A STUDY OF MICROBIAL CRUDE OIL DEGRADATION IN WATER AND SEDIMENT FROM THE GREENLAND SEA

Scientific Report from DCE – Danish Centre for Environment and Energy No. 347 2019

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Data sheet

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Abstract:	This project evaluated the capacity for microbial oil degradation in the marine environment offshore NE Greenland.

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Preface

This study is part of the Northeast Greenland Environmental Study Program.

The Northeast Greenland Environmental Study Program is a collaboration between DCE – Danish Centre for Environment and Energy at Aarhus University, the Greenland Institute of Natural Resources, and the Environmental Agency for Mineral Resource Activities of the Government of Greenland.

Oil companies operating in Greenland are obliged to contribute to knowledge regarding environmental matters. The Strategic Environmental Impact Assessment and the background study program were funded under these commitments administered by the Mineral License and Safety Authority and the Environmental Agency for Mineral Resource Activities.

The study was carried out as a collaboration between The Geological Survey of Denmark and Greenland (GEUS), Department of Geochemistry; Copenhagen University (CU), Section for Environmental Chemistry and Physics; and Aarhus University (AU), DCE – Danish Centre for Environment and Energy.

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1 Summary

This project evaluated the capacity for microbial oil degradation in the marine environment offshore NE Greenland. Offshore oil exploration and exploitation in this area will be associated with an increased risk of accidental oil spills due to sea ice, drifting icebergs, and the depth at which the drillings may take place. Natural oil pre-exposure from seeps presumably induces microbial degrader communities with broad substrate diversity by selecting and enriching rare degraders with the necessary metabolic machinery to "handle" a great number of different oil compounds. Any indications of oil pre-exposure would therefore be important for the strategic environmental impact assessment.

Sediment and / or sea water were sampled at 11 stations within and outside of the license areas. We carried out four sub-experiments. 1) Sediment from 10 stations was analysed for the contents of natural oil compounds and the contents of oil degrader microorganisms. 2) Six sediments from the continental shelf were spiked with crude oil and / or mineral nutrients to investigate the potential for oil degradation and to which extend this potential is dependent on the addition of mineral nutrients. 3) Crude oil degradation was investigated in seawater from a selected station by comparing the intrinsic degradation and degradation in water spiked with mineral nutrients and / or a dispersion agent. 4) The metabolism of microbial oil degraders at different depths of the water column at three stations was qualitative characterized by extracting microorganisms from water samples and incubating them with crude oil under optimized conditions.

The results from our simplified laboratory experiments can only be extrapolated to the complex environment of the Greenland Sea with great caution, but some microcosms did show a large potential for biodegradation of many different oil compounds in the water column, even "difficult" compounds such as C1-pyrene and C3-phenanathrene was partially degraded after 70-90 days. This suggests, that bioremediation of surface spills may have a large potential if the intrinsic microbial degraders can be activated.

It was also clear that in-situ concentrations of mineral nutrients are strongly limiting for oil degradation. Oil biodegradation will thus be very limited in the water column without addition of mineral nutrients. Contingency strategies based on the intrinsic potential for microbial oil removal should therefore include strategies for applying mineral nutrients for degradation to be efficient. The dispersing agent Slickgone NS was efficient at dispersing the oil under the tested conditions, but may have little effect on oil biodegradation when mineral nutrients are limiting, probably because degradation of the dispersant itself requires mineral nutrients.

The concentration of alkyl-PAHs was low in sediments from the shelf break, but light alkyl-PAHs were detectable, suggesting that the shelf break stations may have had some form of limited oil pre-exposure. Alkyl-PAHs were non-detectable in the tested shelf sediments suggests that the shelf stations have had no oil pre-exposure. The potential for biodegradation of weathered PAHs in the shelf sediments was very low, even when mineral nutrients were not a limiting factor, which is in line with the absence oil pre-exposure.

The applied methods all had benefits and drawbacks, but they were strong tools when combined. The knowledge gained from this project will be important for evaluating the potential for oil removal based on the natural microbial communities that are present in the marine environment of NE Greenland.

2 Sammenfatning

Dette projekt evaluerede potentialet for mikrobiel olienedbrydning i havmiljøet ud for Nordøstgrønland. I dette område vil offshore olieefterforskning og olieproduktion være forbundet med øget risiko for utilsigtede olieudslip på grund af havis, drivende isbjerge, og havdybden hvor boringerne vil finde sted. Præ-eksponering med olie fra naturlige olie-udsivninger (seeps) forventes at give de mikrobielle nedbrydersamfund en bred substratdiversitet ved at udvælge og berige sjældne nedbrydere, som har det nødvendige metaboliske maskineri til at "håndtere" et stort antal forskellige olieforbindelser. Indikationer på præeksponering med olie vil derfor være vigtige for den strategiske vurdering af miljøpåvirkningen.

Sediment og/eller havvand blev udtaget på 11 stationer indenfor og udenfor licensområderne. Vi udførte fire delforsøg: 1) Sediment fra 10 stationer blev analyseret for det naturlige indhold af olieforbindelser og olienedbrydende mikroorganismer. 2) Seks sedimenter fra kontinentalsoklen blev tilsat råolie og/eller næringssalte for at undersøge potentialet for nedbrydning af olie, og i hvilket omfang dette potentiale afhænger af tilsætning af næringssalte. 3) Nedbrydning af råolie blev undersøgt i havvand fra en udvalgt station ved at sammenligne nedbrydning i ubehandlet vand med nedbrydningen i vand som var behandlet med næringssalte og/eller et dispergeringsmiddel. 4) Ved tre stationer blev de mikrobielle nedbryderes oliemetabolisme kvalitativt karakteriseret ved at udtage prøver fra forskellige dybder, opkoncentrere mikroorganismerne og inkubere dem med råolie under optimale forhold.

Resultaterne fra vores forholdsvis simple laboratorieforsøg kan kun med stor forsigtighed ekstrapoleres til Grønlandshavets komplekse miljø, men det var tydeligt, at nogle mikrokosmer udviste et stort potentiale for bionedbrydning af mange forskellige olieforbindelser i vandsøjlen. Selv "svære" forbindelser som fx C1-pyrener og C3-phenanathrener blev delvist nedbrudt efter 70-90 dage. Dette viser, at der kan være et stort potentiale for bioremediering af overfladespild, hvis de naturlige mikrobielle nedbrydere kan aktiveres.

Det var også tydeligt, at in situ koncentrationer af næringssalte er stærkt begrænsende for olienedbrydningen. Mikrobiel olienedbrydning vil således være meget begrænset i vandsøjlen uden tilsætning af næringssalte. Beredskabsstrategier baseret på det naturlige potentiale for mikrobiel fjernelse af olie bør derfor omfatte strategier for anvendelse af næringssalte, for at nedbrydningen kan blive effektiv. Dispergeringsmidlet Slickgone NS dispergerede effektivt olien under de testede forhold, men vil have ringe virkning på den biologiske olienedbrydning, når næringssalte er begrænsende, sandsynligvis fordi nedbrydning af dispergeringsmidlet i sig selv kræver næringssalte.

Koncentrationen af alkyl-PAH'er var lav i sedimenter fra overgangen mellem kontinentalsoklen og kontinentalskrænten, men lette alkyl-PAH'er kunne påvises, hvilket tyder på, at stationerne på overgangen mellem kontinentalsoklen og kontinentalskrænten måske har haft en eller anden form for begrænset olieeksponering. Alkyl-PAH'er kunne ikke påvises i sedimenter fra kontinentalsoklen, hvilket tyder på at stationerne på kontinentalsoklen ikke har været eksponerede for olie. Potentialet for biologisk nedbrydning af forvitrede

PAH'er i sedimenter fra kontinentalsoklen var meget lavt, selv hvis næringssalte ikke var en begrænsende faktor, hvilket er i overensstemmelse med den manglende olieeksponering.

De anvendte metoder havde hver især fordele og ulemper, men kombineret var de stærke værktøjer. Den viden, der er tilvejebragt i dette projekt, vil være vigtig for at kunne vurdere de naturlige mikrobiologiske samfunds potentiale for oliefjernelse i Grønlandshavet ud for Nordøstgrønland.

3 Eqikkaaneq

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4 Introduction

With increasing oil exploration activities in the Greenland Sea more information is needed on the ecology and sensitivity of this very little studied marine ecosystem. An interdisciplinary survey with the research ship Dana was therefore conducted in August/September 2017 to integrate oceanographic and biological surveys. Offshore oil exploration and exploitation in this area will be associated with an increased risk of accidental oil spills due to sea ice, drifting icebergs, and the depth at which the drillings may take place (Figure 1). There is, however, no knowledge on the fate of oil pollution in the different compartments of the Greenland Sea such as spills on the surface, dispersed oil in the water column and sediment polluted by sinking oil. The blow out at BP's Macondo Deepwater Horizon drilling rig in the Gulf of Mexico in 2010 exemplified the risks of offshore drillings for oil. One major lesson learned was that knowledge on intrinsic potentials for oil biodegradation in waterbodies and sediment in oil drilling areas is crucial to predict the fate of oil released to the water column. This may be especially important for the cold, pristine and oligotrophic waters and sediments offshore Northeast Greenland, where the environmental conditions for microbial oil degradation may be much less favourable than in the Gulf of Mexico with respect to temperature, mineral nutrients and oil pre-exposure. It is therefore critical to assess the intrinsic oil biodegradation potential, as biodegradation may often be the only remediation strategy possible for offshore spills in remote areas such as the Greenland Sea. This biodegradation potential depends on a number of environmental factors including the presence of microorganism that can degrade the multitude of structurally different oil compounds, sufficient levels of mineral nutrients, and possibly also the oil bioavailability, which may be manipulated by adding dispersants.

Figure 1. Iceberg in a Greenland Sea snowstorm on September 2, 2017. Weather, drifting icebergs and sea ice limit the possible oil remediation strategies.

The focus of our experiments was therefore to determine to which extend oil-degrading bacteria are present in the different marine compartments, i.e., in surface water, at different depths in the water column, and in the upper sediment offshore Northeast Greenland. This type of analyses has often been based on chemical measurements of oil degradation by GC-FID, which measures reductions in the total concentration of petroleum hydrocarbon over time. The GC-FID measurements have often been supplemented with counts of total hydrocarbon-degrading bacteria, i.e., changes in the concentration of bacteria that can grow on crude oil or diesel. The drawback of these approaches is that they have a strong bias towards the straight-chain alkanes, which comprise the largest oil fraction, but the alkanes are also easily degraded by microbes and show relatively little toxicity. The aromatic fraction, however, is much less biodegradable and has the highest toxic potential. Counts of microbial oil degraders should therefore cover representative compounds from various oil substance groups including representative aromatics to get a measure of the metabolic diversity of the oil-degrading bacteria, i.e., to test if they have the metabolic capacity to grow on a wide range of structurally different oil compounds or only the ability to grow on simple alkanes. Oil degradation should also be determined by chemical measurements of the degradation of a range of structurally different oil compound classes by GC-MS. Biodegradation may then be distinguished from physical removal by calculation of diagnostic ratios. The principle of diagnostic ratios relies on the assumption that bacteria degrade isomers within a compound class, typically alkylated aromatics, at different rates, whereas physicochemical processes such as evaporation and dissolution affect the isomers equally. This means that biodegradation rates depend on the positions of the alkyl side chains and biodegradation is therefore reflected in changing isomer ratios. Physicochemical oil removal will not change the diagnostic isomer ratios; changes over time in diagnostic isomer ratios are therefore a very strong indication of oil biodegradation.

The overall aim of our study was to determine the microbial capacity for degradation of different oil compound classes in sediment and in the water column (surface, middle and bottom) of the Greenland Sea.

To this end, we conducted a series of experiments on-board the research vessel Dana as well as back in the laboratories. Initially, we characterized sediment samples with respect to the content of aromatic hydrocarbons and hydrocarbon degrader microorganisms. This was to determine the level and variation of natural oil pre-exposure in the area. If detected, exposure would presumably be the result of oil seeps that could seed downstream sediments with microbial aromatics degraders as well as increased concentrations of recalcitrant and alkylated polycyclic aromatic hydrocarbons (PAHs). In a second experiment, selected sediments were spiked with crude oil and/or mineral nutrients to investigate the potential for oil degradation in the sediment, and to which extend this potential is dependent on the addition of mineral nutrients. A third experiment investigated in detail the oil degradation in seawater from a selected station. This was done by comparing the intrinsic degradation and degradation in water spiked with mineral nutrients and/or a dispersion agent. A fourth experiment was set up to determine the general efficiency and variation in the metabolism of microbial oil degraders at different depths of the water column at different stations. This was done by extracting microorganisms from water samples and incubating them with crude oil under optimized conditions.

We used different methods to evaluate the experiments. The first approach was to determine the population size of different types of microbial oil degraders and how these populations changed over time. This was done by using a most-probable-number (MPN) method that counts the concentration of microbes that can utilize different selected oil compounds as source of carbon and energy. MPN was followed by different ways of interpreting the concentration of specific oil compounds determined by GC-MS. For characterization of sediment samples, we determined the absolute concentrations of a range of PAHs to investigate in-situ oil pre-exposure. For the incubation experiments with water or sediments spiked with crude oil, we determined how the relative concentration of alkanes and series of alkylated PAHs changed over time, i.e., the efficiency of oil degradation. Finally, the changes in oil concentration were related to biodegradation by calculating diagnostic oil compound ratios that can discriminate between physical oil removal and microbial oil removal, which may be the only realistic remediation process for many types of oil spill in this area.

On top of this, differences and changes in the prokaryotic communities were quantified at the DNA level. This was done by extracting DNA from the original water- and sediment samples, from selected microcosm samples and from growth-positive MPN-plate wells. The communities in these samples were characterized by sequencing of 16S rRNA genes. The original samples represented the in-situ prokaryotic communities, the microcosms represented the changes induced by oil and mineral nutrients, and the MPN plate wells represented the dominant, cultivable hydrocarbon degraders.

5 Materials and Methods

5.1 Sampling and transport

Water and sediments was sampled during a three-week campaign on-board the research ship Dana during August-September 2017. The campaign was part of an interdisciplinary survey to integrate oceanographic and biological surveys. Reports from the other projects as part of the interdisciplinary study can be found here: http://projects.au.dk/neg/. In Figure 2 the survey area, sampling stations for our samplings as well as license areas in the Greenland Sea is shown.

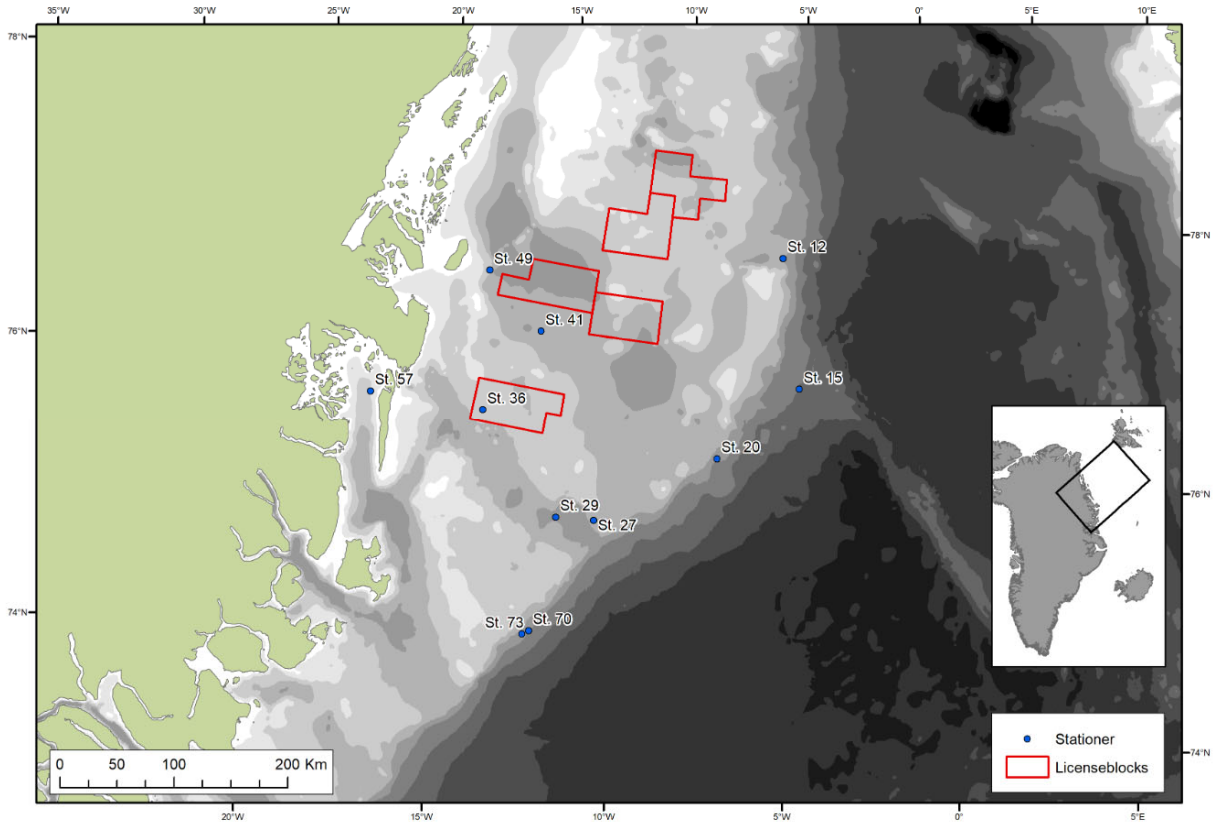


Figure 2. Map of the survey area, sampling stations for our samplings as well as license areas in the Greenland Sea.

Eleven representative stations were selected within the surveyed area of the Greenland Sea. The location of the selected stations was partly determined by the distribution of sea ice, which hindered access to many of the planed stations within the license areas. Sediment was sampled from ten stations by using a HAPS corer (KC HAPS bottom corer, KC Denmark, one core from each station, Figure 3). The sediment sampling was coordinated with another project as part of the campaign to reduce the amount of resources spend on sediment sampling (Subproject 5.2, Benthic macro-fauna and habitats, http://projects.au.dk/neg/theme-5-benthic-flora-and-fauna-biodiversity-productivity-food-chain/). The cores had a total length of up to 40 cm. The upper 6-7 cm of sediment were transferred to 0.5-L plastic containers, sealed, and stored at 2 °C until return to the lab in Copenhagen. For coastal sediments, all of the oxidized top layer was sampled, which for a few stations was not quite enough sample material and resulted in fewer replicates in some sediment experiments.

Water from the bottom, middle and surface of the water column was sampled at four station using Danas' CTD-rosette (SBE-32) with Niskin bottles (Figure 4). The bottles were emptied into new 10-L foldable PE water bags. At each station, the optimal sampling depths were determined from depth-profiles of temperature, salinity and fluorescence (chlorophyll). The origins of the sampled water bodies were tentatively identified from temperature and salinity profiles according to Rudels et al. (2002). Water samples were either processed immediately onboard DANA or stored at 2 °C and processed upon arrival at Copenhagen. Temperature loggers were placed together with the samples to monitor the temperature from sampling to the lab in Copenhagen. Bacteria in the water phase may have a quick population turn-over due to continuous predation and virus infection (bacteriophages). We therefore initiated the water experiments on-board Dana. The bacterial populations in sediments are presumably more stable, sediment experiments were therefore initiated upon arrival in Copenhagen.

Figure 3. HAPS corer for sediment sampling.

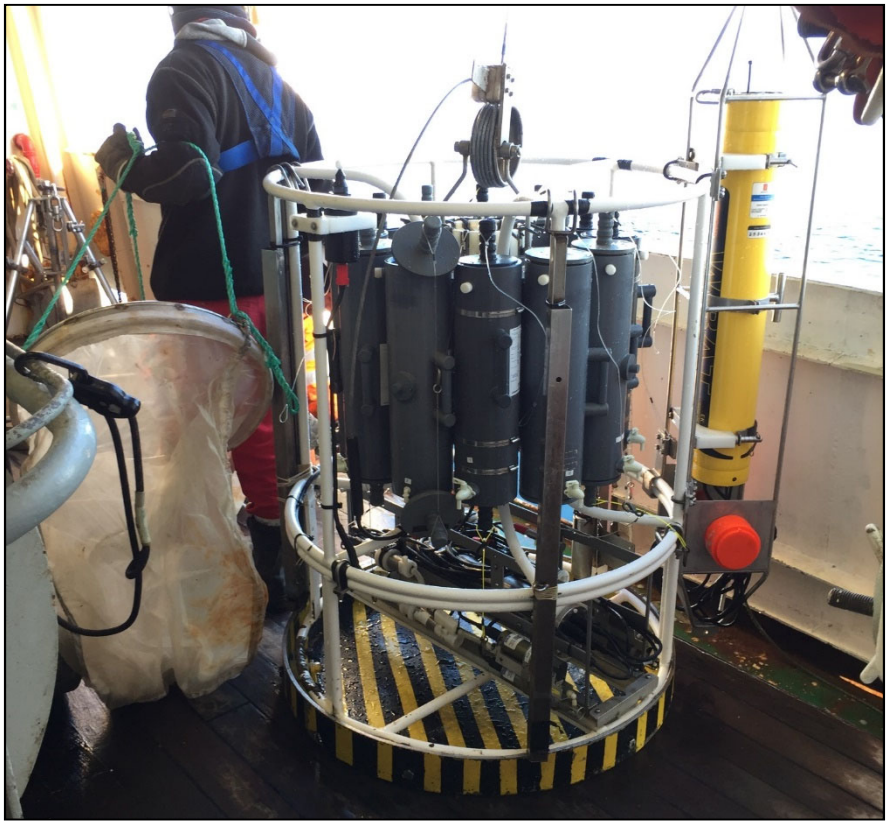


Figure 4. Danas' CTD rosette for water sampling and measurements of depth, salinity, temperature, fluorescence, etc.

5.2 Characterization of sediments.

This characterization determined the natural oil pre-exposure by screening the sediment samples for natural in-situ presence of oil compounds (GC-MS) and oil degrader microorganisms (MPN). Sediment samples were homogenized upon arrival at the GEUS labs in Copenhagen. Subsamples of 0.4 g (wet weight) were transferred to 50-mL centrifuge tubes and half-strength Bushnell Haas mineral medium (Bushnell and Haas, 1941) with 25 g/L NaCl (BH-medium) was added to a total volume of 40 mL. The sediment suspensions were used for MPN enumeration of different types of microbial hydrocarbon degrader as described below. Sediment subsamples (20 g) were frozen in glass scintillation vials for later quantification of PAH content (described below) and extraction of total genomic DNA.

5.3 Microcosm experiments

5.3.1 Metabolic capacity for degradation of structurally diverse oil components in sediment microcosms with and without added mineral nutrients

This experiment determined the response of indigenous oil-degrading microorganisms when oil entered the sediment. Sediments from the stations 27, 29, 36, 41, 49 and 57 were tested for oil degradation and the development in populations of oil-degrading microorganisms. We used the paraffinic Statfjord crude as our model oil source as this crude is expected to resemble oil from the area (Boertmann and Mosbech 2011). One µL of the crude oil was streaked in duplicate on tryptic soy broth plates, and 50 µL was streaked in duplicate on half-strength Bushnell Haas mineral medium (added 25 g/L NaCl) to test for sterility. No colonies formed during eight weeks incubation at room temperature, which confirmed that the aromatics content was sufficient to keep the crude oil sterile. For the sediment incubations, the Statfjord crude was artificially weathered by evaporating 30 % (v/v) under a gentle stream of nitrogen.

Subsamples of 25 g sediment (wet weight) were transferred to 250-mL red cap glass bottles with Teflon liners in the lids. 25 mg BH salts were added to half of the flasks from each station. Bottom water from Station 76 (sampled 2-4 m above the bottom) was sterile-filtered through 0.2 µm filters. Aliquots of 10 mL filtered bottom water was added to the flasks followed by 25 µL of artificially weathered crude oil. The flasks were immediately closed, shaken heavily and incubated in the dark at 4 °C. Triplicate microcosms were terminated after 0, 21 and 70 days incubation by adding 10 mL HPLC-grade methanol followed by shaking. The microcosms were frozen for later oil analyses (see below). Negative controls for oil analysis and DNA analysis were prepared without sediment and frozen on day zero. Microcosms terminated on day 21 and 70 were also analysed by MPN for different subpopulations of microbial oil degraders by taking 0.2 mL subsamples (after 1 min manual shaking) before addition of methanol. The subsamples were mixed with 19.8 mL cold BH-medium and used for MPN dilution series as described below. The reduction in oxygen concentration was followed in controls with sediment from the stations 27, 29, 36 and 41. The controls were prepared with and without BH salts as described above, but with the exception that a small OxyDot (non-invasive oxygen sensor) was placed inside each flask, so that the oxygen concentration could be read from the outside with an OxyDot reader.

5.3.2 Water microcosms to describe the effects of nutrients and oil dispersion

A series of experiments were set up to elucidate whether the in-situ concentrations of nutrients limited oil degradation and to elucidate how dispersing chemicals may interfere with oil degradation and nutrients since degradation of the dispersant will itself require nutrients. We used the chemical dispersant Slickgone NS provided by Greenland Oil Spill Response A/S. Slickgone NS is composed of hydrotreated light petroleum distillates and sodium dioctyl sulphosuccinate (DASIC International, 2017). A pre-experiment showed that a 1:10 Slickgone/oil ratio was sufficient to disperse 10 µL of Statfjord crude in 350 mL of seawater. Dispersion was demonstrated by highly increased turbidity after shaking and the absence of a visible slick as compared to controls without Slickgone. Seawater was sampled at Station 70 from 10 meters depth and 340 meters depth. We chose 10 m depth instead of surface water to avoid surface film, cooling water, exhaust from the ship etc. Aliquots of 350 mL were distributed in 1-L glass flasks on-board Dana. Half of the microcosm flasks contained 40 mg of Bushnell-Haas mineral salts (mineral nutrients), and half were controls without added mineral nutrients. Half of the flasks were spiked with 10 µL Statfjord crude, the other half were spiked with 11 µL of a 1:10 mixture of Slickgone NS dispersant and Statfjord crude. We thus compared four treatments: 1. control flasks without dispersant and without mineral nutrients, 2. flasks with added mineral nutrients, 3. flasks with added dispersant, and 4. flasks with added nutrients and added dispersant. The flasks were closed with Teflon-lined caps and shaken vigorously to disperse the oil and incubated in the dark at 2-4 °C with weekly shaking. Triplicate microcosms from each of the four treatments were inactivated by adding 0,130 L 96% ethanol after 0, 28 or 90 days incubation followed by freezing for later oil analyses (see below). Microcosms terminated on day 28 and 90 were also analysed for different subpopulations of microbial oil degraders (MPN) by taking 0.4 mL subsamples (after 1 min manual shaking) before addition of the ethanol. The subsamples were mixed with 9.6 mL cold BH-medium and used for MPN dilution series as described below. Time zero reagent blanks were prepared with and without BH salts as described above, but with the exception that oil was not added.

5.3.3 Characterization of the metabolic capacity for degradation of structurally diverse oil components in the water column.

These experiments were designed to determine the diversity and thus the potential of the oil compounds that can be metabolized by the oil-degrading bacteria. Water was sampled from the top (10 m), the middle and the bottom of the water column at three stations (29, 41 and 57), that were also tested for oil degradation in the sediment (above). The bottom samples were sampled approximately 40 meters above the bottom to stay clear of the bottom boundary layer that may contain suspended material from the sediment. As hydrocarbon degraders are rare in the water phase at pristine places, we concentrated the water samples by filtration through sterile 10-mL filter funnels (0.2 µm MicroFunnel, Pall Corporation). Each filter contained the microorganisms present in 1L of seawater (the filtride). The filters were transferred to 100-mL red-cap bottles containing 20 mL of half-strength BH-medium supplemented with 25 g/L of NaCl. Ten µL of crude oil was added, the flasks were sealed with Teflon-lined caps and incubated at 2-4 °C with manual shaking twice a week in addition to the shaking from the ship's movement. The mineral salts and the large headspace ensured that the microbial communities were not limited by neither oxygen, nor nutrients. Duplicate samples were inactivated

by adding 8 mL 96% ethanol after 0, 35 or 84 days incubation followed by freezing for later oil analyses (see below). Microcosms terminated on day 35 and 84 were also analysed for different subpopulations of microbial oil degraders (MPN) by taking 0.4 mL subsamples (after 1 min manual shaking) before addition of the ethanol. Time zero reagent blanks with sterile filters and with and without oil were prepared on-board Dana as described above. A second series of sterile blanks with oil were prepared and incubated under sterile conditions in Copenhagen to determine the non-biotic changes in oil compounds over time, i.e., to account for non-biological oil removal.

5.4 Analysis of alkanes and PAHs by gas chromatography mass spectrometry (GC-MS)

5.4.1 Sample preparation for full quantification of PAHs in sediment samples

All samples were homogenized with equal amounts hydromatrix in an IKA 11® sample mill and extracted through pressurized liquid extraction (PLE) in an ASE200 system. The extraction cell for the sediment samples consisted of 4 g of activated silica, bottom layer, 4 g of acidified copper powder (to bind elemental sulphur), ca. 5 g of sample mix (equivalent to ca. 3g dry weight), and topped off with Ottawa sand. The samples were added 200 µL 8 µg / mL internal standard mix (see Table 1 for content and application). The following extraction parameters were used: Pressure: 1500 psi, preheat time: 2 minutes, static time: 5 minutes, flush volume: 70%, purge time: 60 seconds, static cycles: 2, temperature: 100°C, solvent mixture: *n*-pentane:dichloromethane (90:10). Each cell was extracted twice into separate collection vials, and after concentrating under elevated temperature (40°C), the two extracts were combined and evaporated to below 5 mL, added 200 µL 8 µg / mL recovery standard mix (see Table 1 for content and application) and filled to 5 mL with *n*-pentane:dichloromethane (90:10).

5.4.2 Sample preparation for semi-quantification of alkanes and PAHs in sediment microcosms

The sediment microcosms were extracted in the original bottles in which they were provided. The samples were added 1 g NaCl, 20 mL 0.05 M $Na_4P_2O_7$, 20 mL *n*-pentane, and 200 µL 8 µg / mL internal standard mix (see Table 1 for content and application). The samples were shaken overnight and the supernatant / pentane phase was transferred to a vial. The extractable amount was very low for these samples and it was not possible to add the recovery standard mix.

5.4.3 Sample preparation for semi-quantification of alkanes and PAHs in seawater microcosms

The water microcosms were extracted in the original bottles in which they were provided. The samples were added 30 g NaCl, 20 mL *n*-pentane, and 200 µL 8 µg / mL internal standard mix (see Table 1 for content and application). The samples were shaken overnight and the supernatant / pentane phase was transferred to a vial, added 200 µL 8 µg / mL recovery standard mix (see Table 1 for content and application) and filled to 20 mL with *n*-pentane. For the initial ten samples in the sample preparation procedure, NaCl was not added and these samples were stirred with a magnetic stirrer overnight. The sample numbers for these ten samples are: 203, 205, 209, 213, 229, 240, 243, 245, 246, and 255.

5.4.4 Sample preparation for semi-quantification of alkanes and PAHs in filter microcosms

The filter microcosms were extracted in the original red cap bottles in which they were provided. All samples were added 20 mL 0.05 M $Na_4P_2O_7$, 20 mL *n*-pentane, and 200 µL 8 µg / mL internal standard mix (see Table 1 for content and application). The samples were shaken overnight and the supernatant / pentane phase was transferred to a vial, filled to 20 mL with *n*-pentane and added 200 µL 8 µg / mL recovery standard mix (see Table 1 for content and application).

Table 1. List of internal and recovery standards for the quantification of PAHs.

PAH	Internal standard	Recovery standard
Naphthalene, Acenaphthylene	Naphthalene-d8	Acenaphthylene-d8
Acenaphthene	Acenaphthene-d10	Anthracene-d10
Fluorene	Fluorene-d10	Anthracene-d10
Dibenzothiophene	Dibenzothiophene-d8	Anthracene-d10
Anthracene, phenanthrene	Phenanthrene-d10	Anthracene-d10
Pyrene, fluoranthene	Pyrene-d10	Fluoranthene-d10
Chrysene, benzo(a)anthracene	Chrysene-d12	Benzo(a)anthracene-d12
Benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene	Benzo(k)fluoranthene-d12	Benzo(a)pyrene-d12
Dibenzo(a,h)anthracene, Benzo(ghi)perylene, indeno(1,2,3-c,d)pyrene	Benzo(ghi)perylene-d12	Indeno(1,2,3-cd)pyrene-d12

5.4.5 GC-MS analysis

The extracts were analysed for PAHs and alkyl PAHs (Figure 5) on an Agilent 5975C inert XL MSD with electron ionization operating in selected ion monitoring (SIM) mode. The GC parameters were as follows: 1 µl sample was injected in split less mode (inlet: 300°C) to a 60 m HP-5 capillary column with 0.25 mm inner diameter, 0.25 µm film thickness. The flow rate was 1.1 ml / min. The initial temperature (40°C) was held for 2 minutes, ramped with 25 °C / min to 100 °C, followed by 5 °C / min to 315 °C and held for 14 minutes (total run time: 61.4 minutes). Temperatures: transfer line: 315 °C, ion source: 230 °C, quadrupole: 150 °C. 55 m / z values were monitored in 12 groups with 13 m / z's each, with a dwell time of 25 ms, according to Table 2. Peaks were quantified using MassHunter Quantitative Analysis version B.07.00 (Agilent technologies, Inc.). Internal standard normalization was used for all sediments and microcosms except for the filter microcosms where hopane normalization was used because 0.4% of the sample volume was used for MPN at day 35 and day 84, but not at day zero.

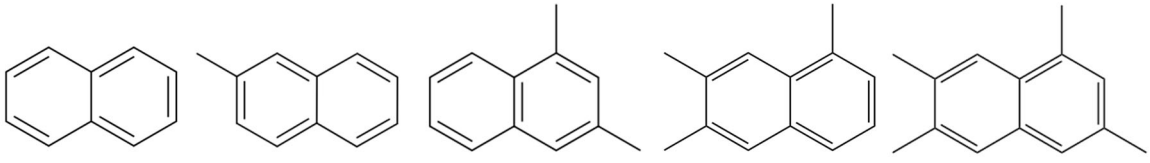


Figure 5. PAHs are grouped in categories with the same ring structure, but with different numbers and positions of alkyl side groups. Examples of naphthalenes with (left to right) zero (C0-), one (C1-), two (C2-), three (C3-) or four (C4-) carbon atoms in alkyl side groups.

Table 2. List of compounds, selected ion chromatogram (SICs) and corresponding groups of GC-MS/SIM as described by Gallotta and Christensen (2012).

