Microbial biodegradation of Alaska North Slope crude oil and Corexit 9500 in the Arctic marine environment

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ABSTRACT

This project investigated the biodegradation of Alaska North Slope crude oil and Corexit 9500 (Corexit) in Arctic seawater and sediments to identify the microbes and genes actively involved in the biodegradation process. Results showed degradation of non-ionic surfactants in Corexit occurred before biotic degradation of petroleum hydrocarbons, but Corexit did not suppress oil biodegradation. The presence of oil appeared to delay mineralization of the Corexit component dioctyl sodium sulfosuccinate (DOSS). Some taxa responded selectively to the presence of oil (*Thalassolituus, Sedimentalea*) or Corexit (*Colwellia*, *Polaribacter*, *Moritella*, *Octadecabacter*, and *Amylibacter*); other taxa (*Oleispira, Pseudofulvibacter,* and *Roseobacter*) responded to both, suggesting the ability to utilize compounds from oil and Corexit. Metatranscriptomics (gene expression) analyses of experimental seawater microbial communities revealed that oil and Corexit stimulated different metabolic gene expression profiles in the microbial community. However, communities exposed to either oil or Corexit both showed upregulation of the pathway for fatty acid degradation. These observations, and evidence of increased metabolic activity in treatments containing oil with Corexit, support the theory that dispersants stimulate oil-degrading activity by the microbial community. Expression of fatty acid degradation genes was upregulated in the presence of Corexit without oil, indicating the pathway in degradation. Thus, the authors propose a degradation pathway that utilizes the fatty acid β-oxidation pathway to break down the non-ionic and DOSS surfactant components of Corexit.

Mesocosm incubation tests were performed with Arctic marine sediments to identify indigenous oil-degrading microbes and assess oil biodegradation potential, the latter of which was not complete at the time of this report. Mesocosm treatments included fresh and weathered crude oil under anaerobic and aerobic conditions. Molecular analyses revealed that oiled communities differed from those in non-oiled treatments, significant microbial community shifts occurred in oiled treatments under aerobic conditions, and distinct community structures emerged following exposure to fresh versus weathered oil. Oil-associated shifts were characterized by increases in the relative abundance of several taxa, implicating them in oil biodegradation. *Cycloclasticus, Neptuniibacter, Oleispira, Roseobacter clade NAC11-7,* and *Zhongshania*, known oil-degraders, were only present in oiled treatments. Oil-degrading taxa identified in the Chukchi Sea sediment incubation studies were compared to taxa data from Beaufort Sea sediments. The majority were present in very low abundances, indicating a broad distribution of degradation potential in the Beaufort Sea benthos.

Oil stimulated different hydrocarbon-degrading organisms in seawater *(Thalassolituus*, *Sedimentalea*, and *Pseudofulvibacter)* than in sediment (*Cytoclasticus*, *Neptuniibacter*, and *Zhongshania*). *Oleispira* and *Roseobacter* were stimulated in both environments. This finding highlights the diversity and ubiquity of hydrocarbon-degrading microbes and the strong influence of environmental parameters on potential post-spill microbial community succession, even in the same geographic region.

INTRODUCTION

Rapid change is occurring in the Arctic marine environment due to climbing atmospheric temperatures, and projections state that the summer Arctic will be nearly ice-free by 2030 (Overland & Wang, 2013). Historically ice-covered waters are becoming more accessible to human activities, increasing the likelihood of anthropogenic disturbance and contaminant exposure through oil and gas development, the expansion of commercial shipping, and other activities. Microbial biodegradation is the primary means by which oil is naturally removed from the marine environment (Braddock et al., 1995; McFarlin et al., 2014), so understanding oil biodegradation potential of the indigenous benthic microbial communities may help in accurately predicting the fate and effects of oil in the environment and developing spill response strategies.

Chemical dispersants, such as Corexit 9500 (Corexit), have been widely applied in temperate and sub-tropical regions as an oil spill response strategy for open water slicks, and Corexit is currently under consideration for use in Alaskan waters. Some early dispersant formulations were toxic to wildlife (Lessard & DeMarco, 2000), but newer formulations, like Corexit, were designed to be less toxic (Word et al., 2015). While Corexit is credited with protecting shorelines and facilitating biodegradation, concerns remain regarding its fate and potential ecological consequences from its use.

Fate, effects, and interactions of oil and Corexit in seawater

The 2010 Gulf of Mexico Deepwater Horizon oil spill (DWH) initiated one of the most notable large-scale applications of chemical dispersants. Corexit and Corexit 9527 were used in unprecedented amounts to disperse surface oil and help prevent surfacing of wellhead oil (National Research Council, 2001). The spill prompted numerous studies investigating Corexit, particularly its effects on microbial hydrocarbon biodegradation (Atlas & Hazen, 2011; Gonzalez-Gaya et al., 2019; Hazen et al., 2016; Prince & Butler, 2014; Prince et al., 2013; McFarlin et al., 2014) and the fate of dispersant components (Prince et al., 2013; McFarlin et al., 2014; Brakstad et al., 2014).

Biodegradation of oil may be limited by environmental factors such as the bioavailability of oil (Hazen et al., 2016) and access to nutrients (Atlas, 1981; Atlas & Bartha, 1972). Corexit aids in the formation of small oil droplets, which may increase the bioavailability of oil to indigenous microorganisms and increase access to nutrients for microbes as droplets disperse through the water column. The majority of studies of Corexit reported increased oil biodegradation in seawater when compared to non-dispersed oil (Prince & Butler, 2014) or physically-dispersed oil (Brakstad et al., 2014; Brakstad et al., 2018; McFarlin et al., 2014; Prince et al., 2013). However, at least two studies have reported that Corexit inhibited oil-degrading bacteria (Kleindienst et al., 2015; Rahsepar et al., 2016). Biodegradation of oil, including the genes and pathways involved, has been characterized in a variety of environmental conditions (Das & Chandran, 2011;

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Harayama et al., 1999), but less is known about oil degraders in the Arctic marine environment or those associated with Corexit degradation.

The interactions between oil and dispersants are not well understood in Arctic marine ecosystems, and the biochemical pathways and bacterial taxa involved in the biodegradation of dispersants are unknown. Combining oil and Corexit may affect their respective biodegradation processes in Arctic waters because preferential degradation, enrichment, and suppression of select microbes may change the fate of these chemicals when mixed. Several studies have reported increased rates of petroleum biodegradation with the addition of chemical dispersants using either enrichment cultures (Campo et al., 2013; Techtmann et al., 2017; Venosa & Holder, 2007; Zahed et al., 2010) or indigenous microbial communities (Brakstad et al., 2014; Brakstad et al., 2018; McFarlin et al., 2017; Prince et al., 2013; Prince et al., 2014). However, contrasting research has shown dispersants negatively affecting oil biodegradation in indigenous (Kleindienst et al., 2015) and cultured (Rahsepar et al., 2016) seawater by suppressing the growth of oil-degrading bacteria.

The degradation of crude oil and chemical dispersants in the Alaskan Arctic marine environment has been investigated primarily through laboratory studies. Mesocosm studies by McFarlin et al. (2014) demonstrated that biodegradation of low concentrations of crude oil (2.5 and 15 ppm) by indigenous microorganisms in Chukchi Sea surface waters occurred with and without the addition of Corexit, with the dispersant slightly enhancing biodegradation. McFarlin et al. (2017) also conducted a survey of the microbial community structure and genetic potential for hydrocarbon degradation in the Burger oil and gas lease area in the Chukchi Sea. They observed the presence of bacterial genera known to include oil degraders and oil biodegradation genes in surface waters and at depth. An investigation of the biodegradation of oil and Corexit incubated separately in seawater found distinct microbial community shifts and evidence of Corexit component degradation (McFarlin et al., 2018).

The major constituents of Corexit include the anionic surfactant dioctyl sodium sulfosuccinate (DOSS, 18% w/w) and the non-ionic surfactants Span 80 (4.4% w/w), Tween 80 (18% w/w), and Tween 85 (4.6% w/w) in a petroleum distillate solvent (Gray et al., 2014; Parker et al., 2014; Place et al., 2010; Place et al., 2014). Ethylhexyl sulfosuccinate (EHSS) has also been identified as an abiotic hydrolysis product of DOSS (Campo et al., 2013; Ramirez et al., 2013) and is present in detectable quantities (0.28% w/w) in Corexit 9500 formulations (Place et al., 2016). Studies that have directly or indirectly measured Corexit loss suggest that some components may be readily biodegraded (Campo et al., 2013; Kleindiest et al., 2015; McFarlin et al., 2014; McFarlin et al., 2018; Techtmann et al., 2017). However, most of these studies limited their focus to DOSS or provided only generalized evidence of mineralization of Corexit as a whole (e.g., via CO_2 evolution), so less is known about the fate of the individual non-ionic surfactant and solvent components.

Following the application of Corexit at the DWH spill, *in situ* measurements showed persistence of DOSS (Kujawinski et al., 2011; Perkins et al., 2017; White et al., 2014), indicating that some components of Corexit may be recalcitrant to degradation. The biodegradation of DOSS and the non-ionic surfactants in Corexit was observed when Corexit was incubated in Arctic seawater mesocosms with and without the addition of oil (McFarlin et al., 2018). A Geochip assay of Arctic seawater showed an increase in the abundance of hydrocarbon degradation genes for both treatments relative to controls (McFarlin et al., 2018). These results suggest that Corexit biodegradation may share genes or pathways with oil biodegradation, possibly through the cleavage of long alkyl chains present in the surfactant components. However, no studies have identified the specific genes or metabolic pathways associated with Corexit biodegradation.

While specific genes and metabolic pathways have not yet been identified for Corexit biodegradation, associated shifts in microbial community structure have been well documented. Different bacterial taxa have been shown to proliferate in the presence of Corexit alone (e.g., *Moritell:* McFarlin et al., 2018) or oil alone (e.g., *Thalassolituus*, Coulon et al., 2007; McKew et al., 2007 and *Cytoclasticus*, Brakstad et al., 2018; Coulon et al., 2007; Kleindienst et al., 2015; McKew et al., 2007; Tremblay et al., 2017). Taxa have also been shown to respond positively to the presence of either oil or Corexit under some experimental conditions (e.g., *Colwellia,* Brakstad et al., 2008; Bælum et al., 2012; Kleindienst et al., 2015, *Oleispira,* McFarlin et al., 2018; Coulon et al., 2007, and *Polaribacter,* McFarlin et al., 2018; Prabagaran et al., 2007*).* Techtmann et al. (2017) found that chemically-dispersed oil enriched a mixture of taxa that were observed to proliferate in both oil-only and Corexit-only treatments, which suggests they biodegrade components of both oil and Corexit. It is possible that the same genes and pathways are employed to biodegrade components of both mixtures. Given the functional genetic diversity among species and strains within the same genus, it is difficult to address this question with 16S rRNA data. Studies have focused on identifying taxa that become enriched following an oil spill (e.g., Brakstad et al., 2015; Hazen et al., 2010; Kleindienst et al., 2015; McFarlin et al., 2018), but less is known about the effects on functional gene expression, which may yield insights into the specific roles performed by members of the consortium.

In this study, the authors identified microbial taxa putatively involved in biodegradation in the Arctic marine environment and investigated the interactive effects of Alaska North Slope (ANS) crude oil and Corexit on the microbial community and sequential and co-degradation. We identified genes associated with biodegradation of Corexit or oil (alone and combined), identified organisms that expressed degradation genes, and constructed a putative pathway for the biodegradation of Corexit surfactant components. We predicted that some taxa only degrade oil or Corexit, while other taxa utilize the same pathways and alkane hydrocarbon-degradation genes to degrade both.

Oil biodegradation in the benthos

Following an oil spill, the cleanup effort generally focuses on treating visible oil slicks on the sea surface or shorelines through techniques like containment, recovery, *in situ* burning or dispersion, and washing or bioremediation of shorelines. Generally, less attention is afforded to sediments despite conservative estimates that 20-30% of total oil from a spill event reaches benthic marine sediments (Muschenheim & Lee, 2002). Oil in the benthos can persist for many years and impact the health of the marine ecosystem, including adverse effects on benthic and pelagic food webs. Oil can be toxic to a multitude of demersal fishes and invertebrates and persist in tissues of exposed organisms. For example, oil compounds remained in marine sediments and at toxic levels within the tissues of organisms such as Pacific Halibut, mussels, and clams for ten years following the Exxon-Valdez oil spill (Jewett et al., 2002). In the Arctic, benthic fishes and invertebrates compose much of the diet for the walruses, seals, and whales that are essential subsistence species for Alaska Native communities (Laidre et al., 2008). Thus, understanding the fate of oil in Arctic marine sediments is important for assessing the potential environmental and human health impacts of an oil spill in this ecosystem.

Oil is initially buoyant in seawater; however, multiple mechanisms help transport it to the seafloor, including aggregate formation and weathering. Aggregate formation occurs through the adhesion of oil droplets to suspended particulate material, such as sediment and detritus, which increases the density of oil and promotes sedimentation to the benthos. Oil weathering processes occur via photooxidation, evaporation, biodegradation, dissolution, emulsification, or through a combination of these processes. Typically, the lighter and more volatile oil compounds are the first lost during the process of weathering, leaving the heavier and more metabolically recalcitrant hydrocarbons and other compounds. Weathered oil components are the most persistent in marine environments and include many of the polycyclic aromatic hydrocarbons (PAHs).

Oxygen availability can be limiting to biodegradation in sediments, but evidence of anaerobic petroleum biodegradation, such as the presence of anaerobic biodegradation metabolites, has been documented in contaminated sediments, including those from the DWH oil spill (Kimes et al., 2013). Marine sediment environments exhibit local oxic and anoxic pockets, even within the upper 1 cm layer of sediment (Rysgaard et al., 2004). Anoxic conditions can slow or stop oil biodegradation or provide the necessary conditions for anaerobic biodegradation of more recalcitrant, weathered oil compounds (Rysgaard et al., 2004). Due to the presence of more labile components, fresh oil is generally broken down through microbial processing much more quickly than weathered oil (Rysgaard et al., 2004). Once oil has been heavily weathered, and primarily recalcitrant chemical compounds remain, oil biodegradation is very limited. However, some anaerobic microbes, which require low or no oxygen environments, are capable of breaking down some of these recalcitrant compounds (Braddock et al., 1995; Rysgaard et al., 2004).

Three studies assessed aspects of microbial biodegradation of oil in sediments from the North American Arctic region (Braddock et al., 2004; Dong et al., 2015; Sharma et al., 2016). Braddock et al. (2004) sought to establish a baseline for microbial communities in Arctic marine sediments and associated oil biodegradation potential in several sites from Barrow to Prudhoe Bay, Alaska. Their research provided estimates of the abundance of total and oil-degrading bacteria in the region using direct counts and culture-based methods (Most Probable Number– MPN). They also examined the mineralization potential of native microbes on two specific chemical components of oil (hexadecane and phenanthrene) and assessed how sediments affect the bioavailability of adsorbed oil. They found that biodegradation of hexadecane and phenanthrene by indigenous microbes was relatively slow, and it was unlikely that the bioavailability of oil was hindered by adhesion to sediments. Dong et al. (2015) identified microbes that degrade specific PAH components of oil in deep-sea sediments from the Chukchi Plateau. They used cultivated oil-degraders and, due to the limited culturability of many organisms, this method can underestimate the microbial diversity present in the environment. The use of indigenous microbial communities is more likely to reflect rates observed for *in situ* conditions (McFarlin et al., 2014). Seeking to inform bioremediation efforts for Arctic shorelines, Sharma at al. (2016) investigated the effects of salinity, temperature, and oil concentration on biodegradation in intertidal sediments from Utqiaġvik (formerly Barrow).

This study expanded on the work of Braddock et al. (2004) by providing high-resolution information on sediment microbial community composition and the identity of oil-degrading microbes using modern molecular methods. Comprehensive quantification of biodegradation of the major components of crude oil was accomplished using gas chromatography coupled to mass spectrometry (GC-MS). Using high-throughput DNA sequencing techniques, we tracked microbial community shifts in response to oil exposure and identified key taxonomic groups that increased in abundance during biodegradation of fresh and weathered oil, identifying them as putative oil degraders. Identifying active oil degraders in the Arctic facilitates better predictions of degradation potential throughout the region. Additionally, this study assessed oil biodegradation rates and the associated microbial communities involved in both oxic and anoxic conditions to better understand the optimal oxygen conditions required for oil breakdown and bioremediation efforts.

Objectives and hypotheses

The goal of this project was to investigate the biodegradation of ANS crude oil in Arctic seawater and sediments to better predict the fate and effects of the chemical dispersant Corexit in such environmental conditions. We quantified the biodegradation of oil and Corexit components in Arctic seawater and applied in-depth analyses of microbial community structure and gene expression to gain a comprehensive perspective of microbial biodegradation. Mesocosm incubation experiments were conducted using Arctic marine sediments. Fresh and weathered crude oil were added under both oxic and anoxic conditions to assess aerobic and anaerobic

biodegradation potential and to identify oil-degrading microbes in the benthos. Following the identification of putative oil-degrading bacteria in incubation tests using Chukchi marine sediments, we queried our existing sediment microbial community datasets from the Beaufort Sea region to assess the biodegradation potential present in indigenous sediment microbial communities. Our findings provide an in-depth analysis of the crude oil biodegradation potential in the Arctic marine environment.

Reporting on Objectives 1 and 2 includes new data from our previous CMI project that focused on the biodegradation of oil and Corexit (contract number M15AC00008).

Biodegradation and interactions of oil and Corexit in Arctic seawater

Objective 1. Quantify the extent of oil and Corexit biodegradation over time and the effects of their co-presence in Arctic seawater on their fate.

- Hypothesis: Some Corexit components will degrade more slowly than others, such as DOSS, which is suspected to be among the most persistent surfactant components based on prior literature.

- Hypothesis: Despite the predicted lability of some of Corexit's components, their presence will not retard oil biodegradation through processes such as inhibition or competitive biodegradation in seawater.

Objective 2. Identify microorganisms important to biodegradation of oil and dispersant chemical components in Arctic seawater and their overlaps and/or potential interactions.

- Hypothesis: Distinct microbial populations will be stimulated by the presence of crude oil or Corexit, and the co-presence of oil and Corexit will stimulate both populations together. Although the majority of taxa may degrade either oil or Corexit, some individual taxa will be capable of biodegrading components of both mixtures.

