessing seasonal changes in carbonate parameters across small spatial gradients in the Northeastern Chukchi Sea. *Cont. Shelf. Res.* 67:42-51.

McCune B, Grace JB. (2002). Analysis of Ecological Communities. MjM Software Design. Gleneden Beach, Oregon

McCune B, Mefford MJ. (2011). PC-ORD v. 6.255 beta. MjM Software Design. Gleneden Beach, Oregon.

McFarlin KM, Prince RC, Perkins R, Leigh MB. (2014). Biodegradation of dispersed crude oil in Arctic seawater at -1˚C. *PLoS One.* 9:e84297.

Menezes-Blackburn D, Jorquera MA, Greiner R, Gianfreda L, de la Luz Mora M. (2013). Phytases and phytase-labile organic phosphorus in manures and soils. *Crit. Rev. Env. Sci. Tec.* 43:916-954.

Michel C, Bluhm B, Gallucci V, Gaston AJ, Gordillo FJL, Gradinger R, et al. (2012). Biodiversity of Arctic marine ecosystems and responses to climate change. *Biodiversity.* 13:200-214.

Mielke PW. (1984). Meteorological Applications of Permutation Techniques Based on Distance Functions. In: Krishnaiah PR, Sen PK (eds). Handbook of Statistics, Vol. 4: Nonparametric Methods. Elsevier North-Holland: Amsterdam. pp 813-830.

Mielke PW, Berry KY. (2001). Permutation Methods: A Distance Function Approach. Springer Series in Statistics: New York.

Miller DN, Bryant JE, Madsen EL, Ghiorse WC. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *App. Env. Micro.* 65:4715-4724.

Monier A, Findlay HS, Charvet S, Lovejoy C. (2014). Late winter under ice pelagic microbial communities in the high Arctic Ocean and the impact of short-term exposure to elevated CO2 levels. *Front. Microbiol.* 5:490.

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, Giovannoni SJ. (2002). SAR11 clade dominates ocean surface bacterioplankton communities. *Nature.* 420:806-810.

Nielsen KM, Calamai L, Pietramellara G. (2006) Stabilization of extracellular DNA and proteins by transient binding to various soil components, in: Nannipieri P, Smalla K (Eds.) Nucleic Acids and Proteins in Soil. Springer; Berlin Heidelberg; pp. 141–157.

NRC (National Research Council), *Oil in the Sea III: Inputs, Fates and Effects,* Washington DC, National Academy Press, 2003.

Pedrós-Alió C, Potvin M, Lovejoy C. (2015). Diversity of planktonic microorganisms in the Arctic Ocean. *Prog. Oceanogr.* 139:233-243.

Pommier T, Canbäck B, Riemann L, Boström KH, Simu K, Lundberg P, Tunlid A, Hagström Å. (2007). Global patterns of diversity and community structure in marine bacterioplankton. *Mol. Ecol.* 16:867-880.

Prince RC, Gramain A, McGenity TJ. (2010). Prokaryotic hydrocarbon degraders. In *Handbook of Hydrocarbon and Lipid Microbiology*, (Timmis, K. N., ed.) Springer-Verlag, Berlin, Heidelberg, pp. 1672-1692.

Questel JM, Clarke C, Hopcroft RR. (2013). Seasonal and interannual variation in the planktonic communities of the northeastern Chukchi Sea during the summer and early fall. *Cont. Shelf Res.* 67:23-41.

Rao NN, Kornberg A. (1996). Inorganic polyphosphate supports resistance and survival of stationary-phase Escherichia coli. *J. Bacteriol.* 178:1394-1400.

Rappé MS, Giovannoni SJ. (2003). The uncultured microbial majority. Annu Rev Microbiol 57:369-394.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *App. Env. Microbiol.* 75:7537-7541.

Schloss PD, Gevers D, Westcott SL. (2011). Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PloS One*. 6:e27310.

Schneiker S, dos Santos VAM, Bartels D, Bekel T, Brecht M, Buhrmester J, Chernikova TN, Denaro R, Ferrer M, Gertler C, Goesmann A. (2006). Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium Alcanivorax borkumensis. *Nature Biotechnol.* 24:997-1004.

Serreze MC, Holland MM, Stroeve J. (2007). Perspectives on the Arctic's shrinking sea-ice cover. *Science.* 315:1533-1536.

Shell Gulf of Mexico Inc. (2015). Revised Outer Continental Shelf Lease Exploration Plan. Submitted to: U.S. Department of the Interior Bureau of Ocean Energy Management. http://www.boem.gov/shell-chukchi/

Tabita FR. (1999). Microbial ribulose 1,5-bisphisphate carboxylase/oxygenase: a different perspective. *Photosynth. Res.* 60:1-28.

Tabita FR, Hanson TE, Li H, Satagopan S, Singh J, Chan S. (2007). Function, structure, and evolution of the RubisCO-like proteins and their RubisCO homologs. *Microbiol. Molecul. Biol. R.* 71:576-599.

Van Nostrand JD, Yin H, He Z, Zhou J. Hybridization of environmental microbial community nucleic acids by GeoChip. In *Microbial Environmental Genomics (MEG), Methods in Molecular Biology*; Martin F, Uroz S, Eds.; Springer Science+Business Media: New York, 2016; pp183.

Wang Q, Garrity GM, Tiedje JM, Cole JR. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73:5261–5267.

Wang W, Zhong R, Shan D, Shao Z. (2014). Indigenous oil-degrading bacteria in crude oil-contaminated seawater of the Yellow Sea, China. *Appl. Microbiol. Biotechnol.* 98:7253-7269.

Weingartner T, Aagaard K, Woodgate R, Danielson S, Sasaki Y, Cavalieri D. (2005). Circulation on the north central Chukchi Sea shelf. *Deep Sea Res. II.* 52:3150-3174.

Weingartner T, Dobbins E, Danielson S, Winsor P, Potter R, Statscewich H. (2013a). Hydrographic variability over the northeastern Chukchi Sea shelf in summer-fall 2008–2010. *Cont. Shelf Res.* 67:5-22.

Weingartner T, Danielson S, Dobbins L, Potter R. (2013b). Physical oceanographic measurements in the northeastern Chukchi Sea: 2012. viewed January 2015. https://www.chukchiscience.com/science/physical-oceanography

Wu L, Liu X, Schadt CW, Zhou J. (2006) Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. *Appl. Environ. Microbiol.* 72:4931–4941.

Yakimov MM, Gentile G, Bruni V, Capello S, D'Auria G, Golyshin PN, Giuliano L. (2004). Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. *FEMS Microbiol. Ecol.* 49:419-432.

Yakimov MM, Timmis KN, Golyshin PN. (2007). Obligate oil-degrading marine bacteria. *Curr. Opin. Biotech.* 18:257-266.

Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-Cohen L. (2015). High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. mBio 6:e02288-14.

Zinger L, Amaral-Zettler LA, Fuhrman JA, Horner-Devine MC, Huse SM, Welch DBM, *et al.* (2011). Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. *PLoS One*. 6: e24570.s

Figures

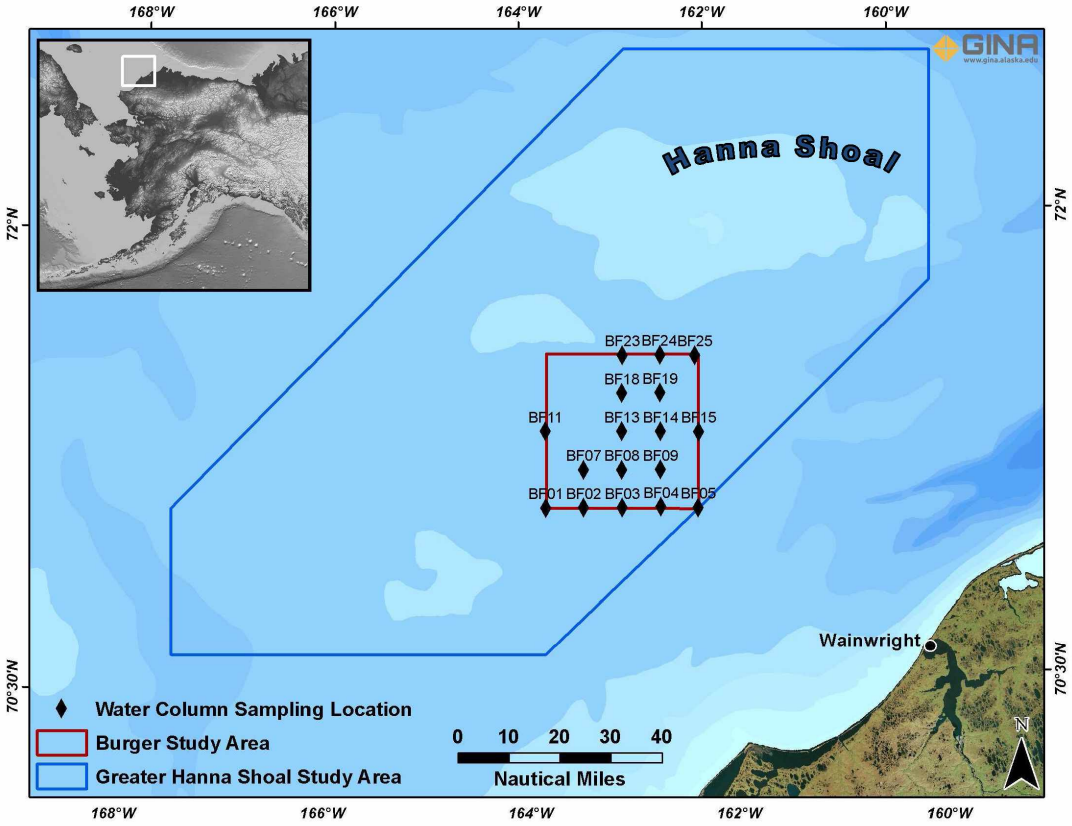

Figure 4-1. The Burger lease area in the northeastern Chukchi Sea sampled during August 12-18 and September 20-22, 2012. Sampled stations are designated as black diamonds. At each station, water was collected from three depths: surface, middle (20 m from surface) and bottom (2-3 m from seafloor).

