Fate and Persistence of Oil Spill Response Chemicals in Arctic Seawater

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List of Acronyms

ANS = Alaska North Slope crude oil

DEC= Alaska Department of Environmental Conservation

DOSS = bis-(2-ethylhexyl) sulfosuccinate, also known as dioctyl sodium sulfosuccinate

DWH = Deepwater Horizon (Macondo) oil spill

EHSS = ethylhexyl sulfosuccinate

EVOS= *Exxon Valdez* oil spill

GC/MS = gas chromatography mass spectrometry

GOM = Gulf of Mexico

LC-MS/MS = liquid chromatography tandem mass spectrometry

OSU = Oregon State University

PAHs = Polyaromatic Hydrocarbons

qPCR = quantitative polymerase chain reaction

TPH = total petroleum hydrocarbons

UAF = University of Alaska Fairbanks

UA BLaST = University of Alaska Biomedical Learning and Student Training

US EPA = United States Environmental Protection Agency

Abstract

In the event of an oil spill in the Arctic Ocean, the use of chemical dispersants is one potential option for oil spill response. Corexit 9500A is currently the principal chemical dispersant stockpiled and is likely to be applied to an oil spill in the Arctic, should it receive regulatory approval. Before its risks can be fully assessed, there is a need to determine the fate and persistence of Corexit 9500A in the Arctic marine environment. In this project, we quantified biodegradation of the chemical dispersant Corexit 9500A in Arctic seawater and determined how the presence of crude oil alongside dispersants affects the biodegradation of both dispersants and oil. Using advanced molecular tools, we identified microbes active in oil and dispersant biodegradation in Arctic seawater. We found that crude oil and surfactant components of Corexit 9500A can undergo substantial biodegradation within 28 days in Arctic surface seawater: 36–41% of oil; 33–77% of bis-(2-ethylhexyl) sulfosuccinate (DOSS); 96% of Span 80. In near-shore seawater, crude oil degraded slightly more extensively than in offshore waters. Overall, the nonionic surfactant components of Corexit 9500A (Tween 80+85 and Span 80) were more labile than DOSS, with the nonionics dropping to below detection limits within 2–5 days, while DOSS was more persistent, especially in the presence of oil. Molecular analyses of the microbial communities were performed in the 2013–14 incubations. The microbial population size grew more extensively in response to Corexit 9500A than oil alone within 28 days, suggesting that some components are more readily biodegraded and utilized as growth substrates. In general, different members of the microbial community appear to be responsible for Corexit 9500A and oil biodegradation; however, throughout the incubation, a subset of taxa (*Oleispira*, *Colwellia*, *Lutibacter*, and an unclassified Flavobacteriaceae spp.) and functional genes associated with oil biodegradation (*alkB*, *nagG*, and *pchCF*) increased in response to both oil and Corexit 9500A, suggesting that some organisms may biodegrade both Corexit 9500A components and oil. Together, these findings indicate that the indigenous microbial community in the Chukchi Sea in summer and fall can perform substantial oil and dispersant biodegradation within 28 days, although the longer-term fate of the residual components is yet-to-be-determined.

Introduction

Shipping activities and offshore oil development present environmental risks to the Arctic marine ecosystem. It is important to base the operational plans for offshore exploration and spill response on a scientific understanding of the potential environmental impacts to Arctic ecology. The fate and effects of chemical dispersants are key factors in need of consideration, yet there have been no thorough studies on their biodegradation and persistence in the Arctic marine environment, nor are the effects of dispersants on oil biodegradation in the Arctic marine environment well understood.

If an oil spill occurs in the Arctic, the chemical dispersant formula marketed as Corexit 9500A will likely be the dispersant of choice due to its efficacy (Belore et al., 2009; SL Ross, 2007), relatively low toxicity (United States Environmental Protection Agency [US EPA], 2010), reported effectiveness during the Deepwater Horizon (DWH) oil spill (Bejarano et al., 2013), and prior approval for use in sub-Arctic Alaskan waters (Alaska Regional Response Team, 2004). In previous studies, it was demonstrated that Corexit 9500A enhanced oil biodegradation in the early stages of oil exposure to Arctic seawater (McFarlin et al., 2014). However, these studies were limited to a few small-scale experiments at low oil concentrations under lab-simulated open water conditions. Further tests are needed to quantify the intrinsic biodegradation of oil under different conditions as well as the effects of Corexit 9500A. In addition to its effects on oil biodegradation, the fate of Corexit 9500A in seawater is an important question (both alone and in the presence of oil) since non-target dispersant application often occurs in the field.

After the *Exxon Valdez* oil spill (EVOS), Exxon proposed to use Corexit 958OM2 to facilitate cleanup of contaminated beaches. However, the Alaska Department of Environmental Conservation (ADEC) and US EPA were hesitant to approve spraying of Corexit 958OM2 due to insufficient data on its toxicity, especially for long-term exposures, as well as doubts about its operational efficiency. Authorities allowed only localized testing of Corexit 958OM2 at selected sites (Major and Wang, 2012). Spraying with Corexit 958OM2, followed by hot water washing, was successful in removing oil from rock surfaces, although efficient recovery of the oil-Corexit-mix released into the water was regarded as a challenge by governmental authorities. After Corexit 958OM2 was applied in response to the *Exxon Valdez* oil spill in 1989, numerous toxicity studies were conducted. However, no analytical methods for the detection of the surface-active agents (surfactants) in Corexit were developed, and few studies were performed to determine the fate of the surfactants in Corexit products (Place et al., 2010).

During the Gulf of Mexico Deepwater Horizon (DWH) oil spill, ~771,000 gallons (>2,900,000 L) of dispersants were injected into the flow of oil near the sea floor. Due to the limited information on the composition of Corexit 9500A and fate of the surfactants, Dr. Jennifer Field at Oregon State University (OSU), developed an analytical method that ultimately revealed the quantitative composition of the surfactant components of Corexit 9500A and 9527A (Place et al., 2014). The primary components of Corexit 9500A are the ionic surfactant bis-(2-ethylhexyl) sulfosuccinate (DOSS) (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). These methods were used to study the spatial distribution of Corexit 9500A surfactants in Gulf of Mexico (GOM) seawater and are being used to study the biodegradation of Corexit 9500A surfactants in aerobic and anaerobic laboratory microcosms. Methods for the extraction of DOSS from solid matrices and the quantification of DOSS in these extracts have been developed by Dr. Field and are currently being applied to sediments and marine snow collected in the GOM as part of the Gulf of Mexico Research Initiative (GOMRI). Other analytical methods for the analysis of the surfactant components of Corexit 9500A are limited to the analysis of DOSS in aqueous (Mathew et al., 2012; Ramierz et al., 2013; Kujawinski, 2011; Gray, 2014; Parker et al., 2014) and solid matrices (White et al., 2014) and dipropylene glycol butyl ether, which is the solvent carrier in Corexit 9500A (Parker et al., 2014).

In the DWH spill, Kujawinski et al. (2011) monitored DOSS and found that it was conservatively transported in the deep-sea oil/dispersant plume, and showed little to no sign of biodegradation. Dr. Field's research group detected DOSS in Gulf of Mexico seawater associated with the DWH spill ranging from 71–13,000 ng/L, also suggesting persistence, while the nonionic surfactants (Span 80, Tween 80, Tween 85) were detected infrequently in seawater (Place et al., 2016). Yet, Corexit 9500A components were more frequently detected in deep-sea sediments compared to the water column, further suggesting that they are not rapidly biodegraded in the water column as previously thought, but rather that they persisted alongside oil in sediments for ~4 years in the Gulf of Mexico (White et al., 2014). Corexit 9500A 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 the metabolic functions of these temperate deep-sea microbial communities may not be representative of Arctic communities. The mineralization of Corexit 9500A (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 9500A components and oil degrade in Arctic environments are still unknown (NRC, 2014).

McFarlin et al. (2014) demonstrated that Corexit 9500A undergoes some biodegradation in Arctic seawater, as evidenced by increased respiration rates upon addition of the dispersant to seawater in the absence of oil. Respiration rates increased rapidly in response to Corexit 9500A addition, with much less of a lag time than we observed for a comparable quantity of Alaska North Slope (ANS) crude oil, suggesting that at least some components of the dispersant are very readily biodegraded by the indigenous microbes, even at extremely low temperatures (-1°C) (McFarlin et al., 2014). In the current project, we partner with Dr. Field (Oregon State University) to track the biodegradation as well as abiotic/physical losses of the surfactant components of Corexit 9500A in mesocosm incubations containing chemically dispersed oil in Arctic seawater.

Using advanced metagenomic analyses of oil/seawater incubations, we identified organisms and genes important to oil and dispersant biodegradation to determine what microbial community changes might occur in response to an oil spill. High throughput 16S rRNA sequencing using next-generation DNA sequencing (Illumina MiSeq) technology was used to taxonomically identify bacteria that grow in response to dispersants (putative dispersant-degraders) with and without crude oil and to assess how shifts in the community caused by dispersants may affect microbially-mediated ecological functions.

This project provides an improved fundamental understanding of an offshore marine environment potentially affected by oil and gas exploration, specifically the fate and effects of oil spills and of oil spill response methods, on the Arctic marine environment. This study also provides valuable information to regulators, industry, stakeholders, the scientific community, and the public regarding the fate, biodegradation, persistence, and effects of chemical dispersants (i.e., Corexit 9500A) in the Arctic marine environment.

Background

Oil biodegradation in Arctic seawater and the effects of Corexit 9500A

Several laboratory studies have investigated sub-Arctic oil biodegradation in Alaska (Lindstrom and Braddock, 2002; Venosa and Holder, 2007) and Canada (Siron et al., 1995), as well as Arctic and sub-Arctic Norway (Deppe et al., 2005; Brakstad, 2006; Davies et al., 2001). These studies indicate the potential for appreciable oil biodegradation exists in coldwater environments. However, given the geographic differences in biogeochemistry and microbiology of seawater, it is essential to obtain assessments of biodegradation potential for a specific region to accurately predict the fate of an accidental oil release. Having accurate oil biodegradation rates is essential to generating accurate predictive models. Existing oil biodegradation models, (e.g., the Oil Spill Contingency and Response model; OSCAR), rely primarily on Q10 calculations calibrated with data above the temperature range of the Arctic, and thus may not function properly at the prevailing temperatures of Arctic seawater where microbes and oil may behave in a nonlinear fashion.

McFarlin et al. (2014) reported the chemical loss due to the biodegradation of ANS crude oil in mesocosms simulating the water column following the successful dispersion of a surface oil slick, both in the presence and absence of Corexit 9500A. 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 9500A), with oil losses ranging from 46–61% and up to 11% mineralization to CO_2 over 60 days.

These studies revealed that microorganisms indigenous to Arctic seawater are capable of performing biodegradation of chemically and physically dispersed oil at an environmentally relevant temperature (-1˚C). When compared to oil biodegradation in other environments, our experiments suggest that in the Arctic, ANS crude oil degrades much more slowly than oil in

temperate regions (Prince et al., 2013).

When we assessed the impacts of Corexit 9500A on oil biodegradation, we found that the dispersant enhanced the biodegradation of oil during the early stages of incubation at low oil concentrations intended to simulate a successfully dispersed plume of oil (Figure 1) (McFarlin et al., 2014), although, at later stages of the incubation (e.g., 63 days), the extent of oil loss was comparable regardless of the presence of Corexit. Our use of extremely low oil concentrations (2.5–15 ppm) stirring in flasks resulted in a comparison between physically dispersed oil with and without Corexit 9500A, which likely underestimates the effects of chemical dispersion on biodegradation rates of an oil slick. We did not compare higher concentrations of oil or a concentrated surface slick with dispersed oil, which may provide a more accurate representation of the comparison between biodegradation of oil with or without dispersant application in the field. We also did not compare how the presence of oil affects the biodegradation of the dispersant, nor did we quantify the biodegradation of dispersant components with or without oil.

Molecular genetic methods for identifying contaminant-degrading microbes in Arctic seawater

Identifying microbes and genes responsible for oil biodegradation is valuable in providing a fundamental understanding of the potential biodegradation processes of a particular environment. Molecular characterization enables monitoring of relevant microbial activities during spill events and may prove useful in modeling predictions about unstudied environments.

In our previous oil biodegradation studies, we applied high-throughput DNA sequencing of 16S rRNA genes to identify microorganisms that increased in relative abundance in response to oil in microcosm studies of seawater collected offshore and nearshore in the Burger Lease area in the Chukchi Sea. Of the 19 most abundant genera that responded to oil in our studies, four genera are known to include oil degraders: *Pseudoalteromonas* (Deppe et al.*,* 2005; Brakstad et al., 2008)*, Polaribacter* (Pradagaran et al., 2006; Gerdes et al., 2005)*, Oleispira* (Kube et al., 2013; Prabagaran et al., 2007; Brakstad et al., 2008)*,* and *Colwellia* (Brakstad et al., 2008; Baelum et al., 2012), and *Ulvibacter* (Prabagaran et al., 2007) is tentatively associated with oil biodegradation. *Oleispira* were the first to respond markedly, and this genus includes a known oil degrader that was actively involved in the degradation of MC252 oil in the Gulf of Mexico during the Deepwater Horizon blowout and includes known or suspected degraders of oil in Antarctic seawater and Arctic marine ice (Kube et al., 2013; Prabagaran et al., 2007; Brakstad et al., 2008). *Colwellia* were found to increase in relative abundance after the *Oleispira* in our incubations, which were also reportedly abundant in enrichment incubations containing water from the Gulf of Mexico and Macondo oil (100 ppm) at 5°C over 20 days and so is a suspected oil degrader (Baelum et al., 2012). *Colwellia* has also been found to be associated with the biodegradation of oil in Antarctic seawater and Arctic marine ice (Brakstad et al., 2008). The sequential blooms of *Oleispira* and *Colwellia*, combined with the sequence of petroleum hydrocarbon losses, suggest that *Oleispira* may biodegrade labile hydrocarbons (low molecular weight alkanes), while *Colwellia* attack higher molecular weight oil components.

Our previous studies were limited in scope but generated some of the first data regarding oil biodegradation in Chukchi Sea water. In the current study, we integrate these earlier findings with those from new incubations to develop a stronger picture of the Arctic marine microbial community structure and identity of organisms that respond to oil and/or Corexit.

