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Characterisation of microbial communities of drill cuttings piles from offshore oil and gas installations

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ABSTRACT

Drill cuttings (DC) are produced during hydrocarbon drilling operations and are composed of subsurface rock coated with hydrocarbons and drilling fluids. Historic disposal of DC at sea has resulted in the formation of large piles on the seabed that may be left *in situ* following infrastructure decommissioning. This study provides a first insight into the microbial abundance, diversity and community structure of two DC piles from North Sea oil and gas installations. The abundance of both bacteria and archaea was lower in DC than in surrounding natural sediments. Microbial diversity and richness within DC were low but increased with distance from the piles. Microbial community structure was significantly different in DC piles compared to nearby natural sediments. DC bacterial communities were dominated by *Halomonas*, *Dietzia* and *Dethiobacter*. The presence of such organisms suggests a potential function of hydrocarbon degradation ability and may play an active role in DC pile remediation.

1. Introduction

Offshore hydrocarbon exploration and production requires drilling into marine subsurface rock. Drilling muds (DM, hereafter) enable this operation and are used to lubricate the drill bit and carry subsurface rock debris, also known as drill cuttings (DC, hereafter), back to the surface. DC have been historically disposed of at sea, forming piles at platform footings. DC piles are a heterogeneous mixture of subsurface rock, crude oil and a mixture of DM. Hydrocarbon concentrations in DC piles can be highly variable depending on the DM type used (oil-, synthetic- or water-based; further information on these can be found in Breuer et al., 2004) and have been detected at 1000 times the background level (Davies et al., 1984). Metal concentrations (*e.g.* Cr, Cu and Pb) in DC piles are also highly variable (0–100 s $\mu g\ g^{-1}$; Breuer et al., 2008). The Oslo-Paris commission (OSPAR) proposed complete removal of DC piles in the 2006/5 recommendation. This recommendation holds unless it can be determined that the maximum hydrocarbon leaching rate does not exceed the threshold of 10 t $year^{-1}$. If leaching is below the threshold rate and the pile is left *in situ*, contaminants are expected to degrade naturally due to the presence of hydrocarbon-degrading microorganisms. Therefore, a better understanding of the microbial composition, structure and function in DC piles is required to explore the intrinsic microbial degradation potential should DC piles remain *in situ*; a realistic option as previously proposed by decommissioning net environmental benefit analysis (NEBA) reports (BP, 2011; Shell UK Ltd., 2016).

Hydrocarbon biodegradation as a means of reducing oil pollution in the environment have been performed in a range of environments such as soils, beaches and marine sediments (Atlas, 1995; Head and Swannell, 1999; Leahy and Colwell, 1990; Prince, 2010). However North Sea DC sediment matrices have not been studied to the same extent (Gerrard et al., 1999). DC are expected to contain microorganisms indigenous to the subsurface and those tolerant to the toxic effects of high oil and DM chemical concentrations. Additionally, total microbial abundance and biodiversity are expected to be lower in chemical perturbed piles compared to less-perturbed sediments distant to piles (Acosta-Gonzalez and Marques, 2016). Therefore, information regarding degradation potential gathered from previous hydrocarbon degradation studies (as mentioned above) may not be transferable to DC piles. Within DC piles, oxygen availability is expected to decrease with depth due to limited oxygen diffusion and increased biological oxygen demand caused by the presence of carbon-rich substrates (*e.g.* hydrocarbons and certain DM components (Struchtemeyer et al., 2011)). Anoxia selects for obligate/facultative anaerobic microbial

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communities leading to community composition stratification in the DC pile (Gerrard et al., 1999). For example, aerobic organisms would dominate surface layers of the pile, whereas anaerobes such as sulphate reducers, denitrifiers and possibly methanogens, are expected to reside in deeper layers, as recorded in redox gradient-defined marine sediments (Acosta-Gonzalez and Marques, 2016). It was previously suggested that only surface-layer contaminants in DC piles would be degraded due to oxygen availability (Massie et al., 1985); however anaerobic hydrocarbon degradation has since been well documented (Widdel et al., 2010). Cold temperatures in offshore drilling environments such as the North Sea and deep-sea areas reduce microbial activity, as previously demonstrated for hydrocarbon metabolism (Coulon et al., 2007; Potts et al., 2018). The type of DM used has also been shown to influence microbial activity and composition, with the high aromatic content present in diesel-based DM being less likely to undergo biodegradation when compared to the less toxic kerosene-based DM (Sanders and Tibbetts, 1987). Similarly, growth of *Bacillus* species was depressed when exposed to a range of drilling fluid concentrations (25, 50, and 75 $\mu g\,ml^{-1}$), whereas Baroid mineral had no effect (Okpokwasili and Nnubia, 1995). These factors; anoxia, temperature and DM type all affect the rate at which biodegradation may occur.

Very few studies have characterised microbial communities within DC piles, primarily due to the logistic difficulty and expense of acquiring samples. A study analysing DC from the Beryl field, North Sea, identified several bacterial strains capable of mineralising hydrocarbons (Artz et al., 2002) but did not provide detailed information on the community composition. The aim of this work was to evaluate the microbial abundance, diversity and composition of two North Sea DC piles where oil- and synthetic-based DMs have been used. Comparisons to native seabed sites located up to 130 m from the centre of piles were undertaken to assess the effect of DC on natural seabed communities. It was hypothesised that total microbial diversity would increase with distance from DC piles due to lower concentration of hazardous materials. It was also predicted that DC piles would be dominated by hydrocarbon-degrading bacteria while submerged sediments beneath the DC pile would be dominated by anaerobic microbes (*e.g.* sulphate reducers and methanogens) due to limited oxygen availability caused by smothering. Finally, it was hypothesised that microbial community composition and abundance would be spatially variable (both between and within DC piles) due to extensive small-scale variability of hydrocarbon, metal concentrations and DM types.

2. Methods

2.1. Site and sample collection

DC piles of two platforms from the North Sea (Alpha; 400408E, 6507251N and East; 415072E, 6527347N, Supplementary Fig. 1), were investigated. The Alpha and East DC piles are located immediately below the platforms. The DC piles cover areas of approximately 12,700 and 6900 m^2, respectively, and deposited DC material volumes are estimated at 27,900 and 22,500 m^3, respectively (Marathon Oil, 2013). To evaluate the influence of DC accumulation under the platforms on seabed microbial communities, three types of sediment were collected: (1) seabed surface samples from around the platforms (controls, hereafter) by van Veen grab ($2 \times 0.1\ m^2$) from a vessel (April 2015), (2) shallow push-core samples were collected from the outskirts of the pile (transects, hereafter) by ROVs (two for Alpha and three for East) (April 2015), and (3) two replicate core samples from the centre of each pile (piles, hereafter) by deploying a piston sampler tool through the drill string on a wire line into the DC piles (May and June 2015). Core sections were cut at ~50 cm intervals. Samples were deep-frozen and stored at −20 °C until transportation to the laboratory where they were stored at −80 °C.

2.2. Sediment characterisation

For particle size distribution analysis, hydrogen peroxide (30% v/v) was added to sediment samples daily for one week and oven-dried at 60 °C thereafter. Sediment was then rinsed with distilled water to remove salt traces and sieved for determination of particles with diameter larger than 1 mm. For smaller particles, 1–2 g of sediment was analysed by laser diffraction using a Malvern Mastersizer 2000 (Malvern Panalytica, UK; detail on the laser diffractometer configuration can be found here, Marathon Oil, 2015a, 2015b).