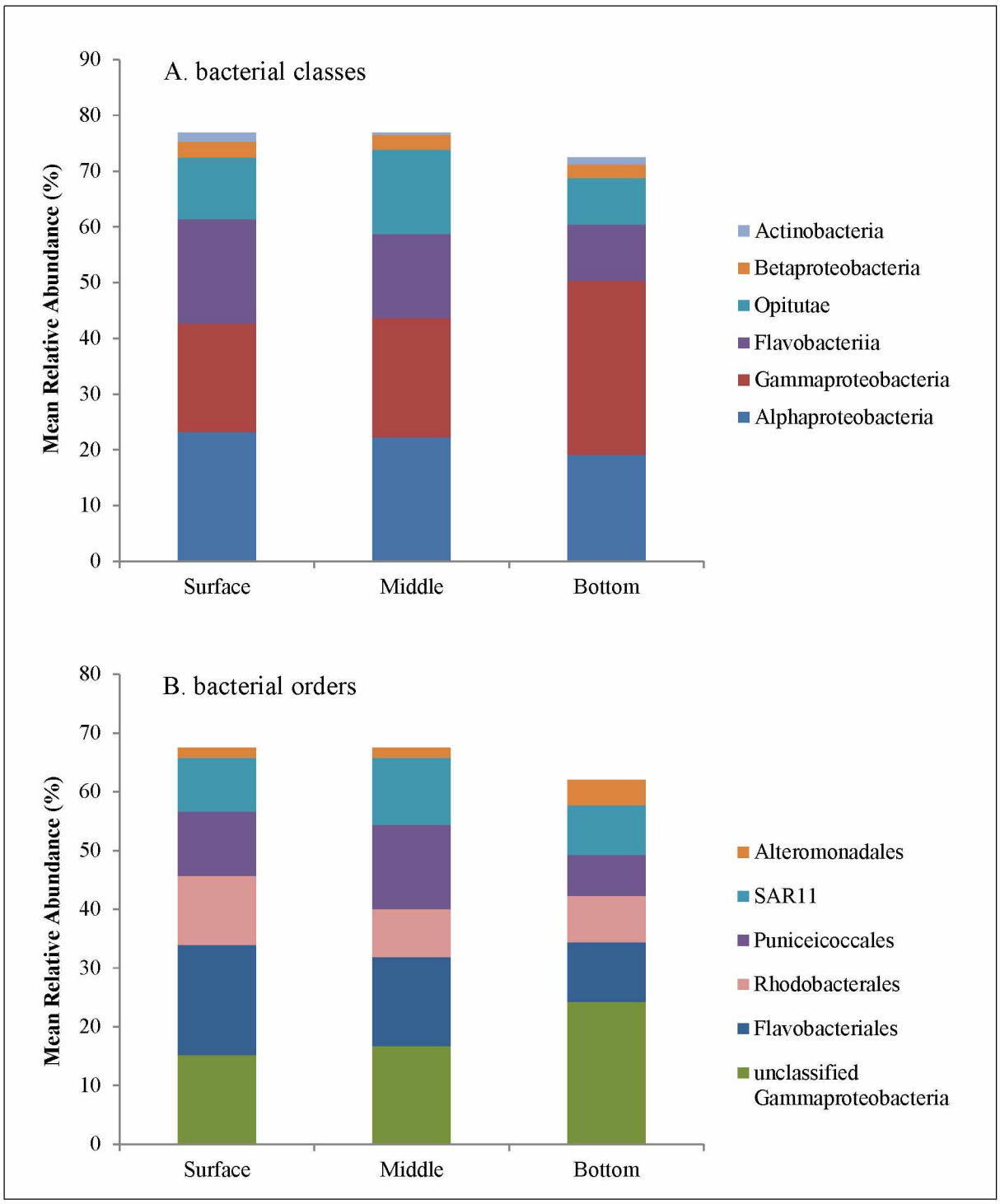

Figure 4-2. Dominant bacterial classes (A; >0.8% of total bacterial community) and orders (B; >1.6% total bacterial community) in the Chukchi Sea's Burger region during August and September of 2012. Values are averages of normalized relative abundances from samples obtained from each depth (n = 23).

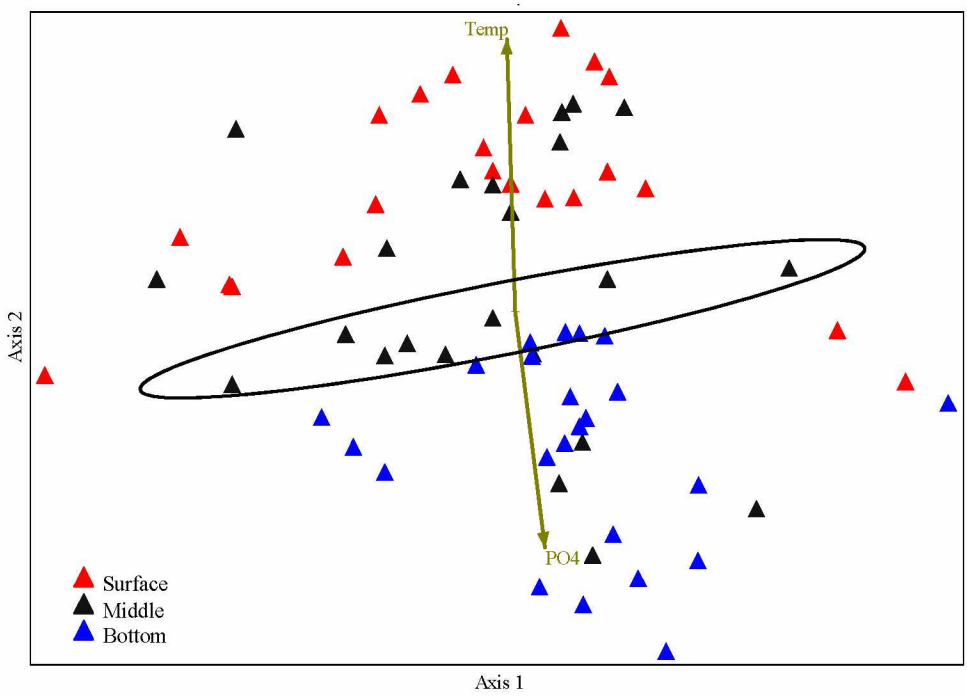

Figure 4-3. NMDS ordination of 16S rRNA gene sequences from seawater collected from the Burger lease area, Chukchi Sea, in August and September 2012. Middle samples within the black oval were sampled at the pycnocline, while middle samples above and below the oval were sampled above and below the pycnocline, respectively.

Tables

Table 4-1. Differences in bacterial community structure based on MRPP analysis of 16S rRNA gene amplicon sequencing. The associated *p*-value, test statistic, and A-value are shown for each water column location. On the left, statistical analyses were calculated between August and September from surface, middle, and bottom seawater samples for sequences with relative abundances >0.01% and >1.0%. On the right, analyses were calculated between water column locations (surface, middle, and bottom) for August and September. Different data sets are indicated in bold and with an asterisk ($p < 0.05$).

	August vs. September (Surface)	**August vs. September (Middle)**	**August vs. September (Bottom)**	**Surface vs. Middle**	**Surface vs. Bottom**	**Middle vs. Bottom**
	Relative abundance >0.01%			**August**		
p-value	**0.03***	0.06	**0.04***	0.09	**< 0.00***	**< 0.00***
Test statistic	-2.2	-1.73	-2.27	-1.39	-7.54	-5.44
A -value	0.03	0.02	0.03	0.02	0.15	0.09
	Relative abundance >1.0%			**September**		
p-value	0.07	0.14	0.14	**0.02***	**< 0.00***	**< 0.00***
Test statistic	-1.58	-1.03	-1.03	-2.79	-11.41	-6.79
A -value	0.03	0.02	0.02	0.03	0.17	0.10

Table 4-2. Functional gene sequences at different depths in the Chukchi Sea during August and September 2012. The average relative abundance of functional genes in each category is shown for each water column location (± standard error of the mean) and different letters correspond to significant differences in relative abundance of genes between different water column depths. The difference among individual functional genes between water column locations is also shown and significantly different data sets are indicated in bold and with an asterisk ($p < 0.05$; MRPP multiple comparisons).

