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Bacterial Community Response in Deep Faroe-Shetland Channel Sediments Following Hydrocarbon Entrainment With and Without Dispersant Addition

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Deep sea oil exploration is increasing and presents environmental challenges for deep ocean ecosystems. Marine oil spills often result in contamination of sediments with oil; following the Deepwater Horizon (DwH) disaster up to 31% of the released oil entrained in the water column was deposited as oily residues on the seabed. Although the aftermath of DwH was studied intensely, lessons learned may not be directly transferable to other deep-sea hydrocarbon exploration areas, such as the Faroe-Shetland Channel (FSC) which comprises cold temperatures and a unique hydrodynamic regime. Here, transport of hydrocarbons into deep FSC sediments, subsequent responses in benthic microbial populations and effects of dispersant application on hydrocarbon fate and microbial communities were investigated. Sediments from 1,000 m in the FSC were incubated at 0°C for 71 days after addition of a 20-hydrocarbon component oil-sediment aggregate. Dispersant was added periodically from day 4. An additional set of cores using sterilized and homogenized sediment was analyzed to evaluate the effects of sediment matrix modification on hydrocarbon entrainment. Sediment layers were independently analyzed for hydrocarbon content by gas chromatography with flame ionization detection and modeled with linear mixed effects models. Oil was entrained over 4 cm deep into FSC sediments after 42 days and dispersant effectiveness on hydrocarbon removal from sediment to the water column decreased with time. Sterilizing and homogenizing sediment resulted in hydrocarbon transport over 4 cm into sediments after 7 days. Significant shifts in bacterial populations were observed (DGGE profiling) in response to hydrocarbon exposure after 42 days and below 2 cm deep. Dispersant application resulted in an accelerated and modified shift in bacterial communities. Bacterial 16S rRNA gene sequencing of oiled sediments revealed dominance of *Colwellia* and of *Fusibacter* when dispersant was applied over oiled sediments. The increased relative abundance

of anaerobic hydrocarbon degraders through time suggests creation of anoxic niches due to smothering. The study showed that hydrocarbons can entrain deep sediments to over 4 cm in a short time and that FSC indigenous bacteria are able to respond to a contamination event, even at a low temperature, reflecting the *in situ* conditions.

Keywords: oil spill, deep-sea sediment, hydrocarbon degradation, hydrocarbon entrainment, bacteria, dispersant, pollution, Faroe-Shetland Channel

INTRODUCTION

Oil and gas exploration in the deep sea is increasing as shallow and more accessible sources become depleted (Leffler et al., 2011; Ramirez-Llodra et al., 2011). The Faroe-Shetland Channel (FSC) is an area of deep-water hydrocarbon exploration (Smallwood and Kirk, 2005; Gallego et al., 2018) where hydrocarbon prospecting is occurring at depths of up to 1,500 m (Lagavulin well: 1,567 m depth, 62°39′N, 1°7′W). There are numerous concerns over environmental implications stemming from oil drilling activity, production and potential spillages/release to areas such as the FSC which require further investigation (Cordes et al., 2016).

The exposure of the marine environment to hydrocarbons can cause serious detriment to localized and wide-scale regional ecosystems (Mearns et al., 2010). Oil can be released to the water column directly, as witnessed following the *Deepwater Horizon* (DwH) well blowout in the Gulf of Mexico (GoM) in 2010 (Atlas and Hazen, 2011; Schrope, 2011; Montagna et al., 2013; Joye et al., 2016). More commonly oil is released to surface waters in shipping accidents such as the *Exxon Valdez* spill in 1989 (Atlas and Hazen, 2011) and *Prestige* spill in 2002 (Acosta-González et al., 2015). Following an oil spill in the water column or sea surface hydrocarbons are transported to the seabed, often in large quantities and across vast areas (Valentine et al., 2014). The quantity of oil transported to the seabed during the DwH was estimated to range between 1.8 and 31% of the water column-entrained oil (Lubchenco et al., 2010; Chanton et al., 2014; Valentine et al., 2014). There have been several oil-to-seabed transportation mechanisms proposed (Romero et al., 2015): (1) a combination of advective transport and oceanic currents carried the oil entrained plume into the continental slope leaving a "bathtub" ring of oily residue (Valentine et al., 2014); (2) the formation of oil-mineral aggregates and marine oil snow were deposited to the seafloor (Passow et al., 2012; Ziervogel et al., 2012); (3) a loss of buoyancy of oil droplets and oil-mineral aggregates in the water column resulted in deposition (Gong et al., 2014a) and (4) the ingestion of oil or oil-mineral aggregates by zooplankton and excretion as fecal pellets that settled on the seabed (Muschenheim and Lee, 2002).

The sedimentation pulses that took place following the DwH spill (Chanton et al., 2014; Valentine et al., 2014; Romero et al., 2015) led to the formation of flocculent oily material on the seabed which covered corals (White et al., 2012), reduced natural bioturbation (Brooks et al., 2015) and altered the microbial community (Yang et al., 2016). Although it was determined that the indigenous microflora and fauna were capable of responding to and degrading the influx of hydrocarbons (Kimes et al., 2014), major shifts within sediment microbial populations in the months following the spill had varying effects on geochemical cycling and redox conditions (Kimes et al., 2013; Scott et al., 2014; Hastings et al., 2016). Anaerobic conditions often prevailed in normally aerated sediments due to smothering by floc and rapid oxygen consumption (Yang et al., 2016), yet Mason et al. (2014a) and Kimes et al. (2013) both detected aerobic and anaerobic hydrocarbon degraders in sediments surrounding the Macondo well following DwH. The shift in redox state to anaerobic conditions meant that deposited hydrocarbons would undergo slower degradation compared to aerobic processes (Head et al., 2006; Widdel et al., 2010), leading to persistence of harmful and toxic oil components in the environment (Hylland, 2006; Marini and Frapiccini, 2013). Seabed-bound hydrocarbons are subjected to physical and biological processes which can translocate them (Konovalov et al., 2010; Zuijdgeest and Huettel, 2012). Solutes can be transported into sediments by diffusive and advective pore water fluxes (Huettel et al., 2014) and hydrocarbons may desorb from sediment, dissolve in the water column and be transported to remote locations (Zhao et al., 2015) where they may be degraded within the water column. The environmental conditions in the deep sea vary with location and a greater understanding of how hydrocarbons entrain and are removed from deep sea sediments is required to assess environmental risks in the event of an oil spill similar to DwH in a different location such as the FSC.

Deepwater hydrocarbon exploration in the FSC has been underway for over 20 years (Austin et al., 2014; Gallego et al., 2018) with fields such as Schiehallion (350–450 m) in 1993 and more recently the North Uist prospect (∼1300 m) in 2012. Increased exploration in this region at great depths presents a potential risk of oil spills in the FSC and an outcome analogous to the deep sea intrusion layers and sedimentation pulses observed following DwH is certainly possible. Direct application of lessons learned from DwH may, however, be misguided as the environmental conditions differ to those in the GoM (The Energy and Climate Change Committee, 2011). Temperatures at depths > 1000 m in the FSC are ∼0°C, thus colder than temperatures in the GoM (∼5°C), and are accompanied by an extreme hydrodynamic regime comprising complex multidirectional water masses (Berx et al., 2013). The transport processes of hydrocarbons in the FSC are likely to be more complex than those observed in the GoM and oil would inevitably be dispersed over a vast area (Main et al., 2016). The FSC is an important region hosting diverse benthic habitats and

parts of it have been designated as marine protected areas (e.g., the Faroe-Shetland Sponge Belt Nature Conservation Marine Protected Area; Joint Nature Conservation Committee, 2014). The FSC is known to have intense benthic ecological activity and bioturbation (Jones et al., 2007; Gontikaki et al., 2011), potentially enhancing hydrocarbon transport into sediments. Unlike GoM sediments, FSC microflora and bacterial communities have not been pre-exposed to hydrocarbons, a factor which contributed to the efficiency of microbial oil degradation following DwH (Hazen et al., 2010; Joye et al., 2014). Hydrocarbon degradation in the FSC may be slower compared to that in the GoM due to ∼0°C temperatures prevailing below 600 m (Ferguson et al., 2017). The FSC is of high environmental interest as a proxy for potential oil spills in deep arctic ecosystems since North Atlantic Deep Water formed in the Arctic flows southwards at depths over 600 m in the channel (Berx et al., 2013). In particular, ∼50% of North Atlantic Deep Water flows through the FSC. It follows that an oil spill in this location would entail the risk of contaminating the North Atlantic's deep water supply.