2.3. Hydrocarbon and metal characterisation

Hydrocarbons in sediments were extracted as described by Marathon Oil (2015a, 2015b) by three sequential ultrasound extractions in a mixture of 50 ml methanol and 60 ml dichloromethane for 30 min. Extracts were then filtered (Whatman Glass microfiber filters, Grade GF/C) into a separating funnel where the dichloromethane layer was transferred to a round bottom flask. The ultrasound extraction process was repeated twice with 50 ml dichloromethane for 15 min. The combined extracts were evaporated to ~1 ml. Extracts were then cleaned in a silica gel column with 35 ml dichloromethane: pentane (1:2 v/v), with activated copper and evaporated to ~1 ml. Total petroleum hydrocarbons (TPH) were analysed by gas chromatography with flame ionisation detector (GC-FID; HP 6890 Series GC with a 7673 auto-injector) and a 100%-dimethylpolysiloxane bonded fused silica column (60 m, 0.25 μm film thickness, 0.32 mm internal diameter). Hydrogen was used as the carrier gas (3.5 $ml\,min^{-1}$) and an injection volume of 2 μl was performed on-column. The oven temperature was held at 80 °C for 2 min, ramped to 320 °C at 18 $°C\,min^{-1}$ and held at 320 °C for 13 min before a final ramped increase to 350 °C at 30 $°C\,min^{-1}$. The detector temperature was held at 350 °C. Polyaromatic hydrocarbon (2–6 ring PAH, PAH hereafter) analysis was carried out by gas chromatography with mass spectrometry (GC–MS; ThermoFinnigan Trace GC-DSQ mass selective detector with AS3000 auto-injector) and a (5% phenyl)-methylpolysiloxane bonded fused silica column (30 m, 0.25 μm film thickness 0.25 mm internal diameter). Helium was used as the carrier gas (0.7 $ml\,min^{-1}$) and an injection volume of 1 μl was performed in an injector (splitless, 280 °C, split flow 40 $ml\,min^{-1}$, vent time 1.5 min). The oven temperature was held at 60 °C for 0.5 min, ramped to 180 °C at 25 $°C\,min^{-1}$, then ramped to 330 °C at 6 $°C\,min^{-1}$ and finally held for 6 min. The detector temperature was 250 °C, electron energy was set to 70 eV and selected ion monitoring for 8 groups (6 per ion group).

Sediment samples for determination of arsenic and barium content were dried at 30 °C and mechanically milled. Thereafter samples were digested by hot reflux with nitric acid and analysed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500i) and inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 5300 DV), respectively. Details on the analysis of polychlorinated biphenyls, alkylphenol ethoxylates, organotonins, and other heavy metals can be found in the cuttings pile characterisation surveys (Marathon Oil, 2015a, 2015b).

2.4. DNA extraction and microbial 16S rRNA gene abundance

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. Eluted DNA was stored at −80 °C until further analysis. DNA was quantified using a spectrophotometer (NanoDrop ND-1000).

The abundance of total bacteria and archaea was estimated by quantitative PCR of 16S rRNA genes using primers 344f and 907r (Muyzer et al., 1993) and 344f and 915r (Raskin et al., 1994), respectively. Targeting the 16S rRNA gene provides a good representation of bacteria and archaea present in the system. Reactions were performed

in a 25 µl volume containing 12.5 µl of QuantiFast™ qPCR master mix (Qiagen), 2 µl of 10 µM of each primer, 9 µl of sterile PCR grade water and 2.5 µl of nucleic acid (~5 ng µl^{-1}). For bacteria, cycling conditions were 5 min denaturation at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 56 °C, and first plate read for 1 min at 72 °C followed by hold of 95 °C for 15 s and a melt curve from 60 °C to 95 °C. Bacterial standards consisted of a dilution series (10^1–10^9) of a known quantity of target gene isolated from a strain of *Halomonas neptunia*. Amplification efficiencies of > 99% were obtained, with R^2 > 0.99. Archaeal cycling conditions were 15 min at 95 °C, 30 cycles of 15 s at 94 °C, 45 s at 67 °C, and 8 s at 72 °C followed by a hold of 95 °C for 15 s and a melting curve from 60 to 95 °C. Archaeal standards consisted of a dilution series (10^1–10^9) of a known quantity of target gene isolated from a strain of *Nitrosotalea devanaterra*. Amplification efficiencies of > 98% were obtained, with R^2 > 0.98. Amplification was performed using an Eppendorf Mastercycler Realplex Real-Time PCR System (Hamburg, Germany). For all qPCR assays, melting curve analysis and agarose gel electrophoresis (1.2% w/v) of amplicons were checked at the end of each run.

2.5. Next Generation sequencing

DNA extracts were PCR amplified using the KAPA Hi-Fidelity enzyme (Roche Diagnostics Ltd. UK) across the universal bacterial and archaeal V4 region of the 16S rRNA gene using primers 515F (5′ GTG CCAGCMGCCGCGGTAA 3′) and 806R (5′ GGACTACHVGGGTWTCTAAT 3′) (Caporaso et al., 2012). PCR products were prepared for sequencing using Nextera DNA library preparation kit (Illumina, San Diego, USA) and paired-end (2 × 300 bp) amplicon sequencing were performed on the Illumina MiSeq platform (Centre for Genome Enabled Biology and Medicine, University of Aberdeen) using V3 Illumina chemistry.

Average sequence read depth per sample was 52,720 (± 4866 standard error of the mean; 40 samples). Three samples (QQ8, QQ12, E3) from within the cores had low read depth (< 3000) and were omitted from further analysis. Bioinformatics analysis was performed on the Maxwell high performance computing cluster at the University of Aberdeen, using Mothur v1.39.5 (Schloss et al., 2009). Chimera detection and removal was performed with VSEARCH (Rognes et al., 2016) and taxonomic assignment executed with the May 2013 release of GreenGenes (gg_13_5_99). OTU clustering was performed at 97% similarity. Bacterial and archaeal sequences were separated for taxonomic downstream analysis. The raw sequencing data is available in the European Nucleotide Archive (ENA) under the accession number PRJEB31062.

2.6. Statistical analysis

Abundance of bacterial and archaeal 16S rRNA genes (B16S and A16S, respectively, hereafter) were modelled using locally weighted regressions (loess) where either B16S or A16S was the response variable and depth was the explanatory variable. The models were performed separately for samples of each platform. The loess model fits a polynomial curve determined by either B16S or A16S values using local polynomial fitting (Cleveland et al., 1992). Both B16S and A16S were log-transformed for ease of visualisation. Partial Least Squares (PLS) regression was carried out to compare the presence of B16S and A16S (log-transformed) in surface sediments (depth = 0 cm, n = 17). Candidate response variables (25 in total, including pollutant concentrations, distances from platform, platform name, B16S and A16S) were tested for correlation using Pearson's correlation coefficient to detect collinearity. Variables were dropped until no significant correlation was detected (p < 0.05). Resulting response variables were platform (factor), distance (m), bearing (degrees), 16S rRNA gene copy number (either B16S or A16S depending on explanatory variable), TPH, PAH, arsenic and barium concentrations (all in µg kg^{-1}). Cross-validation was performed to reduce the number of components to two. Homogeneity of residuals was verified graphically. All statistical analysis was undertaken using the statistical software R (R Core Team, 2017) and the packages *corrplot* (for the correlation analysis and model simplification) (Wei and Simko, 2017), *plsdepot* (for the PLS regressions) (Sanchez, 2012) and *ggplot2* (for the locally weighted regression analysis) (Wickham and Chang, 2009).