Functional Gene Category	Relative Abundance of Functional Gene Categories			Difference in Individual Functional Genes (p-values)		
	Surface	Middle	Bottom	Surface vs. Middle	Surface vs. Bottom	Middle vs. Bottom
Carbon Cycling	6654 ± 422 [a]	6682 ± 164 [a]	7293 ± 316 [a]	0.33	**0.03***	0.51
Carbon Degradation	6356 ± 395 [a]	6465 ± 193 [a]	6924 ± 302 [a]	0.12	**0.03***	0.18
Carbon Fixation	219 ± 33 [ab]	151 ± 7 [a]	226 ± 5 [b]	0.11	0.21	0.41
Electron Transfer	129 ± 18 [a]	133 ± 12 [a]	158 ± 11 [a]	0.34	0.18	0.96
Nitrogen Cycling	1617 ± 105 [a]	1518 ± 87 [a]	1638 ± 37 [a]	0.21	**0.03***	0.10
Organic Remediation	2960 ± 57 [a]	2942 ± 54 [a]	3627 ± 50 [b]	0.20	**0.03***	0.07
Oil Degradation	232 ± 5 [a]	236 ± 6 [a]	264 ± 22 [a]	0.49	0.06	0.43
Phosphorus Cycling	814 ± 90 [a]	905 ± 60 [a]	1004 ± 99 [a]	0.27	**0.04***	0.05
Secondary Metabolism	1479 ± 54 [a]	1331 ± 23 [b]	1636 ± 94 [a]	0.19	**0.04***	0.53
Sulfur Cycling	1148 ± 87 [a]	1051 ± 54 [a]	1261 ± 73 [a]	0.20	**0.03***	0.40
Virulence	7197 ± 324 [ab]	6874 ± 240 [a]	8141 ± 332 [b]	0.15	**0.03***	**0.04***
Viral	363 ± 19 [a]	272 ± 14 [b]	299 ± 24 [ab]	**0.02***	**0.02***	**0.03***

Table 4-3. Prominent functional genes associated with the biodegradation of oil in the Chukchi Sea (August and September 2012). The genes *alkB*, *catB*, and *hbn* encode the proteins alkane monooxygenase, muconate cycloisomerase, and p-hydroxybenzoate hydroxylase, respectively. The GenBank accession number is included after each gene. Genes are normalized to mean signal intensity. Error bars represent standard error of the mean. Different letters correspond to significant differences between different water column locations ($p < 0.05$; MRPP multiple comparisons).

Genes ($n = 3$)	**Surface**	**Middle**	**Bottom**
*alkB*_54018095	23 ± 1.9^{a}	14 ± 3.1^{ab}	7.0 ± 1.2^{b}
*catB*_326951594	13 ± 2.9^{a}	7.0 ± 1.4^{a}	6.9 ± 0.5^{a}
*alkB*_88863253	7.5 ± 1.7^{a}	4.2 ± 0.4^{a}	14 ± 6.4^{a}
*alkB*_83026028	6.9 ± 1.8^{a}	5.3 ± 1.3^{a}	10 ± 0.7^{a}
*alk*B_114542542	6.0 ± 2.0^{a}	5.2 ± 0.9^{a}	15 ± 7.0^{a}
*hbh*_126734307	4.9 ± 1.7^{a}	2.5 ± 0.3^{a}	3.2 ± 1.0^{a}
*catB*_42627730	2.6 ± 0.8^{a}	4.0 ± 0.8^{a}	3.7 ± 0.7^{a}
*hbh*_21106432	1.8 ± 0.2^{a}	8.0 ± 2.6^{b}	5.8 ± 2.4^{ab}
*alk*B_124004655	0.12 ± 0.0^{a}	0.36 ± 0.2^{a}	12 ± 7.0^{a}
*alk*B_357590453	0.23 ± 0.0^{a}	5.5 ± 3.0^{a}	5.3 ± 1.8^{a}

Table 4-4. Nutrient levels and other properties of samples collected in the Chukchi Sea during August and September 2012 that were subjected to GeoChip analyses. Table displays mean values ± standard error of the mean and different letters correspond to significant differences between different water column locations ($p < 0.05$; MRPP multiple comparisons).

Environmental Variables	Mean Values		
	Surface	Middle	Bottom
Phosphate, PO_4^- (µM)	0.47 ± 0.08[a]	0.46 ± 0.06[a]	1.37 ± 0.28[b]
Nitrate, NO_3^- (µM)	0.06 ± 0.01[a]	0.04 ± 0.01[b]	4.56 ± 3.64[c]
Nitrite, NO_2^- (µM)	0.01 ± 0.00[a]	0.01 ± 0.01[a]	0.08 ± 0.02[b]
Ammonium, NH_4 (µM)	0.36 ± 0.55[a]	0.65 ± 0.47[a]	3.77 ± 1.27[b]
Chlorophyll *a* (µg/L)	0.35 ± 0.09[a]	0.47 ± 0.18[a]	0.32 ± 0.12[a]
Phaeophytin (µg/L)	0.13 ± 0.03[a]	0.17 ± 0.04[a]	0.69 ± 0.22[b]
Salinity (PSU)	29.8 ± 1.00[a]	31.0 ± 0.50[a]	32.9 ± 0.20[b]

Supplementary Information

Figures and Tables

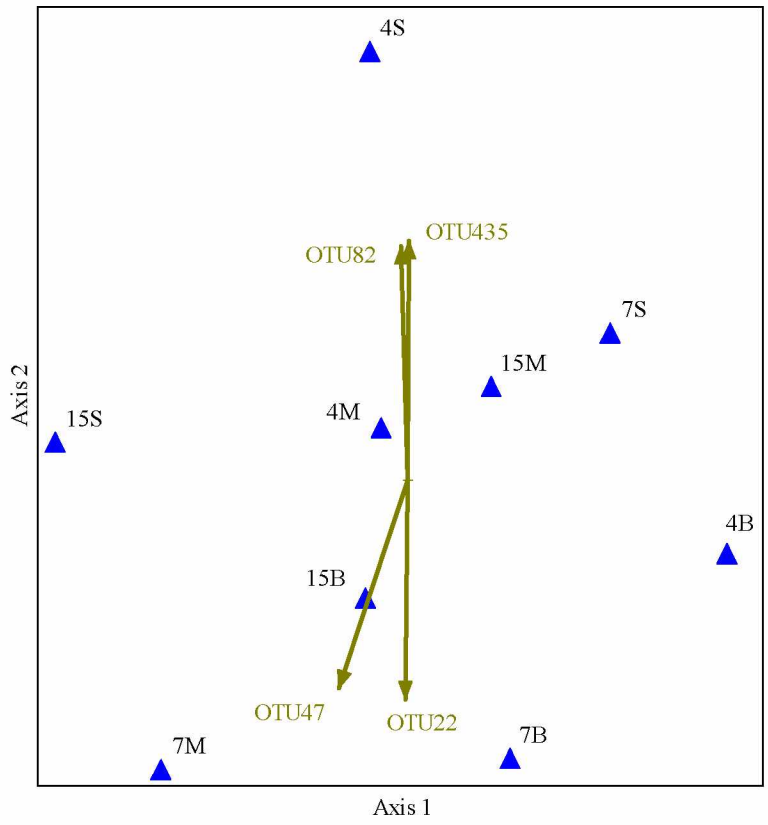

Figure S4-1. NMDS ordination plot illustrating the distribution of petroleum degradation genes within the Burger lease area, Chukchi Sea. Seawater was sampled from the surface (S), middle (M), and bottom (B) in September 2012 (n = 3; station ids: BF04, BF07, and BF15 on Figure 1). Vectors indicate individual OTUs with the strongest influence on the distribution of petroleum degradation genes throughout the water column ($r^2 > 0.58$).