In response to the DwH blowout, 7 million liters of chemical dispersant (mainly Corexit 9500 and 9527A) were applied to both the surface slick and deep sea plume. Dispersant reduces the surface tension of the oil-water interface, transforming large globules of oil into smaller droplets enhancing solubilization and dissolution; this is proposed to enhance biodegradation rates as hydrocarbons become more bioavailable (Kleindienst et al., 2015b). However, there are concerns over the toxicity and degradability of dispersants (Scarlett et al., 2005) and their efficacy is inconclusive with studies reporting both suppression (Kleindienst et al., 2015b) and stimulation of microbial oil degradation (Baelum et al., 2012). The effect of dispersants on oil-sediment interactions is currently under investigation and contrasting effects on hydrocarbon sorption to marine sediments have been observed (Zhao et al., 2015). Oil dispersants selectively enhance sorption of aliphatic and aromatic hydrocarbons to marine sediments at different dispersant concentrations (Zhao et al., 2016). These effects varied with type of oil dispersant used, highlighting that an understanding of the effects of commercial dispersants on oil-sediment-microbe interactions requires further investigation.

The aims of this study were to evaluate the propensity of hydrocarbons to be transported into and out of FSC sediments following oil-sediment particle deposition and the subsequent bacterial community response over time. An additional aim was to determine the effects of a commercial oil dispersant (Superdispersant-25, SD25 hereafter) on these processes, and identify bacteria responding to oil in the presence or absence of SD25 treatments in deep sea sediments. To achieve this, the following hypotheses were developed: (1) post-depositional transport of hydrocarbons in surficial sediments at *in situ* temperatures triggers a stratified bacterial community response, (2) chemical dispersant increases hydrocarbon mobility and accessibility to microbial communities enhancing shifts to a hydrocarbon-degrading population and (3) sedimentary matrix modification results in enhanced mobility of hydrocarbons through surficial sediments. To our knowledge, this is the first study to evaluate hydrocarbon transport and subsequent bacterial community response in naturally stratified deep sea sediments of subarctic origin at *in situ* temperatures.

MATERIALS AND METHODS

Sediment and Seawater Collection, Transportation, and Preparation

Sediment and seawater samples were collected on May 2014 and May 2015 on-board *MRV Scotia* (cruise numbers: 0514S and 0515S, respectively) in the FSC from two stations (Supplementary Figure 1). Sediments from station A (1000 m deep; 61°35.02′N, 4°14.64′W), collected in 2015 cruise using a maxi-corer (OSIL, UK) fitted with acrylic cores (internal diameter = 10 cm, length = 60 cm), were used in "undisturbed sediments" experiments. Four sub-cores (acrylic, internal diameter = 3.6 cm, length = 30 cm) of ∼10 cm sediment depth were collected from each Maxi-corer core on-board. The sub-cores were stored fully submerged in seawater baths inside a temperature-controlled unit at 0°C that allowed water circulation and were individually aerated via air stones attached to an air pump to prevent anoxia. Following transport to the laboratory, the sub-cores were allowed to acclimatize for 10 days at 0°C until initiation of the experiments. Seawater was collected from station A using a rosette equipped with Niskin bottles during the 2015 cruise to be used in the undisturbed experiment reservoir system. Sediments were collected using a van Veen grab from station B at 180 m depth (69° 49.08′N, 5° 21.03′W) during the 2015 cruise and used in the production of oil-sediment pellets (see below).

A second set of sediments, collected using a van Veen grab during the 2014 cruise from station A, were sterilized and homogenized, and used in the transport experiment ("modified sediments," hereafter). Once transported to the lab modified sediments were mechanically homogenized and autoclaved at 120°C and 100 kPa for 21 min. Thereafter, sub-cores were made up to 10 cm with the modified sediment and filled up with UV-filtered seawater (0.5 μm filter) collected from the Ythan estuary. Sediment and seawater characterization was performed using methods described in (Supplementary Methods 1).

Model Oil and Artificial Oil-Sediment Pellets

A model oil was prepared with 20 hydrocarbons with a resulting density of 880 kg m^{-3}, similar to a medium crude oil (Ferguson et al., 2017). The model oil was composed of 64.9% aliphatic hydrocarbons, 30.0% combined monoaromatic and PAHs and 5.1% resin fractions. The hydrocarbons used to make the model oil, and their characteristics are listed in Supplementary Table 1. Model oil was filter-sterilized through a 0.22 μm PTFE filter (VWR).

Oil-sediment pellets (OSPs) were made using the 300–350 μm fraction of station B sediments after removal of organic carbon (450°C, 12 h). This ensured that the sediment used was homogenous in terms of organic matter and particle size. To make the 3-cm OSPs, 4 g of treated sediment were placed in tin foil and 1 ml of model oil and 1 ml of MilliQ water (18.2 MΩ cm, 25°C) were added. The OSPs were stored at −20°C

until use. To evaluate the exact amount of model oil components retained in individual pellets, three replicate OSPs were extracted by Soxhlet. On average, 0.41 ± 0.09 g (error = standard deviation, n = 3) of the oil was retained in the OSPs (Supplementary Table 1).

Incubation of Oiled Seawater-Sediment Systems

Undisturbed sediment sub-cores were removed from the water baths and lined around a rosette fitted with magnets. The cores were subject to three treatments; no oil (control), oil only (O-treatment) and oil and dispersant (OD-treatment). O- and OD-treatments were inoculated with artificial OSPs. All sub-cores were sealed with modified rubber stoppers on the upper end, leaving no headspace (Supplementary Figure 2). The modified stoppers were fitted with magnetic stirrers and two hollow steel pipes ∼8 cm above the sediment surface to enable water exchanges to take place throughout the experiment. The rosette rotated to move the magnetic stirrers inside the sub-cores and simulated advection of supernatant water (20 rpm). The system was kept in darkness and held at 0°C for the duration of the experiment. Supernatant water was periodically replaced to emulate replenishment of seawater in the water column and prevent anoxia in surficial sediments. Each sub-core was connected to a water reservoir (225 ml) and water was exchanged between sub-cores and reservoirs using a 520S peristaltic pump (Watson Marlow) at a rate of 25 ml min^{-1} for 20 min twice per week. SD25 (Oil Technics, UK) was added to OD-treatment reservoirs (33 µl, 1:30 SD25:oil ratio, based on manufacturer's recommendation) when water exchanges were performed to evaluate the effect of SD25 on transport and solubilization of hydrocarbons. Triplicate sub-cores were analyzed per time point, treatment and sediment type.

Hydrocarbon Extraction and Analysis

At specified time intervals (7, 21, 42, and 71 days) hydrocarbons were quantified from each of the 4 depth layers (0–1, 1–2, 2–4, and 4–10 cm). Approximately 10 g of sediment from each section was stored in glass vials with PTFE caps at −20°C prior to hydrocarbon extraction. Supernatant seawater was collected and stored in amber vials with PTFE caps and maintained at −20°C until extraction. Hydrocarbons were extracted from sediment by Soxhlet extraction for ∼40 cycles (5 h in total) using 100 ml dichloromethane. Hydrocarbons were liquid-liquid extracted from the total volume of seawater of each core (∼150 ml) with 3 × 10 ml dichloromethane and the resulting extractions combined. Extraction recovery rates for all components were performed in triplicate and can be found in Supplementary Table 1. Hydrocarbon extractions were analyzed by gas chromatography fitted with a flame ionization detector (GC-FID) using a previously described system and method (Ferguson et al., 2017). Calibration curves (6-point) were determined for each compound of the model oil. Laboratory control samples were analyzed to establish the effect of the sediment matrix and extraction procedure on the recovery of model oil compounds. Toluene was added as an internal standard to account for injection error (1 µl ml^{-1}). The limits of detection and quantification were defined as chromatographic signal to noise ratios of 3 and 10, respectively.

Bacterial Community Analysis

DNA Extractions

Total genomic DNA was extracted from 0.4 g sediment using the FastDNA™ SPIN Kit for Soil and FastPrep®-24 instrument (both MP Biomedicals, Cambridge, UK), according to manufacturer's instructions. Purified DNA was stored at −20°C until further analysis.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed on all sediment layers and shows differences in bacterial profile through depth. The conserved V3-V5 region of bacterial 16S rRNA gene was amplified using the primer pair 341F (with GC clamp) (Muyzer et al., 1993) and 907R (Muyzer and Smalla, 1998). Each PCR reaction contained 1 µl of target DNA extract (diluted to < 20 ng μl^{-1}), 2 µl PCR water, 45 µl Red Taq DNA Polymerase Master Mix with 1.5 mM $MgCl_2$ and 1 µl of each primer (10 µM). Amplification was carried out on a Techne thermal cycler (Techne, UK) using a step-down PCR programme as follows: initial denaturation at 95°C for 4 min, followed by 10 cycles of 94°C for 30 s, 62°C for 45 s, and 72°C for 1 min, followed by 25 cycles of 94°C for 30 s, 57°C for 40 s, and 72°C for 1 min, plus final extension for 10 min at 72°C. The PCR products were checked on a 1.2% (wt/v) agarose gel with gel electrophoresis for 40 min at 90 V. Gels were stained with GelRed™ and visualized on a UV transilluminator (UGenius 3, Syngene, UK).