All Illumina sequencing analysis was performed using the statistical software R. The package *phyloseq* (McMurdie and Holmes, 2013) was used to import the biom file produced with mothur. Alpha diversity measures were performed using plot_richness() in package *phyloseq* and analysis of variance between sample types and environmental variables calculated within R. Beta diversity metrics and nMDS plots were calculated using the package *vegan* (Oksanen et al., 2017) and function metaMDS() with the Bray-Curtis index (Bray and Curtis, 1957). Isolines were fitted to nMDS plots to illustrate correlation with distance using a generalised additive model with function ordisurf(). For multivariate testing of sample type effect on community composition permutation ANOVA was used with function adonis(). Canonical correspondence analysis (CCA) was performed with function cca() and significance testing with function anova(cca). R visual outputs were generated using package *ggplot2*. Biomarker analysis and identification was performed using Linear Discriminant Effect Size analysis (LEfSe) within the galaxy environment (available at https://huttenhower.sph.harvard.edu/galaxy/; Segata et al., 2011). Specifically, LEfSe identified taxa (OTUs) that were significantly differently abundant between sample site (piles and controls) using non-parametric factorial Kruskal-Wallis sum-rank tests, followed by Linear Discriminant Analysis (LDA) to determine the effect size. Significance was determined with a Kruskal-Wallis cutoff of 0.05 and LDA score of 4.0.

3. Results

3.1. Drill cuttings pile characterisation

Most sediment contaminant concentrations were variable with distance and between the two platforms although the general trend was that concentrations of contaminants decreased with distance from DC piles. The DC piles were analysed for a wide range of chemicals. In the interest of simplicity, only the contaminants that did not show collinearity and used in the analysis of microbial data are described here. Concentration of all compounds by platform and distance in surface sediments (depth = 0 cm) can be found in Supplementary Fig. 2. For more detail of each contaminant please refer to the pile characterisation surveys (Marathon Oil, 2015a, 2015b). TPH concentrations ranged from 3.70 × 10^4–1.35 × 10^5 µg g^{-1} within 25 m of the centre of platforms to 1.10 × 10^1–3.48 × 10^3 µg g^{-1} within 100–130 m (maximum sampling distance) from the platforms. PAH concentrations ranged from 30.0 to 74.3 µg g^{-1} within 25 m of the centre of platforms to 0.2–2.7 µg g^{-1} within 100–130 m from the platforms. Particle size was smallest nearer the piles. Silt percentage ranged from 14.8 to 45.2% within 25 m of the centre of platforms to 6.0 to 19.7% within 100–130 m from the platforms. Clay percentages ranged from 3.0 to 17.3% within 25 m of the centre of platforms to 1.5–5.9% within 100–130 m from the platforms.

3.2. Microbial abundance

There was a distinct pattern of B16S distribution in the East-DC pile, with the highest abundance recorded for both surface and deepest strata (Supplementary Fig. 3). In the Alpha-DC pile, B16S was highest at the surface too, but the trend with depth was variable and unclear. A16S patterns were highly variable with depth in both DC piles and A16S genes were below detection limits in some samples (see Supplementary Fig. 3). Control sites had the highest B16S and A16S abundance overall (> 10^8 and > 10^5 16S rRNA gene copies g

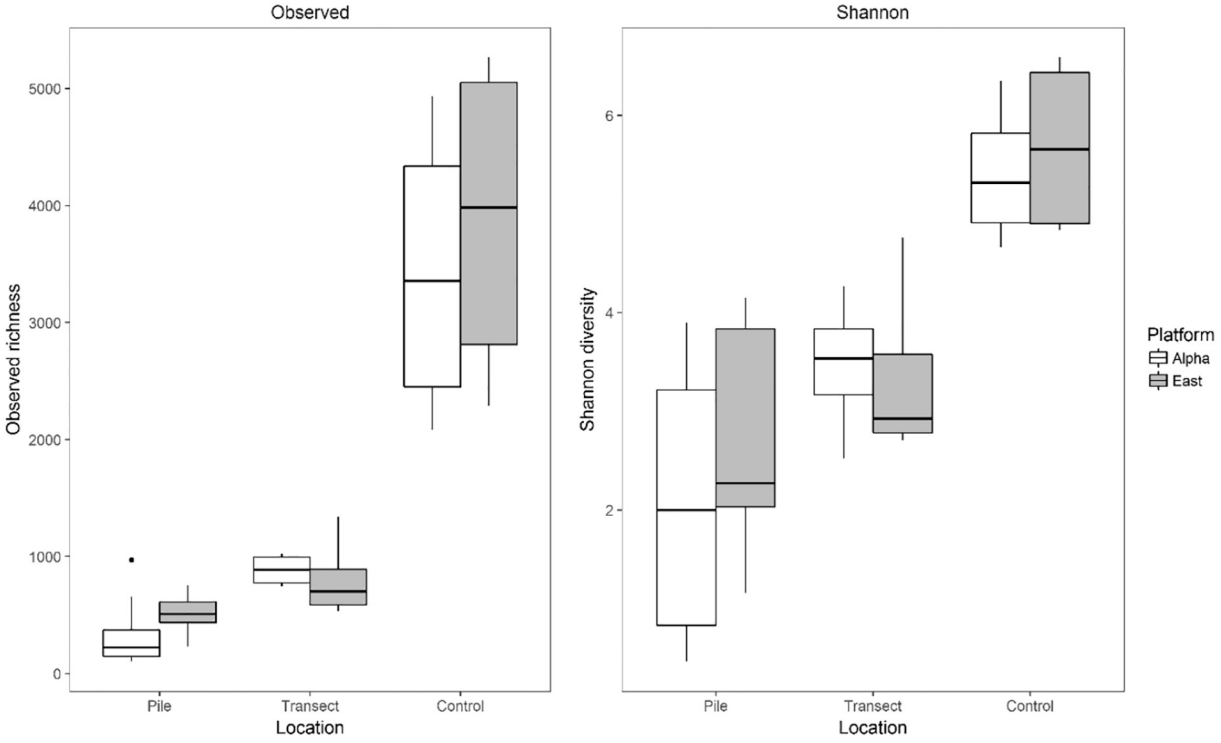

Fig. 1. Alpha diversity measures of observed richness (A) and Shannon diversity (B) of all samples and depths. Solid plot points indicate outliers.

sediment^{-1}, respectively).

In the B16S, PLS model axes 1 and 2 explained 55.7 and 7.8% of the variation, respectively. B16S was positively correlated with concentration of PAH, A16S, and TPH (Supplementary Fig. 4 A). Correlation with platforms and metal concentrations was weak in axis 1, which explained most of the variation. In the A16S PLS model, axes 1 and 2 explained 59.8 and 2.3% of the variation, respectively. A16S was positively correlated with distance and B16S (Supplementary Fig. 4 B). Unlike in the B16S PLS, PAH concentration did not affect A16S. The rest of the variables did not influence A16S either.

3.3. Microbial diversity

The number of OTUs (97% clustering) recorded at control sites was 3657 ± 466 (error = standard error of the mean, $n = 6$) compared to 410 ± 45 ($n = 25$) and 832 ± 77 ($n = 10$) at pile and transect sites, respectively (Fig. 1.A). Estimated microbial diversity (Shannon index) at control sites was significantly higher than at DC piles and transect sites (Fig. 1.B; ANOVA, $p < 0.01$). Furthermore, diversity significantly increased with increasing distance from the centre of the DC piles to distant control sites (Supplementary Fig. 5; ANOVA, $R^2 = 0.523$, $p < 0.01$). Alpha diversity also increased with decreasing concentrations of TPH and arsenic (along with other co-correlated heavy metals; data not shown).