Biodegradation of Corexit 9500A

Our previous studies provided evidence that Corexit 9500A undergoes biodegradation in Arctic seawater (Figure 1; McFarlin et al., 2014). Using respirometry, a basic test was run in which Corexit 9500A was added to Arctic seawater (50 mg/L) and incubated at -1°C. A rapid microbial response was observed, in which respiration rates increased in flasks amended with Corexit 9500A. The indigenous microorganisms in Arctic seawater initiated Corexit 9500A degradation more rapidly than that of fresh crude oil (Figure 1; McFarlin et al., 2014). The results indicated that Arctic seawater does possess the capability to degrade Corexit 9500A at least partially, and detectable microbial biodegradation of Corexit 9500A actively occurs in Arctic seawater at temperatures of -1°C within 1–2 days. We hypothesized that the nonionic surfactants are responsible for the microbial response; nonionics make up 27% (w/w) of Corexit 9500A compared to 18% (w/w) DOSS. Detailed chemical analyses are needed to test this hypothesis and to determine the fate of other Corexit 9500A components in the Arctic marine environment. The current study was designed to investigate this further.
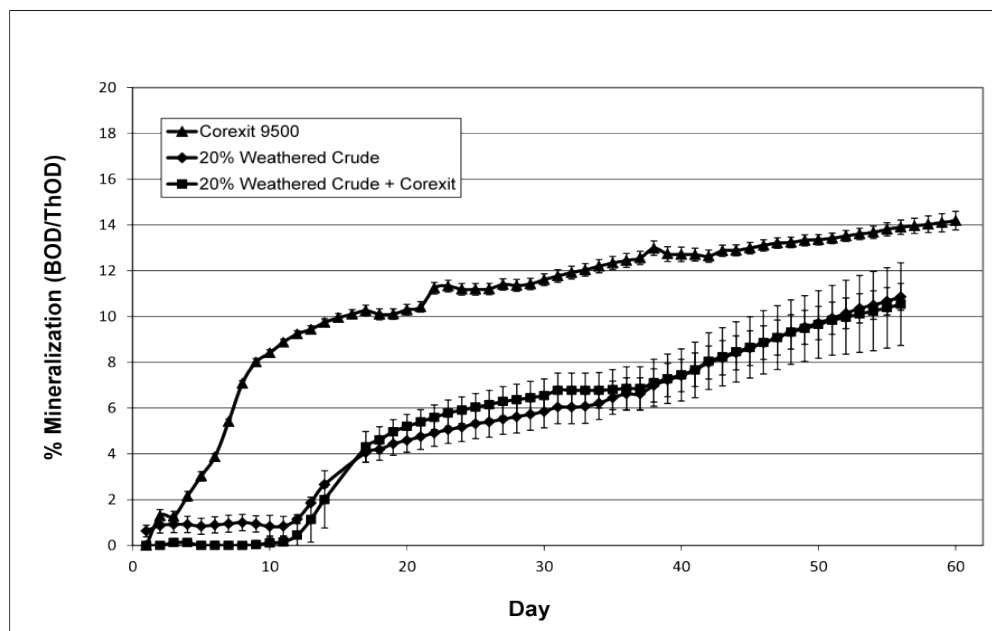
Figure 1. Percent mineralization at of Corexit 9500A and weathered ANS crude oil +/- dispersant in Arctic seawater at -1˚C (error bars ±1 SD from the mean). Sealed respirometer experiments contained Corexit 9500A (50 ppm) alone or 20% weathered ANS crude (15 ppm) with and without Corexit 9500A (1:20 dispersant to oil ratio). Reprinted from McFarlin et al., 2014.

Objectives/Hypotheses

In this project, our collaborative team performed a scientific study to assess the biodegradation of Corexit 9500A and crude oil in the Arctic marine environment as detailed below.

Specific aims

- Quantify the fate and persistence of the chemical dispersant, Corexit 9500A, including its individual chemical constituents, in Arctic seawater.

- Determine if dispersant biodegradation is slowed or accelerated by the presence of crude oil.

- Assess the effects of dispersants on crude oil biodegradation rates.

- Identify microorganisms important to biodegradation of dispersant chemical components in Arctic seawater.

- Evaluate shifts in microbial communities in response to dispersants in the context of potential changes in ecological function.

Hypotheses

- Dispersant chemical components biodegrade more slowly in the Arctic marine environment than in temperate or tropical regions.

- Corexit 9500A biodegradation will be subject to seasonal variability, due to differences in temperature, nutrient availability, and microbial communities.

- Nonionic surfactants are more biodegradable than DOSS in Arctic seawater, as has been observed in temperate regions.

- Corexit 9500A will elicit a shift in microbial community structure that favors labile hydrocarbon-degrading bacteria, which will initially outcompete those that degrade more recalcitrant components of oil and Corexit 9500A and bacteria that perform nutrient cycling reactions in the marine environment.

Methods

Incubation Tests

We conducted a series of laboratory incubation tests using Arctic seawater to assess Corexit 9500A and crude oil biodegradation and evaluate the microbial community response. We applied our previously established laboratory incubation protocols (adapted from McFarlin et al., 2014) for assessing the biodegradation of dispersants in freshly-collected Arctic seawater (including incubations with and without crude oil present), using replicate 1-L incubation vessels stirring in a temperature controlled cold room held at environmentally relevant temperatures as determined at the time of seawater sampling with a light/dark cycle that also replicated environmental conditions at the time of sampling. 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 quantity of nutrients (16 mg/L Bushnell Haas), and ANS crude oil or Corexit 9500A, or both. In order to simulate a surface oil slick, oil and Corexit 9500A was directly added to the surface of the seawater. We also incubated seawater and nutrients in the absence of oil or Corexit 9500A as a negative control to reveal incubation-associated microbial community shifts. Replicates (at least 3 per time point and type of analysis) were destructively harvested over a time course for analyses of petroleum, Corexit 9500A components, and microbial community structure. For one of the tests (summer 2016), we added a series of 6-L incubations in larger containers to enable repeated sampling for nonionic surfactants in addition to the series of destructively harvested 1-L containers. Details regarding each of the incubations are presented below.

Fall (September and October) 2013 offshore seawater + Corexit 9500A or Oil (Incubation A)

Prior to the start of this project, we conducted a preliminary biodegradation incubation test using offshore Chukchi Sea water microcosms amended with either Corexit 9500A or Alaska North Slope crude oil. Seawater was collected from approximately 90 km offshore from Wainwright, Alaska, in October 2013. Seawater was transferred to the University of Alaska Fairbanks within 24 hours of collection and placed in a cold room set to the temperature of the ocean at the time of collection (2°C). Upon receipt, seawater was aerated with a nutrient medium (16 ppm Bushnell Haas; Bushnell & Haas, 1941). These nutrients provided 75 µM phosphate, 49 µM nitrate and 76 µM ammonium per liter of seawater and were within a magnitude of background nutrient concentrations (Codispoti et al., 2005). The oil was not intentionally weathered prior to the start of the experiment. The test series consisted of triplicate mesocosms containing 800 mL of surface seawater with and without 15 mg/L of Corexit 9500A or 15 mg/L of oil. Mesocosms were incubated for 28 days. At day 0 and day 28, mesocosms were treated with isopropyl alcohol and frozen at -20°C for later analysis. Abiotic controls were also conducted in parallel to biotic mesocosms.

Summer (August) 2014 nearshore seawater + Corexit

We completed an additional biodegradation incubation test using nearshore Chukchi Sea water and Corexit 9500A prior to the start of this project. Seawater was collected from near shore in Utqiagvik (formerly Barrow), Alaska and was aerated with the same nutrient medium as the 2013 offshore incubation (i.e., 16 ppm Bushnell Haas). The test series consisted of triplicate mesocosms containing 800 mL of surface seawater with and without 15 mg/L of Corexit 9500A. Mesocosms were incubated for 10, 28 and 60 days at 1°C, which was the temperature of the ocean at the time of water collection. At each time point the mesocosms were treated with isopropyl alcohol and frozen at -20°C for later analysis. Abiotic controls were conducted in triplicate, alongside live mesocosms.

Crude oil biodegradation and nutrient limitation preliminary incubation test

In August of 2015, we conducted an additional preliminary incubation test to evaluate the role of nutrient limitation in oil biodegradation using Chukchi Sea water collected from open water near the shoreline of Utqiagvik. Sample storage and transport protocols previously described were followed. Microcosms (800 ml) were amended with crude oil (15 mg/kg) and different nutrient treatments (± 16 mg/kg Bushnell Haas) and incubated at 4°C. In addition, this experiment was also used to further refine the extraction procedure and GC/MS methods for crude oil in the presence of a seawater matrix.

Summer 2016 nearshore seawater ± Corexit 9500A (Incubation B)

A temporal incubation series of Arctic seawater amended with crude oil, dispersant, or both was conducted in August 2016. The incubation series was conducted at UAF and consisted of replicate 1-L media bottles containing 800 mL of seawater that were destructively harvested to analyze petroleum and dispersant loss, microbial communities, and nutrient shifts over time at 0, 5, 10, 20, and 30 days of incubation. An additional set of 6-L subsampled incubations were concurrently performed in order to study the rapid degradation of the nonionic surfactant components of Corexit 9500A within the first 10 days. Treatments included the addition of 50 ppm of Alaska North Slope crude oil, 5 ppm of Corexit 9500A (1:10 dispersant to oil ratio) or both, as well as autoclaved sterile controls to account for abiotic contaminant loss. Microbial controls were also run using seawater without the addition of any oil or dispersant substrate to determine how the microbial community shifts due to bottle effects. All incubations were performed in triplicate.

Winter seawater incubation

We originally proposed to conduct both summer (open water) and winter (under-ice) incubations, but we chose to increase the size and scope of summer incubation experiments at the expense of winter incubations. We increased the scope by performing a substantially larger set of summer incubations and by adding the detailed nonionic surfactant analyses to the summer 2016 incubation test.

Chemical Analyses of Petroleum

For incubations containing oil, whole replicate microcosms were harvested for petroleum hydrocarbon analyses. The entire flask contents were solvent-extracted, and total petroleum hydrocarbons (TPH), n-alkanes (C9-C40) and polyaromatic hydrocarbons (PAHs) were analyzed using gas chromatography and mass spectrometry (GC/MS) methods adapted from existing protocols (McFarlin et al., 2014; Prince et al., 2013).

Petroleum analyses for the first incubation (fall 2013, performed prior to the initiation of this project) were performed by a third-party laboratory. In that case, the entire contents of each flask were extracted and analyzed for 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 measurable hydrocarbons (Brakstad et al., 2008; Venosa & Holder, 2007; Stewart et al., 1993).

Chemical Analyses of Corexit 9500A

Oregon State University (OSU) performed advanced chemical analyses of Corexit 9500A components to quantify losses due to biodegradation over the course of incubation studies. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used to quantify the four surfactant classes (DOSS, Span 80, Tween 80 and Tween 85) and the degradation product of DOSS (ethylhexyl sulfosuccinate, EHSS) as described in Place et al., 2016. 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 OSU and stored at -20°C until analysis. Aliquots were diluted (0– 100 fold) with a combination of instant ocean 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. 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 ($[C] = [C_0]e^{-kt}$).

Quantification

Standards of DOSS (98.1%), Span 80 (70.5%), Tween 80 (74%), and Tween 85 (67%) were purchased from Sigma Aldrich Corporation. The stable-isotope labeled form of DOSS (^{13}C-DOSS; Cambridge Isotope Laboratories) and a custom-made ^{13}C-EHSS (Barsamian et al., 2014) were spiked as internal standards into all samples. DOSS was quantified using one quantitative (421-81 M/z) and one qualitative transition (421-227 M/z), while EHSS was quantified using a single quantitative transition (M/z 309). DOSS and EHSS were acquired in negative ion mode,

and concentrations were determined by internal standard calibration. Span 80 was determined from one quantitative transition (429-411 M/z) and one qualitative transition (446-429 M/z) while Tween 80 and Tween 85 were homolog series captured by precursor ion scanning for masses that fragment to give a common ion of m/z 309 (Place et al., 2016). Span and the Tweens were acquired in positive mode and were quantified by external calibration since no stable-isotope labeled internal standards are available for these surfactant classes. Calibration curves were made daily, and all samples were analyzed within 8 hours (Place et al., 2016). Calibration curves range from $0.2 - 25$ µg L^{-1} for DOSS and EHSS, and $60 - 300$ µg L^{-1} for Span 80, Tween 80 and Tween 85 with the lower limit set to equal the lower limit of quantification (LLOQ). The LODs were 0.08, 0.02, 20, 10, and 10 µg L^{-1} for DOSS, EHSS, Span 80, Tween 80, and Tween 85, respectively.

Accuracy and precision

For each analytical sequence containing microcosm samples for analysis, within-day accuracy was determined by over-spiking a solution of blank Instant Ocean and isopropanol (IO:IPA, 75:25) 75:25 matrix with all analytes of interest and analyzing this mixture four individual times. Accuracy was reported as a percentage of the observed to expected analyte concentration ± 95% confidence interval (CI). Within-day precision (% RSD) was reported as the standard deviation divided by the mean multiplied by 100. Because the number of microcosm samples was greater than what can be analyzed in a single analytical sequence, the between-day accuracy of the method was also determined. The between-day error was calculated as described by Gonzalez and Herrador (2007). Briefly, a 250 mL solution of Instant Ocean and isopropanol (IO:IPA, 75:25) was spiked with standards to yield a concentration of 0.5 µg L^{-1} DOSS and EHSS and 80µg L^{-1} of Span 80, Tween 80, and Tween 85. This solution was divided into 50 ml aliquots and stored at -20 °C until analyses. These aliquots were further divided and analyzed (5 mL, n = 4) on four different days to calculate an accuracy term that accounts for within-day and between-day variability. On each day of analysis, standards (all analytes) and internal standards (DOSS and EHSS only) were made fresh and used to calibrate the instrument.

Quality control (QC)

Several types of QC samples were employed within each analytical sequence to ensure data integrity. Matrix blanks consisting of Instant Ocean-isopropanol were analyzed to show that analytes below the limit of detection (LOD). Matrix spikes were performed to demonstrate the instrument capability to detect the analyte in the sample matrix within established performance metrics. For matrix spikes, DOSS and EHSS were spiked into Instant Ocean:isopropanol (75:25) to give a concentration of 0.5µg L^{-1} to test that the determined concentration was within 20% of the expected mass. Tween 80, Tween 85, and Span 80 were spiked into Instant Ocean:isopropanol (75:25) to give concentrations of 100 µg L^{-1} and the computed values had to be within 35% of the spiked mass to meet QC requirements. The higher acceptance rate for nonionic surfactants derives from the fact that no internal standards for these analytes are available and the standards for these analytes are less pure than those for DOSS and EHSS.

Microbial Community Analyses

We applied next-generation DNA sequencing (Illumina MiSeq) to determine the composition and structure of the bacterial community throughout our incubation tests. An increase in the relative abundance of organisms in response to oil or Corexit 9500A implicates them as putative degraders of those substrates.

We extracted total DNA from filters containing microorganisms from each mesocosm based on the extraction methods of Miller et al. (1999). We sequenced 16S rRNA genes to determine the taxonomic identity and relative abundance of Bacteria and Archaea. 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). 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.

Quantitative real-time PCR (qPCR)

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

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

To visualize relationships between bacterial community composition and environmental variables (season, exposure time with Corexit, oil, etc.), we ordinated community data using nonmetric multidimensional scaling (NMS) and Sorensen (Bray-Curtis) distance measures. Detrended correspondence analysis (DCA) was used to examine the heterogeneity of the functional community composition. Nonmetric multidimensional scaling (NMS), clustering

analysis, indicator species analysis (ISA), and multi-response permutation procedures (MRPP) were all conducted with PC-ORD V6 (McCune & Mefford, 2011). 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 % loss of oil and Corexit 9500A among their respective time points and reported the p-value.