Objective 3. Characterize the pathways and genes and taxonomic affiliations involved in the biodegradation of petroleum hydrocarbons and Corexit in Arctic seawater.

- Hypothesis: The presence of oil in seawater will result in a significantly increased expression of hydrocarbon degradation genes by indigenous microbes. Incubation with crude oil will first stimulate increased expression of genes associated with labile petroleum components (e.g., alkanes and other aliphatics) and, as they become depleted due to biodegradation, expression of genes for more recalcitrant oil components (e.g., aromatics) will increase.

- Hypothesis: Microorganisms that biodegrade components of both oil and Corexit utilize some of the same alkane-degradation genes and pathways since some Corexit components contain structurally similar hydrocarbon side-chains.

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Biodegradation of oil in Arctic marine sediments

Objective 4. Assess the capacity for biodegradation of fresh and weathered ANS crude oil by indigenous microorganisms in subtidal Arctic marine sediments under aerobic and anaerobic conditions.

- Hypothesis: Arctic sediment microbial communities are capable of biodegrading oil both aerobically and anaerobically, with aerobic biodegradation being more rapid.

- Hypothesis: Oil biodegradation in Arctic sediments follows trends observed for oil biodegradation in other environments where increased oil weathering reduces biodegradation rates compared to fresh oil.

Objective 5. Identify microorganisms indigenous to Arctic marine sediments that are important to oil biodegradation.

- Hypothesis: Oil-degrading microbes are ubiquitous; a variety of oil-degrading microbial taxa will be present in Arctic marine sediments.

- Hypothesis: Arctic benthic microorganisms are not well studied; our analyses of community shifts in response to oil will implicate species in oil biodegradation that were not previously reported to perform this function.

Objective 6. Utilize taxonomic information from sediment incubation studies to assess the distribution of putative hydrocarbon-degrading microbes more broadly in Arctic sediments.

- Hypothesis: Putative oil-degrading microorganisms are widespread in the environment; oil degradation potential is broadly distributed in North American Arctic sediments.

Seawater-sediment comparison

Objective 7. Identify and compare microorganisms involved in biodegradation of oil in both Arctic seawater and sediments and use taxonomic information from sediment incubation studies to assess the distribution of putative hydrocarbon-degrading microbes more broadly in Arctic sediments.

- Hypothesis: Indigenous microorganisms involved in oil biodegradation in Arctic sediments are more diverse than those reported for seawater from the same region, due to the higher biomass and associated diversity of microbes associated with sediments.

METHODS

Seawater incubations

Temporal incubation series of Arctic seawater with crude oil, dispersant, or both were completed in August 2016. Arctic surface seawater was collected ~1 km offshore of Utqiaġvik, Alaska, from the Chukchi Sea in August 2016. The 150 liters of collected seawater were stored at 5°C overnight and immediately transported by air to Fairbanks, Alaska, where it was aerated overnight at the temperature recorded at the time of collection (4°C) prior to the initiation of the incubation experiment. Before aliquoting seawater into mesocosms, it was supplemented with 16 ppm of Bushnell-Haas media (McFarlin et al., 2014) to prevent potential nutrient limitations that may occur as an artifact of the small-scale incubations. The supplementation with Bushnell-Hass provided an additional measured 62 µM phosphate, 49 µM ammonia, 42 µM nitrate, and estimated 1.5 µM iron. Since these nutrient concentrations are much higher than those expected in Arctic regions (Codispoti et al., 2009; Pisareva et al., 2015), the oil and Corexit component degradation reported here represents a best-case scenario.

Mesocosm incubation experiments were performed in a cold room set to a temperature of 4°C and the lights set to a 19-hour day/5-hour night cycle to mimic the conditions at the sampling site at the time of collection. Mesocosms were constructed by aliquoting 800 mL of seawater into acid-washed and pre-autoclaved 1-L glass bottles containing Teflon-coated magnetic stir bars and treated with either 50 ppm ANS crude oil, 5 ppm Corexit (1:10 dispersant-to-oil ratio), both, or neither, the latter of which served as a negative control. The bottles were stirred at a low speed to allow movement of the oil slick at the surface while preventing the formation of a large vortex, and the lids were left slightly ajar to allow air exchange. Sterile controls consisting of autoclaved seawater were also used to account for abiotic losses of oil and Corexit from processes such as evaporation, volatilization, hydrolysis, and photooxidation. Treatments were replicated in triplicate and destructively harvested at 0, 5, 10, 20, and 30 days for crude oil and microbial analyses.

There were no 5- or 20-day mesocosms for Corexit analyses constructed because of space limitations; however, an additional triplicate series of larger 6-L mesocosms were created and subsampled at high frequency to capture the relatively rapid degradation of the non-ionic surfactant components of Corexit as reported by McFarlin et al. (2018). Treatments for the subsampled series included live and sterile abiotic treatments of seawater amended with 5 ppm Corexit with or without 50 ppm ANS crude oil. These large-sized incubations were subsampled through a Teflon tube with a syringe at 0, 1, 2, 3, 4, 5, 6, 7, 10, 20, and 30 days. In addition to acid washing and autoclaving, all vessels used for the analysis of Corexit were baked at 400°C for 12 hours to remove any surfactant contaminants present as a result of manufacturing or other contamination sources.

Sediment incubations

Marine sediment and seawater samples for the sediment oil-incubation experiment were collected on the AMBON cruise in August 2017 at 45 m water depth via Van Veen grab for sediments and an intake hose for seawater (69.91°N, -166.05°W; Figure 1). The sediments and seawater were collected in sterile, acid-washed containers and kept at *in situ* temperature, 5°C until shipped on ice to the University of Alaska Fairbanks, where they were stored in a cold room set at 5°C. All collected seawater was vacuum filtered through 0.2-um filters, autoclaved, cooled to 5°C, and spiked with a 1% resazurin solution (with a final concentration of 0.002% per bottle) before the experiment. These measures were implemented to prevent the seawater microbial signal from interfering with the sediment microbial signal, to maintain *in situ* water properties, and to allow determination of oxygenation states (oxic, suboxic, anoxic) for individual serum bottles.
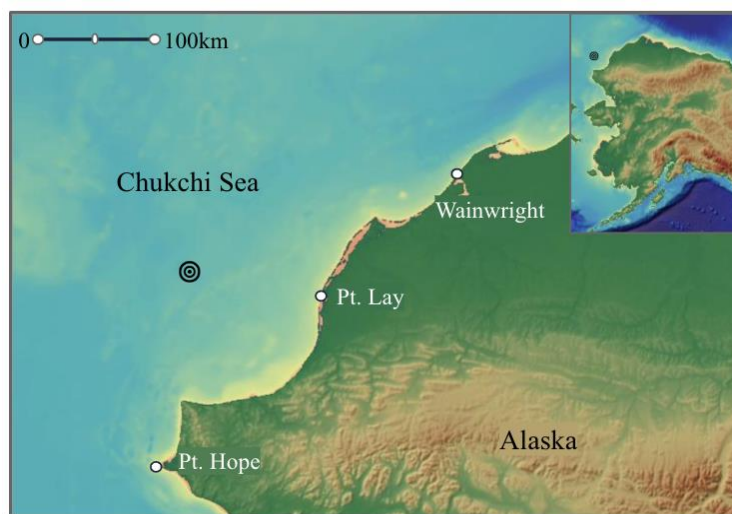
Figure 1. Map of the study area and sediment collection location (~ 130 km offshore of Pt. Lay, Alaska).

The ANS crude oil was obtained from Polar Tankers Inc., in Valdez, Alaska, on July 12, 2017. Upon acquisition of the ANS crude, half of the oil was sealed and stored in an amber glass bottle in a dark area of the cold room at 5°C for use as the fresh oil treatment. For use in the weathered oil treatment, half of the oil was dispensed into a large graduated cylinder, weighed, and set on a shaker table loosely capped with sterile foil until 20% of its mass was lost to volatilization (Prince et al., 2012).

Serum bottles were washed with lab-grade detergent, acid-washed in a 10% HCL solution, and autoclaved to ensure sterility and the removal of any potential contaminants. Based upon similar incubation studies, we chose to set up each 100-mL incubation serum bottle with 40 mL of seawater and 8 mL of sediment, and oil treatments with 1 mL of oil dispensed via positive displacement pipette for a total oil concentration of 2% (Braddock et al., 2004; Ferguson et al.,

2017; Zanaroli, personal communication, June 28, 2017). Sediment was weighed ten times to establish an average mass of 12.2 g sediment per 8 mL. After homogenization of source marine sediments, a sterile stainless-steel funnel was used to transfer 12.2g of sediment to each serum bottle. Residual sediment was rinsed from the funnel into the serum bottle with 40 mL of resazurin-spiked seawater. Bottles for abiotic quantification of oil loss were autoclaved, re-oxygenated, and cooled down to $5^{\circ}C$. Fresh or weathered ANS crude oil (1 Ml) was dispensed to bottles, which were then homogenized. A total of 333 bottles were prepared; the aerobic treatment bottles remained loosely capped with sterile foil as to remain oxic and anaerobic treatment bottles were plugged with blue butyl rubber stoppers and crimped with aluminum seal caps to foster natural anoxic conditions. All serum bottles were encased in a foam-lined holder on a shaker table.

The oxic incubation bottles were harvested every 12 days. With the letter T representing time in days, our coding system is as follows: T1 = 12 d, T2 = 24 d, T3 = 36 d, T4 = 48 d, and T5 = 60 d, including a T0 harvest on the first day of the incubation. The bottles designated for anaerobic degradation of crude oil went anoxic after 5 days, as indicated by resazurin dye. These samples were allowed to incubate for 26 months as anaerobic oil degradation typically occurs at much slower rates than aerobic degradation. The anaerobic incubations were analyzed at two time points, T0 and Tfinal. An overview schematic of the experimental design is depicted in Figure 2. Bottles harvested for oil quantification were immediately frozen at $-80^{\circ}C$, and samples for DNA extraction were transferred to DNA/RNA-ase-free 50 mL falcon tubes and immediately frozen at $-80^{\circ}C$ until extraction. Oil degradation rates and community composition shifts were observed at six points at 12-day intervals over 60 days. There were three replicates for each of the incubation treatments: control (no-oil), fresh oil, weathered oil, oxic, and anoxic. Oxic treatments were analyzed at time 0 (T0), 12 (T1), 24 (T2), 36 (T3), 48 (T4), and 60 (T5) days. Anoxic incubations were only analyzed at T0 at Tfinal (26 months).
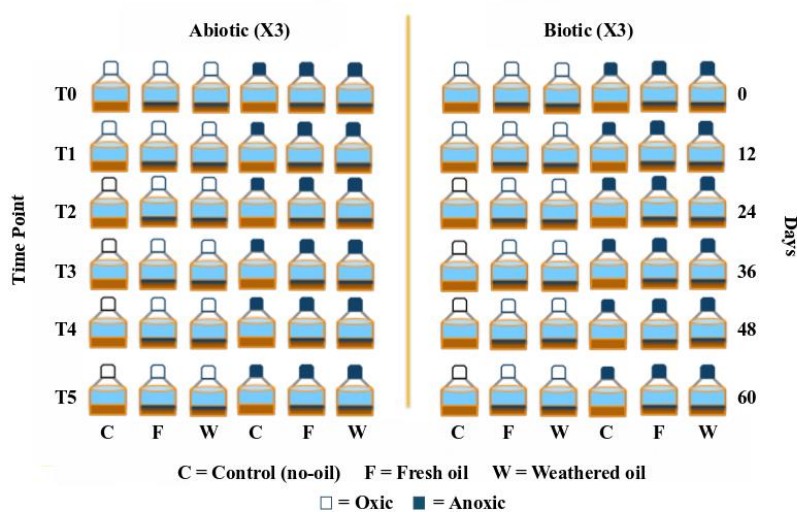

Figure 2. Experimental design for sediment oil biodegradation incubation tests.

Oil quantification

Seawater incubations

Chemical extraction and analyses of petroleum hydrocarbons were performed on seawater incubations using gas chromatography mass spectrometry (GC/MS) based on the methods of Prince & Douglas (2005). Three 20-mL aliquots of dichloromethane were added to each seawater mesocosm and mixed with a magnetic stir bar for a short time. After stirring, the dichloromethane droplets re-coalesced under the water layer and were pipetted out and combined. The extracts were dried with anhydrous sodium sulfate and stored at -20°C until analysis. Hydrocarbon analysis was performed on an HP 5890/5973 GC/MSD in scan mode with all signals normalized to the internal marker compound 17α(H),21β(H)-Hopane, which is naturally present in oil and does not biodegrade under normal environmental conditions (Aeppli et al., 2014; Prince et al., 1994). Total Petroleum Hydrocarbons (TPH) were measured, as well as individual n-alkanes, branched alkanes, and PAHs using the respective primary and secondary ions to identify those compounds (Douglas et al., 1994).

Sediment incubations

Several steps were employed to quantify oil degradation in sediment incubation mesocosms. Designated serum bottles were inoculated with 300 uL of 1000-ppm D8-naphthalene/methanol surrogate and homogenized to ascertain oil extraction efficiency accurately. Oil was then extracted from each bottle by adding 12.2 of anhydrous sodium sulfide to curb emulsions and three 20 mL aliquots of dichloromethane (DCM). After each aliquot of DCM, the sample was immediately sealed and gently swirled on a shaker table for 20 minutes, left to settle for 5 minutes, and ~20 mL of the DMC oil mixture was pipetted out and transferred to an acid-washed collection flask and tightly capped. Once all 60 mL of DMC-oil mixture was recovered, 2 g of anhydrous sodium sulfide was added to ensure the solution was devoid of water. The DCM-oil solution was then transferred to a 40-mL amber VOA vial, and 1000 uL transferred to an amber GC vial. Both vials were stored at -20°C until analysis on the GC-MS. Once samples were run on the GC-MS, peak integration of chemical profiles were manually conducted using the Agilent Chemstation software, and TPH concentrations were calculated using extraction efficiency, internal standard, and surrogate values.

Corexit component quantification in seawater

Dispersant components were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) in whole-bottle mesocosm and subsampled 6-L incubations for the following compounds using the methods previously described by McFarlin et al. (2018): DOSS, Tween 80 and 85, and Span 80, all known constituents of Corexit (Place et al., 2016), and EHSS, a degradation metabolite of DOSS (Campo et al., 2013; Place et al., 2014). The Tweens could not be quantitated individually and were quantitated as the sum of the concentrations of both (Place

et al., 2016). Percent recovery for this method ranged from 88-119%, and precision reported as the relative standard deviation ranged from 1.4-23%, depending on the analyte (Place et al., 2016).

Molecular microbial analyses

Seawater microbial community structure analyses

Seawater from mesocosms was vacuum filtered on 0.22-µm filters to separate cells from the bulk solution and immediately frozen at -80°C until analysis. Microbial community DNA was extracted from frozen filters using a DNeasy Powerwater (Qiagen, Venlo, Netherlands) commercial extraction kit following the manufacturer's protocol. To study the prokaryotic community structure, the V4 region of the 16S rRNA gene was amplified using indexed 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806RB (5'-GGACTACNVGGGTWTCTAAT-3') primers (Apprill et al., 2015) and sequenced on an Illumina MiSeq using a 2 x 250 bp format.

Sequences were filtered, trimmed, dereplicated into 100% similarity amplicon sequence variants (ASVs), and assigned taxonomy from the SILVA rRNA database (v. 132) using the dada2 bioinformatics pipeline (Callahan et al., 2016; Callahan et al., 2017; Quast et al., 2012). Microbial community data analyses were performed using the PC-ORD V6 statistical software package (PC-ORD v. 6.255 Beta. Gleneden Beach, OR: MjM Software Design; McCune et al., 2002). Non-metric multidimensional scaling (NMDS) plots were used to visualize differences in community structure between treatments and over time (Clarke & Ainsworth, 1993). Statistical significances were determined using PERMANOVA (Anderson, 2001) and non-parametric multi-response permutation procedure (MRPP) tests (Mielke & Berry, 2001). Correlations and modeling of chemical data to community structure were performed using Mantel tests and backward step-wise model selection (Mantel, 1967). All statistical analyses were performed using a 95% confidence interval

Sediment microbial community structure analyses

16S ribosomal (rRNA) amplicon sequencing was conducted on lyophilized sediments to assess the diversity, structure, and composition of prokaryotes in the Beaufort Sea benthos. To prepare samples for sequencing, total genomic DNA was extracted from sediment samples using the Qiagen PowerSoil kit, revised forward (515FB) and reverse primers (806RB) from the Earth Microbiome Project (EMP) were used to amplify the V4 region of the 16S rRNA gene. Library preparation with iTru adapters for sequencing was completed following the standard protocol used by the EMP - a one-step PCR protocol with indexed primers (Apprill et al., 2015; Caporaso et al., 2012; Parada et al., 2016; Walters et al., 2016). The processed samples were sequenced on an Illumina MiSeq at the UAF Institute of Arctic Biology Genomics Core Lab.

The 16S rRNA amplicon sequences were de-multiplexed using the Mr. Demuxy package (Cock et al., 2009). Demultiplexed sequences were run with mothur v1.40.0 on a high performance-computing cluster through UAF Research Computing Systems using a modified MiSeq standard operating procedure (Schloss et al., 2009). Operational taxonomic units (OTUs) were clustered at 100% similarity using OptiClust option in mothur, taxonomy was assigned to OTUs using the SILVA 132 mothur formatted reference database with a bootstrap cutoff of 100%, and the samples in the resulting OTU table were converted to relative abundances (Edgar, 2018; Glöckner et al., 2017; Wang et al., 2007; Westcott & Schloss, 2017). The table comprising the relative abundances of OTUs identified for each sample was used to identify prokaryotes involved in biodegradation of oil in Arctic marine sediments. A combination of nMDS ordination, hierarchical clustering analysis, and area plots was used to assess patterns in microbial communities between different experimental treatments.

Seawater microbial metatranscriptomics analyses

Metatranscriptomics analyses were performed on seawater incubations to examine the gene expression profiles of the microbial communities and their shifts in response to exposure to oil, Corexit, or both. Due to the cost of sequencing, the metatranscriptomic analyses were performed only at 0-, 5-, and 30-day time points. RNA was extracted from filters using a Qiagen RNeasy Lipid tissue kit following the manufacturer's protocol; this kit was selected to help ensure extracts were free of oil. RNA extracts were held at -80°C and shipped on dry ice to the Oregon State University Center for Genome Research and Biocomputing. There, they were quantified, eukaryote ribodepleted, normalized to all contain the same amount of RNA, pooled, and sequenced on an Illumina HiSeq 3000 using a 100-bp paired-end format.