DGGE using a TV-400 DGGE system (Scie-Plas, UK) was performed on the PCR amplified products. PCR product was loaded onto a 6% (wt/vol) polyacrylamide gel with a denaturant gradient of 30–70% (100% corresponds to 7 M urea and 40% vol/vol formamide). Electrophoresis was performed in 1 × TAE buffer at 60°C for 16 h at 100 V. The gel was stained with GelRed™ for 60 min and visualized on a UV transilluminator. All three incubation replicates for each time point were analyzed by DGGE to ensure reproducibility but a single replicate for each treatment was loaded on a single DGGE gel to evaluate shifts over time between all treatments. DGGE bands that potentially represented different strains were excised with a sterile scalpel and transferred to sterile 1.5 ml micro-centrifuge tubes containing 50 µl sterile molecular water. DNA was eluted from bands over 48 h at 4°C. Eluted DNA was re-amplified using the same primer pair (without GC clamp) and PCR programme. PCR products were then purified (E.Z.N.A cycle purification kit according to manufacturer's instructions; Omega) and quantified using a Genova Nano spectrophotometer (Jenway). Purified DNA was then diluted to a concentration of 6 ng μl^{-1} using sterile PCR water and sequenced by paired-end Sanger sequencing (Source Bioscience, Bellshill, Scotland). Sequences were quality checked using SeqTrace (Stucky, 2012) and run through the Basic Local Alignment Search Tool (BLASTn) for nucleotide closest match.

Next-Generation Sequencing

Paired-end (2 × 300 bp) Illumina MiSeq sequencing of the 16S rRNA gene V3–V4 variable region was performed on the upper sediment layer as previously described (Ferguson et al., 2017). Average read depth was 39,838 ± 2,206 (SEM) per sample for 39 samples, except for 3 samples (<10,000) which were omitted from downstream analysis. Bioinformatics analysis was carried out on the Maxwell High Performance Computing Cluster at the University of Aberdeen, using Mothur v 1.39.0. Chimera detection was performed using UCHIME (Edgar et al., 2011). OTU clustering was performed at 97% similarity and taxonomic assignment obtained with SILVA (Quast et al., 2012). The raw sequencing data is available in the European Nucleotide Archive (ENA) under the accession number PRJEB25813.

Statistical Analysis and Calculations

Statistical analysis was carried out using the package *nlme* (Pinheiro et al., 2017) within R environment (R Core Team, 2017). Preliminary data exploration was undertaken to establish the best approach to analyse the sediment profile data. To model the entrainment of hydrocarbons in the sediments, linear mixed effects models were used due to the correlation of hydrocarbon concentrations at each depth interval within individual cores. [Hydrocarbon] was used as the response variable ($x^{0.4}$-transformed to ensure normality of residuals), core identity was specified as a random effect and hydrocarbon identity (HCID) was nested within core identity to allow for variation in total [hydrocarbon] content between cores and variation in average [hydrocarbon] between hydrocarbons within cores. An autoregressive residual correlation term (AR1) was included in the model, such that the concentration of a hydrocarbon was allowed to depend on the concentration of the same hydrocarbon directly above it in each core. Treatment, HCID, depth and time (both as factors) were included as fixed effects in the model and all possible interactions between them. Model selection was performed by stepwise elimination of non-significant terms (from higher to lower order terms) using the likelihood ratio test and maximum likelihood estimation. Once a minimal adequate fixed effect model structure was determined, it was refitted using restricted maximum likelihood estimation (Zuur et al., 2009). Separate models were fitted for undisturbed and modified sediment incubations.

To evaluate the partitioning of hydrocarbons between sediment and water phases, a distribution coefficient was developed that describes the ratio of total hydrocarbon mass in supernatant water to total hydrocarbon mass in sediment. This is not a true partition coefficient as this typically refers to distribution between two immiscible liquids. It is a distribution coefficient due to the presence of dissolved hydrocarbons in interstitial water but in this work, the sediment "phase" encapsulates the hydrocarbons dissolved and dispersed in the interstitial water as well as those adsorbed to and absorbed into sediment particles, while the supernatant "phase" encapsulates the hydrocarbons dissolved and dispersed in the water column. The distribution coefficient was defined as:

$$K_{ws} = \log \frac{m_w}{m_s}$$

Where m_w is the total hydrocarbon mass in supernatant phase and m_s the total hydrocarbon mass in the sediment phase. Where no hydrocarbon was detected in either phase, the minimum detected concentration of that hydrocarbon in that phase was used instead of zero. K_{ws} values below and above zero indicate preferential partitioning to the sediment and the supernatant water phase, respectively. The effect of SD25 application and time was evaluated using a linear mixed effects model following the same procedure as for the sediment transport models above with the exception that no autoregressive correlation was implemented into the model because [hydrocarbon] in one core did not depend on [hydrocarbon] in other cores. Separate models were developed for undisturbed and modified sediment incubations. Underlying model assumptions were validated graphically for hydrocarbon transport and K_{ws} models (Supplementary Figures 3–6).

Effective diffusivities (D_{eff}) for model oil compounds were calculated following Thibodeau and Mackay (2011) assuming the sediments were porous and saturated. Briefly, D_{eff} is calculated as a ratio of aqueous diffusivity divided by physical and chemical resistance terms which take into account sediment properties and organic matter content.

Based on the analysis of DGGE images using Phoretix 1D analysis software (version 4.0; TotalLab Ltd), bacterial community analysis was represented by the relative band intensities within lanes as previously performed (McCaig et al., 2001). The statistical analysis of microbial communities was performed using the package *vegan* (Oksanen et al., 2017) in R. A distance matrix was generated using the Bray-Curtis method from the relative band intensity data (function vegdist) and the treatment effects on the community structure were visualized by non-parametric multi-dimensional scaling (nMDS) (function metaMDS). Hierarchical clustering (function hclust) was performed using the unweighted pair group method with arithmetic mean (UPGMA). Statistical differences between bacterial communities between treatments and times were analyzed using permutational multivariate analysis of variance (PerMANOVA) (function adonis; 999 restarts).

The operational taxonomic unit (OTU) table resulting from the Illumina sequencing analysis was imported into R with the package *phyloseq* (function import_biom) (Mcmurdie et al., 2013). Singletons from the whole database were removed and samples rarefied to the smallest sample read depth (function rarefy_even_depth). Plots representing the bacterial structure were visualized using the *ggplot2* package (Wickham and Chang, 2009). Alpha diversity analysis was performed in package *phyloseq* (function estimate_richness). To assess differences in alpha diversity over time and between treatments, analysis of variance was performed where diversity estimates was the response variable and the interaction between treatments and day were the explanatory variables after log transformation of the data. For beta diversity analysis, a distance matrix was produced (function vegdist) with the Jaccard index and community structure differences between samples were visualized by nMDS as described above. PerMANOVA was performed to detect significant differences in community composition. In order to identify the taxa

whose changes of abundance between treatments are more significant, differential abundance testing (function deseq) was carried out on non-subsampled data as recommended by package developers in package *DESeq2* (Love et al., 2014).

RESULTS

Sediment Properties and Hydrocarbon Effective Diffusivities

The sediment from station A was silt dominated (81–82%) and contained relatively low quantities of carbon (1.7–1.9% TOC and 0.9% TIC) (Supplementary Table 2). The BTEX components and naphthalene had the highest effective diffusivities ($D_{eff;} > 29$ cm^2 $year^{-1}$) (Supplementary Table 1). For aliphatics, D_{eff} decreased with increasing carbon chain length. Similarly, for PAHs, as the number of rings increased, D_{eff} decreased (Supplementary Table 1).

Transport Into Sediments

In undisturbed sediments, hydrocarbon entrainment varied with HCID over time (Supplementary Figures 7–9, Supplementary Table 4, Supplementary Data 1), interaction: HCID × Depth (factor) × Time (factor): $LR_{df=271} = 240.74$, $P = 0.0001$). SD25 application significantly affected hydrocarbon entrainment over time (**Figures 1**, **2**, Supplementary Figures 7–9, Supplementary Table 4, Supplementary Data 1, Treatment × Depth (factor) × Time (factor), $LR_{df=9} = 162.9$, $P < 0.0001$). Hydrocarbons were detected over 4 cm deep on day 42 for both treatments (**Figures 1**, **2**, Supplementary Figures 7–9, Supplementary Data 1, $[TPH]_{O-Treatment} = 73.1 \pm 96.0$ $\mu g\ g^{-1}$, $[TPH]_{OD-Treatment} = 124.0 \pm 161.9$ $\mu g\ g^{-1}$, error = standard deviation) but not in the OD-treatment on day 71 ($[TPH]_{O-Treatment} = 22.3 \pm 22.2$ $\mu g\ g^{-1}$, error = standard deviation).

