Microbial response to oil enrichment in Gulf of Mexico sediment measured using a novel long-term benthic lander system

Beth N. Orcutt*, Laura L. Lapham†, Jennifer Delaney‡, Neha Sarode‡, Kathleen S. Marshall†, Kelly J. Whaley-Martin§, Greg Slater§, C. Geoff Wheat‖ and Peter R. Girguis‡

Weathered crude oil sank to the seafloor following the Deepwater Horizon disaster in 2010, removing this oil from further physical and photo-chemical degradation processes and leaving benthic processes as the mechanisms for altering and remediating this hydrocarbon source. To quantify potential microbial oil degradation rates at the seafloor, and associated changes in sediment microbial community structure and pore fluid composition, we used a benthic lander system to deploy novel sediment flow-through chambers at a natural hydrocarbon seep in the Gulf of Mexico (at a depth of 1226 m in lease block GC600) roughly 265 km southwest of the Deepwater Horizon wellhead (at 1500 m depth). Sediment amended with 20% unweathered crude oil had elevated rates of sulfate reduction over the course of the 5-month-long experiment as compared to an unamended control, yielding potential rates of sulfate reduction (600–800 mmol m⁻² d⁻¹) among the highest measured in hydrocarbon-influenced seafloor sediment. Oil amendment also stimulated methane production towards the end of the experiment, and led to slightly higher cell densities without significant changes in microbial community structure, based on 16S rRNA gene sequence libraries and fatty acid profiles. Assuming a link between sulfate reduction and hydrocarbon degradation, these results suggest that electron acceptor availability may become limiting in heavily oiled deep-sea environments, resulting in minimal degradation of deposited oil. This study provides unique data on seafloor sediment responses to oil deposition, and reveals the value of using observatories to fill the gap in understanding deep-sea microbial processes, especially for ephemeral and stochastic events such as oil spills.

Keywords: Gulf of Mexico; Deepwater Horizon; oil degradation; sulfate reduction; methane; geomicrobiology

1. Introduction

A massive amount of oil was released into the Gulf of Mexico after the Deepwater Horizon blowout from April to July 2010 (Crone and Tolstoy, 2010; MacDonald, 2010; Joye et al., 2011; McNutt et al., 2012), with roughly 5–15% of that oil sinking to the seafloor (Valentine et al., 2014; Chanton et al., 2015). Sedimentary bacteria and archaea are known to break down natural and human-supplied oil and gas in oxic and anoxic deep-water systems (Head et al., 2003; Miralles et al., 2007; Bowles and Joye, 2010; Orcutt et al., 2010b). However, it is not understood how natural sediment communities respond to such significant loading of oil as occurred during the Deepwater Horizon event. As the Gulf of Mexico has many natural oil seeps (MacDonald et al., 1996, 2002), it has been suggested that some Gulf of Mexico sediment microorganisms are “primed” to begin oil degradation quickly following an oil-loading event (Valentine et al., 2010; Kessler et al., 2011); however, a lag in microbial response to hydrocarbon inputs might also be expected based on slow growth rates of microorganisms involved in anaerobic hydrocarbon consumption (Nauhaus et al., 2007). Moreover, it is unclear if microbial communities degrading sedimented oil would exhaust the available pool of electron donors (i.e., oxygen, nitrate, sulfate) needed for complete hydrocarbon oxidation.

Since the Deepwater Horizon event, studies have aimed to determine rates of oil degradation and the oil-degrading microorganisms in bottle experiments using coastal sands and muds (Mortazavi et al., 2012; Singh et al., 2014).
and surface waters of the spill site (Edwards et al., 2011; Ziervogel et al., 2012). In addition, there have been some in situ analyses of microbial community structure in oil-impacted beach sands (Kostka et al., 2011). By comparison, no work has been reported in the continental shelf and deep-sea environments to determine rates of microbial oil degradation in situ, especially after a rapid deposition of oil as might be seen during an oil spill. The lack of studies is due in no small part to the technical challenges associated with working in the deep sea, and in collecting samples with the appropriate spatial and temporal resolution to address such questions. One approach is to collect samples with high spatial resolution during a single oceanographic expedition; however, this approach lacks a temporal domain, which has been documented as important in other settings (MacNaughton et al., 1999; Röhling et al., 2002). Similarly, conducting long-term laboratory experiments with collected materials may introduce biases to the microbial communities involved in hydrocarbon transformations by removing critical physical and other constraints (currents, pressure, grazing, episodic events such as storms, etc.) that shape the ecosystem.