Sequences were processed using the MG-RAST pipeline (v.4.0.3) and analyzed against the RefSeq phylogenetic and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional databases (Glass & Meyer, 2011; Kanehisa et al., 2016; O'Leary et al., 2016). All rRNA sequences were removed from the dataset during processing to focus on metabolic mRNA transcripts. Sequencing efforts generated 2,092,023,528 sequences that passed quality-control filters across 27 individual samples for an average of 76,340,561 sequences per sample.

Statistical analyses were performed using the PC-ORD V6 statistical software package (McCune et al., 2002), which included non-metric multidimensional scaling (NMDS) plots to visualize metatranscriptomes, PERMANOVA tests to evaluate the statistical significance of observed differences in the metatranscriptomes, Mantel tests to correlate the abundance of metabolism gene classes with the overall metatranscriptomes, and ANOVA and Tukey's honest significant difference tests to determine if the relative abundances of genes were different between treatments at a given time point. All statistical tests were performed using a 95% confidence interval.

RESULTS AND DISCUSSION

Oil biodegradation in seawater

Biodegradation of crude oil and the influence of Corexit on oil degradation rates were assessed by quantifying the TPH (C_{10}-C_{30}), n-alkane, branched alkane, and PAH losses over time (Figure 3). While the majority of the alkanes biodegraded within the first ten days, the remaining TPH and PAHs degraded much more slowly over the course of the 30-day incubation. There were no significant differences observed for any of the measured petroleum losses with the addition of Corexit. Evidence of biodegradation contributing to TPH loss was detected on day 10, which was the first time point in which live treatments experienced greater loss ($20\pm3\%$) than abiotic treatments ($7\pm4\%$) ($p=0.0002$; Figure 3a). By the end of the experiment, biotic TPH loss had reached $29 \pm 4\%$ and was significantly higher ($p<0.0001$) than the TPH loss in abiotic treatments ($12\pm2\%$). Based on these findings, both biotic and abiotic processes are important in the early loss of crude oil in the Arctic, with biodegradation becoming significant between 5-10 days for TPH.

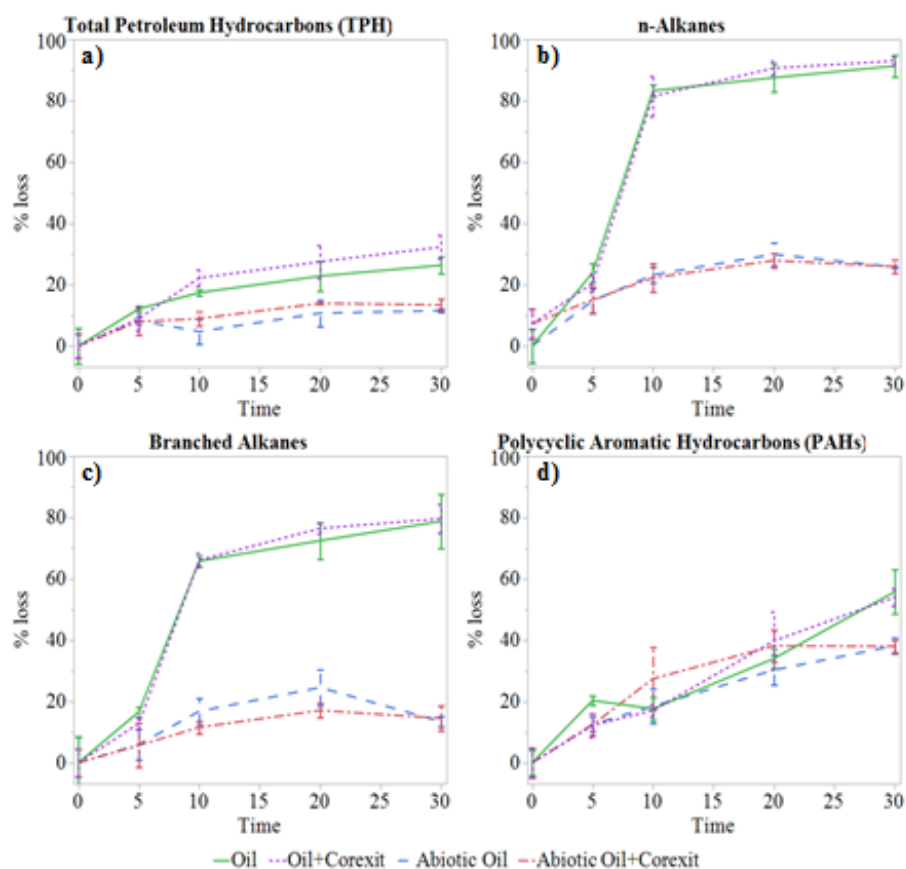

Figure 3. Loss of oil fractions over time in seawater incubations. Mean (n=3) percent loss of a) total petroleum hydrocarbons (TPH), b) n-alkanes, c) branched alkanes, and d) polycyclic aromatic hydrocarbons (PAHs). The error bars denote one standard deviation from the mean.

The loss of individual petroleum compounds followed the well-documented pattern of degradation, with smaller, simpler, and saturated hydrocarbon compounds degrading more readily than larger, complex, and conjugated compounds, such that loss of n-alkanes>branched alkanes>PAHs (Table 1). When comparing the loss of the different hydrocarbon compound classes to TPH loss, the hydrocarbons remaining in live treatments at 30 days were largely composed of PAHs and other recalcitrant hydrocarbons present in the unresolved complex mixture. While linear and branched alkanes showed relatively rapid degradation by Arctic microorganisms, the slower degradation of the remaining oil components raises concern for the long-term effects in the environment due to the toxic and bioavailable nature of these compounds (Booth et al., 2007; Gardiner et al., 2013; Petersen et al., 2017; Thomas et al., 1995).

Table 1. Mean and standard deviation of total (biotic+abiotic) and abiotic percent losses of crude oil compounds in Arctic seawater incubations.

Treatment	Time (days)	Total Petroleum Hydrocarbons		n-alkanes	
		Total loss	Abiotic loss	Total loss	Abiotic loss
Oil	5	12.1±0.9	8.3±1.3	23.9±3.1	14.7±4.1
	10	17.4±1.0	4.7±4.1	83.4±2.0	23.2±2.5
	20	22.8±4.9	10.7±4.3	87.6±4.7	29.9±3.7
	30	26.3±2.8	11.6±0.6	91.4±3.6	25.8±0.4
Oil+Corexit	5	9.0±3.9	8.0±4.4	20.4±2.9	15.1±4.3
	10	22.2±2.7	8.9±2.3	81.6±6.6	22.3±4.6
	20	27.5±5.4	13.9±0.3	90.7±2.2	27.9±2.3
	30	32.3±3.8	13.4±1.9	93.1±1.3	25.9±2.2

Treatment	Time (days)	Branched alkanes		Polycyclic Aromatic Hydrocarbons	
		Total loss	Abiotic loss	Total loss	Abiotic loss
Oil	5	16.6±1.7	6.0±5.0	20.3±1.6	12.6±2.3
	10	65.9±1.8	16.8±4.3	17.6±3.8	18.5±5.6
	20	72.5±6.0	24.6±5.9	34.0±3.1	30.3±4.9
	30	78.8±8.8	13.5±1.6	55.8±7.2	38.2±2.6
Oil+Corexit	5	12.8±1.9	5.7±7.1	12.2±3.8	12.4±3.5
	10	66.2±2.4	11.6±1.8	17.1±2.0	27.5±10.3
	20	76.5±1.8	17.1±2.3	39.9±9.5	38.2±5.3
	30	79.7±4.7	14.5±4.1	54.1±2.8	38.0±2.0

These findings support previous studies reporting that crude oil biodegradation can occur in the Arctic marine environment but more slowly than in temperate regions. In a similarly designed experiment by McFarlin et al. (2014), TPH percent losses from 2.5 ppm oil in -1°C Alaskan Arctic seawater (obtained from the same location as this study) were 36% and 45% after 10 and

28 days, respectively. Percent loss was slightly lower in this study; 20±3% after 10 days and 29±4% after 30 days. The lower percentages of TPH loss observed here can be attributed to the higher concentrations of oil used (50 ppm in this study vs. 2.5 ppm by McFarlin et al., 2014) and, potentially, to increased weathering of the stock oil supply over time. Despite these differences, both experiments show lower extents of oil loss than in temperate regions. For example, a similarly designed incubation of New Jersey seawater with 2.5 ppm of ANS crude oil at 8°C observed 51% and 69% TPH loss at 11 and 24 days, respectively (Prince et al., 2013).

While no differences in TPH, n-alkane, branched alkane, or PAH loss were observed with the addition of Corexit in this experiment, similar seawater incubation studies have noted some dispersant-associated effects. McFarlin et al. (2014) observed increased TPH loss with the addition of Corexit at 10 days (47% loss with Corexit, 36% loss without) and 28 days (54% TPH loss with Corexit, 45% loss without). Similarly, Prince et al. (2013) observed increased TPH loss with the addition of Corexit at 11 days (64% loss with Corexit, 51% without) and 24 days (77% loss with Corexit, 69% without). In those studies, oil and Corexit were pre-mixed prior to their addition to seawater, which may influence the effect of Corexit on oil degradation. In this study, oil and Corexit were added separately. Studies have reported inhibition of oil degradation with the addition of Corexit (Kleindienst et al., 2015; Rahsepar et al., 2016); however, the experimental conditions varied considerably from this study (e.g., use of very low oil concentrations, oil fractions, and enrichment cultures), precluding direct comparisons. The incubation test methods applied here were not optimized to replicate a comparison of an oil slick to dispersed oil (see Prince & Butler, 2014). Instead, this study mimicked the relatively low concentrations of oil and dispersant associated with a dispersed oil plume to provide more realistic estimates of whole-oil biodegradation following dispersion and assessment of the sequence and potential interactions of petroleum and dispersant component biodegradation.

Dispersant degradation in seawater

Analysis of the surfactant constituents of Corexit revealed the rapid degradation of the non-ionic surfactants but was inconclusive for DOSS due to high analytical variability (Figure 4). Relative to the non-ionic surfactants, DOSS measurements showed substantial variation between replicates, compared to the other Corexit components. Concentrations of DOSS in individual samples were consistent with the previously reported accuracy for the analytical methods (Place et al., 2016), yet were variable even in the whole-bottle mesocosms. This variability occurring even when entire mesocosms were solvent extracted suggests that DOSS measurement variation may occur as an innate property of the compound, such as its surface-active behavior.

DOSS concentrations were comparable in both destructively harvested and subsampled incubations and did not show significant differences between treatments over time until 30 days (Figure 4a). By the end of the 30-day incubation, a 91±13% loss of DOSS was observed for Corexit alone and 48±41% loss for Corexit in the presence of oil. The loss of DOSS in Corexit-only treatments was significantly higher (p=0.0018) than the abiotic controls (0±23% loss),

suggesting biodegradation as a mechanism for loss. However, in treatments containing both oil and Corexit together, there was no difference in DOSS degradation between the biotic and abiotic conditions. It was also noted that, during analysis, recovery and measurement of DOSS in treatments containing oil was more challenging than with Corexit alone, contributing to the observed variation. EHSS, a metabolite of DOSS, accumulated significantly more in treatments containing both crude oil and Corexit than in other treatments by day 6 ($p<0.0001$) in the large-volume subsampled incubations and remained high throughout the remainder of the experiment in both incubation types (Figure 4b). This increase in EHSS concentrations suggests that, although direct quantitation of DOSS itself was inconclusive due to variation in the data, DOSS transformation may be occurring. EHSS concentrations increased for treatments containing both crude oil and Corexit but did not increase for treatments containing only Corexit. The prolonged presence of EHSS and decreased DOSS loss suggests that DOSS mineralization beyond EHSS was delayed or incomplete in the presence of oil.

In contrast, the non-ionic surfactants (Tween 80, Tween 85, Span 80) showed rapid losses with and without the presence of oil. Tween 80 and 85 concentrations (Figure 4c) had fallen below limits of quantitation (LOQ) in both biotic treatments by day 5 (60,000 ng/L) and Span 80 (Figure 4d) dropped below LOQ (60,000 ng/L) by day 3, suggesting that rapid degradation of both components can occur in Arctic environments. Decreases of all non-ionic surfactants in abiotic treatments by 30 days were also observed, suggesting that abiotic transformation and loss of these analytes were occurring in addition to biotic processes.
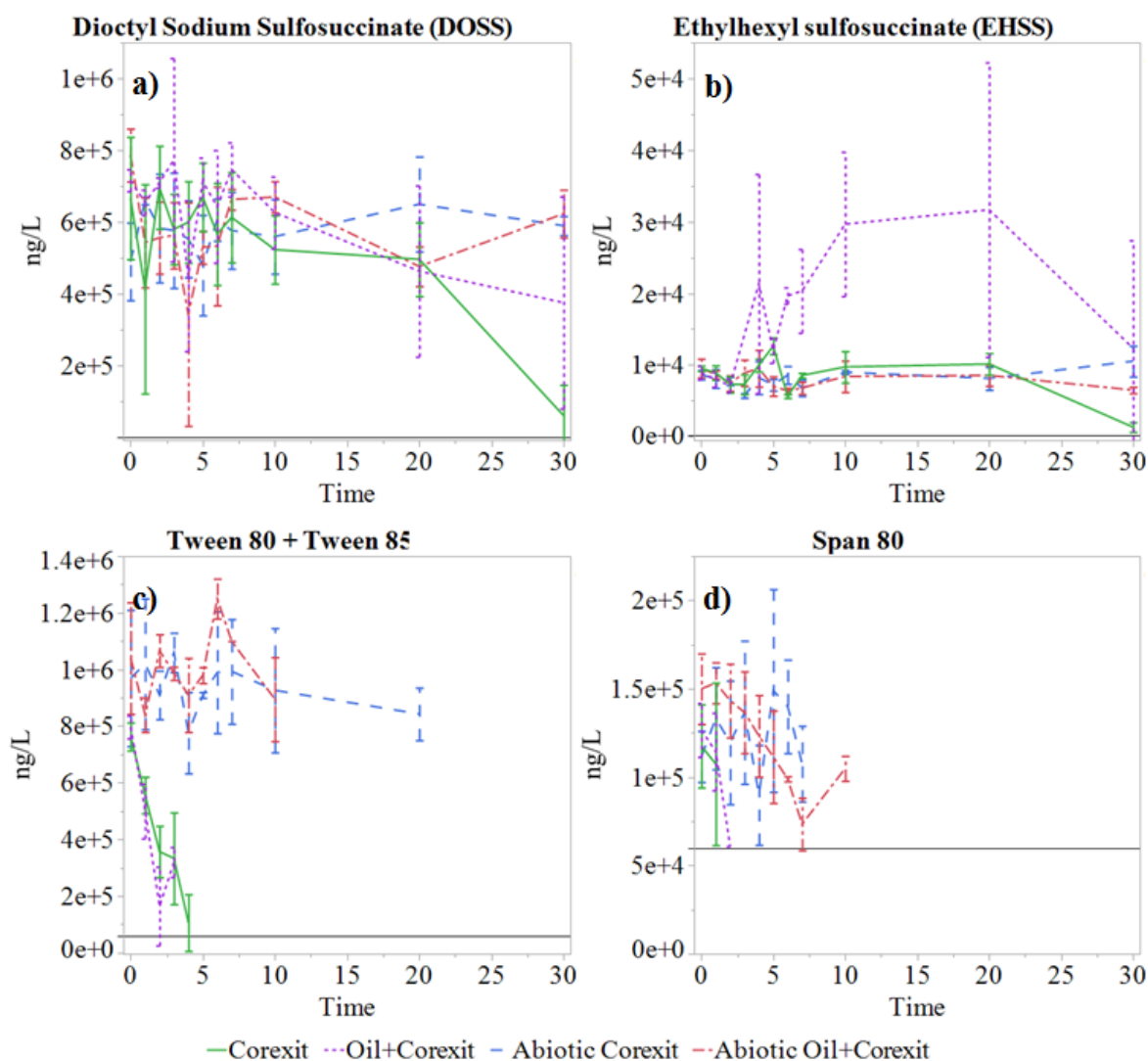
Figure 4. Corexit surfactant component loss in seawater incubations. Mean (n=3) concentrations in subsampled 6-L mesocosms over time of Corexit constituents or suspected metabolites a) DOSS, b) EHSS, c) Tweens 80 and 85, and d) Span 80. The error bars denote one standard deviation from the mean, and the horizontal lines in each panel represent the limit of quantitation for that analyte (200 ng/L for DOSS and EHSS, 60,000 ng/L for the Tweens and Span 80).

Previous studies of the fate of Corexit have primarily focused on the degradation of the most abundant constituent, DOSS. As in our study, large variations for DOSS measurements over time have been observed by others (Kleindienst et al., 2015; McFarlin et al., 2018), underscoring the difficulty of reliably quantifying it in experiments. McFarlin et al. (2018) performed Arctic seawater incubations at 2°C and observed 98% total loss and 21% abiotic loss of DOSS in offshore seawater at 28 days but only 35% total and 2% abiotic loss in nearshore seawater. In experiments using Gulf of Mexico seawater at 8°C, Kleindienst et al. (2015) found an 8% loss of DOSS for Corexit alone and ~30% loss of DOSS for Corexit with oil at four weeks. In an

experiment using cultures isolated near the Macondo wellhead in the Gulf of Mexico, Campo et al. (2013) found that DOSS did not undergo substantial degradation within 28 days at 5°C; however, at 25°C, the cultures exhibited rapid and complete DOSS degradation within 14 days. Similar results were observed by Techtmann et al. (2017), who used the same cultures and experimental conditions, with the majority of degradation at 25°C occurring within 20 days, but no observable degradation at 5°C over the course of the 56-day experiment. Our current and previous studies (McFarlin et al., 2018) demonstrated little abiotic loss of DOSS in Arctic seawater, which is supported by evidence that DOSS does not undergo significant hydrolysis or photolytic degradation under simulated solar conditions (Glover et al., 2014; Perkins, 2017).