In modified sediments, hydrocarbons from all fractions were detected over 4 cm depth in the sediment on day 7 (Supplementary Figures 10–13). As in undisturbed sediments, SD25 application significantly affected [hydrocarbon] by time and depth in modified sediments (Supplementary Figures 10–14, Supplementary Table 3, Supplementary Data 2, Interaction Treatment × Time (factor) × Depth (factor), $LR_{df=9} = 167.21$, $P < 0.0001$). However, during model simplification, [hydrocarbon] across hydrocarbons over time was found to be non-significant (Interaction HCID × Treatment × Time (factor): $LR_{df=57} = 74.31$, $P = 0.0615$). In contrast to undisturbed sediments, there was no evidence that hydrocarbon entrainment varied across hydrocarbons over time (Interaction HCID × Time (factor) × Depth (factor): $LR_{df=171} = 92.59$, $P = 1$) or for the effect of SD25 on hydrocarbon entrainment to vary with hydrocarbon (Interaction HCID × Treatment × Depth (factor): $LR_{df=57} = 39.73$, $P = 0.9603$). The two-way interactions of HCID with treatment and time were also found to be not significant in modified sediments (HCID × Treatment: $LR_{df=57} = 25.68$, $P = 0.1392$ and HCID × Time (factor): $LR_{df=57}$: 70.25, $P = 0.1117$).

Water-Sediment Distribution Coefficients (K_{ws})

In undisturbed sediments, there was no evidence of K_{ws} values changing over time ($LR = 4.06$, d.f.$_3$, $P = 0.2555$). However, SD25 application significantly affected K_{ws} values differently between hydrocarbons (**Figure 3**, Supplementary Table 5, Supplementary Data 3, interaction HCID × Treatment: $LR_{df=19} = 80.12$, $P < 0.0001$). SD25 application increased K_{ws} values of all hydrocarbons except BTEX components and naphthalene. In modified sediments, the effect of SD25 application varied with hydrocarbon and time (Supplementary Figure 15, Supplementary Table 6, Supplementary Data 4, Interaction HCID × Time × Treatment, $LR_{df=19} = 101.63$, $P = 0.0001$). For the O-treatment, K_{ws} increased in day 21 and decreased thereafter. For the OD-treatment, K_{ws} was lower than in the O-treatment for most components for day 7 but increased in day 21, remained higher than for the O-treatment in day 42 and decreased in day 71 (Supplementary Figure 15).

Depth Profile of Bacterial Community Structure in Hydrocarbon and Superdispersant-25 Treated Sediments

According to DGGE analysis the bacterial community structure in the upper layer (0–1 cm) of the controls remained relatively unchanged over time (Supplementary Figures 16A,B). Bacterial communities from layer 1 controls days 0–21 and day 0 O-treatment were grouped together by cluster analysis (Supplementary Figure 16C). Ordination analysis of DGGE patterns of the top layer revealed differences in bacterial community structure between C- and both O- and OD-treatments (Supplementary Figure 16B). The presence of oil (O- and OD-treatments) significantly changed community composition compared to controls (PerMANOVA; $R^2 = 0.46$, $P = 0.0009$). There were successional shifts in band patterns of O- and OD-treatments from day 7 to 71. The bacterial structure on day 42 in the OD-treatment was similar to O-treatment on day 71. However, this structure had changed in the parallel OD-treatment, indicating similar trajectories but at significantly different rates (PerMANOVA; $R^2 = 0.93$, $P = 0.0009$).

Bacterial community structure of the control in the upper layer was similar to both C- and O-treatments in the deeper layers (Supplementary Figure 17A). The composition in these treatments was dominated by the presence of a core group of organisms (hereinafter referred to as "core group"). The core group was consistently present regardless of time or treatment. However, within the top layer of O-treatment incubations where [hydrocarbon] was highest, bands decreased in density and abundance in days 21–71. Moreover, there was a shift in bacterial structure at 1–2 cm deep in the O-treatment at days 42–71 (when hydrocarbons had migrated into the sediment) that clustered together with 0–1 cm deep O-treatment days 21–71 (Supplementary Figures 17B,C). Below 2 cm, there was dominance of the core group with no noticeable shifts. There was a significant difference between bacterial communities as a function of depth across both treatments (PerMANOVA; $R^2 = 0.48$, $P = 0.0009$).

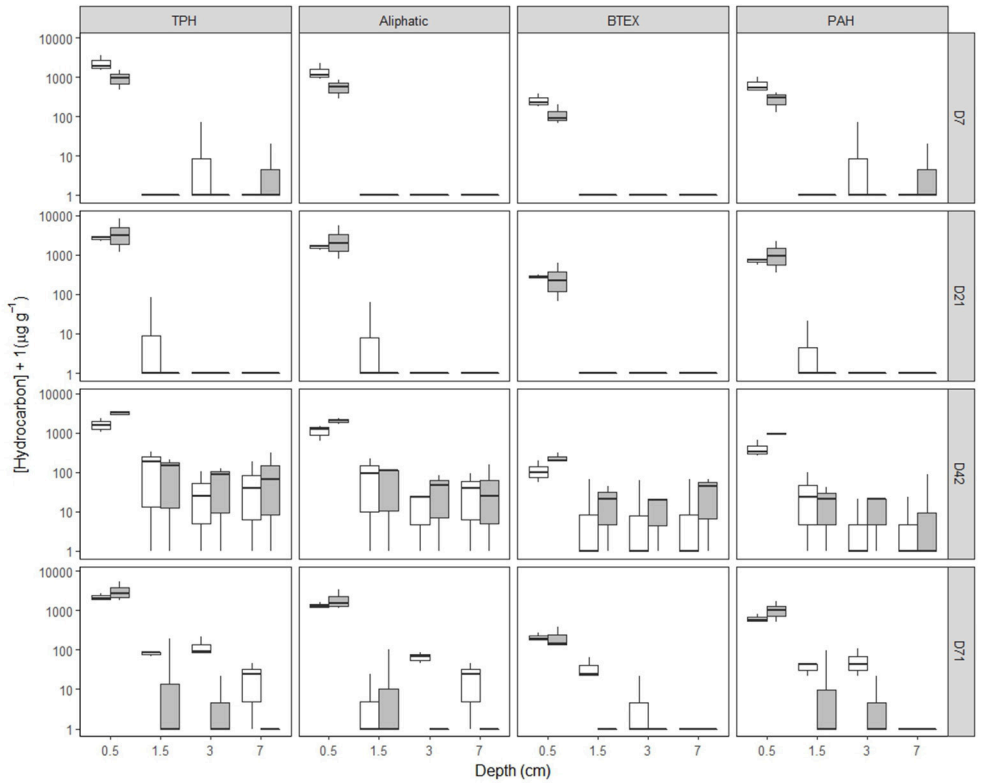

FIGURE 1 | [Hydrocarbon] of hydrocarbon fractions by time, depth and treatment in Faroe-Shetland Channel undisturbed sediments. From top to bottom, days 7, 21, 42, and 71 and left to right total petroleum hydrocarbons (TPH), aliphatic, BTEX and polyaromatic hydrocarbons (PAH) fractions. Box plots represent [hydrocarbon] for oil (white) and oil and dispersant (gray) treatments ($n = 3$). Depths shown are the average depth of the sections analysis (i.e. 0–1, 1–2, 2–4, and 4–10 cm correspond to 0.5, 1.5, 3, and 7 cm deep, respectively). Note [hydrocarbon] has been log(x+1) scaled.