Here we present the results of a five-month seafloor experiment designed to determine rates of microbial oil degradation in the deep sea; evaluate the time it takes deep-sea microorganisms to consume oil; elucidate oil degradation pathways, mechanisms, and the key microbial groups that contribute to degradation; and constrain limiting factors that might control degradation rates, such as the lack of electron acceptors, nutrients, or excess trace element concentrations. These studies were enabled by our development and deployment of a novel oil-enrichment experiment on a benthic “lander” system intended for long-term analysis of in situ sediment microbial oil degradation. This experiment utilized sampling systems that allow for parallel simultaneous collection of samples for geochemical and microbiological analyses (Jannasch et al., 2004; Lapham et al., 2008; Orcutt et al., 2010a; Wheat et al., 2011; Robidart et al., 2013) coupled to flow-through sediment reactors deployed at the seafloor for months at a time. We refer to this experiment system as the Microbial Methane Observatory for Seafloor Analysis (MIMOSA). This deployment provides the first quantification of in situ microbial responses to oil enrichment in a deep-sea sediment environment, highlighting the geochemical and microbial ecological implications of an oil sedimentation event.

2. Materials and methods

2.1. MIMOSA concept, configuration, and sampling strategy

The goal of MIMOSA was to examine the dynamics of hydrocarbon cycling and microbial community structure in deep-sea sediment to better understand the response of microorganisms to oil release, as happened following the Deepwater Horizon event. To meet this goal, we developed a novel sediment flow-through reactor coupled to fluid sampling technology (used extensively in deep-sea borehole environments; Wheat et al., 2011) for experimentation in sediment seafloor environments. The flow-through reactors allow for side-by-side comparisons of sediment microbial activity and community composition under amended conditions versus non-amended controls. In this study, crude oil with a similar composition to that from the Macondo site (defined below) was used as the amendment substrate with sediment derived from a natural oil and gas seep in the Gulf of Mexico. Accordingly, the sediment flow-through reactors were designed to retain oil and sediment, allow seawater and/or microbial infiltration without loss of oil, and allow continuous pore fluid sampling via osmotic pump systems described below (Figure 1).

Comprehensive details about the design of the experiment are available in the Supplemental Materials, with brief summaries provided below. Each reactor consisted of a column open at both ends, constructed from commercially available polyvinyl chloride (PVC) trap adapters and couplers (Figure 1). Each end was covered with a Nylon mesh screen to retain oil and sediment but allow exchange of unfiltered seawater. The internal volume of the PVC chambers was roughly 100 cm³. For this deployment, one enrichment chamber was filled with sediment that was collected from a natural oil seep in the Green Canyon lease block (GC600, collected on cruise PE13–31 in June 2013 from 27° 21.885N, 90° 33.791W; see Johansen et al., 2017, for more site details) and stored at 4°C in a sealed glass Ball® jar until use, with no amendments to the sediment. A second enrichment chamber was filled with the same sediment that had been mixed in a 5:1 sediment:oil [v/v] ratio with Macondo well-head surrogate crude oil provided by BP (SO-201116-MPDF-003 OL-OIL A0057T, density 0.856 g ml⁻¹) and stored in the dark at 4°C until use.