Community composition (assessed by nMDS across all sites; Fig. 2) was significantly different across control, transect and pile samples (perMANOVA; $p < 0.01$). Community clustering was dependent upon TPH concentration (perMANOVA; $p < 0.05$); however, composition was not significantly dependent on other geochemical variables (perMANOVA; $p > 0.05$). A generalised additive model representing distance from the centre of piles was fitted to the nMDS to illustrate the effect of distance on community dissimilarity (Fig. 2). Control samples clustered together, away from pile and transects, indicating similar community composition which correlated with increasing distance away from piles. When community composition ordination was constrained by the effect of environmental variables (CCA) similar results

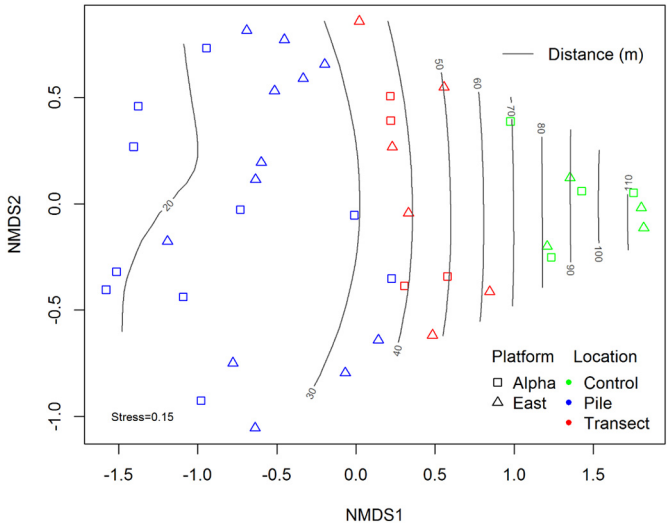

Fig. 2. Ordination analysis (nMDS) of control (green), transect (red) and DC pile core (blue) samples from both platforms (Alpha: squares and East: triangles), and effect of distance from the centre of piles on community composition using a generalised additive model.

were seen (data not shown). In addition, CCA was adopted to explore community composition patterns within DC mounds (pile and transect samples combined) and the influence of environmental variables (Supplementary Fig. 6). Distance and direction from the centre of DC piles significantly influenced community composition ($p = 0.001$ and 0.048, respectively). Furthermore, TPH and PAH concentration significantly affected community composition ($p = 0.019$ and 0.030, respectively), as did depth ($p = 0.001$).

3.4. Microbial community composition

Control sites were used as a proxy for natural communities in

sediments surrounding platforms, which allowed assessment of (1) the effects of smothering by DC on natural seabed communities and (2) the microbial community composition of DC compared to natural sediment. Control sediments were composed of Proteobacteria (α = 7%; β = 25%; δ = 17% and ε = 7%), Flavobacteria (10%) and Clostridia (6%). Due to the high richness and diversity of control sediments, no particular genus dominated. However, prominent members include taxa from the families Piscirickettsiaceae, Flavobacteriaceae and Desulfobacteraceae. *Pseudoalteromonas* was present in higher relative abundance in one site only (BRA7, 10%). Archaeal populations were dominated by Crenarchaeota, particularly Thorarchaeota (49%), Bathyarchaeota (19%) (recently proposed changes from MBGB and MCG, respectively; Adam et al., 2017) and Thaumarchaeota, specifically the genus *Nitrosopumilus* (12%). The exception to this was the dominance of order Methanobacteriales at one site (BRA6, 85%). Compared to control sites, natural sediments smothered by DC (deepest section of pile cores) consisted of Bacilli (18%), although γ-Proteobacteria dominated (50%). Archaea present in smothered sediments were similar to those detected at control sites.

Community composition of DC piles varied with depth. However, γ-Proteobacteria dominated most samples. In particular, *Halomonas* comprised > 50% of all genera within 8 separate sections of the four deep cores (Supplementary Fig. 7). Within the same class, *Marinobacter*, *Pseudomonas* and *Thiomicrospira* were frequently present in high relative abundance. Other notable taxa within cores included *Dietzia* (Actinobacteria), *Planomicrobium* (formerly *Planococcus*; Bacilli) and *Dethiobacter* (Clostridia). Transect samples were similar in composition to cores, with the additional presence of *Bacillus* and *Dethiosulfatibacter* of the classes Bacilli and Clostridia, respectively. Within Archaea, Thermoplasmata often prevailed. Members from the classes Methanomicrobia (genera *Methanocalculus* and *Methanosarcina*) and *Methanobacteria* (order Methanobacteriales), both Euryarchaeota, were also detected. Thorarchaeota and Bathyarchaeota were present in transect samples. Lefse analysis indicated that *Halomonas*, *Marinobacter*, *Dietzia*, *Bacillus* and *Pseudomonas* may represent potential biomarkers of DC (LDA score ($\log_{10}$) > 4; Fig. 3).

4. Discussion

4.1. Inherent geochemical variability of drill cuttings piles

The DC piles were characterised by high variability in both chemical composition and particle size distribution. This is likely a consequence of the piles' history of deposition of oil-based and synthetic-based DM as well as cuttings from various locations above and within the underlying oil reservoir. For example diesel-based DM have been found to contain up to 17% residual hydrocarbons (Sanders and Tibbetts, 1987), whereas synthetic DM have been found to contain less toxic compounds (Breuer et al., 2004). In-pile samples in this study evidently had higher concentrations of TPH and PAHs than surrounding control sites indicating chronic pollution. Similarly, particle size distribution analysis revealed higher proportion of silt and clay in DC piles and coarser sediments in surrounding control sites. This suggests that oxygen penetration into the piles may be reduced in comparison to the surrounding sediments, potentially extending the half-lives of the pollutants found within the pile.

4.2. Effect of drill cuttings contaminants on natural seabed sediment microbial communities

Sites that surrounded DC piles were used as controls to assess the effect of dispersed and transported DC on sediment microbial communities. Diversity indices and microbial abundance estimates at control sites were similar to those of other unperturbed marine sediments (Zinger et al., 2011) indicating relatively undisturbed microbial communities. Overall, these sites were all similar in composition and populated by common marine microbial taxa such as γ- and δ-Proteobacteria, Flavobacteria, Thorarchaeota, Bathyarchaeota and Thaumarchaeota (Kubo et al., 2012; Pester et al., 2011; Zinger et al., 2011). However, there were variations in relative abundance of dominant taxa indicating a degree of deterministic variable selection processes (Dini-Andreote et al., 2015). For example, bacteria of the Helicobacteraceae family were most dominant at the control site with the highest TPH concentration (BRA6; 73,700 $\mu g\, g^{-1}$) and have previously been detected in oil contaminated coastal sediments (Korlević et al., 2015; Quero et al., 2015). The increased relative abundance of δ-proteobacteria at sites BRA6 and 7 (highest TPH concentrations) suggests anaerobic sulphate-reduction, potentially indicative of smothering and agrees with previous findings (Nguyen et al., 2018). Overall, these changes were small-scale compared to the general composition of control sites.

Sediments sampled from beneath the DC piles, within natural seabed, showed the lowest TPH concentrations throughout the pile core profiles (< 100 $\mu g\, g^{-1}$). In some of these samples, community composition was relatively even. For example, in pile Alpha, at a core depth of 8 m (natural sediment) the TPH concentration was 31 $\mu g\, g^{-1}$ and comprised several taxa, including *Psychrobacter*, which are ubiquitous in cold marine environments (Brinkmeyer et al., 2003) and numerous strains have been isolated from Antarctic environments (Bozal et al., 2003). Indeed, this genus has also been detected in hydrocarbon-contaminated waters in a laboratory setup (Deppe et al., 2005; Prabagaran et al., 2007). Therefore, it may have utilised hydrocarbons that leached from the pile into the subsurface, a process recently evidenced in mesocosms evaluating hydrocarbon transport processes (Perez Calderon et al., 2018). Known hydrocarbon-degraders were present in other subsurface samples too, particularly *Halomonas* and *Dietzia*, which were present in pile East at 6.8 m deep, despite low TPH concentrations, again suggesting leaching of hydrocarbon from the piles.