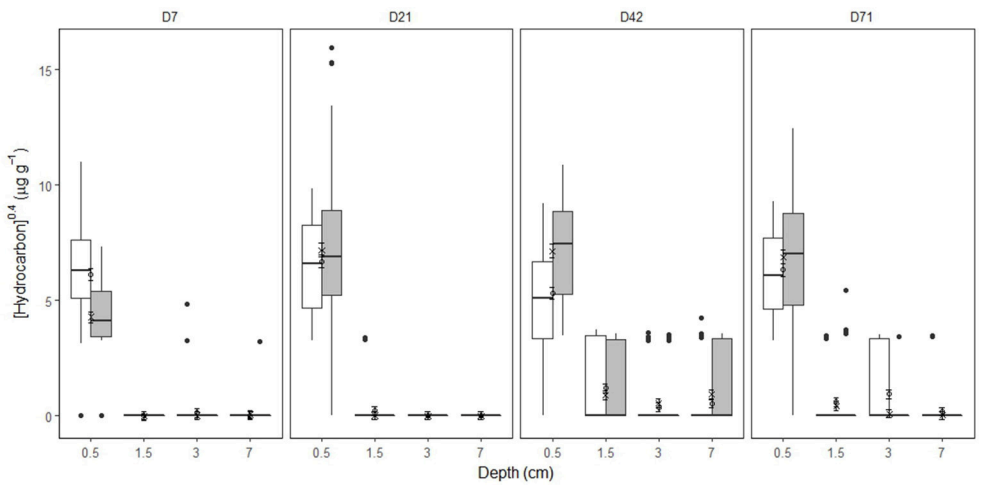

FIGURE 2 | [Hydrocarbon] by time, depth and treatment in Faroe-Shetland Channel undisturbed sediments. From left to right, days 7, 21, 42, and 71. Box plots represent [hydrocarbon] for oil (white) and oil and dispersant (gray) treatments ($n = 60$). Linear mixed effects predictions are shown for oil (circles) and oil and dispersant (crosses) treatments. Error bars represent standard error. Depths shown are the average depth of the sections analysis (i.e. 0–1, 1–2, 2–4, and 4–10 cm correspond to 0.5, 1.5, 3, and 7 cm deep, respectively). Note [hydrocarbon] has been $x^{0.4}$- transformed.

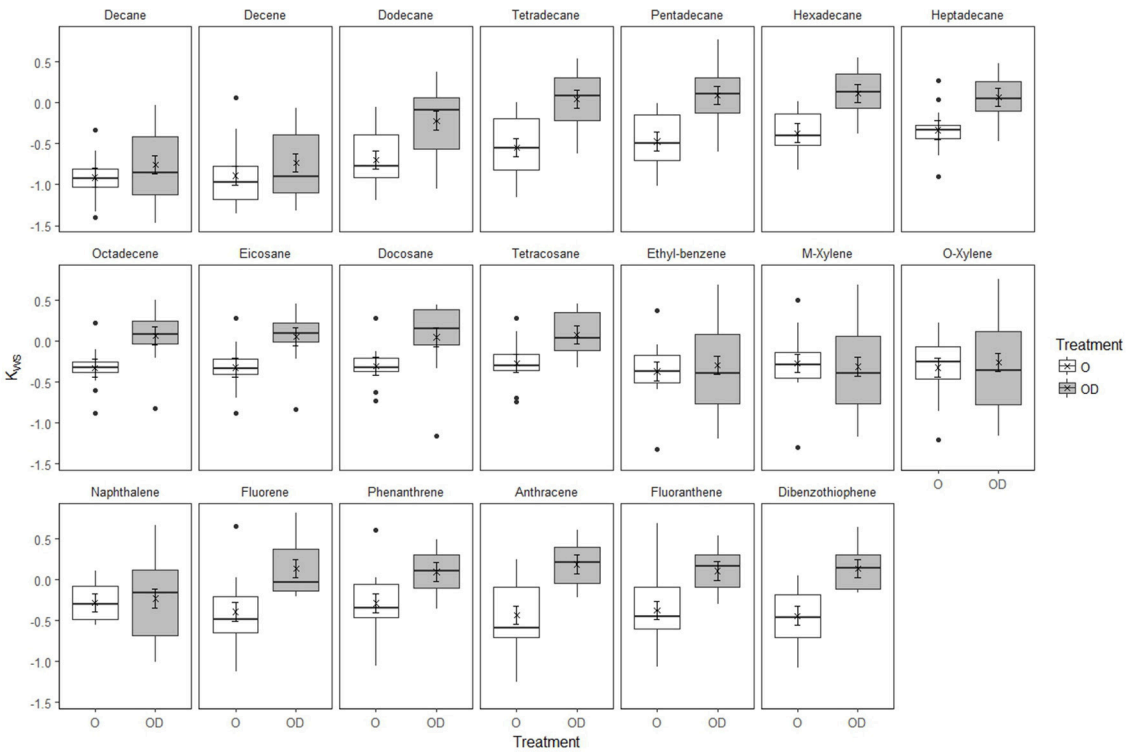

FIGURE 3 | Effect of Superdispersant-25 on K_{WS} by hydrocarbon in undisturbed Faroe-Shetland Channel sediments. Box plots represent the raw data for oil (white) and oil and dispersant (gray) treatments ($n = 12$). Linear mixed effects model predictions are shown for the oil (circles) and oil and dispersant (crosses) treatments. Error bars represent standard error.

Differences between C- and O-treatment communities were only identified within 0–1 cm deep (PerMANOVA; $R^2 = 0.59$, $P = 0.0009$).

To identify the bacterial members of communities DGGE bands were excised and sequenced. All bands were classified as members of Proteobacteria, except band A, which was part of the Firmicutes phylum (Supplementary Table 7). Bands classified as Proteobacteria were characterized as γ-Proteobacteria, except bands K and L which were categorized as δ-Proteobacteria. Bands B and C showed similar homology to *Colwellia rossensis* strain ANT9279 (100% identity) and *Colwellia psychrerythraea* strain 34H (99%), respectively. *Colwellia* related strains were most prominent in layer 1 in O-treatment incubations but also present in other treatments and depths at a lower density. Bands H and J showed high sequence homology to *Pseudoalteromonas* sp. K8 (99%) and *Pseudoalteromonas translucida* KMM 520 (99%), respectively. *Pseudoalteromonas* related strains appeared to be stimulated by oil exposure within 0–1 cm, particularly band J which was only present on day 71. The core group was mainly represented by bands D to I. Band D diminished in the presence of oil whereas band G was more prominent in the O-treatment; both matched to uncultured γ-Proteobacteria. Band F initially increased in density from days 0–21 but then decreased from days 42–71 showing potential succession to *Pseudoalteromonas* spp.

Bacterial Community Structure at the Sediment/Water Interface

Illumina sequencing of the top centimeter of sediment was performed to examine the O- and OD-treatments induced bacterial shifts in more detail. The relative abundance of taxa within the bacterial communities was consistent among replicates early in the study but became increasingly divergent over time (**Figures 4**, **5**). Bacterial communities from all treatments were dominated by Proteobacteria across all time points (46–66%) except for OD-treatment day 71 where composition was shared between Proteobacteria (41%) and Firmicutes (28%). Firmicutes was represented in the other two treatments but only with maximum contribution of 9% within the controls and 17% in the O-treatment. The phylum Parcubacteria represented <1% at all time points and treatments except O-treatment day 71 where it averaged 5%. The phylum Actinobacteria was found at consistent levels throughout all time points within controls. Within Proteobacteria, the majority of organisms were assigned to the orders Alteromonadales and Xanthomonadales.

The presence of oil selected for Alteromonadales, which averaged 14% in day 0 of both C- and O-treatments. By day 71 this reduced to 10% in controls but increased to 39% by day 71 in the O-treatment. In contrast, in the OD-treatment it peaked at 32% on day 21 but decreased to 8% by day 71

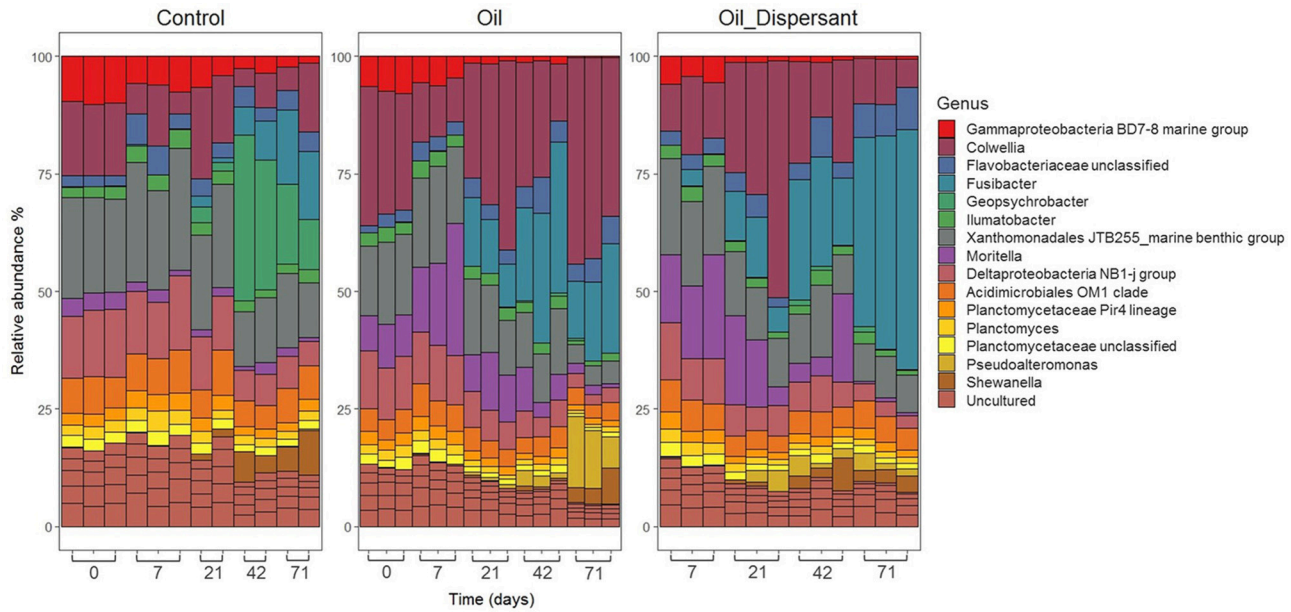

FIGURE 4 | Relative abundance plots visualizing bacterial community composition and dynamics within the top centimeter of Faroe-Shetland Channel sediment over time. Time in days is displayed left to right along the x axis. Treatment is grouped as control (left box), oil (middle) and oil and dispersant (right). Bars represent the top 20 OTUs for each sample grouped at the genus level (clustered at 97%).