RESULTS

2013–14 Incubation Tests

Biodegradation of crude oil

The indigenous microorganisms biodegraded $16 \pm 4\%$ (mean $\pm$SD) of ANS crude oil within the first 5 days in both offshore incubations (Table 1). The extent of oil biodegradation at 28 days was similar between September and October, with extents ranging from $36 \pm 6\%$ to $41 \pm 0.0\%$ (Table 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.

Table 1. Mean percent loss of total measurable hydrocarbons in Arctic surface seawater. All incubations contained whole ANS crude oil ($n = 3$). Letters correspond to significant differences among time points (MRPP, $p < 0.05$). Errors are the 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 ± 0.02^c	nm
Nearshore (Feb.)	McFarlin et al., 2014*	2.5	-1	0	nm	36 ± 3	45 ± 3.6	58 ± 10

* Percent losses includes abiotic losses

Biodegradation of Corexit 9500A

The concentration of DOSS significantly decreased between day 0 and 28 in the offshore (by $77 \pm 0.5\%$) and nearshore (by $33 \pm 7\%$) incubations, while the abiotic controls showed no significant difference in DOSS ($p > 0.05$; Table 2). The nearshore incubation included an earlier time point and indicated that the majority of DOSS was biodegraded within the first 10 days (23 $\pm 9\%$ loss; $p > 0.05$; Table 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 nonionic 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 (LOD), 5.5 µg/L, 15 µg/L, and 6.5 µg/L, respectively (Table 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 2). Out of all surfactants, Tweens were the only ones that exhibited any abiotic loss, with significant abiotic losses observed at day 10 (82% loss) and at day 28 (87% loss) in the offshore incubation (Table 2).

Table 2. Mean concentration, % loss, and % biodegraded (biodeg.) of the surfactant components of Corexit 9500A in offshore and nearshore seawater at 2°C ($n = 3$, ± standard deviation) in 2013–14 incubations. Percent loss is relative to time zero and based upon mean values. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas Broth), and Corexit 9500A (15 mg/L). Abiotic controls are designated with an 'A' after the time point. Superscript letters correspond to significant differences within each surfactant (MRPP, $p < 0.05$). The limit of detection (LOD) for Span 80, Tween 80, and Tween 85 was 5.5 µg/L, 15 µg/L, and 6.5 µg/L, respectively.

	DOSS			SPAN			Tweens		
Offshore	**µg/L**	**% Loss**	**% Biodeg.**	**µg/L**	**% Loss**	**% Biodeg.**	**µg/L**	**% Loss**	**% Biodeg.**
Day 0	2880 ± 697^a			244 ± 30^e			2010 ± 20^q		
Day 28A	2270 ± 400^a	21		132 ± 91^{efg}	46		1230 ± 570^q	39	
Day 28	71 ± 15^d	98	77	<LOD	>98	52–54	<LOD	>99	60–61
Nearshore									
Day 0	2260 ± 210^a			179 ± 23^{efg}			2240 ± 110^r		
Day 10A	2190 ± 120^a	3		150 ± 10^f	16		409 ± 33^s	82	
Day 10	1660 ± 200^b	27	24	44 ± 2^h	75	59	<LOD	>99	17–18
Day 28A	2210 ± 220^a	2		177 ± 8^g	1		298 ± 41^t	87	
Day 28	1460 ± 170^c	35	33	<LOD	>97	96–99	<LOD	>99	12–13

Prokaryotic Population Size

Throughout the offshore and nearshore incubation tests, we quantified prokaryotic (bacterial and archaeal) 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 9500A within 28 days (Figures 2 & 3). At 28 days, 69% more 16S rRNA genes were present in offshore seawater with Corexit 9500A than with oil (1.1×10^{10} vs. 3.5×10^9; Figures 2 & 3). In nearshore seawater with no oil or Corexit, prokaryotes increased in abundance between day 0 (3.3×10^9 gene copies) and day 10 (9.2×10^9 gene copies), but only 2% of the initial abundance remained (7.0×10^7 gene copies; Figures 2 & 3) at day 28. In both offshore and nearshore incubations, 16S rRNA gene abundance decreased in the seawater-only treatments between day 10 and 28 (Figure 3).
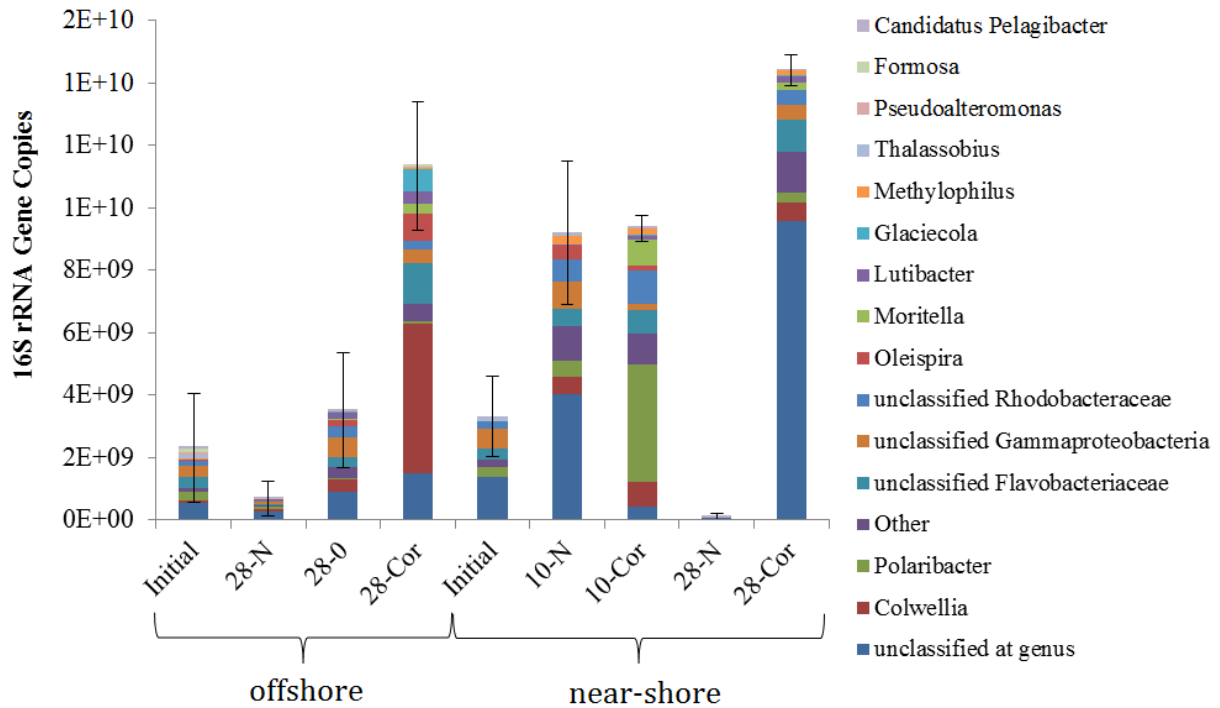
Figure 2. Mean relative abundance of bacterial taxa as a portion of mean prokaryotic abundance in experiments with (a) offshore and (b) nearshore seawater at 2°C ($n = 3$) (error bars ±1 SD from the mean). Total 16S rRNA copies were determined by qPCR (per 800 mL incubation), and community structure was identified using 16S rRNA amplicon sequencing. Bottles contained surface seawater (800 mL) with no amendment (N, biotic control), ANS crude oil (O; 15 mg/L) or Corexit (Cor; 15 mg/L).
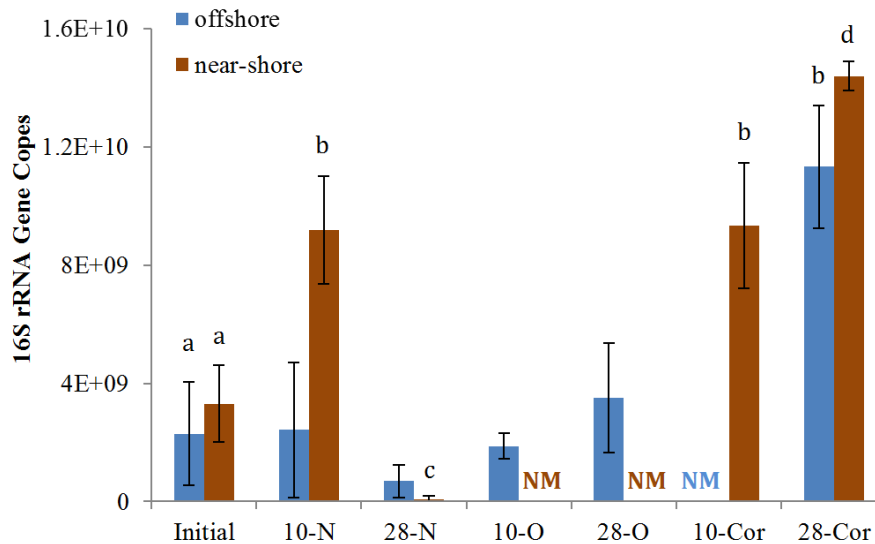

Figure 3. Mean abundance of prokaryotes in offshore and nearshore experiments at 2°C (error bars ±1 SD from the mean). Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500A (Cor; 15 mg/L). Letters correspond to significant differences among treatments containing three replicates (MRPP, $p < 0.05$). NM: not measured.

Microbial Community Analysis

The response of indigenous microbial communities to ANS crude oil and Corexit 9500A 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 of all samples combined 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 9500A incubations at day 28 (Figures 4 & 5). 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 4 & 5). Consistent grouping was also observed in the ordination with nearshore seawater, where bacterial sequences from replicate incubations grouped together and control incubations (seawater in the absence of Corexit) grouped separately from Corexit 9500A treatments (Figure 6).

MRPP was conducted to determine if microbial community shifts over the course of Corexit 9500A incubations were statistically significant. Each treatment contained a significantly different microbial community at each time point (Figure 6; MRPP, p-value < 0.05). In nearshore seawater, the biotic control at day 10 contained a similar abundance of prokaryotes as the Corexit 9500A treatment at day 10, but the structure of the microbial community was drastically different (Figure 6; MRPP, p-value < 0.05). The microbial communities in all incubations with Corexit 9500A were significantly different from communities without Corexit 9500A (i.e., the biotic controls; MRPP, p-value < 0.05).
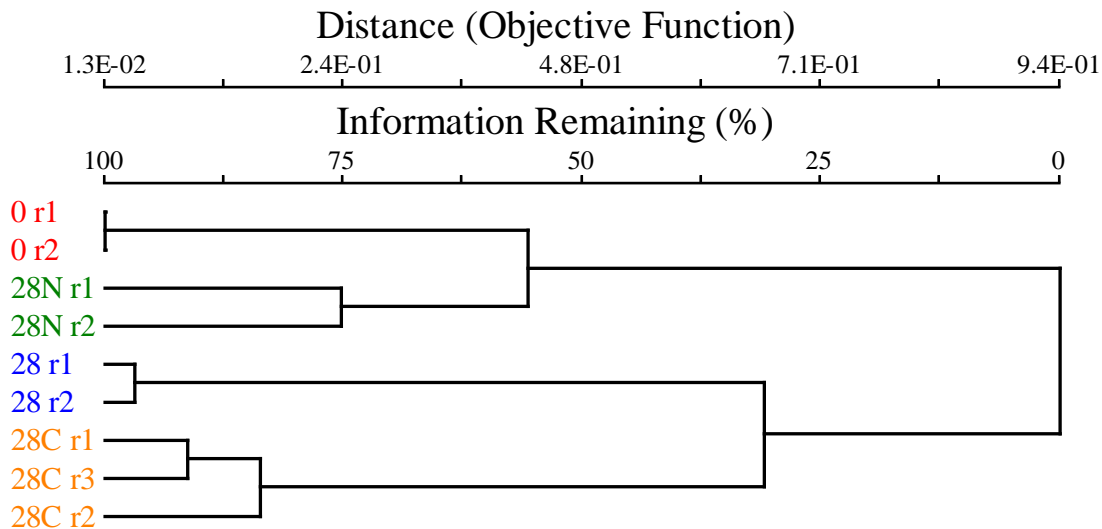

Figure 4. Dendrogram of bacterial sequences (16S rRNA genes) in offshore incubation experiment at day 0 and 28. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no-amendment (N; biotic control), oil (15 mg/L), or Corexit 9500A (C; 15 mg/L), and were incubated at 2°C. The codes r1, r2, and r3 refer to replicates of the same treatment type.
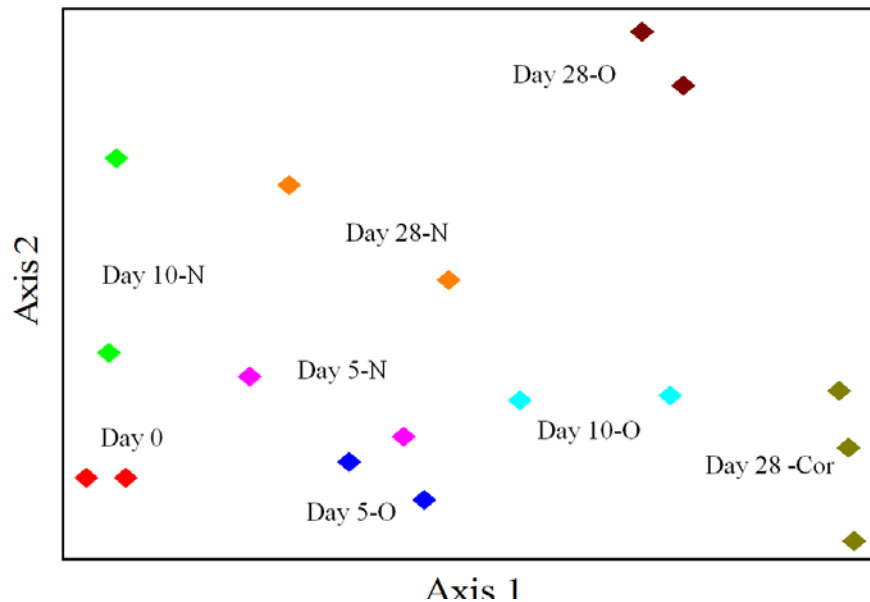
Figure 5. NMS ordination of bacterial 16S rRNA sequences in incubations containing offshore seawater. Bottles contained seawater (800 mL), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500 (Cor; 15 mg/L), and were incubated at 2°C for 0 (red), 5 (N = pink; O = blue), 10 (N = green; O = turquoise), and 28 days (N = orange; O = brown; Cor = dark green).
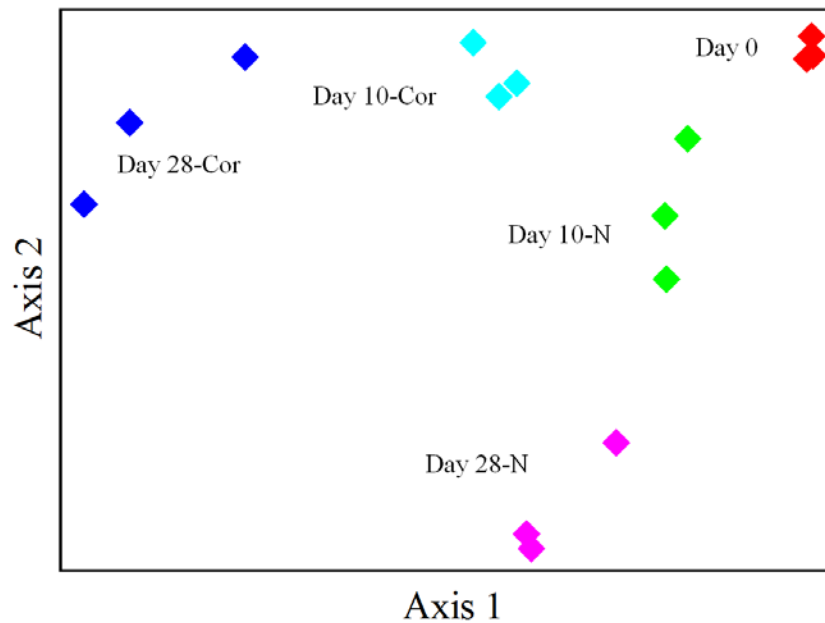

Figure 6. NMS ordination of bacterial 16S rRNA sequences in incubation experiment containing near-shore seawater (*n* = 3). Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), or Corexit 9500 (Cor; 15 mg/L), and were incubated for 0 (red), 10 (Cor= turquoise; N = green), and 28 days at 2°C (Cor = blue; N = pink).