To assess microbial activity and diversity over time, sediment pore-fluids were continuously collected from these reactors (described below) and preserved in situ for subsequent laboratory analysis using three different OsmoSampler systems (Figure 1E). Each is based on autonomous, non-mechanical, non-powered osmotically-driven pumps (OsmoPumps; Jannasch et al., 2004) to sample fluids or deliver fixatives using the osmotic potential between salt-saturated and distilled water reservoirs separated by a semi-permeable membrane, with pump rates based on temperature and the surface area of membranes that separate the salt and freshwater reservoirs. In this experiment, temperature did not vary appreciably during the deployment (4.5 ± 0.9 °C; Chris Martens and Howard Mendlovitz, personal communication) and the pumping rate was roughly 0.5 ml d⁻¹, as described in the Supplemental Materials. The pumps continuously pull fluid into long coils of small (~ 1.2 mm inner diameter) plastic or copper tubing over time, as described below. Concurrently, an adjacent slower pump delivers a small volume of fixative into the sample stream, enabling in situ preservation. Upon recovery, tubing is cut at distinct intervals and the fluid is extracted into desired sampling containers.

The three types of OsmoSamplers had different configurations for geochemical and microbiological analyses (Figure 1E). The “CH₂” OsmoSampler collected filtered fluids into gas-tight copper tubing to provide a continuous record of methane and sulfate concentrations and
methane stable carbon isotopic composition, based on published designs (Lapham et al., 2008, 2013). This sampler included a high-pressure valve that was closed on the seafloor to maintain in situ pressure. The “acid” OsmoSampler collected and acidified filtered pore fluids for determining dissolved major, minor and trace elements, based on published designs (Wheat et al., 2010, 2011). The third “BOSS” OsmoSampler in MIMOSA was designed to preserve nucleic acids to study changes in the pore fluid microbial community composition, based on designs tested in deep-sea hydrothermal vent systems (Robidart et al., 2013). Details about the OsmoSampler fluid collection, preservation, and analysis procedures are available in Supplemental Materials. Each of these samplers housed in boxes on a benthic lander was attached to the reaction chamber using polyether ether ketone (PEEK) tubing, and the reaction chambers were attached to a lance made of PVC pipe, enabling targeted deployment on the seafloor by remotely operated vehicle (ROV) manipulator (Figure 1B, 1D). Coupled together, the three OsmoSampler systems connected to the sediment flow-through reactors provide a long-term time series of chemical and community changes in amended and non-amended sediment incubated at the seafloor in deep-water settings.

2.2. Benthic lander deployments and recoveries

One benthic lander equipped with MIMOSA (Figure 1A, 1D; more detail on lander design available in Supplemental Materials) was deployed on 11 October 2013 during cruise PE14–09 of the RV Pelican (Louisiana University Marine Consortium) at a known natural oil seep at a depth of...
1226 m on the seafloor. The site was located at 27.36484 N and 90.56430 W in the Green Canyon lease block (GC600) about 265 km southwest of the Macondo wellhead site (Figure 2; Johansen et al., 2017). The lander was deployed using the I-SPIDER camera-guided release system (Lowe et al., 2013); once on the seafloor, the flow-through reactors mounted to the PVC probe tip were put in place using the Station Service Device ROV (Figure 1B, 1D). The flow-through reactors were positioned to have the bottom open-end in contact with surficial sediment, and the top open-end exposed to bottom seawater. The lander remained in position for 149 days, at which time (9 March 2014) it was recovered during RV Pelican cruise PE14–14 with the aid of the ROV Global Explorer MK3 (Deep Sea Systems International [DSSI], Oceaneering International).

Upon recovery of the lander, sample coils within the MIMOSA were disconnected and closed for cold transport back to shore-based laboratories for processing. Sediment slurries from the flow-through experiment chambers were transferred using flame-sterilized instruments into sterile plastic Whirlpak® bags and frozen for DNA and bulk hydrocarbon analysis. In addition, a plug of oily sediment that was serendipitously collected in one of the MIMOSA probe tip PVC legs was also preserved for DNA analysis.