	Group number												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
nCx	**0**	**13**	**14**	**15**	**16**	**18**	**19**	**21**	**23**	**24**	**27**	**32**	**n-alkane (retention time of n-alkane to be used for group division)**
SIC													**Compound name**
83	x	x	x	x	x	x	x	x	x	x	x	x	n-Alkyl cyclo hexanes
85	x	x	x	x	x	x	x	x	x	x	x	x	Alkanes
105	x	x	x	x	x	x	x	x	x	x	x	x	Alkyl toluenes
123	x	x	x	x	x	x							Sesquiterpanes
128	x												Naphthalene
134	x												Benzo(b)thiophene
136	x												d8-Naphthalene
138	x												Decalin
142		x											C_1-Naphthalenes
148	x	x											C_1-Benzo(b)thiophenes
152	x	x	x										C_1-Decalins, Acenaphthylene
154		x	x	x									Acenaphthene
156			x										C_2-Naphthalenes
160			x										d8-Acenaphthylene
162		x	x										C_2-Benzo(b)thiophenes
164			x	x									d10-Acenaphthene
166	x	x			x								C_2-Decalins, Fluorene
168		x	x	x									Dibenzofuran
170				x	x								C_3-Naphthalenes
176				x	x								C_3-Benzo(b)thiophenes, d10-Fluorene
178						x							Phenanthrene, Anthracene
180	x	x	x		x								C_3-Decalins, C_1-Fluorenes
182				x	x	x							C_1-Dibenzofurans
184				x	x	x							C_4-Naphthalenes, Dibenzothiophene
188						x							d10-Phenanthrene, d10-Anthracene
190				x	x								C_4-Benzo(b)thiophenes
191									x	x	x	x	Tricyclic terpanes, Hopanes
192					x	x	x						C_1-Phenanthrenes/anthracenes, d8-Dibenzothiophene
194	x	x	x	x		x	x						C_4-Decalins, C_2-fluorenes
196					x	x	x						C_2-Dibenzofurans
198						x	x						C_1-Dibenzothiophenes
202							x	x	x				Fluoranthene, Pyrene
206							x	x	x				C_2-Phenanthrenes/anthracenes
208						x	x						C_3-Fluorenes
212							x	x					C_2-Dibenzothiophenes, d10-Fluoranthene, d10-Pyrene
216								x	x				C_1-Fluoranthenes/pyrenes
217								x	x	x	x	x	Steranes
218										x	x	x	Steranes
220							x	x	x				C_3-Phenanthrenes/anthracenes
226							x	x	x				C_3-Dibenzothiophenes
228										x			Benzo(a)anthracene, Chrysene
230									x	x			C_2-Fluoranthenes/pyrenes
231										x	x	x	Triaromatic steranes
234								x	x	x			C_4-Phenanthrenes/anthracenes, Retene, Benzonaphtothiophene

240								x	x	x			C_4-Dibenzothiophenes, d12-Benzo(a)anthracene, d12-Chrysene
242										x	x		C_1-Chrysenes
244								x					d14-p-Terphenyl
248										x	x		C_1-Benzonaphtothiophenes
252											x	x	5 Rings PAHs
256											x		C_2-Chrysenes
264											x	x	d12-Benzo(k)fluoranthene, d12-Benzo(a)pyrene, d12-Perylene
270											x	x	C_3-Chrysenes
276												x	6 Rings PAHs
278												x	6 Rings PAHs
288												x	d12-Indeno(1,2,3-cd)pyrene, d12-Benzo(ghi)perylene
Total	13	13	13	13	13	13	13	13	13	13	13	13	

5.5 Most-Probable-Number (MPN) enumeration of hydrocarbon degrading microorganisms.

The size of microbial subpopulations that could utilize specific hydrocarbons as sole source of carbon and energy were estimated with a modified version of a previously published microplate method (Johnsen and Henriksen, 2009) where hydrocarbon growth substrates are added to the microplate wells in a separate silicone phase (silicone oil AR20, Sigma Aldrich) to avoid toxic effects. The concentrations in the silicone phase were as follows: hexadecane: 10 %; 2,2,4-trimethylpentane: 10 %; 1,3-dimethylcyclohexane: 10 %; isobutylbenzene: 2 %; 2-methylnaphthalene: 2 % phenanthrene: saturated solution. The molecular structure of the substrates is depicted in Figure 6. The substrates were pasteurized for 5 min in a 70 °C water bath in tightly closed glass scintillation vials with aluliners in the lids. Half strength Bushnell-Haas minimal medium (Bushnell and Haas, 1941) was supplemented with 25 g/L of NaCl (BH-medium) and autoclaved for 20 minutes at 121 °C in tightly closed red-cap bottles. The precipitate was removed after autoclavation by decanting into sterile blue-cap bottles. For MPN enumerations, 0.4 mL subsamples were sampled from each microcosm. Five-fold dilution series were prepared in cold BH-medium. From six dilution levels, four times 200 µL were added to seven microtiter plates. The first dilution was 25-fold for water samples and 2500 fold for sediment samples because of the high content of suspended clay and silt in lower dilutions. Then, 15 µL of hydrocarbon substrate solution was added to each microplate well (one substrate per microplate), a microplate lid was put on and the microplates were placed in airtight plastic boxes. A small vial with extra substrate solution (1 mL) was placed on top of the plates to compensate for evaporation. Plates with sample dilutions and silicone oil, but no hydrocarbons, served as negative controls. The plates were incubated at 10 ºC in the dark. After 10 weeks incubation, absorbance of the microplate wells were read at 450 nm on a microplate reader. Wells were scored growth-positive when having an absorbance higher than 0.1. MPN estimates were calculated according to Hurley and Roscoe (1983) from the distribution of growth-positive and growth-negative microplate wells (Figure 7). The detection limit was calculated as one growth-positive well in the lowest dilution, giving a MPN of 26 cells/mL for water samples and 2600 cells/mL for sediment samples.

Figure 6. Hydrocarbons used as substrates in MPN-enumeration of specific oil degrader microorganisms.

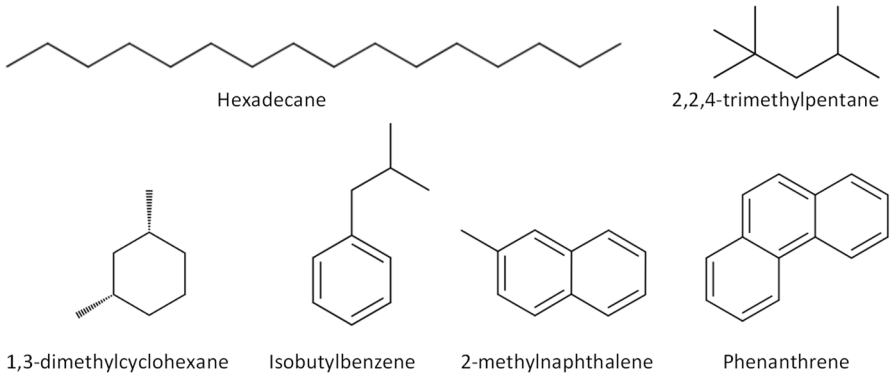
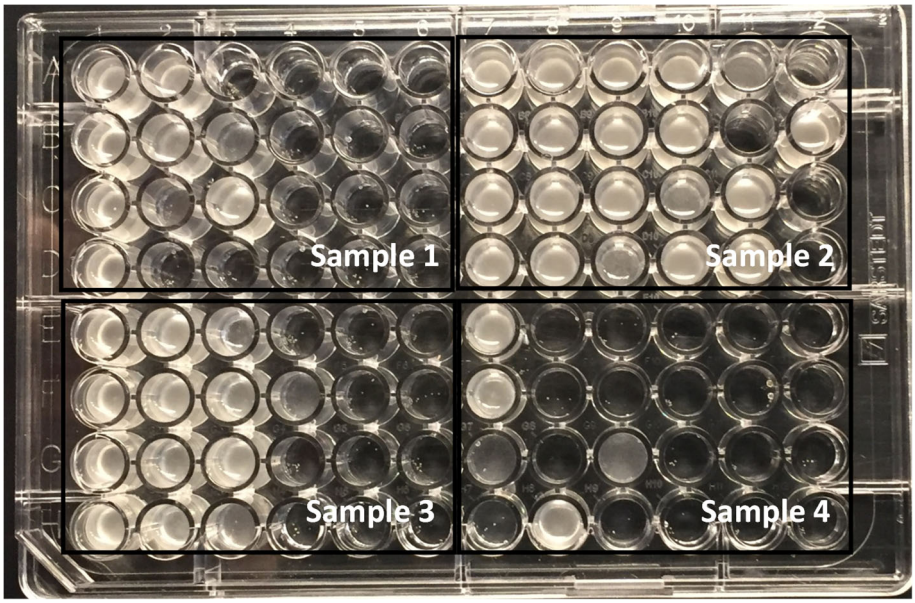


Figure 7. MPN-plate for enumeration of hexadecane degraders. The plate contains four samples, each with 6 dilution levels and four replicates per dilution level. Turbid wells (white) are growth-positive.



5.6 Phylogenetic characterization of the microbial communities from selected samples.

To evaluate the bacterial communities DNA was extracted from selected samples of water, sediment, microcosms and MPN plates. The following samples were chosen:

1. Sediment samples from all sediment stations sampled in section 4.2.
2. Sediments microcosms when the incubation was terminated after 70 days enrichment of oil degraders (section 5.3.1). The samples used were subsamples of the first dilution (100-fold dilution) from the MPN dilution series.
3. MPN plates from the sediment microcosms to characterize the dominant cultivable oil degraders that had emerged when the incubations were terminated (section 4.3.1, day 70). For each MPN sample, 4 replicate wells of the highest growth-positive dilution were pooled.
4. Filters containing the microorganisms from 1L of seawater; these were replicas of the filters used to initiate microcosms (section 5.3.3)
5. Filter microcosms (section 4.3.3) subsampled when the incubation was terminated after 84 days incubation and enrichment of oil degraders. The samples used were subsamples of the first dilution (25-fold dilution) from the MPN dilution series.

6. MPN plates from the filter microcosms to characterize the dominant cultivable oil degraders that had emerged when the incubations were terminated (section 4.3.3, day 84). For each MPN sample, 4 replicate MPN plate wells of the highest growth-positive dilution were pooled.

All samples were frozen at minus 18 °C until DNA extraction. Based on previous tests, we decided to extract all samples by using the custom-built fusion kit II:MP BIOMEDICALS - FastDNA SPIN Kit for Soil, 116560200 MP BIOMEDICALS + G2 tubes (G2 DNA / RNA Enhancer 0,1 mm beads), AMPLIQON - PCR enzymes and reagents. Illumina 16S rRNA gene amplicon library preparation was done using the 341F and 806R primers and conditions described in Feld et al. (2016), and sequencing was carried out on the MiSeq platform at AU-ENVS. Bioinformatics analyses were done using Qiime2 pipeline. In short, demultiplexed reads from Illumina sequencing were analysed using QIIME 2 v. 2018.11. Reads were filtered, denoised, merged, chimera checked and dereplicated using DADA2. Alignment and phylogenetic tree generation were followed by inspection of rarefaction curves to check for saturation. The output was rarefied at 7.400 (water samples) and 7.900 (sediment samples) reads and one sample with low number of reads (37) was discarded.

The beta diversity of the samples, based on Bray-Curtis beta-diversity measure, was visualised by PCoA plots. Taxonomic classification was done using qiime feature-classifier in which a pre-trained Naïve-Bayes classifier with Silva v. 132 (silva-132-99-nb-classifier) was applied.

5.7 Maps

Maps showing sample locations and bathymetry were constructed as follows: Projection: WGS84 IBCAO Polar Stereographic, EPSG 3996, rotated 25°. Bathymetry data: IBCAO V3.0: (https://www.ngdc.noaa.gov/mgg/bathymetry/arctic/ibcaoversion3.html. Onshore map Greenland modified from Escher and Pulvertaft (1995).

6 Results and discussion

6.1 Characterization of sediments

Sediment from 10 stations (Figure 8) was analysed for the contents of natural oil compounds and the contents of oil degrader microorganisms. The stations were distributed on the continental shelf ranging from close to the coast (stations 36, 41, 49 and 57) over stations close to the shelf break (stations 27 and 29) to stations at the shelf break (stations 12, 15, 20 and 73) (Figure 8). The depth ranged from relatively shallow at Station 49 (117 m) to much deeper at the shelf break (1441 m, Station 15). Details for each station are presented in Table 3. The stations closest to the coast (stations 36, 49 and 57) were oxidized only in the top layer, whereas sediment from the stations further from the coast were oxidized at least down to 25 cm.

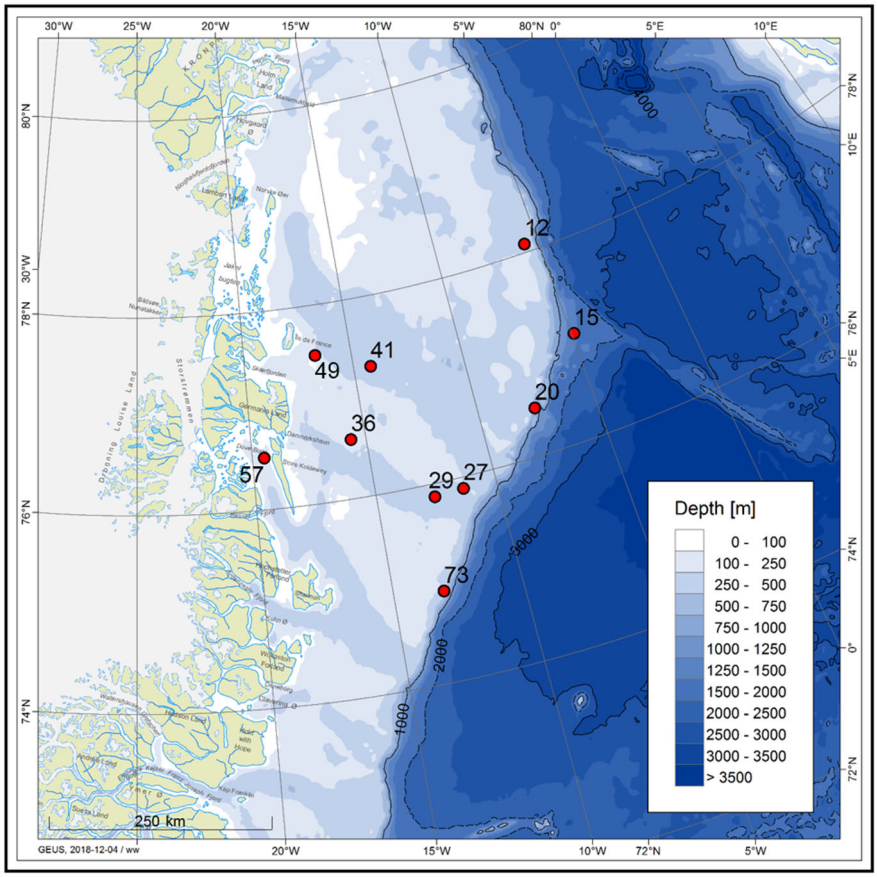


Figure 8. Map of the Greenland Sea showing stations sampled for sediment characterization

Table 3. Stations sampled for sediment characterization.

Station	Date	Depth (m)	Latitude	Longitude	Comment
12	26/8-2017	334	78.04.987N	005.39.617W	Core all oxidized.
15	28/8-2017	1441	77.03.770N	004.53.460W	Core all oxidized.
20	29/8-2017	757	76.28.524N	007.34.770W	Core all oxidized.
27	30/8-2017	300	75.53.168N	011.23.400W	Core all oxidized.
29	31/8-2017	402	75.52.114N	012.36.564W	Core all oxidized.
36	1/9-2017	191	76.35.715N	015.39.074W	Top 0-5 cm fully oxidized sample), partly reduced from 6 cm downwards.
41	1/9-2017	271	77.17.403N	014.12.661W	Core all oxidized.
49	2/9-2017	117	77.40.401N	16.29.502W	Top 0-4 cm fully oxidized (sample), 4-6 cm partly reduced, and 6 cm downwards greyish-blue and fully reduced.
57	4/9-2017	318	76.31.387N	019.28.635W	Top 0-3.5 cm fully oxidized (sample), greyish-blue and fully reduced from 4 cm downwards.
73	5/9-2017	450	74.55.168N	013.03.141W	Core all oxidized.

Concentrations of non-substituted PAHs and series of oil alkyl-PAHs were quantified using the internal standard method and corrected for recoveries. Concentrations for C1-naphthalene and C1-phenanthrene are presented in The sediments were also tested by an MPN-method for the concentration of microorganisms that can utilize specific oil compounds for growth. Oil-degrading microorganisms were not detected in any of the samples, not even degraders of the easily degradable compound *n*-hexadecane. The negative results were to some degree a consequence of a high detection limit (2600 cell / g), caused by the high content of silt and clay that interfered with optical density readings. The results nevertheless indicate that it is unlikely that oil seeps have enriched the sediments with high concentrations of biodegradable hydrocarbons at any of the stations, as in-situ oil enrichment would most likely have given detectable concentrations of degrader organisms. . The concentration of alkyl-PAHs (oil PAHs) were below the detection limit except for C1-naphthalenes and C1-phenanthrenes. All stations with detectable C1-naphthalenes (stations 12, 15, 20, 27, 29 and 73) were situated at the shelf break (stations 12, 15, 20 and 73) or on the shelf close to the shelf break (stations 27 and 29). The three stations with detectable concentrations of the less mobile C1-phenanthrene (stations 15, 20 and 73) were the deepest stations right at the shelf break. The low concentrations of C1- naphthalenes and C1-phenanthrenes together with the absence of heavier alkyl-PAHs, suggest that the six stations may have had only little pre-exposure to oil PAHs. The absence of detectable alkyl-PAHs in the shelf sediments suggests that these stations have had no oil pre-exposure.

The sediments were also analysed for the content of non-substituted PAHs. The results are presented as bar plots for concentrations of 2.5- to 6-ring PAHs (Figure 8). Non-substituted PAHs were present in all of the sediments (Figure 8) with total concentrations ranging from 50 to 129 µg / kg. Some sediments were depleted in the low-molecular weight 2.5-ring PAHs (stations 12, 27, 49 57 and 73) and two stations were enriched in the high-molecular-weight

PAHs with five or six rings. There was no obvious correlation to the geographical distribution of the sediments with respect to neither the composition of non-substituted PAHs, nor the total concentration.

The sediments were also tested by an MPN-method for the concentration of microorganisms that can utilize specific oil compounds for growth. Oil-degrading microorganisms were not detected in any of the samples, not even degraders of the easily degradable compound *n*-hexadecane. The negative results were to some degree a consequence of a high detection limit (2600 cell / g), caused by the high content of silt and clay that interfered with optical density readings. The results nevertheless indicate that it is unlikely that oil seeps have enriched the sediments with high concentrations of biodegradable hydrocarbons at any of the stations, as in-situ oil enrichment would most likely have given detectable concentrations of degrader organisms.

Table 4. Sediment content of alkyl-PAHs (mg/kg dry weight, n=1). Numbers indicate values smaller than the quantification limit, but larger than the detection limit (DL).

Sample name	C1-naphthalenes	C1-phenanthrenes
Station 12	(0.020)	<DL
Station 15	0.035	0.018
Station 20	0.023	0.020
Station 27	(0.010)	<DL
Station 29	(0.028)	<DL
Station 36	<DL	<DL
Station 41	<DL	<DL
Station 49	<DL	<DL
Station 57	<DL	<DL
Station 73	(0.021)	(0.011)

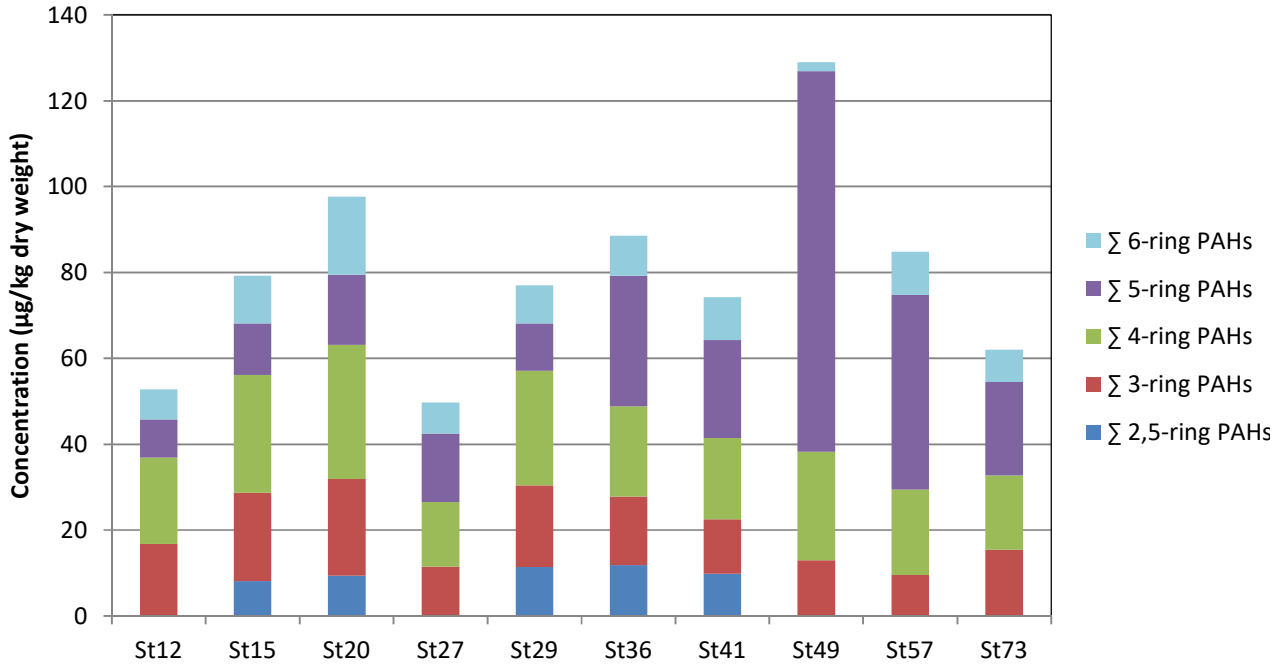


Figure 8. Sediment content of pyrogenic PAHS (mg/kg dry weight (n=1.). **2,5-ringPAHs**: acenaphthylene, acenaphthene, fluorene; **3-ring-PAHs**: anthracene, phenanthrene; **4-ring PAHs**: fluoranthene, pyrene, benz[a]anthracene, chrysene; **5-ring PAHs**: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene+benzo[a]pyrene, perylene, dibenz[a,h]anthracene; **6-ring PAHs**: indeno(1,2,3-c,d)pyrene, benzo[g,h,i]perylene.

The prokaryotic microbial communities (bacteria and archaea) in the sediments were characterized by DNA extraction followed by amplification and sequencing of prokaryotic (bacteria and archaea) 16S rRNA genes. The microbial communities in the sediment samples were greatly dominated by the archaean class *Nitrososphaeria* with 56 - 81% relative abundance (Figure 9). The lowest values for the relative abundance of this class were at station 27 and station 29, while the highest were found at station 12 and station 73. Gammaproteobacteria was the second most dominant class with 10 - 24% relative abundance (Figure 9).

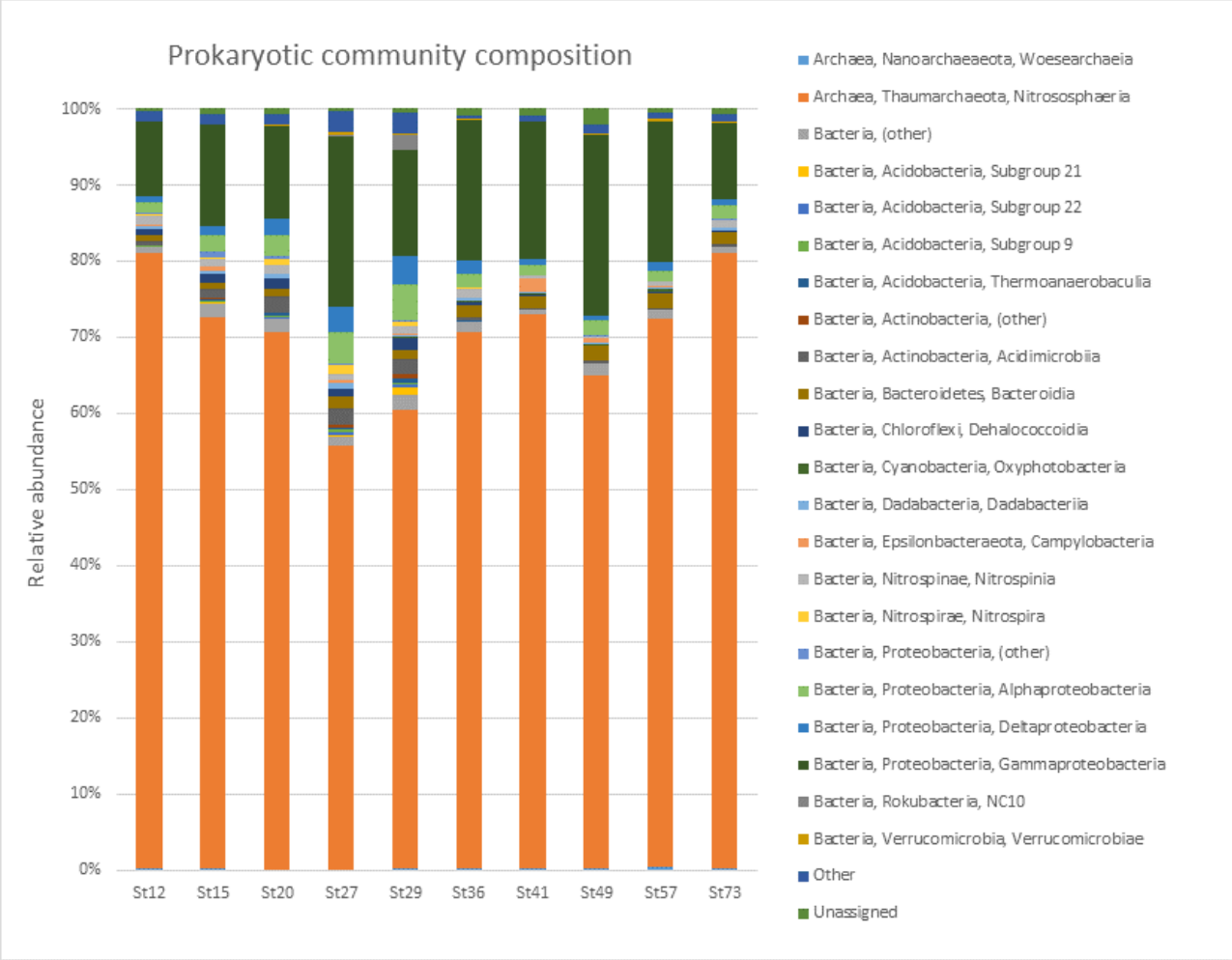


Figure 9. Prokaryotic community composition (i.e. bacteria and archaea) of the raw sediment samples from the 10 sampling stations. The taxonomy (16S rRNA gene) of the groups are determined until the class level where possible.

Differences and similarities in the microbial community structures are visualized in a beta-diversity plot (Figure 10). According to R. Lappan (2018) “Beta diversity refers to the diversity *between* samples. This is essentially a measure of how similar or dissimilar the samples are, and is usually represented by a distance matrix which is then used to do Principal Coordinates Analysis (PCoA). The result of this is an ordination plot of multiple dimensions, where each sample is a point and the distance between the points represents the similarity of those samples (closer together = more similar)”. Axis-1 explains most of the variation between the sediment samples (42.7%). The distribution of stations along axis-1 represents the geographical location of the stations. Microbial communities from the shelf break stations (St12, St15, St20 and St73) are more similar and well separated from the other samples along axis-1. Microbial communities from the coastal shelf stations (St36, St41, St49 and St57) are also more similar and well separated from the other samples along axis-1.

The communities from the shelf stations close to the shelf break (St27 and St29), on the other hand, are found in-between the two groups.

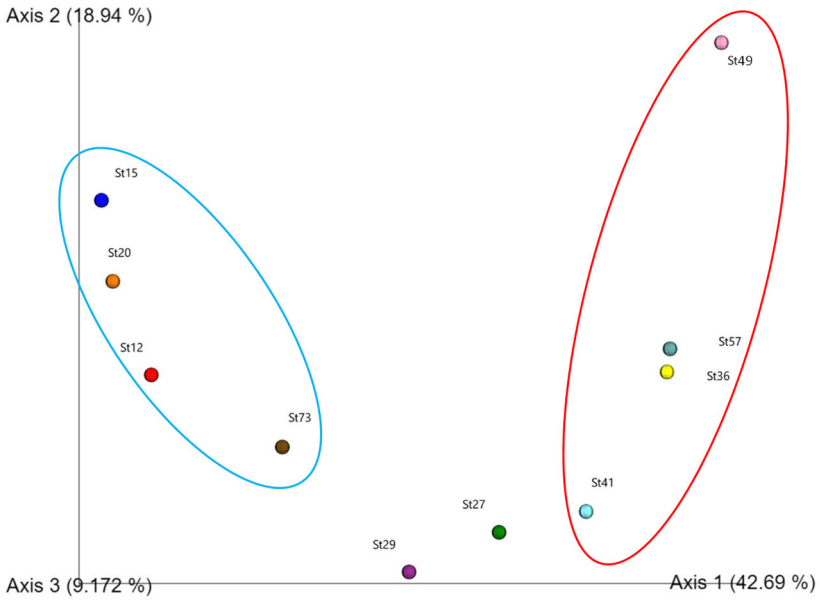


Figure 11. Betadiversity plot (16S rRNA) for visualization of differences in prokaryotic communities of the raw sediment samples. The blue group shows shelf stations, and the red group shows shelf break stations. Stations 27 and 29 are on the shelf but close to the shelf break.

6.2 Characterization of the metabolic capacity for degradation of structurally diverse oil components in top sediments

Six sediments were spiked with weathered crude oil to test how the potential for oil degradation and the evolution of different populations of degrader microorganisms evolve over time after an oil spill. Figure 12 shows the geographical distribution of the six tested sediments. They were all situated on the continental shelf, but at different distances from the coast and at different depths (Table 3). The sediments were tested both as they were and with addition of mineral nutrients.

Our first concern was that the oxygen consumption in the closed sediment microcosms would be too high so that oxygen would become limiting. We were especially concerned with the samples that were added mineral nutrients as these may increase the degradation of organic matter and other reduced compounds in the sediment. The microcosm with OxyDots showed that oxygen tensions did not drop to critical levels during incubation. The oxygen tension decreased only from 21 % to 16.4- 17.7 % and most of the oxygen consumption took place during the first ten days of incubation (Figure 13). There was furthermore almost no difference in oxygen consumption between microcosms with and without added mineral nutrients.

The degradation of oil compounds showed little variation between the six sediments. Most of the variation is covered by comparing results from Station 29 and Station 36 below. The degradation of *n*-alkanes was slow in station 29 sediment without addition of mineral nutrients, and only the smallest alkanes were efficiently degraded at day 70 (Figure 14). Degradation was a little faster in Station 36 sediment without mineral nutrients, showing more degradation of medium-chain-length *n*-alkanes. The branched isoprenoids phytane and pristane and the long-chain *n*-alkanes showed very little degradation without added mineral nutrients. Addition of mineral nutrients stimulated alkane degradation in both sediments showing that oil degradation was limited by

the in-situ concentrations of mineral nutrients. The branched isoprenoids were in all cases more recalcitrant than the corresponding *n*-alkanes. Appendix 0 shows the degradation of alkanes for all six sediments.

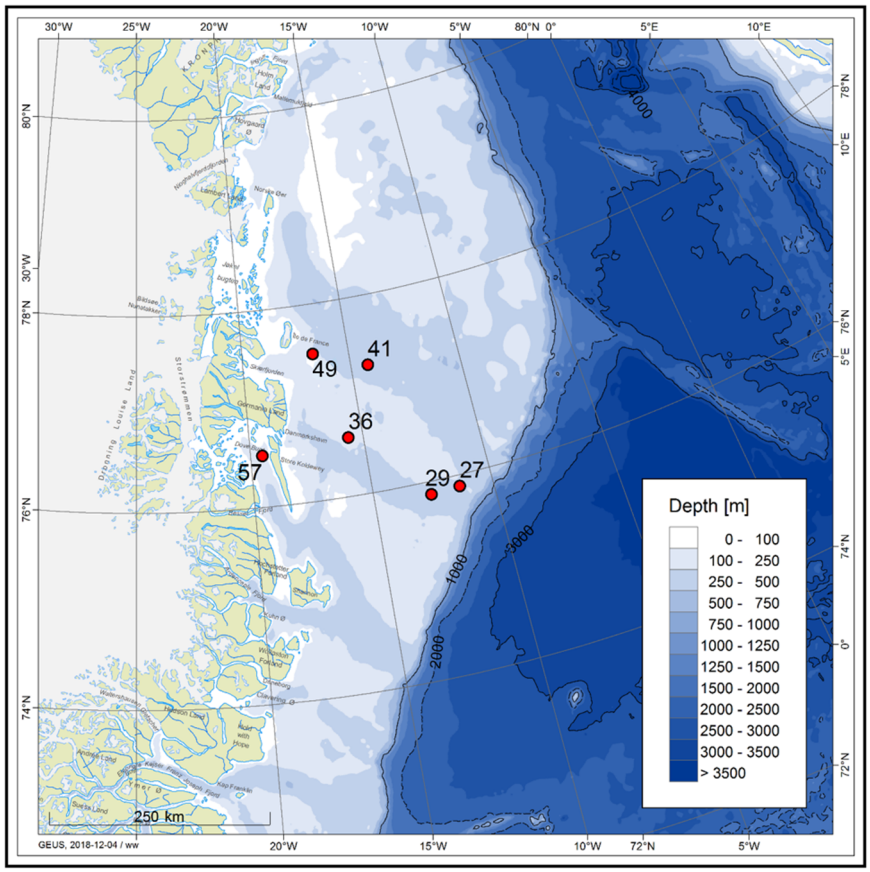


Figure 11. Map of the Greenland Sea showing stations sampled for sediment incubations.

The disappearance of series of alkyl-PAHs (Figure 14) was very different from the disappearance of the alkanes. Most of the microcosms showed a little removal of almost all alkyls-PAHs over time, which was probably caused by sorption or other non-biological removal processes. Without added mineral nutrients, there was slightly more removal of C0-fluorene in station 29 sediment and C0-naphthalene, C0-fluorene and C1-fluorenes in the station 41 sediment. Addition of mineral nutrients in the station 29 sediment increased the removal of C0-, C1- and C2-fluorenes. Mineral nutrients had a larger effect on PAHs in the station 41 sediment where several compound classes showed some removal most clearly for C0-, C1- and C2-naphthalenes, C0- and C1-fluorenes, and C0-phenanthrene. The general effects of mineral nutrients was, however, much smaller than for the alkanes. Appendix 0 shows the degradation of alkyl-PAHs for all six sediments.

The cultivable populations of oil degrader microorganisms showed a strong response to addition of mineral nutrients. Figure 15 shows the development over time in hexadecane degraders in sediment microcosms with and without added mineral nutrients. There was almost no detectable hexadecane degraders in microcosms without added nutrients (<2600 cells / g). At time zero, there were also no detectable degraders in the microcosms with nutrients. This is in line with the results from the characterization of the sediments where all degraders were below the detection limit. Hexadecane degraders grew to around 10^6 cells / mL on day 70 in station 27 and station 29 sediment microcosms. The hexadecane degrader populations were detectable, but at very different concentrations between replicates in station 36 and station 57 sediments, and remained

undetectable throughout the incubation in station 41 and station 49 sediments. Isobutylbenzene degraders were detected in a two samples). There were no cultivable degraders that could grow on 2,2,4-trimethylpentane, 1,3-dimethylcyclohexane, 2-methylnaphthalene or phenanthrene.