Taxa that responded to oil or Corexit 9500A

In the offshore incubation, *Oleispira* (Figures 7 & 8a), *Colwellia* (Figures 7 & 9a), *Lutibacter* (Figure 10a), and an unclassified member of Flavobacteriaceae (OTU83; Figure 10a) 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 9500A at day 28 compared to the biotic controls ($n = 2$; Figure 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 9500A at day 28 (by 22%) compared to the biotic control (Figure 9a). Overall, the relative abundance of *Colwellia* increased in response to oil within 10 days and increased in response to Corexit 9500A between 10 and 28 days in the offshore incubation; however, more *Colwellia* grew in response to Corexit 9500A than oil (relative abundance; Figure 9a).

In the offshore incubation, three *Colwellia* OTUs (OTUs 12, 19, and 21) and an individual unclassified Flavobacteriaceae (OTU83) were identified as indicator species for the presence of Corexit 9500A at day 28, with indicator values and *p*-values of IV = 88, $p = 0.013$; IV = 67, $p = 0.012$; IV = 56, $p = 0.013$; IV = 88; $p = 0.013$, respectively. In response to oil-only, *Sulfitobacter* (Figure 11), an unclassified Flavobacteriaceae (OTU50; Figures 10a), and an unclassified Rhodobacteraceae (OTU4; Figures 11) increased in relative abundance compared to the biotic controls within 28 days. ISA identified an individual *Sulfitobacter* (OTU72) as an indicator species for 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 9500A compared to the biotic controls. *Polaribacter*, a member of Flavobacteriaceae, increased in relative abundance in response to Corexit 9500A at day 10 (by 34%; Figures 2 & 10b), while Oceanospirillaceae (Figure 8b) and Colwelliaceae (Figure 9b) increased in response to Corexit 9500A at day 28.

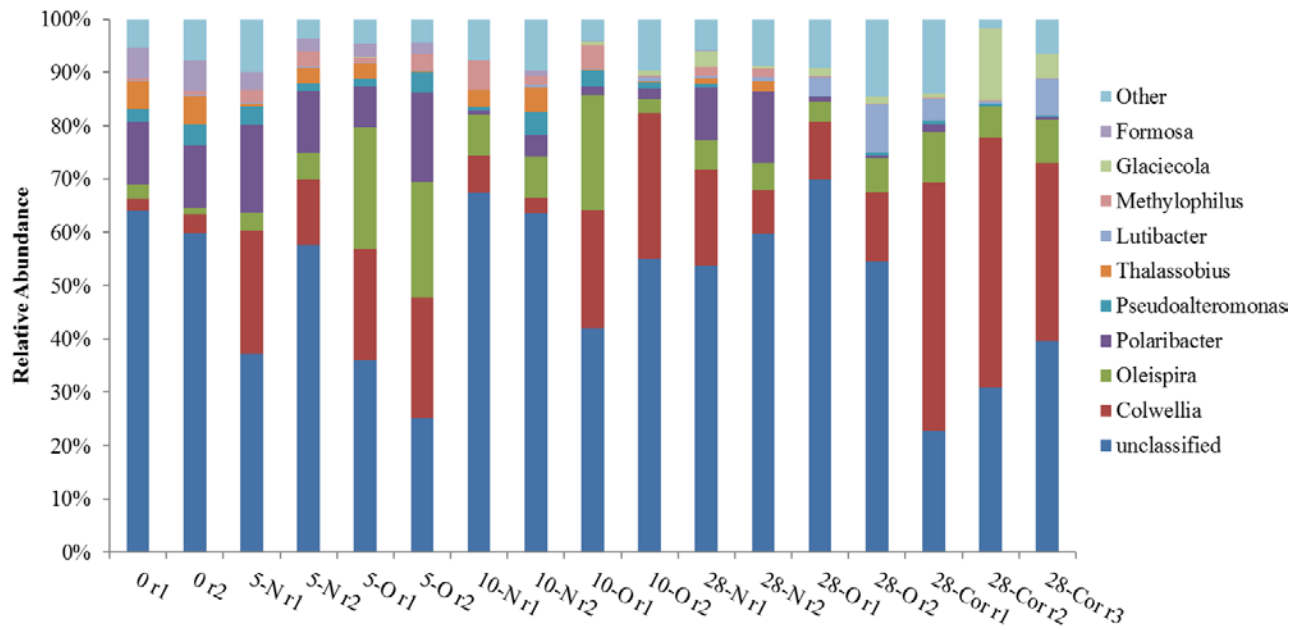Figure 7. Relative abundance of bacterial genera in the offshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500A (Cor; 15 mg/L), and were incubated for 0, 5, 10, and 28 days at 2°C. The Corexit 9500A treatment (28C, $n = 3$) was only incubated for 28 days in the offshore experiment.

Figure 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 9500A (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 9500A treatments.
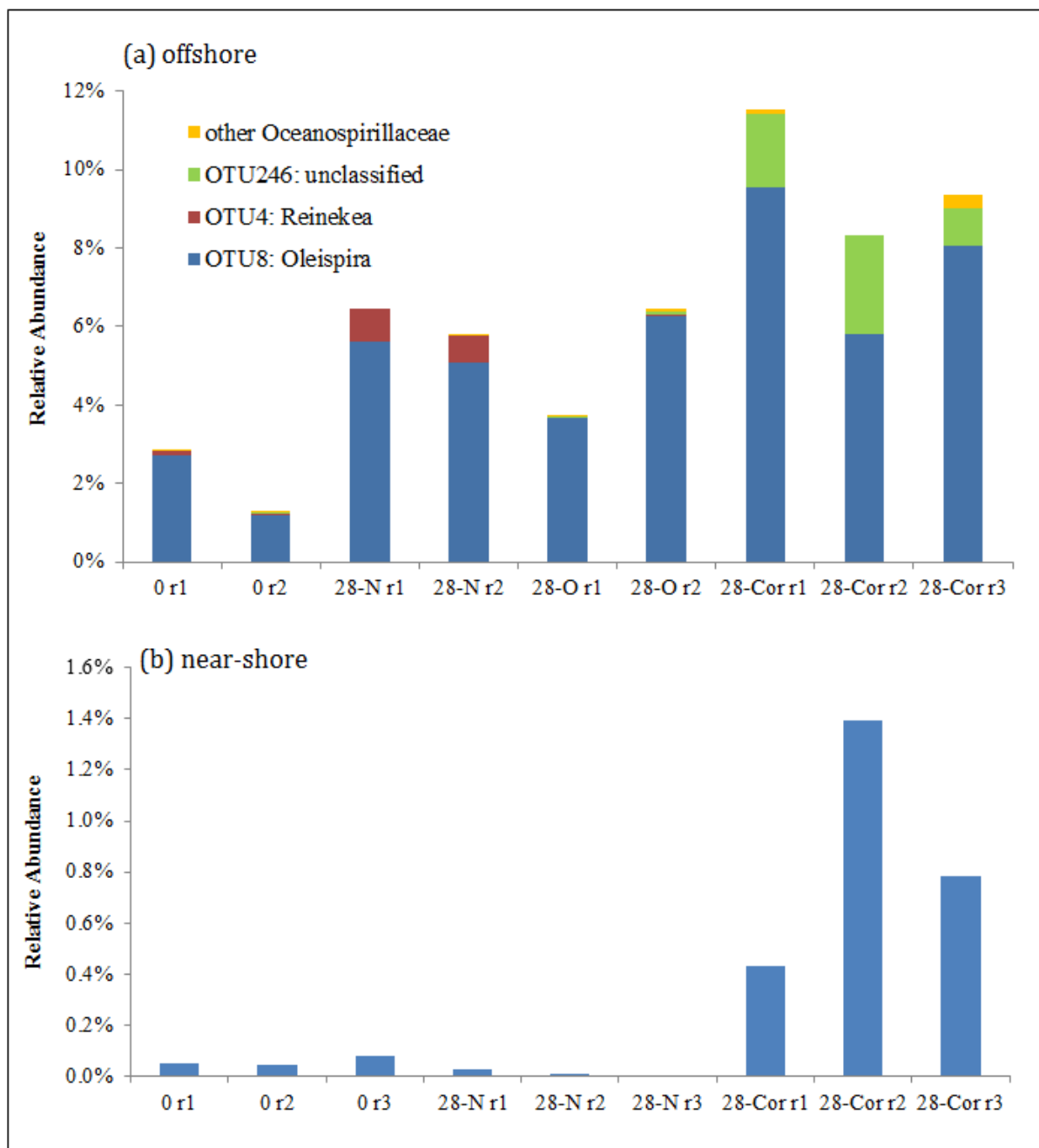
Figure 9. Relative abundance of bacterial sequences classified in the Colwelliaceae family in the (a) offshore experiment and (b) nearshore experiment. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas) and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500A (Cor; 15 mg/L) 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 9500A treatments.
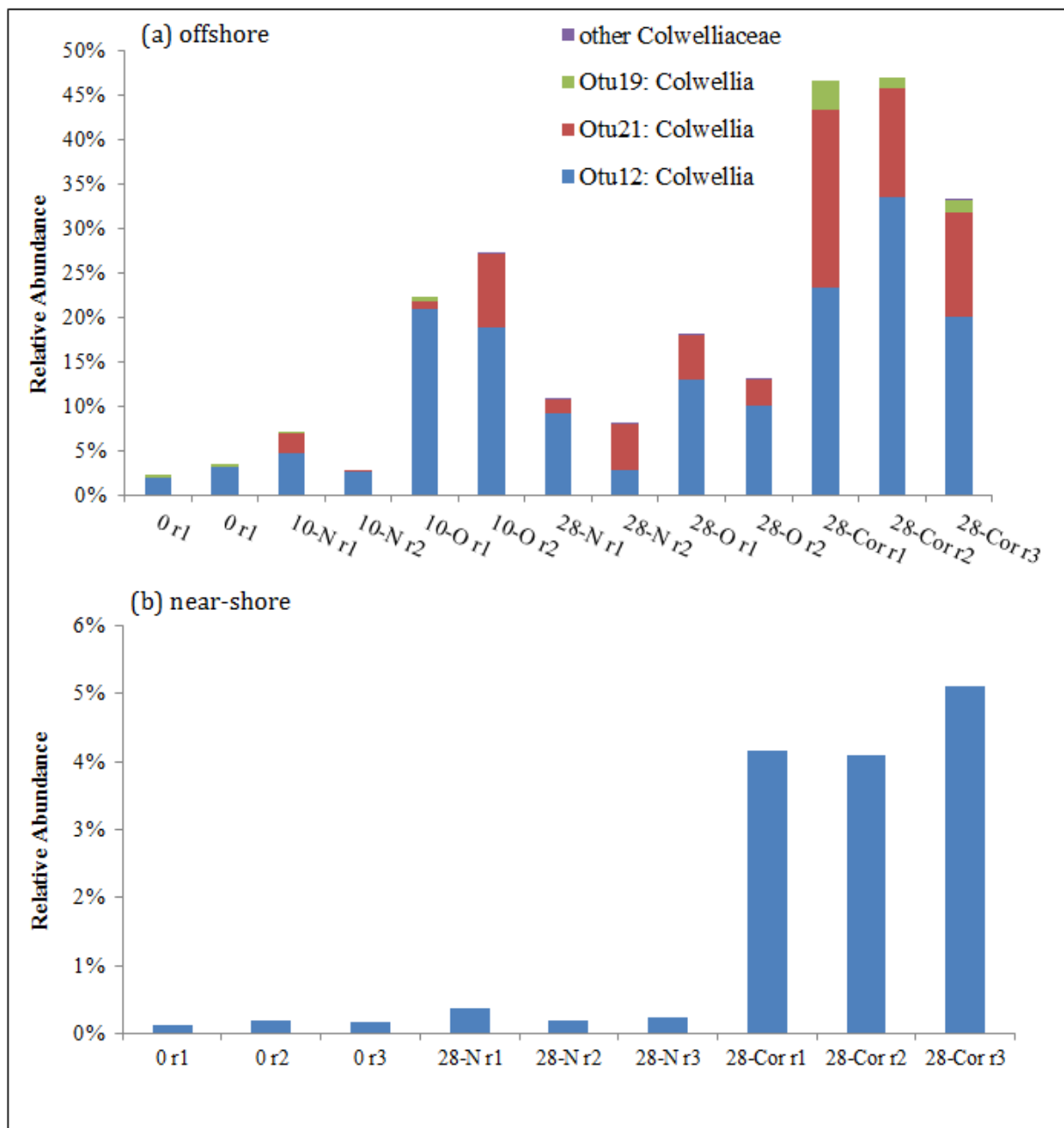
Figure 10. Relative abundance of bacterial sequences classified in the Flavobacteriaceae family in the (a) offshore experiment and (b) nearshore experiment at day 0, 10, and 28. Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; biotic control), oil (O; 15 mg/L), or Corexit 9500A (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 9500A treatments.
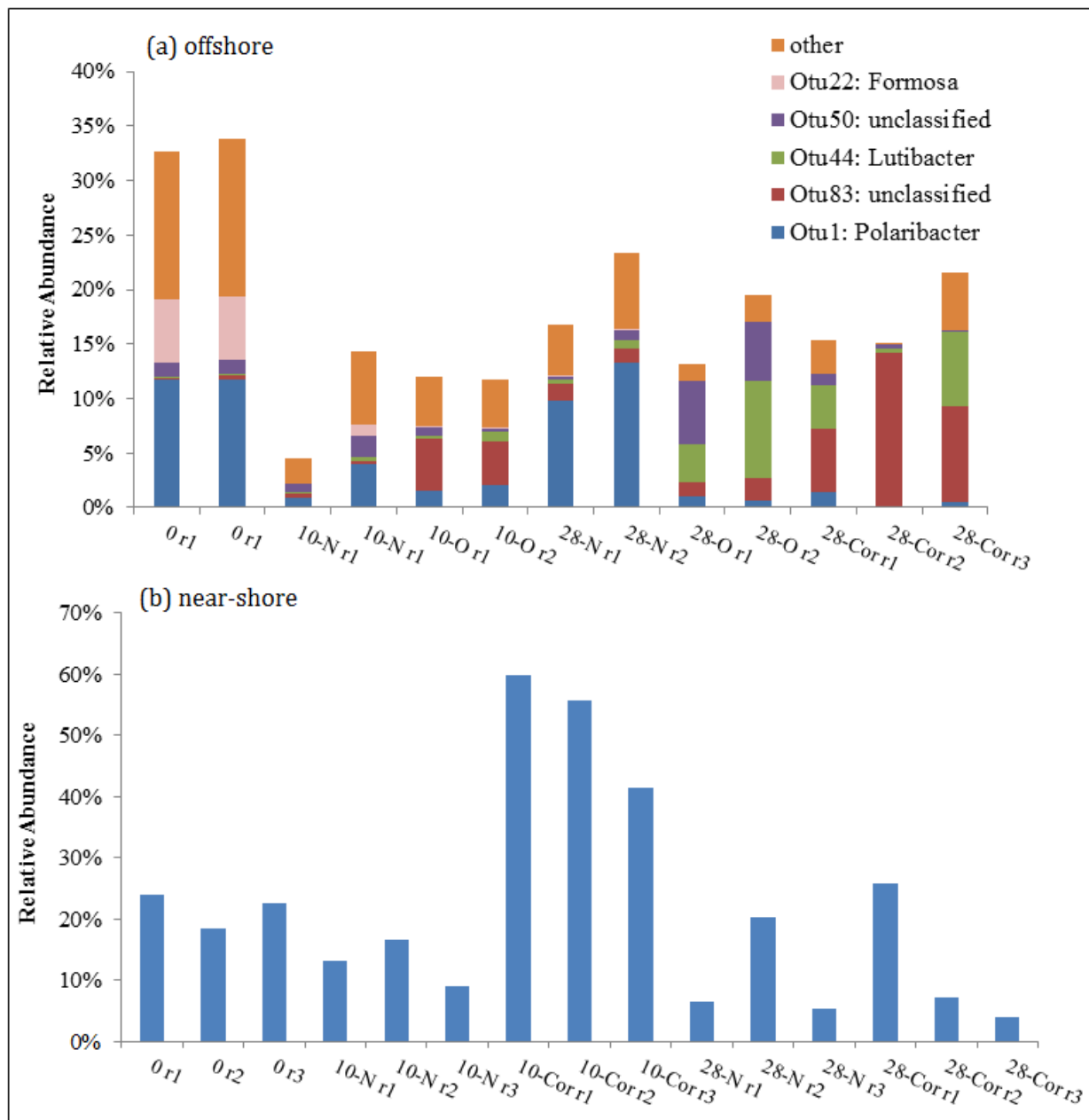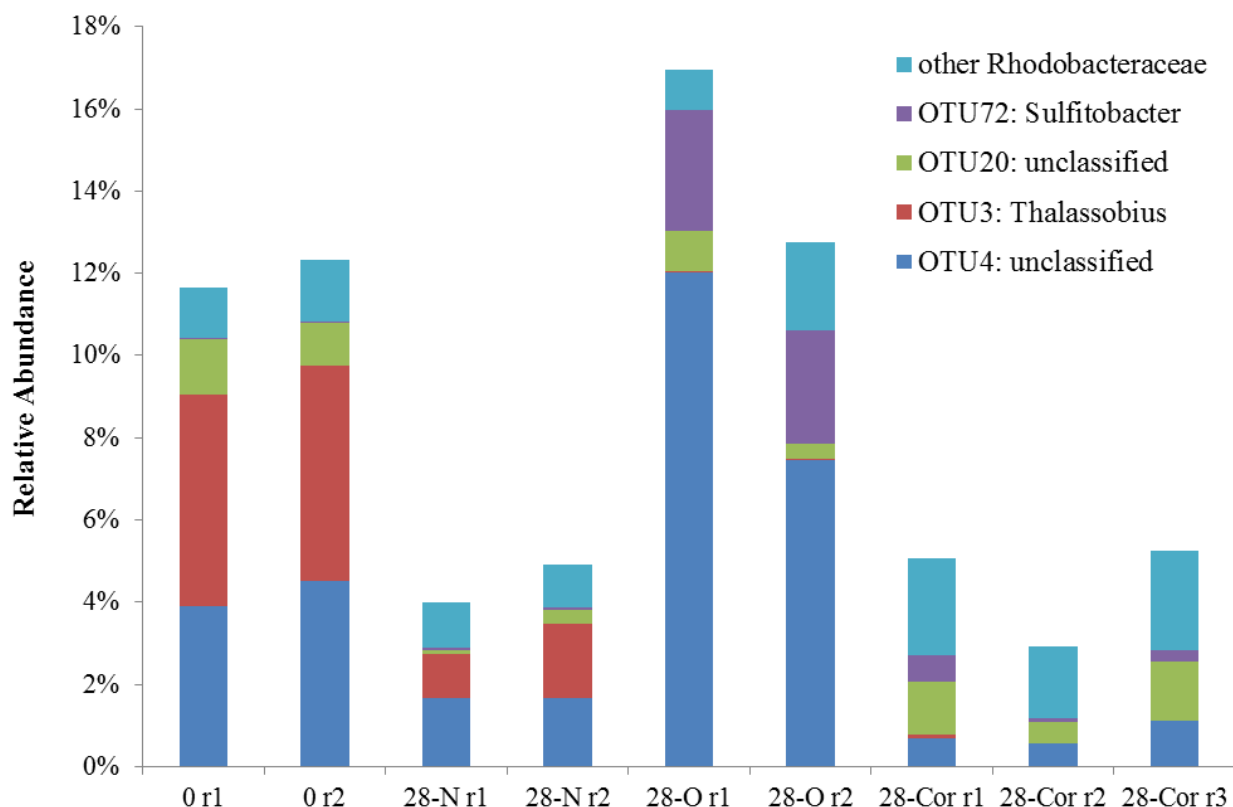
Figure 11. Relative abundance of sequences classified in the Rhodobacteraceae family at day 0 and 28. Incubations contained offshore seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500A (Cor; 15 mg/L) at 2°C. Individual OTUs (sequences) are identified to provide a specific comparison between oiled and Corexit 9500A treatments.