4.3. Microbial community composition of DC piles

To date, knowledge of DC microbial composition is extremely limited. Several studies have reported effects of DC and DM on surrounding seabed microbial composition and function (Dow et al., 1990; Nguyen et al., 2018; Sanders and Tibbetts, 1987), but there is no systematic characterisation of DC piles through depth attained by coring. Here, a first insight into the microbial communities of DC piles is provided.

High-throughput sequencing of DC piles revealed the prevalence of both aerobic and anaerobic bacteria. Oxygen penetration within DC piles is believed to be limited (Bakke et al., 2013) and it has been hypothesised that sulphate-reducing microbes dominate microbial communities in DC (Gerrard et al., 1999). All 4 sections of the deep core analysed from the DC pile at platform Alpha were dominated by *Halomonas*. The presence of *Halomonas* in hydrocarbon-contaminated environments has been consistently documented (Cai et al., 2014; Chronopoulou et al., 2015; Curtis et al., 2018; Ferguson et al., 2017; Hassanshahian et al., 2012). Members of this genus are renowned for their ability to tolerate hypersaline conditions; a strain capable of utilising crude oil as a carbon source was isolated from production water (Mnif et al., 2009). Despite being commonly cultured in aerobic conditions, certain strains of *Halomonas* are also capable of anaerobic growth using nitrate as an electron acceptor (Wang et al., 2007). Recent bacterial profiling of a subsurface oil reservoir core revealed dominance of an OTU related to facultative anaerobic *Halomonas* spp. (28% of all OTUs; Gales et al., 2016). The versatility of *Halomonas* has been evidenced by studies describing its ability to degrade a range of saturated and aromatic hydrocarbons (Corti Monzón et al., 2018; Mnif et al., 2011), and has been found to harbour genes which are functional in arsenic resistance (Gasperotti et al., 2015). Therefore, *Halomonas*-related strains may be functional hydrocarbon-degraders within cutting piles. Recently a bacterial consortium enriched with *Halomonas* degraded TPH by 40% over 3 months in a lab-based DC bioremediation

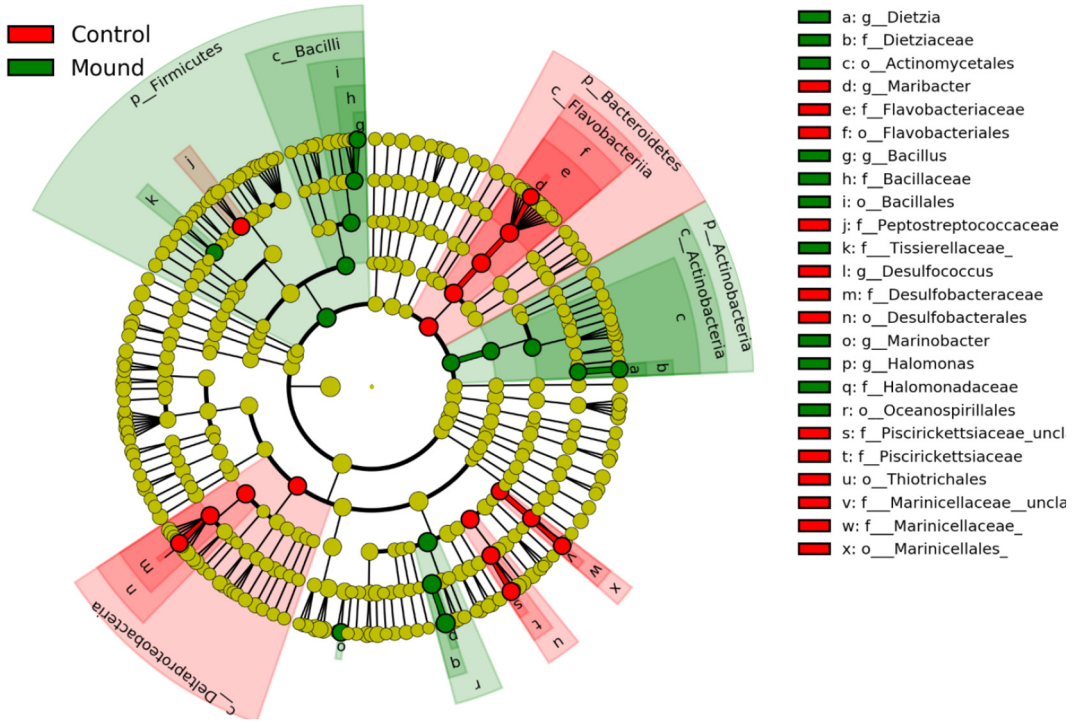

Fig. 3. LEfSe analysis of all samples and identification of biomarkers within the DC mound (pile cores and transects) and controls. Cladogram (left) indicates levels of taxonomic classification from kingdom (inner yellow circles) through phylum, class, order, family, and genus to species (outermost circles). Taxa found to be significantly more abundant in control samples are indicated in red, and in DC mound samples in green. Table on the right indicates the letter shown in the cladogram to the matching taxa. For example, within the phylum and class of Actinobacteria (p_ Actinobacteria) and letters describing: c, order of Actinomycetales; b, family of Dietziaceae; a, genus Dietzia, was found to be significantly more abundant in mound (green) samples than control (red).

survey (Rezaei Somee et al., 2018). Biomarker analysis by LEfSe identified *Halomonas* to be significantly more abundant in DC piles than control sites (Fig. 3). Within the Alpha pile, an increase in relative abundance of *Halomonas* was, in some cases, associated with decreasing bacterial abundance (Supplementary Fig. 7). This may indicate selection of *Halomonas* by the conditions presented in DC piles, or that it is simply more tolerant to high hydrocarbon and DM chemicals concentrations. Further research into the activity and function of *Halomonas* spp., including degradation of DC-associated hydrocarbons and use of genus-specific primers to quantify its abundance, will elucidate its role in natural attenuation processes.

Hydrocarbon contamination of North Sea sediments typically results in the proliferation of a predictable group of bacteria (*e.g. Alcanivorax*, *Cycloclasticus*, *Oleispira etc.*; see Head et al., 2006 and Yakimov et al., 2007 for reviews), which were not detected in this study. Instead, a diverse range of alternative organisms were detected in hydrocarbon contaminated DC. The consistent presence of *Dietzia*, a hydrocarbon-degrader and biosurfactant producer (Wang et al., 2014), was determined to be significantly more abundant in piles than control sites according to LEfSe analysis. Isolated *Dietzia* strains have been previously associated with hydrocarbon degradation (Alonso-Gutiérrez et al., 2011; Zhang et al., 2017) and are often detected at hydrocarbon polluted sites (Alonso-Gutiérrez et al., 2009; Dong et al., 2015). Similarly, another gram-positive bacterium, *Planomicrobium* (formerly *Planococcus*; Bacilli) has been implicated in the degradation of straight and branched alkanes (Engelhardt et al., 2001) and was detected in the upper layers of cores in this study. Although there was detection of taxa associated with hydrocarbon-degrading properties within piles, low estimates of bacterial abundance determined by qPCR suggest low biomass and would have negative implications for degradation rates.