2.3. Fluid chemical composition

Methane, sulfate and chloride concentrations were determined from fluid samples collected into the “CH4” copper tubing and analyzed via gas chromatography, isotope ratio mass spectrometry, and ion chromatography, as described in detail in Supplemental Materials. Concentrations of major and minor ions (i.e., B, Ca, Fe, K, Li, Mg, Mn, Na, S, Si, and Sr) and trace elements (i.e., Ba, Cd, Co, Cr, Cs, Cu, Mo, Ni, Pb, Rb U, V) were measured in the acid-fixed fluid samples (Wheat et al., 2010, 2011). Concentrations were measured via inductively coupled plasma (ICP) atomic emission spectroscopy and ICP-mass spectrometry, with all values normalized by the sodium concentration for subtle variations in the rate of acid addition. Reported bottom seawater values were averaged across samples.

2.4. Sulfate reduction model

The behavior of sulfate in the oil-amendment experiment was simulated with a simple reaction-transport box model. Conceptually, the sulfate concentration over time would be a function of the amount of sulfate in the chamber at the beginning of the experiment, the input of sulfate due to pulling in bottom seawater (with an assumed sulfate concentration of 28 mM), the removal of sulfate in the fluid exiting the system and stored in the OsmoSampler coils, and any reactions that consume or produce sulfate within the chamber. The box model dimensions are set by the chamber size (3.5 cm length x 1.5 cm radius). Another controlled parameter includes the rate at which bottom seawater is pulled into the chamber, which is equivalent to the pumping speed of the three OsmoSampler systems connected to the chamber (i.e., roughly 1.4 ml d⁻¹). Several assumptions were invoked. First, we assume that bottom seawater flows into the chamber through the upper column opening, even though the chamber is open on both ends, because flow through the bottom end would have been limited by connection to the seafloor. Thus, the volume of fluid affected is calculated based on the half volume of the chamber and an estimated porosity.

Figure 2: Location of the benthic lander deployment in the “GC600” lease block in the northern Gulf of Mexico. The lander was deployed at a water depth of 1226 m, roughly 265 km southwest of the Deepwater Horizon well-head (at 1500 m depth). Contour lines indicate water depth intervals of 500 m. DOI: https://doi.org/10.1525/elementa.129.f2
of 75%, resulting in a fluid volume of 38 ml. Given that the advective flux of seawater sulfate is much higher than potential diffusive fluxes due to the OsmoSampler pumping, diffusion was excluded from the box model. The starting sulfate concentration in the model is 15 mM, which matches the first measured concentration, and is a reflection of the initial starting conditions of mixing sediment with depleted sulfate concentrations with 20% oil [sediment volume:oil volume]. Three model profiles are presented: one excludes the reaction term, another assumes a linear reaction rate constant for sulfate reduction, and the third assumes an exponential rate constant for sulfate reduction. Modeled sulfate concentrations were compared to measured sulfate concentrations to adjust rate parameters and determine best fit to measured data.

2.5. Microbial community composition

DNA was extracted from 5-m-long sections from the BOSS sampling coils, representing roughly biweekly time resolution. Samples were filtered onto 0.2 μm mesh Whatman polycarbonate filters (GE Healthcare Bio-Sciences), and DNA was extracted from the filters using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) with the following modification to the manufacturer’s protocol: the cell lysis procedure consisted of two rounds of heating the sample (85°C for 5 min) and then bead beating (6 m s⁻¹ for 60 sec on a FastPrep-24™ Instrument; MP Biomedicals). DNA concentration in the extracts was below the detection limit with the Qubit® HS Assay Kit (Life Technologies) according to manufacturer instructions. DNA was extracted from the filters using the REPLI-g® Mini Kit (Qiagen) according to the manufacturer’s instructions, using 5 μl template and a 16-hr incubation period. Amplified DNA was purified according to a Supplementary Protocol for the QIAamp® DNA Mini Kit (Qiagen) and then sent to Research and Testing Laboratory (Lubbock, TX, USA) for sequencing. Illumina MiSeq sequencing was performed using the 2 × 300 bp kit and primers sets specific for 16S rRNA genes of Bacteria (assay b.2: primers 28F (5’-GAGTTTGATCNTGGCTCAG) and 519R (5’-GTNTACNGCGCGKCTG)) and Archaea (assay a.9: primers Arch519wF (5’-CAGCMGCCCGGTAA) and Arch1017R (5’-GGCCATGCACWCCCTTCT)) as described elsewhere (Hand et al., 2011).