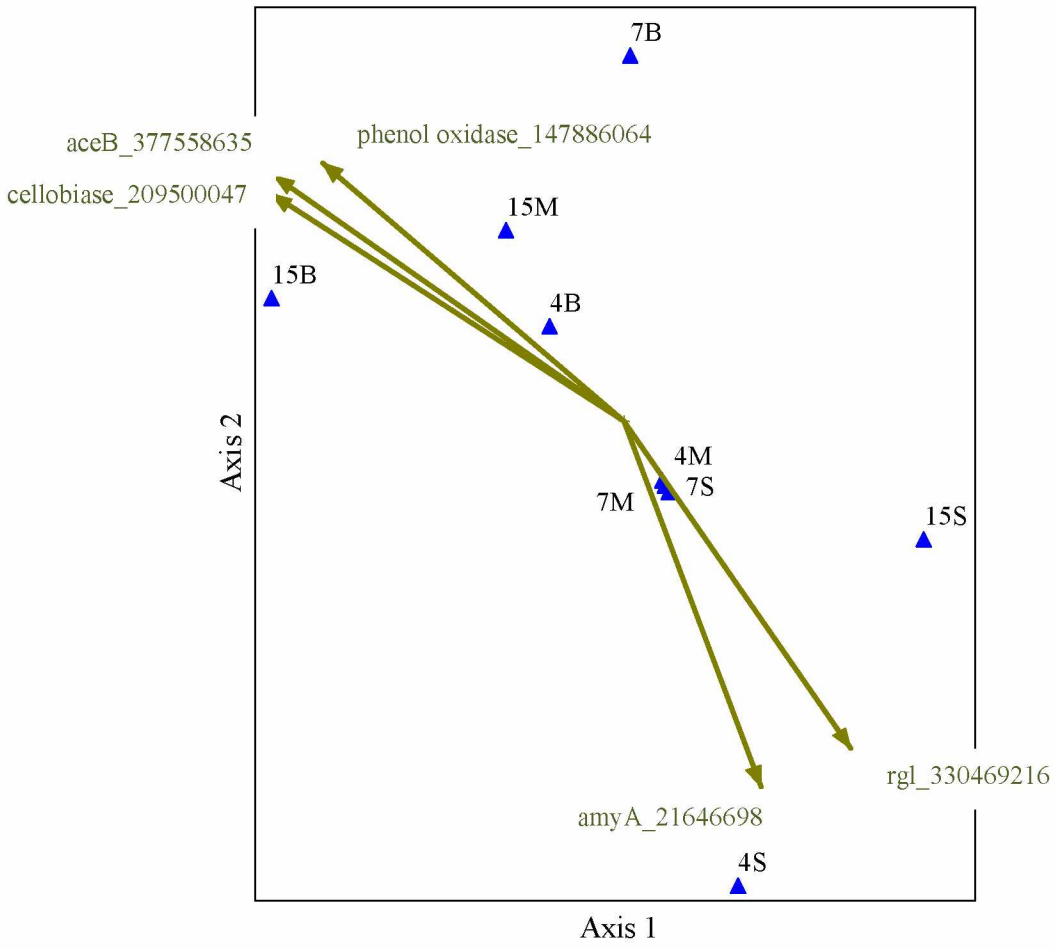

Figure S4-2. NMDS ordination plot illustrating the distribution of carbon degradation (non-petroleum) genes within the Burger lease area, Chukchi Sea. Seawater was sampled from the surface (S), middle (M), and bottom (B) in September 2012 (n = 3; station ids: BF04, BF07, and BF15 on Figure 1). Vectors indicate individual carbon degradation genes with the strongest influence on the distribution of total carbon degradation genes throughout the water column (r^2 > 0.73). Each individual gene is followed by its GenBank accession number.

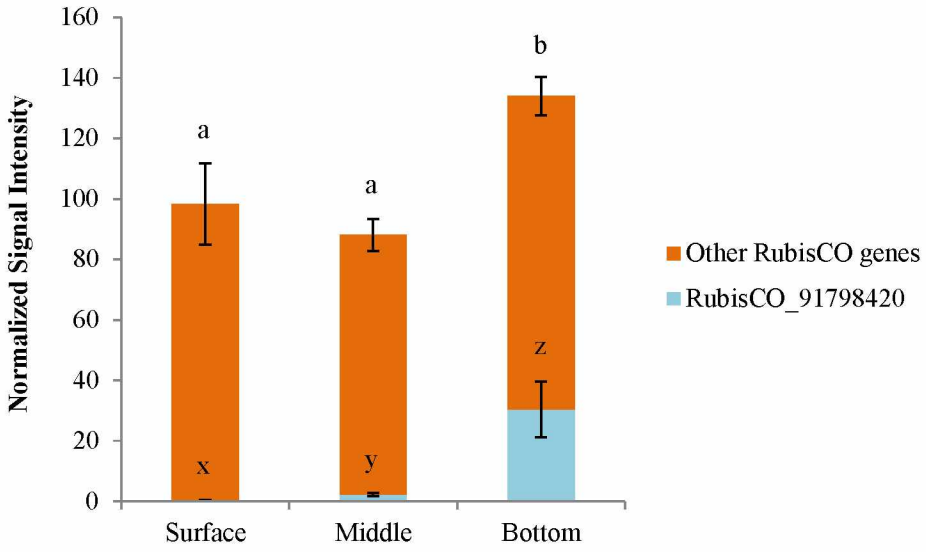

Figure S4-3. RubisCO genes in surface, middle and bottom seawater samples. The RubisCO-like protein (RLP; GenBank ID: 91798420) is shown as a portion of total RubisCO genes. Error bars represent standard error of the mean. Different letters correspond to significant differences between different water column locations ($p < 0.05$; MRPP).

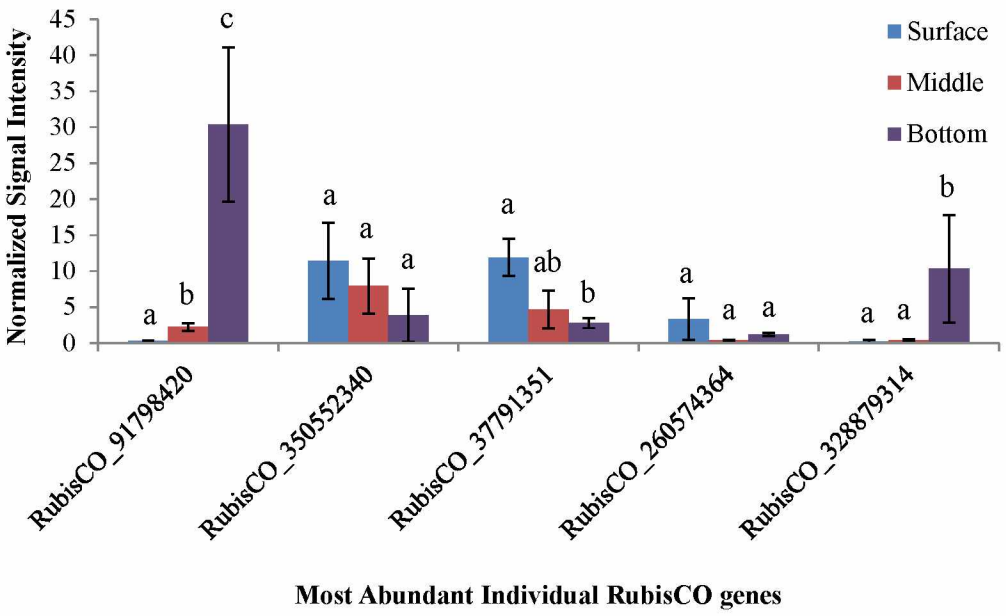

Figure S4-4. Most abundant individual RubisCO genes in GeoChip samples (n = 3; station ids: BF04, BF07, and BF15 on Figure 1) collected from the Burger lease area, Chukchi Sea, in September 2012. Genes with > 3% abundance of total RubisCO genes are shown. Different letters correspond to significant differences between water column locations for each gene (p < 0.05; MRPP). Error bars correspond to standard errors of the mean. Gene name is followed by its GenBank accession number.

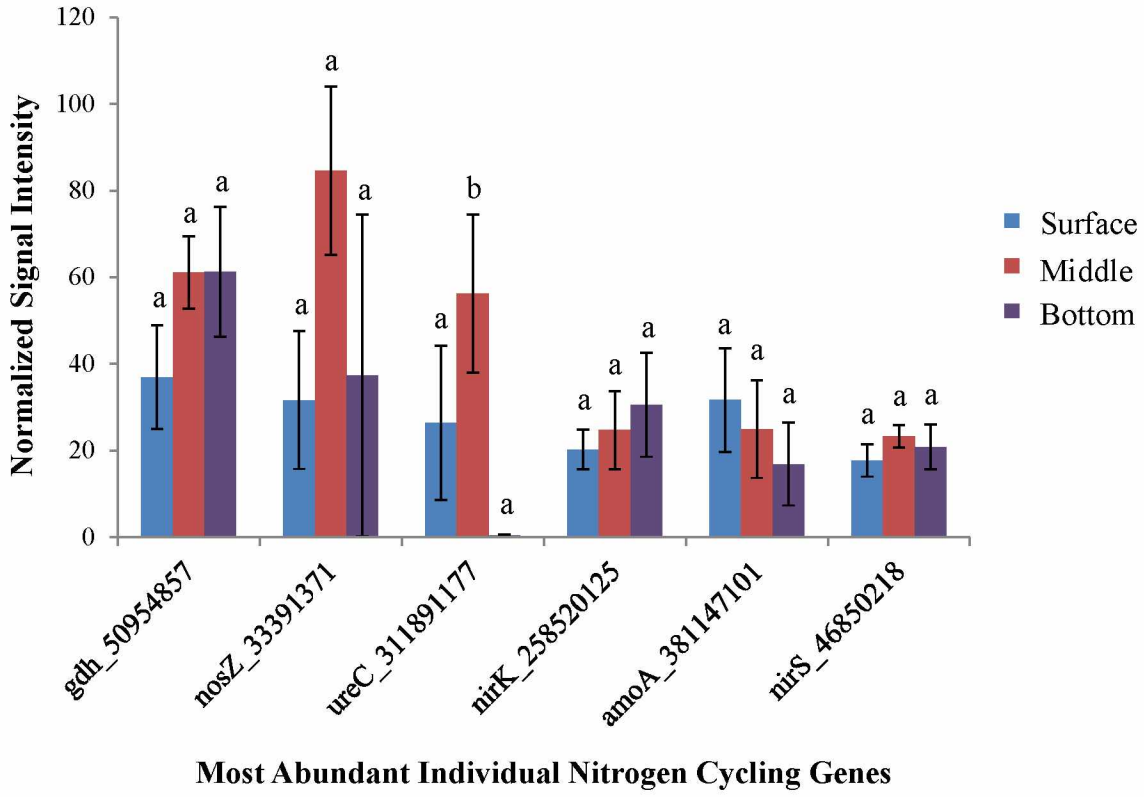

Figure S4-5. Most abundant individual nitrogen cycling genes in GeoChip samples ($n = 3$; station ids: BF04, BF07, and BF15 on Figure 1) collected from the Burger lease area, Chukchi Sea, in September 2012. Genes with > 1.3% abundance based on total nitrogen cycling genes are shown. Different letters correspond to significant differences between water column locations for each gene ($p < 0.05$; MRPP). Error bars correspond to standard errors of the mean. Each individual gene is followed by its GenBank accession number.