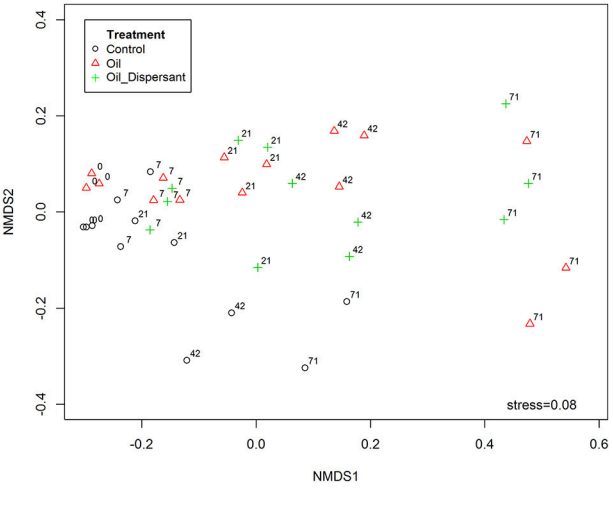

FIGURE 5 | Effect of treatment and time on bacterial community composition within the top centimeter of Faroe-Shetland Channel sediment, assessed using non-metric multidimensional scaling (nMDS), based on Jaccard index following square root transformation of sample data. Treatments are indicated as control (black circles), oil (red triangles) and oil and dispersant (green crosses). Numbers placed next to treatments indicate time points in days.

indicating succession to other organisms. At the genus level, *Colwellia* represented the most prominent OTU in both O- and OD-treatments on day 21 (**Figure 4**). It then decreased in the OD-treatment but increased to 27% in O-treatment. *Moritella* was present at 3% on day 0 in all treatments. However, by day 7 it had risen to 10% in both O and OD-treatments before decreasing at similar rates down to 1% by day 71. *Pseudoalteromonas*, *Pseudomonas* and *Oleispira* were present in oiled treatments at a lower abundance (1–10%). Oil selected for Candidatus Campbellbacteria, the predominant order within Parcubacteria, which was not present above 0.05% in any treatment other than O-treatment day 71 where it had increased to 6% in two of the three replicates. Application of dispersant resulted in increased abundance of the order Clostridiales, with relative abundance of 1% at day 7 before increasing to 29% at day 71 in the OD-treatment (Supplementary Figure 18). The most prominent member in the OD-treatment by day 71 was *Fusibacter* (27%), which was also present in O-treatment (11%).

Oil exposure had negative effects on Xanthomonadales which was consistently present in controls but decreased in relative abundance from 10% in both oiled treatments at day 7, to 5% by day 71 (Supplementary Figure 18). Genera which markedly increased in relative abundance in controls include *Geopsychrobacter* which was <0.5% at day 0 yet increased to 18% by day 42 and Desulfuromonadales which increased in relative abundance from day 42 onwards in controls. A group of taxa were seen to withstand oil contamination such as Acidimicrobiales OM1_clade and Planctomycetaceae Pir4_lineage and may represent the aforementioned core group (**Figure 4**).

Statistical Analysis of Illumina Sequenced Bacterial Communities

Ordination analysis revealed dissimilarity of oil treated (both O- and OD-) communities with controls (**Figure 5**). The effect

of treatment significantly explained variation in community structure (PerMANOVA; $R^2 = 0.16$, $P = 0.001$). There was clustering of day 71 incubations of O- and OD-treatments away from earlier time points. Furthermore, there appeared to be gradual community divergence over time within replicates across all treatments. The overall effect of time was significant in community composition (PerMANOVA; $R^2 = 0.38$, $P = 0.001$). The richness (observed OTUs) and diversity (Shannon index, Supplementary Figure 19) of the microbial communities across all treatments significantly decreased with time (ANOVA; $F = 196.053$, $P = 0.001$; $F = 60.586$, $P = 0.001$, respectively). When considering the interacting effects of treatment and time there were significant variations in community richness and diversity (ANOVA; $F = 3.776$, $P = 0.006$; $F = 5.124$, $P = 0.001$ respectively).

Differential abundance testing using *DESeq2* determined which taxa were significantly more abundant between treatments (**Figure 6**). *Pseudoaltermonas*, *Oleispira*, *Moritella*, Candidatus Campbellbacteria, *Pseudomonas*, *Colwellia* and *Fusibacter* were more significantly abundant (all adjusted $p < 0.001$) in O- and OD-treatments. Members of the genera *Geopsychrobacter* and *Desulfuromonas* were more significantly (adjusted $p < 0.001$) abundant in controls.

DISCUSSION

The experimental design was unique in the way that it was developed to replicate a scenario following an oil spill in the deep sea where oil residues would be deposited to the seabed. Natural sediment structure was carefully maintained within cores and kept under *in situ* temperatures, an important factor in understanding and comparing contaminated sediments as was recently stressed (Acosta-González and Marqués, 2016). The use of OSPs enabled retention of oil within the top centimeter of sediment and aimed at replicating heavy oiling of sediments. This likely resulted in the formation of anoxic micro-niches as witnessed at previous spill sites (Yang et al., 2016) and represents a realistic microcosm. SD25 use aimed to simulate continuous application of dispersant at the wellhead as may be performed in a future oil spill response. To replicate appropriate field conditions, whole-core incubations were run and subjected to seawater flushing to ensure an aerobic water column and sediment-water interface, and replenishment of natural seawater nutrients. Flushing would have resulted in mobilization of oil from the sediment and removal of oil from the system, effectively diluting the oil concentration within the water column over time as expected *in situ*.

Hydrocarbon Transport Into Sediments

Hydrocarbons from all fractions were detected below 4 cm deep from day 7 to day 71 suggesting that the hydrocarbons in the model oil have the capability of entraining undisturbed FSC sediments relatively rapidly (**Figures 1**, **2**, Supplementary Figures 7–9). Limited transport of C20–24 aliphatics and higher ring-number PAHs can be attributed to two key factors: (1) interactions with the minerals and organic matter in the upper centimeters of sediment (Stoffyn-Egli and Lee, 2002) and (2) continuous water replacement of supernatant water promoting the partition to the water column over intra-sediment transport. However, on day 71 C10 aliphatics were not detected below 2 cm in either treatment and C12+ aliphatics were not detected over 3 cm in the OD-treatment suggesting that these hydrocarbons may have been biodegraded in deep FSC sediments within 71 days. Metagenomic profiling of sub-surface sediments at a depth of 1.5–3 cm within 3 km of the well following the DwH spill revealed the dominance of known hydrocarbon degraders from the class δ-Proteobacteria (Kimes et al., 2013). The effect of SD25 was not found to be significant for individual hydrocarbons, it was significant for the Treatment × Depth × Time interaction (**Figures 1**, **2**, Supplementary Figures 7–9). Therefore, the influence of SD25 on post-depositional transport of hydrocarbons in this work was unclear. C22–24 aliphatics were not detected below 2 cm deep at any time point suggesting limited transport of longer chained aliphatics. This is in agreement with the corresponding calculated D_{eff}, which are the lowest of the model oil components (12.8–12.9 cm year^{-1}). As found for aliphatics, BTEX and PAHs were also more abundant in day 42 than in day 71 over 1 cm deep (Supplementary Figures 7–9) and may have been degraded during the experiment. There is evidence for local 1000 m deep sediment microbial communities degrading these hydrocarbons (Ferguson et al., 2017). This is further supported by findings of microbial shifts at 1–2 cm from day 42 (Supplementary Figure 17). The entrainment of hydrocarbons over 4 cm deep into FSC sediments is of significance because it will encourage the consumption of oxygen by aerobic microbes and deplete available oxygen in the sediment rendering it anoxic. This suggests that hydrocarbon biodegradation beyond this will be limited by oxygen availability and dominated by anaerobic microbial communities (Widdel et al., 2010). There was no detected shift in the microbial community structure below 2 cm deep suggesting slower rates of metabolism than in surficial sediments. The half-life of PAHs in the GoM has been shown to be twice as long in sediments over 1000 m deep than at 100–150 m deep (Tansel et al., 2011). Due to the cold deep water temperatures in the FSC, PAH residence times are expected to be higher than in the GoM. Discussion on hydrocarbon transport in modified sediments can be found in the Supplementary discussion.