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 9500A in the NMS ordination (Figure 12). In regards to total petroleum degradation genes, the NMS ordination did not indicate a strong separation between oil and Corexit 9500A incubations (Figure 12); however, the relative abundance of several individual genes did shift over the course of incubation with oil or Corexit 9500A (Figure 13). 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 biotic control (Figure 13).
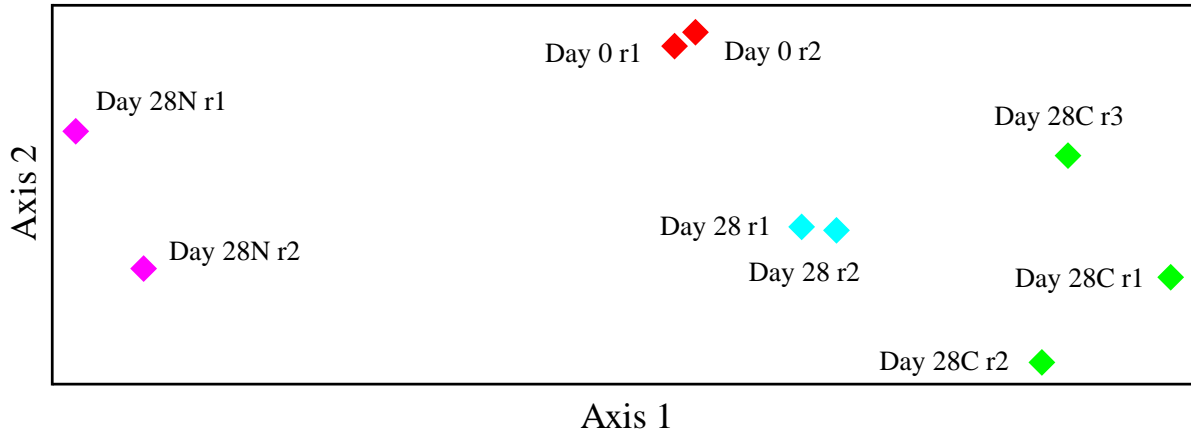
Figure 12. NMS ordination of petroleum degradation genes in incubations containing offshore seawater. Bottles were incubated at 2°C for 28 days and contained offshore seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N; pink), oil (15 mg/L; turquoise), or Corexit 9500 (C; 15 mg/L; green). Day 0 is the initial seawater prior to any addition (red). The codes r1 and r2 refer to replicates of the same treatment.

Figure 13. Relative abundance of *alkB*, *nagG*, and *pchCF* genes in an offshore experiment at day 0 and 28 (error bars ±1 SD from the mean). Bottles contained seawater (800 mL), nutrients (16 mg/L Bushnell Haas), and either no amendment (N), oil (O; 15 mg/L), or Corexit 9500A (Cor; 15 mg/L) and were incubated at 2°C. Error bars are standard deviations.

Summer 2016 Incubations

Petroleum biodegradation

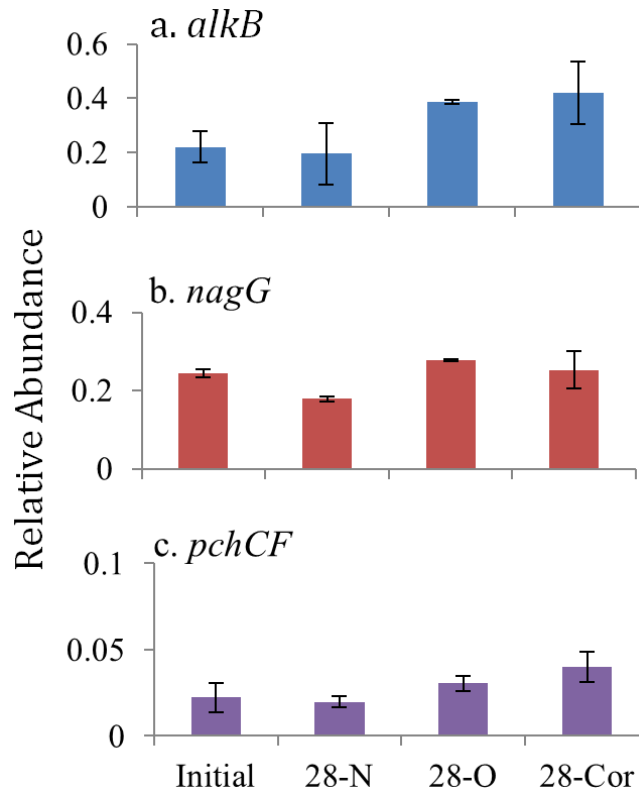TPH losses in incubations containing oil with and without Corexit 9500A were measured over the course of the 30-day incubation and were compared to that in abiotic (sterile) replicate mesocosms to distinguish between abiotic losses and biodegradation (Figure 14). Abiotic loss of TPH due to evaporation or volatilization appear to occur most appreciably within the first 5-10 days, after which time any additional loss is attributable to biodegradation (Figure 14). The extent of biodegradation was similar, yet slightly lower than that we observed in the offshore experiments conducted in summer 2013–14 (Table 1), which may be a function of the differences in microbial communities and conditions in nearshore versus offshore waters.

Biotic total petroleum hydrocarbon (TPH) loss was significantly higher with the addition of Corexit 9500A at t=5 days (p=0.0290) but not at subsequent time points (Figure 14). This indicates that the addition of Corexit 9500A may enhance biodegradation of TPH early on in a spill, though the low oil concentrations used in this study do not capture the comparison of a dispersed vs. non-dispersed oil slick accurately.
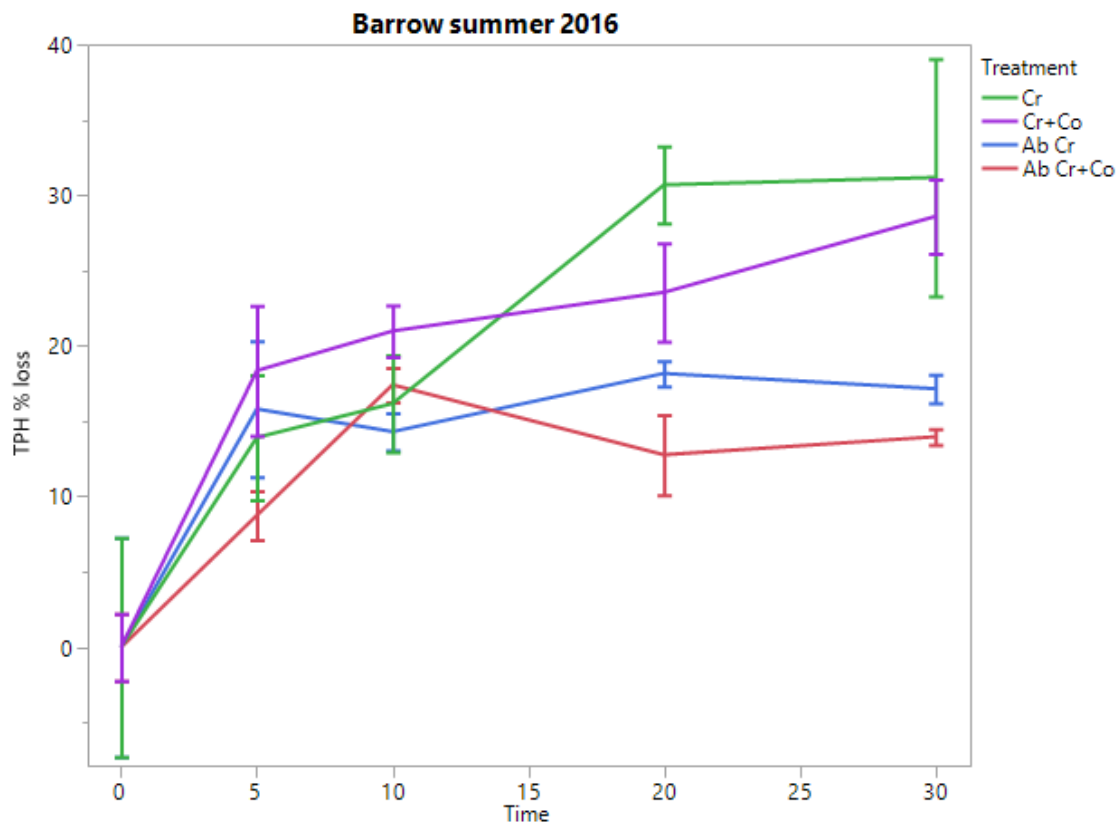

Figure 14. Comparison of total petroleum hydrocarbon loss due to biodegradation in treatments with crude oil only (Cr) and crude oil with Corexit 9500A (Cr+Co) (error bars ±1 SD from the mean). TPH loss is significantly higher with the addition of Corexit 9500A at t=5 days (p=0.0290) but not at subsequent time-points.