While some mesocosm and culture-based experiments have observed rapid degradation of DOSS, *in situ* measurements in the Gulf of Mexico following the DWH spill suggest that DOSS may be more recalcitrant in the environment. Water samples collected by Kujawinski et al. (2011), 64 days after dispersant applications had ceased for the DWH spill, demonstrated the persistence of DOSS, which was present in concentrations significantly higher than those that were predicted by dilution and transport. Additionally, White et al. (2014), observed DOSS concentrations ranging from 6-9,000 ng/g in coral communities six months after the spill and 1-260 ng/g in beach sands 26-45 months after. The presence of DOSS in Gulf of Mexico sediments was confirmed as well as the discovery of DOSS in settling particles (Perkins et al., 2017). This disparity between the results of laboratory experiments and *in situ* measurements may be due to laboratory conditions not accurately replicating environmental conditions. Based on current findings, it remains unclear if DOSS degrades, either biotically or abiotically, to an appreciable extent in the environment when large quantities are applied following an oil spill.

In contrast to DOSS, the non-ionic surfactant components of Corexit (Tween 80, Tween 85, and Span 80) underwent rapid losses, falling below LOQs within 3-5 days. Based on initial concentrations, this represents a ≥93% loss for the Tweens at 5 days in biotic treatments and 30 days for abiotic treatments. However, the extent of the loss of Span 80 below the LOQ was more uncertain, with the LOQ representing ≥53% loss based on initial concentrations. This LOQ was reached at 3 days in biotic treatments and 20 days in abiotic treatments. The rapid loss of the non-ionic surfactants demonstrated here has been observed in other seawater incubation experiments. Kleindienst et al. (2015) observed concentrations of Tweens and Span 80 falling below detection limits (20 μg/L for Tweens and 36 μg/L Span 80) within one week using Gulf of Mexico seawater at 8°C; this represented ~99.7% of the Tweens and ~87% of Span 80. McFarlin et al. (2018) also observed near complete (>97%) loss of Span 80 in 28 days at 2°C using both nearshore and offshore Arctic seawater. The loss of Span 80 was attributed to biodegradation since much lower losses were observed for abiotic treatments (46% offshore and 1% nearshore at 28 days). The Tweens also underwent rapid loss (>99% total loss) for nearshore seawater at 10 days, but more was attributed to abiotic loss (82%) (McFarlin et al., 2018). These findings suggest that the non-ionic surfactant components of Corexit are rapidly lost from the

environment through both biotic and abiotic processes, though the pathways and mechanisms underlying this have yet to be determined.

During TPH analysis, an unexpected set of peaks was detected by GC/MS in treatments of oil with the addition of Corexit (not present in oil-only treatments). The compound represented by these peaks was identified based on its mass spectra as 1-(2-Butoxy-1-methylethoxy)propane-2-ol (Figure 5), which is also known as dipropylene glycol n-butyl ether (DGBE). DGBE is an industrial chemical used as a solvent, chemical reaction intermediate, insecticide, and surfactant that has been identified as a solvent component of Corexit (Parker et al., 2014). Relative to the components of the crude oil, significant DGBE remained at the end of the incubation series. Due to its complex chemical structure, DGBE may be resistant to biodegradation in the presence of more labile compounds in crude oil and Corexit, such as n-alkanes and non-ionic surfactants. Loss of this compound could not be accurately quantitated from the analytical methods used here, which were intended to measure the loss of compounds present in oil. DGBE concentrations likely had high variability in mesocosms due to the extremely small volumes of Corexit used in this experiment. However, it is noteworthy that DGBE was detectable in significant amounts relative to crude oil compounds by the end of the 30-day incubation, which is an important environmental consideration. While there have not been any mesocosm-based experiments performed to study DGBE degradation, *in situ* persistence was observed in nearshore water and sediment samples and offshore water samples four months after the DWH spill (Operational Science Advisory Team, 2010). Although DGBE has low acute toxicity compared to oil, it has acute effects on the liver, and the implications of chronic exposure are unknown (Johnson et al., 2005; Organization for Economic Co-operation and Development, 2003). This component of Corexit has not been studied as extensively as other constituents, and future study is warranted.
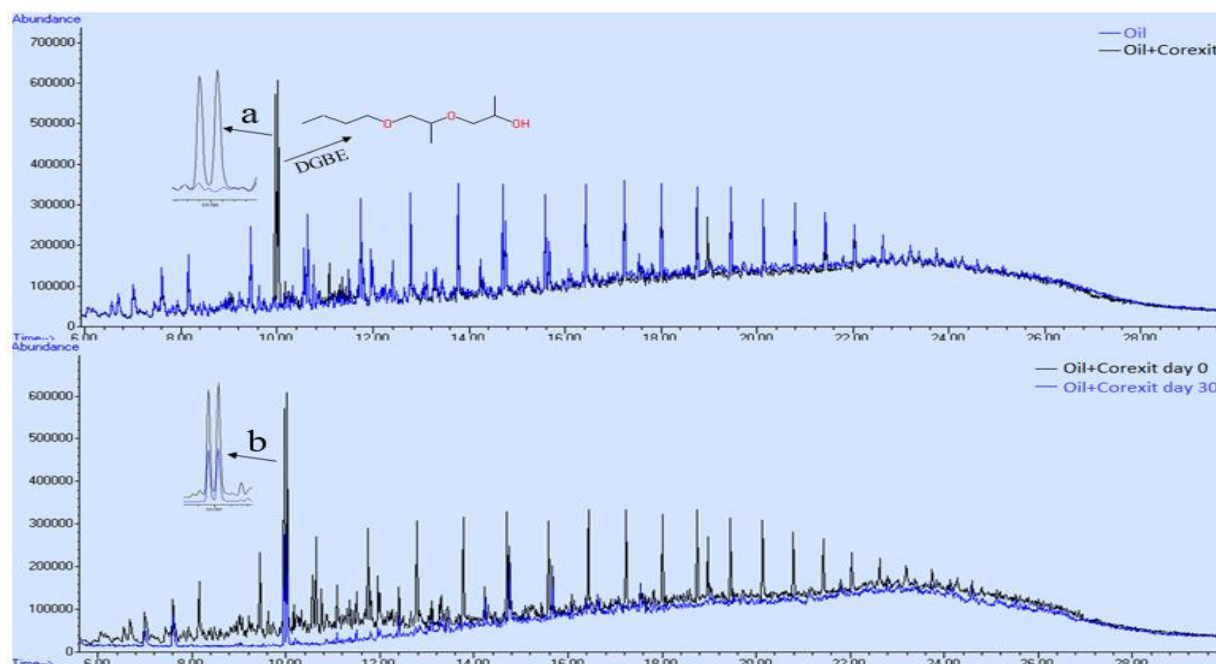
Figure 5. GC/MS chromatogram of oil+Corexit from seawater incubation samples. A peak (a) identified as dipropylene glycol n-butyl ether (DGBE) was not present in oil-only samples and remained present in significant amounts by the end of the 30-day incubation (b).

Seawater microbial community structure

Changes in the prokaryotic microbial community structure were evaluated by analyzing bacterial and archaeal 16S rRNA gene amplicon sequences. Experimental treatments and time both had significant effects on microbial community succession (Figure 6). Communities associated with the co-presence of oil and Corexit (oil+Corexit) treatment began with structures similar to Corexit-only treatments and became more similar to oil-only treatments as the incubation progressed (Figure 7). Generally, different taxa and individual ASVs responded to the presence of either oil or Corexit, although some taxa and ASVs responded to multiple treatments. Parameters correlated with community structure and the proliferation of specific taxa included the presence of petroleum, Corexit, nitrogen compounds, dissolved oxygen, and pH (Figure 6, Table 2).
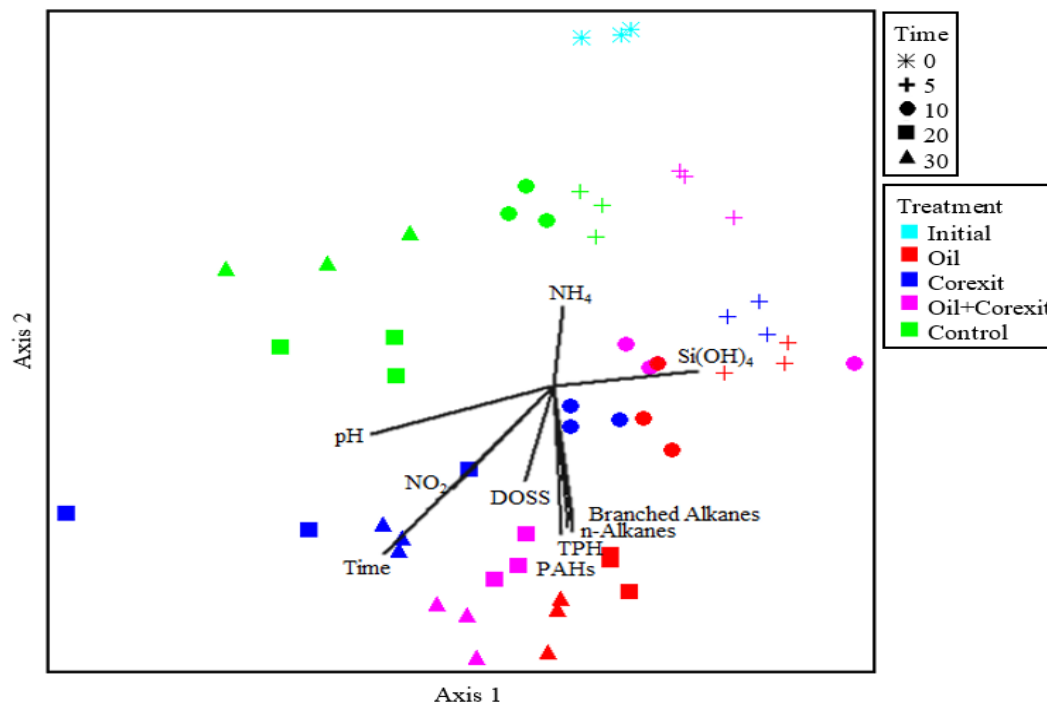
Figure 6. Prokaryotic community structure in seawater incubations with oil +/- Corexit. Bray-Curtis NMDS ordination of the prokaryotic microbial community structure and correlating environmental parameters over time exposed to crude oil, Corexit, or oil+Corexit.
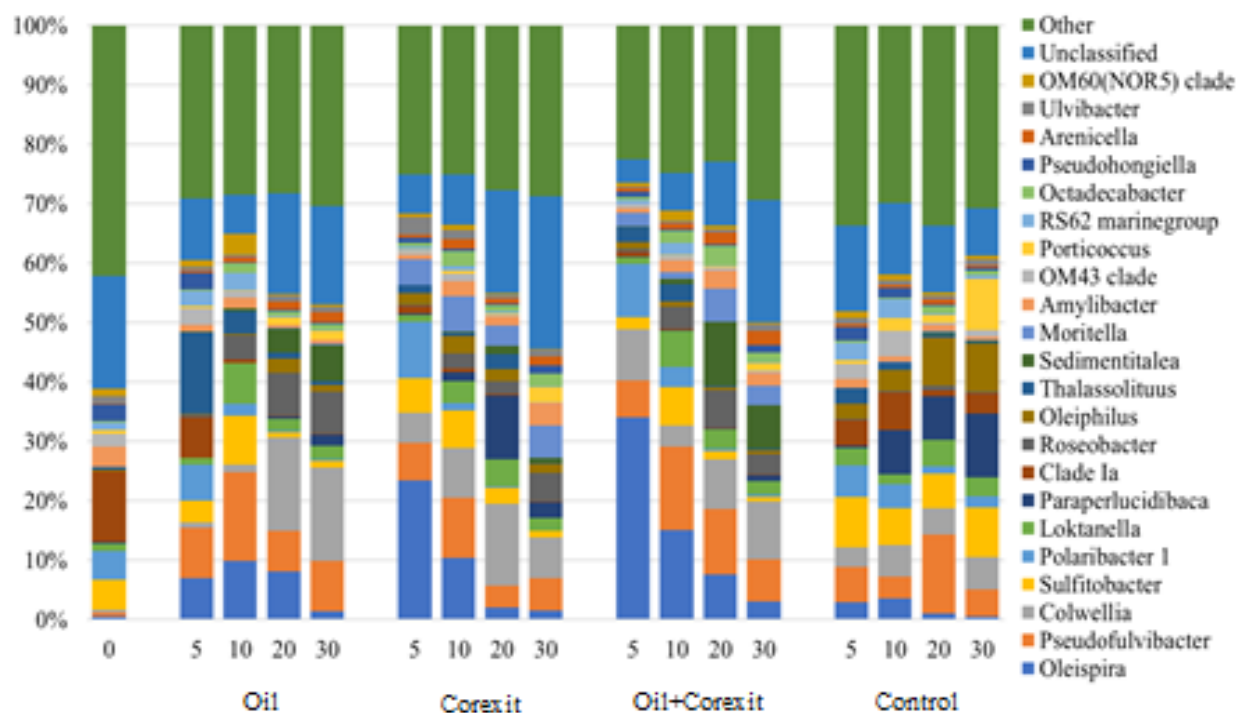
Figure 7. Prokaryotic community structure based on 16S rRNA sequence analyses associated with different treatments in seawater incubations. Mean (n=3) relative abundance of prokaryotic microbial community taxa at the genus level for experimental treatments over time.

Table 2. Relationships of environmental parameters to the prokaryotic microbial community structure in Arctic seawater mesocosms determined using Mantel tests. All relationships in the table below are significant (95% confidence interval). Moderate to strong correlations (r>0.5) are in bold. Note that the following parameters are strongly correlated with time: TPH (r=0.775, p<0.0001), n-alkanes (r=0.573, p<0.0001), branched alkanes (r=0.638, p<0.0001), PAHs (r=0.978, p<0.0001), and DOSS (r=0.801, p<0.0001).

|	Mantel correlation coefficient (r)	p-value		Mantel correlation coefficient (r)	p-value
All samples			*Oil-containing treatments*		
Time	**0.597**	**0.0010**	**Time**	**0.774**	**0.0010**
Si(OH)$_4$	0.182	0.0070	**TPH**	**0.758**	**0.0010**
NO$_2$	0.268	0.0010	**n-Alkanes**	**0.681**	**0.0010**
NH$_4$	0.236	0.0020	**Branched alkanes**	**0.728**	**0.0010**
pH	0.347	0.0010	**PAHs**	**0.669**	**0.0010**
DO	0.101	0.0200	PO$_4$	0.253	0.0250
			Si(OH)$_4$	0.281	0.0071
			NO$_3$	0.315	0.0050
Day 5			NO$_2$	0.396	0.0010
No significant (p<0.05) relationships observed			**pH**	**0.620**	**0.0010**
Day 10					
NH$_4$	0.372	0.0030	*Corexit-containing treatments*		
pH	0.391	0.0090	**Time**	**0.588**	**0.0010**
DO	0.374	0.0170	EHSS	-0.164	0.0160
			DOSS	0.273	0.0320
Day 20			NO$_3$	0.416	0.0010
NO$_2$	0.345	0.0460	NO$_2$	0.355	0.0330
			pH	0.384	0.0020
Day 30			DO	0.205	0.0470
Si(OH)$_4$	0.289	0.0330			
NO$_3$	0.233	0.0400			
DO	**0.619**	**0.0010**			

The community structures of all samples appear to cluster by treatment (Figure 6), with the oil+Corexit community representing a mixture of the oil-only and Corexit-only communities. An MRPP test demonstrated the observed clustering was statistically significant (p<0.0001) and all pairwise comparisons significant (p<0.032), indicating that the microbial community of each treatment was significantly different from one another. A PERMANOVA test of all treatments and time points found significant effects of treatment (p=0.0002), time (p=0.0002), and the interactions of both (p=0.0002). The influence of time as a significant factor driving the microbial community structure is indicative of succession. Comparisons were also made at each time point to isolate the influence of the treatments applied. MRPPs showed a significant difference between treatments (p<0.0004 for each time point), and all pairwise comparisons were significant (p<0.033) except for oil and oil+Corexit at day 10 (p=0.8920).

The communities associated with the oil+Corexit treatment contained a combination of the genera stimulated by the presence of oil or Corexit alone (Figure 7). At day 5, the oil+Corexit community was more similar to the Corexit-only treatments, and, after 10 days, it was more similar to the oil-only treatments. The chemical analyses mirror this trend, showing a rapid loss of the non-ionic surfactants in Corexit due to degradation within the first 5 days and oil not undergoing extensive degradation until after 10 days. The patterns of community change and chemical losses suggest that labile Corexit components are initially preferentially degraded over the less labile oil compounds (Kleindienst et al., 2015). However, this did not appear to impact oil degradation negatively. Previous studies also found a distinct separation of community structure between incubations containing oil or Corexit separately (Kleindienst et al., 2015; McFarlin et al., 2018; Techtmann et al., 2017), with an overlap in the oil+Corexit communities (Techtmann et al., 2017).

Several taxa increased significantly ($p<0.05$) in relative abundance in response to Corexit compared to controls, including *Colwellia*, *Polaribacter*, *Moritella*, *Octadecabacter*, and *Amylibacter* (Figure 7). Taxa that experienced significant ($p<0.05$) increases in response to oil included *Thalassolituus* and *Sedimentitalea*. Taxa that significantly increased ($p<0.05$) in abundance in the presence of oil, Corexit, or oil+Corexit included *Oleispira, Pseudofulvibacter,* and *Roseobacter.* Backward, step-wise model selection for individual genera revealed that the relative abundances of organisms stimulated by the presence of oil or Corexit were predicted by concentrations of those respective chemical components and nutrients (Table 3). Genera stimulated by both oil and Corexit were predicted by compounds from both, implicating these taxa as potential biodegradative organisms. For these taxa, individual ASVs were frequently shared across all experimental treatments and enriched relative to controls. ASVs that were identified to be unique to either oil or Corexit treatments by indicator species analysis were found to be present in treatments containing both, indicating that the addition of Corexit did not suppress the proliferation of oil-degrading bacteria.

Many of the taxa stimulated by oil or Corexit here have been observed in previous *in situ* measurements and incubation studies from a variety of marine environments, including *Oleispira* (Bælum et al., 2012; Coulon et al., 2007; Hazen et al., 2010; Ribicic et al., 2018a), *Colwellia* (Bælum et al., 2012; Brakstad et al., 2008; Dubinsky et al., 2013; Kleindienst et al., 2015; McFarlin et al., 2018; Redmond & Valentine, 2012; Tremblay et al., 2017), *Moritella* (McFarlin et al., 2018), *Octadecabacter* (Brakstad et al., 2008), *Thalassolituus* (Coulon et al., 2007; Crisafi et al., 2016; Hazen et al., 2010; McKew et al., 2007; Tremblay et al., 2017), and *Roseobacter* (Coulon et al., 2007; McKew et al., 2007).