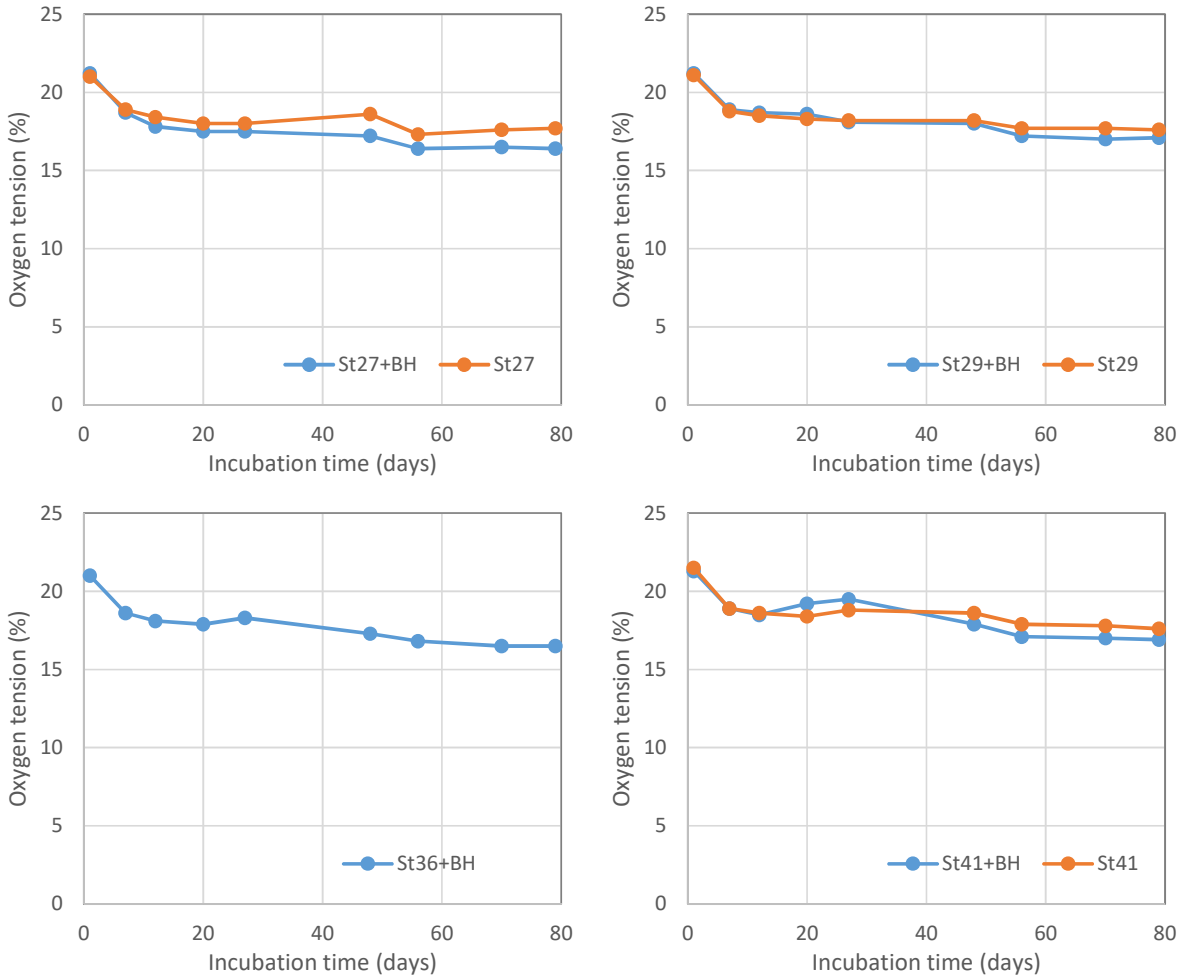


Figure 12. Sediment microcosms. Changes over time in oxygen tension in sediment microcosms from different stations (27, 29, 36 and 41) incubated with weathered crude oil with (+BH) or without Bushnell Haas mineral nutrients.

Changes in diagnostic ratios over time (Figure 16) is, together with increased numbers of cultivable degraders, a strong sign of oil biodegradation. The nC17 / pristane and nC18 / phytane ratios changed a little in station 29 sediment without mineral nutrients and slightly more in station 36 sediment without mineral nutrients. Substantial changes were seen for station 29 sediment with added mineral nutrients, and very large changes took place in station 36 sediment with added mineral nutrients. This corresponds well with the changes in alkane concentrations seen in Figure 13 and shows that the alkane removal was due to biodegradation. Biodegradation of PAHs was almost non-existing. There were no changes in alkyl-PAH ratios without mineral nutrients in the station 29 and station 36 microcosms, and the ratios changed only a little when mineral nutrients were added. 2+3-methylfluorene / 4-methylfluorene and 1-methylfluorene / 4-methylfluorene increased slightly in the station 29 sediment with mineral nutrients and 1-methylfluorene / 4-methylfluorene increased slightly in the station 36 sediments with mineral nutrients, but the changes in alkyl-PAH ratios were in most cases hard to distinguish from experimental uncertainty. The increased fluorene ratios were on the other hand seen in most samples incubated with mineral nutrients, especially station 49 sediment (Appendix 0), and also in some of the sediments without nutrients, suggesting that the small changes in these ratios

were reproducible. The absence of cultivable 2-methylnaphthalene degraders and phenanthrene degraders together with the very small changes in diagnostic alkyl-PAH ratios (if any) suggest that the potential for biodegradation of PAHs in the sediment is very low, even when mineral nutrients are not a limiting factor. Appendix 0 shows the development in diagnostic ratios for all six sediments.

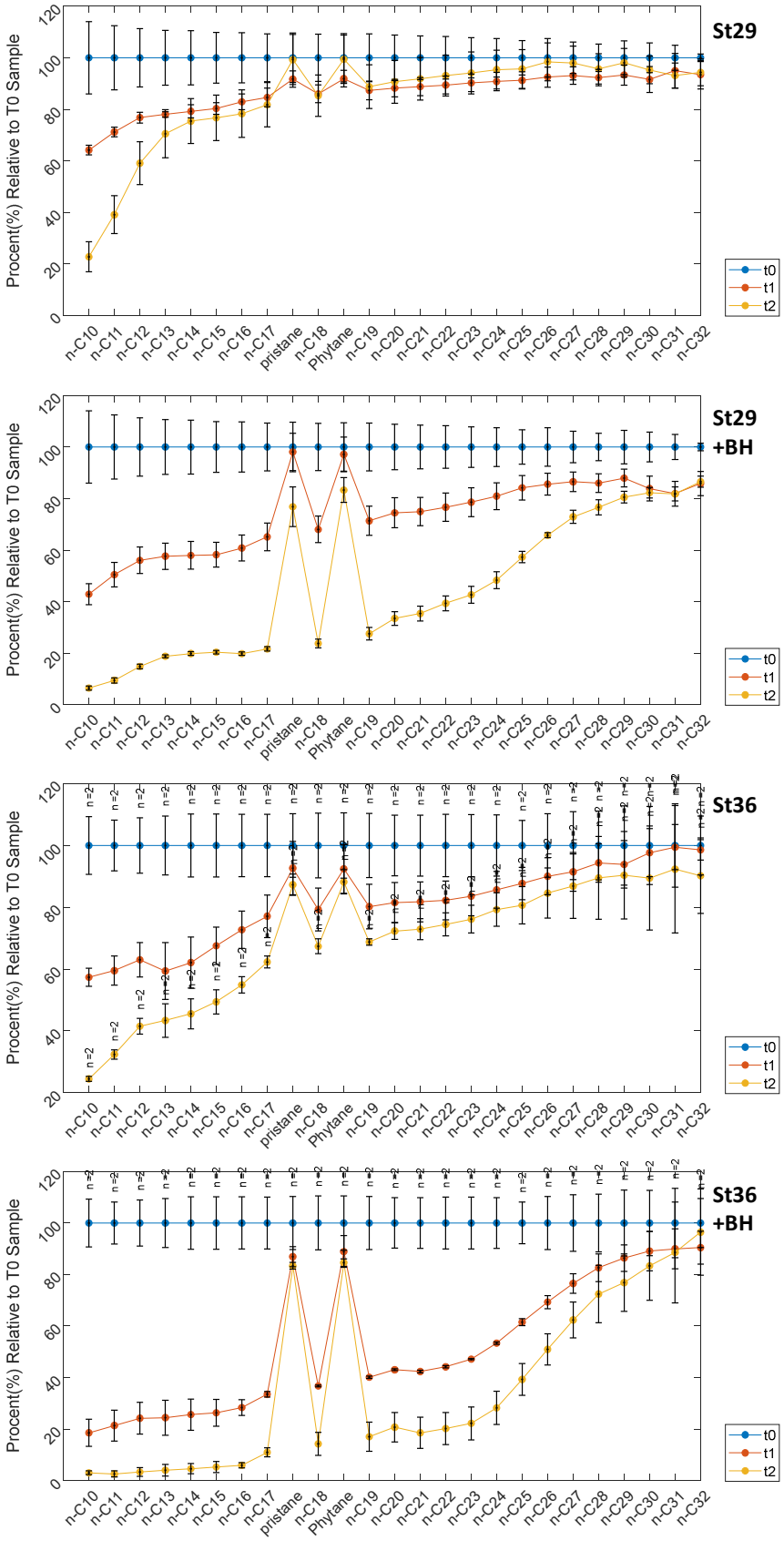


Figure 13. Sediment microcosms. Degradation of oil alkanes in sediments incubated with weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients.n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. Internal standard normalization.

Figure 14. Sediment microcosms. Degradation of oil PAHs in sediments incubated with weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients. C0-, C1-, C2, C3- and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. Internal standard normalization.

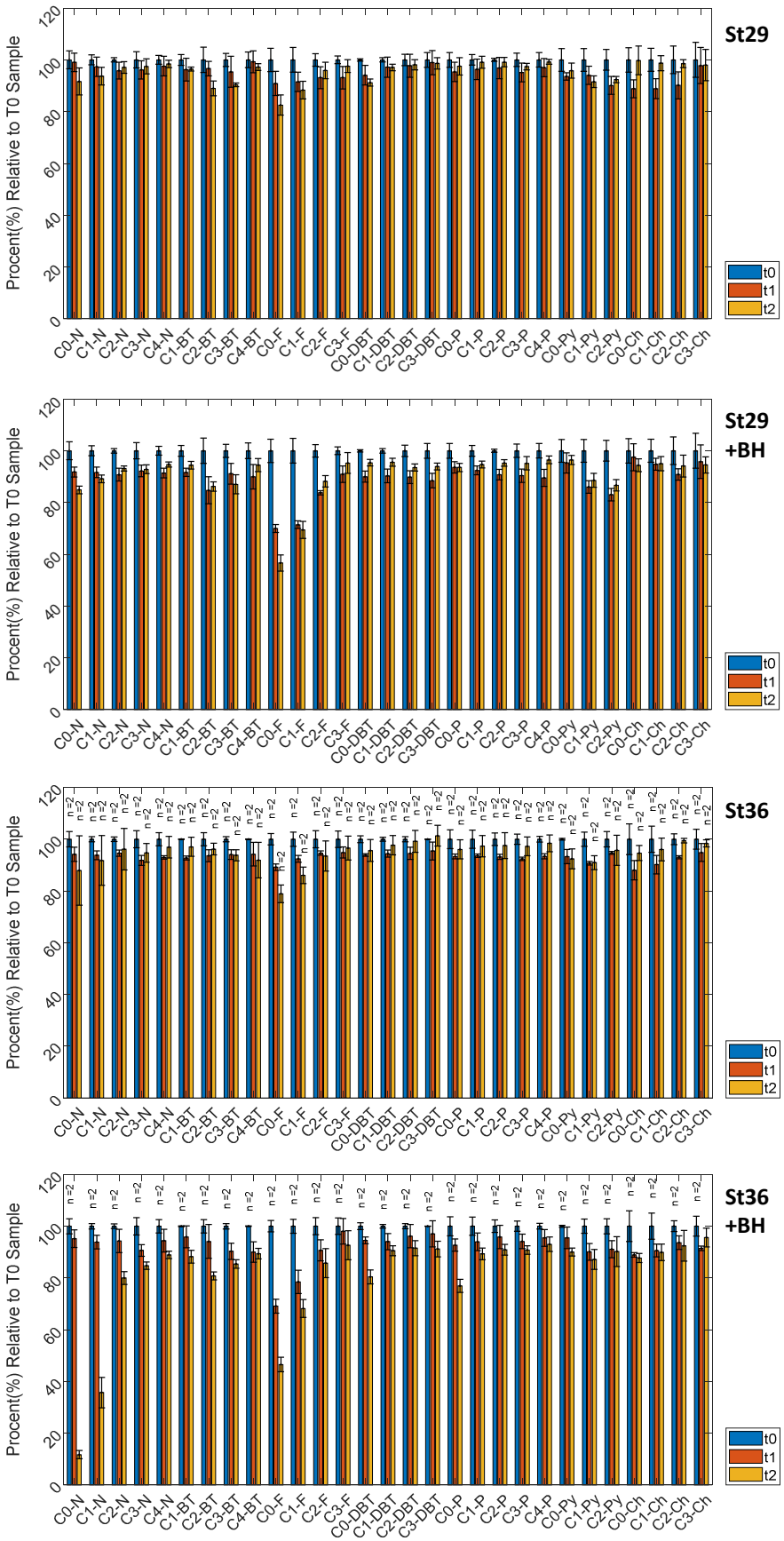


Figure 15. Sediment microcosms. Populations of microbial hexadecane degraders (MPN, cells/mL) in sediment incubated with weathered crude oil with (+BH) or without Bushnell-Haas mineral nutrients for 21 days or 70 days. Replicates are shown with the same colour. The dotted line indicate the lower detection limit. n=3.

The prokaryotic communities in the oil-enriched sediment microcosms were dominated by the archaean class *Nitrososphaeria*, as for the "raw" sediments in the previous section. This group represented 36 - 73 % in relative abundance in sediment microcosms enriched only with crude oil (Figure 17). For the microcosms enriched with both crude oil and BH mineral nutrients, the relative abundances of *Nitrososphaeria* was 21 - 47%, except for the samples originating from St. 27 with 0% of this class (Figure 17). As *Nitrososphaeria* was also dominant in the "raw" sediments before oil addition, it probably is not connected to oil degradation at all.

We also identified oil degrading prokaryotes in MPN plates from sediment incubated with crude oil and Bushnell-Haas mineral nutrients with either hexadecane or isobytylbenzene as carbon source (Appendix 10). The dominant microorganisms in the MPN plates presumably represented the numerically dominant oil degraders in the oil-enriched sediment from which the MPN samples originated, but only those degraders that were cultivable. *Nitrososphaeria* was not represented in any of the MPN samples. Instead, gammaproteobacteria

dominated across the MPN samples representing 89 - 100% in relative abundance (Appendix 10). Within this class, the MPN samples all contained an unidentified gammaproteobacterium in high abundance. This bacterium seems to be the most important cultivable alkane degrader. The MPN plates also contained other members of the proteobacterial genera *Marinobacter*, *Pseudoalteromonas*, *Shewanella Paraglaciecola*, *Alkanivorax*, *Pseudomonas* and *Paraglaciecola* in high abundance in some samples, as well as *Rhodococcus* (Actinobacteria) in one sediment sample. Many of these are well-known marine hydrocarbon degraders (e.g. Cui et al., 2016; Lattuati et al., 2002; Chronopoulou 2015; Bej et al., 2000). MPN counts with 2-methylnaphthalene and phenanthrene as carbon source were all growth-negative, indicating that there was no potential enrichment for PAHs degraders such as the obligate aromatics degrader *Cycloclasticus* (e.g. Dyksterhouse et al., 1995; Kasai et al., 2002).

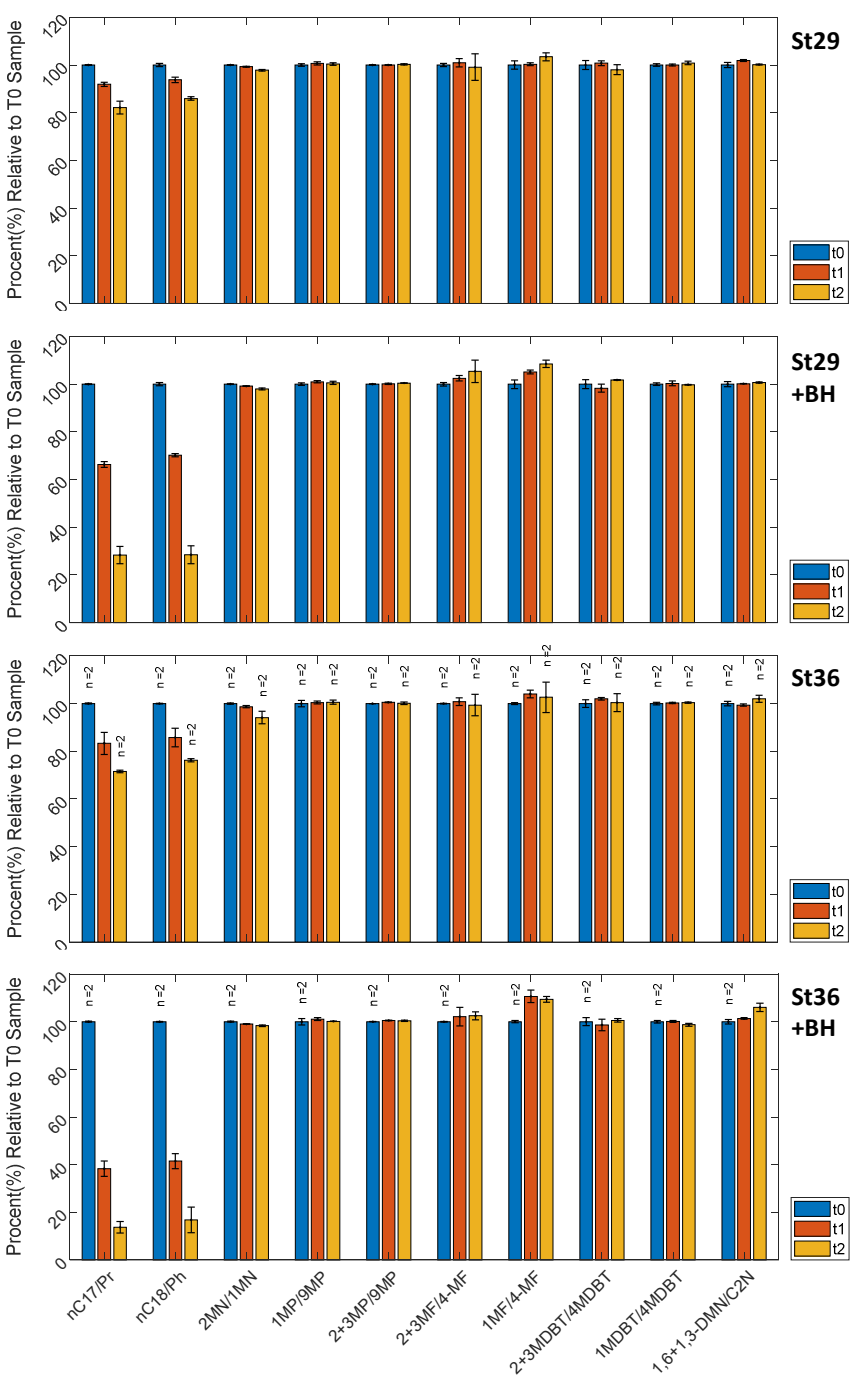


Figure 16. Sediment microcosms. Diagnostic ratios for degradation of weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients. Pr: pristane, Ph: phytane, MN: methylnaphthalene, MP: methylphenanthrene, MF: methylfluorene, MDBT: methyldibenzothiophene, DMN: dimethylnaphthalene. n=3. Internal standard normalization.

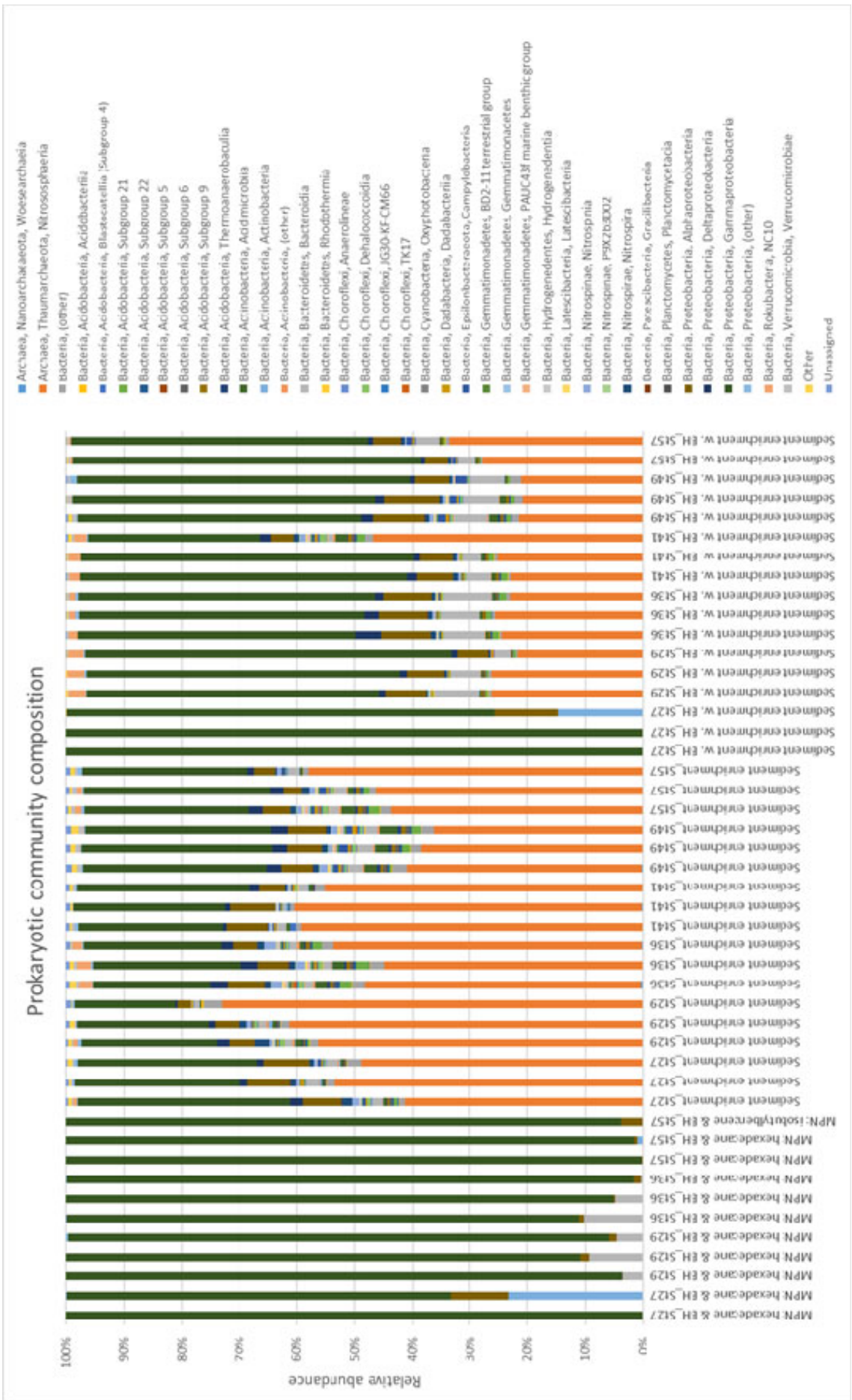


Figure 17. Sediment microcosms. Prokaryotic community composition (i.e. bacteria and archaea) of the oil-enriched sediment samples (triplicate samples), with or without addition of BH mineral nutrients. The composition of growth-positive MPN wells (dominant cultivable hexadecane degraders) is shown for comparison. The taxonomy (16S rRNA gene) of the groups were determined until the class level where possible.

6.3 Effects of nutrients and oil dispersion in sea water

The effects of added mineral nutrients and the dispersing agent Slickgone was investigated with water from station 70. This station is situated close to the shelf break in the southern part of the surveyed area (Figure 18). The station was sampled on September 5, 2017 (74.57.235 N, 12.51.927 W) and had a total depth of 555 m. Water was sampled from 10 m depth (warm Polar Surface Water) and at 340 m depth (probably lower Return Atlantic Water). Station 70 is close to station 73 where the sediment contained both C1-naphthalene and C1-phenanthrene (The sediments were also tested by an MPN-method for the concentration of microorganisms that can utilize specific oil compounds for growth. Oil-degrading microorganisms were not detected in any of the samples, not even degraders of the easily degradable compound *n-hexadecane*. The negative results were to some degree a consequence of a high detection limit (2600 cell / g), caused by the high content of silt and clay that interfered with optical density readings. The results nevertheless indicate that it is unlikely that oil seeps have enriched the sediments with high concentrations of biodegradable hydrocarbons at any of the stations, as in-situ oil enrichment would most likely have given detectable concentrations of degrader organisms.).

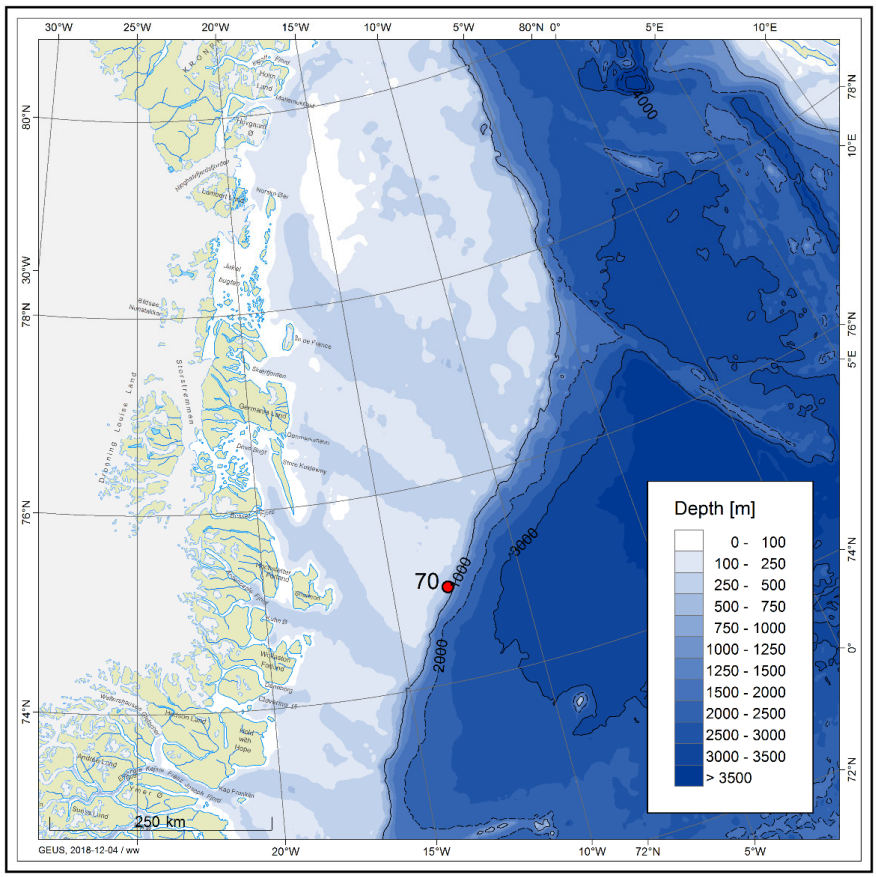


Figure 18. Map of the Greenland Sea showing the station where the water column was sampled for testing the effects of mineral nutrients and oil dispersion.

The alkane data show a strong contamination with alkanes with even numbers of carbon atoms. The contamination was strongest for the lighter alkanes (Figure 19). The contamination was furthermore only present in microcosms that were incubated for 28 or 90 days, which shows that the contamination took place after the experiment was initiated. It is tempting to think that the contamination could have come from a source of light oil product, gasoline for instance, but that is very unlikely as technical grade oil products contain

alkanes with even and uneven carbon numbers in almost equal portions. Alkane sources with only even-chain alkanes are rare. The only possible source, that we can imagine, is polyethylene plastic. Polyethylene is synthesized from ethene (ethylene) monomers to form (-CH_2-CH_2-). As ethene is a two-carbon monomer, only polymers with even carbon numbers are synthesized during the polymerization process. The microcosms were "stopped" by addition of ethanol instead of methanol. This was because the handling and incubation of samples took place on-board Dana, often during bad weather, where accidental spills of methanol would be a problem. The microcosms stopped at days 28 and 90 were added ethanol from another producer than the time zero samples. As the ethanol was purchased in PE-bottles, it is likely that the PE-bottle from the second batch may have contained traces of small PE-polymers with even numbers of carbon that migrated from the PE-bottle into the ethanol. All even-numbered *n*-alkanes were therefore removed from the data set before data analysis.

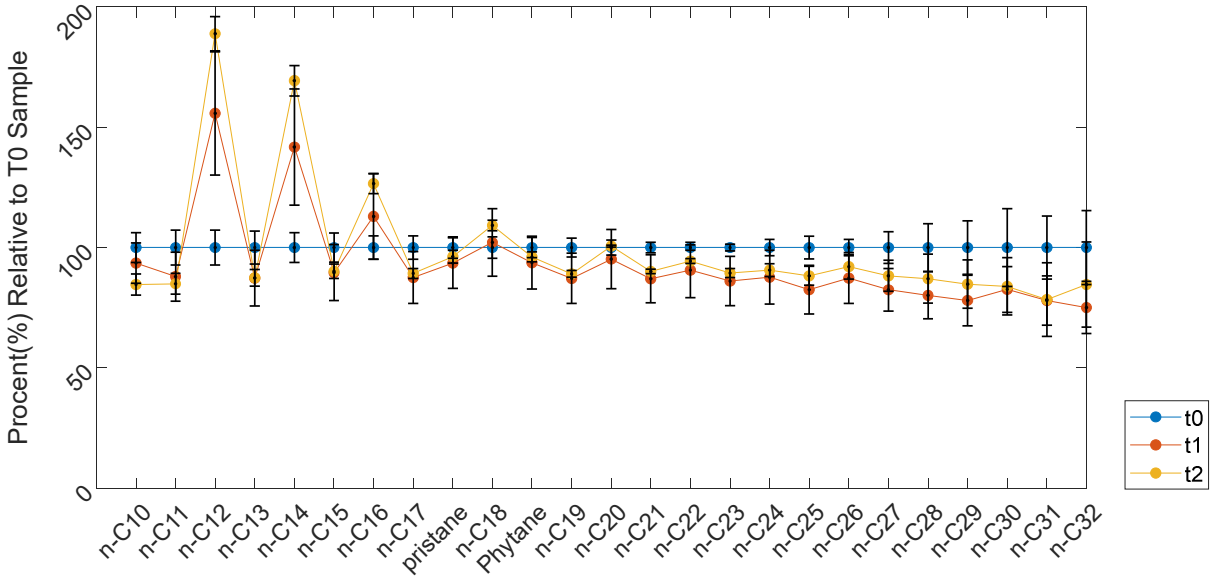


Figure 19. Seawater microcosms. Example of contamination with even-number n-alkanes (t1 and t2) in seawater samples from 10 meters depth incubated with crude oil and Slickgone for 0 (t0), 28 (t1) or 90 days (t2).

Figure 20 shows how alkane degradation was affected by addition of mineral nutrients and / or dispersant. Microcosms with water from 10 m showed little degradation of alkanes in controls without any addition of mineral nutrients or Slickgone. Almost all *n*-alkanes were removed already on day 28 when mineral nutrients were added, and pristane and phytane were also degraded, but still present in some quantity. The pristane and phytane was completely removed on day 90 together with almost complete degradation of all *n*-alkanes. The dispersant Slickgone did not increase alkane removal compared to the control without additions for the 10-m microcosms. Removal of alkanes was on the other hand very efficient when mineral nutrients and Slickgone were added together in the 10-m microcosms. The removal was in this case similar to the mineral nutrients only microcosms except that phytane and pristane were degraded slightly slower in the presence of Slickgone. Alkane removal was slightly different in the microcosms containing water from 340 m depth (Figure 21). Controls without any addition showed some removal of the lighter *n*-alkanes at day 28 and some removal of heavy *n*-alkanes at day

90. Addition of Slickgone and mineral nutrients together showed faster removal than addition only of mineral nutrients, but Slickgone alone was similar to the control without any additions.

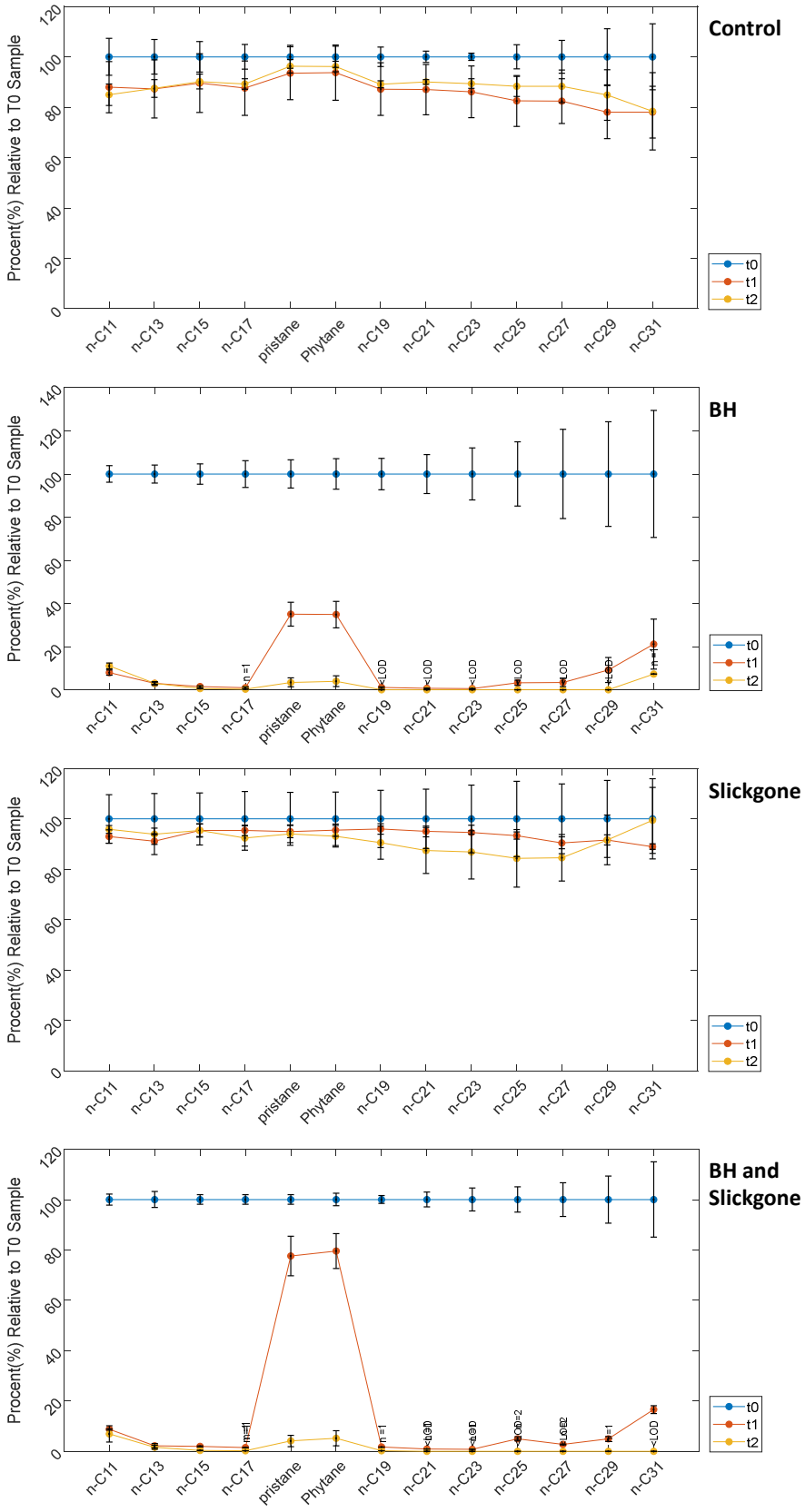
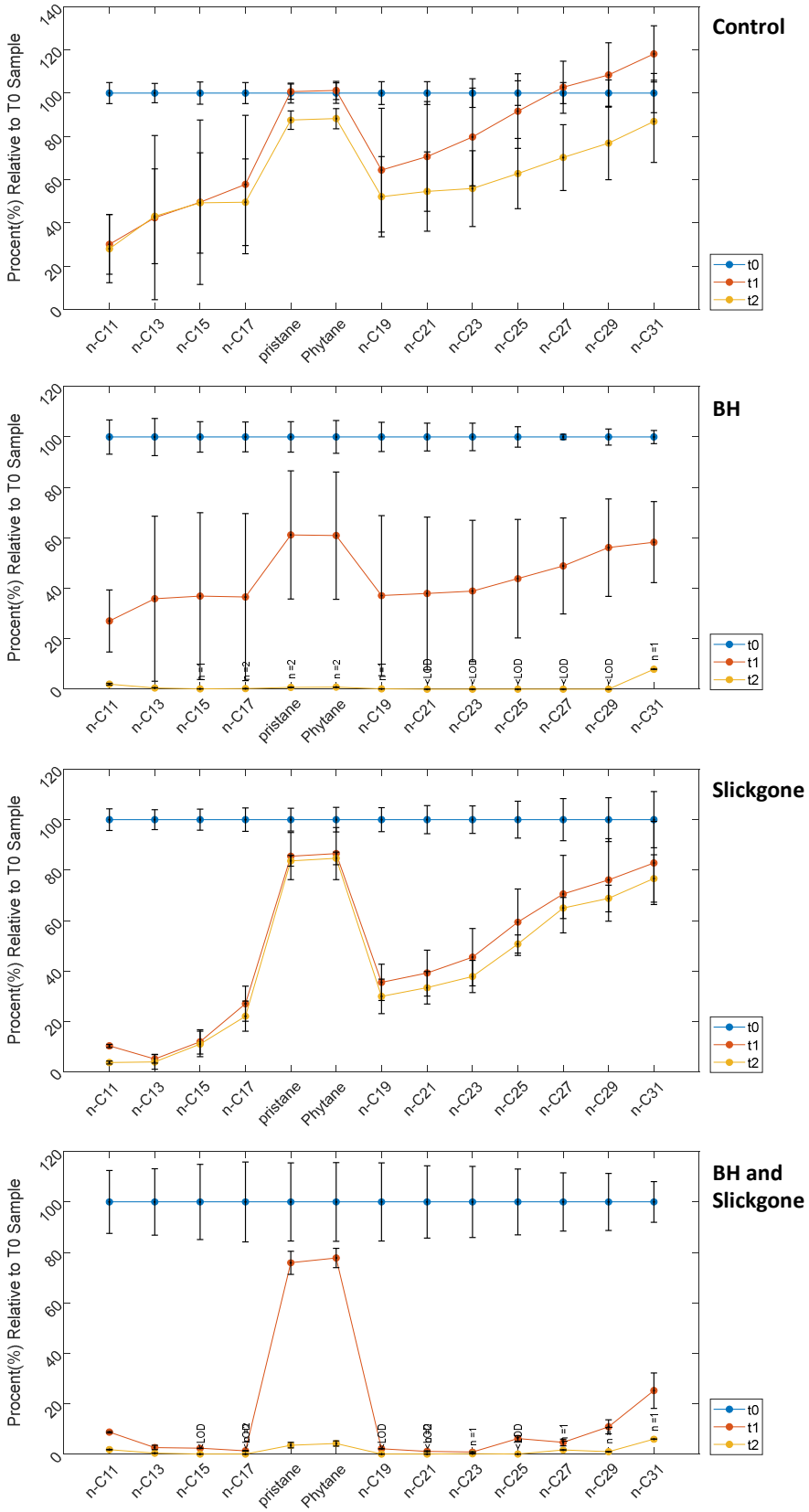


Figure 20. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of odd-number n-alkanes and isoprenoids in seawater from 10 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2). n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. n=3. Internal standard normalization.