Biodegradation of Corexit 9500A components

Two types of incubation series were performed to study the degradation of the dispersant Corexit 9500A over time: sacrificed whole bottle microcosms (800 ml) and subsampled mesocosms (6 L). The sacrificed whole bottle microcosms were executed with success in prior Corexit 9500A experiments, but we elected to add parallel mesocosms that were subsampled to enable more frequent quantitation of the nonionic surfactant components of Corexit 9500A during the first 7 days, since in previous studies they had dropped to near or below detection limits within this timeframe (Table 2). The whole-bottle sacrifices serve as a comparison to prior studies and also were used to assess the reliability of subsampled incubations. Dispersants were analyzed at OSU by LC-MS/MS in whole bottle and subsampled incubations for the following compounds: dioctyl sodium sulfosuccinate (DOSS), Tweens 80 and 85, Span 80, and ethylhexyl sulfosuccinate (EHSS). DOSS, Tweens 80 and 85, and Span 80 are the constituents of Corexit 9500A; the Tweens were unable to be quantitated separately and were quantitated as the sum of their concentrations. EHSS is a suspected degradation metabolite of DOSS.

In both types of incubations, DOSS concentrations did not show significant differences between treatments over time (Figures 16 & 17) and show a great amount of variation between replicates, which is likely a limitation of the analytical methods to quantitate DOSS compared to the other Corexit 9500A components. However, EHSS concentrations increased over time in incubations containing both crude oil and Corexit 9500A and were significantly higher in those treatments compared to time zero by day 5 in subsampled incubations (Figure 16) and remained high throughout the remainder of the experiment in both incubation types. This increase in EHSS concentrations suggests that DOSS degradation may, in fact, be occurring since EHSS is a DOSS degradation product. Interestingly, EHSS concentrations increased for treatments containing both crude oil and Corexit but did not increase for treatments containing Corexit 9500A only.
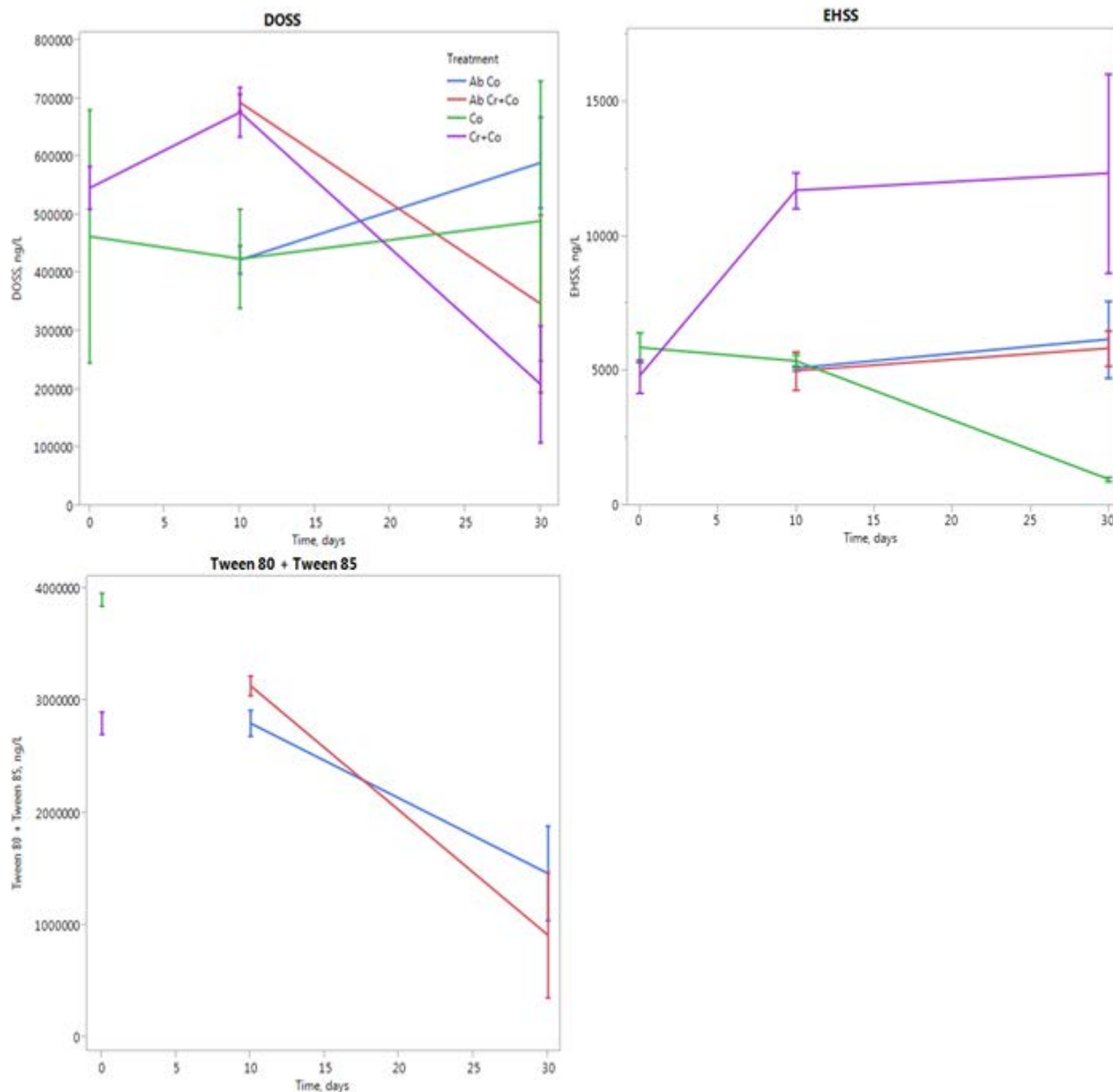
Figure 15. Concentrations of dispersant components DOSS, EHSS, and Tween 80 + Tween 85 in destructively harvested whole bottle (800-ml) incubations (error bars ±1 SD from the mean). Incubations of Arctic seawater contained Corexit 9500A (Co) alone, Corexit 9500A together with crude oil (Cr+Co), or contained no added substrate as microbial controls (Mc). Abiotic controls (Ab) were also run to reveal differences between abiotic and biotic losses.

The nonionic surfactants (Tween 80, Tween 85, Span 80) showed rapid loss across both incubation types (Figure 15). Tween 80 and 85 concentrations were below limits of quantitation for OSU (LOQ) in both biotic treatments by day 5 (60,000 ng/L) and Span 80 was below LOQ (60,000 ng/L) by day 2, suggesting that rapid degradation of both components can occur in Arctic environments. In whole bottle incubations, a decrease in concentration in abiotic treatments by 30 days was observed, suggesting that there is both abiotic loss and transformation

of these analytes occurring or that the abiotic treatments became contaminated with microbes over the course of the incubation. Analyses of the nonionic surfactants for these time points and also for additional time points early in the incubation are in progress by collaborators at Duke University (Dr. Lee Ferguson and Ph.D. student Sarah Choyke), who can achieve lower limits of detection than OSU for these analytes.

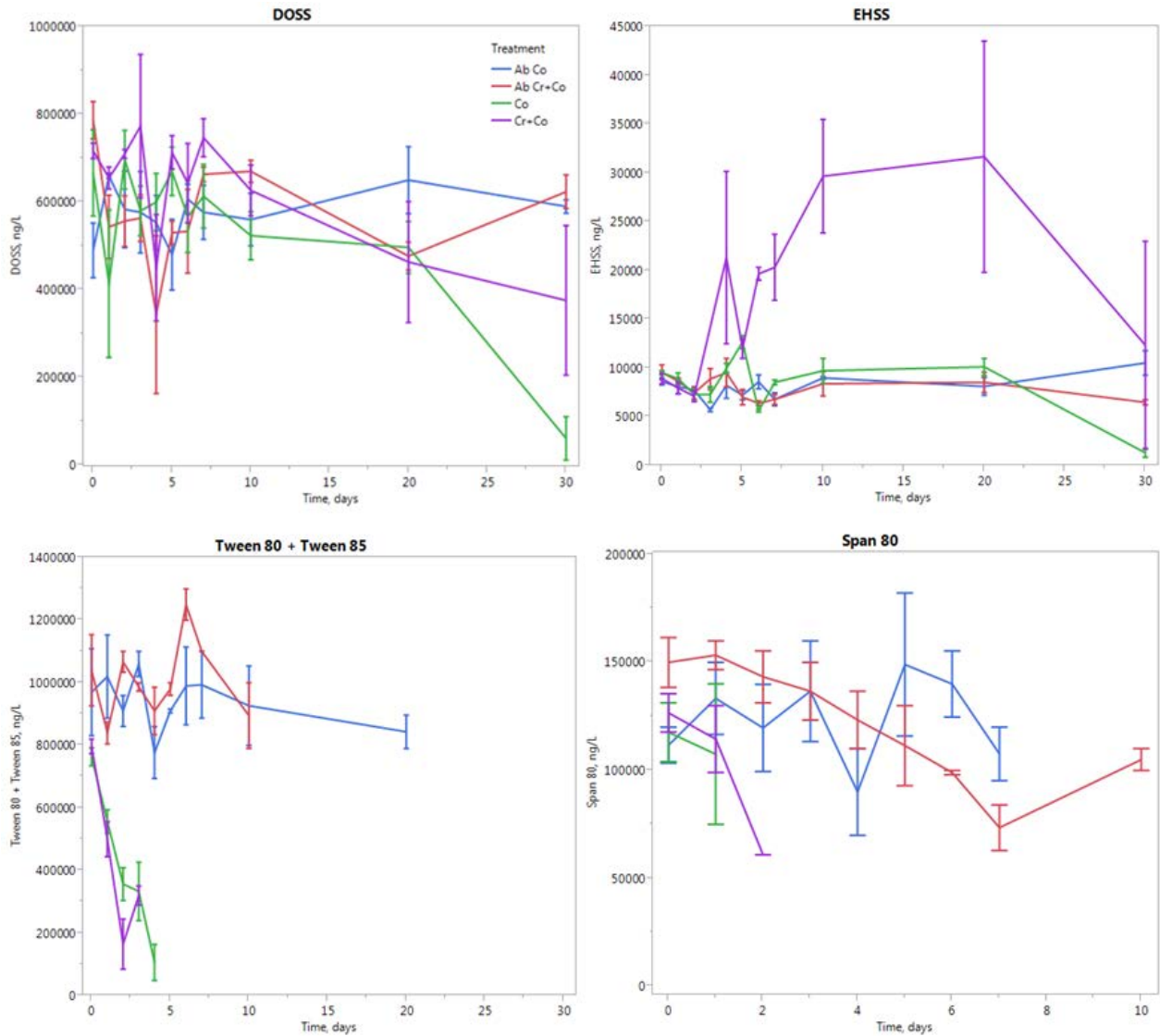Figure 16. Dispersant concentrations from subsampled 6-L incubations (error bars ±1 SD from the mean). LOQ is 200 ng/L for DOSS and EHSS and 60,000 ng/L for Span 80 and Tweens. Legend code key: Cr = Crude oil only, Co = Corexit 9500A only, Cr+Co = Crude oil and Corexit 9500A, Mc = Microbial control (no substrate), Ab Cr = Abiotic crude oil only, Ab Co = Abiotic Corexit 9500A only, Ab Cr+Co = Abiotic crude oil and Corexit 9500A.

During TPH analysis, an unexpected set of peaks was detected by GC/MS in treatments containing Corexit 9500A that were not present in oil-only treatments. This suggests that these peaks are associated with a component of Corexit. The compound represented by these peaks was identified based on its mass spectra as 1-(2-Butoxy-1-methylethoxy)propan-2-ol (Figures 17 & 18), also known as Dowanol dipropylene glycol n-butyl ether (DPnB). DPnB is an industrial chemical used as a solvent, chemical reaction intermediate, insecticide, and surfactant. This compound remains present in substantial concentrations at the end of the incubation series relative to the components of the crude oil (Figure 19). Loss of this compound appears to be solely due to abiotic means such as evaporation and volatilization, with a majority of the loss occurring within the first 5 days (Figure 19). Due to its chemical structure, this compound may be recalcitrant to biodegradation in the presence of more labile compounds in crude oil such as simple alkanes. The differences observed between the biotic and abiotic treatments can be attributed to error associated with the analytical method, which is intended to measure the loss of compounds present only within crude oil, as well as possible evaporation and recondensation over time within the mesocosms at cold temperatures. However, it is still noteworthy that this compound is still detectable in appreciable amounts relative to crude oil by the end of the 30-day incubation, which is an important environmental consideration.
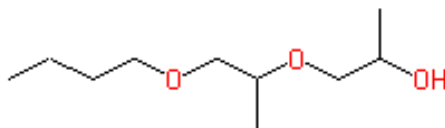

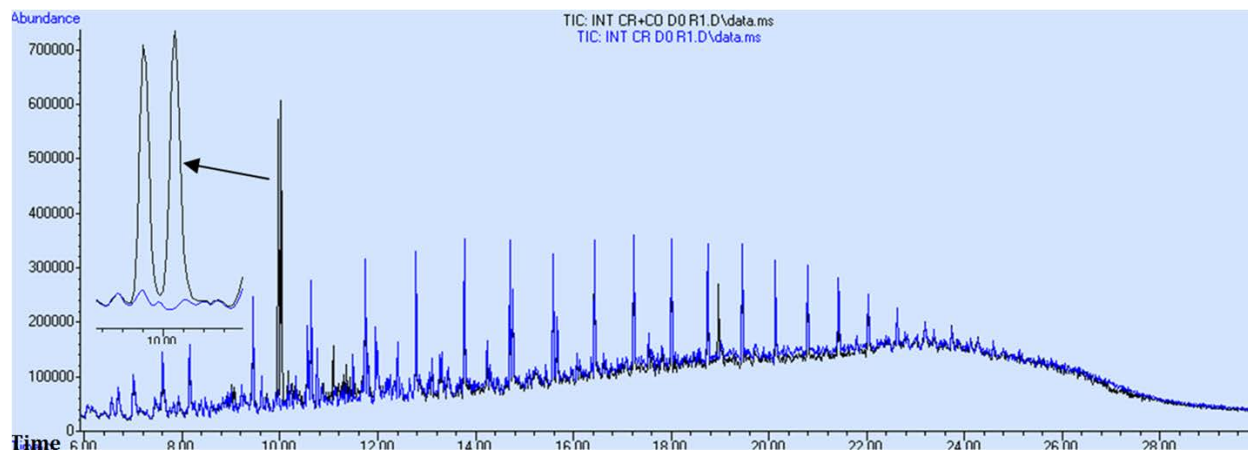
Figure 17. DPnB chemical structure.