Table 3. Backward stepwise models selected for individual genera using the lowest corrected Akaike and Bayesian Information Criterion from Arctic seawater mesocosm microbial community data.

Final model	R^2	Adjusted R^2	P values
Amylibacter = DOSS+Time+(DOSS*Time)	0.65	0.59	Overall<0.0001 DOSS=0.0007 Time=0.0433 DOSS*Time=0.0189
Colwellia = PAHs	0.30	0.27	Overall=0.0051
Moritella = Treatment	0.71	0.69	Overall<0.0001
Octadecabacter = DOSS+(DOSS*PAHs)	0.88	0.85	Overall<0.0001 DOSS=0.0002 DOSS*PAHs<0.0001
Oleispira = n-alkanes+DOSS	0.95	0.94	Overall<0.0001 n-alkanes=0.0009 DOSS=0.0118
Polaribacter = DOSS+(DOSS*pH)	0.80	0.78	Overall<0.0001 DOSS<0.0001 DOSS*pH<0.0001
Pseudofulvibacter = EHSS+NO_3+PO_4	0.71	0.66	Overall<0.0001 EHSS<0.0001 NO_3=0.0024 PO_4=0.0427
Roseobacter = PAHs+DOSS+NH_4	0.93	0.90	Overall<0.0001 PAHs<0.0001 DOSS<0.0001 NH_4=0.0051
Sedimentalea = PAHs+EHSS+(PAHs*EHSS)	0.90	0.86	Overall=0.0002 PAHs<0.0001 EHSS=0.0024 PAHs*EHSS=0.0081
Thalassolituus = TPH+NO_3+PO_4	0.90	0.88	Overall<0.0001 TPH=0.0003 NO_3<0.0001 PO_4=0.0003

The response of *Amylibacter* to Corexit and *Pseudofulvibacter* (previously unclassified) to both oil and Corexit described here has not been reported elsewhere. While some taxa have been demonstrated to be stimulated only by oil (e.g., *Thalassolituus,* Coulon et al., 2007; McKew et al., 2007, and *Cytoclasticus,* Brakstad et al., 2018; Coulon et al., 2007; Kleindienst et al., 2015; McKew et al., 2007; Tremblay et al., 2017) or Corexit (e.g., *Moritella*, McFarlin et al., 2018), others have been shown to be stimulated by both (e.g., *Colwellia*, Bælum et al., 2012; Brakstad

et al., 2008; Kleindienst et al., 2015; McFarlin et al., 2018; Tremblay et al., 2017, *Oleispira,* Bælum et al., 2012; Brakstad et al., 2008; Coulon et al., 2007; McFarlin et al., 2018, and *Polaribacter,* Brakstad et al., 2018; McFarlin et al., 2018; Prabagaran et al., 2007). The variety of different responsive taxa observed may be due to the number of environmental conditions that can influence community succession. For example, Redmond & Valentine (2012) observed that the abundance of *Colwellia* in the Gulf of Mexico samples was inversely proportional to temperature. Techtmann et al. (2017) also observed a dramatic influence of temperature on community composition. Yakimov et al. (2007) noted that biogeography influences communities, with *Cycloclasticus* and *Thalassolituus* widely distributed and *Oleispira* more common at high latitudes. The sharing of phylogenetic units (oligotypes, OTUs, ASVs) across oil and Corexit treatments has been observed in temperate (Kleindienst et al., 2015) and Arctic regions (McFarlin et al., 2018), suggesting that some organisms may be capable of degrading both oil and Corexit. This agrees with findings (McFarlin et al., 2018) that the expression of some oil degradation genes (e.g., *alkB*) increased in response to exposure to oil or Corexit in Arctic seawater incubations.

Microbial metagenomics analyses of seawater communities

Overview

The metatranscriptomes of seawater microbial communities exposed to crude oil, Corexit, and oil+Corexit were all significantly different from an unexposed community and to each other (Figure 8). Visualization of the metabolic metatranscriptomes with an NMDS plot and a PERMANOVA test showed significant grouping by the treatments applied (p=0.0002), time (p=0.002), and the interaction of both of these factors (p=0.0016; Figure 8), with all pair-wise comparisons significant (p<0.02). The total number of reads originating from bacteria was significantly different between treatments, with reads from treatments containing Corexit significantly higher than oil and the control at day 5, and oil+Corexit reads significantly higher than Corexit alone (p=0.0002; Figure 9). RNA polymerase B (rpoB) expression was also significantly higher for Corexit and oil+Corexit treatments than the oil-only and the control at day 5 (p=0.0010; Figure 10), with reads for oil+Corexit higher than Corexit-only (although not statistically different, p>0.05), indicating that overall gene expression was significantly increased for treatments containing Corexit early in the incubation relative to oil-only and the control.
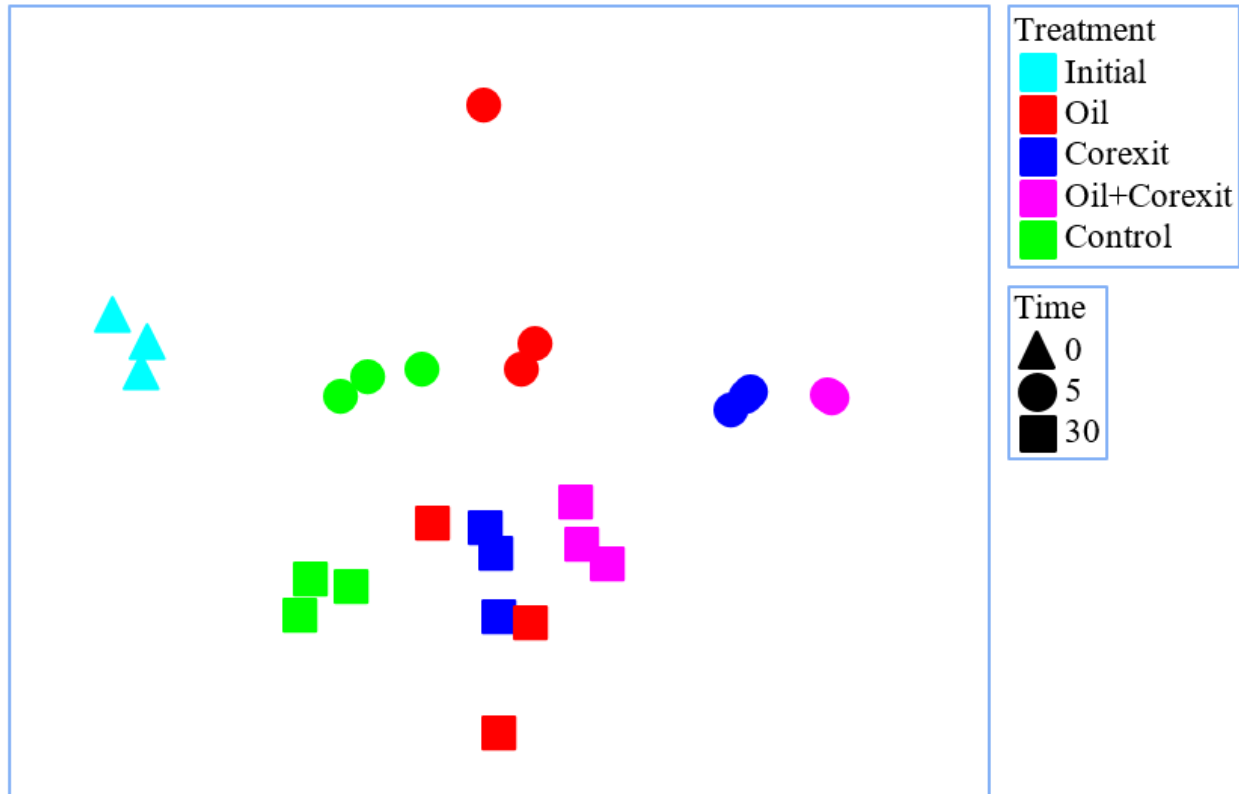
Figure 8. Bray-Curtis NMDS of metatranscriptomes from seawater oil/Corexit mesocosms. The NMDS shows the metatranscriptomes observed for each sample at 0, 5, and 30 days in Arctic seawater mesocosms amended with Corexit and/or oil or no treatment (control). A PERMANOVA test was performed for the effects of the treatments applied (p=0.0002), time (p=0.002), and the interaction of both of these factors (p=0.0016), with all pair-wise comparisons between different treatments significant (p<0.02).

The presence of Corexit appears to markedly increase microbial activity soon after application, particularly when present with oil (Figure 9, Figure 10). Stimulation of bacterial communities by Corexit alone or with oil has been observed in other seawater incubation studies using respirometry (McFarlin et al., 2014), cell counts (Hazen et al., 2010; Kleindienst et al., 2015; Lindstrom & Braddock, 2002), and qPCR of the 16S rRNA gene (McFarlin et al., 2018), which all increased in the presence of Corexit relative to controls. The observation that microbial communities become more active when incubated with oil+Corexit than oil alone agrees with the theory that Corexit can enhance oil biodegradation by making oil more bioavailable to microorganisms (Lee et al., 2013; Lessard & DeMarco, 2000; Prince, 2015). In this experiment, the number of total and metabolic transcripts returned to basal levels by 30 days. However, this may be different *in situ*, where much larger water, oil, and dispersant volumes are involved.
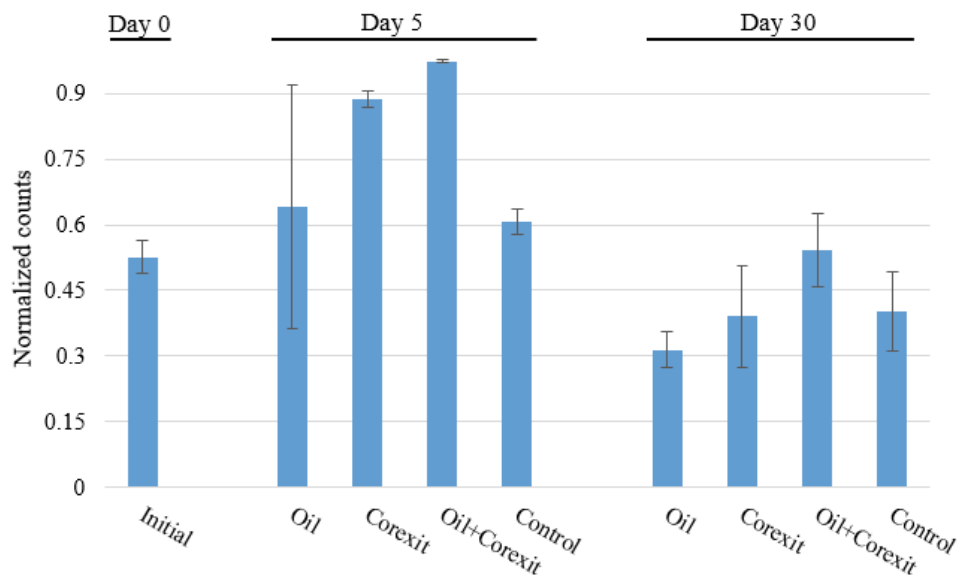
Figure 9. Normalized transcript counts originating from bacteria. The histogram above depicts the total numbers of reads for bacterial gene transcripts in Arctic seawater mesocosms amended with Corexit and/or oil or no treatment (control) at 0, 5, and 30 days incubation. Wald tests with Benjamini-Hochberg adjusted p-values were performed to assess the relative expression of gene transcripts between treatments, revealing p=0.0145 at 5 days. Error bars represent one standard deviation from the mean.
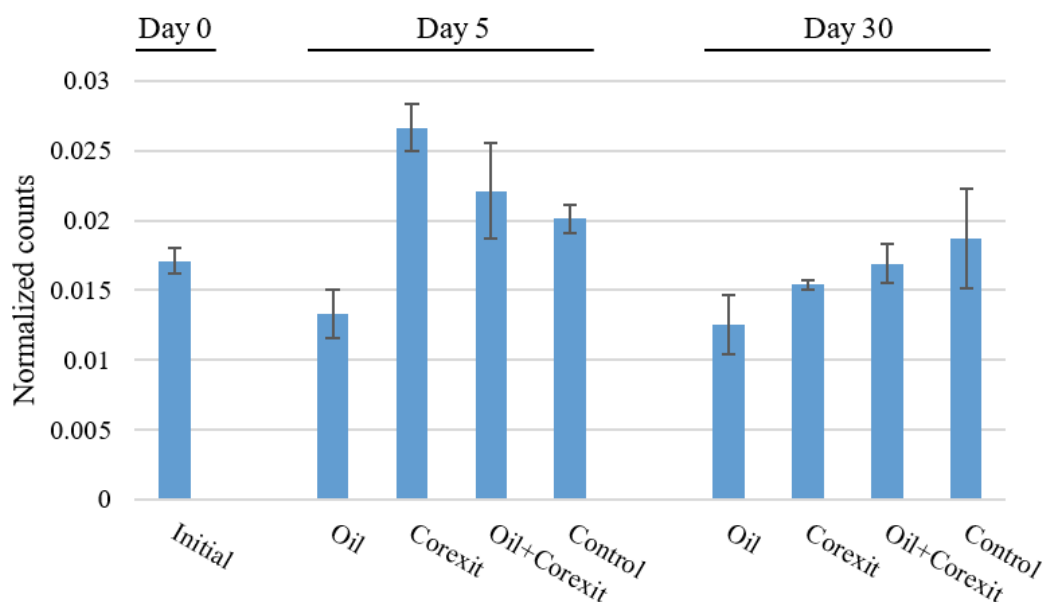
Figure 10. Normalized counts of RNA polymerase B (rpoB). The histogram above depicts the normalized counts of RNA polymerase B gene transcripts at 0, 5, and 30 days in Arctic seawater mesocosms amended with Corexit and/or oil or no treatment (control). Wald tests with Benjamini-Hochberg adjusted p-values were performed to assess the relative expression of gene transcripts between treatments, revealing p<0.0001 at 5 days. Error bars represent one standard deviation from the mean.