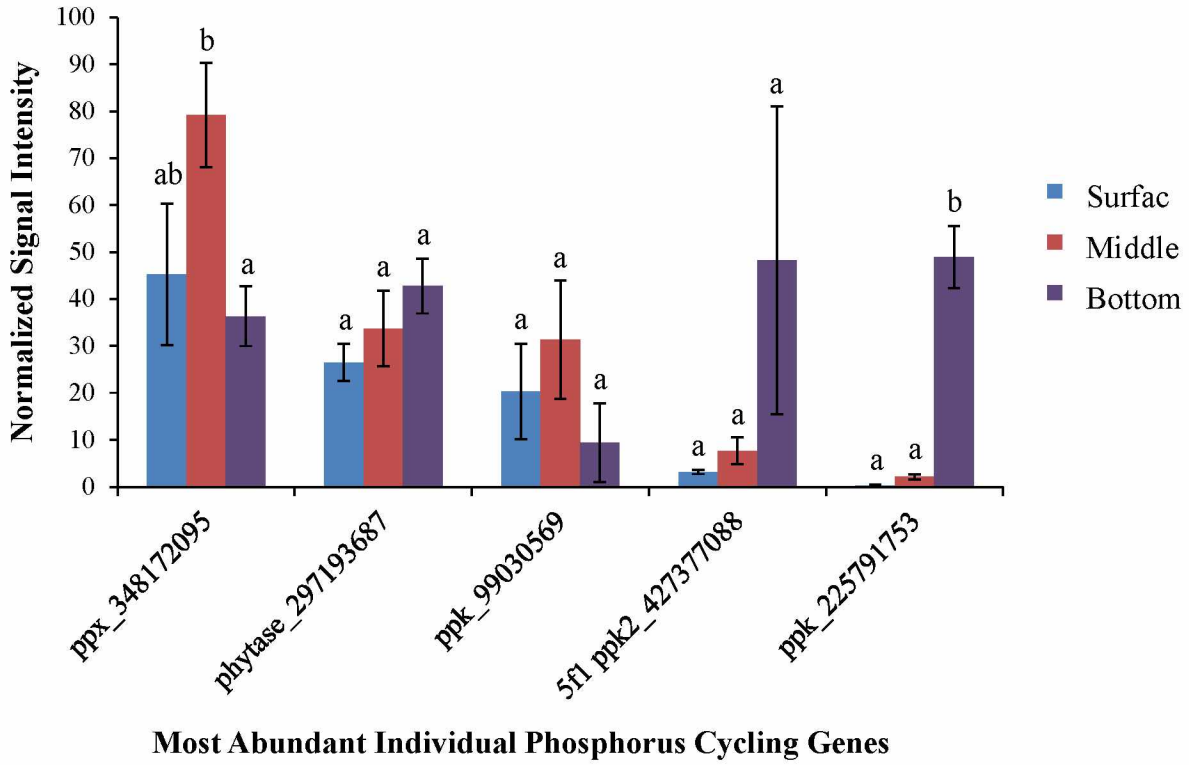

Figure S4-6. Most abundant individual phosphorus cycling genes in GeoChip samples ($n = 3$; station ids: BF04, BF07, and BF15 on Figure 1) collected from the Burger lease area, Chukchi Sea, in September 2012. Genes with > 1.0% abundance of total phosphorus genes are shown. Different letters correspond to significant differences between water column locations for each gene ($p < 0.05$; MRPP). Error bars correspond to standard errors of the mean. Each individual gene is followed by its GenBank accession number.

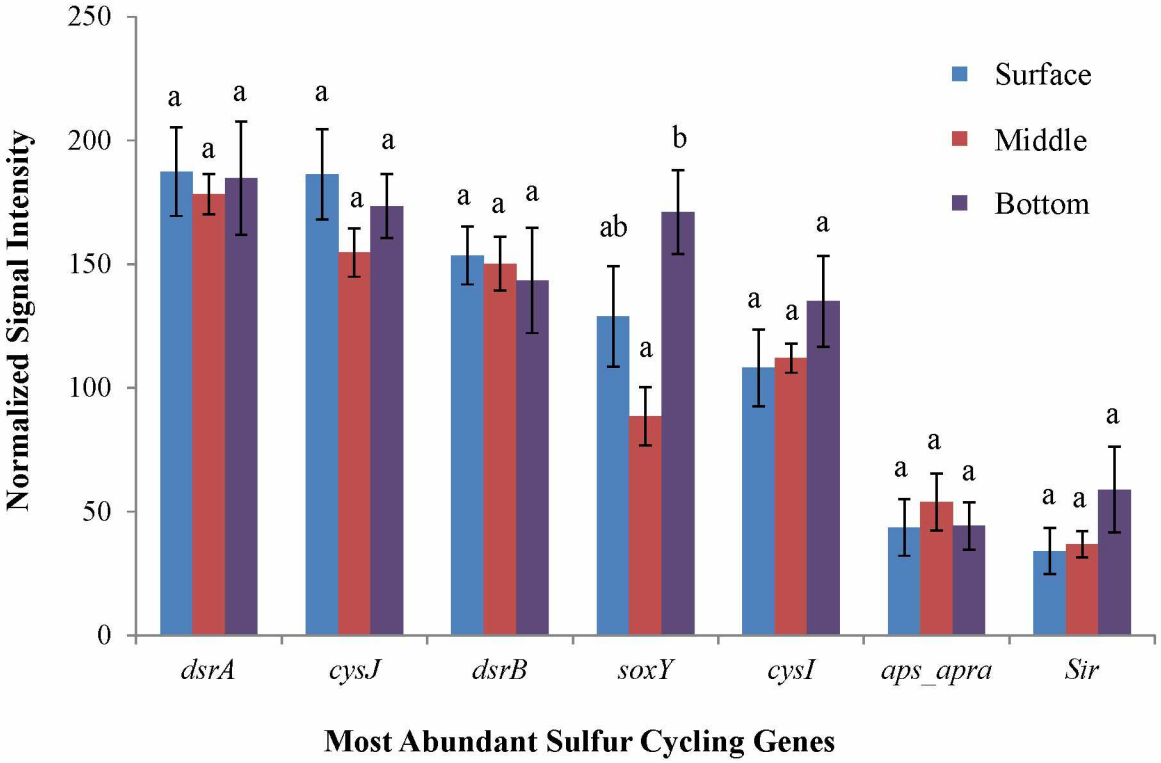

Figure S4-7. Most abundant sulfur cycling genes in GeoChip samples (n = 3; station ids: BF04, BF07, and BF15 on Figure 1) collected from the Burger lease area, Chukchi Sea, Arctic Ocean from September 20-22. Average signal intensities for the most abundant sulfur cycling genes are shown for surface, middle and bottom samples. Genes with > 4.7% abundance of total sulfur cycling genes are shown. Significant differences were calculated using MRPP with a Bray-Curtis distance measure. Different letters correspond to significant differences between water column locations for each gene ($p < 0.05$). Error bars correspond to standard errors of the mean.

Table S4-1. Most abundant OTUs in surface, middle and bottom seawater collected from the Burger lease area, Chukchi Sea, Arctic Ocean, in August and September of 2012. OTUs that are >1% of total abundance in at least one water column location are shown. OTUs are organized by phyla in alphabetical order. $n = 23$ for each water column location. Different letters correspond to significant differences between water column locations for each OTU that is a member of a taxonomic group previously reported to be associated with oil.