The surface of the transect samples closer to the centre of pile Alpha were colonised by bacteria that were similar to those found in cores, such as *Halomonas*. At the same pile, the more distant transect sample was dominated by *Marinobacter*. Genomic analysis of the strain *Marinobacter aquaeolei* VT8 revealed the presence of gene clusters for alkane degradation and some strains are believed to be obligate hydrocarbon-degraders, *e.g.* they can only metabolise hydrocarbons for growth (Yakimov et al., 2007). Further investigation of the hydrocarbon-degrading properties of *Marinobacter* spp. revealed its ability to utilise PAH (Bonin et al., 2015). Moreover, *Marinobacter* usually prevail in the latter stages of hydrocarbon-degrading community dynamics and coincide with recalcitrant heavy PAH removal (Potts et al., 2018; Vila et al., 2010).

Many of the taxa identified in the DC piles of this study are often detected in subsurface environments such as Firmicutes, which are typically anaerobic and many can form spores as a survival mechanism; this may allude to their presence in DC. For example *Dethiobacter* and *Dethiosulfatibacter* were frequently present in DC piles and were recently enriched from fracture fluid obtained from a sub-surface depth of 967 m (Purkamo et al., 2017). *Bacillus* spp. represented a large proportion of the bacterial community in deep transect samples and some core sections and are also Firmicutes. The genus *Bacillus* is extremely diverse and ubiquitous in nature. Despite the renowned ability of certain strains (*e.g. B. subtilis*) to degrade hydrocarbons (Kim et al., 2000), their ability to produce spores may explain detection within DC. The same may apply to archaeal members detected in DC, such as *Thermoplasmata*, a strain commonly detected in the marine environment, in particular, deep sediments (Oni et al., 2015). There are no cultivated members of this genus at present, so little is known of their function. *Thermoplasmata* have been detected in both oil-contaminated and non-contaminated sediments suggesting a tolerance factor (Jurelevicius et al., 2014).

4.4. Application of the findings

Leaving DC piles *in situ* in perpetuity appears to be the most

common outcome of decommissioning NEBA reports because (1) it causes the least damage on the environment in the short-term, (2) does not require expenditure of energy resulting in carbon emission production and (3) can prove to be economical. However, the long-term impacts within the marine system are largely unknown. It is expected that harmful contaminants, such as hydrocarbons, contained within DC piles will degrade naturally. Indeed, it is established that hydrocarbons can be degraded, both aerobically and anaerobically (Head et al., 2006) and shows promise for natural attenuation of DC piles. However, degradation rates within DC are difficult to estimate considering the variability of DC piles; and therein lies the challenge. Without significant coverage of DC pile characterisation, which is demanded by the intrinsic heterogeneity the piles present, it is not possible to accurately estimate hydrocarbon degradation rates and apply them to discharge models. It has been proposed that degradation of contaminants within piles could take decades if not centuries (Artz et al., 2002). Thus, further research is required to refine such timescales.

In a recent attempt to develop a microbial consortium to be used in a bioaugmentation effort, diesel-polluted soil was enriched in a saline media on diesel fuel (Rezaei Somee et al., 2018). From the enrichment, *Halomonas* and *Dietzia* were prominent members and capable of degrading diesel (40% removal) when DC were diluted with sand (1,1). This is promising considering these microbes were among the most abundant members in the DC piles in this study. However, the use of a diluting agent such as sand or soil to facilitate bioremediation by increasing oxygen penetration requires pile intervention. Given that the most appropriate strategy for managing DC piles, as decided by NEBA reports, is to leave these piles *in situ*, it is not a realistic option. Therefore, more research should focus on natural attenuation potential. Pioneering research provided evidence for mineralisation of hydrocarbons within cutting piles (Massie et al., 1985), suggesting that microbes have the potential to reduce the long-term fate of hydrocarbons in North Sea DC piles. More recently, an *ex situ* study demonstrated degradation of *n*-alkanes sampled from DC (Artz et al., 2002). Degradation rates estimated from *ex situ* studies are important for modelling hydrocarbon half-lives and degradation potential. However, without an understanding of *in situ* communities, it is not possible to realistically determine DC pile intrinsic degradation capability. Here, microbial community composition and estimated abundances based on 16S rRNA genes were characterised. The information gained from this study should be used as a platform to effectively direct further research on DC pile degradation potential by indigenous microbes.

5. Conclusion

While there is accumulating evidence describing hydrocarbon degradation in sediments, the unique environment of DC has not been studied to the same extent. This study provides an account on the microbial communities residing in DC piles and of natural sediments from nearby locations. The key findings are:

1. Drill cutting piles are heterogeneous in terms of geochemical characteristics and microbial community abundance and structure.
2. Microbial diversity is significantly reduced within piles compared to nearby natural sediments.
3. Community composition within piles is dominated by taxa such as *Halomonas* and *Dietzia*, which may provide hydrocarbon degradation services.

The findings from this research should encourage further investigation on the ability of micro-organisms detected here, to degrade hydrocarbons at *in situ* conditions. This would allow increased accuracy when modelling hydrocarbon residence times and natural attenuation potential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.03.014.

References

Acosta-Gonzalez, A., Marques, S., 2016. Bacterial diversity in oil-polluted marine coastal sediments. Curr. Opin. Biotechnol. 38, 24–32. https://doi.org/10.1016/j.copbio.2015.12.010.

Adam, P.S., Borrel, G., Brochier-Armanet, C., Gribaldo, S., 2017. The growing tree of Archaea: new perspectives on their diversity, evolution and ecology. ISME J. 11, 2407–2425. https://doi.org/10.1038/ismej.2017.122.

Alonso-Gutiérrez, J., Figueras, A., Albaigés, J., Jiménez, N., Viñas, M., Solanas, A.M., Novoa, B., 2009. Bacterial communities from shoreline environments (costa da morte, northwestern Spain) affected by the prestige oil spill. Appl. Environ. Microbiol. 75, 3407–3418. https://doi.org/10.1128/AEM.01776-08.

Alonso-Gutiérrez, J., Teramoto, M., Yamazoe, A., Harayama, S., Figueras, A., Novoa, B., 2011. Alkane-degrading properties of Dietzia sp. H0B, a key player in the Prestige oil spill biodegradation (NW Spain). J. Appl. Microbiol. 111, 800–810. https://doi.org/10.1111/j.1365-2672.2011.05104.x.

Artz, R.R.E., Semple, K.T., Killham, K., Prosser, J.I., Paton, G.I., 2002. The potential for anaerobic mineralisation of hydrocarbon constituents of oily drill cuttings from the North Sea seabed. J. Environ. Monit. 4, 553. https://doi.org/10.1039/b203991p.

Atlas, R.M., 1995. Petroleum biodegradation and oil spill bioremediation. Mar. Pollut. Bull. 31, 178–182. https://doi.org/10.1016/0025-326X(95)00113-2.

Bakke, T., Klungsøyr, J., Sanni, S., 2013. Environmental impacts of produced water and drilling waste discharges from the Norwegian offshore petroleum industry. Mar. Environ. Res. 92, 154–169. https://doi.org/10.1016/j.marenvres.2013.09.012.

Bonin, P., Vieira, C., Grimaud, R., Militon, C., Cuny, P., Lima, O., Guasco, S., Brussaard, C.P.D., Michotey, V., 2015. Substrates specialization in lipid compounds and hydrocarbons of Marinobacter genus. Environ. Sci. Pollut. Res. 22, 15347–15359. https://doi.org/10.1007/s11356-014-4009-y.

Bozal, N., Montes, M.J., Tudela, E., Guinea, J., 2003. Characterization of several Psychrobacter strains isolated from Antarctic environments and description of Psychrobacter luti sp. nov. and Psychrobacter fozii sp. nov. Int. J. Syst. Evol. Microbiol. 53, 1093–1100. https://doi.org/10.1099/ijs.0.02457-0.