The trend of increased gene expression for treatments containing Corexit between days 0 and 5 was observed not only for the total number of metabolic reads but also for the relative abundance of several specific classes of functional transcripts, including lipid, fatty acid (Figure 11), respiration, nitrogen, and sulfur metabolism (Figure 12). A vector analysis of these metabolic gene classes relative to the functional metatranscriptome of each sample (Figure 8) revealed strong correlations (Mantel correlation coefficient, $r>0.70$) associating energy metabolism genes, including oxidative phosphorylation and photosynthesis, with the microbial metatranscriptomic structure (Table 4). Moderate correlations ($0.45<r<0.70$) to metatranscriptomes also occurred for lipids, fatty acids, nitrogen, and sulfur metabolism genes (Table 4). The correlations of these functional gene classes to the overall metatranscriptome suggest that energy metabolism and lipid genes are largely responsible for the differences observed in the metatranscriptomes of the different treatments. Interestingly, total reads of photosynthesis-related transcripts were significantly lower ($p=0.0002$) in all experimental treatments relative to the control, indicating that photosynthesis was depressed during post-spill metabolism, which has been reported for oiled (González et al., 2009) and dispersed oil (Hsiao et al., 1978; Lewis & Pryor, 2013) mesocosms, and *in situ* following the DWH spill (Mishra et al., 2012).
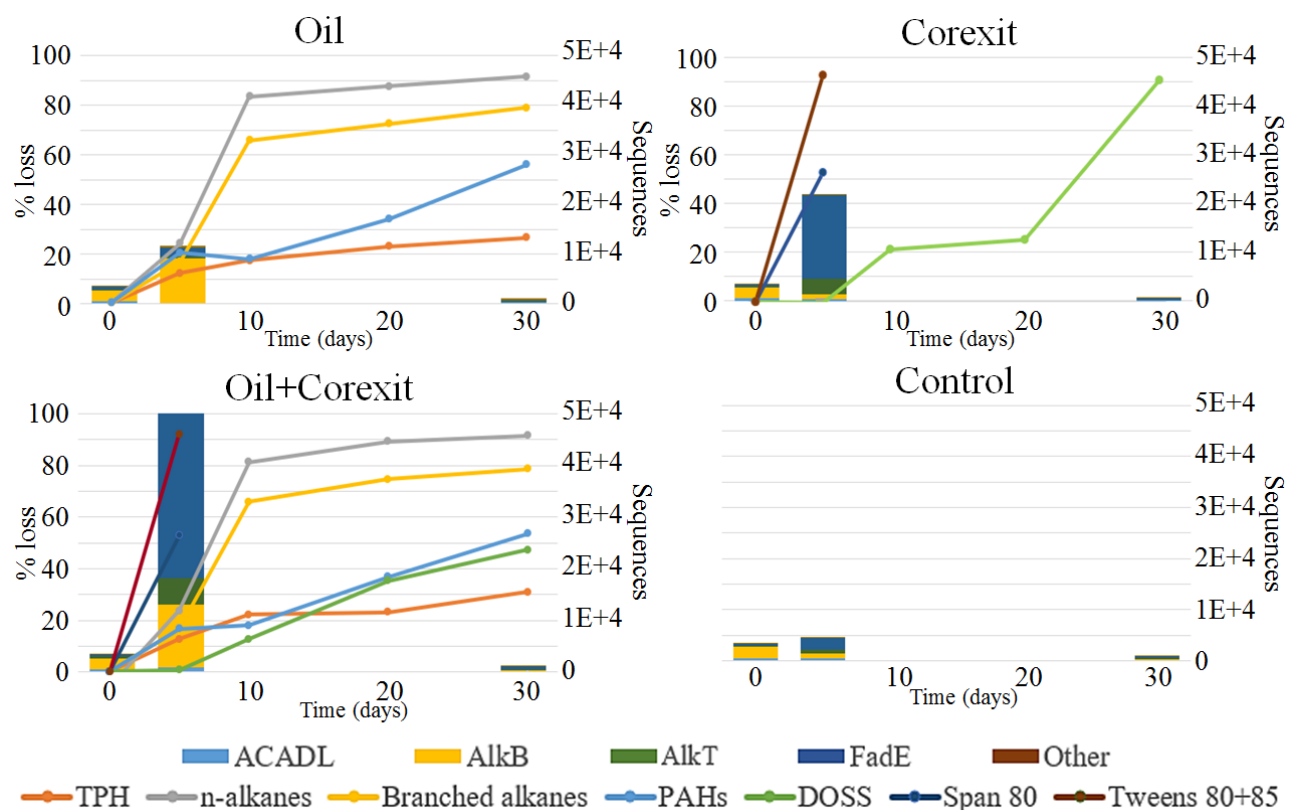
Figure 11. Total reads of fatty acid degradation gene transcripts and chemical loss. The figure above shows fatty acid degradation gene transcripts (bars) and the extent of chemical loss (lines) at 0, 5, and 30 days for four treatments: oil only, Corexit only, oil+Corexit, and the unamended control in Arctic seawater mesocosms.
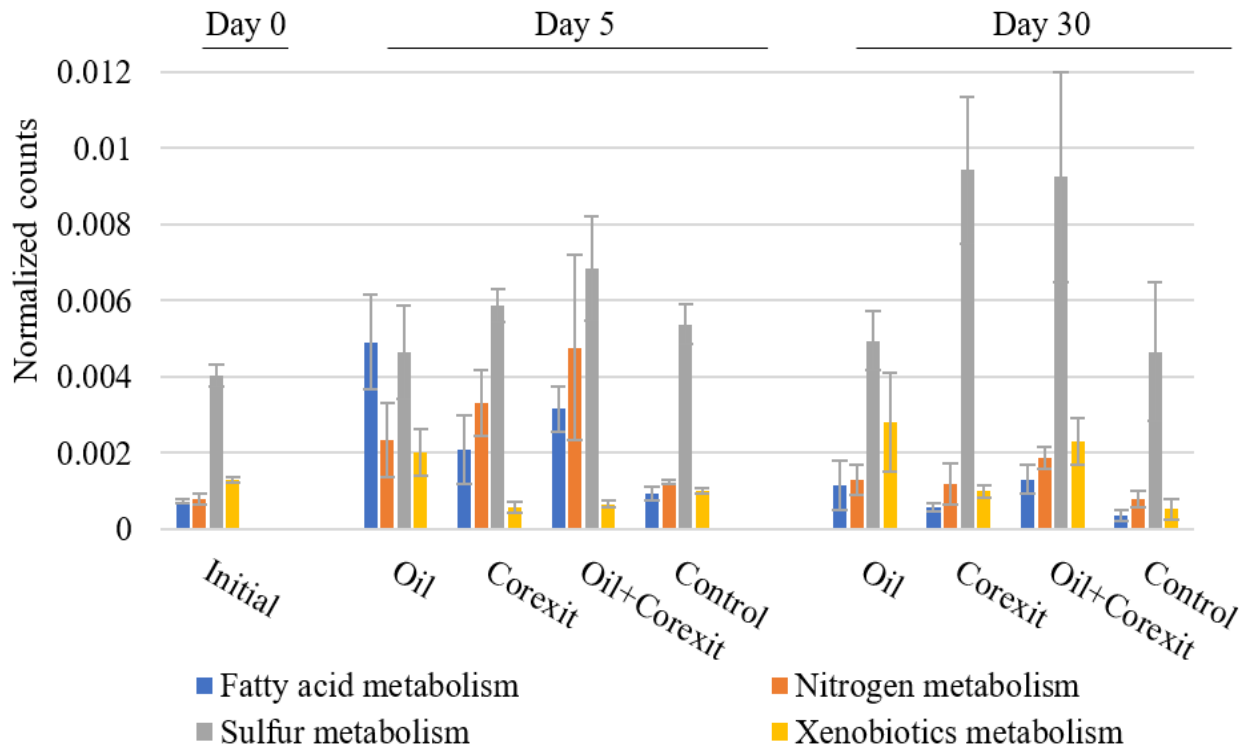
Figure 12. Normalized counts of select metabolic gene transcripts. The barplot above shows the distribution of several metabolism gene transcripts by treatment at 0, 5, and 30 days in Arctic seawater mesocosms amended with Corexit and/or oil or no treatment (control). Error bars represent one standard deviation from the mean.

Table 4. Mantel test statistics and associated p-values for the vector analysis of specific KEGG metabolic gene classes to the overall metatranscriptomic profile in Arctic seawater mesocosms.

	Mantel test statistic, r	p-value
Time	0.336	0.0010
Lipids	0.539	0.0010
Fatty acids	0.429	0.0010
Glycerolipids	0.061	0.1460
Glycerophospholipids	0.158	0.0320
Energy metabolism	0.776	0.0010
Oxidative Phosphorylation	0.742	0.0010
Photosynthesis	0.789	0.0010
Carbon fixation	0.130	0.1131
Nitrogen	0.489	0.0010
Sulfur	0.530	0.0010

Functional gene expression

Microbial communities exposed to oil and/or Corexit had increased expression of fatty acid degradation genes relative to the control, but each substance stimulated different genes. The presence of oil resulted in increased expression of hydrocarbon degradation gene alkane 1-monooxygenase (*alkB*), and the presence of Corexit resulted in increased expression of β-oxidation gene acyl co-A dehydrogenase (*fadE*). The oil+Corexit treatments showed the expression of both, suggesting that different genes from the same pathway are responsible for oil and Corexit component degradation.

The numbers of total lipid and fatty acid gene reads, and their relative abundance within the metatranscriptome were higher in substrate-amended treatments than the control at five days (p=0.0021 and p=0.0001) (Figure 11). Oil-only treatments had significantly (p=0.0001) higher total reads of alkane 1-monooxygenase (*alkB;* a key enzyme in hydrocarbon alkane degradation) than the Corexit-only and control treatments (Figure 11). The total *alkB* reads in the oil+Corexit treatment were significantly higher than in the oil-only treatment. The relative abundance of *alkB* for oil-only treatments was also significantly higher (p<0.0001) than for other treatments at day 5 (Figure 11). Functional gene analyses performed following the DWH oil spill showed that the presence (Lu et al., 2012) or expression (Mason et al., 2012) of alkane degradation genes such as *alkB* were enriched in contaminated waters, although it is unknown which genes were associated with oil vs. Corexit. However, in one study, increased intensity and richness of *alkB* genes on a GeoChip microarray assay was observed for Arctic seawater exposed to either oil or Corexit alone relative to controls, indicating that this gene or pathway may be involved in the degradation of both (McFarlin et al., 2018).

In contrast, acyl-CoA dehydrogenase (*fadE*), which catalyzes the first step of fatty acid β-oxidation, had higher numbers of total reads and relative abundances in treatments containing Corexit compared to oil-only and the control at 5 days (p=0.0068 and p<0.0001, respectively). The oil+Corexit treatment was significantly higher than Corexit-only. Additionally, the total reads and relative abundances for the β-oxidation genes acetyl-CoA acetyltransferase (*fadA*) and 3-hydroxyacyl-CoA dehydrogenase (*fadB*) were all significantly higher (p<0.0005) in treatments containing Corexit at day 5. The increased expression of all of these β-oxidation genes was associated with a significant loss of Corexit non-ionic surfactant components to below detection limits at day 5 (Figure 11). However, the total reads and relative abundances were not different between treatments at 30 days, likely due to the extensive loss of Corexit compounds by this time point (Figure 11). The presence or expression of β-oxidation genes such as *fadE*, *fadA*, and *fadB* was reported for waters exposed to dispersed oil *in situ* (Rivers et al., 2013) and in incubation experiments (Ribicic et al., 2018b), but it is unclear whether those findings were associated with the oil, dispersant, or both.

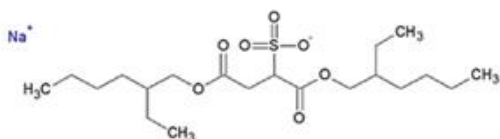*Proposed Corexit surfactant component degradation pathway*

Early in the incubation, gene expression in the oil-only treatment was dominated by *alkB*, which catalyzes alkane degradation, whereas, Corexit-only and oil+Corexit treatments were dominated by *fadE*, which catalyzes the initial step of β-oxidation of fatty acids (Figure 11). Additionally, the subsequent β-oxidation step enzymes *fadA* and *fadB* showed increased expression in the presence of Corexit. The oil+Corexit treatment appeared to be an amalgamation of the oil-only and Corexit-only treatments, with the abundances of *alkB* and *fadE* genes falling between the two groups. Based on the high abundances of these genes at the 5-day time point (relative to oil-only and the control), increased β-oxidation and subsequent mineralization of compounds was likely occurring in the presence of Corexit and oil+Corexit. This agrees with previously reported chemical loss data from this experiment that showed the majority of Corexit surfactant loss occurred by 5 days, and the majority of oil loss occurred at 10 days (Figure 11). When examining the constituents of Corexit, their chemical components contain multiple ester groups (Figure 13A), which may lead to them being more labile than the alkanes present in crude oil due to fewer steps required to prepare esters to undergo β-oxidation (Figure 13B).

The above finding led to the construction of a proposed pathway for the biodegradation of the major surfactant components in Corexit (Figure 13B). The pathway involves hydrolysis of the ester groups with either lipase or abiotic hydrolysis to form fatty acids, which can then undergo β-oxidation beginning with *fadE*. The alcohols produced from the ester hydrolysis can also undergo further oxidation to fatty acids to enter the β-oxidation pathway (Figure 13B). In support of biotic ester hydrolysis, the lipid ester-hydrolyzing enzyme phospholipase A1 (*pldA*) was found to have significantly higher numbers of reads (p=0.0002) in treatments containing Corexit, with oil+Corexit reads higher than Corexit-only. Compared to the degradation of alkanes, which must undergo several oxidation steps to be transformed into fatty acids to enter β-oxidation, this proposed pathway for Corexit compounds funnels directly into the central metabolism that is widespread in prokaryotes. The general use and prevalence of these enzymes in the environment may serve to explain the rapid degradation of some Corexit components and the variety of organisms observed in response to Corexit (Kleindienst et al., 2015; McFarlin et al., 2018; Techtmann et al., 2017).

Based on the previously described chemical data, which showed rapid degradation of the non-ionic surfactants within 5 days, the community shifts and functional genes observed at 5 days are most likely associated with degradation of the remaining Corexit components, including early stages of DOSS degradation and/or biotransformation. However, the non-ionic components of Corexit also contain many ester functional groups, as does DOSS, which allows them to enter the proposed degradation pathway described here.
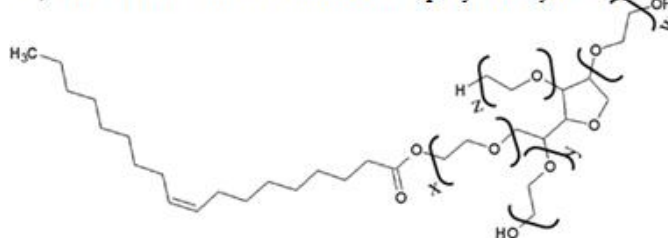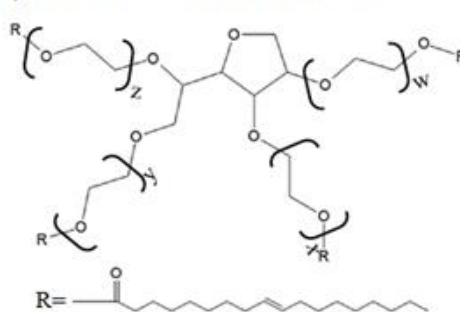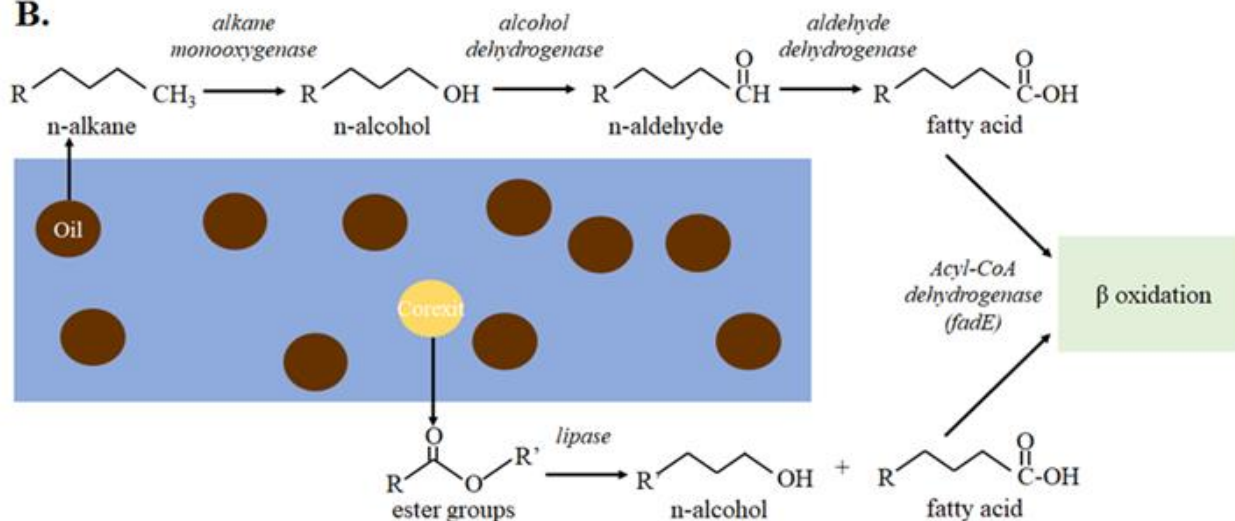
Figure 13. (A) Chemical structures of the Corexit surfactant components (Place et al., 2016) and (B) proposed bacterial biodegradation pathway for the major surfactant components and comparison to petroleum hydrocarbon alkane degradation.

Taxonomic identity of putative biodegrading organisms and genes in seawater

The taxa previously described in this study as proliferating in response to oil and/or Corexit were also observed (Table 5) to simultaneously take part in several key metabolic and biogeochemical processes relevant to contaminant degradation, including fatty acid degradation, nitrogen cycling, and sulfur cycling. Based on the taxa that each metabolic transcript originated from, different organisms became metabolically active in response to oil and/or Corexit (Figures 14 and 15).

Table 5. Summary of genera associated with the expression of different genes or metabolic pathway transcripts in Arctic seawater mesocosms.

Gene or metabolic pathway	Associated genera
Alkane 1-monooxygenase (*alkB*)	*Alcanivorax, Bukholderia, Marinobacter, Pseudomonas*
Acyl Co-A dehydrogenase (*fadE*)	*Chromohalobacter, Marinobacter, Pseudoalteromonas, Shewanella*
Lipases	*Aeromonas, Burkholderia, Colwellia, Rhodopirellula, Pseudomonas, Shewanella*
Oxidative phosphorylation	*Alcanivorax, Chromohalobacter, Colwellia, Flavobacterium, Marinobacter, Pseudomonas, Shewanella*
Nitrogen cycling	*Alcanivorax, Alteromonas, Colwellia, Marinobacter, Marinomonas, Pseudoalteromonas, Pseudomonas, Psychromonas*
Sulfur cycling	*Colwellia, Marinobacter, Marinomonas, Pseudoalteromonas, Pseudomonas, Saccharophagus, Shewanella*
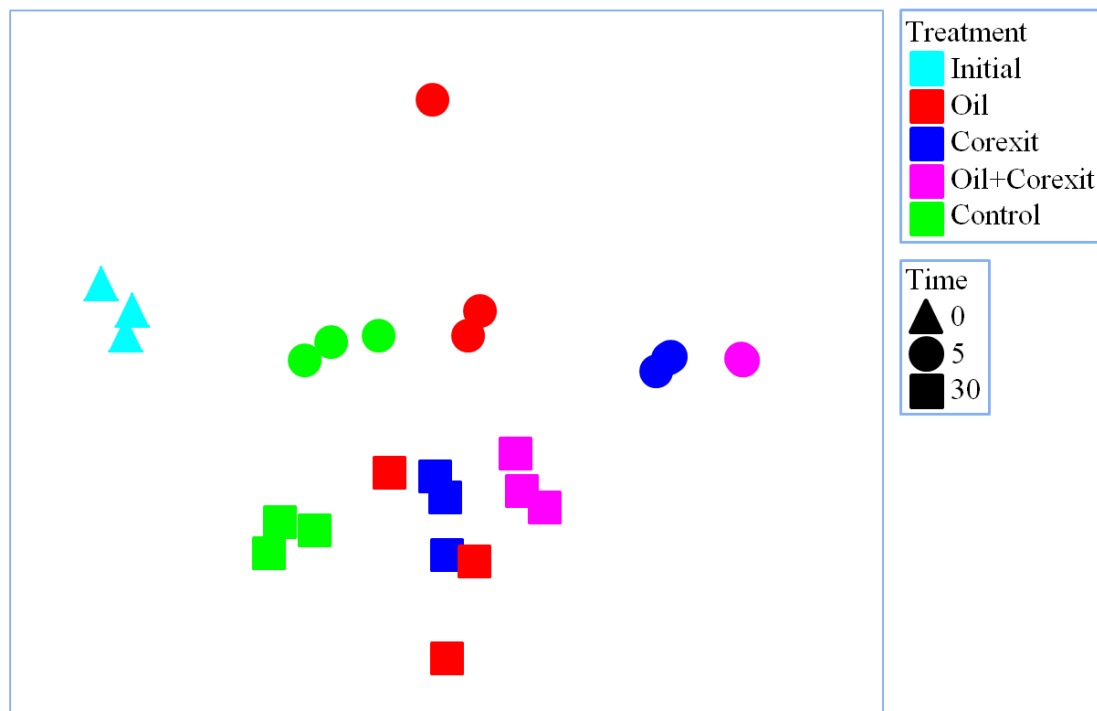
Figure 14. Bray-Curtis NMDS plot of the phylogenetic derivation of RNA transcripts. The NMDS plot above shows the phylogenetic derivation of RNA transcripts in Arctic seawater mesocosms amended with Corexit and/or oil or no treatment (control) at 0, 5, and 30 days incubation. A PERMANOVA test was performed to assess the effects of the treatments applied (p=0.0002), time (p=0.0002), and the interaction of both of these factors (p=0.0002). All pair-wise comparisons between different treatments were significant (p<0.05).
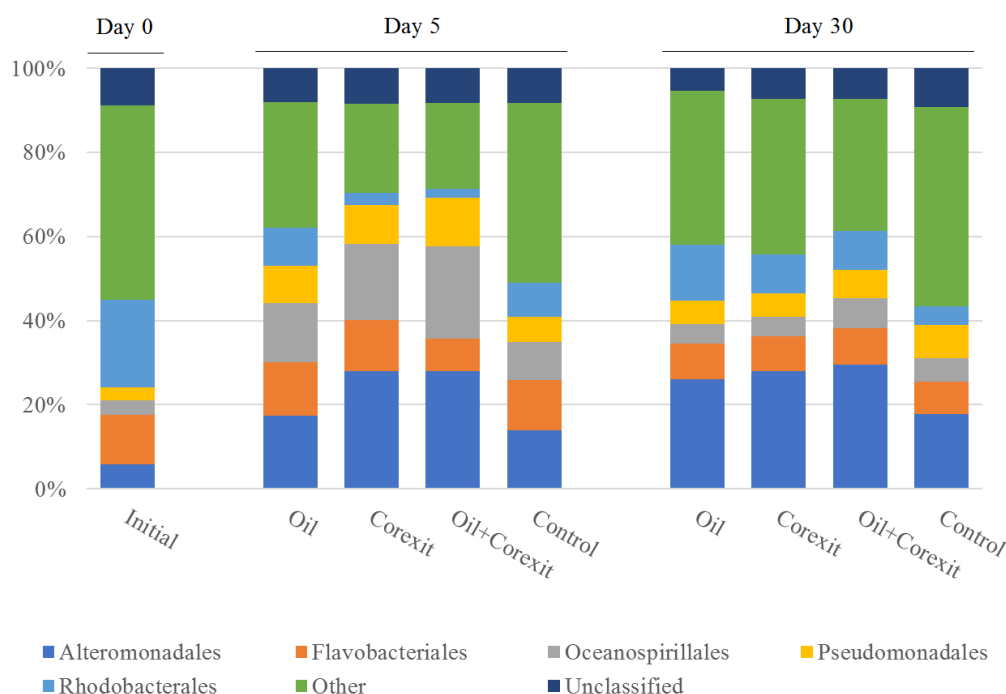
Figure 15. Relative abundance of bacterial taxa associated with metabolic gene expression. Bacterial taxa associated with gene expression in the Arctic seawater mesocosm treatments at 0, 5, and 30 days incubation are identified to order.