Figure 21. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of odd-number n-alkanes and isoprenoids in seawater from 340 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2). n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. . n=3. Internal standard normalization.



The disappearance of series of alkyl-PAHs was also very dependent on the addition of mineral nutrients. Very little happened in the control microcosms containing water from 10 m without any additions (Figure 22). Addition of mineral nutrients resulted in almost complete removal (<LOD) of most 2-, 2.5- and 3-ring PAHs. Only some of the most alkylated compounds within the fluorenes, benzothiophenes and phenanthrenes remained. The pyrenes (4-rings) showed some removal with decreasing removal with increasing degree of alkylation. Only the chrysenes (4-rings) showed almost no removal over time. The removal of PAHs in the water microcosms with mineral nutrients was thus much faster than in the sediments and removal took place for many different alkyl-PAHs in the seawater compared to the few alkyl PAHs in the sediments. Addition of Slickgone to the 10-m microcosms had almost no effect when compared to the control without any additions, that is, almost no removal of PAHs. Addition of both mineral nutrients and Slickgone to 10-m microcosms gave a result that in many ways was similar to the mineral nutrients, except that C4-naphthalenes, C2-fluorenes and C2-phenanthrenes showed little removal when Slickgone was added. This showed that Slickgone had a small negative effect on PAH removal when added together with mineral nutrients. The 340-m microcosms (Figure 23) were in most respects similar to the corresponding 10-m microcosms, except that more PAHs were removed in the controls without additions, and Slickgone alone had a small negative effect on PAH removal compared to the controls without additions.

Figure 24 shows the concentrations of degraders that could grow on different types of oil compounds. The data is from day 90 to allow enrichment of rare degraders of the more recalcitrant compounds. Degraders were detected for hexadecane, isobutylbenzene, 2-methylnaphthalene, phenanthrene and 1,3-dimethylcyclohexane, but at very different concentrations and very different fractions of the microcosms. Hexadecane degraders were the most common degraders and were present at high concentrations in almost all microcosms, which is to be expected since *n*-alkanes are easily degradable and constitute a large fraction of the crude oil. The concentration of hexadecane degraders varied between treatments and between 10-m and 340-m microcosms, but this is probably because light alkanes were completely degraded in most microcosms at day 90 so that there was no direct relation between removal of light alkanes and the concentration of hexadecane degraders. The population of some of the treatments was actually higher at day 28 (appendix 0). Degraders of 2-methylnaphthalene were also found in all treatments in microcosms from both depths, but they were detected in all three replicates only in microcosms with mineral nutrients or mineral nutrients and Slickgone (Figure 24), that is, in the treatments with the highest removal of alkyl-naphthalenes. Degraders that could grow on phenanthrene or isobutylbenzene were rare, occurring only in a few microcosms. Cultivable degraders of that could grow on 1,3-dimethylcyclohexane were detected only in one microcosm (day 28, appendix 0) and degraders that could grow on 2,2,4-trimetylpentane were not detected at all.

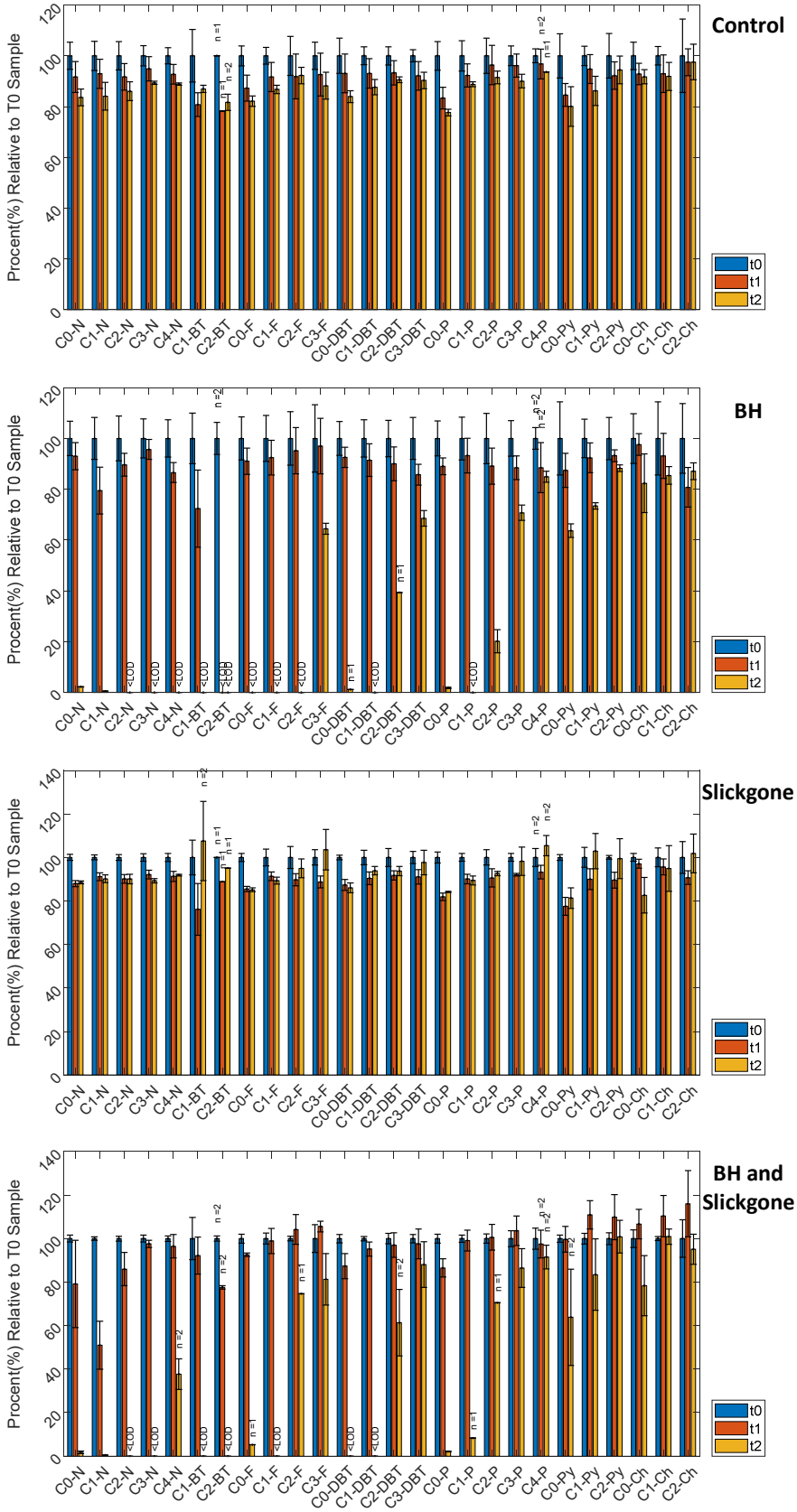


Figure 22. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of oil PAHs in seawater from 10 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2).C0-, C1-, C2, C3- and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=3. Internal standard normalization.

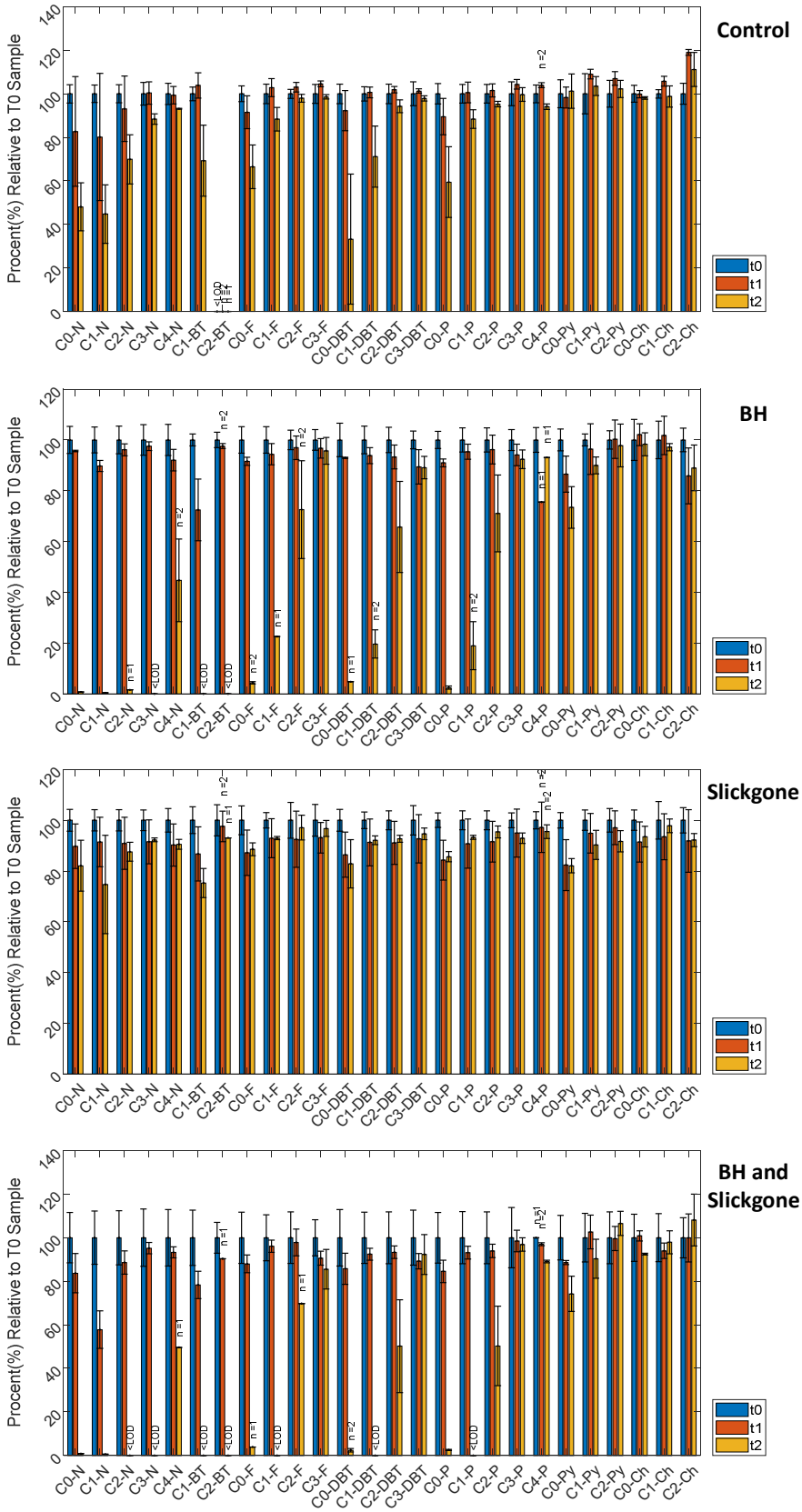
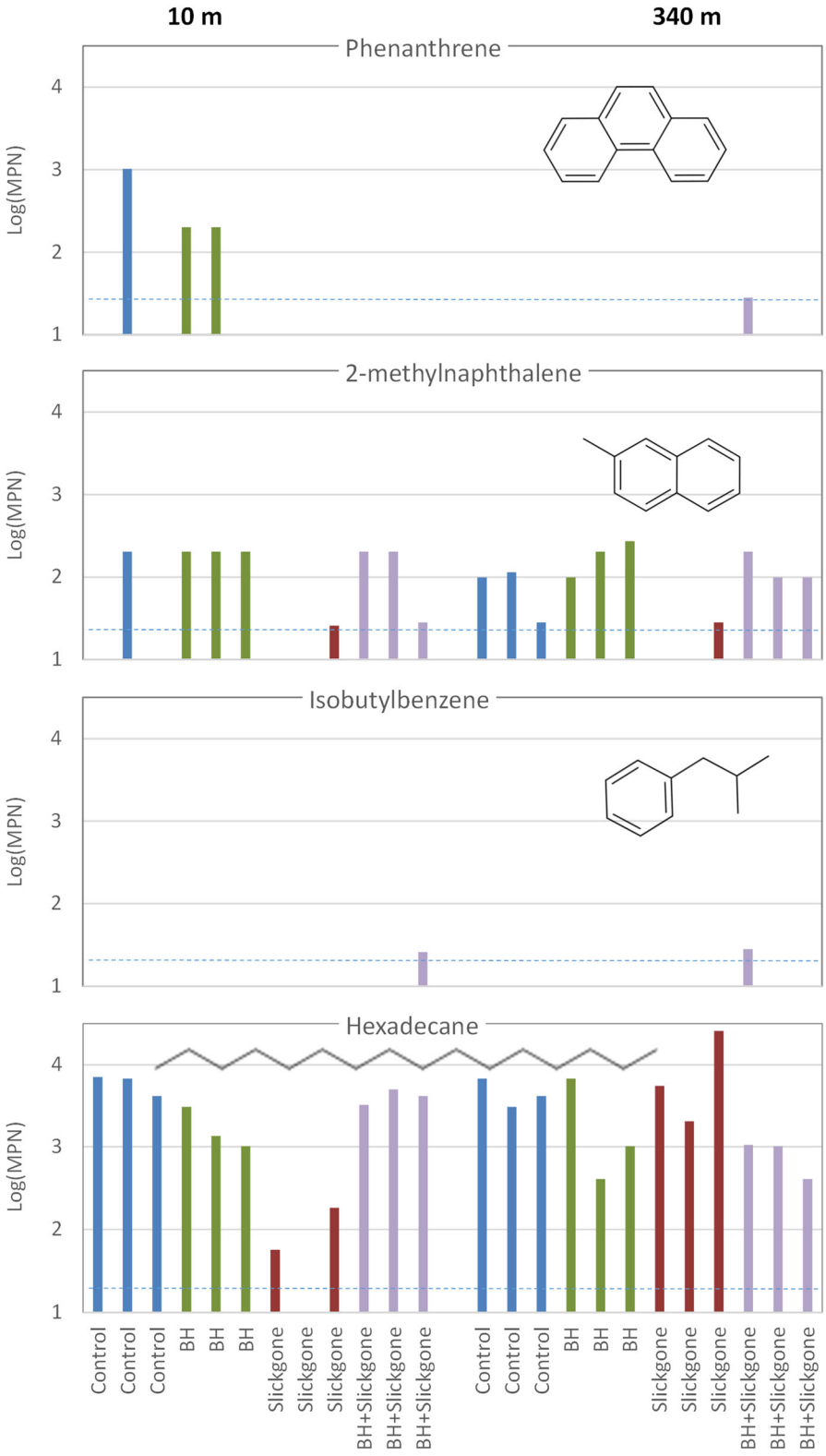


Figure 23. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of oil PAHs in seawater from 340 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2). C0-, C1-, C2, C3- and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=3. Internal standard normalization.

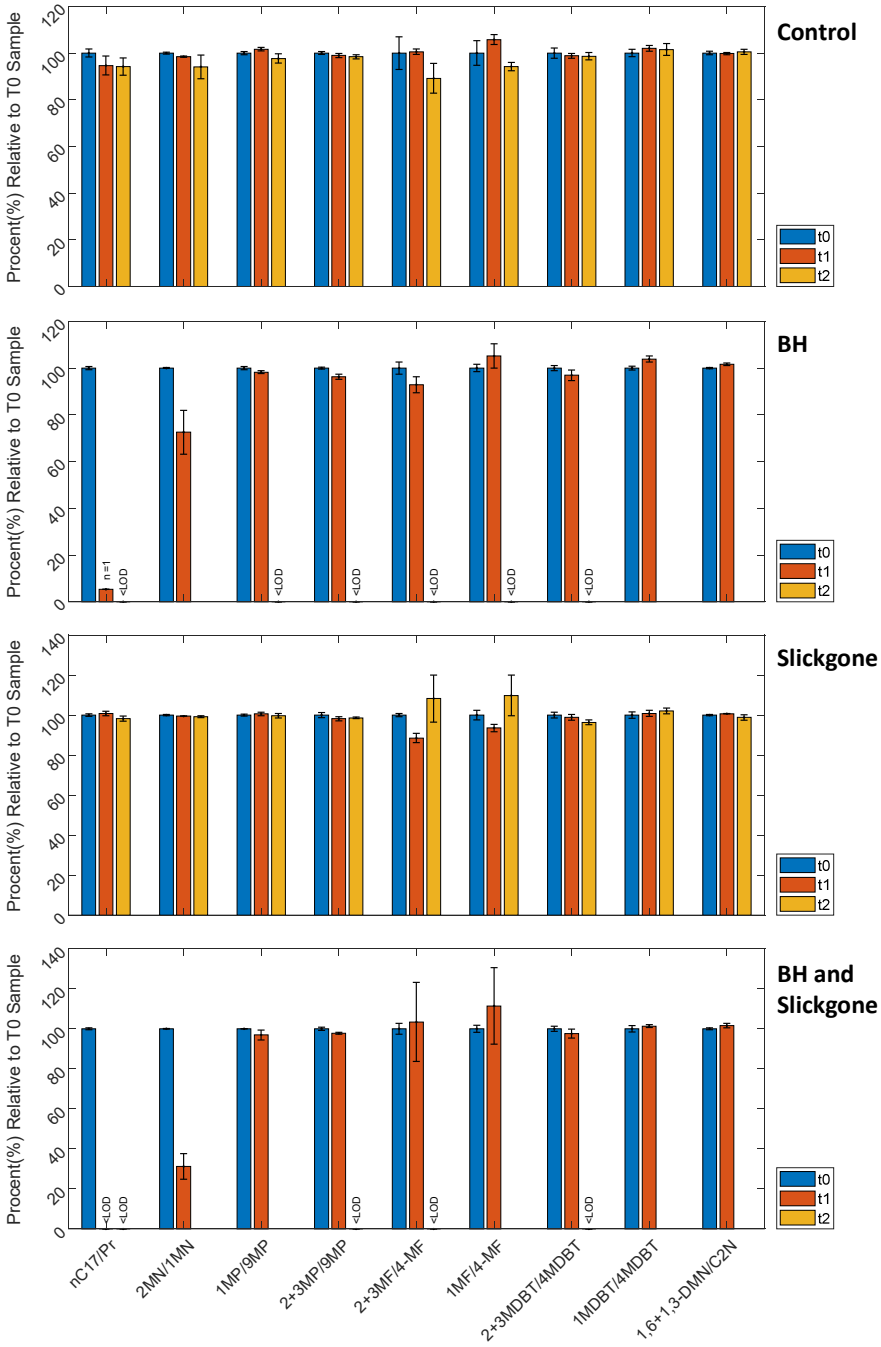
Figure 24. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the size of microbial oil degrader populations (MPN, cells/mL) in seawater incubated with crude oil for 90 days. The dotted line indicate the lower detection limits. Left: water from 10 meters depth. Right: water from 340 meters depth. Replicate samples are shown with the same colour for each depth.



The above observations were compared to the changes in isomer ratios that are diagnostic of oil biodegradation. The ratios from the 10-m microcosms (Figure 25) were very stable over time in the controls without any additions, which indicated that oil biodegradation was almost negligible. It is interesting that even the nC17 / pristane ratio remained almost constant, which indicates that alkane degradation was very limited and that the small general removal of all alkanes (Figure 20) was non-biotic and probably an experimental artefact such as sorption or reduced extraction efficiency. This also explains why the removal was highest for the heavy alkanes, which is opposite to removal by biodegradation where the lighter alkanes are first degraded. Addition of Slickgone alone had no effect on biodegradation in the 10-m microcosms as the ratios were similar to the controls without any additions. The only difference was slightly different C1-fluorene ratios (2+3MF / 4MF and 1MF / 4MF) indicating a little biodegradation of C1-fluorenes. Addition of mineral nutrients had a dramatic effect of the diagnostic ratios. For the 10-m microcosms with mineral nutrients (with and without Slickgone), the nC17 / pristine ratio dropped sharply already at day 28 (Figure 25) suggesting extensive biodegradation of alkanes, which was also seen in the alkane concentrations (Figure 20). The 2MN / 1MN ratio started to drop already at day 28, especially when both mineral nutrient and Slickgone were added, suggesting fast biodegradation of C1-naphthalenes, which is consistent with the reduced C1-naphthalene concentrations in these microcosms (Figure 22). C2-naphthalenes did not show any isomer-specific degradation (no changes in the 1,6+1,3DMC / C2N ratio) at day 28. However, at day 90 both the nominator and denominator of the ratio was below DL and the ratio could not be calculated, suggesting complete biodegradation when mineral nutrients were added. The other alkyl-PAH ratios were quite similar to the C2-naphthalenes, indicating extensive biodegradation of alkyl-PAHs from day 28 to day 90 after the alkanes were degraded.

Biodegradation in the 340-m microcosms were somewhat different from the 10-m microcosms (Figure 26). The controls without additions and the Slickgone microcosms (without mineral nutrients) were again similar, but in contrast to the 10-m microcosms there was substantial biodegradation of the alkanes (reduced nC17 / pristane ratio) and the C1-naphthalenes (reduced 2MN / 1MN ratio), which was also clearly seen in the removal of these compounds (Figure 21, Figure 23). Addition of mineral nutrients (without Slickgone) had a smaller effect on biodegradation than in the 10-m microcosms. The 1MDBT / 4MDBT remained unchanged which conflicts with the removal of C1-dibenzothiophenes. Whereas changing ratios are a clear indication of biodegradation, constant ratios may in rare cases be caused by equal removal of the isomers (Lamberts et al., 2008). The other alkyl-PAH ratios showed substantial reductions though not as great and in the 10-m microcosms. Addition of both mineral nutrients and Slickgone gave the largest reduction of all the diagnostic ratios, suggesting that removal of both alkanes (Figure 21) and alkyl-PAHs (Figure 23) were caused by biodegradation. The degradation of alkyl-phenanthrenes, indicated by changes in the 1MP / 9MP and 2+3MP / 9MP diagnostic ratios in the presence of mineral nutrients and Slickgone, however, was not as efficient as for the 10-m microcosms.

Figure 25. Seawater microcosms. Diagnostic ratios. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of oil PAHs in seawater from 10 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2). Pr: pristane, Ph: phytane, MN: methylnaphthalene, MP: methylphenanthrene, MF: methylfluorene, MDBT: methyldibenzothiophene, DMN: dimethylnaphthalene. n=3. Internal standard normalization.



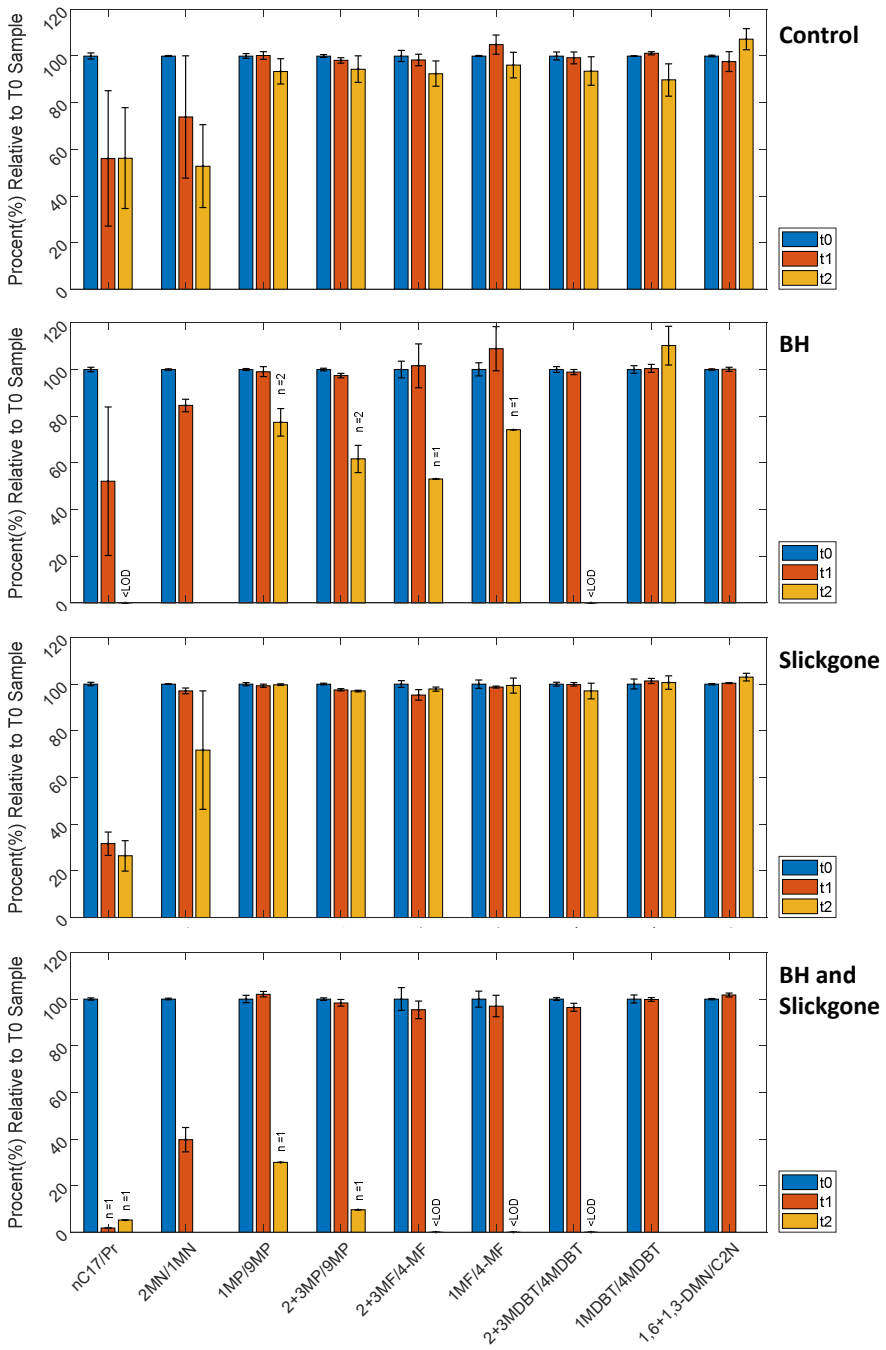


Figure 26. Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the degradation of oil PAHs in seawater from 340 meters depth incubated with crude oil for 0 (t0), 28 (t1) or 90 days (t2). Pr: pristane, Ph: phytane, MN: methylnaphthalene, MP: methylphenanthrene, MF: methylfluorene, MDBT: methyldibenzothiophene, DMN: dimethylnaphthalene. n=3. Internal standard normalization.

6.4 Characterization of the metabolic capacity for degradation of structurally diverse oil components in the water column

Water was sampled at three stations (station 29, station 41, and station 57) and at three depths at each station. Samples from the top of the water column were preferably sampled at the chlorophyll maximum. Bottom water was sampled approximately 40 m above the sediment to avoid the sediment boundary layer that may contain suspended sediment. A third sample was sampled at selected depth in the middle of the water column. The exact depth of interest was determined from the CTD temperature data. All three stations were on the continental shelf (Figure 27).

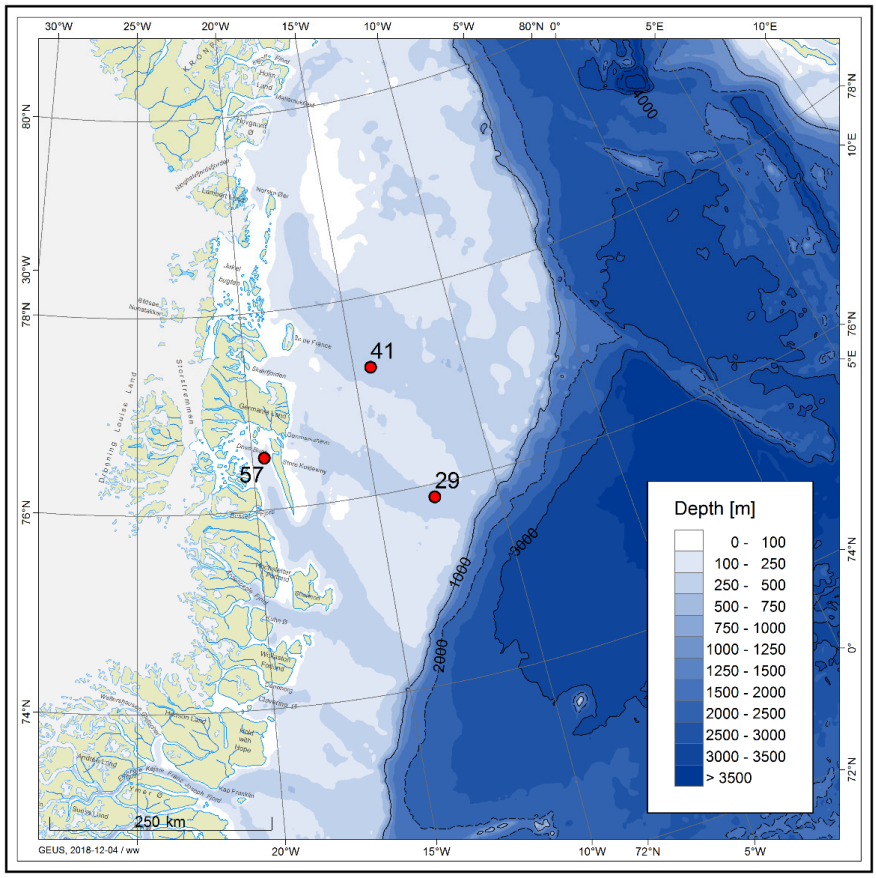


Figure 27. Map of the Greenland Sea showing stations where the water column was sampled for characterization of the metabolic oil degradation capacity.

Station 29 was sampled on August 30, 2017. The top sample was from the weak chlorophyll maximum at 28 m (total depth 402 m) and was Polar Surface Water. The middle sample was from the temperature maximum at 240 m (total depth 388) and was Return Atlantic Water. The bottom sample was from 340 m (total depth 388) at the transition to Upper Polar Deep Water. Station 41 was sampled on September 1, 2017. The top sample was from the chlorophyll maximum at 30 m (total depth 265 m) and was Polar Surface Water. The middle sample was from the temperature minimum at 100 m (total depth 265) and was probably also Polar Surface Water. The bottom sample was from 210 m (total depth 265) at the transition between Polar Surface Water and warmer bottom water (possibly Return Atlantic Water). Station 57 was sampled close to the coast on September 3. Top water was sampled at 16 m and was Polar Surface Water. Chlorophyll concentrations in the top water were very low without a clear maximum. The middle sample was from the temperature minimum at 100 m (total depth 320 m) and was Polar Surface Water. The bottom sample was from 280 m (total depth 320) and was probably Atlantic water.

The microorganisms were concentrated by filtration (the filtride) and incubated with crude oil in mineral medium, i.e., there was no mineral nutrient limitation for the evolving microbial communities in the microcosms. This sub-experiment is less realistic compared to the previous sub-experiment, and the purpose was to qualitatively determine the range of oil substrates that could potentially be degraded in the different water samples. Controls without crude oil were included to determine if there was any development of oil-degrading microorganisms in the absence of added crude oil. The results from the three station were similar, so only results from station 57 is discussed in detail, but the results from the other two stations are presented in appendices

0-0. Alkane degradation was not only similar between stations; it was also quite similar for the different depths (Figure 29). The *n*-alkanes showed varying degrees of removal on day 35 depending on chain length so that the shortest *n*-alkanes were completely removed whereas there was no removal of the longest *n*-alkanes. Almost all *n*-alkane was removed on day 84. The branched isoprenoids (pristane and phytane) were more recalcitrant with slower removal than the corresponding *n*-alkanes in the top- and middle water microcosms from station 57 and very little or no removal in the bottom microcosms from station 57 and all of the microcosms from stations 29 and 41.