BP, 2011. Miller decommissioning programme. In: Igarss 2014 September, pp. 1–5. https://doi.org/10.1007/s13398-014-0173-7.2.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349. https://doi.org/10.2307/1942268.

Breuer, E., Stevenson, A.G., Howe, J.A., Carroll, J., Shimmield, G.B., 2004. Drill cutting accumulations in the Northern and Central North Sea: a review of environmental interactions and chemical fate. Mar. Pollut. Bull. 48, 12–25. https://doi.org/10.1016/j.marpolbul.2003.08.009.

Breuer, E., Shimmield, G., Peppe, O., 2008. Assessment of metal concentrations found within a North Sea drill cuttings pile. Mar. Pollut. Bull. 56, 1310–1322. https://doi.org/10.1016/j.marpolbul.2008.04.010.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., Helmke, E., 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol. 69, 6610–6619. https://doi.org/10.1128/AEM.69.11.6610.

Cai, Q., Zhang, B., Chen, B., Zhu, Z., Lin, W., Cao, T., 2014. Screening of biosurfactant producers from petroleum hydrocarbon contaminated sources in cold marine environments. Mar. Pollut. Bull. 86, 402–410. https://doi.org/10.1016/j.marpolbul.2014.06.039.

Caporaso, J.G., Lauber, C.L., Walters, W. a, Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. a, Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Chronopoulou, P.-M., Sanni, G.O., Silas-Olu, D.I., van der Meer, J.R., Timmis, K.N., Brussaard, C.P.D., McGenity, T.J., 2015. Generalist hydrocarbon-degrading bacterial communities in the oil-polluted water column of the North Sea. Microb. Biotechnol. 8, 434–447. https://doi.org/10.1111/1751-7915.12176.

Cleveland, W.S., Grosse, E., Shyu, W.M., 1992. Local regression models. In: Chambers, J.M., Hastie, T.J. (Eds.), Statistical Models in S, (Wadsworth & Brooks/Cole).

Corti Monzón, G., Nisenbaum, M., Herrera Seitz, M.K., Murialdo, S.E., 2018. New findings