Figure 18. Initial treatments (day 0) of ANS crude oil with (black) and without (blue) the addition of Corexit 9500A.
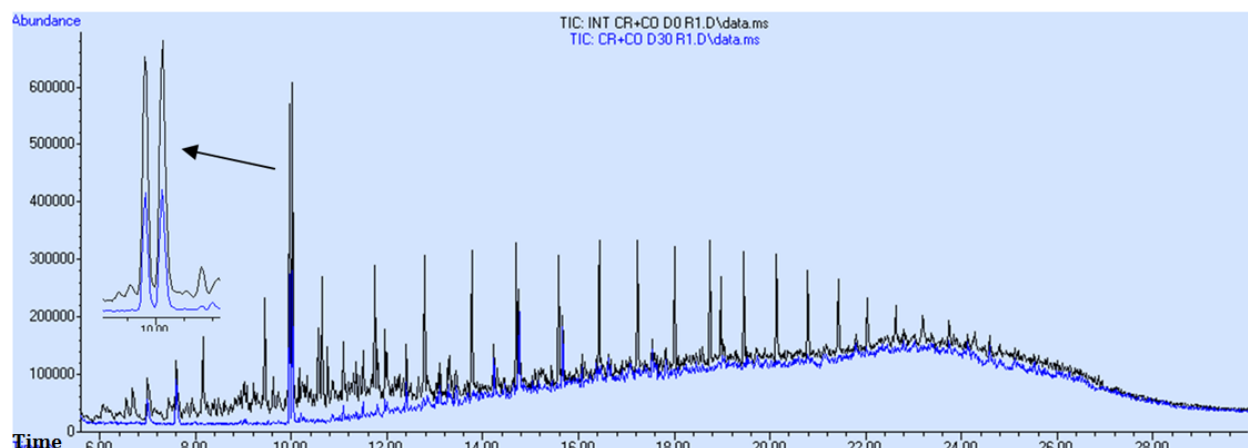
Figure 19. Comparison of ANS + Corexit 9500A treatments at 0 (black) and 30 days (blue).

Nutrient Dynamics

Nutrient concentrations for phosphate, silicate, nitrate, nitrite, and ammonia were measured in destructively harvested whole bottle incubations performed in summer 2016 in order to gain greater insight into nutrient dynamics and potential limitations (Figure 20). Overall patterns across all treatments include a decrease of ammonia and silicate, an increase of nitrite, and concentrations of phosphate and nitrate over time. Ammonia decreased the most and was likely the most limiting macronutrient in this system. Of note was a relatively large change in nutrient consumption for the crude oil only (Cr) treatment at day 5; however, overall nutrient loss for this treatment is not substantial and quickly normalizes over time. Additionally, there was also a significant decrease in ammonia concentrations for treatments containing oil at days 5 (p=0.0148) and 10 (p<0.0001), which suggests there is a greater nitrogen requirement for oil biodegradation than for Corexit 9500A alone. While nutrients were rapidly consumed early on, the system did not appear to be nutrient limited, suggesting that the conservative rates of crude oil biodegradation observed may either be a function of genetic potential or temperature.

The pH and dissolved oxygen (DO) concentrations were also measured (Figure 21) in destructively harvested whole bottle incubations. Both pH and DO dramatically decrease at 5 days, which coincides with TPH and nonionic surfactant loss, which may indicate microbial activity that draws down oxygen and generates acidic products by 5 days. The pH then increases over time to levels above initial values and DO changes vary with treatment and time.
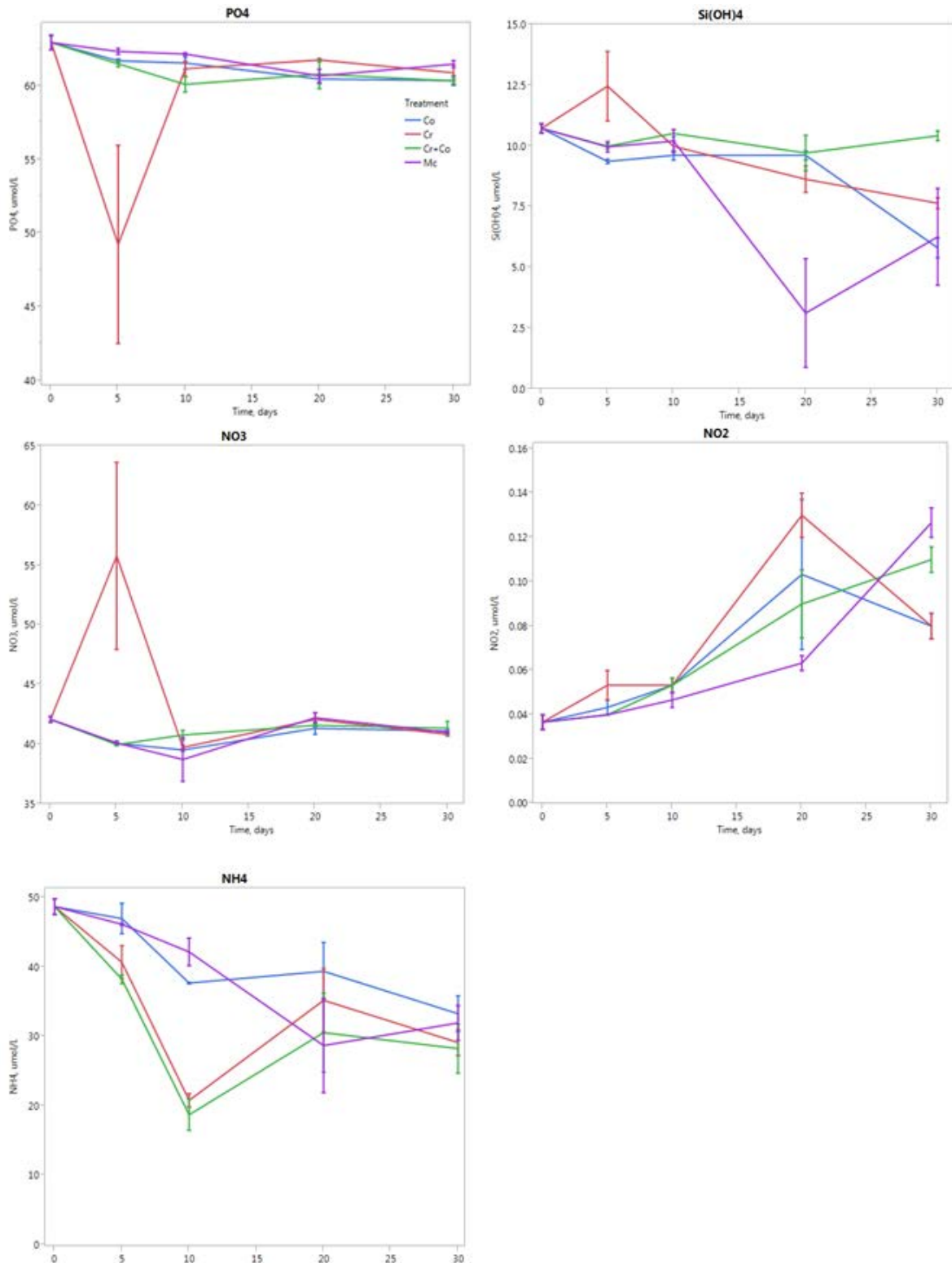
Figure 20. Nutrient concentrations from destructively harvested whole-bottle incubations (error bars ±1 SD from the mean). Cr = Crude oil only, Co = Corexit 9500A only, Cr+Co = Crude oil and Corexit 9500A, Mc = Microbial control (no substrate), Ab Cr = Abiotic crude oil only, Ab Co = Abiotic Corexit 9500A only, Ab Cr+Co = Abiotic crude oil and Corexit 9500A.
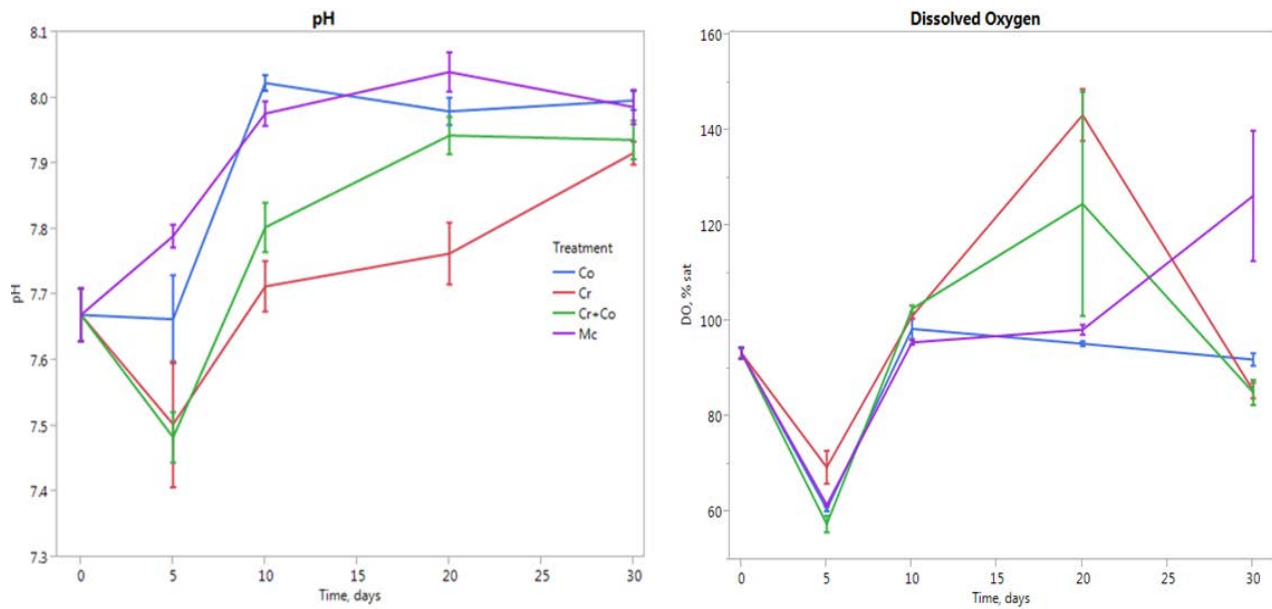
Figure 21. pH and dissolved oxygen measurements from destructively harvested whole bottle incubations (error bars ±1 SD from the mean). Legend code key: Cr = Crude oil only, Co = Corexit 9500A only, Cr+Co = Crude oil and Corexit 9500A, Mc = Microbial control (no substrate), Ab Cr = Abiotic crude oil only, Ab Co = Abiotic Corexit 9500A only, Ab Cr+Co = Abiotic crude oil and Corexit 9500.

Discussion

We report here that crude oil and surfactant components of Corexit 9500A can undergo substantial biodegradation (i.e., 36–41% of oil; 33–77% of DOSS; 96% of Span 80) within 28 days in Arctic surface seawater (Table 1, Figures 14 & 16). In nearshore seawater, crude oil degraded slightly more extensively than in offshore waters (Table 1, Figure 14). Overall, the nonionic surfactant components of Corexit 9500A (Tween 80+85 and Span 80) were more labile than DOSS, with the nonionics dropping to below detection limits within 2–5 days, while DOSS was more persistent, especially in the presence of oil (Table 2, Figure 16).

Corexit 9500A enriched a different overall microbial community than crude oil in offshore surface seawater at 28 days (Figures 2, 4 & 5), indicating that different organisms are largely responsible for their biodegradation; however, throughout the incubation a subset of taxa (*Oleispira*, *Colwellia*, *Lutibacter*, and an unclassified Flavobacteriaceae spp. OTU83; Figures 1, 7, 8a, 9a, & 10a) and functional genes associated with oil biodegradation (*alkB*, *nagG*, and *pchCF*; Figure 13) 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.

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 2014, respectively. While there is a lack of literature reporting 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 temperate seawater (0.011 gC/m^3*d; 18°C, Atlas & Bartha, 1972) and sub-Arctic seawater (0.015 gC/m^3*d; 1°C, Laake et al., 1984; Stewart et al., 1993). 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 microbial metabolic rates (Bagi et al., 2013). The rate of abiotic factors such as evaporation and diffusion increase with increasing temperature (Honrath & Mihelcic, 1999), which can result in more oil lost in temperate (Prince et al., 2013) vs. Arctic environments (McFarlin et al., 2014). Biodegradation rates are fairly similar across temperatures and regions, and the combination of biodegradation and abiotic losses are the most relevant to predicting the fate of oil in the environment. Such comparative studies show lower extents of oil loss in the Arctic than in temperate regions within the same time periods (Prince et al., 2013; McFarlin et al., 2014).

The surfactant constituents of Corexit 9500A underwent substantial biodegradation (33–77% loss of DOSS, 96% of Span 80) within 28 days in both offshore and nearshore incubations. Complete consumption of Tweens was observed within 10 days in offshore seawater (Table 2), which was also observed by Kleindienst et al. (2015) in a laboratory microcosm study using only

Corexit 9500A and Gulf of Mexico deep-seawater at 8℃. Nearshore incubations, which were sampled more frequently than our offshore tests, demonstrated a complete loss of Tweens and Span within 2–5 days and also an increased persistence of DOSS when in the presence of oil (Figure 16). Degradation rates of DOSS have yet to be reported in other cold environments; however, the loss of DOSS (33–77% loss over 28 days) observed in our study (2℃) is in contrast to Kleindienst et al. (2015) who reported an 8% loss of DOSS over 28 days in their Corexit-only incubation of Gulf of Mexico deep seawater at 8℃. When Corexit 9500A biodegradation was incubated along with oil in the form of chemically enhanced water accommodated fractions (CE-WAFs), Kleindienst et al. (2015) reported that DOSS biodegraded 30% within 28 days. These differences between Corexit 9500A biodegradation observed in our studies compared to those of Kleindienst et al. (2015) may be a function of geographic differences in seawater microbial communities and/or different methodologies, such as the use of aerated mesocosms (stirred with lids ajar) created with freshly collected seawater compared to their use of sealed bottles on roller tables using seawater stored for over 1 month prior to the initiation of incubations.

Campo et al. (2013) reported the only other published DOSS biodegradation rate constant in the absence of oil. In microcosms (100 mL) containing artificial seawater and cultured microorganisms from the Gulf of Mexico incubated at 25℃, Campo et al. (2013) reported that DOSS biodegraded to extents > 99% after 8 days with a first-order rate constant of 0.30 day^{-1}. Here we report a substantially lower rate of DOSS biodegradation in Arctic seawater at 2℃, with a first-order rate constant of 0.015 day^{-1}. These data may simply suggest that DOSS biodegrades slower at 2℃ than 25℃ (Campo et al., 2013); however, our experimental methods were substantially different from those used by Campo et al. (2013) since they used cultures rather than intact seawater communities, which prevents a direct comparison of our results. Furthermore, the extent of DOSS biodegradation differed between our nearshore and offshore incubations (33 ± 7% loss vs. 77 ± 0.5% loss, respectively; Table 2), which were both conducted at the same temperature (2℃), suggesting that other variables (e.g., microbial community structure) may have a stronger impact on the extent of DOSS biodegradation than temperature.