Many alkyl-PAHs seemed to be removed in microcosms from all three depths (Figure 30), but the removal patterns were puzzling and very different from comparable microcosms with seawater and mineral nutrients from station 70 (Figure 23 and Figure 24). We did not see the usual pattern that PAH compound series with few rings, e.g. the naphthalenes, are biodegraded faster than compound series with more rings (e.g. phenanthrenes). Also, biodegradation usually decreases with increasing degree of alkylation, this was not the case for the naphthalenes, the benzothiophenes and the fluorenes. Overall, this suggests that much of the observed removal may not have been caused by biodegradation, but was instead an experimental artefact. Sorption processes (non-extractable sorption) would indeed produce ”removals” where larger compounds are “removed” to a larger degree than smaller compounds. What was also intriguing was the almost lack of removal from day 35 to day 84, since the opposite would be expected from biodegradation when mineral nutrients are added in surplus. Such constant concentrations are on the other hand consistent with sorption that has reached equilibrium between the solid phase and the water phase at day 35. To investigate this effect, we carried out an additional control experiment to quantify the non-biological removal of oil compound. Completely sterile microcosms with sterile filters (no filtration of sea water) and mineral medium were prepared in a sterile flow-bench and incubated, stopped by addition of ethanol and stored frozen as for the “real” microcosms. The sterile controls produced removal patterns that were similar to the active microcosms (Figure 30). This indicated that PAH removal patterns were indeed experimental artefacts rather than biodegradation, and that the potential for biodegradation of PAHs was very low.

Hexadecane degraders were present in all the oil microcosms from the three stations at very high concentrations, often exceeding the upper detection limit of 5.4×10^5 cells / mL. Interestingly, hexadecane degraders were also present in the negative controls without oil, but at much lower concentrations, probably because the hexadecane degraders grew on particulate organic matter and decaying microorganisms co-extracted from the water. The substrate spectrum of the cultivable oil degraders was, however, very low for all stations and all depths. Except for the hexadecane, we detected only 2-methylnaphthalene degraders in two microcosm (station 57, bottom, 28 cells / mL and station 41, middle, 219 cell / mL) and phenanthrene degraders in one microcosm (station 41, top, 28 cell / mL). Degraders that could grow on isobutylbenzene, 1,3-dimethylcyclohexane or 2,2,4-trimethylpentane were absent in all microcosms (<26 cells / mL).

Figure 28. Seawater filtride from **station 57**. Degradation of alkanes in Seawater filtride incubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. n-C11, n-C12, n-C13, etc. indicate the number of carbon atoms in specific n-alkanes. n=2, hopane normalization.

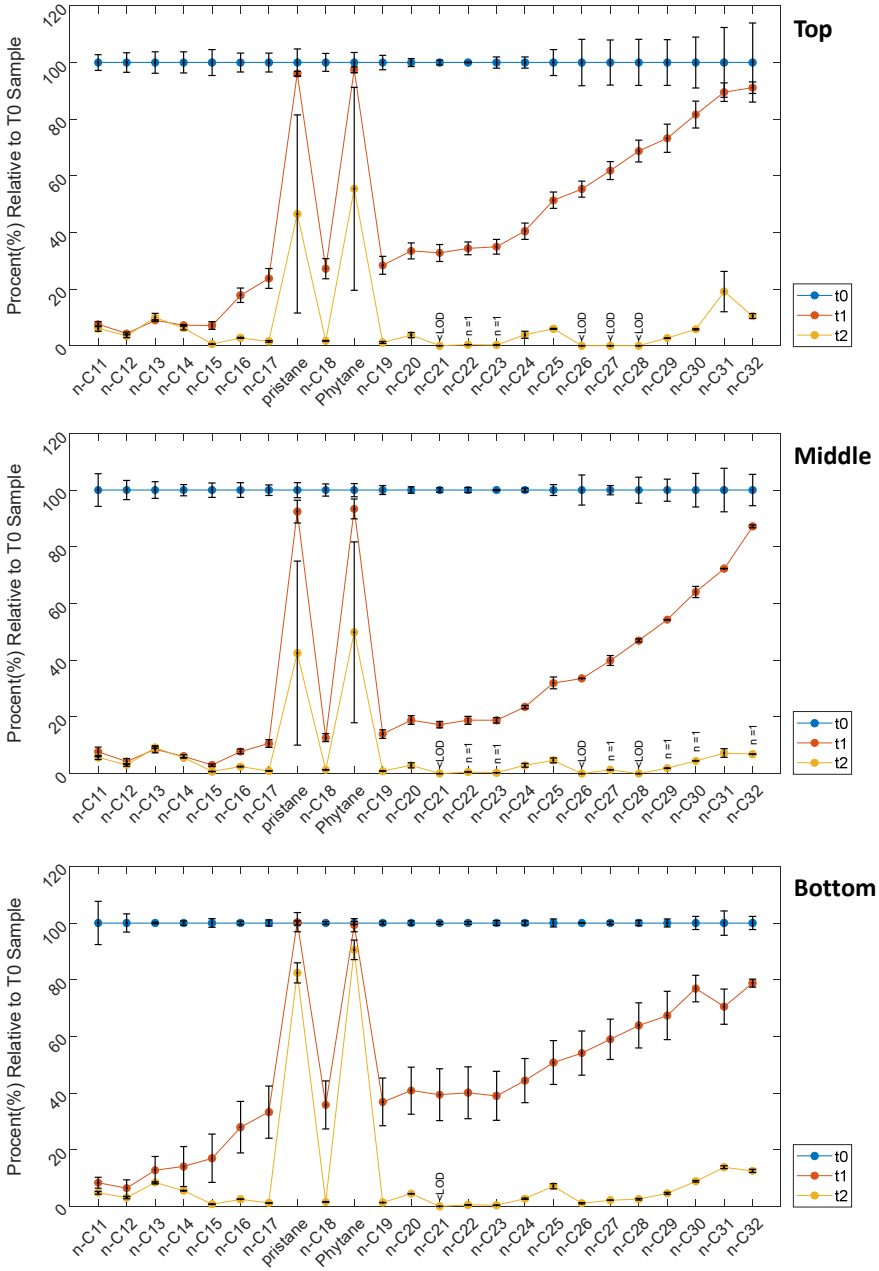
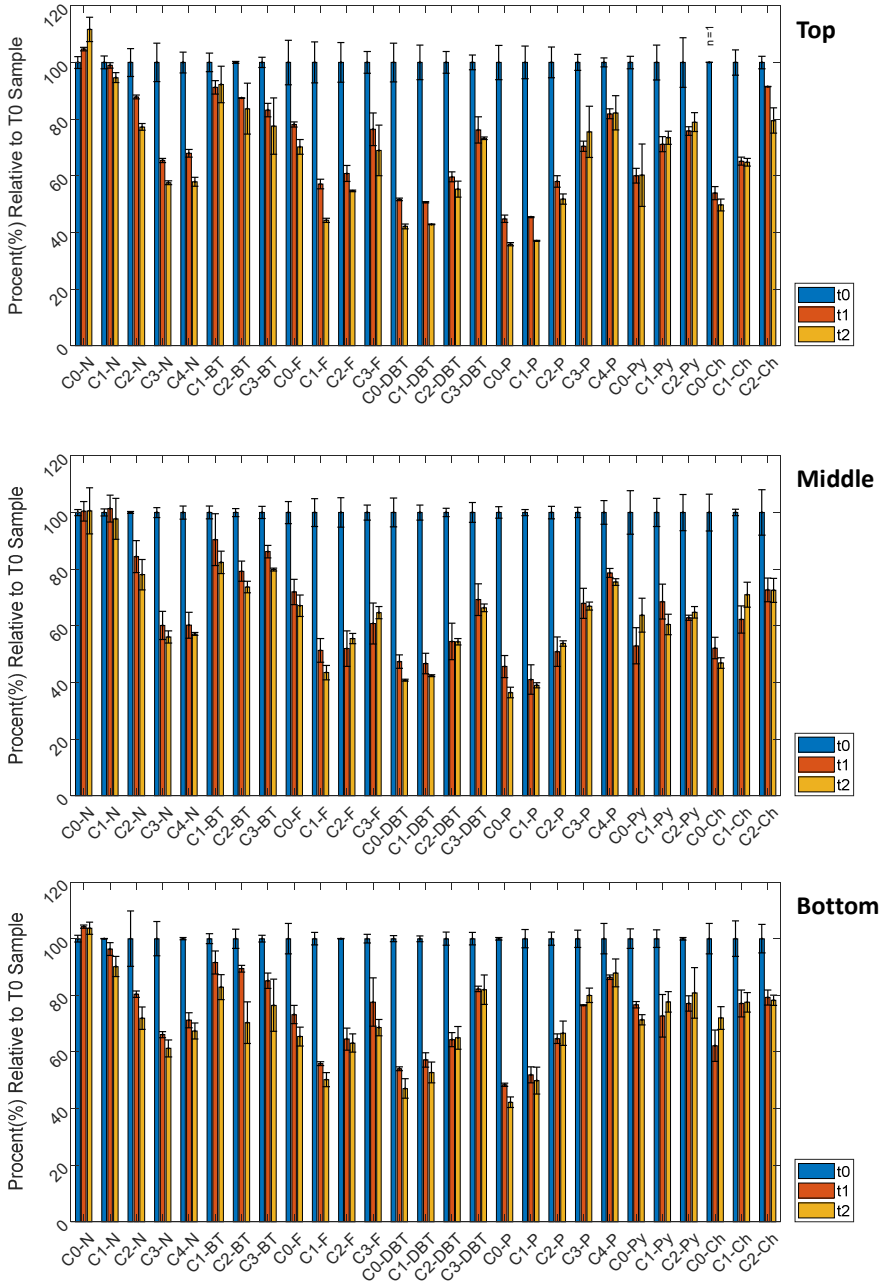


Figure 30. Seawater filtride from **station 57**. Degradation of oil PAHs in Seawater filtride incubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. C0-, C1-, C2-, C3-, and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalization.



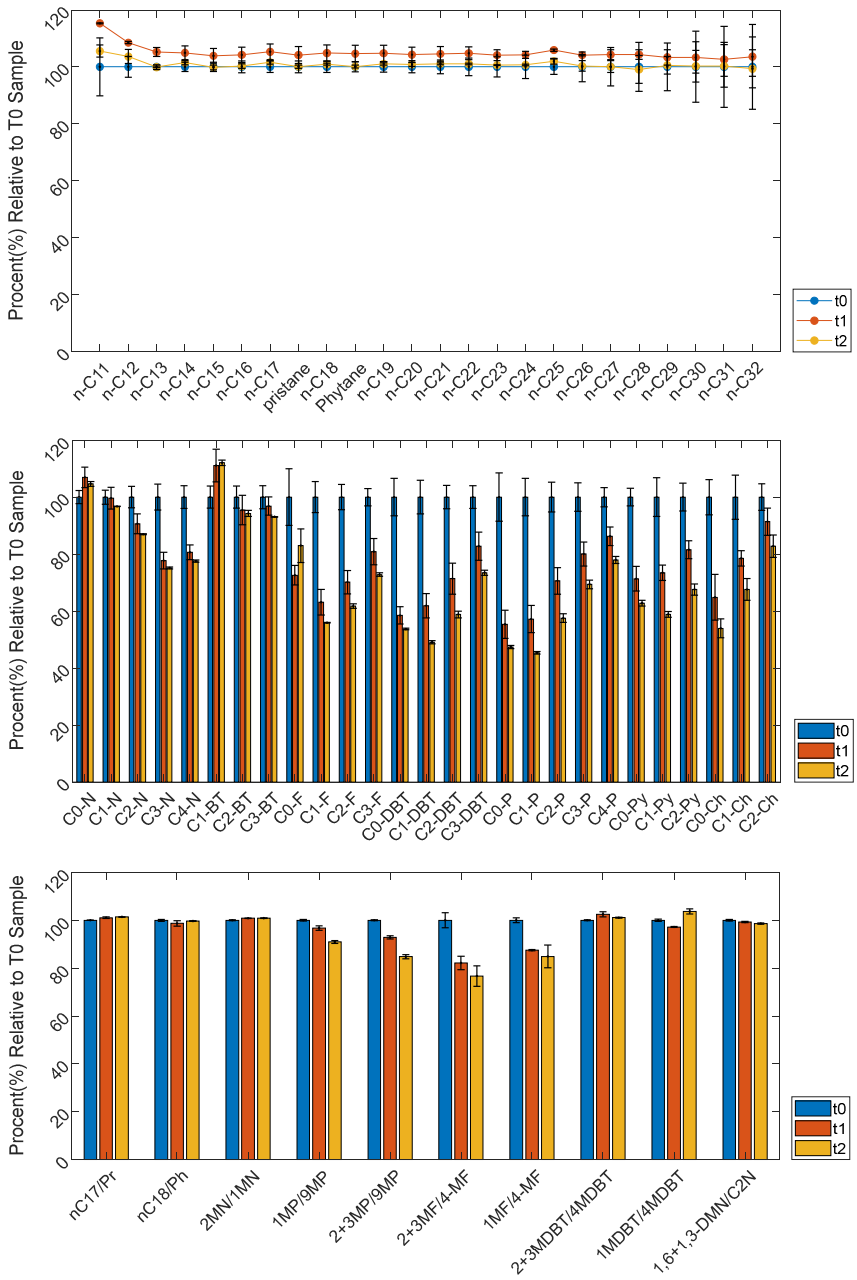
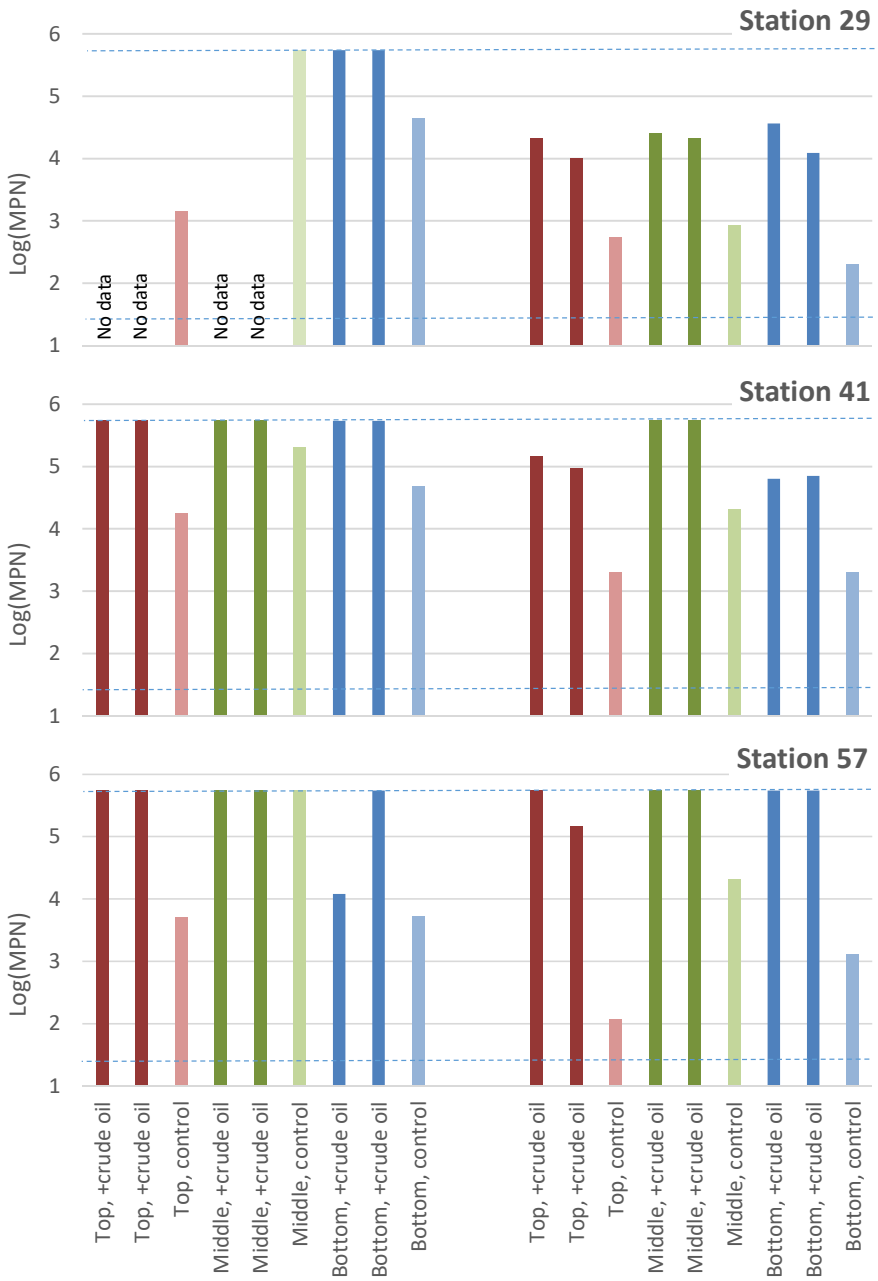


Figure 29. Results of sterile control incubations. Top panel: Changes in alkane concentrations. n-C11, n-C12, n-C13, etc. indicate the number of carbon atoms in specific n-alkanes. Mid panel: Changes in PAH concentrations. C0-, C1-, C2-, C3- and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. Bottom panel: changes in ratios for diagnostic degradation of crude oil. Pr: pristine, Ph: phytane, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. t0: 0 days, t1: 28 days, t2: 90 days. n=2, hopane normalization.

The isomer ratios showed extensive biodegradation of *n*-alkanes (Figure 31) which is in line with the removal of these compounds (Figure 28) and the high concentrations of hexadecane degraders (Figure 30). The alkyl-PAH isomer ratios showed only small changes over time (Figure 31 and Appendix 0), that were similar to changes in the sterile controls (Figure 29). Most PAH removal therefore seems to be abiotic and an experimental artefact, confirming that that biodegradation of alkyl-PAHs was very limited.

Figure 30. Seawater filtride. Populations of microbial hexadecane degraders (MPN, cells/mL) in concentrated sea water incubated with Bushnell-Haas mineral nutrients with crude oil (+crude oil) or without crude oil (control). The blue lines indicate the lower and upper detection limits. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Left: 35 days incubation. Right: 84 days incubation. Replicates are shown in the same colour.



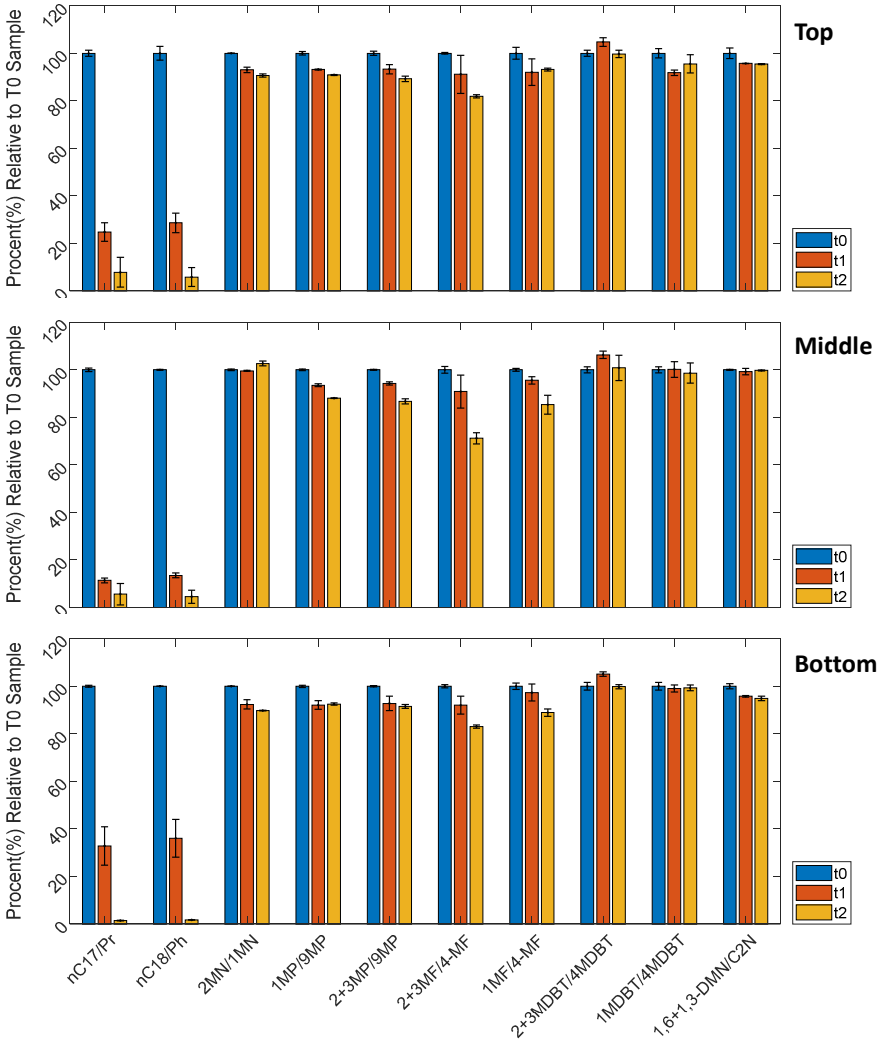


Figure 31. Seawater filtride from **station 57**. Diagnostic ratios for degradation of crude oil in the presence of Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. Pr: pristine, Ph: phytane, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalization.

The prokaryotic communities differed depending on were in the water column the samples were taken. The bottom and middle samples (before incubation) had high relative abundance of the archaean class *Nitrososphaeria* representing 48 - 57 % of the communities (Figure 32). The class *Nitrososphaeria* therefore seem to be dominant in all marine compartments tested at these stations (middle- and bottom of the water column and oxic sediment). The top samples show a somewhat higher abundance of *Alphaproteobacteria* and *Gammaproteobacteria* compared to the bottom- and middle samples. In addition, the top samples also contained a relatively higher abundance of *Oxyphotobacteria* (*Cyanobacteria*) and *Bacteroidia* compared to the bottom and middle samples (Figure 32).

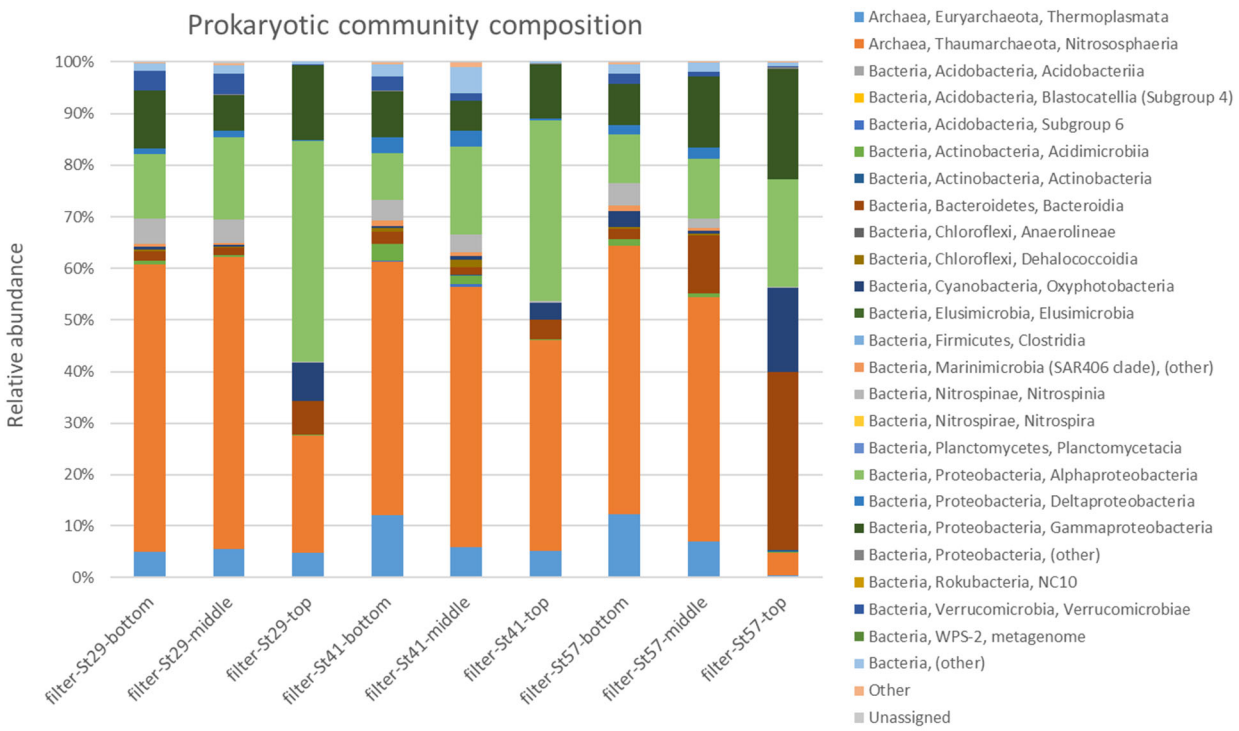


Figure 32. *Prokaryotic community composition (i.e. bacteria and archaea) of the filtride samples. The taxonomy (16S rRNA gene) of the groups are determined until the class level where possible.*

The *Bacteroidia*, *Alphaproteobacteria* and *Gammaproteobacteria* were the most dominating classes in the microcosms and the MPN wells (Figure 33). Dominant sequences in the MPN wells presumably represent the cultivable alkane degraders as hexadecane was the only carbons source in the MPN wells. The most common, cultivable hexadecane degrader found in almost all MPN plates was, as for the sediment MPNs, an uncultured gammaproteobacterium (Appendix 11). The second most common hexadecane degraders belonged to the genera *Pseudomonas* and *Shewanella* that were also found in the sediment MPNs. Some MPN wells were also enriched in other sequences, especially from the genera *Halomonas*, *Thalassospira*, *Flavobacterium* and *Marinomonas* of which *Halomonas* (Pepi et al., 2005) is well known for alkane degradation, and *Thalassospira* (Kodama et al., 2008) and *Marinomonas* (Dong et al., 2015) are capable of marine PAH degradation. *Flavobacterium* are common marine bacteria, but some species within this genus are also known to degrade oil (e.g. Chaudhary and Kim, 2017) though these isolates are psycrophilic, terrestrial bacteria. The content of the MPN wells from one of the St57 bottom replicates was very different from the other St57 replicate and also different from all other samples in that the dominant sequences in the MPN wells all belonged to four uncultured archaea. Notably, there were no cultivable *Proteobacteria* in the sequenced MPN wells of this sample. Presumably, this means that the alkanes in this microcosm were predominantly degraded by archaea.

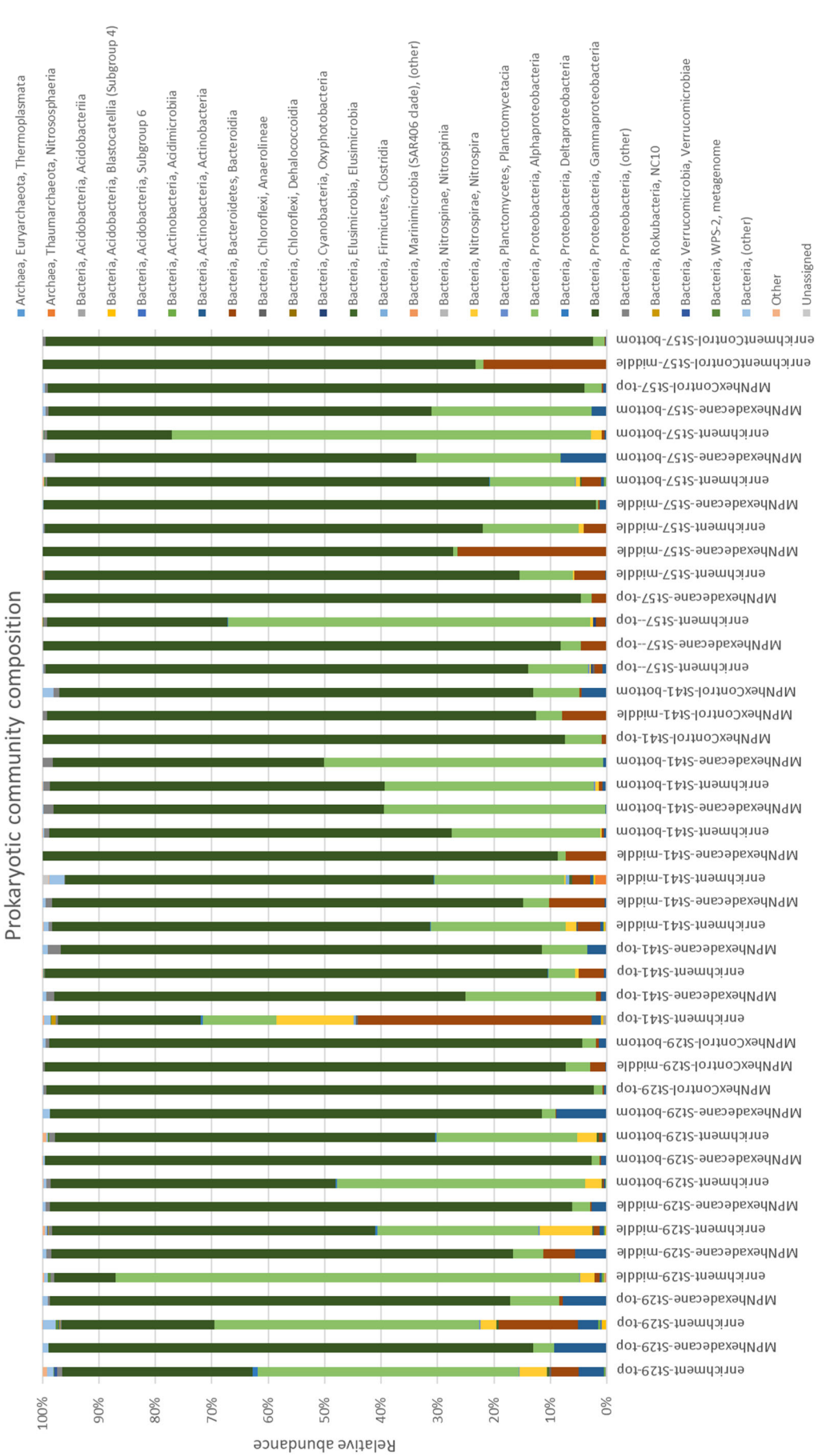


Figure 33. Prokaryotic community composition (i.e. bacteria and archaea) of filtride microcosms added either crude oil and BH mineral nutrients (enrichment) or BH mineral nutrients only (control). The communities were sampled after 84 days incubation. The composition of growth-positive MPN wells (dominant cultivable hexadecane degraders) is shown for comparison. The taxonomy (16S rRNA gene) of the groups were determined until the class level where possible.

6.5 General discussion

The total absence of bacterial strains with hydrocarbon degradation capability is unlikely in any location (e.g. Roubal and Atlas, 1978), but the metabolic diversity of the hydrocarbon degrader community may range from degradation of only simple *n*-alkanes to very diverse communities that can degrade a multitude of structurally different oil compounds. Pre-exposure presumably induces microbial degrader communities with broad substrate diversity by selecting and enriching rare degraders with the necessary metabolic machinery. A central point in marine oil degradation is therefore the degree of pre-expose and the consequent adaptation, so that the degrader community may be able to "handle" a great number of oil compounds with widely different molecular structures. The sampling locations and the experimental design should therefore take into account that pre-exposure, and thereby metabolic diversity and numbers of degraders, may vary from location to location. This is particularly important when natural oil seeps are expected, which they were for stations within the license areas. It is unlikely that we should sample water or sediment right at a seep, but sediment and water will probably be enriched in degraders and PAHs downstream of the seeps. We therefore expected to find heavy recalcitrant alkyl-PAHs and specialized oil degraders at some of the shelf stations within the license areas, where oil might seep from the underlying reservoir rock. This hypothesis was not confirmed.

Alkyl-PAHs indicate a petrogenic source as the concentration of these compounds is low in PAH-mixtures derived from combustion processes (pyrogenic PAHs). The sediment samples were therefore screened for both non-substituted PAHs that are primarily pyrogenic and alkylated PAHs that are primarily petrogenic. The low concentrations of only a few light alkyl-PAHs in the tested sediments indicate that oil pre-exposure ranged from very low to absent. Pre-exposure to alkyl-PAHs was furthermore demonstrated only at stations situated at the shelf break. These alkyl-PAHs might hypothetically have been released from sources on the continental slope. Stations on the shelf did not show indications of alkyl-PAH pre-exposure. This was unexpected as the license areas are on the shelf, which is where we would expect seeps. The seeps would not have to be right at the sample station, as we would expect a plume of slightly oil-contaminated water that could enrich down-steam sediments with alkyl-PAHs and different types of oil-degrading microorganisms. This seems not to be the case as cultivable oil degraders were below the detection limit in all sediment samples.

All of the sediments contained a variety of non-substituted PAHs from combustion processes, especially the high-molecular-weight PAHs with five rings or more. This indicated a background level of pyrogenic compounds from distant sources such as traffic, combustion of wood, forest fires etc. Comparable results were shown in a study of the distribution of PAHs and PAH-degrading bacteria in deep-sea sediments of the high latitude Arctic Ocean (Dong et al., 2015). Sediment cores were taken at four places along a north-south transect north of Canada where water depth ranged from 2500 m to 4000 m. The sediment samples were analysed for the content of non-substituted PAHs (Σ16PAHs: 2-42 ng / g) and degraders of non-substituted low-molecular weight PAHs. The overall conclusions of Dong et al. were that PAHs and PAH-degrading bacteria are widespread in the deep-sea sediments of the Arctic Ocean. A notable limitation of their study is that both the PAH analyses (EPA16 PAHs) and the degrader enrichments focused on pyrogenic PAHs. The results therefore cannot be generalized with respect to degradation of petrogenic oil PAHs since these comprise a high number of different, alkylated isomers.

The sediment potential for degradation of alkyl-PAHs and other oil compounds was tested in our second experiment where sediments from the shelf were incubated with weathered oil with or without addition of mineral nutrients to see the difference between in-situ nutrient conditions and a situation where mineral nutrients were not a limiting factor. We chose to artificially weather the oil (i.e., enrich in the heavier components by evaporation), since oil that enters sediments at greater depths have often lost the light compounds due to evaporation, photo-oxidation, dissolution and biodegradation. It was clear from the results, that the sediments had very low contents of available mineral nutrients so that there was almost no degradation without addition of extra nitrogen, phosphorous, magnesium and iron. This was demonstrated by using several complementary methods. The first approach was to measure the removal over time of a large number of alkane and PAHs compounds. Only the lighter alkanes were efficiently removed and mostly when mineral nutrients were added, which to some degree indicate biodegradation. To better differentiate between biodegradation and physical processes, we also investigated changes over time in diagnostic isomer ratios. The principle of diagnostic ratios relies on the assumption that bacteria degrade isomers within a compound class, typically methylated aromatics, at different rates, whereas physicochemical processes such as evaporation and dissolution affect the isomers equally. This means that degradation rates depend on the positions of the alkyl side-chains, and biodegradation is therefore reflected in changing isomer ratios, whereas the ratios will remain almost constant during physicochemical removal. Only ratios indicative of *n*-alkane biodegradation changed in the sediments and mostly when mineral nutrients were added.

Isomer ratio analyses and semi-quantification of oil compounds indicate only the disappearance of oil compounds. This may in many cases be a simple hydroxylation of a methyl group that produces another compound rather than complete degradation of the oil. We therefore also determined changes in the concentration of specific oil-degrading microorganisms that have the capacity to fully degrade different types of oil compounds to CO_2 and water by using them as a sole source of energy and carbon for growth. The drawback of this method is that only cultivable microorganisms can be detected. The presence of specific degraders is a clear indication, but the absence of cultivable degraders should not be over-interpreted as some degraders might be active in the microcosms, but in a non-cultivable state. *n*-alkane degraders were enriched in many of the sediment microcosms when mineral nutrients were added. The other oil compounds that were used to test for specific degraders were all growth-negative except two detections of isobutyl degraders. The metabolic spectrum of the cultivable degraders was thus very narrow which is not what we would expect if there was extensive in-situ pre-exposure for instance from seeps. The slow or absent PAHs removal in the shelf sediments without mineral nutrient limitation was probably due to oil degraders with narrow metabolic capabilities because different degraders have not been enriched by long-term exposure to oil components from seeps. The weathering of the oil probably aggravated this effect, as degraders may co-metabolize many of the more recalcitrant compounds when they grow on lighter oil compounds, which are removed during weathering.