Microbial communities within the oiled and unamended experiment chambers at the beginning and end of the experiment were analyzed by extracting environmental DNA from ~ 0.5 g aliquots using a modified protocol for the PowerSoil® DNA Isolation Kit (MO BIO Laboratories) that included a phenol-chloroform extraction step. DNA extracts were cleaned using the CleanAll Purification Kit (Norgen) according to manufacturer instructions. DNA concentrations were determined by fluorometry using the Qubit® dsDNA HS Assay Kit (Life Technologies Corporation). Cleared DNA extracts were sent to Research and Testing Laboratory for Illumina MiSeq sequencing using the same conditions described above. To assess the abundance of Bacteria in the enrichment experiment sediment slurries as compared to ambient sediment at the beginning and end of the experiment, quantitative PCR (qPCR) of the 16S rRNA gene was performed using the same DNA extracts described above. Sequence processing and PCR methods are described in detail in the Supplemental Materials.

2.6. Compound-specific hydrocarbon analyses

To assess the change in the hydrocarbon pool from the beginning to the end of the oil-amendment experiment, sediment samples from the flow-through reactors from the beginning and end of the deployment were extracted with methylene chloride to concentrate hydrocarbons for analysis by gas chromatographic/mass spectrometry (GC/MS) in the laboratory of Dr. Christoph Aeppli, Bigelow Laboratory for Ocean Sciences. Analysis was performed using an Agilent 8977 MS and an Agilent 7890B GC equipped with a Rx-1ms column (30 m length, 0.25 mm I.D., 2.5 μm film; Restek Corp., Bellefonte, PA). The GC oven was kept at 40°C for 10 min, then ramped to 320°C at 5°C min⁻¹ (held for 10 min). Splitless injection (injector temperature 320°C) of 1 μl sample volume was used, and the carrier gas was He. The MS was operated in full scan mode.

2.7. Phospholipid fatty acid concentrations and isotopic compositions

To assess for changes in phospholipid content and isotopic composition between the oiled and non-amended experiments, samples from the end of the deployment from both chambers were extracted and purified for analysis by GC/MS. Fatty acids were extracted from the sediment, purified, and analyzed, focusing on fatty acid methyl esters (FAMEs), as detailed in Supplemental Materials.

3. Results

3.1. Changes in fluid composition with time in oiled versus unamended experiments

Time-series samples from the oiled and unamended experiments revealed dynamic processes (Figure 3). For example, sulfate concentrations increased to a maximum of 16.7 mM before decreasing with time to 3.4 mM in the oiled experiment, whereas sulfate concentrations did not change in the unamended experiment (29.2 ± 0.2). Concomitant with the sulfate decrease, methane concentrations rose in the oiled experiment, from less than 100 nM to a maximum of 772 nM, whereas methane concentrations in unamended experiment increased with time but remained below 215 nM. Methane concentrations were below the detection limit from the beginning of the time series until mid-November. The increase in methane concentrations in the oiled experiment corresponded to increasing depletion of the δ¹³C stable carbon isotopic composition of the methane, from ~49 % at lower concentrations to ~56 % at the higher concentrations. This trend is indicative of methane produced from methanogenesis (Whiticar, 1999). Only one methane sample from the unamended experiment had sufficient mass to measure the stable carbon isotopic composition (δ¹³C = −44 %).

Similarly, some ion concentrations revealed temporal trends in the oiled and unamended conditions, whereas other ions did not change (Figure 4). For example, boron and lithium concentrations were initially elevated in the
Figure 3: Time series of dissolved methane, sulfate and chloride in the MIMOSA experiment. Dissolved methane (A, C) and sulfate and chloride concentrations (B, D) in the oiled chambers (left panels) compared to the control chambers (right panels) in the MIMOSA experiment at site GC600, covering the period of October 2013–March 2014. Dissolved methane in nanomolar concentrations in A and C plotted as red circles, with the methane stable carbon isotopic signature in per mil plotted as blue triangles. Chloride and sulfate concentrations, in millimolar, plotted in B and D as red circles and blue triangles, respectively. Methane concentrations were below the detection limit from the beginning of the time series until mid-November, so no methane data are shown for this time interval. DOI: https://doi.org/10.1525/elementa.129.f3