The different extents of DOSS biodegradation in our different incubation tests may be due to differences in the community structure, (i.e., dominated by *Colwellia* in offshore vs. *Polaribacter nearshore*; Figure 2). This may be explained by different water masses harboring distinct communities 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 was also correlated with a greater response of *Colwellia* (42% of the community in offshore seawater at day 28 vs. 4% of the community in nearshore). At day 0, *Colwellia* was also present at a greater relative abundance in the offshore incubation (3% of the total community) than the nearshore incubation (0.2%). Genus *Colwellia*

showed the most increase in abundance in the offshore incubations; however, *Polaribacter* showed the greatest change in the nearshore incubations, increasing by 30% within the first 10 days (Figure 2). Within our dataset, psychrotrophs *Colwellia* and *Polaribacter* (Deming & Junge, 2005; Moyer & Morita, 2007) are likely the most influential in the biodegradation of Corexit 9500A in Arctic seawater. These findings also indicate that the extent of Corexit 9500A biodegradation in Arctic seawater is determined by microbial properties that may vary with season and geographic location.

 Colwellia may have a greater role in degrading Corexit 9500A components than crude oil in Arctic marine environments. Based on indicator species analysis, *Colwellia* spp. (OTUs 12, 19 and 21) indicated of the presence of Corexit 9500A in offshore seawater at day 28, and incubations containing Corexit 9500A had a higher relative abundance of *Colwellia* (Figure 9a) and total prokaryotes (Figures 2 & 3) than incubations containing oil. *Colwellia* spp. are known for their psychrophilic members isolated from deep sea and polar marine ice (Deming & Junge, 2005), and they 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* populations increasing in response to oil or Corexit 9500A 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 9500A (Figure 9) together with the increase in total prokaryotic abundance (Figure 3) supports prior reports of their rapid response to dispersed oil in temperate environments (Kleindienst et al., 2015) and labile carbon substrates in Arctic environments (Collins & Deming, 2013).

 The abundance of *Polaribacter* coincided with the biodegradation of nonionic surfactant components of Corexit 9500A at day 10 (Figure 2; Table 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). Some microorganisms use dispersants as growth substrates (Chakraborty et al., 2012). A higher abundance of microorganisms was enriched by Corexit 9500A than oil in our incubations (Figures 2 & 3), which was also observed

by Lindstrom & Braddock (2002) and Kleindienst et al. (2015). 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). McFarlin et al. (2014) previously reported that indigenous Arctic marine microorganisms mineralized more Corexit 9500A than crude oil (20% weathered) continuously throughout a 60-day respirometer experiment at subzero temperatures (-1°C). Corexit 9500A contains more water-soluble components than oil (Corexit 9500AA SDS), thus making the mixture more bioavailable to bacteria as a carbon and energy source. Therefore, in addition to its physical effects (i.e., movement of oil into the water column; Prince & Butler, 2014; Brakstad et al., 2015), Corexit 9500A also impacts the Arctic marine microbial community by increasing the abundance of total microorganisms (Figures 2 & 3), while enriching some oil-degradation genes (Figure 13) and taxa known to include oil-degrading bacteria (Figure 2).

These results suggest that taxa known to include oil degraders also have the ability to biodegrade components in Corexit. Microorganisms may use some of the same metabolic pathways to biodegrade Corexit 9500A as oil. Known oil degradation genes, most notably *alkB* and *nagG*, increased in abundance in both oil and Corexit 9500A incubations (Figure 13). 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 9500A coincides with the abundance of *Colwellia* (Figure 9), a taxon that has shown a preference for Corexit 9500A (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 9500A 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 9500A in surface waters of the Arctic Ocean.

Correlating observed losses of Corexit 9500A and oil with increases in prokaryotic abundance and relative abundances of specific taxa and functional genes enable the identification of microorganisms likely to be active in degrading oil and Corexit 9500A compounds, as well as the relevant biodegradation genes they possess that may be involved. Our results support prior research indicating that microbes indigenous to seawater can perform substantial oil and Corexit 9500A biodegradation without the need to biostimulate with added nutrients or bioaugment with cultures of oil-degrading microorganisms (Mearns, 1997; Head et al., 2006). The inclusion of biodegradation rates reported from this project may be useful for improving the accuracy of trajectory models that predict the fate of spilled oil and Corexit 9500A in nearshore and offshore Arctic environments may be improved.

Study Products

Outreach

UAF Ph.D. student Taylor Gofstein, whose work was supported by this project with co-funding by an Oil Spill Research Institute Graduate Fellowship and the UA BLaST program, delivered community outreach and education in Utqiagvik, Alaska, in the summer of 2017. Gofstein designed and delivered an oil spill clean-up outreach activity for K–12 students that included a hands-on component and discussion of chemical dispersants and biodegradation. The activity required only household materials (e.g., cooking oil, etc.) and can be modified to accommodate different age levels, making it ideal for use in rural communities throughout Alaska. The activity was offered at a microbiology summer camp for high school students who were visiting Utqiagvik from a variety of rural communities across Alaska. The activity was very well received by the students, and we hope to deliver it again in the near future. Additionally, we will be working with educators in order to adapt the activity to meet the state of Alaska science standards and Iñupiaq Learning Framework for future outreach efforts.

Manuscript

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Presentations

McFarlin KM, Leigh MB. Biodegradation of Oil and Corexit EC9500AA in Arctic Seawater. Alaska Oil Spill Technology Symposium (AOSTS), Fairbanks, AK, March 2015. (Oral presentation)

McFarlin KM, Perkins M, Field J, Leigh MB. Biodegradation of Oil and Corexit EC9500AA by Arctic Marine Microorganisms. Battelle International Symposium on Bioremediation and Sustainable Environmental Technologies, Miami, FL, May 2015. (Oral presentation)

McFarlin KM, Perkins M, Field J, Leigh MB. Biodegradation of Oil and Corexit EC9500AA by Arctic Marine Microorganisms. Society for Environmental Toxicology and Chemistry North America 37th Annual Meeting, Orlando, FL, November 2016. (Oral presentation)

McFarlin KM, Perkins M, Field J, Leigh MB. Biodegradation of Oil and Corexit 9500A by Arctic Marine Microorganisms. Alaska Regional Response Team Meeting, Fairbanks, AK, January 2017. (Oral presentation)

McFarlin KM, Perkins M, Field J, Leigh MB. Biodegradation of oil and Corexit 9500A by Arctic marine microorganisms. Coastal Marine Institute Annual Research Review, Anchorage, AK, January 2017. (Oral presentation)

Gofstein TR, Leigh MB. Fate and Influence of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. Week of the Arctic, Fairbanks, AK, May 2017. (Poster presentation)

Gofstein TR, Leigh MB. Fate and Influence of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. UAF Biomedical Research Conference Fairbanks, AK, May 2017. (Poster presentation)

Leigh MB, McFarlin KM, Gofstein TR, Walker A, Hardy S, Perkins M, Field J. Biodegradation of oil and Corexit 9500A in the Arctic Marine Environment. Coastal Marine Institute Annual Research Review, Anchorage, AK, January 2018. (Oral presentation)

Gofstein TR, Perkins M, Field J, Leigh MB. Biodegradation of Crude Oil and Corexit EC9500A in Arctic Seawater. Alaska Oil Spill Technology Symposium, Anchorage, AK, March 2018. (Poster presentation)

Leigh MB, McFarlin K, Gofstein TR, Schuette U, Perkins M, Field J. Investigating Oil Spill Response Products Corexit 9500 and Oil Spill Eater II: Composition, Fate, and Effects on Oil Biodegradation in Alaskan Waters. Alaska Oil Spill Technology Symposium. Anchorage, AK, March 2018. (Invited oral presentation)

Gofstein TR. Fate and Effects of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. UAF Annual Environmental Chemistry Symposium, Fairbanks, AK, April 2018. (Oral presentation: Received Outstanding Graduate Presentation award)

Acknowledgments

References

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

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.

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

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.; Zemla, M.; Bill, M.; Conrad, M.E.; Malfatti, S.A.; Tringe, S.G.; Holman, H.; Hazen, T.C.; Jansson, J.K. 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.

Barsamian, A.; Perkins, M.; Field, J.; Blakemore, P. Regioselective Syntheses of [13C]4-Labelled Sodium 1-Carboxy-2-(2-ethylhexyloxycarbonyl)ethanesulfonate and Sodium 2-Carboxy-1-(2-ethylhexyloxycarbonyl)ethanesulfonate from [13C]4-Maleic Anhydride. *J. Labelled Compd. Radiopharm.* **2014**. doi:10.1002/jlcr.3189.

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 9500A and 9527 dispersants. *Marine Poll. Bull.* **2009**, *58*, 118-128.

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.; 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.; 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.

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, QC, Canada **1983**; pp 96.

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

Campo, P.; Venosa, A.D.; Suidan, M.T. Biodegradability of Corexit 9500A 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.; Jason Betley, J.: Fraser, L.: Bauer, M.; Gormley, N.; Gilbert, J.A.; Smith, G.; Knight, R. 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 9500A in the Gulf of Mexico. *Front. Microbiol*. **2012**, *3*, 357.

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.

Corexit 9500AA Safety Data Sheet; COREXIT ® EC9500AA; Nalco Company, Naperville, IL. https://dec.alaska.gov/spar/ppr/docs/dispersant_MSDS/Corexit%209500AA%20-MSDS.pdf.

Davies, L.; Daniel, F.; Swannell, R.; Braddock, J. *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); **2001**, http://dec.alaska.gov/spar/perp/r_d/aeat.pdf

Day, R.H.; Weingartner, T.J.; Hopcroft, R.R..; Aerts, L.A.; Blanchard, A.L.; Gall, A.E.; Gallaway, B.J.; Hannay, D.E.; Holladay, B.A.; Mathis, J.T; Norcross, B.L.; Questel, J.M.; Wisdom, S.S. The offshore northeastern Chukchi Sea, Alaska: a complex high-latitude ecosystem. *Cont. Shelf Res.* **2013**, *67*, 147-165.

Deming, J.W.; Junge, K. Genus *Colwellia* Deming 1988 159AL. In *Bergey's Manual of Systematic Bacteriology;* Krieg, N.R.; Brenner, D.J.; Staley, J.T.; Eds.; Bergey's Manual Trust, Springer: New York **1988**.

Deppe, U.; Richnow, 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.; Mason, O.U.; Piceno, Y.M.; Reid, F.C.; Stringfellow, W.T.; Tom, L.M.; Hazen, T.C.; Andersen, G.L. 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.

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. doi:10.1093/femsec/fiw130.

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

González, A.G.; Herrador, M.Á. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *TrAC Trends Anal. Chem.* **2007**, *26*(3), 227-238.

Gray, J.L., Kanagy, L.K., Furlong, E.T., Kanagy, C.J., McCoy, J.W., Mason, A.; Lauenstein, G. Presence of the Corexit component dioctyl sodium sulfosuccinate in Gulf of Mexico waters after the 2010 Deepwater Horizon oil spill. *Chemosphere*. **2014**, *95*, 124-130.

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., Ed.; 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.

Kube, M.; Chernikova, T.N.; Al-Ramahi, Y.; Beloqui, A.; Lopez-Cortez, N.; Guazzaroni, M.E.; ... & Golyshin, P. N. Genome sequence and functional genomic analysis of the oil-degrading bacterium *Oleispira antarctica*. *Nature Comm*. **2013**, *4*, 2156. doi:10.1038/ncomms3156.

Kujawinski, E.B.; Kido Soule, M.C.; Valentine, D.L.; Boysen, A.K.; Longnecker, K.; Redmond, M.C. Fate of dispersants associated with the Deepwater Horizon oil spill. *Environ. Sci. Technol.* **2011**, *45*(4), 1298-1306.

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.

Lindstrom, J.E.; Braddock, J.F. Biodegradation of petroleum hydrocarbons at low temperatures in the presence of the dispersant Corexit 9500A. *Mar. Pollut. Bull.* **2002**, *44*, 739-747.

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.

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

Major, D.N.; Wang, H.; How public health impact is addressed: a retrospective view on three different oil spills. *Toxicol. Environ. Chem.* **2012**, *94*, 442-467.

Mathew, J.; Schroeder, D.L.; Zintek, L.B.; Schupp, C.R.; Kosempa, M.G.; Zachary, A.M.; Schupp, G.C.; Wesolowski, D.J. Dioctyl sulfosuccinate analysis in nearshore Gulf of Mexico water by direct-injection liquid chromatography tandem mass spectrometry. *J. Chromatogr. A*. **2012**, *1231*, 46-51.

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*, 332. doi:10.3389/fmicb.2014.00332

McCune, B.; Mefford, M.J. *PC-ORD v. 6.255 beta;* MjM Software Design: Gleneden Beach, OR **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*(1), p.e84297. https://doi.org/10.1371/journal.pone.0084297

McFarlin, K.M.; Perkins, M.J.; Field, J.A.; Leigh, M.B. Biodegradation of crude oil and Corexit 9500 in Arctic seawater. *Front. Microbiol.* **2018**, *9*, 1788.

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

Mielke, P.W. 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.; Ed.; 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 Research Council (NRC). *Oil Spill Dispersants: Efficacy and Effects*; National Academies Press: Washington, D.C. **2005**.

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

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

Place, B.; Anderson, B.; Mekebri, A.; Furlong, E. T.; Gray, J. L.;Tjeerdema, R.; Field, J. A role for analytical chemistry in advancing our understanding of the occurrence, fate, and effects of Corexit oil dispersants. **2010**, *44*, 6016-6018.

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 Microbiology Ecology*. **2007**, *59*(2), 342-355.

Prince, R.C.; Elmendorf, D.L.; Lute, J.R.; Hsu, C.S.; Haith, C.E.; Senius, J.D.; Dechert, G.J.; Douglas, G.S.; Butler, E.L. 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.Y.; 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.

Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley B.B.; Parks, D.H.; Robinson, C.J.; Sahl, J.W.; Stres, B.; Thallinger, G.G.; Van Horn, D.J.; Weber, C.F. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *App. Env. Micro.* **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*(12): 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.

Siron, R.; Pelletier, E.; Brochu, C. Environmental factors influencing the biodegradation of petroleum hydrocarbons in cold seawater. *Arch. Environ. Contam. Toxicol*. **1995**, *28*, 406-416.

SL Ross Environmental Research Ltd.; MAR Inc. *Corexit 9500A Dispersant Effectiveness Testing In Cold Water On Four Alaskan Crude Oils.* Report to US Department of the Interior, Mineral Management Service, Herndon, VA **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.

US EPA (United States Environmental Protection Agency). *Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species;* Office of Research and Development: Washington, D.C. **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.; Humana Press: New York **2016**.

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

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.

White, H.K.; Lyons, S.L.; Harrison, S.J.; Findley, D.M.; Liu, Y.; Kujawinski, E.B. Long-term persistence of dispersants following the Deepwater Horizon oil spill. *Environ. Sci. & Technol. Lett.* **2014**, *1*(7), 295-299.

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.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the sound use of our land and water resources, protecting our fish, wildlife and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island communities.

The Bureau of Ocean Energy Management

The Bureau of Ocean Energy Management (BOEM) works to manage the exploration and development of the nation's offshore resources in a way that appropriately balances economic development, energy independence, and environmental protection through oil and gas leases, renewable energy development and environmental reviews and studies.