In a third series of experiments, we investigated in details how degraders in the water from one station responded to addition of oil, mineral nutrients and a dispersing agent. Specialized oil-degrading bacteria usually occur in low numbers in pristine environments. Efficient biodegradation of spilled oil therefore requires massive growth of these bacteria. Degrader growth and

hence oil degradation may, however, be limited by lack of mineral nutrients, especially in the photic zone where the availability and cycling of mineral nutrients vary greatly and nitrogen can be almost absent during the spring algal bloom (Thingstad et al., 2008). The nutrient limitation was indeed stronger in the surface water than in the deeper water, probably due to such nutrient depletion. Degrader growth may also be limited by low bioavailability of the oil compounds, a parameter that can be reduced by dispersing the oil in the water. The dispersion agent was very efficient at dispersing the oil, which formed a turbid suspension in the microcosms. The increased oil surface area itself, however, did not stimulate degradation in the surface water, and stimulation was barely detectable when the dispersion agent was added together with mineral nutrients in microcosms containing water from greater depths. Without mineral nutrients, there was very little alkane removal and hardly any PAH removal in surface water and substantial removal of only light *n*-alkanes in deeper water. Addition of mineral nutrients had a strong effect leading to complete *n*-alkane and isoprenoid removal and removal of a large proportion of the alkylated PAHs. Degradability followed the normal patterns with decreasing removal with increasing number of rings in the PAH molecule, and decreasing removal with increasing number of alkyl substituents within classes of PAHs (Wang and Fingas, 1995; Wang et al., 1998; Kristensen et al., 2015). The removal results were supported both by changes in isomer ratios (diagnostic of biodegradation) and by the counts of cultivable oil degraders.

Some types of degraders were common in the water used for the microcosms (hexadecane degraders). Some were rare (e.g., 1,3-dimethylcyclohexane degraders) and occurred only in one microcosm, but the presence of many different types of degraders in the original water samples showed that the degrader community in the water at station 70 had a broad substrate spectrum with the ability to utilize hydrocarbons with different molecular structures that requires many different metabolic pathways. This corresponds well with the fact that petrogenic PAHs (C1-naphthalenes and C1-phenanthrenes) were present in sediments from the shelf break, suggesting some natural oil preexposure at these stations. But what about the stations at the shelf, where petrogenic PAH were not detected in the sediments, did the microbial degrader community at these stations have the necessary metabolic pathways for degradation of a range of structurally diverse oil compound as seen for the microbial community in water sampled at station 70? To answer this question, we carried out a fourth experiment with microorganisms that were extracted from the water columns at three shelf stations and incubated with oil under optimal conditions.

The purpose of the fourth experiment was to determine the diversity of metabolic oil degradation and thus the potential diversity of oil compounds that can be metabolized by the oil-degrading bacteria at the top, middle and bottom of the water column on the continental shelf. The extracted microorganisms were exposed to oil in a mineral nutrient medium that has proved optimal for growth of oil degrading bacteria (Bushnell and Haas, 1941). All *n*-alkanes were efficiently removed, which was supported both by changes in alkane isomer ratios and large populations of cultivable hexadecane degraders. The results were more ambiguous for the alkyl-PAHs. Many compounds were removed, but the removal was only slightly mirrored in shifting isomer ratios or in detection of cultivable degraders. A sterile control experiment produced PAH “removals” and isomer ratios that were similar to the filtride microcosms, indicating that PAH removal in the filtride microcosms were an experimental artefact. Also, there were only few types of degraders compared

to station 70. Many bacteria from environmental samples are considered uncultivable, which means that they may not show up in MPN counts that rely on growth in defined mineral media. It is however unlikely that oil degrader bacteria in the filtride microcosms could not be MPN-enumerated as they were enriched in the flasks in exactly the same mineral medium as used in the MPN plates. A possible explanation is that the water concentrated on the filter originated from shelf stations with no oil pre-exposure, whereas the water at station 70 was from the shelf break where the sediment analyses indicated a bit of pre-exposure.

A central point in our reasoning is that the Statfjord model oil should simulate oil spills from oil exploration and production. According to the strategic environmental impact assessment of hydrocarbon activities (SEIA) for Northeast Greenland (Boertmann and Mosbech 2011), geologists from GEUS suggest the paraffinic Statfjord crude oil as a representative crude for the area. Paraffinic crudes like Statfjord contain a relatively high volume percentage of linear and branched paraffins (alkanes), whereas other types of crude oil may have a high content of naphthenes (cycloalkanes) and / or aromatics. The high content of paraffins make the Statfjord relatively easy for bacteria to degrade, given that there is sufficient mineral nutrients. This is true for the *n*-alkanes and the smaller aromatics, but the lack of isoprenoid (phytane and pristine) degradation in many microcosms and the complete lack of cultivable 2,2,4-trimethylpentane degraders suggest that branched paraffins may show higher persistence even when mineral nutrients are not limiting degradation. The lack of cultivable 1,3-dimethylcyclohexane degraders also suggests that the potential for naphthenic compounds is very limited even when the microbial communities have plenty of mineral nutrients and have been exposed to naphthene compounds for up to three months. The result cannot be used directly to predict the fate of spills of naphthenic or aromatic crudes since the microbial metabolism may show other limitations for oils of other compositions. Our results, however, do suggest that oils with a high content of branched alkanes, naphthenes or heavy aromatics may be less susceptible to biodegradation.

6.6 Conclusions

Results from our simplified laboratory experiments can only be extrapolated to the complex environment of the Greenland Sea with great caution, but some microcosms did show a large potential for biodegradation of many different oil compounds in the water column, even “difficult” compounds such as C1-pyrene and C3-phenanthene was partially degraded after 70 days. This suggests, that bioremediation of surface spills may have a large potential if the intrinsic microbial degraders can be activated.

It was also clear that in-situ concentrations of mineral nutrients are strongly limiting for oil degradation, both in the water column and in the sediments. Biodegradation will be very limited without addition of mineral nutrients. Contingency strategies based on the intrinsic potential for microbial oil removal should therefore include strategies for applying mineral nutrients for degradation to be efficient. The dispersing agent Slickgone NS was efficient at dispersing the oil under the tested conditions, but may have little or no effect on oil degradation, or even a slightly negative effect on PAH degradation when mineral nutrients are limiting, probably because degradation of the dispersant itself requires mineral nutrients.

The absence of detectable alkyl-PAHs in the tested shelf sediments suggests that the tested shelf stations have had no oil pre-exposure. The concentration of alkyl-PAHs was low for the tested stations along the shelf break, suggesting that these stations may have had only very little pre-exposure to oil PAHs, if any. The absence of cultivable 2-methylnaphthalene degraders and phenanthrene degraders together with the very small changes in diagnostic alkyl-PAH ratios suggest that the potential for biodegradation of weathered PAHs in the shelf sediments is very low, even when mineral nutrients are not a limiting factor. The applied methods all had benefits and drawbacks, but they were strong tools when combined. Together they showed that we could not detect any signs of oil pre-exposure in the tested shelf sediments.

7 References

Bej AK, Saul D, Aislabie J. 2000. Cold-tolerant alkane-degrading Rhodococcus species from Antarctica. Polar Biology 23: 100–105

Boertmann D, Mosbech A. (eds). 2011. The western Greenland Sea, a strategic environmental impact assessment of hydrocarbon activities. Aarhus University, DCE – Danish Centre for Environment and Energy, 268 pp. - Scientific Report from DCE – Danish Centre for Environment and Energy no. 22.

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

Chaudhary DK and Kim J. 2017. Flavobacterium olei sp. nov., a novel psychrotolerant bacterium isolated from oil-contaminated soil. Int J Syst Evol Microbiol. 67: 2211-2218

Chronopoulou PM, Sanni GO, Silas-Olu DI, van der Meer JR, Timmis KN, Brussaard CP, McGenity TJ. 2015. Generalist hydrocarbon-degrading bacterial communities in the oil-polluted water column of the North Sea. Microb Biotechnol. 8: 434-47

Cui Z, Gao W, Xu G, Luan X, Li Q, Yin X, Huang D, Zheng L. 2016. Marinobacter aromaticivorans sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from sea sediment. International Journal of Systematic and Evolutionary Microbiology 66: 353–359

DASIC International Ltd. 2017. Safety data sheet Slickgone NS, Revision date: 5/04/2017. http://oil.dasicinter.com/oil-dispersants/slickgone-ns-1

Dong C, Bai X, Sheng H, Jiao L, Shao Z. 2015. Distribution of PAHs and PAH-degrading bacteria in the deepsea sediments of the high-latitude Arctic Ocean. Biogeosciences 12: 2163-2177

Dyksterhouse SE, Gray JP, Herwig RP, Lara JC, Staley JT. 1995. Cycloclasticus pugetii gen. nov., sp. nov., an Aromatic Hydrocarbon-Degrading Bacterium from Marine Sediments. International Journal of Systematic Bacteriology 45: 116-123

Escher JC and Pulvertaft TCR. 1995. Geological map of Greenland, 1:2 500 000. Copenhagen, Geological Survey of Greenland.

Feld L, Nielsen TK, Hansen LH, Aamand J, Albers CN. 2016. Establishment of bacterial herbicide degraders in a rapid sand filter for bioremediation of phenoxypropionate-polluted groundwater. Appl Environ Microbiol 82:878 – 887. doi:10.1128/AEM.02600-15

Gallotta FDC and Christensen JH. 2012. Source identification of petroleum hydrocarbons in soil and sediments from Iguacu River Watershed, Parana, Brazil using the CHEMSIC method (CHEMometric analysis of Selected Ion Chromatograms). Journal of Chromatography A, *1235*:149-158

Hurley MA and Roscoe ME. 1983. Automated statistical-analysis of microbial enumeration by dilution series. Journal of Applied Bacteriology *55*:159-164

Johnsen, AR and Henriksen S. 2009. Microplate MPN-enumeration of monocyclic- and dicyclic-aromatic hydrocarbon degraders via substrate phase-partitioning. Biodegradation 20:581-589

Kasai Y, Kishira H, Sasaki T, Syutsubo K, Watanabe K, Harayama S. 2002. Predominant growth of Alcanivorax strains in oil-contaminated and nutrient-supplemented sea water. Environ Microbiol. 4: 141-147

KodamaY, Stiknowati LI, Ueki A, Ueki K, Watanabe K. 2008. Thalassospira tepidiphila sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater. International Journal of Systematic and Evolutionary Microbiology 58: 711–715

KristensenM, AR Johnsen and JH Christensen. 2015. Marine biodegradation of crude oil in temperate and Arctic water samples. J. Hazard. Mat. 300:75–83.

Lamberts RF, Johnsen AR, Andersen O and Christensen JH. 2008. Univariate and multivariate characterization of heavy fuel oil weathering and biodegradation in soil. Environ. Pollut. 156:297-305

Lappan R. 2018. 16S rRNA analysis. https://rachaellappan.github.io/16S-analysis/index.html

Lattuati A, Metzger P, Acquaviva M, Bertrand JC, Largeau C. 2002. n-Alkane degradation by Marinobacter hydrocarbonoclasticus strain SP 17: long chain β-hydroxy acids as indicators of bacterial activity. Organic Geochemistry 33: 37-45

Pepi M, Cesàro A, Liut, G and Baldi F. 2005. An antarctic psychrotrophic bacterium Halomonas sp. ANT-3b, growing on n-hexadecane, produces a new emulsyfying glycolipid. FEMS Microbiology Ecology 53: 157–166

Roubal G and RM Atlas. 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaskan continental shelf areas. Appl Environ Microbiol 35:897-905

Rudels B, E Fahrbach, J Meincke, G Budeus and P Eriksson 2002. The East Greenland Current and its contribution to the Denmark Strait overflow. ICES Journal of Marine Science, 59:1133–1154. doi:10.1006/jmsc.2002.1284

Thingstad TF,Bellerby R, Bratbak G, Børsheim KY, Egge JK, Heldal M, Larsen A, Neill CC, Nejstgaard JC, Norland S, Sandaa R-A, Skjoldal Evy F, Tanaka T, Thyrhaug R, Tøpper B. 2008. Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem. Nature 455: 387-391 .

Wang ZD, Fingas M.1995. Study of the effects of weathering on the chemical composition of a light crude oil using GC/MS GC/FID. Journal of Microcolumn Separations 7:617-639

Wang ZD, Fingas M, Blenkinsopp S, Sergy G, Landriault M, Sigouin L, Foght J, Semple K, Westlake DWS. 1998. Comparison of oil composition changes due to biodegradation and physical weathering in different oils. Journal of Chromatography A 809:89-10

8 Appendices

Appendix 1. Degradation of alkanes in sediment microcosms

Degradation of alkanes in sediments incubated with weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients.n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. Internal standard normalization. n=3.

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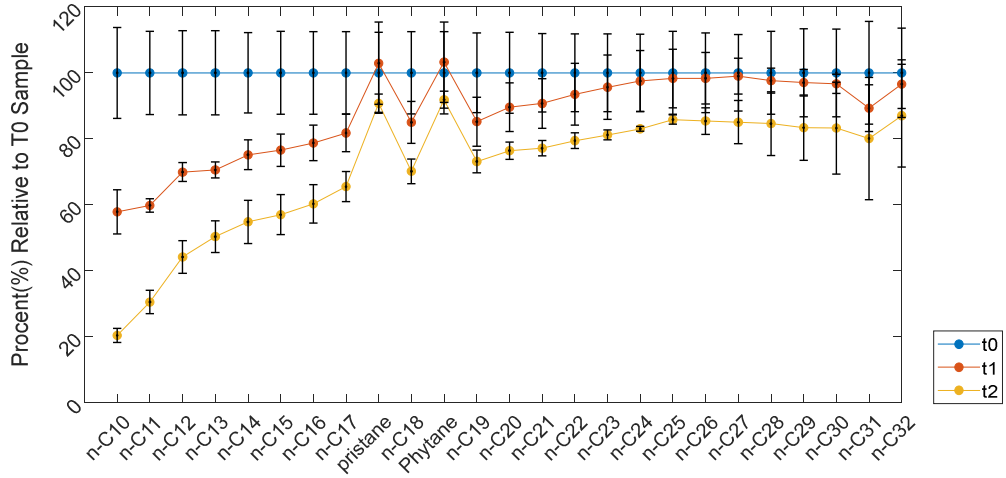

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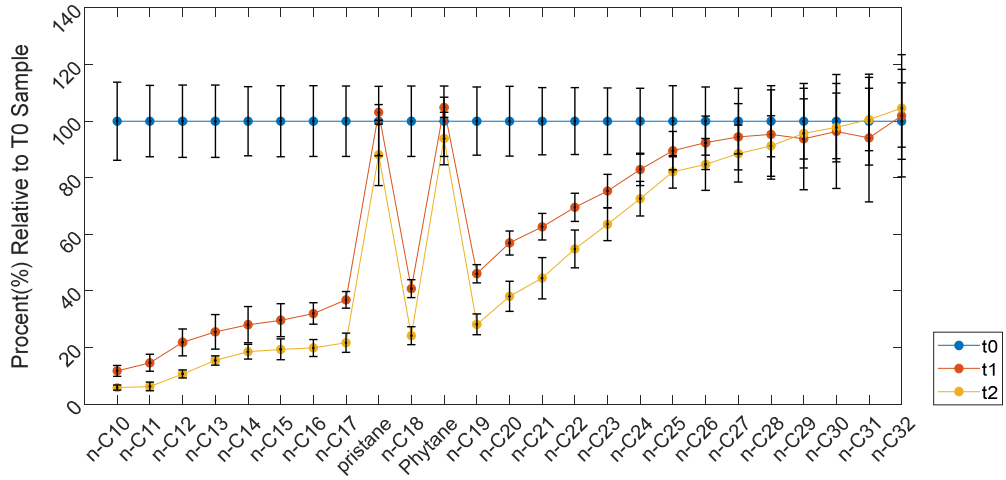

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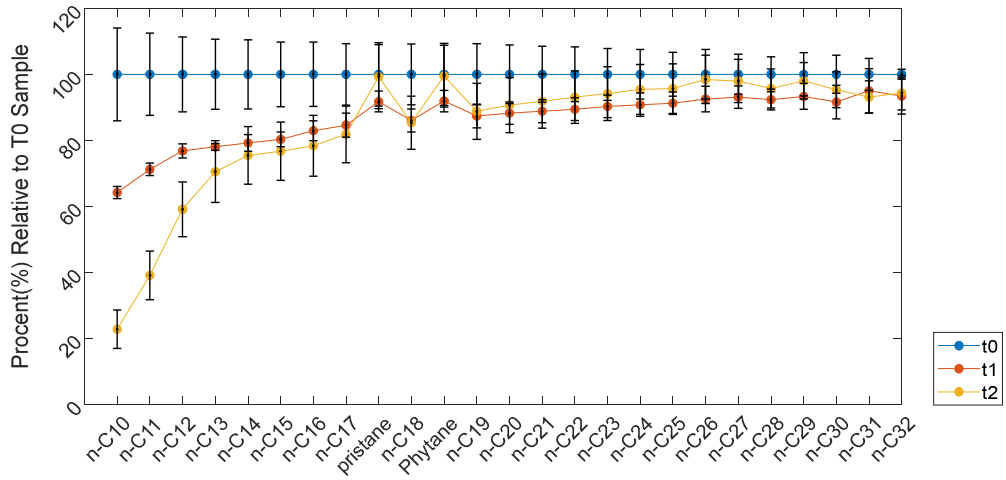

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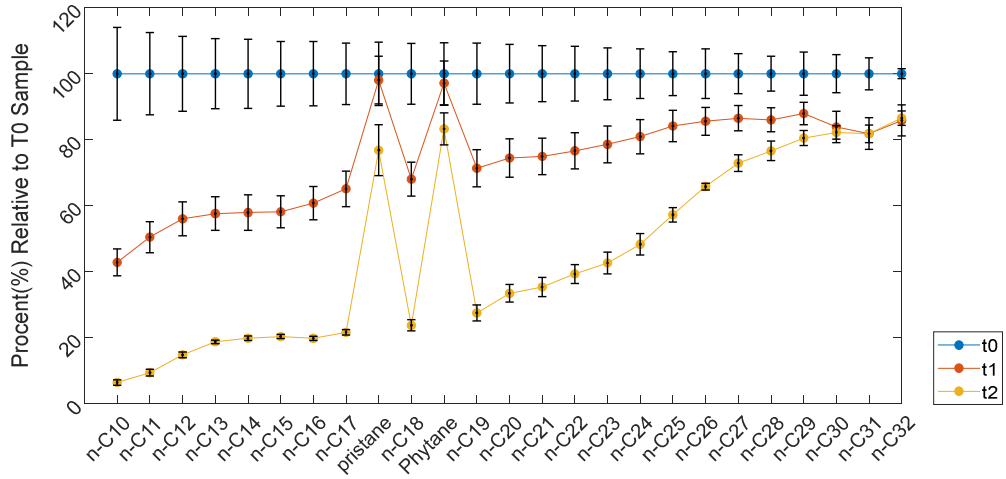

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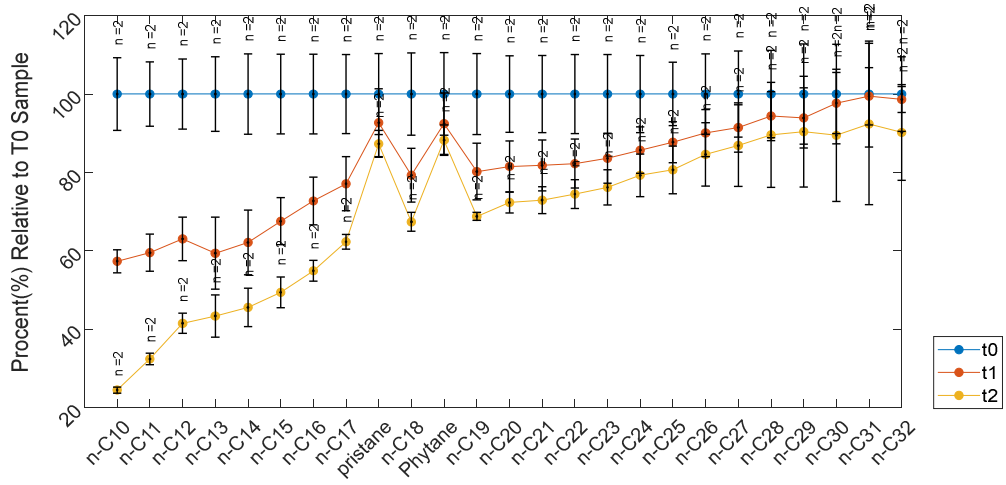

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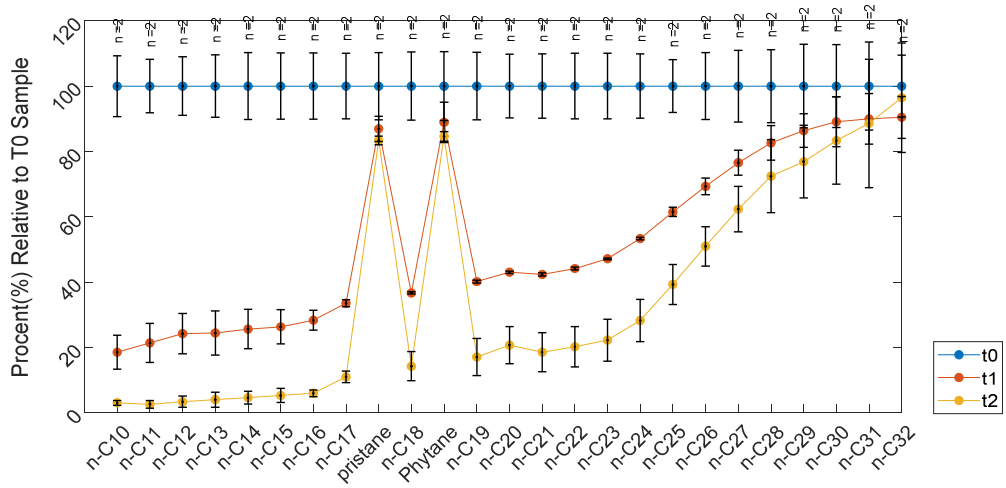

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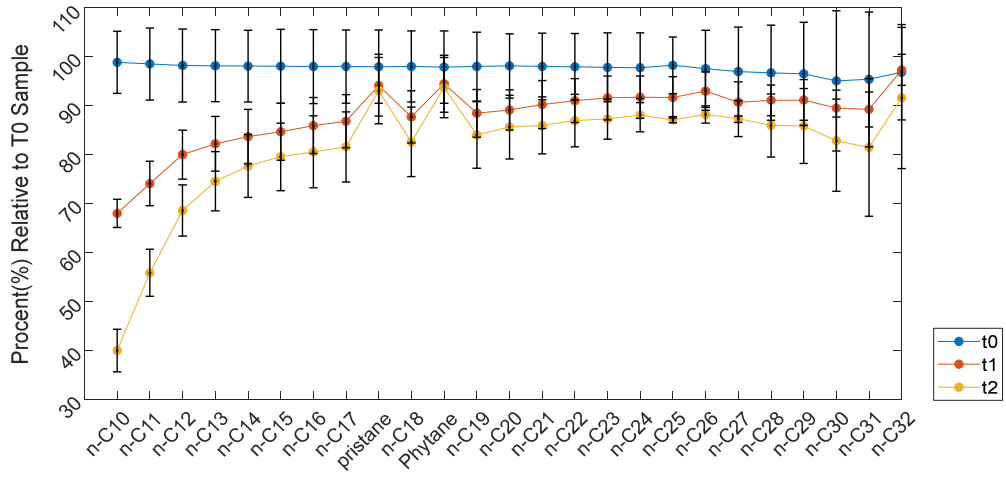

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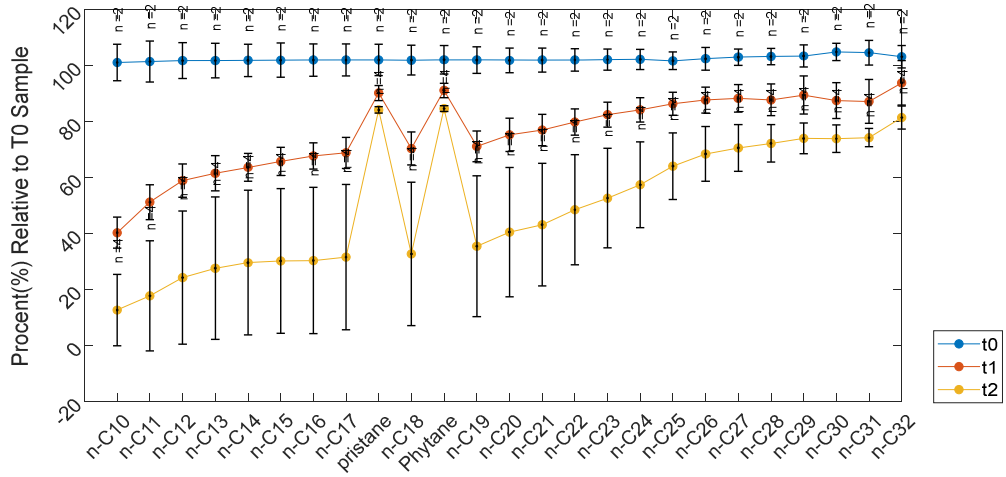

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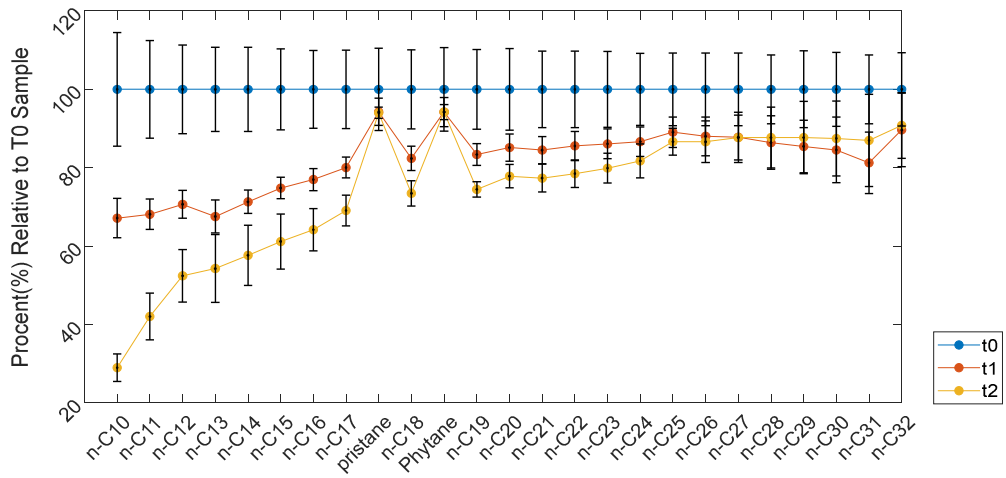

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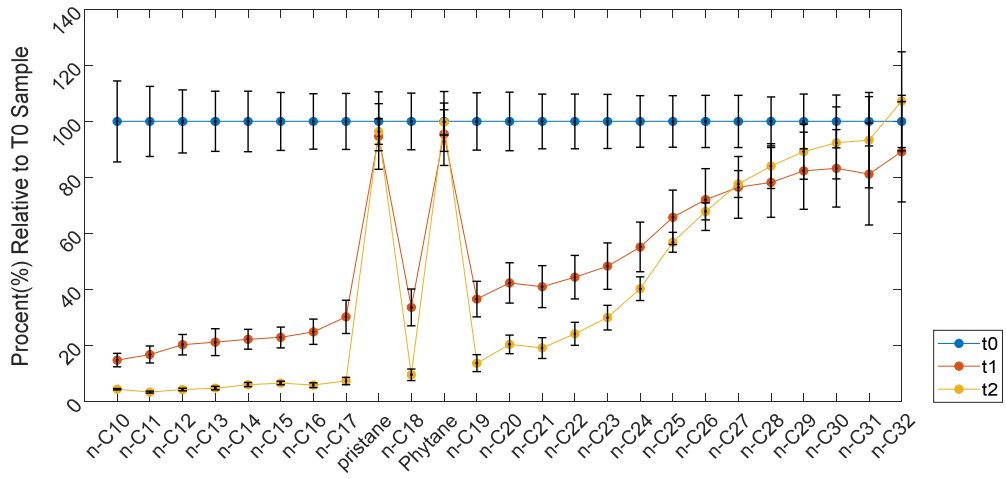

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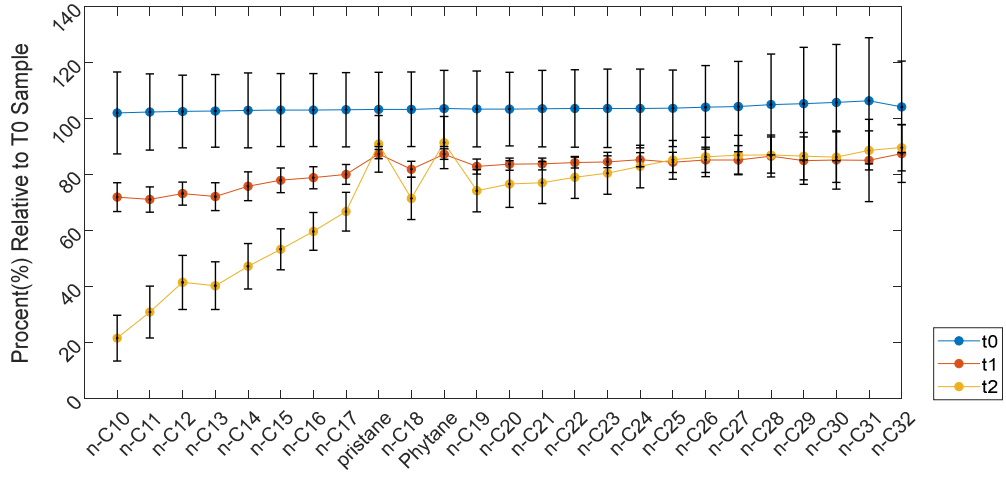

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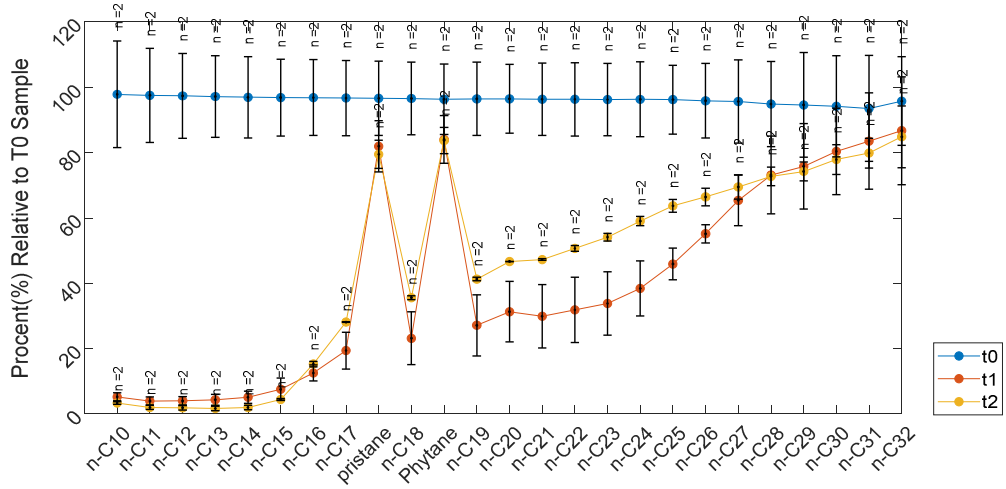

Appendix 2. Degradation of oil PAHs in sediment microcosms

Degradation of oil PAHs in sediments incubated with weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients.C0-, C1-, C2- and C3- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=3. Internal standard normalization.