Figure 4: Time series of dissolved elements in the MIMOSA experiment. Dissolved elements in the oiled chambers (OC, black circles) and control chambers (CC, red triangles) in the MIMOSA experiment at the study site (Figure 2), covering the period of October 2013–March 2014, as compared to bottom seawater (BW, blue line). DOI: https://doi.org/10.1525/elementa.129.f4
oiled experiment, but decreased with time; concentrations of these elements in the unamended experiment matched bottom seawater values. Conversely, barium concentration increased over time in the oiled experiment, whereas barium concentrations in the unamended experiment matched bottom seawater values. Temporal variations were also observed in the molybdenum, silica, and manganese data. In contrast, there were no apparent changes in concentrations of cobalt, nickel, or vanadium in the oiled and unamended experiments (0–10, 0–50, and 0–40 nM, respectively; data not shown), even though these elements are potential tracers of oil contamination (Roeder et al., 2011).

3.2. Modeled sulfate concentration profiles and potential sulfate reduction rates

Sulfate concentrations from the oil-amendment experiment provide a measure of sulfate reduction within the chamber. At the start of the experiment, the chamber was filled with a 5:1 [volume:volume] mixture of sulfate-depleted sediment and crude oil, resulting in a starting sulfate concentration that was lower than bottom seawater. During the initial 40 days of the experiment, the flux of sulfate into the chamber exceeded the removal of sulfate, resulting in an increase in concentration. After this initial period, the flux of bottom seawater into the chamber was less than the removal flux and concentrations decreased with time. On the basis of the model of sulfate concentrations, the lack of a reaction term results in fluids with seawater sulfate concentrations (28 mM) by the middle of the experiment. Both linear and exponential reaction rates can approximate the observed profiles, with a linear reaction rate of 0.0008 per day providing the best fit to the earliest parts of the deployment, and an exponential reaction rate of 0.009 per day raised to the exponent of 0.024 per day providing the best fit towards the end of the deployment (Figure 5). Thus, the volumetric rate of potential sulfate reduction is 600–800 nmol cm⁻³ sediment d⁻¹ by the end of the experiment (Figure 5). Accounting for the area of the reaction chamber, the calculated corresponding areal potential sulfate reduction rate is roughly 700 mmol m⁻² d⁻¹ (Figure 6). These potential rate values are at the high end of the range of areal sulfate reduction rates in naturally oiled sediment measured ex situ with radiotracers (Bowles et al., 2011).

Figure 5: Model predictions of sulfate concentration (A) and reduction rates (B) over time in the oil-amended enrichment. Lines indicate predicted values assuming no sulfate reduction [SR, blue], linear sulfate reduction rates (red), and exponential sulfate reduction rates (black). Yellow symbols in A indicate measured values. Time is indicated as month/day/year. DOI: https://doi.org/10.1525/elementa.129.f5

Figure 6: Comparison of sulfate reduction rates from this study with prior studies. Comparison of the estimated potential in situ areal sulfate reduction rates in the oil-amendment experiment from this study (yellow) to ex situ radiotracer measurements of sulfate reduction rates from other oily and non-oily environments. Comparison data derive from a previously published summary of rates from Gulf of Mexico (GoM) and other sediments (Bowles et al., 2011). DOI: https://doi.org/10.1525/elementa.129.f6
3.3. Hydrocarbon profiles