A PERMANOVA test of taxonomic affiliation of transcripts showed there were significant effects of treatment (p=0.0002), time (p=0.0002), and the interaction of both (p=0.0002) on the bacterial phylogenetic community make-up represented by the origins of metabolic transcripts, with all pair-wise comparisons showing significant differences (p<0.05). This agrees with prior 16S-rRNA-gene amplicon sequence analyses from this incubation experiment, which showed that the overall microbial community structure and relative abundance of specific taxa differed significantly between treatments, and oil+Corexit incubations resulted in communities representing a mixture of the distinct communities that emerged in response to oil-only and Corexit-only treatments. Functional gene expression was dominated by gammaproteobacteria and included the orders Oceanospirillales, Pseudomonadales, and Alteromonadales (Figure 15). Genera belonging to the Oceanospirillales and Pseudomonadales were both stimulated by the presence of oil, Corexit, and oil+Corexit at 5 days, including *Alcanivorax, Bermanella, Marinomonas,* and *Pseudomonas*. Some Alteromonadales genera were stimulated by the presence of either oil or Corexit (*Alteromonas, Marinobacter, Saccarophagus, Shewanella*); however, total Alteromonadales counts and some genera were only stimulated by the presence of Corexit (*Colwellia, Psychromonas, Moritella*). There was no indication that the addition of Corexit had a negative impact on the presence or abundance of sequences belonging to genera enriched in the presence of oil alone, as previously reported for the oil-degrading taxa

Marinobacter and *Cytoclasticus* in Gulf of Mexico seawater mesocosm incubations (Kleindienst et al., 2015).

When examining the organisms associated with metabolic gene expression, the upregulation of biodegradation processes in response to oil and/or Corexit (lipid metabolism, oxidative phosphorylation, nutrient cycling) was performed by multiple taxa (Table 5), as observed in other studies (Brakstad et al., 2015; Dombrowski et al., 2016; Handley et al., 2017). The *alkB* and *fadE* genes expressed originated from different genera, with the exception of *Marinobacter,* which expressed both. Broader metabolic functions were otherwise generally associated with the same groups of genera regardless of treatment (Table 5).

The genus *Colwellia* garnered substantial attention due to its increased abundance in the aftermath of the 2010 DWH spill (Bælum et al., 2012; Dubinsky et al., 2013; Hazen et al., 2010; Kleindienst et al., 2015; Mason et al., 2012, 2014; Rivers et al., 2013), and there has been speculation that *Colwellia* are stimulated by the presence of Corexit. *Colwellia* are facultatively anaerobic psychrophilic heterotrophs that typically found in deep marine environments (Deming & Junge, 2015). As a result of cold-environment adaptations, *Colwellia* possess unique metabolic capabilities such as the ability to degrade polyhydroxyalkanoate (PHA), cyanophycin, and chitin-like compounds (Deming & Junge, 2015; Methé et al., 2005). PHAs are a family of polyesters that can be degraded and used as a carbon source or energy reserve. The ability to degrade PHAs is linked with a significant capacity to produce and degrade fatty acids using *fadE* and *fadB* genes (Methé et al., 2005). Additionally, *Colwellia* possess coenzyme F_{420}, which is linked to polynitroaromatic compound degradation (i.e., 2,4 dinitrophenol), the ability to respond to reactive oxygen species, and enzyme structural plasticity. These characteristics give *Colwellia* a metabolic advantage over their mesophilic counterparts (Methé et al., 2005), and this metabolic niche may link *Colwellia* to Corexit degradation. While not linked to *fadE* expression in this experiment, *Colwellia* were involved in the expression of lipase and oxidative phosphorylation transcripts as well as those for nitrogen and sulfur cycling (Table 5) in both Corexit and oil+Corexit treatments. Further studies are needed to gain a better understanding of the roles *Colwellia* and other genera play in both dispersant and dispersed oil biodegradation. The use of cultures or stable isotope probing (SIP) of Corexit components can confirm that the proposed pathway (Figure 13b) is being utilized for Corexit degradation and to determine the organisms involved in the different steps.

Oil biodegradation in Arctic marine sediments

The sediment incubation experiment resulted in 333 samples, each requiring extraction and GC-MS analysis. Only half of the extractions were completed by the time of this report due to methodological issues; specifically, the formation of emulsions during methylene chloride extraction of the wet sediments. We subsequently modified our method by separating the water from the sediment for each sample (via certified glass serological pipette) and then extracting oil

from the water and sediment fractions independently. The addition of 2 grams of anhydrous sodium sulfate per 1 gram of sediment to the sediment fraction dehydrated the sample such that an emulsion could not occur. Methylene chloride was then added, mixed, and removed three times for each fraction, both water and sediment, to extract crude oil from the entire sample. GC-MS analysis will proceed after all samples are extracted. Data from these analyses will be reported in forthcoming publications (see Study Products). The information will provide quantitation of the % loss of oil in sediments under aerobic and anaerobic incubation conditions over time, providing an indication of degradation rates in the benthos. Percent loss values will be determined for incubations containing live microbes as well as sterile controls, which will enable us to distinguish between losses due to abiotic processes such as weathering and those associated with biodegradation.

Sediment microbial community response to oil

Hierarchical clustering analysis and nMDS ordination indicate that there were seven different clusters of prokaryotic communities represented in the sediment-oil incubation experiment (Figure 16). Cluster 1 contains all samples, weathered, fresh, and non-oiled sediments, from time zero, the start of the incubation experiments. Cluster 2 contains all oil-free samples after 12 days of incubation and *in situ* community samples collected immediately upon acquisition of sediments in the Chukchi Sea. Cluster 3 encompasses all but one of the fresh and weathered oil samples from the first destructive harvest (12 days) of the incubation experiment. Cluster 4 contains all non-oiled samples between the second harvest (24 days) and the final harvest (60 days). Cluster 5 is represented by all weathered oiled samples from the second and third harvest, 24 and 36 days, respectively. Cluster 6 contains all but one fresh oiled sample from the second harvest through the end of the incubation, 24-60 days. Cluster 7 encompasses all weathered oil samples from the fourth and final harvests, 48-60 days into the incubation experiment. The nMDS indicates that differences between non-oiled samples and oiled are largely exhibited on the nMDS1 axis, and within-cluster differences are largely exhibited on the nMDS2 axis.
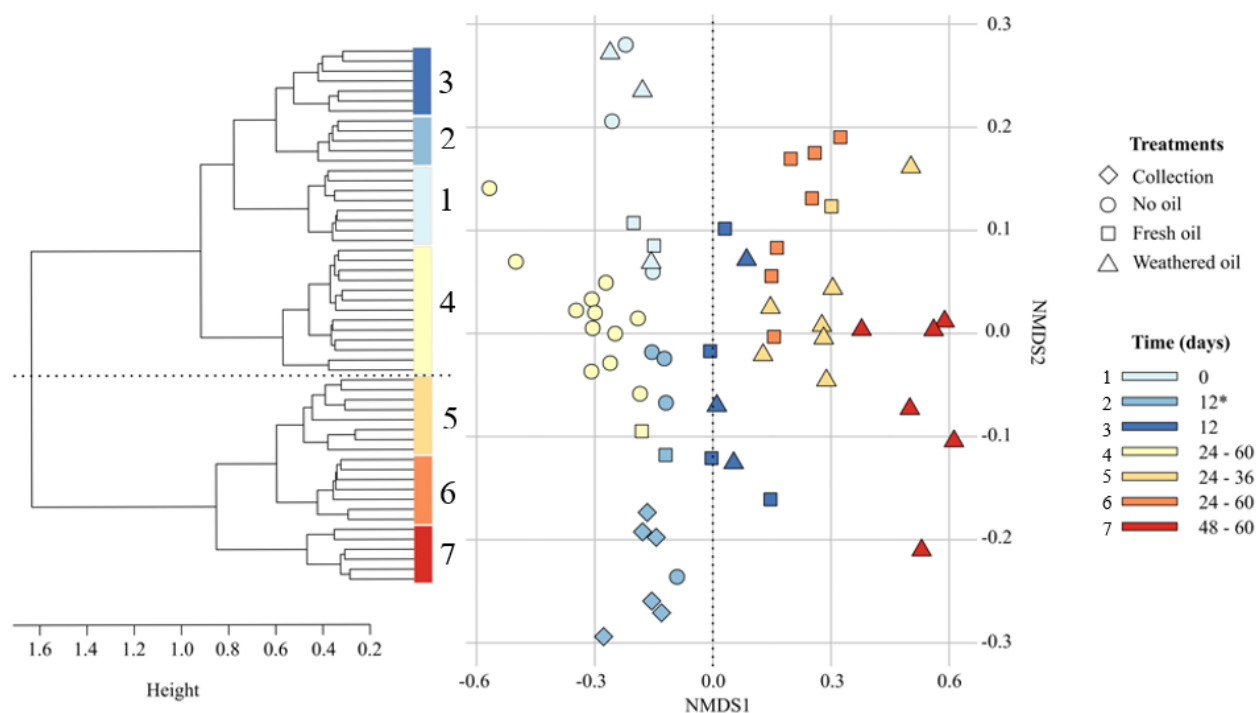
Figure 16. Hierarchical cluster dendrogram and nMDS ordination of the prokaryotic community from sediment-oil incubation experiment. The figure above depicts the results from the hierarchical cluster analysis on the left-hand side and nMDS plot on the right-hand side; both are representations of prokaryotic community structure across treatments. Shapes identify treatments and colors identify incubation times in days. The dotted lines on both the cluster dendrogram and nMDS delineate the non-oiled samples from the oiled samples. The * highlights the clustering of the non-oiled samples with the *in situ* samples after 12 days, indicating that after the initial disturbance of putting sediments in experimental bottles, it took up to 12 days for the microbial community to reflect that of the *in situ* community.

To explore differences in prokaryotic community structure that explain the clustering patterns, change in taxa abundance was measured from the time of collection *in situ* to the end of the experiment for each treatment (no-oil, fresh-oil, and weathered-oil) (Figure 17). The 14 most abundant taxa observed in the study were identified to genus-level. *Woesia* occurred solely in no-oil samples, but eight taxa were highly abundant in all treatments and are considered the core community in this study: *Aquibacter, Candidatus Nitrosopumilus, Halioglobus, Lutibacter, Lutimonas, Maritmimonas, Persicirhabdus,* and *Psychromonas.* Five taxa were abundant only in the oiled samples; *Cycloclasticus, Neptuniibacter,* and *Oleispira* in fresh and weathered treatments, *Zhongshania* in fresh-oil samples, and *Roseobacter clade AC117* in weathered-oil samples. Though the relative abundances of these five taxa varied and exhibited different trends over time within each treatment, they are considered putative oil-degraders because of their select occurrence in oiled treatments.

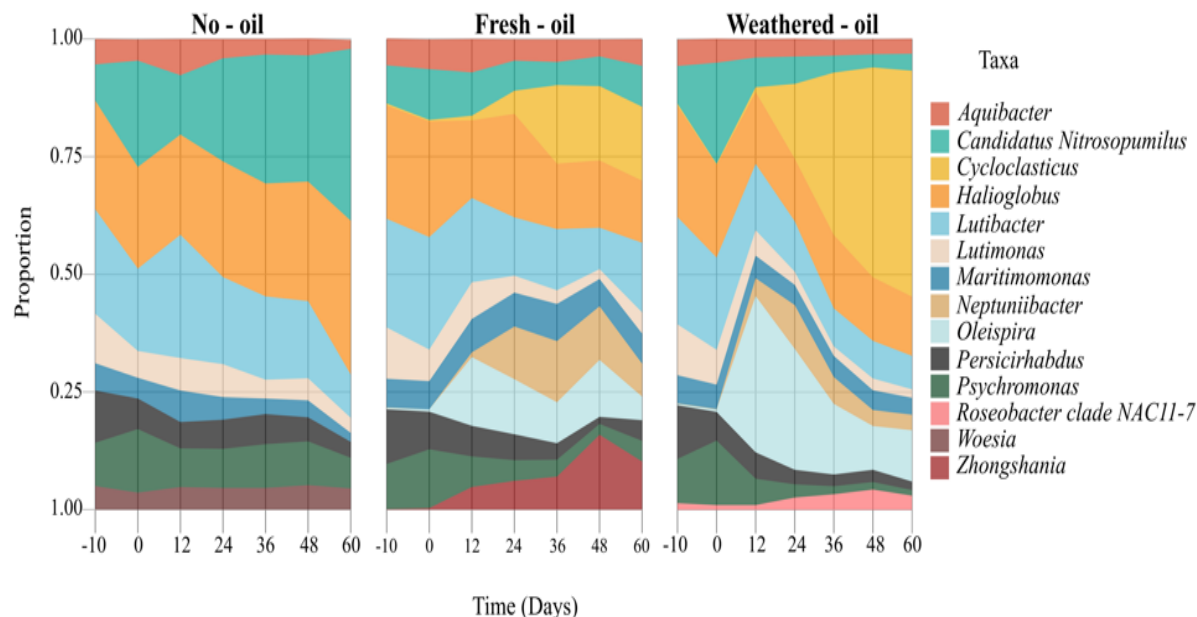Figure 17. Area charts of top genus-level taxa over time with no-, fresh-, and weathered-oil treatments in sediment incubations. The charts above show the most abundant taxa (upper 14) identified to genus-level represented in no-oil, fresh-oil, and weathered-oil samples over time. It is important to note that with area charts, the change in thickness of the color associated with individual taxa is a metric of change in relative abundance over time.

The first putative oil-degrading taxonomic group to increase in relative abundance after 12 days of incubation in both the fresh- and weathered-oil samples was the genus *Oleispira*. *Oleispira spp.* are among the most well-known marine oil-degrading microbes and have been implicated in aerobic oil biodegradation, primarily of branched and straight-chain alkanes, in both seawater and marine sediments (Guibert et al., 2012; Ribicic et al., 2018a; Yakimov et al., 2003, 2007). The early increase in abundance of *Oleispira* in the oiled samples is congruent with the typical succession of chemical compounds in oil. Branched and straight-chain alkanes (saturated hydrocarbons) are generally the first compounds to be biodegraded (Head et al., 2006). The matching trend in *Oleispira* in both fresh- and weathered-oil samples indicates that the weathered-oil still contained saturated hydrocarbons. In the fresh-oil samples, *Oleispira* decreased in abundance from day 12 to day 48 when it exhibited a second spike in relative abundance. However, in the weathered-oil samples, *Oleispira* showed a consistent decline in abundance following the initial spike. The second peak in fresh-oil samples may indicate byproducts of oil degradation favorable to *Oleispira* that were not present in the weathered-oil samples.

The initial spike in *Oleispira* relative abundance was followed by an increase of *Neptuniibacter* and *Cycloclasticus* at 24 days of incubation in both fresh- and weathered-oil samples. The increase in *Neptuniibacter* was smaller in the weathered oil samples, where the increase in *Cycloclasticus* was more pronounced, particularly over time. Some studies have indicated that

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specific strains of *Neptuniibacter* can degrade carbazole and phenanthrene (Ahmad et al., 2015; Dombrowski et al., 2016; Maeda et al., 2010; Nagashima et al., 2010). Carbazole and phenanthrene are both PAHs containing three rings, though carbazole is also a heterocyclic compound in which one of the carbons has been replaced with nitrogen (Head et al., 2006; Salam et al., 2017). In the fresh-oil samples, *Neptuniibacter* reached its peak abundance at 36 days, which coincided with a trough in *Oleispira*. Following the peak in *Neptuniibacter* abundance, *Oleispira* exhibited a second peak of higher abundances at day 48, indicating that degradation products of fresh-oil from *Neptuniibacter* may be labile or favorable to *Oleispira*. This trend was not observed in the weathered-oil samples, where *Neptuniibacter* had relatively stable abundance throughout the remainder of the experiment. Meanwhile, *Oleispira* continued to decline, suggesting that similar compounds present in the fresh-oil samples were not available during the weathered oil incubation at day 48.