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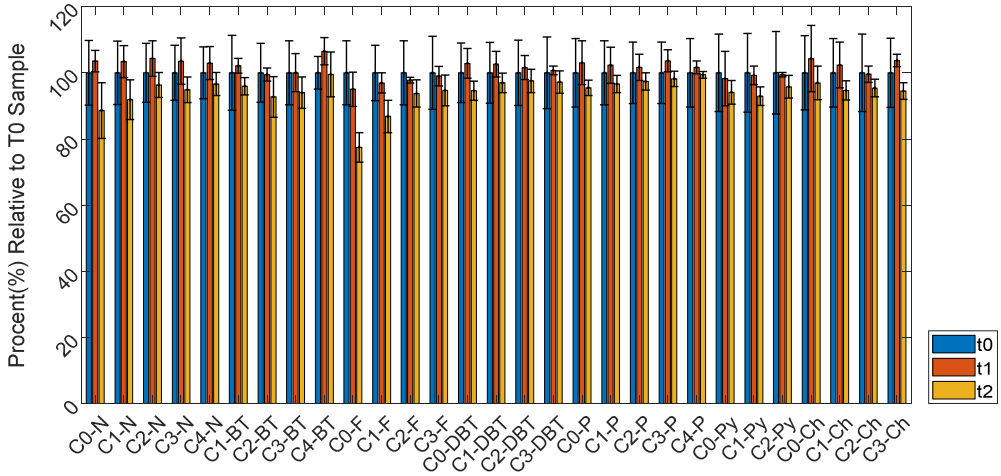

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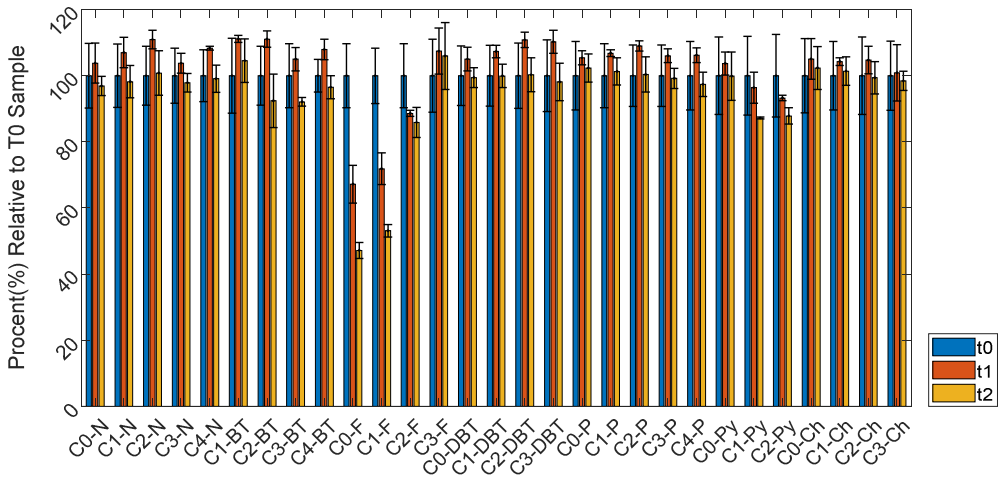

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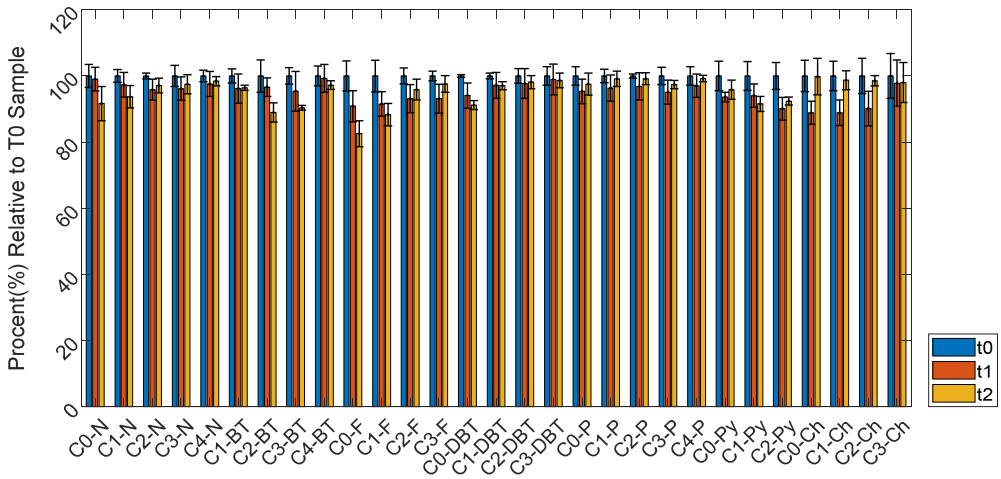

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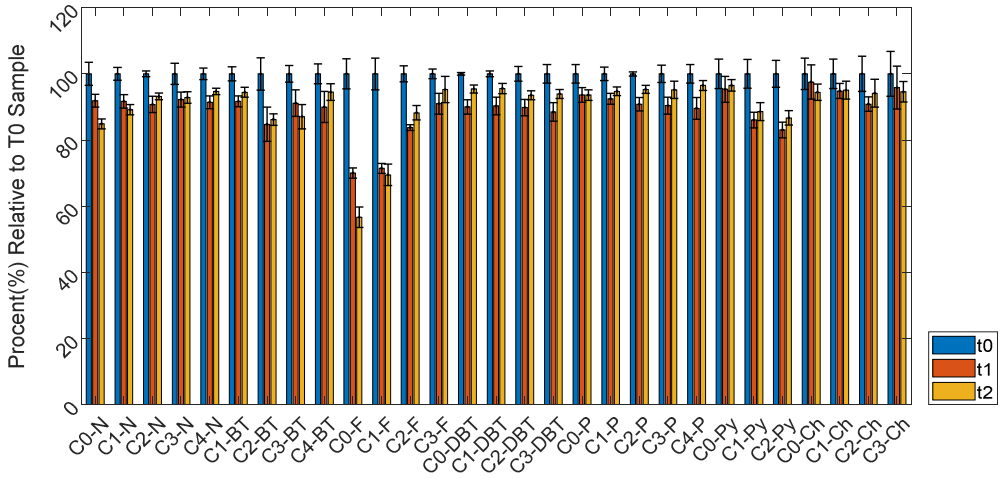

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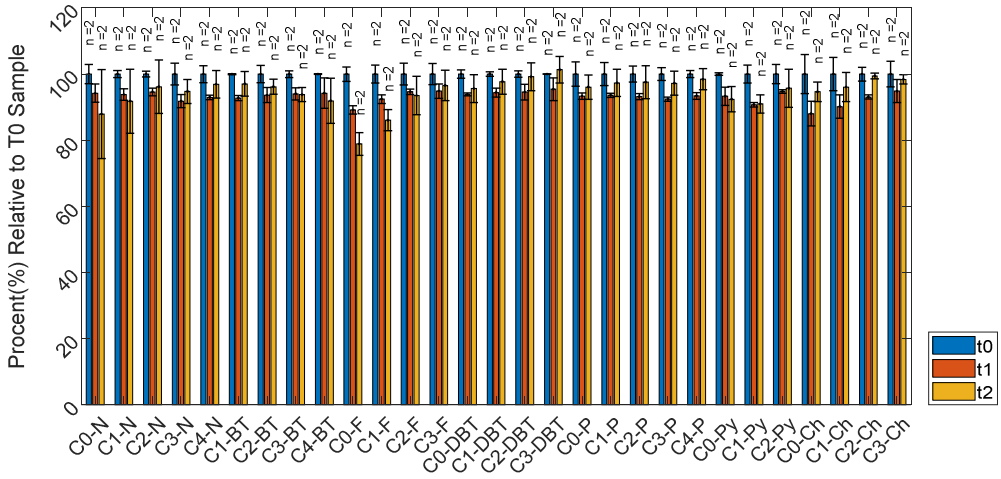

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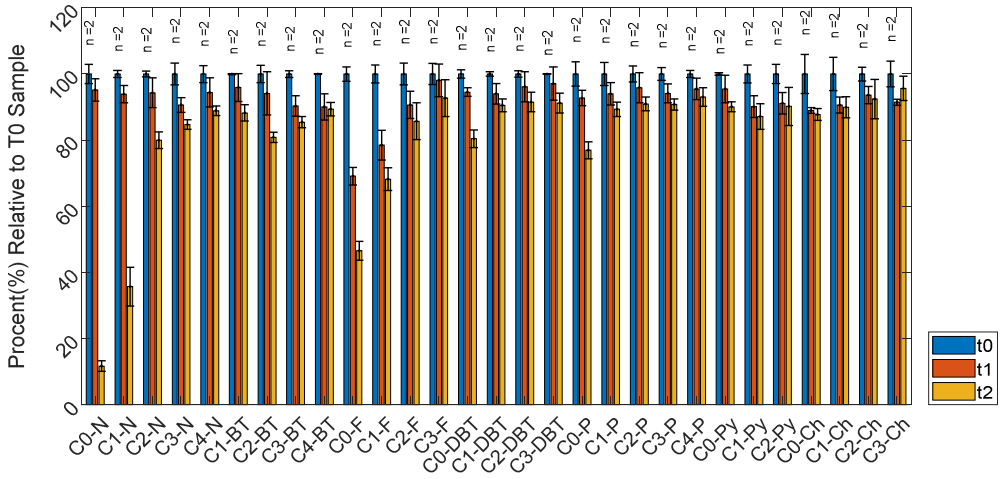

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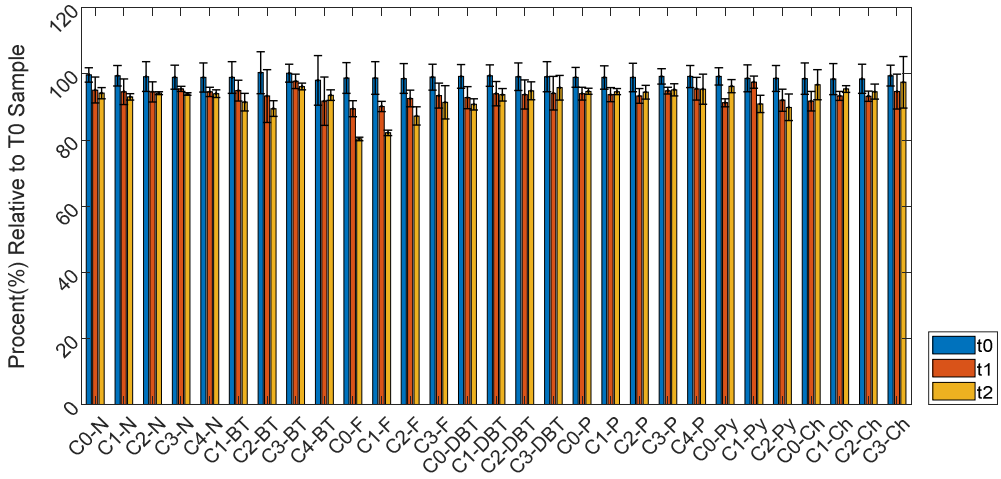

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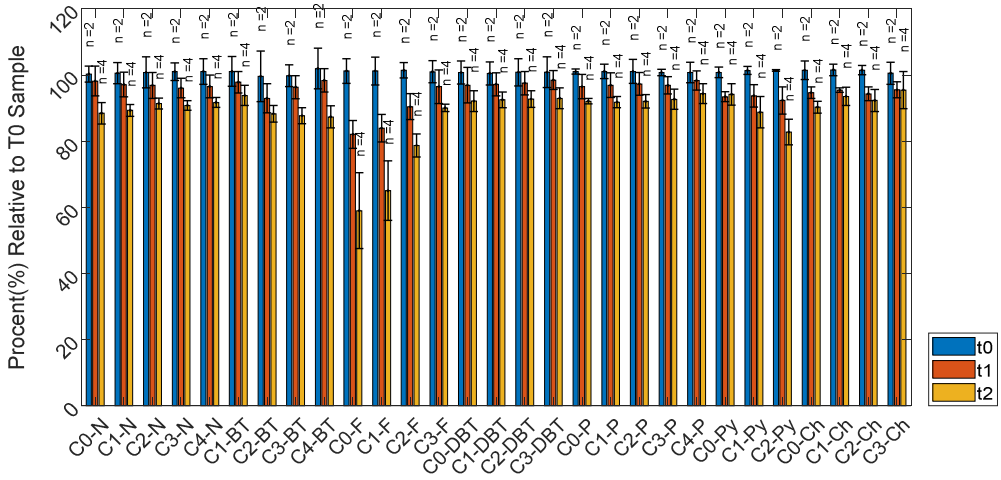

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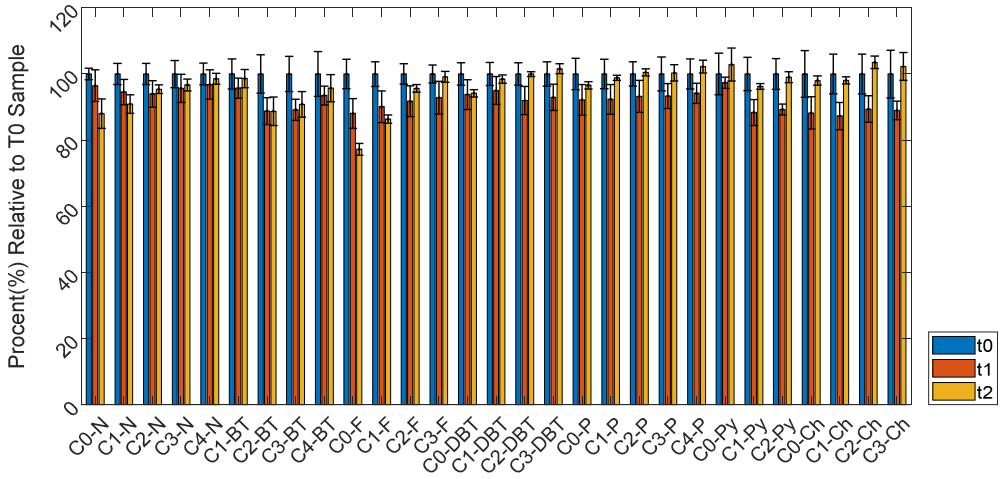

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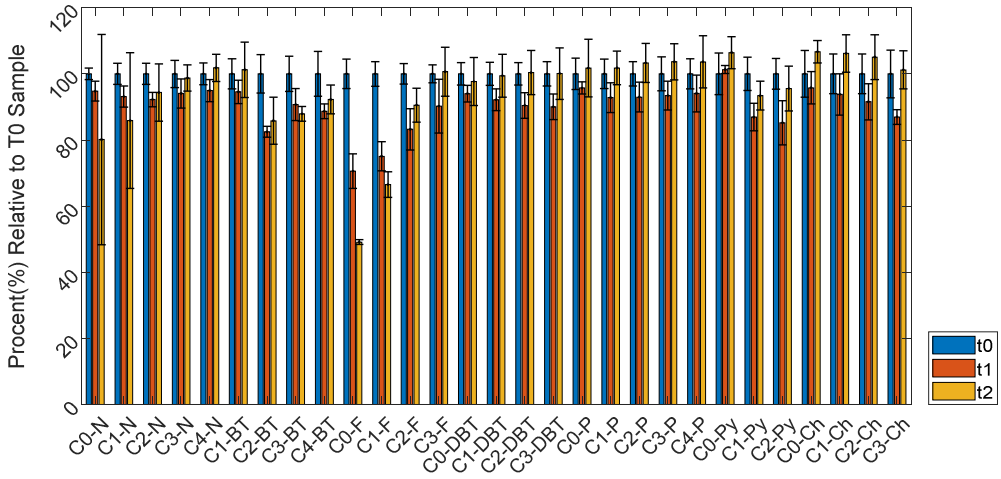

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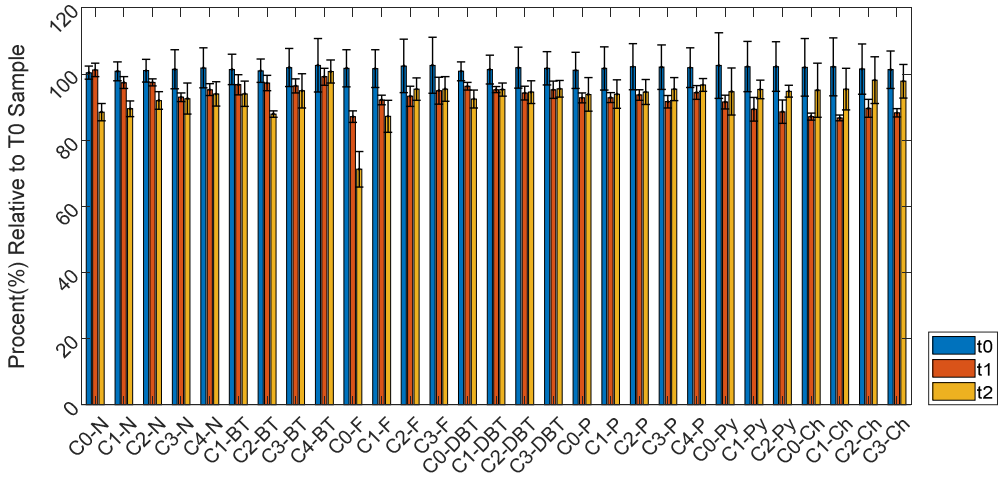

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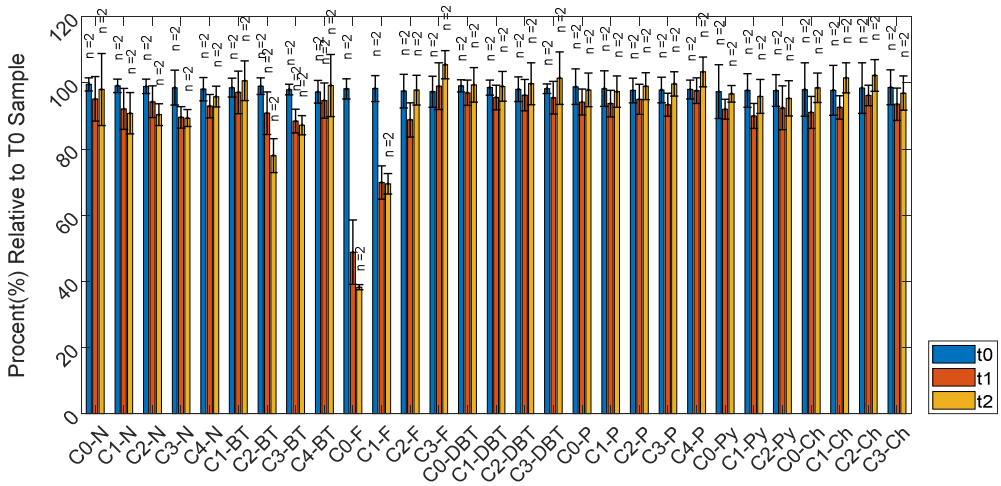

Appendix 3. Diagnostic ratios in sediment microcosms

Sediment. Diagnostic ratios for degradation weathered crude oil for 0 (t0), 21(t1) or 70 days (t2) with (+BH) or without Bushnell Haas mineral nutrients. n=3. Internal standard normalization.

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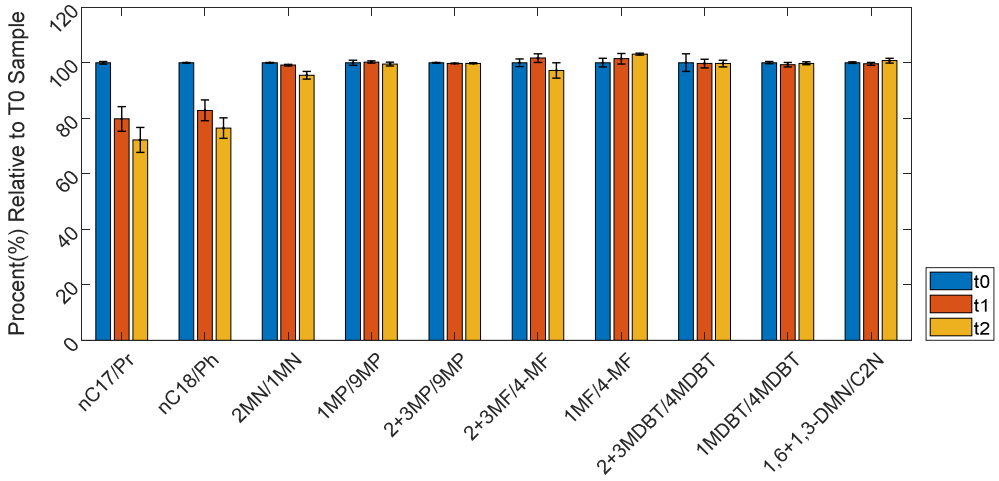

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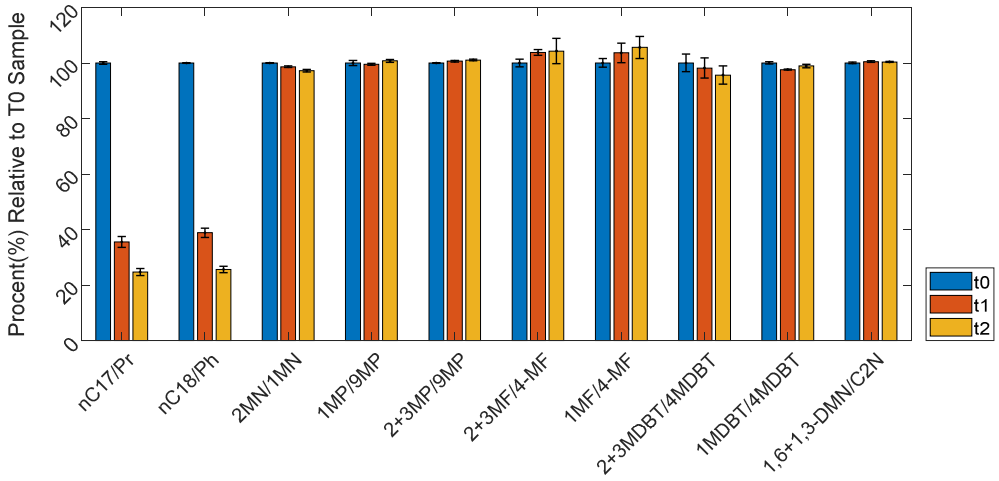

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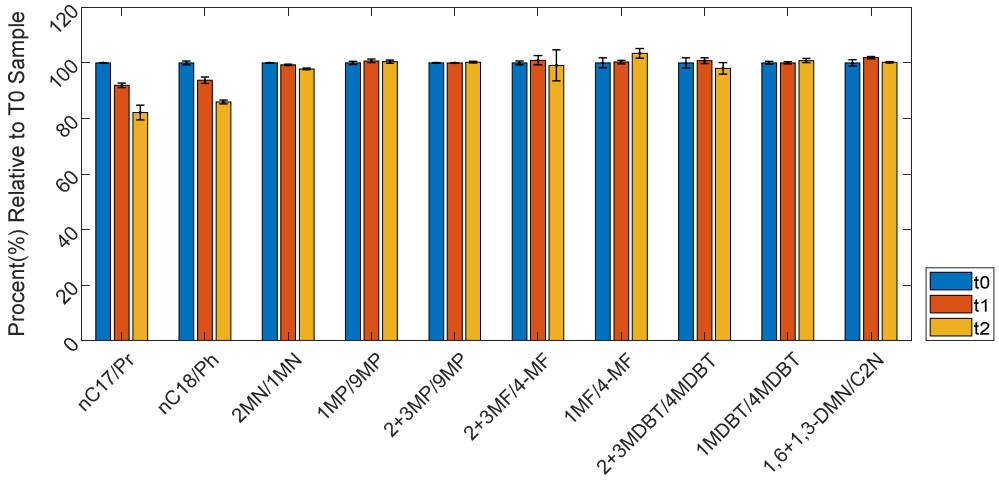

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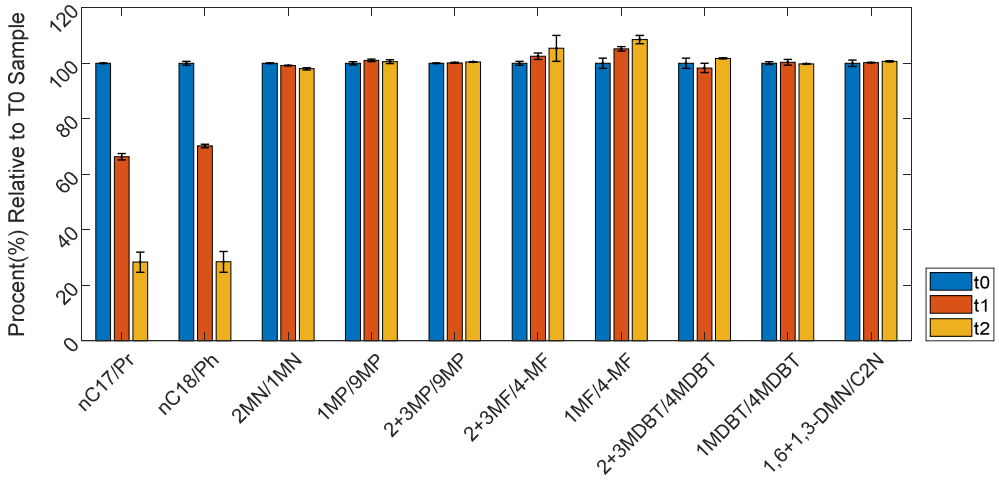

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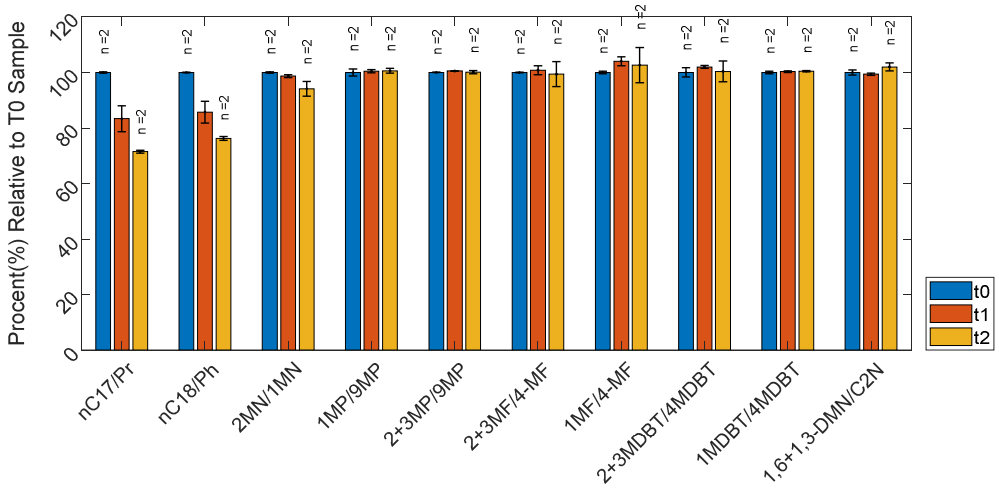

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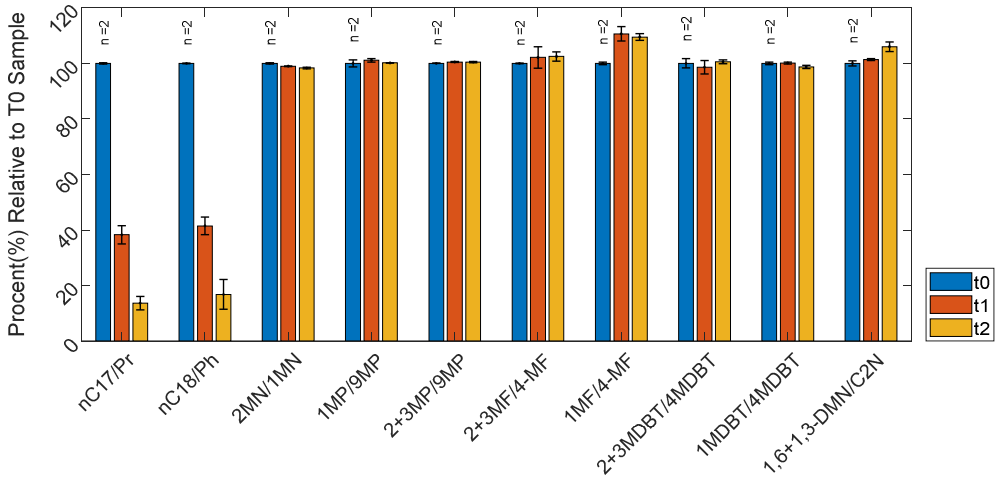

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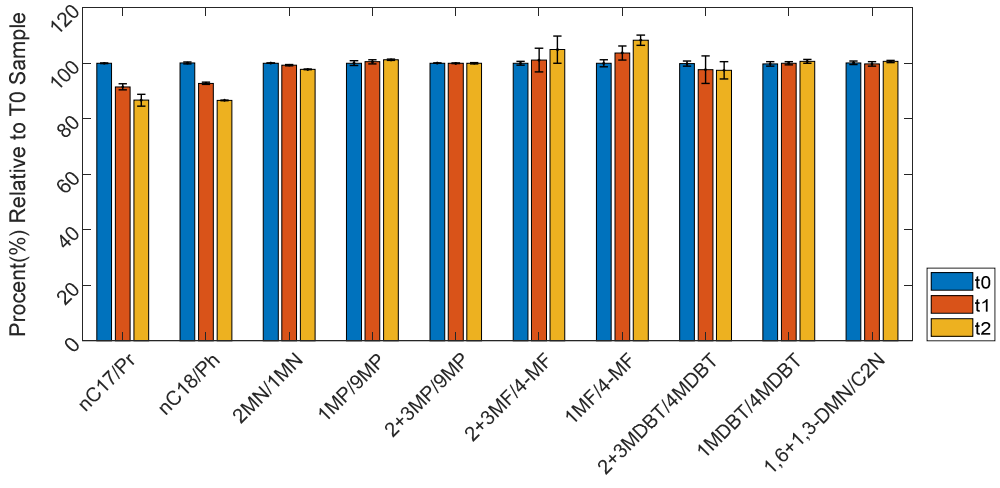

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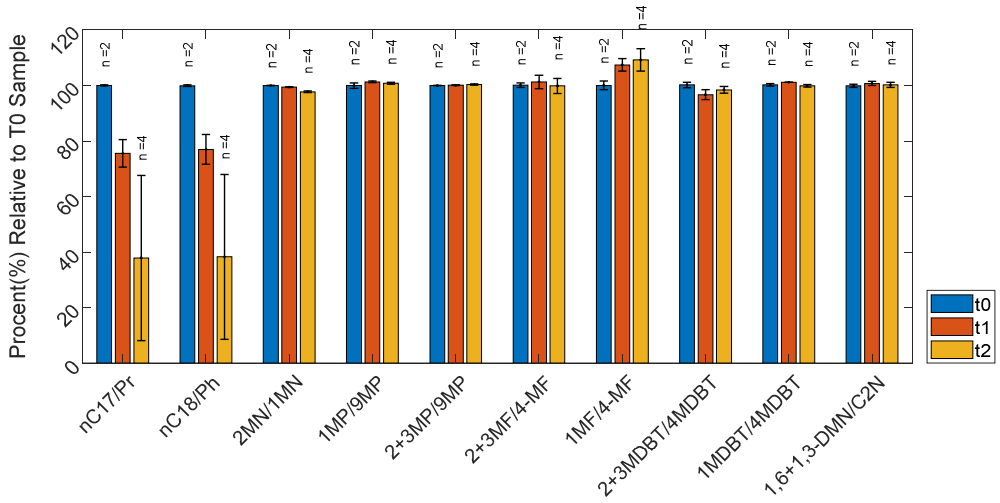

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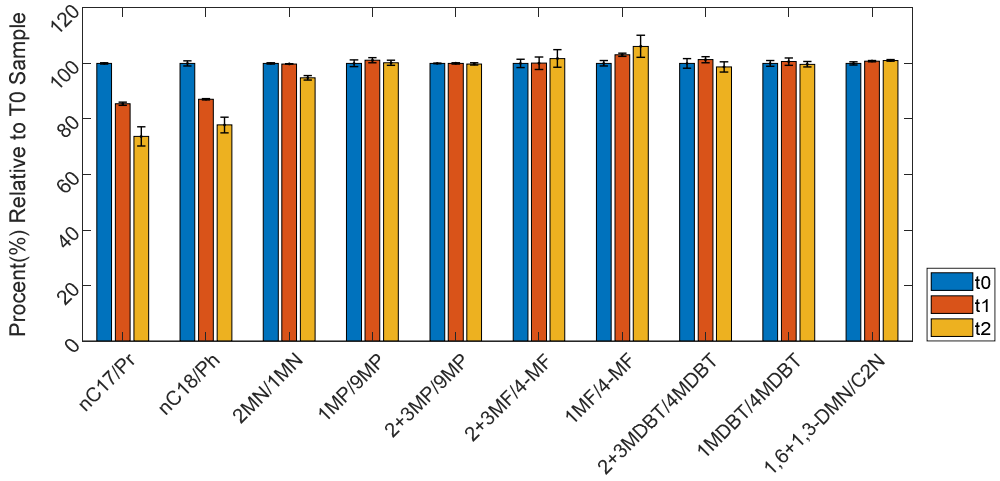

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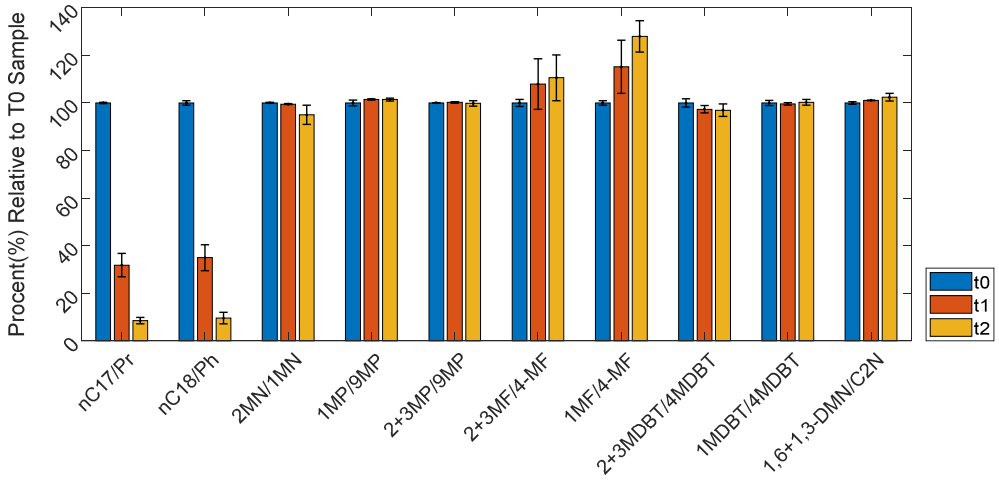

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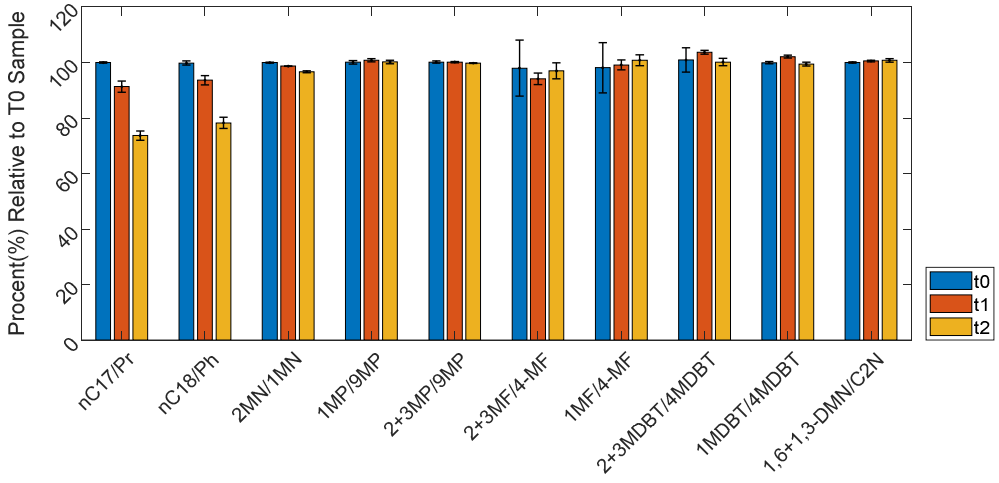

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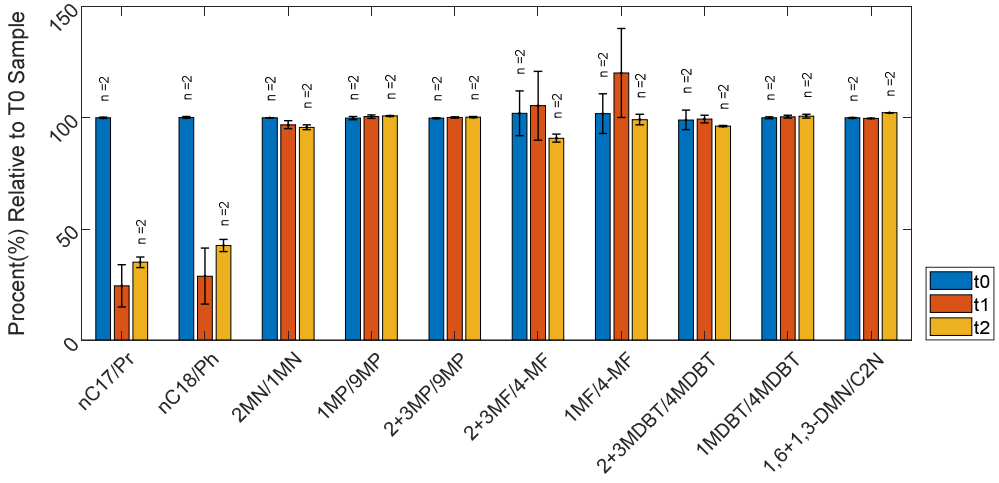


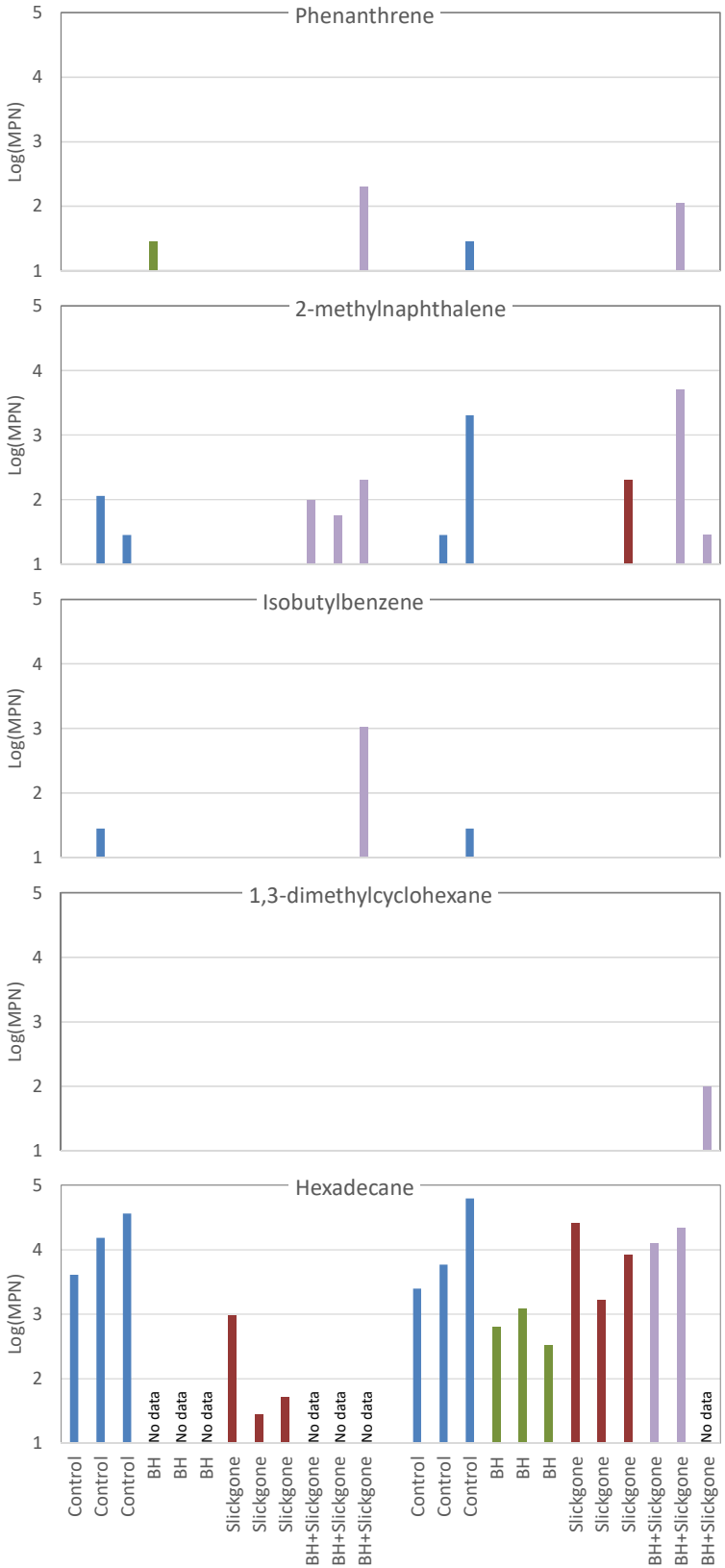
Appendix 4. MPN in sediment microcosms

Populations of microbial alkane degraders (MPN, cells / mL) in sediment incubated with weathered crude oil in the presence (+BH) or absence of Bushnell-Haas mineral nutrients. Degraders that could grow on 2-methylnaphthalene, 1,3-dimethylhexane or 2,2,4-trimethylpentane were below the limit of detection for all samples (<26 cells / g).