In undisturbed sediments, the application of SD25 increased K_{ws} values of most hydrocarbons with the exception of BTEX and naphthalene, the most water-soluble components of the model oil, but the interaction was not significant over time (**Figure 3**, Supplementary 10–12). In contrast, the effect of SD25 application in modified sediments changed significantly over time and followed a similar trend for most hydrocarbons revealing a time lag in K_{ws} which was consistent across hydrocarbons (Supplementary Figure 15). K_{ws} values increased from day 7 to day 21 to equilibrate with the supernatant water phase in both treatments. In 42-day incubations, K_{ws} values decreased for the O- but not for the OD-treatment. The decrease in K_{ws} in the O-treatment can be explained by desorption hysteresis, whereby hydrocarbons adsorbed onto fine particles and organic matter can remain adsorbed despite experiencing conditions

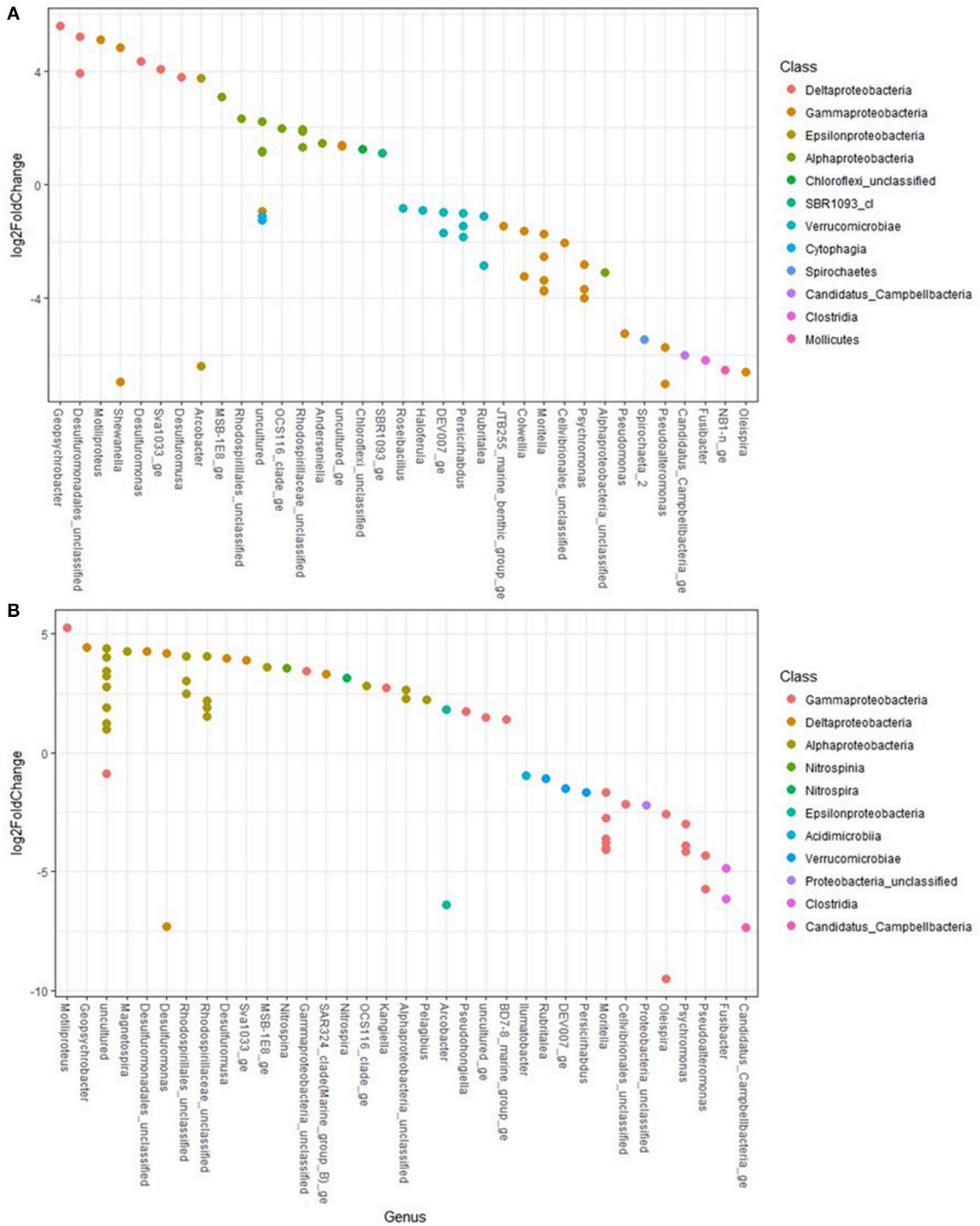

FIGURE 6 | Differential abundance testing of taxa between treatments using *DESeq2* found in **(A)** control (+log2FoldChange) and oil treatments (-log2FoldChange) and **(B)** control (+) and oil and dispersant treatments (–).

which would favor partition to the water column (Gong et al., 2014b). The sustained K_{ws} values in the OD-treatment suggest that SD25 application facilitates the partitioning of hydrocarbons to the water column and reduces the impact of hysteresis. This contradicts the findings of Zhao et al. (2015) where they show that PAH uptake increases with SD25 application. In their experimental setup the [hydrocarbon] used are much lower than those used here (0–6000 mg l^{-1} for naphthalene and 1-methylnaphthalene, 0–280 mg l^{-1} for pyrene) suggesting that this effect is only prevalent at low [hydrocarbon].

Bacterial Community Response Following Hydrocarbon Entrainment

Diverse microbial communities inhabit deep sea sediments and their structure and diversity is dependent upon environmental conditions such as depth and organic carbon content (Biddle et al., 2011). These microbes are believed to make up a "seed bank" of taxa which are niche-dependent (Gibbons et al., 2013). Certain bacterial taxa capable of utilizing hydrocarbons are present within the seed bank in very small numbers and bloom once provided with their preferred carbon source (Syutsubo et al., 2001; Head et al., 2006). Differential abundance testing identified certain taxa that were undetectable in controls but were able to respond to oil exposure. Shifts in relative abundance resulted in a modification to the richness, evenness and subsequently diversity of microbial communities. A significant reduction in diversity of oil contaminated samples compared to control samples over time in this study agrees with previous work (Hazen et al., 2010; Baelum et al., 2012; Dubinsky et al., 2013). Yet, the continued presence of a "core" group revealed by both molecular analyses suggests tolerance of selected sediment bacterial communities. This could be caused by the incubation design, whereby the environment/sediment provided a buffering effect, mitigating the impact of oil as opposed to intrusive incubation methods that more readily expose communities to toxic fractions of oil such as slurries and liquid incubations.

Following the sedimentation pulse during DwH, metagenomic analysis of surficial sediments revealed OTU dominance of uncultured γ-Proteobacteria and *Colwellia* spp. (Mason et al., 2014b). *Colwellia* was identified here as most responsive to hydrocarbon exposure in oiled treatments. *Colwellia* is believed to play an active role in hydrocarbon degradation throughout the oceans (Valentine et al., 2010; Redmond and Valentine, 2012; Mason et al., 2014a) and within sediments (Mason et al., 2014b). Strains identified in this study had similarity of 100% to *C. rossensis* strain ANT9279 isolated from Arctic sea ice (Brinkmeyer et al., 2003) and 99% to *C. psychrerythraea* isolated from Arctic sea sediment, confirming its ability to function in cold environments. Both of these strains were matched to clones from a library constructed from cold DwH plume waters and linked to short chain alkane degradation (Valentine et al., 2010) and PAH degradation in surface slick and plume samples (Gutierrez et al., 2013). Single cell genomic analysis of a *Colwellia* strain from the DwH hydrocarbon plume (matching 84% to *C. psychrerythraea* 34H) revealed the organism has genes for denitrification, adaptations to cold environments, nutrient acquisition and hydrocarbon degradation (Mason et al., 2014a). The presence of denitrification genes suggest anaerobic respiration capability, which would allow growth in areas of oxygen depletion as witnessed within the DwH plume (Joye et al., 2011; Kessler et al., 2011) and in anoxic sediments (Yang et al., 2016). It is plausible to find both aerobic and anaerobic organisms in the same location; Yang et al. (2016) identified both aerobic α-Proteobacteria and anaerobic δ-Proteobacteria within the same site surrounding the Macondo well. This is concurrent with evidence in this study where from days 21 in both O- and OD-treatments over 50% of dominant taxa were a combination of *Colwellia* and *Fusibacter* genera which are capable of anaerobic respiration. Yang et al. (2016) showed this may be the result of heterogeneous redox conditions forming within the sediment due to smothering by oil residues and the development of anoxic micro-niches. *Fusibacter* are generally halotolerant fermentative anaerobes capable of reducing a range of sulfur species and often isolated at mesophilic temperatures (Serrano et al., 2017). Several strains have been isolated from hydrocarbon related environments including the type strain *Fusibacter paucivorans* isolated from an oil-producing well (Ravot et al., 1999) and *Fusibacter bizertensis* isolated from a corroded kerosene storage tank (Smii et al., 2015). However, this genus is yet to be directly linked to PAH degradation (Kappell et al., 2014) and the ability of Firmicutes to form endospores may infer an ability to survive when other organisms cannot.