Cycloclasticus exhibited the most dramatic increase between days 12 and 36 in the fresh-oil samples and remained relatively stable through the end of the experiment. In the weathered-oil samples, however, *Cycloclasticus* continued to increase in abundance substantially from day 12 to day 60 and showed no sign of plateauing. Bacteria belonging to the *Cycloclasticus* genus have been indicated physiologically, experimentally, and inferentially in the aerobic degradation of PAHs (Dyksterhouse et al., 1995; Kasai et al., 2002). Members of *Cycloclasticus* are considered the prevalent, aerobic PAH-degrader in marine sediments and Pacific Arctic deep-sea sediments. Dong et al. (2015) found it was one of the most abundant taxa in PAH-enriched marine sediments and exhibited utilization of naphthalene and phenanthrene as sole carbon sources. Studies by Yakimov et al. (2003, 2007) indicated that *Cycloclasticus* specializes in degrading the crude oil compounds that remain after more labile components have been degraded.

The remaining putative oil-degrading taxa detected in this experiment were treatment-specific and only present in high abundances in either fresh- or weathered-oil samples. *Zhongshania*, representative of the fresh-oil samples, gradually increased in abundance from day 12 to day 36, spiked at day 48, and gradually declined. Li et al. (2011) proposed *Zhongshania* as a genus isolated from land-fast ice and seawater from the Antarctic. Since then, several *Zhongshania* species have been described within the genus, all of which were isolated from marine environments and one of which is capable of degrading straight- or branched-chain alkanes, otherwise known as aliphatic hydrocarbons (Li et al., 2011; Naysim et al., 2014). *Zhongshania aliphaticivorans,* isolated from oil-contaminated marine sediments, has been implicated as an aliphatic hydrocarbon degrader based on the presence of several alkane degradation genes within its genome, including alkane 1-monooxygenase and haloalkane dehalogenase (Jia et al., 2016; Naysim et al., 2014). This was substantiated by Ribicic et al. (2018a), who indicated that *Zhongshania* played a significant role in the initial alkane degradation of chemically dispersed oil in Norwegian seawater. Metagenome analysis also suggested that *Zhongshania* may be capable of degrading aromatics such as biphenyl, benzoate, catechol, and cyclopentanol (Jia et

al., 2016). To our knowledge, *Zhongshania* has not yet been identified or isolated from Arctic marine sediments. Those OTUs affiliated with *Zhongshania* in this study matched equally at 100% identity in the BLAST database to *Zhongshania aliphaticivorans* and an uncultured clone. The peak in abundance of *Zhongshania* occurred with the second peak in *Oleispira* abundance at day 48 in the experiment suggesting that oil degradation products generated by *Cycloclasticus, Neptuniibacter*, or both were favorable to *Zhongshania* as well as *Oleispira.*

The *Roseobacter NAC11-7* lineage, which is representative of the weathered-oil samples, began to increase in abundance starting at day 12 and exhibited a gradual increase until day 48. It gradually declined until day 60. The increase in abundance of the *Roseobacter* lineage in weathered-oil incubations is subtle, yet apparent, as it was not found to be abundant in non-oiled or fresh-oil samples. The *Roseobacter NAC11-7* lineage is one that is commonly associated with phytoplankton blooms and has only been identified and described from seawater (Hahnke et al., 2013; Luo & Moran, 2014; Teeling et al., 2016; West et al., 2008). There are few studies pertaining to this lineage with respect to oil degradation; however, Brakstad et al. (2004) reported that *Roseobacter* NAC11-7 was abundant during oil biodegradation in low-temperature (5.9-7.4°C) seawater. Additionally, Netzer et al. (2018) found that bacteria in the *Roseobacter* NAC11-7 lineage were prevalent only in oiled seawater (versus non-oiled), with slightly higher abundances associated with oiled diatom aggregates. Based upon these and other studies correlating *Roseobacter* with oil degradation, genomic studies have investigated the metabolic capabilities of 24 representatives of the *Roseobacter* lineage and found different pathways pertaining to the aerobic degradation of aromatic compounds (Buchan et al., 2000; Buchan et al., 2019; Giebel et al., 2016).

Distribution of putative oil-degrading taxa in Beaufort Sea sediments

A Beaufort Sea sediment microbial community survey dataset was queried to assess the distribution of putative oil-degrading microbes in that region (Objective 4). Putative oil-degrading taxa identified in this study were plotted on a map via ArcGIS (Figure 18). All taxa except *Neptuniibacter* were found in low abundances in the Beaufort Sea sediments. *Roseobacter NAC11-7* clade exhibited the highest abundances at more sites, followed by *Oleispira, Cycloclasticus,* and, lastly, *Zhongshania,* which was found at just one site.
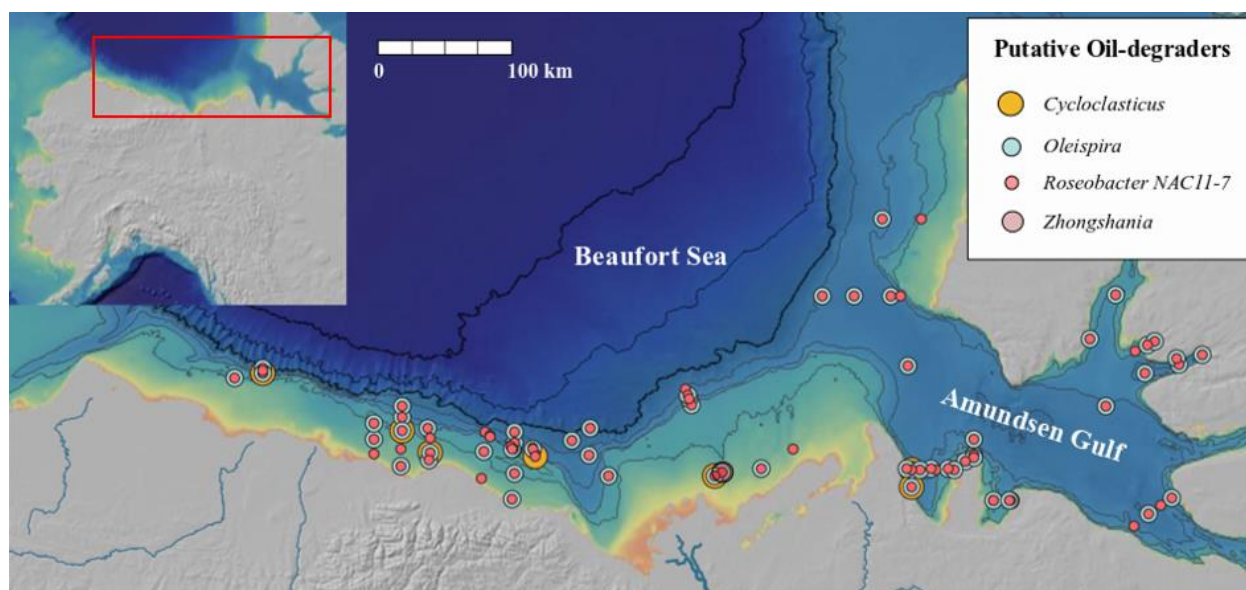
Figure 18. Distribution of putative oil-degraders found in Beaufort Sea marine sediments. This map shows where the same bacterial genera identified in the experiment conducted in this study on Chukchi Sea sediments were found in another study where a survey of prokaryotes was conducted on Beaufort Sea surface sediments. The inset shows the sampling region.

Comparison of putative oil-degrading taxa detected in seawater vs. sediments

A comparative analysis of putative oil-degrading bacteria identified in our seawater and sediment studies was conducted to determine if similar taxa potentially play important roles in biodegradation in the pelagic versus the benthic zones of the Chukchi Sea environment. The sediments and seawater samples used in our experiments did not originate from the same time or geographic location in the Chukchi Sea; seawater was collected in 2016 approximately 1 km offshore of Utqiagvik and sediments were collected in 2017 at 130 km offshore of Pt. Lay, Alaska (Figure 1). Nonetheless, some similarities were detected among putative oil degraders. Members of the genera *Oleispira* and *Roseobacter* were found to increase in relative abundance in both seawater and sediment incubations, suggesting that they play an active role in the biodegradation of petroleum hydrocarbons in these settings. Putative oil-degraders *Cytoclasticus* and *Neptuniibacter* were only detected in sediments, while *Thalassolituus*, *Sedimentalea*, and *Pseudofulvibacter* were only detected in seawater. *Zhongshania* was most abundant and responsive to oil in sediments but was detected in very low numbers in some seawater incubation samples. In our original hypothesis, we predicted that the diversity (taxonomic richness) of hydrocarbon-degrading microbes would be higher in sediment than seawater due to the much greater concentration of biomass in sediments. However, the richness of the most abundant organisms stimulated by the presence of oil was found to be comparable, although some different taxa were observed to respond in each sample type. It is important to note that this finding focuses exclusively on aerobic incubation tests, and potential anaerobic oil degraders may differ taxonomically. Results from this comparative analysis underscore the ubiquity of hydrocarbon-

degraders in the marine environment, as well as the influence of specific environmental conditions (i.e., nutrients, oxygen, temperature, etc.) on microbial community succession following an oil spill.

CONCLUSIONS

The findings of this study are consistent with prior studies indicating that the indigenous microbial community in Arctic seawater has the potential to biodegrade crude oil and components of Corexit (McFarlin et al., 2014, 2018). This study is the first to closely examine the interactions between oil and Corexit degradation when co-present in Arctic seawater. It provides no evidence that Corexit suppressed crude oil biodegradation or oil-degrading microorganisms, as reported by Kleindeinst et al. (2015). Our experiments revealed that, when oil and Corexit were present in seawater together, the non-ionic surfactants (Span 80, Tween 80, Tween 85) were rapidly degraded, followed by subsequent biodegradation of oil components. This apparent preferential degradation of non-ionic dispersant components did not result in significant reductions in oil loss when Corexit was present. This may be due to the reduction in oil droplet size and increased bioavailability generated by Corexit application outweighing the effects of competitive degradation, or due to the relatively small quantity of carbon contributed by Corexit compared to oil when applied in the 1:10 dispersant: oil ratio being insufficient to outcompete the utilization of petroleum hydrocarbons.

Analytical variability when quantifying the Corexit component DOSS precluded definitive determination of its degradation extent during the study but indicated that it is more persistent than the non-ionic surfactants. DOSS appeared to undergo some biotransformation to EHSS concurrent with the degradation of oil and other Corexit components.

In Arctic seawater, some taxa responded only to the presence of oil (*Thalassolituus, Sedimentalea*) or Corexit (*Colwellia*, *Polaribacter*, *Moritella*, *Octadecabacter*, and *Amylibacter*) alone, implicating them as degraders of those mixtures. Some members of the taxa *Oleispira, Pseudofulvibacter,* and *Roseobacter* became enriched in response to both oil and Corexit (when provided separately or together), suggesting that these organisms are capable of utilizing components of both mixtures.

Metatranscriptomics (gene expression) analyses of the seawater microbial communities revealed that oil and Corexit stimulate different overall metabolic gene expression profiles in the microbial community. It also indicated that oil and Corexit share a common upregulation of the pathway for fatty acid degradation that shows a synergistic response when both oil and Corexit are present. These results and the evidence of increased overall metabolic activity when exposed to oil+Corexit, support the theory that dispersants stimulate existing microbial oil-degrading activity, possibly through mechanisms such as increasing the bioavailability of oil.

The observation that fatty acid degradation gene expression was upregulated in the presence of Corexit alone indicates that this pathway plays a role in the degradation of some Corexit components. We propose a hypothetical degradation pathway for Corexit components that utilizes the fatty acid β-oxidation pathway to break down side-chains of the non-ionic and DOSS surfactant components of Corexit. Fatty acid oxidation is a common metabolic pathway in bacteria, so Corexit surfactant compounds may be readily metabolized, completely or in part, by the many different taxa that responded to the presence of Corexit. Because acid β-oxidation is also used in the biodegradation of alkanes (after several enzymatic transformations), it appears that both petroleum-derived alkanes and the hydrocarbon side-chains of some Corexit components share a common degradation pathway. Corexit components are able to proceed to the pathway more directly since the alkane activation steps are not needed. *Colwellia*-associated genes, which were particularly prominent in seawater exposed to Corexit, were associated with several metabolic processes that were enriched in the presence of Corexit, indicating it may play a key role in its degradation. Further research is needed to confirm the use of the β-oxidation pathway in Corexit surfactant degradation and elucidate which taxa are directly involved in its degradation, which will bolster our understanding of the fate of chemical dispersants such as Corexit in the environment.

Molecular analyses of the sediment microbial community response to oil in aerobic incubations revealed that the core sediment microbial community remained present following oil exposure. However, significant community shifts occurred in the oiled treatments, with fresh and weathered oil treatments producing distinct shifts relative to each other and to non-oiled treatments. The oil-associated shifts were characterized by increases in the relative abundance of several taxa, which implicates these taxa as oil biodegraders. The taxa present in oiled treatments but absent in non-oiled samples, *Cycloclasticus, Neptuniibacter, Oleispira, Roseobacter clade NAC11-7,* and *Zhongshania,* have all been previously linked to oil degradation. Overall the presence of these taxa supports our hypothesis that oil-degrading microbial taxa will be present in Arctic marine sediments, but refute our hypothesis that we might identify abundant taxa that were not previously reported to degrade oil. Among these taxa, there is variation in relative abundances between fresh and weathered-oil samples over time, indicating that certain taxa are likely linked to specific oil compounds present during different stages of oil degradation.

Following our identification of putative oil-degrading taxa in Chukchi Sea sediment incubation studies, we assessed the distribution of these taxa across a geographically distributed series of Beaufort Sea sediment samples. We found that the majority of putative oil-degrading taxa found in Chukchi marine sediments were also present in very low abundances in Beaufort Sea marine sediments. The presence of these microbes indicated that oil degradation potential exists in Beaufort Sea sediments, supporting our hypothesis that putative oil-degraders are broadly distributed in North American Arctic sediments. This hypothesis was predicated on previous findings, which suggest that oil-degrading bacteria are ubiquitous and generally present in very

low abundances in the natural environment, then becoming enriched following a spill event (Yakimov et al., 2007).

The presence of oil stimulated different hydrocarbon-degrading organisms in seawater *(Thalassolituus*, *Sedimentalea*, and *Pseudofulvibacter)*, versus sediment (*Cytoclasticus*, *Neptuniibacter*, and *Zhongshania*), with some taxa being stimulated in both environments (*Oleispira* and *Roseobacter*). This further underlines both the diversity and ubiquity of hydrocarbon-degrading microbes and the strong influence of environmental parameters (i.e., temperature, nutrients, oxygen, biomass, etc.) on post-spill microbial community succession even in the same geographic region.

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STUDY PRODUCTS

Presentations

2018 – CMI Annual Review (oral presentation) – Mary Beth Leigh
2019 – CMI Annual Review (oral presentation) – Alexis Walker
2020 – CMI Annual Review (oral presentation) – Mary Beth Leigh

Gofstein, T. R. & Leigh, M. B. Fate and Influence of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. 2017 Week of the Arctic. Fairbanks, AK, May 9-10, 2017. (oral presentation)

Gofstein, T. R. & Leigh, M. B. Fate and Influence of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. UAF Environmental Chemistry Symposium. Fairbanks, AK, April 21, 2017. (oral presentation)

Gofstein, T. R. & Leigh, M. B. Fate and Influence of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. UAF Biomedical Research Conference. Fairbanks, AK, May 18, 2017. (oral presentation)

Leigh, M. B., McFarlin, K., Gofstein, T. R., 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. Invited oral presentation. Alaska Oil Spill Technology Symposium. Anchorage, AK, March 28-29, 2018. (oral presentation)

Gofstein, T.R. Fate and Effects of Petroleum Contamination and Chemical Dispersants in Arctic Marine Environments. UAF Annual Environmental Chemistry Symposium Fairbanks, AK, April 7, 2018. Received Outstanding Graduate Presentation award. (oral presentation)

Leigh, M. B., McFarlin, K., Gofstein, T. R., 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 Branch of the American Society for Microbiology Annual Meeting. Fairbanks, AK, September 29, 2018. (oral presentation)

Leigh, M. B., McFarlin, K., Gofstein, T. R., Walker, A. Schuette, U., Perkins, M., & Field, J. Fate and effects of oil and oil spill response chemicals in Alaskan marine environments. UAF Institute of Arctic Biology Life Sciences Seminar Series. Fairbanks, AK, November 9, 2018. (oral presentation)

Leigh, M. B., McFarlin, K., Gofstein, T. R., Walker, A. Schuette, U., Perkins, M., & Field, J. Fate and effects of oil and oil spill response chemicals in Alaskan marine environments. UAF Institute of Marine Sciences Seminar Series. Fairbanks, AK, November 14, 2018. (oral presentation)

Gofstein, T.R. & Leigh, M. B. Biodegradation of Crude Oil and Corexit EC9500A in Arctic Seawater. Fifth International Symposium on Bioremediation and Sustainable Environmental Technologies. Baltimore, MD, April 18, 2019. (oral presentation)

Leigh, M. B., McFarlin, K., Gofstein, T. R., Walker, A. Schuette, U., Perkins, M., & Field, J. Fate and effects of oil and oil spill response chemicals in Alaskan marine environments. University of Oklahoma Department of Microbiology and Plant Biology. Norman, OK, November 6, 2019. (oral presentation)

Gofstein, T. R., Perkins, M., Field, J. & Leigh, M. B. Biodegradation of Crude Oil and Corexit EC9500A in Arctic Seawater. Alaska Oil Spill Technology Symposium. Anchorage, AK, March 28-29, 2018. (poster presentation)

Gofstein, Taylor R., Matthew Perkins, Jennifer Field, & Mary Beth Leigh. Biodegradation of Crude Oil and Corexit EC9500A in Arctic Seawater. Poster presentation. Alaska Branch of the American Society for Microbiology Annual Meeting. Anchorage, AK, October 18-19, 2019. (poster presentation)

Publications

Gofstein, T., Perkins, M., Field, J. & Leigh, M. B. (Submitted May 2020) Sequential Degradation of Crude Oil and Corexit EC9500A in Arctic Seawater. *Applied and Environmental Microbiology*

Gofstein, T. R. & Leigh, M. B. (in prep.) Microbial metatranscriptome shifts suggest shared biodegradation pathways for Corexit 9500 components and crude oil in Arctic seawater.

Walker, A., Hardy, S., & Leigh, M.B. (in prep.) Biodegradation of Alaska North Slope crude oil in Arctic marine sediments.

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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.