	Hexadecane			Isobutylbenzene		
Station	**0 days**	**21 days**	**70 days**	**0 days**	**21 days**	**70 days**
St27	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St27	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St27	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St29	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St29	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St29	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St36	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St36	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St36	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St41	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St41	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St41	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St49	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St49	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St49	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St57	<2,6E1	<2,6E1	2,80E+03	<2,6E1	<2,8E1	<2,8E1
St57	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St57	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St27+BH	<2,6E1	6,35E+03	2,07E+06	<2,6E1	<2,8E1	<2,8E1
St27+BH	<2,6E1	2,72E+04	7,07E+06	<2,6E1	<2,8E1	<2,8E1
St27+BH	<2,6E1	2,80E+03	1,36E+06	<2,6E1	<2,8E1	<2,8E1
St29+BH	<2,6E1	1,62E+04	4,11E+05	<2,6E1	2,8E1	<2,8E1
St29+BH	<2,6E1	2,82E+04	5,06E+05	<2,6E1	<2,8E1	<2,8E1
St29+BH	<2,6E1	2,60E+03	1,54E+06	<2,6E1	<2,8E1	<2,8E1
St36+BH	<2,6E1	0,00E+00	1,36E+06	<2,6E1	<2,8E1	<2,8E1
St36+BH	<2,6E1	0,00E+00	2,80E+03	<2,6E1	<2,8E1	<2,8E1
St36+BH	<2,6E1	2,80E+03	7,08E+04	<2,6E1	<2,8E1	<2,8E1
St41+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St41+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St41+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St54+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St54+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St54+BH	<2,6E1	<2,6E1	<2,6E1	<2,6E1	<2,8E1	<2,8E1
St57+BH	<2,6E1	<2,6E1	2,82E+04	<2,6E1	<2,8E1	1,14e4
St57+BH	<2,6E1	1,62E+04	5,47E+05	<2,6E1	<2,8E1	<2,8E1
St57+BH	<2,6E1	<2,6E1	no data	<2,6E1	<2,8E1	<2,8E1

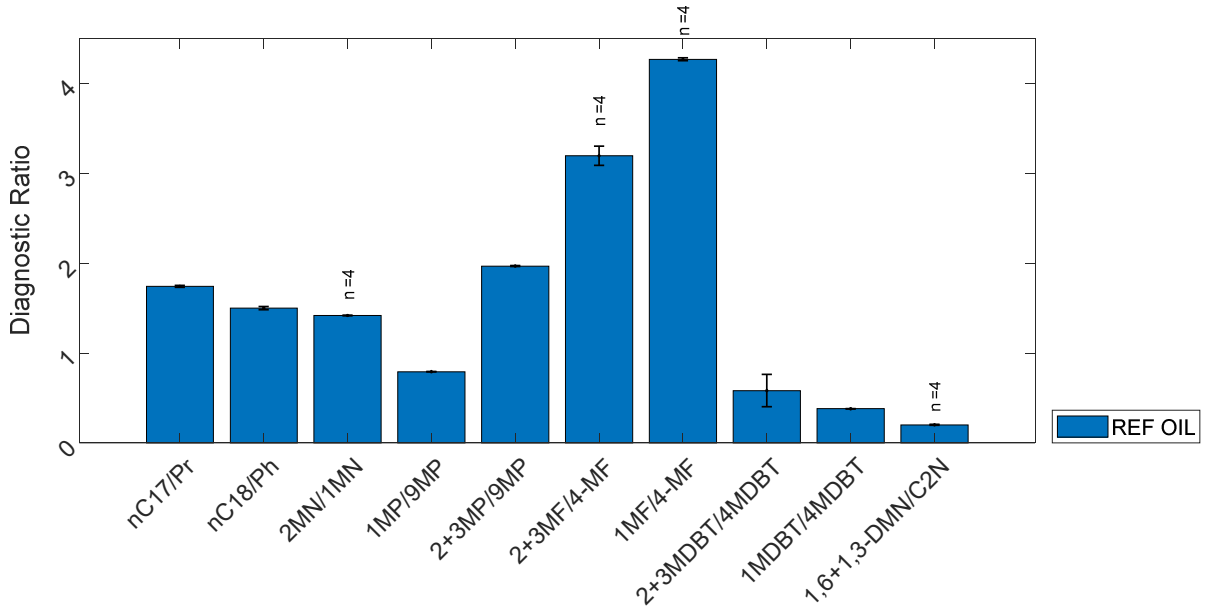
Appendix 5. MPN in seawater microcosms

Seawater microcosms. Effect of Bushnell-Haas mineral nutrients (BH) and Slickgone dispersant on the size of microbial oil degrader populations (MPN, cells / mL) in seawater incubated with crude oil for 28 days. The dotted line indicate the lower detection limits. Left: water from 10 meters depth. Right: water from 340 meters depth.



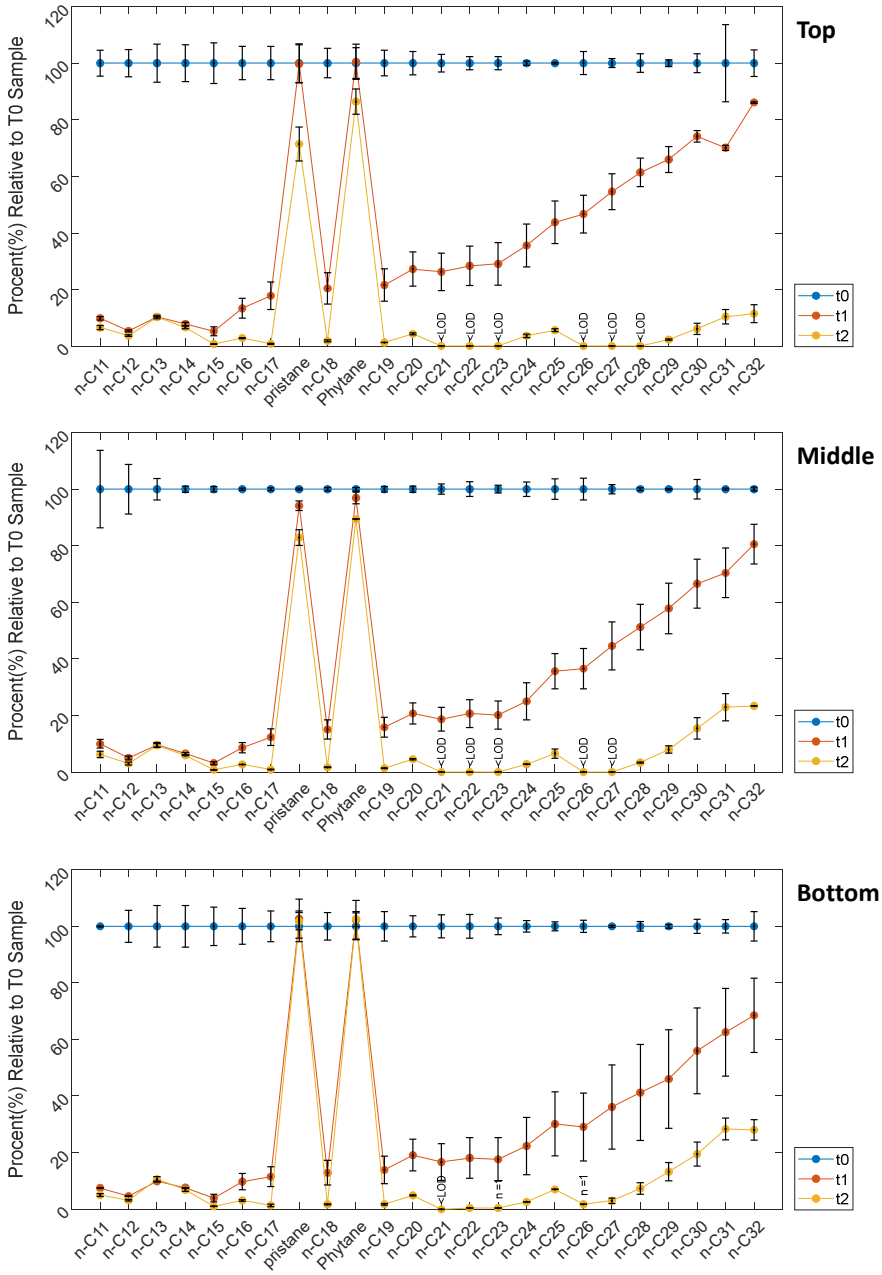
Appendix 6. Diagnostic ratios in the Statfjord reference oil

Diagnostic ration in the reference oil (Statfjord). Experiment with BH mineral salts and dispersion (Slickgone)

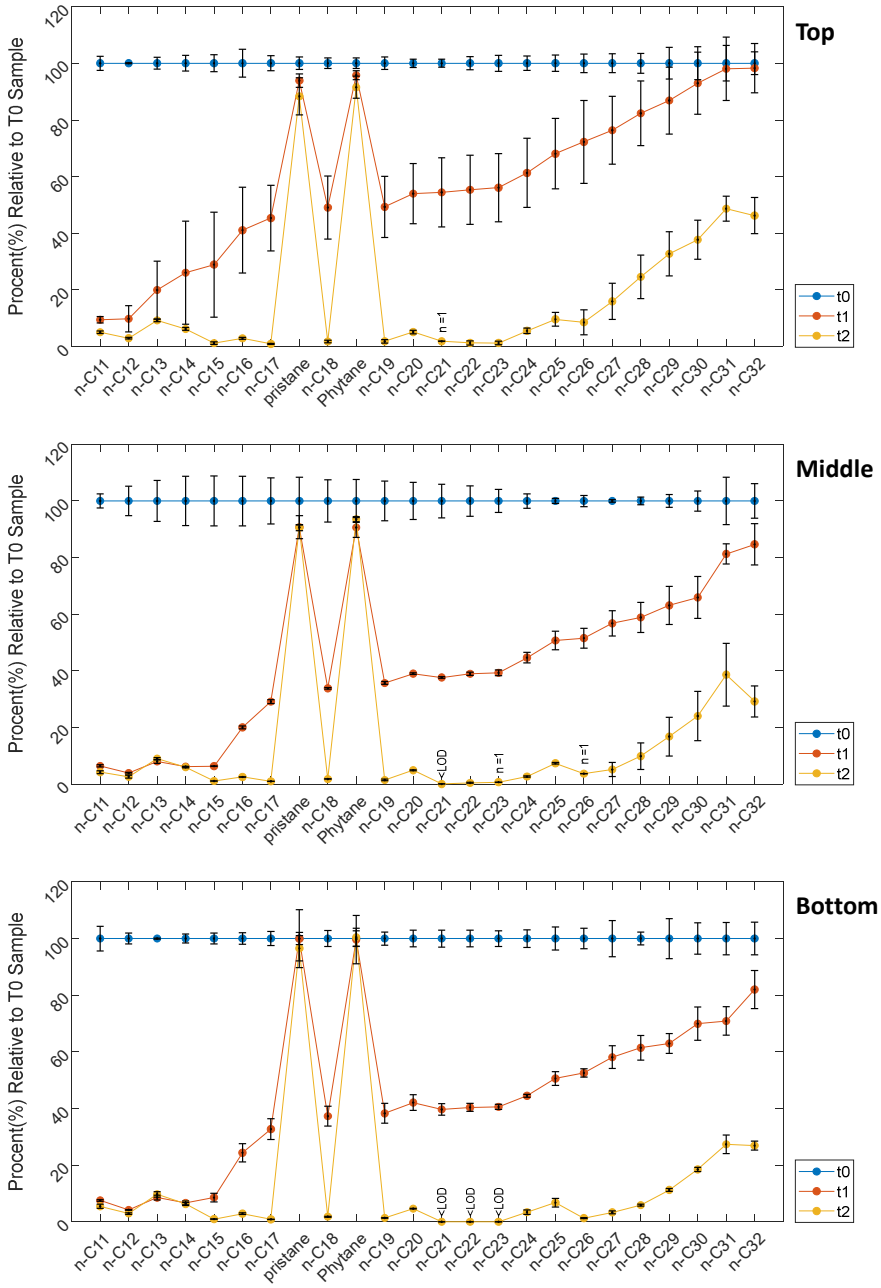


Appendix 7. Degradation of alkanes in filter microcosms

Seawater filtride from **station 29**. Degradation of alkanes in Seawater filtride incubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. n=2, hopane normalization.

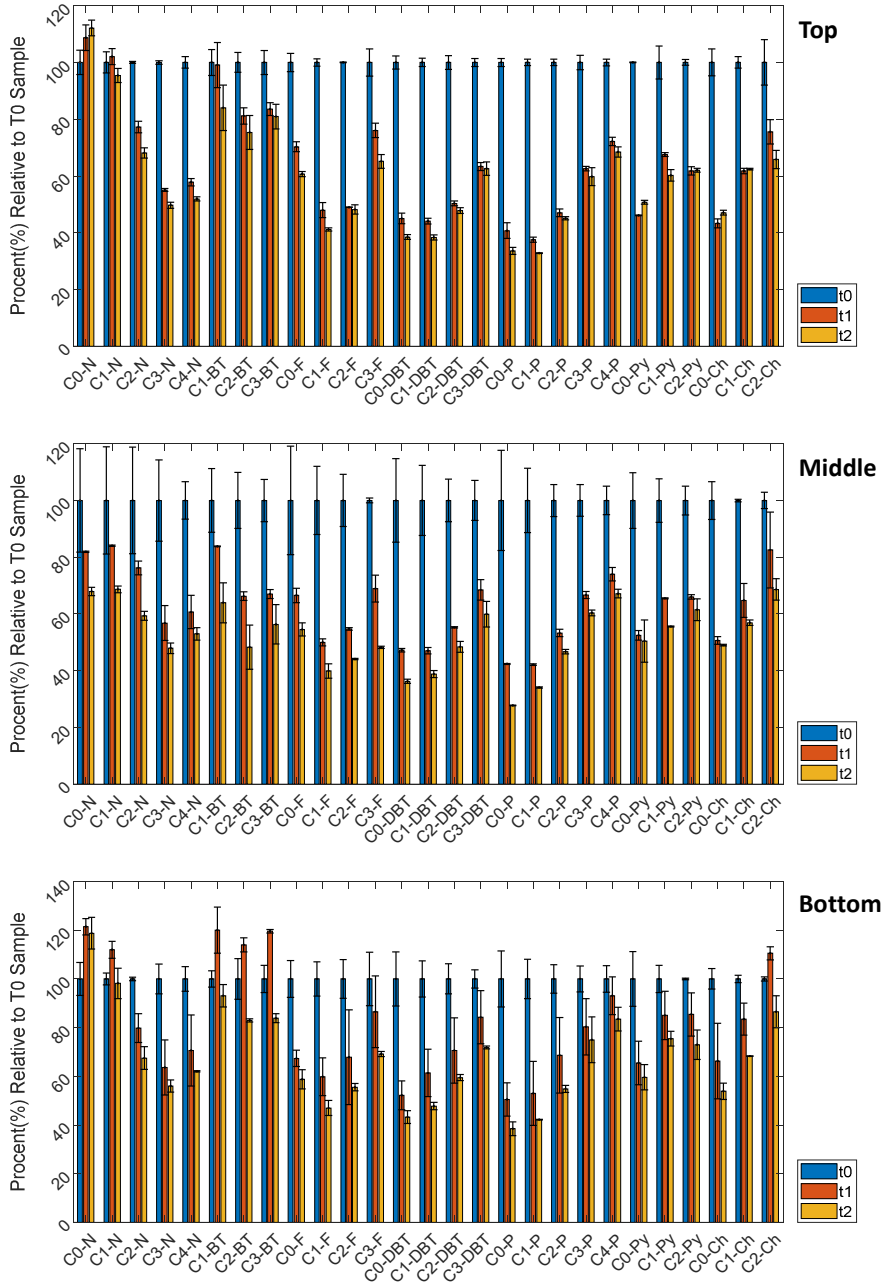


Seawater filtride from **station 41**. Degradation of alkanes in Seawater filtrideincubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. n-C11, n-C12, n-C13 etc. indicate the number of carbon atoms in specific n-alkanes. n=2, hopane normalization.

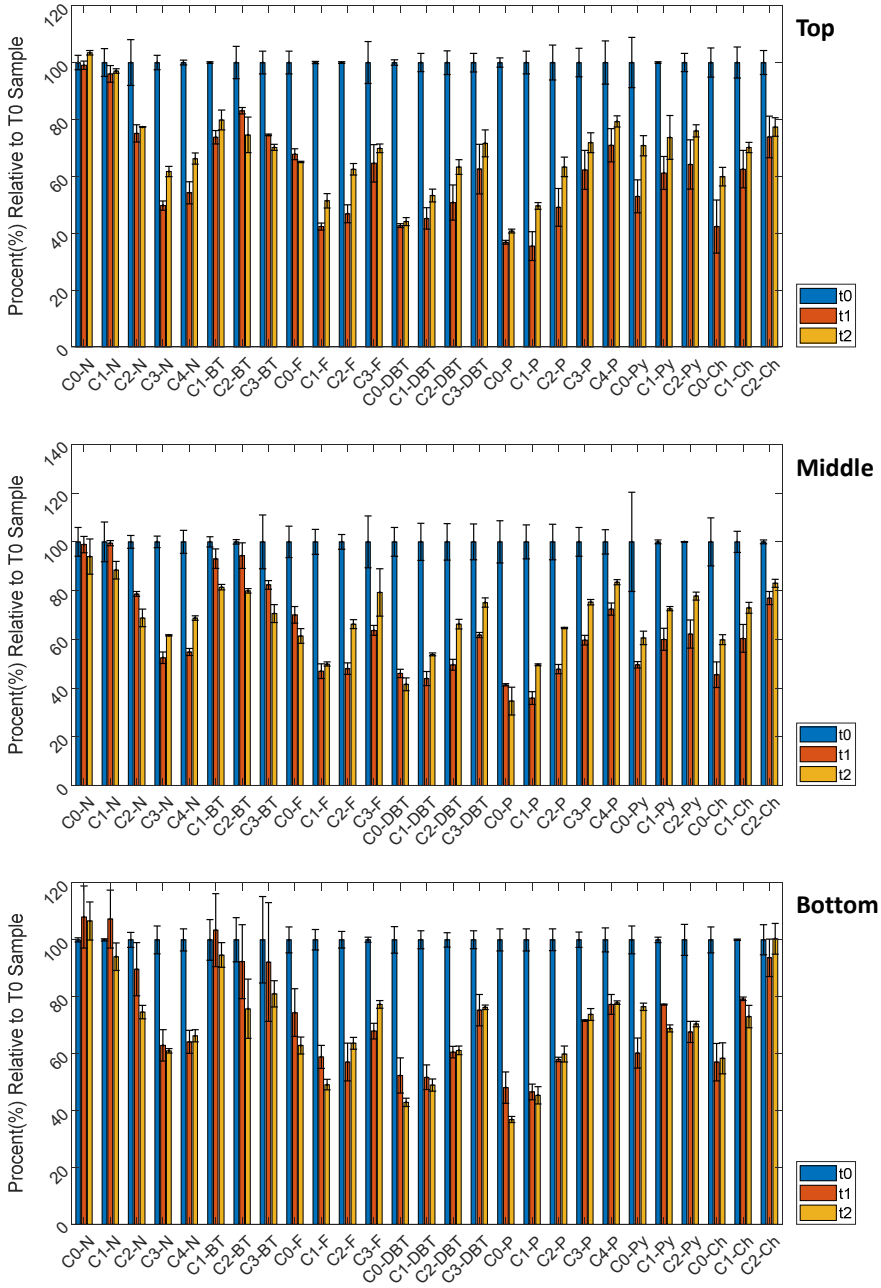


Appendix 8. Degradation of PAHs in filter microcosms

Seawater filtride from **station 29**. Degradation of oil PAHs in Seawater filtrideincubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. C0-, C1-, C2-, C3-, and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalization.

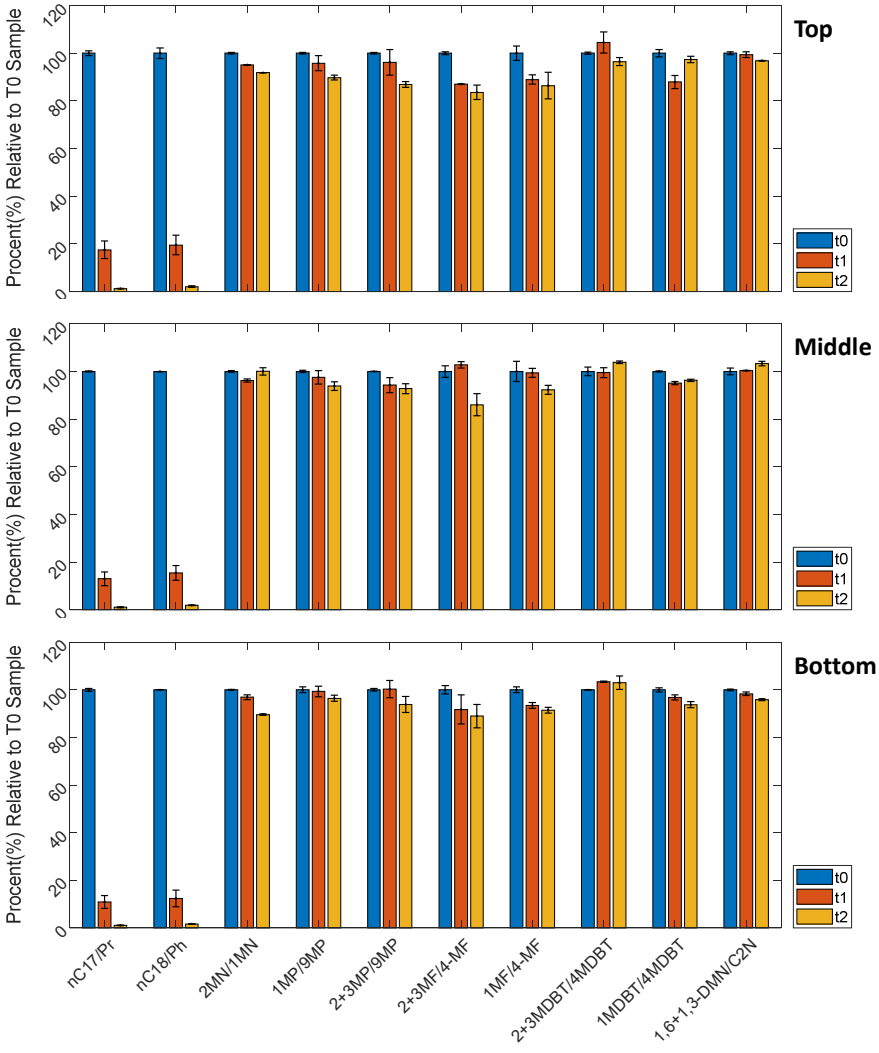


Seawater filtride from **station 41**. Degradation of oil PAHs in Seawater filtrideincubated with crude oil and Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. C0-, C1-, C2-, C3-, and C4- indicate the number of alkyl carbon atoms, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalization.

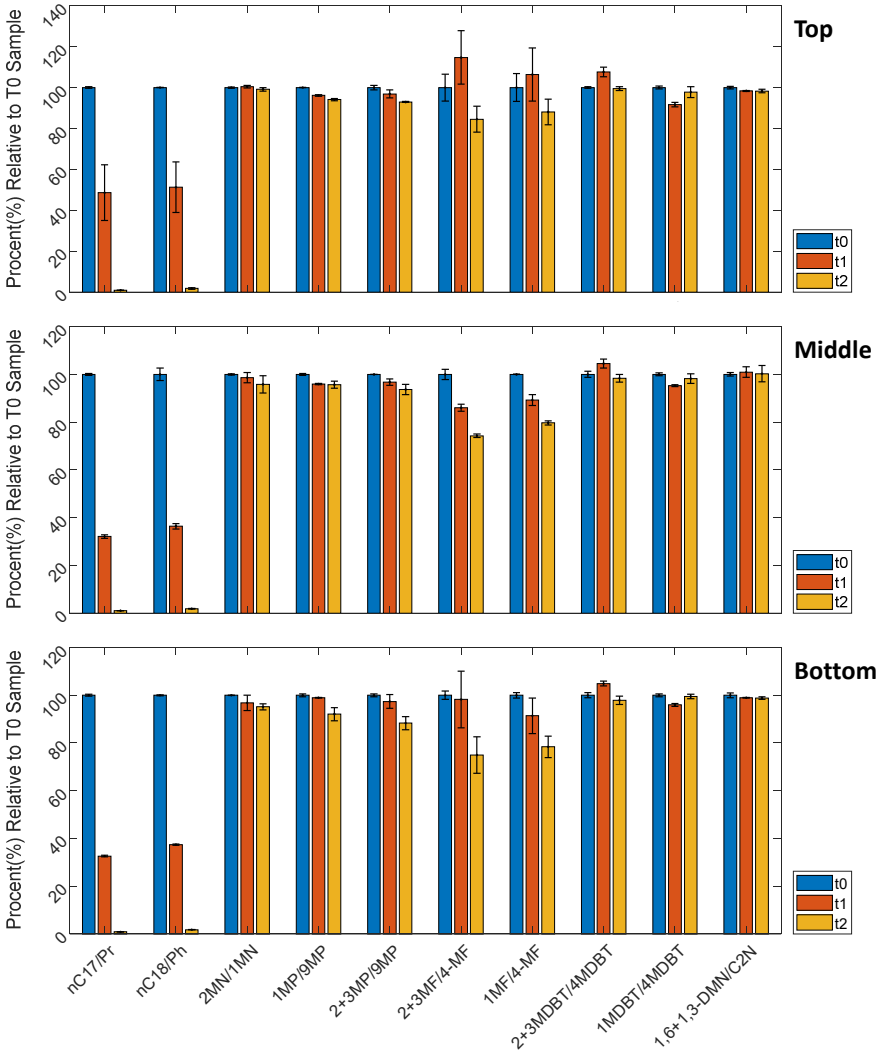


Appendix 9. Isomer ratios in filter microcosms

Seawater filtride from **station 29**. Diagnostic ratios for degradation of crude oil in the presence of Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. Pr: pristine, Ph: phytane, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalized.



Seawater filtride from **station 41**. Diagnostic ratios for degradation of crude oil in the presence of Bushnell Haas mineral nutrients. Top, Middle and Bottom refer to the origin of the filtered samples within the water column. Incubation times: t0: 0 days, t1: 35 days, t2: 84 days. Pr: pristine, Ph: phytane, N: naphthalenes; BT: benzothiophenes, F: fluorenes, P: phenanthrenes, Py: pyrenes, Ch: Chrysenes. n=2, hopane normalization.



Appendix 10. Dominant cultivable hydrocarbon degraders in sediment incubations (section 5.2)

Prokaryotes in MPN wells were identified by 16S rRNA gene sequencing. The content of up to four replicate wells from each microcosm sample was pooled. The MPN plates were from sediment incubated with crude oil and Bushnell-Haas mineral nutrients with either hexadecane (hex) or isobytylbenzene (iso) as carbon source. Dominant sequences (>10%) are indicated in yellow.

	St27 Hex	St27 Hex	St29 Hex	St29 Hex	St29 Hex	St36 Hex	St36 Hex	St36 Hex	St57 Hex	St57 Iso
Bacteria, Proteobacteria, Gammaproteobacteria -- -- --	100.0	54.2	49.5	29.5	45.4	30.2	42.0	41.5	91.1	48.1	14.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, PseudoalteromonadaceaePseudoalteromonas	0.0	0.0	0.0	8.0	2.4	1.9	47.8	30.1	3.2	24.3	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	0.0	0.4	14.0	18.7	9.6	4.7	4.1	8.5	1.1	8.6	6.7
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, Paraglaciecola,	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.9	57.4
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Marinobacteraceae, Marinobacter	0.0	0.0	0.0	0.4	0.6	39.7	0.6	1.1	0.0	0.0	12.2
Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae --	0.0	0.0	0.8	15.3	14.2	0.0	0.0	0.0	2.2	12.9	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Shewanellaceae, Shewanella	0.0	11.7	7.8	7.0	13.4	2.1	0.5	1.1	0.6	0.9	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Shewanellaceae, Shewanella, Shewanella frigidimarina	0.0	0.0	24.4	9.0	7.7	0.0	0.0	1.4	0.9	1.4	0.0
Bacteria, Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae --	0.0	0.0	3.4	9.3	4.5	10.2	4.8	0.3	0.1	0.0	0.0

Bacteria, Proteobacteria, Gammaproteobacteria, Oceanospirillales, Alcanivoracaceae, Alcanivorax	0.0	0.0	0.0	0.0	0.0	9.7	0.0	13.7	0.0	0.0	0.0
Bacteria, Actinobacteria, Actinobacteria, Corynebacteriales, Nocardiaceae, Rhodococcus, R.erythropolis	0.0	20.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae --	0.0	9.2	0.0	0.2	1.1	0.4	0.1	1.1	0.1	0.5	2.6

Appendix 11. Dominant cultivable hydrocarbon degraders in filtride incubations (section 5.4)

Prokaryotes in MPN wells were identified from 16S rRNA gene sequencing. The content of up to four replicate wells from each microcosm sample was pooled. The MPN plates were from sediment incubated with crude oil and Bushnell-Haas mineral nutrients with either hexadecane (hex) as carbon source. Sequences with ≥2,5% abundance are shown. Dominant sequences (>10%) are indicated in yellow.

	St29 top	St29 top	St29 middle	St29 middle	St29 bottom	St29 bottom	St41 top	St41 top	St41 middle	St41 middle	St41 bottom	St41 bottom	St57 top	St57 top	St57 middle	St57 middle	St57 bottom	St57 bottom
Bacteria, Proteobacteria, Gammaproteobacteria, (other), (other), (other), (other)	2.0	2.8	35.1	20.7	5.6	4.7	48.8	52.1	26.9	76.3	31.7	35.3	61.6	46.5	17.7	22.3	10.5	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas, (other)	36.8	25.2	36.1	39.3	41.9	36.9	20.2	11.2	18.5	5.3	4.4	2.8	2.0	25.3	48.0	59.1	19.0	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Shewanellaceae, Shewanella, Shewanella frigidimarina	37.1	28.1	0.5	15.9	7.9	3.4	0.0	10.0	18.1	6.8	11.6	7.5	1.8	0.4	4.0	2.9	11.5	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Oceanospirillales, Halomonadaceae, Halomonas, (other)	8.6	16.5	8.7	7.6	36.4	35.0	2.4	3.6	3.1	0.0	8.3	0.4	1.7	5.2	0.0	0.0	15.5	0.0
Bacteria, Proteobacteria, Alphaproteobacteria, Rhodospirillales, Thalassospiraceae, Thalassospira, uncultured bacterium	3.1	8.5	1.4	0.1	0.0	0.3	22.7	5.1	3.7	0.0	38.8	47.9	1.0	1.2	0.0	0.0	23.6	0.0
Bacteria, Bacteroidetes, Bacteroidia, Flavobacteriales, Flavobacteriaceae, Flavobacterium, (other)	0.0	0.0	5.7	0.3	0.0	0.0	0.1	0.0	9.8	7.3	0.0	0.0	4.2	2.7	26.5	0.3	0.0	0.0

Bacteria, Proteobacteria, Gammaproteobacteria, Oceanospirillales, Marinomonadaceae, Marinomonas, uncultured bacterium	0.0	8.1	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.8	1.0	0.0	16.0	0.0	10.3	1.7	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Shewanellaceae, Shewanella, (other)	0.5	0.4	0.0	7.0	5.0	4.8	0.0	5.2	13.3	2.9	1.4	1.1	0.4	0.0	1.9	1.8	5.3	0.0
Archaea, Euryarchaeota, Thermoplasmata, Marine Group II, uncultured archaeon, uncultured archaeon, uncultured archaeon	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.9
Archaea, Euryarchaeota, Thermoplasmata, Marine Group II, uncultured marine archaeon	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	27.5
Archaea, Euryarchaeota, Thermoplasmata, Marine Group II, uncultured marine group II/III euryarchaeote KM3_53_G07,	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.0
Archaea, Euryarchaeota, Thermoplasmata, Marine Group III, uncultured marine archaeon	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.6
Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Moraxellaceae, (other), (other)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	17.6	0.0	0.0	0.0	0.0	0.0
Bacteria, Actinobacteria, Actinobacteria, Corynebacteriales, Nocardiaceae, Rhodococcus, (other)	7.7	5.9	4.2	2.0	0.5	7.3	0.8	2.1	0.3	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.8	0.0
Bacteria, Proteobacteria, Gammaproteobacteria, Alteromonadales, Pseudoalteromonadaceae, Pseudoalteromonas, (other)	0.2	0.2	0.7	1.7	0.0	0.0	0.4	2.7	3.6	0.0	0.4	0.0	6.4	1.6	1.2	1.4	0.5	0.0

Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiaceae, (other), (other)	0.3	0.0	3.3	0.8	1.0	1.3	0.0	0.4	1.0	0.3	0.3	0.3	1.0	0.4	0.5	0.3	1.3	0.0
Bacteria, Actinobacteria, Actinobacteria, Corynebacteriales, Dietziaceae, Dietzia, (other)	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	5.0	0.0

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