	Relative Abundance		
	Surface	Middle	Bottom
Actinobacteria	**2.1**	**1.8**	**1.2**
Unclassified Actinomycetales (OTU38)	1.60	1.15	0.35
Bacteroidetes	**20**	**17**	**12**
Formosa (OTU22)	7.30	5.71	1.51
Unclassified Flavobacteriaceae (OTU36) †	2.44^{ab}	2.49^{a}	1.74^{b}
Polaribacter (OTU1)*	2.39^{a}	3.51^{a}	2.50^{a}
Unclassified Flavobacteriaceae (OTU98) †	1.30^{a}	0.43^{b}	0.21^{c}
Unclassified Flavobacteriaceae (OTU17) †	1.20^{a}	0.73^{ab}	1.22^{b}
Proteobacteria	**49**	**49**	**58**
Unclassified Candidatus *Pelagibacter* (SAR11) (OTU2)	7.73^{a}	9.27^{a}	7.16^{a}
Unclassified Gammaproteobacteria (OTU6)	3.49	6.56	10.81
Thalassobius (OTU3)	5.00	3.77	3.91
Unclassified Gammaproteobacteria (OTU23)	0.86	2.17	6.44
Unclassified Gammaproteobacteria (OTU18)	3.70	2.82	1.44
Unclassified Rhodobacteraceae (OTU4)	3.97	2.01	1.68
Unclassified Gammaproteobacteria (OTU9)	2.49	2.64	2.39
Pseudoalteromonas (OTU14)*	3.20^{a}	1.66^{a}	1.95^{a}
Unclassified Proteobacteria (OTU13)	1.52	1.65	1.70
Unclassified Betaproteobacteria (OTU11)	1.12	1.03	1.14
Unclassified Rhodobacteraceae (OTU69)	1.44	0.87	0.92
Unclassified Candidatus *Pelagibacter* (SAR11) (OTU43)	1.40^{a}	1.25^{a}	0.56^{b}
Methylophaga (OTU65)	0.72	0.92	1.15
Unclassified Alphaproteobacteria (OTU89)	0.45	0.72	1.40
Unclassified Gammaproteobacteria (OTU29)	0.45	0.76	1.09
Unclassified Proteobacteria (OTU27)	0.30	0.55	1.16
Psychrobacter (OTU144) †	1.08^{a}	0.37^{b}	0.05^{c}
Verrucomicrobia	**12**	**16**	**10**
Unclassified Puniceicoccaceae (OTU7)	11.48	14.16	6.14
Unclassified Bacteria	**16**	**16**	**18**
Unclassified Bacteria (OTU5)	8.00	6.15	5.12
Unclassified Bacteria (OTU32)	4.80	7.65	6.88
Unclassified Bacteria (OTU3)	5.00	3.77	3.91
Unclassified Bacteria (OTU77)	0.26	0.64	1.13

* Taxa known to include oil-degraders.

† Taxa associated with the presence of oil.

Table S4-2. Diversity table showing the number of reads (No. reads), observed OTUs (Obs. OTU), a measure of alpha diversity (Inverse Simpson) and a measure of richness (Chao1). Mean values are shown ± standard deviation. Significant differences in diversity measurements between water column locations are illustrated with different letters ($p < 0.05$; MRPP). The number of reads and observed OTUs were calculated prior to normalization and after sequence processing. Inverse Simpson and Chao1 were calculated after processing and after normalization (i.e. sub.sample) with a cutoff of 2,161 sequences. Sample Ids that end with a 1 (e.g. 11B1) were collected in August and IDs that end with a 2 (e.g. 11B2) were collected in September.

Sample ID	No. reads*	Obs. OTU*	Inverse Simpson	Chao1
Mean Values				
Surface	5611 ± 928	104 ± 11	16 ± 5[a]	171 ± 29[a]
Middle	5797 ± 676	104 ± 7	16 ± 7[a]	168 ± 28[a]
Bottom	7666 ± 1915	201 ± 23	22 ± 8[b]	374 ± 85[b]
Individual Samples				
10B1	8012	253	36	464
10P1	5916	184	31	349
11B1	53488	640	29	380
11B2	4203	220	27	512
11P1	3341	58	8	116
11P2	6337	134	25	232
11S1	2273	60	16	127
11S2	2256	74	12	168
13B1	3957	83	6	189
13P1	3697	48	8	94
13S1	13449	232	8	236
14B1	1595	72	15	340
14B2	6869	247	26	358
14P1	5106	76	12	151
14P2	3191	101	24	249
14S1	8736	91	9	182
14S2	3148	94	22	187
15B1	2933	111	16	350
15B2	14029	378	19	493
15P1	10815	112	12	174
15P2	3371	77	9	154
15S1	2443	59	12	135
15S2	7856	123	19	176
18B1	4487	129	11	296
18P1	9883	97	9	160
18S1	3826	91	19	167
19B1	6793	210	14	407
19P1	2526	85	13	187
19S1	4410	79	20	158
1B1	7400	227	22	398
1B2	7356	229	26	374
1P1	3875	110	22	215
1P2	16925	147	19	201
1S1	2994	79	14	166
1S2	2655	84	22	180

Table S4-2 continued

23B1	3412	179	20	402
23P1	9396	157	11	196
23S1	2381	68	20	141
24B1	4939	182	19	449
24P1	2044	97	27	179
24S1	3809	77	11	133
25B1	5198	197	8	427
25P1	2499	70	6	148
25S1	5874	94	20	143
2B1	6828	200	28	349
2P1	7535	141	22	249
2S1	9733	142	19	199
3B1	9968	270	23	415
3B2	5876	187	25	415
3P1	7612	100	9	176
3P2	8990	110	12	169
3S1	4387	72	19	159
3S2	1387	68	24	182
4B1	8706	200	29	290
4B2	5850	141	23	321
4B3	1996	96	27	282
4P1	4542	98	18	238
4P2	5495	108	18	162
4P3	7004	103	17	175
4S1	5512	119	16	173
4S2	1747	56	16	135
4S3	6028	142	11	228
5B1	2747	126	30	450
5P1	5136	134	21	288
5S1	7140	112	18	156
7B1	5005	173	32	388
7B2	4618	197	26	427
7P1	5678	84	9	121
7P2	6049	108	14	167
7S1	4918	82	14	161
7S2	6411	114	14	207
8B1	8080	296	31	473
8P1	4029	79	12	178
8S1	5427	103	23	186
9B1	2751	99	24	302
9P1	3825	68	13	151
9S1	3856	82	13	134

*: calculated prior to normalization

Table S4-3. Differences in individual functional genes among Arctic surface, middle, and bottom samples ($n = 3$; station ids: BF04, BF07, and BF15 on Figure 4-1). Seawater samples were collected from September 20-22, 2012, in the Burger lease area, Chukchi Sea, Arctic Ocean. The associated *p*-values, test statistics, and A-values are shown for each water column location comparison. The A-value refers to the chance-corrected within-group agreement and describes within-group homogeneity, compared to random expectation. Different data sets are indicated in bold and with an asterisk ($p < 0.05$). Differences were calculated using MRPP with a Bray-Curtis distance measure.

Functional Gene Category	Surface vs. Middle			Surface vs. Bottom			Middle vs. Bottom		
	p-value	T-statistic	A-value	*p*-value	T-statistic	A-value	*p*-value	T-statistic	A-value
Carbon Cycling	0.33	-0.31	0.03	**0.03***	**-2.37**	**0.38**	0.51	0.15	-0.02
Carbon Degradation	0.12	-1.20	0.03	**0.03***	**-2.26**	**0.06**	0.18	-0.88	0.02
Carbon Fixation	0.11	-1.22	0.01	0.21	-0.82	0.01	0.41	-0.16	0.00
Electron Transfer	0.34	-0.26	0.01	0.18	-0.92	0.01	0.96	1.54	-0.04
Nitrogen Cycling	0.21	-0.64	0.02	**0.03***	**-2.14**	**0.05**	0.10	-1.24	0.02
Organic Remediation	0.20	-0.83	0.01	**0.03***	**-2.24**	**0.05**	0.07	-1.50	0.02
Oil Degradation	0.49	0.10	0.00	0.06	-1.52	0.03	0.43	-0.07	0.00
Phosphorus Cycling	0.27	-0.31	0.03	**0.04***	-2.12	0.25	0.05	-1.57	0.22
Secondary Metabolism	0.19	-0.85	0.02	0.05	-1.83	0.04	0.53	0.04	0.00
Sulfur Cycling	0.20	-0.82	0.02	**0.03***	**-2.11**	**0.03**	0.40	-0.30	0.01
Virulence	0.15	-1.01	0.02	**0.03***	**-2.58**	**0.06**	**0.05***	**-1.89**	**0.03**
Virus	**0.02***	**-2.36**	**0.32**	**0.02***	**-2.73**	**0.43**	**0.03***	**-1.94**	**0.19**

Table S4-4. Environmental data collected in August, 2012, from 10 different locations in the Burger lease area, Chukchi Sea, Arctic Ocean. Location IDs correspond to sampling IDs identified in Figure 4-1.