Numerous known hydrocarbon degraders were detected in this study including *Pseudoalteromonas*, *Oleispira*, *Pseudomonas* and *Moritella*. *Pseudoalteromonas* is regularly found in oil polluted environments such as surface oil slicks (Yang et al., 2014), the water column (Chakraborty et al., 2012; Gutierrez et al., 2013; Chronopoulou et al., 2014), deep sea sediments (Yang et al., 2016) and coastal ecosystems (Kostka et al., 2011; Kappell et al., 2014). *Pseudoalteromonas* is commonly linked to PAH degradation and can thrive once aliphatic resources are low or depleted (McKew et al., 2007; Dubinsky et al., 2013) and increased in relative abundance by day 71 in O-treated incubations. *Oleispira* was significantly more abundant in O-treatment incubations in this study and is active in cold environments (Yakimov et al., 2003; Kube et al., 2013; Gentile et al., 2016). *Pseudomonas* can degrade various structured hydrocarbons from cold environments (Bacosa et al., 2010; Viggor et al., 2013). Relative abundance data suggests *Pseudomonas* phylotypes were most prominent toward the end of the incubation period. However, in a simulation study of the DwH spill *Pseudomonas* were most abundant at 6 days of incubation with crude oil (Hu et al., 2017). *Moritella* is also capable of hydrocarbon degradation (Bagi et al., 2014) and was dominant by day 7 but then decreased in relative abundance over time. In addition, several *Moritella* species have been classified as piezophiles (Yanagibayashi et al., 1999; Xu, 2003) as has *Colwellia* (Oger and Jebbar, 2010) and these physiological traits reflect their sampling environment. Most interesting was the enrichment of Candidatus Campbellbacteria which was undetectable (<0.05% relative abundance) in controls but rose to 6% by day 71 in the O-treatment. Candidatus Campbellbacteria belongs to Parcubacteria and has been detected in marine sediments and anoxic environments including the Mariana Trench (León-Zayas et al., 2017). Although never cultured, it has been detected in hydrocarbon polluted environments (Salam et al., 2017).

Effects of Dispersant Application on Bacterial Community Structure

Commercial dispersants have long been used in oil spill response efforts and there are a wide variety of US EPA approved dispersants on the market (Kleindienst et al., 2015a) most notably Corexit 9500 and 9527, both used in the DwH clean-up strategy (Seidel et al., 2015). In the UK, one of the approved and most readily available dispersants is SD25 which was found to be less toxic than Corexit varieties (Scarlett et al., 2005). Therefore, it was

used in this study as an oil spill remediation treatment to assess the effect on hydrocarbon transport in sediments and bacterial community composition.

The application of SD25 had a significant effect on microbial community composition and selected for a different range of taxa, although there were similarities between O- and OD-treatments. *Fusibacter* was strongly selected for by SD25 application and this trend has never been witnessed before. The presence of an additional carbon-rich substrate may have allowed *Fusibacter* to thrive, outcompeting other taxa. Additionally, the increased solubilization of oil in seawater by SD25 may have allowed *Fusibacter* to utilize hydrocarbons faster than other taxa. Community shifts within the OD-treatment followed a similar pattern to the O-treatment; however, it appeared to occur faster. *Moritella* was initially selected for, followed by *Colwellia,* as was apparent in both O- and OD-treatments. Yet, in OD-treatment the decline of *Colwellia* occurred in day 42 and was succeeded by *Fusibacter*.

Dispersant has been reported to significantly increase hydrocarbon degradation and enrich the presence of hydrocarbon degrading bacteria (Baelum et al., 2012). SD25 was found to significantly enhance oil degradation rates by communities obtained from 1,000 m in the FSC however, there were no changes in community structure (Ferguson et al., 2017). Conversely, addition of dispersant has been shown to have limited, if any, impact on biodegradation (Foght and Westlake, 1982; Macías-Zamora et al., 2014) and in one case dispersant suppressed the activity of natural oil-degrading microorganisms (Kleindienst et al., 2015b). Thus, the notion that SD25 may have modified the bacterial response compared to oil only treatment may not necessarily mean that it enhanced biodegradation. Dispersants dissolve and disperse oil in water, leading to an increased oil droplet surface area and potential biodegradation stimulation (Kleindienst et al., 2015a). However, sediment particles present an increased surface area for oil adsorption and may reduce dispersant effectiveness (Macías-Zamora et al., 2014). It is often not possible to discern whether the microbial community shift is due to increased oil biodegradation or other influencing factors. Lindstrom and Braddock (2002) observed quicker mineralization of Corexit EC9500A than that of crude oil and dispersed oil implying that Corexit dispersant offered an alternative carbon source and was more selectively degraded. Whether this detracts from oil removal or positively "conditions" the microbial community for oil degradation remains to be answered.

CONCLUSION

This study assessed the transport of hydrocarbons and subsequent microbial response in FSC sediments following OSP deposition, in the presence and absence of SD25. This is the first study to replicate heavy oiling of naturally stratified deep sea sediments. Conditions were kept representative by maintaining *in situ* temperatures, replenishing seawater and continuously applying dispersant. A deep water oil spill in the FSC would likely have profound impacts on benthic ecosystems due to its unique oceanographic conditions (Bett, 2012). Biodegradation rates are reduced at low temperatures (Sharma and Schiewer, 2016; Ferguson et al., 2017). Additionally, low temperatures will enhance the extent of adsorption of hydrocarbons to sediments (Delle Site, 2001; Zhao et al., 2015). Adsorption of oil by sediment has been shown to promote biodegradation by capturing hydrocarbons (Yang et al., 2008). However, sequestration of hydrocarbons by sediments may also reduce their bioavailability in the long term through entrainment into organic matter and particle micropores that are inaccessible to microbes (Haritash and Kaushik, 2009).

The results of this study provide realistic data regarding hydrocarbon transport, mobilization and the ability of natural bacterial populations to respond and will aid in oil spill response decision-making in the UK. Key findings were:

(1) Deposited oil was transported to over 4 cm deep into FSC sediments following OSP deposition within the timeframe of the experiment, indicating that hydrocarbons can entrain deep into FSC sediments. Microbial shifts below 2 cm in the sediment were witnessed from 42 days onwards.
(2) The use of modified sediment matrix resulted in enhanced oil transport into FSC sediments with respect to undisturbed sediments. This highlights the importance of considering sediment handling and pre-treatment which may influence patterns of hydrocarbon transport.
(3) Deposition of hydrocarbons on surficial sediments forced bacterial communities to undergo significant shifts. The bacterial community response to oil exposure was related to members of the genera *Colwellia, Fusibacter* and *Pseudoalteromonas*. Microbial profiling demonstrated that shifts were primarily taking place within the top centimeter of sediment from day 7.
(4) SD25 application in undisturbed sediments increased the K_{ws} values of all hydrocarbons except C10 aliphatics, BTEX components and naphthalene indicating that SD25 selectively enhances the water-solubility of oil components. In modified sediments, SD25 application had a similar effect for all hydrocarbons.
(5) SD25 application resulted in a significant change in the bacterial community structure with strong dominance of *Fusibacter*. Shifts at parallel time points suggest an accelerated and modified response at the presence of dispersant.

The results of this study provide insight into hydrocarbon transport and subsequent microbial community shifts in FSC deep sediments following OSP deposition and the role of SD25 on these processes.

AUTHOR CONTRIBUTIONS

LJP, LDP, EG, UW, and JA: conceived the study; LJP, LDP, and AG: collected the samples; LJP and LDP: conducted the experiments; LJP, LDP, and TC: designed the statistical analysis for hydrocarbon transport and LJP, LDP, and CG-R designed the microbial community analyses. LJP and LDP: wrote the manuscript with input from all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00159/full#supplementary-material

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