on aromatic compounds' degradation and their metabolic pathways, the biosurfactant production and motility of the halophilic bacterium Halomonas sp. KHS3. Curr. Microbiol. 75, 1108–1118. https://doi.org/10.1007/s00284-018-1497-x.
Coulon, F., McKew, B.A., Osborn, A.M., McGenity, T.J., Timmis, K.N., 2007. Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. Environ. Microbiol. 9, 177–186. https://doi.org/10.1111/j.1462-2920.2006.01126.x.
Curtis, D., Elango, V., Collins, A.W., Rodrigue, M., Pardue, J.H., 2018. Transport of crude oil and associated microbial populations by washover events on coastal headland beaches. Mar. Pollut. Bull. 130, 229–239. https://doi.org/10.1016/j.marpolbul.2018.03.008.
Davies, J.M., Addy, J.M., Blackman, R.A., Blanchard, J.R., Ferbrache, J.E., Moore, D.C., Somerville, H.J., Whitehead, A., Wilkinson, T., 1984. Environmental effects of the use of oil-based drilling muds in the North Sea. Mar. Pollut. Bull. 15, 363–370. https://doi.org/10.1016/0025-326X(84)90169-3.
Deppe, U., Richnow, H.-H., Michaelis, W., Antranikian, G., 2005. Degradation of crude oil by an arctic microbial consortium. Extremophiles 9, 461–470. https://doi.org/10.1007/s00792-005-0463-2.
Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. 112, E1326–E1332. https://doi.org/10.1073/pnas.1414261112.
Dong, C., Bai, X., Sheng, H., Jiao, L., Zhou, H., Shao, Z., 2015. Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean. Biogeosciences 12, 2163–2177. https://doi.org/10.5194/bg-12-2163-2015.
Dow, F.K., Davies, J.M., Raffaelli, D., 1990. The effects of drill cuttings on a model marine sediment system. Mar. Environ. Res. 29, 103–134. https://doi.org/10.1016/0141-1136(90)90031-I.
Engelhardt, M.A., Daly, K., Swannell, R.P.J., Head, I.M., 2001. Isolation and characterization of a novel hydrocarbon-degrading, Gram-positive bacterium, isolated from intertidal beach sediment, and description of Planococcus alkanoclasticus sp. nov. J. Appl. Microbiol. 90, 237–247. https://doi.org/10.1046/j.1365-2672.2001.01241.x.
Ferguson, R.M.W., Gontikaki, E., Anderson, J.A., Witte, U., 2017. The variable influence of dispersant on degradation of oil hydrocarbons in subarctic deep-sea sediments at low temperatures (0–5 °C). Sci. Rep. 7, 2253. https://doi.org/10.1038/s41598-017-02475-9.
Gales, G., Tsesmetzis, N., Neria, I., Alazard, D., Coulon, S., Lomans, B.P., Morin, D., Ollivier, B., Borgomano, J., Joulian, C., 2016. Preservation of ancestral Cretaceous microflora recovered from a hypersaline oil reservoir. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep22960.
Gasperotti, A.F., Studdert, C.A., Revale, S., Herrera Seitz, M.K., 2015. Draft genome sequence of Halomonas sp. KHS3, a polyaromatic hydrocarbon-chemotactic strain. Genome Announc. 3, e00020-15. https://doi.org/10.1128/genomeA.00020-15.
Gerrard, S., Grant, A., Marsh, R., London, C., 1999. Drill cuttings piles in the North Sea: management options during platform decommissioning. Cent. Environ. Risk Res. Rep. 31, 224.
Hassanshahian, M., Emtiazi, G., Cappello, S., 2012. Isolation and characterization of crude-oil-degrading bacteria from the Persian Gulf and the Caspian Sea. Mar. Pollut. Bull. 64, 7–12. https://doi.org/10.1016/j.marpolbul.2011.11.006.
Head, I., Swannell, R., 1999. Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Curr. Opin. Biotechnol. 10, 234–239.
Head, I.M., Jones, D.M., Röling, W.F.M., 2006. Marine microorganisms make a meal of oil. Nat. Rev. Microbiol. 4, 173–182. https://doi.org/10.1038/nrmicro1348.
Jurelevicius, D., de Almeida Couto, C.R., Alvarez, V.M., Vollú, R.E., de Almeida Dias, F., Seldin, L., 2014. Response of the archaeal community to simulated petroleum hydrocarbon contamination in marine and hypersaline ecosystems. Water Air Soil Pollut. 225, 1871. https://doi.org/10.1007/s11270-014-1871-7.
Kim, H.S., Kim, S. Bin, Park, S.H., Oh, H.M., Park, Y. Il, Kim, C.K., Katsuragi, T., Tani, Y., Yoon, B.D., 2000. Expression of sfp gene and hydrocarbon degradation by Bacillus subtilis. Biotechnol. Lett. 22, 1431–1436. https://doi.org/10.1023/A:1005675231949.
Korlević, M., Zucko, J., Dragić, M.N., Blažina, M., Pustijanac, E., Zeljko, T.V., Gacesa, R., Baranasic, D., Starcevic, A., Diminic, J., Long, P.F., Cullum, J., Hranueli, D., Orlić, S., 2015. Bacterial diversity of polluted surface sediments in the northern Adriatic Sea. Syst. Appl. Microbiol. 38, 189–197. https://doi.org/10.1016/j.syapm.2015.03.001.
Kubo, K., Lloyd, K.G., F Biddle, J., Amann, R., Teske, A., Knittel, K., 2012. Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. ISME J. 6, 1949–1965. https://doi.org/10.1038/ismej.2012.37.
Leahy, J., Colwell, R., 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54, 305–315.
Marathon Oil, 2013. NCS 3D Mapping Survey of Brae Alpha, Brae Bravo and East Brae Cuttings Piles.
Marathon Oil, 2015a. Brae Alpha Cuttings Pile Characterisation Survey UKCS Block 16/7a.
Marathon Oil, 2015b. East Brae Cuttings Pile Characterisation Survey UKCS Block 16/3a.
Massie, L.C., Ward, A.P., Davies, J.M., 1985. The effects of oil exploration and production in the northern North Sea: part 2 - microbial biodegradation of hydrocarbons in water and sediments, 1978–1981. Mar. Environ. Res. 15, 235–262. https://doi.org/10.1016/0141-1136(85)90004-2.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.
Mnif, S., Chamkha, M., Sayadi, S., 2009. Isolation and characterization of Halomonas sp. strain C2SS100, a hydrocarbon-degrading bacterium under hypersaline conditions. J. Appl. Microbiol. 107, 785–794. https://doi.org/10.1111/j.1365-2672.2009.04251.x.
Mnif, S., Chamkha, M., Labat, M., Sayadi, S., 2011. Simultaneous hydrocarbon biodegradation and biosurfactant production by oilfield-selected bacteria. J. Appl. Microbiol. 111, 525–536. https://doi.org/10.1111/j.1365-2672.2011.05071.x.
Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700 (doi:0099-2240/93/030695).
Nguyen, T.T., Cochrane, S.K.J., Landfald, B., 2018. Perturbation of seafloor bacterial community structure by drilling waste discharge. Mar. Pollut. Bull. 129, 615–622. https://doi.org/10.1016/j.marpolbul.2017.10.039.
Okpokwasili, G.C., Nnubia, C., 1995. Effects of drilling fluids on marine bacteria from a Nigerian offshore oilfield. Environ. Manag. 19, 923–929. https://doi.org/10.1007/BF02471943.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. vegan: Community Ecology Package.
Oni, O.E., Schmidt, F., Miyatake, T., Kasten, S., Witt, M., Hinrichs, K.U., Friedrich, M.W., 2015. Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland mud area, North Sea. Front. Microbiol. 6, 1–16. https://doi.org/10.3389/fmicb.2015.01290.
Perez Calderon, L.J., Potts, L.D., Gontikaki, E., Gubry-Rangin, C., Cornulier, T., Gallego, A., Anderson, J.A., Witte, U., 2018. Bacterial community response in deep Faroe-Shetland Channel sediments following hydrocarbon entrainment with and without dispersant addition. Front. Mar. Sci. 5. https://doi.org/10.3389/fmars.2018.00159.
Pester, M., Schleper, C., Wagner, M., 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr. Opin. Microbiol. 14, 300–306. https://doi.org/10.1016/j.mib.2011.04.007.
Potts, L.D., Perez Calderon, L.J., Gontikaki, E., Keith, L., Gubry-Rangin, C., Anderson, J.A., Witte, U., 2018. Effect of spatial origin and hydrocarbon composition on bacterial consortia community structure and hydrocarbon biodegradation rates. FEMS Microbiol. Ecol. 94. https://doi.org/10.1093/femsec/fiy127.
Prabagaran, S.R., Manorama, R., Delille, D., Shivaji, S., 2007. Predominance of Roseobacter, Sulfitobacter, Glaciecola and Psychrobacter in seawater collected off Ushuaia, Argentina, Sub-Antarctica. FEMS Microbiol. Ecol. 59, 342–355. https://doi.org/10.1111/j.1574-6941.2006.00213.x.
Prince, R.C., 2010. Bioremediation of marine Oil spills. In: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 2617–2630. https://doi.org/10.1007/978-3-540-77587-4.
Purkamo, L., Bomberg, M., Nyyssönen, M., Ahonen, L., Kukkonen, I., Itävaara, M., 2017. Response of deep subsurface microbial community to different carbon sources and electron acceptors during ~2 months incubation in microcosms. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.00232.
Quero, G.M., Cassin, D., Botter, M., Perini, L., Luna, G.M., 2015. Patterns of benthic bacterial diversity in coastal areas contaminated by heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Front. Microbiol. 6, 1–15. https://doi.org/10.3389/fmicb.2015.01053.
R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.
Raskin, L., Stromley, J.M., Rittmann, B.E., Stahl, D. a, 1994. Group-specific 16S ribosomal-RNA hybridization probes to describe natural communities of methanogens. Appl. Environ. Microbiol. 60, 1232–1240 (doi:0099-2240/94/$04.00+0).
Rezaei Somee, M., Shavandi, M., Dastgheib, S.M.M., Amoozegar, M.A., 2018. Bioremediation of oil-based drill cuttings by a halophilic consortium isolated from oil-contaminated saline soil. 3 Biotech 8, 229. https://doi.org/10.1007/s13205-018-1261-8.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584.
Sanchez, G., 2012. plsdepot: Partial Least Squares (PLS) Data Analysis Methods.
Sanders, P.F., Tibbetts, P.J.C., 1987. Effects of discarded drill muds on microbial populations. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 316, 567–585. https://doi.org/10.1098/rstb.1987.0039.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.a., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60.
Shell UK Ltd., 2016. Brent Field Drill Cuttings Decommissioning Technical Document.
Struchtemeyer, C.G., Davis, J.P., Elshahed, M.S., 2011. Influence of the drilling mud formulation process on the bacterial communities in thermogenic natural gas wells of the Barnett Shale. Appl. Environ. Microbiol. 77, 4744–4753. https://doi.org/10.1128/AEM.00233-11.
Vila, J., Nieto, J.M., Mertens, J., Springael, D., Grifoll, M., 2010. Microbial community structure of a heavy fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. FEMS Microbiol. Ecol. 73. https://doi.org/10.1111/j.1574-6941.2010.00902.x.
Wang, Y.N., Cai, H., Chi, C.Q., Lu, A.H., Lin, X.G., Jiang, Z.F., Wu, X.L., 2007. Halomonas shengliensis sp. nov., a moderately halophilic, denitrifying, crude-oil-utilizing bacterium. Int. J. Syst. Evol. Microbiol. 57, 1222–1226. https://doi.org/10.1099/ijs.0.64973-0.
Wang, W., Cai, B., Shao, Z., 2014. Oil degradation and biosurfactant production by the deep sea bacterium Dietzia maris As-13-3. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00711.
Wei, T., Simko, V., 2017. R Package "corrplot": Visualization of a Correlation Matrix.

Wickham, H., Chang, W., 2009. ggplot2: Elegant Graphics for Data Analysis.
Widdel, K., Knittel, A., Galushko, F., 2010. Anaerobic Hydrocarbon- Degrading Microorganisms: An Overview. In: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1997–2022. https://doi.org/10.1007/978-3-540-77587-4.
Yakimov, M.M., Timmis, K.N., Golyshin, P.N., 2007. Obligate oil-degrading marine bacteria. Curr. Opin. Biotechnol. 18, 257–266. https://doi.org/10.1016/j.copbio.2007.04.006.
Zhang, K., Sun, Y., Cui, Z., Yu, D., Zheng, L., Liu, P., Lv, Z., 2017. Periodically spilled-oil input as a trigger to stimulate the development of hydrocarbon-degrading consortia in a beach ecosystem. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-12820-7.
Zinger, L., Amaral-Zettler, L.A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, J.B.H., Sogin, M., Boetius, A., Ramette, A., 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS One 6, 1–11. https://doi.org/10.1371/journal.pone.0024570.