ID	Location	Depth, m		Date	Temp (°C)	Salinity (PSU)	PO_4 (µM)	Sil (µM)	NO_3 (µM)	NO_2 (µM)	NH_4 (µM)	DIN (µM)	Chl a (µg/L)	Phaeophytin (µg/L)
1S1	BF001	SURFACE	0	18-Aug	5.56	30.38	0.38	2.37	0.02	0.00	1.16	1.19	0.12	0.06
1P1	BF001	MIDDLE	20	18-Aug	2.29	32.41	0.69	5.03	0.44	0.04	1.40	1.87	1.57	0.42
1P1	BF001	BOTTOM	40	18-Aug	1.49	32.66	0.92	9.33	1.08	0.07	2.12	3.28	0.29	0.28
3S1	BF003	SURFACE	0	16-Aug	4.78	30.72	0.37	3.67	0.95	0.01	2.85	3.80	0.14	0.09
3P1	BF003	MIDDLE	20	16-Aug	2.82	32.15	0.64	3.51	1.35	0.01	2.81	4.17	0.77	0.57
3B1	BF003	BOTTOM	43	16-Aug	1.35	32.51	0.76	5.01	0.61	0.04	4.44	5.09	0.31	0.32
4S1	BF004	SURFACE	0	15-Aug	4.86	30.88	0.55	4.91	0.13	0.01	2.24	2.38	0.14	0.08
4P1	BF004	MIDDLE	20	15-Aug	0.13	32.51	0.82	4.22	0.58	0.02	3.27	3.86	0.37	0.34
4B1	BF004	BOTTOM	42	15-Aug	-0.80	32.72	1.26	11.40	2.82	0.11	4.68	7.60	0.29	0.22
5S1	BF005	SURFACE	0	16-Aug	5.42	31.02	0.40	1.77	0.25	0.00	3.09	3.90	0.24	0.13
5P1	BF005	MIDDLE	20	16-Aug	2.81	31.69	0.79	7.68	0.61	0.03	2.96	3.61	0.58	0.44
5B1	BF005	BOTTOM	44	16-Aug	1.35	32.50	0.60	7.01	0.94	0.05	4.20	5.19	0.26	0.42
7S1	BF007	SURFACE	0	18-Aug	3.84	30.48	0.42	5.23	0.06	0.00	0.59	0.65	0.20	0.08
7P1	BF007	MIDDLE	20	18-Aug	2.07	32.49	0.78	5.61	0.59	0.04	1.63	2.26	1.03	0.42
7B1	BF007	BOTTOM	42	18-Aug	1.25	32.61	0.70	5.78	0.67	0.04	2.13	2.84	0.76	0.46
9S1	BF009	SURFACE	0	17-Aug	3.77	30.67	0.41	3.93	0.16	0.00	2.77	2.93	0.18	0.10
9P1	BF009	MIDDLE	20	17-Aug	-0.45	32.57	0.70	5.72	1.07	0.05	3.65	4.77	1.40	0.64
9B1	BF009	BOTTOM	44	17-Aug	-0.65	32.67	1.25	13.17	2.91	0.12	4.95	7.98	0.59	0.71
11S1	BF011	SURFACE	0	17-Aug	3.76	30.45	0.37	3.71	0.06	0.01	1.53	1.60	0.18	0.08
11P1	BF011	MIDDLE	20	17-Aug	-0.41	32.58	0.87	4.22	0.94	0.06	2.94	3.93	1.34	0.45
11B1	BF011	BOTTOM	43	17-Aug	-0.93	32.76	1.11	6.65	2.04	0.07	3.34	5.45	0.62	0.46
13S1	BF013	SURFACE	0	12-Aug	2.94	30.61	0.42	5.52	0.06	0.00	0.99	1.05	0.24	0.03
13P1	BF013	MIDDLE	20	12-Aug	-0.52	32.69	0.86	4.90	0.59	0.02	0.27	0.88	1.94	0.30
13B1	BF013	BOTTOM	42	12-Aug	-1.04	32.83	1.03	3.28	1.40	0.04	2.83	4.26	2.02	0.57
14S1	BF014	SURFACE	0	17-Aug	2.72	30.52	0.48	5.39	0.26	0.01	3.12	3.38	0.28	0.06
14P1	BF014	MIDDLE	20	17-Aug	-0.75	32.75	0.65	4.72	0.26	0.01	2.80	3.07	1.85	0.44
14B1	BF014	BOTTOM	45	17-Aug	-1.46	33.07	1.86	18.46	7.57	0.18	7.11	14.87	1.65	0.70
15S1	BF015	SURFACE	0	17-Aug	2.18	29.90	0.54	10.59	0.33	0.00	2.14	2.47	0.25	0.11
15P1	BF015	MIDDLE	20	17-Aug	-1.01	32.17	0.86	12.71	0.61	0.03	2.34	2.99	1.44	0.35
15B1	BF015	BOTTOM	44	17-Aug	-1.56	33.23	1.90	19.53	9.69	0.16	6.76	16.61	1.21	0.73

Table S4-5. Environmental data collected in September, 2012, from 13 different locations in the Burger lease area, Chukchi Sea, Arctic Ocean. Location IDs correspond to sampling IDs identified in Figure 4-1.

ID	Location	Depth, m		Date	Temp (°C)	Salinity (PSU)	PO_4 (µM)	Sil (µM)	NO_3 (µM)	NO_2 (µM)	NH_4 (µM)	DIN (µM)	Chl a (µg/L)	Phaeophytin (µg/L)
1S2	BF001	SURFACE	0	21-Sep	2.26	30.87	0.47	5.84	0.08	0.02	0.59	0.69	0.29	0.07
1P2	BF001	MIDDLE	20	21-Sep	2.46	31.09	0.45	4.79	0.00	0.01	0.74	0.75	1.32	0.27
1B2	BF001	BOTTOM	40	21-Sep	0.42	32.72	1.18	10.48	1.67	0.08	3.54	5.28	0.31	0.50
2S2	BF002	SURFACE	0	21-Sep	2.68	31.08	0.39	4.30	0.11	0.01	0.83	0.95	0.41	0.15
2P2	BF002	MIDDLE	20	21-Sep	3.14	31.17	0.58	8.12	0.16	0.01	0.22	0.39	0.65	0.20
2B2	BF002	BOTTOM	43	21-Sep	0.59	32.74	1.20	11.40	1.62	0.07	3.18	4.88	0.22	0.30
3S2	BF003	SURFACE	0	21-Sep	3.42	31.08	0.42	5.70	0.04	0.00	0.35	0.40	0.31	0.11
3P2	BF003	MIDDLE	20	21-Sep	3.66	31.12	0.39	4.23	0.06	0.00	0.67	0.73	0.65	0.20
3B2	BF003	BOTTOM	42	21-Sep	0.16	32.76	1.24	13.35	1.80	0.07	3.07	4.94	0.19	0.40
4S2	BF004	SURFACE	0	21-Sep	3.15	30.92	0.54	9.87	0.06	0.00	0.09	0.15	0.34	0.15
4P2	BF004	MIDDLE	20	21-Sep	4.30	31.17	0.45	6.53	0.03	0.01	0.35	0.39	0.39	0.17
4B2	BF004	BOTTOM	42	21-Sep	-0.36	32.77	1.21	13.42	2.10	0.07	2.76	4.92	0.23	0.48
7S2	BF007	SURFACE	0	20-Sep	1.06	29.62	0.53	8.96	0.05	0.01	0.02	0.08	0.44	0.14
7P2	BF007	MIDDLE	20	20-Sep	3.30	31.23	0.39	3.63	0.04	0.01	1.07	1.12	0.68	0.21
7B2	BF007	BOTTOM	42	20-Sep	0.35	32.77	1.07	10.26	1.37	0.07	2.88	4.31	0.28	0.66
8S2	BF008	SURFACE	0	21-Sep	0.67	29.30	0.46	11.15	0.07	0.00	0.93	1.01	0.21	0.10
8P2	BF008	MIDDLE	20	21-Sep	2.38	31.04	0.74	9.07	0.09	0.01	0.50	0.60	0.29	0.08
8B2	BF008	BOTTOM	42	21-Sep	-0.08	32.78	1.34	16.03	2.12	0.08	3.22	5.42	0.35	0.65
11S2	BF011	SURFACE	0	20-Sep	2.32	30.47	0.45	7.24	0.00	0.00	0.34	0.35	0.33	0.14
11P2	BF011	MIDDLE	20	20-Sep	2.54	30.67	0.39	4.47	0.00	0.01	0.94	0.94	0.42	0.16
11B2	BF011	BOTTOM	42	20-Sep	-0.07	32.79	1.21	13.08	1.62	0.06	3.21	4.89	0.29	0.62
15S2	BF015	SURFACE	0	21-Sep	2.08	30.07	0.42	6.71	0.05	0.00	1.19	1.24	0.26	0.10
15P2	BF015	MIDDLE	20	21-Sep	2.33	30.28	0.52	8.87	0.03	0.00	0.06	0.10	0.35	0.13
15B2	BF015	BOTTOM	42	21-Sep	-1.40	33.09	1.50	22.11	5.42	0.09	3.95	9.47	0.46	0.92
18S2	BF018	SURFACE	0	22-Sep	1.04	28.96	0.46	10.33	0.02	0.00	0.27	0.28	0.18	0.11
18P2	BF018	MIDDLE	20	22-Sep	1.04	28.96	0.46	10.33	0.02	0.00	0.27	0.28	0.19	0.09
18B2	BF018	BOTTOM	41	22-Sep	-1.47	33.12	1.81	23.13	8.39	0.13	4.27	12.79	0.16	0.20
19S2	BF019	SURFACE	0	22-Sep	0.35	28.38	0.34	9.61	0.06	0.02	0.54	0.62	0.28	0.19
19P2	BF019	MIDDLE	20	22-Sep	-0.90	31.44	0.53	11.77	0.05	0.01	0.48	0.54	0.39	0.19
19B2	BF019	BOTTOM	41	22-Sep	-1.55	33.15	2.04	40.34	11.95	0.14	6.14	18.23	0.64	0.69
23S2	BF023	SURFACE	0	22-Sep	0.30	29.19	0.50	14.02	0.22	0.01	0.47	0.69	0.22	0.10
23P2	BF023	MIDDLE	20	22-Sep	0.38	29.31	0.44	9.91	0.02	0.00	0.30	0.33	0.22	0.08
23B2	BF023	BOTTOM	40	22-Sep	-1.43	33.11	1.37	21.39	4.90	0.07	3.87	8.84	0.44	0.69
24S2	BF024	SURFACE	0	22-Sep	0.35	28.74	0.27	5.94	0.03	0.01	1.01	1.05	0.26	0.13
24P2	BF024	MIDDLE	20	22-Sep	-0.87	31.70	0.57	10.36	0.34	0.01	0.55	0.90	0.21	0.10
24B2	BF024	BOTTOM	41	22-Sep	-1.51	33.14	1.40	26.13	5.72	0.06	4.39	10.17	0.48	0.71
25SP	BF025	SURFACE	0	22-Sep	0.31	28.42	0.38	11.76	0.08	0.01	0.14	0.23	0.28	0.16
25P2	BF025	MIDDLE	20	22-Sep	-0.44	31.38	0.48	9.63	0.06	0.02	0.92	0.99	1.16	0.16
25B2	BF025	BOTTOM	41	22-Sep	-1.62	33.13	1.70	43.33	9.33	0.10	5.50	14.92	0.74	0.98

Table S4-6. Environmental variables associated with the NMDS ordination of 16S rRNA genes in surface, middle, and bottom samples. Environmental variables that are correlated ($r^2 > 0.52$) with the distribution of 16S rRNA genes are shown in Figure 4-3.

	Axis 1			Axis 2			Axis 3		
	r	***r*²**	**tau**	***r***	***r*²**	**tau**	***r***	***r*²**	**tau**
PO_4	0.29	0.08	0.23	**-0.72**	**0.52**	**-0.51**	0.26	0.07	0.17
Silica	0.05	0.00	0.08	-0.68	0.46	-0.46	0.15	0.02	0.08
NO_3	0.09	0.01	0.28	-0.66	0.43	-0.48	0.23	0.05	0.13
NO_2	0.29	0.09	0.29	-0.66	0.43	-0.49	0.19	0.04	0.10
NH_4	0.34	0.12	0.27	-0.60	0.36	-0.39	0.17	0.03	0.11
DIN	0.20	0.04	0.27	-0.67	0.44	-0.45	0.22	0.05	0.11
Chlorophyll *a*	-0.19	0.04	-0.05	-0.20	0.04	-0.17	0.27	0.07	0.14
Phaeophytin	0.34	0.12	0.20	-0.66	0.43	-0.46	0.16	0.03	0.11
Temperature	0.04	0.00	0.04	**0.77**	**0.60**	**0.60**	-0.14	0.02	-0.16
Salinity	0.35	0.12	0.26	-0.51	0.26	-0.46	0.33	0.11	0.23

Chapter 5: Conclusions

This research addresses key data gaps concerning the fate and effects of dispersed oil in Arctic marine environments and provides novel insight into the biodegradation of crude oil and Corexit 9500 by Arctic marine bacteria. Prior to this research, the rate and extent of oil and Corexit biodegradation in Arctic seawater were unknown, as was their effect on indigenous Arctic marine microorganisms. In Chapter 2, we reported the extent to which indigenous Arctic microorganisms in nearshore surface seawater primarily biodegraded and/or mineralized oil, Corexit, and oil in the presence of Corexit. We reported that some components within Corexit were readily biodegradable by the indigenous Arctic marine community and that oil biodegradation was not inhibited by the presence of Corexit, with oil losses ranging from 46-61% loss after 63 days at -1°C. In Chapter 3, we paired chemical analyses with genetic analyses to build upon the work reported in Chapter 2 and provided a comprehensive analysis of oil and Corexit biodegradation in nearshore and offshore surface seawater. In Chapter 2, the extents of oil loss in Arctic seawater were lower than extents reported in temperate seawater (Prince et al., 2013); however, in Chapter 3, the rate at which oil biodegraded was comparable to rates in temperate and sub-Arctic seawater (Atlas & Bartha, 1973; Laake et al., 1984). We conclude that physico-chemical properties of oil at low temperature more likely limit oil biodegradation extents than metabolism. Chapter 2 included the first report of a biodegradation rate constant for a Corexit component under Arctic conditions (DOSS; $k = 0.015$ day^{-1}). The extent at which Corexit biodegraded varied between nearshore and offshore Arctic seawater, a likely effect of different microbes dominating different water masses at different times. It was also demonstrated that similar bacterial taxa and oil-biodegradation genes increased in relative abundance in response to both oil and Corexit. This finding suggests that some microorganisms have the ability to biodegrade both oil and Corexit and may use similar metabolic pathways when utilizing these carbon sources for energy. In Chapter 4, we built upon this knowledge by investigating the microbial ecology of an offshore oil lease area and reported that oil-degrading genes and taxa known to include oil-degrading microorganisms were located throughout the Arctic water column.

Since their introduction at the Torrey Canyon wreck, the use of chemical dispersants as an oil spill response option have been contentious, and for good reason, as initial formulations

were determined to be toxic to marine organisms (Smith, 1968). Corexit 9500 is the main dispersant utilized today in oil spill response (Bejarano et al., 2013) and was specifically engineered to be readily biodegradable (Corexit 9500A SDS; Word et al., 2015). The results presented here support prior reports of enhanced oil loss (Brakstad et al., 2015; Prince & Butler, 2014) and bacterial enrichment (Chakraborty et al., 2012) in the presence of Corexit 9500. Here we demonstrate that Corexit enhanced the abundance of prokaryotes and the relative abundance of oil-biodegradation genes and taxa known to include members that degrade oil, even when compared to treatments containing oil-alone. Under our experimental conditions, which were designed to simulate realistic environmental conditions, our results demonstrate that Corexit does not inhibit oil-degrading microorganisms.

The biodegradation extents and rates calculated here were conducted with surface waters; however, Chapter 4 reported that seawater collected just above the seafloor contained a similar relative abundance of oil-biodegradation genes as surface seawater. Because gene presence does not indicate function and environmental conditions may limit biodegradation, future studies with deep-sea water are necessary to ascertain deep-sea oil biodegradation rates. When using mesocosms to mimic open oceans, it is important to note the inherent variability between laboratory and field results and the importance of currents and vertical mixing in open systems. A fundamental understanding of Arctic marine hydrology, oil behavior, and the effect of environmental parameters, such as nutrients and oxygen on biodegradation, will help responders extrapolate these oil biodegradation rates to other locations in the Arctic water column.

Understanding oil and dispersant biodegradation in the Arctic Ocean is important to advancing oil spill response policies. Documenting the microbial community response to oil in Arctic seawater will enable the identification of key species that may assist in monitoring the fate of oil, its impact on the food web, and its subsequent recovery. Here we report that *Colwellia*, *Oleispira*, and *Polaribacter* are key genera involved in the biodegradation of oil and Corexit compounds in Arctic surface seawater. While correlating chemical analyses with genetic analyses allowed us to suggest which bacterial taxa and oil-degradation genes (e.g. *alkB* and *nagG*) are performing biodegradation, we cannot say for certain that these taxa and functional genes are involved. Future studies involving stable isotope probing, transcriptomics, and proteomics, would confirm oil-degrading processes such as microbial assimilation of petroleum

hydrocarbons, synthesis of oil-degrading genes, and production of oil-degrading enzymes, respectively.

References

Atlas RM, Bartha R. (1973). Stimulated biodegradation of oil slicks using oleophilic fertilizers. *Environ. Sci. Technol.* 7:538-541.

Bejarano AC, Levine E, Mearns AJ. (2013). Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. *Environ. Monitor. Assess.* 185:10281-10295.

Brakstad OG, Nordtug T, Throne-Holst M. (2015). Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Marine Poll. Bull.* 93:144-152.

Chakraborty R, Borglin SE, Dubinsky EA, Andersen GL, Hazen TC. (2012). Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. *Front. Microbiol.* 3:357.

Corexit 9500A Safety Data Sheet; COREXIT ® EC9500A; Nalco Company, Naperville, IL, USA; https://dec.alaska.gov/spar/ppr/docs/dispersant_MSDS/Corexit%209500A%20MSDS.pdf.

Laake M, Tjessem K, Rein K. (1984). Fate of a tritiated Ekofisk crude oil in a controlled ecosystem experiment with North Sea plankton. *Environ. Sci. Technol.* 18:641-647.

Prince RC, McFarlin KM, Butler JD, Febbo EJ, Wang FCY, Nedwed TJ. (2013). The primary biodegradation of dispersed crude oil in the sea. *Chemosphere.* 90:521-526.

Prince RC, Butler JD. (2014). A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil. *Environ. Sci. Pollut. Res. Int.* 21:9506-9510.

Smith JE. (1968). 'Torrey Canyon' *Pollution* and *Marine Life*. A *Report* by the Plymouth Laboratory of the Marine Biological Association of the United Kingdom (Cambridge Univ. Press, Cambridge).

Word JQ, Clark JR, Word LS. (2015). Comparison of the acute toxicity of Corexit 9500 and household cleaning products. *Hum. Ecol. Risk Assess.* 21:707-725.