Visual comparison of GC/MS profiles from the beginning and end of the oiled experiment revealed nearly identical profiles of bulk hydrocarbons (Figure 7), suggesting that any hydrocarbon degradation during the 5-month deployment was inconsequential to changing the structure of hydrocarbons present. This finding was confirmed by comparing concentrations of n-alkanes (n-C<sub>30</sub> through n-C<sub>50</sub>) and polyaromatic hydrocarbons (naphthalene, fluorene, phenanthrene, and the alkylated congeners) normalized to the recalcitrant biomarker 17α(H),21β(H)-hopane before and after incubation; no depletion of compounds was observed (Figure 7). Considering the 1:5 ratio of crude oil to sediment, which introduced roughly 17 grams of crude oil to roughly 80 cm<sup>3</sup> of deep-sea sediment, and the nmol cm<sup>-3</sup> d<sup>-1</sup> range for respiration rates (described above), it was unlikely that changes in hydrocarbon structure would have been observed with the amount of oil added. Although higher ratios of oil:sediment may have made changes easier to detect, the chosen oiling level was similar to levels observed after the Deepwater Horizon incident, as described below.

3.4. Bacterial phospholipid profiles and isotopic compositions

The five FAMES with the highest percentage composition (i.e., FAMEs 16:1, 18:1, a-15:0, 16:0, and Br-14:0) were identical for the oiled and the unamended samples (Table 1). No significant difference (p = 1.00) was observed between the two samples in the overall distribution of saturated phospholipid-derived fatty acids (PLFA), saturated-branched PLFA, mono-unsaturated PLFA, poly-unsaturated PLFA and cyclic PLFA (data not shown). Converting the total PLFA content into biomass implied cell densities of 1.2 x 10<sup>6</sup> cells g<sup>-1</sup> sediment in the unamended samples and 1.6 x 10<sup>6</sup> cells g<sup>-1</sup> sediment in the oiled sample, indicating a highly abundant bacterial community in both conditions (Deming and Carpenter, 2008).

The δ<sup>13</sup>C values for bacterially derived FAMES from the unamended and oiled samples ranged from −39.9 ‰ to −39.9 ‰ and −22.7 ‰ to −37.9 ‰, respectively (Table 1). No statistical difference was found between the δ<sup>13</sup>C of the FAMES between the two samples (p = 0.815, paired two-sample t test). The Δ<sup>14</sup>C of the total organic carbon of the unamended sediment was −943 ± 4 ‰ while the oiled sediment was slightly more depleted at −975 ± 4 ‰, which is expected due to the addition of radiocarbon-dead (−1000 ‰) petroleum. Note that the starting sediment used in both chambers was originally collected near natural oil seeps, and likely contained some amount of oil, too. The extracted residue (i.e., the portion of carbon that was not extractable in organic solvent) was considered in this case to be natural organic matter originally found in Gulf of Mexico sediments. The Δ<sup>14</sup>C of the extracted residues for both the control and the oiled samples are within statistical error of each other (−822 ± 4 ‰ and −829 ± 4 ‰), indicating the same age/source of natural organic matter. The Δ<sup>14</sup>C of the PLFA for the unamended sample was −670 ± 20 ‰ while the oiled sample was −698 ± 20 ‰, indicating that, for both conditions, bacteria had metabolized a significant component of a younger carbon pool not captured with these measurements. The bacterial lipids within the oiled sample contained slightly more depleted Δ<sup>14</sup>C, which could indicate either some metabolism of petroleum hydrocarbons or differences in the modern carbon pool being used by the bacteria; however, the age and nature of the modern carbon sources are not constrained and the slight difference in depletion fell within the error for the analysis.

3.5. Assessing microbial community diversity from the oil-amended BOSS time series

One goal of the MIMOSA experiment was to assess potential changes in microbial community structure over time, and how those changes related to oil exposure. To this
end, 8-μm-filtered pore fluid from the interior of the chambers was continuously collected and preserved on the seafloor with a cocktail of mercuric chloride and RNAlater® to arrest biological activity and preserve biomolecules, respectively. Upon return to the laboratory, the BOSS sample coil from the oil chamber was cut and sample fluid pooled in approximate biweekly intervals (for a total of 11 samples). An attempt was made to extract DNA from the pooled samples; however, DNA concentrations were below the limit of detection in the initial extracts. Thus, an attempt was made to amplify DNA in these extracts via whole genome amplification (WGA); a blank extraction was also included in the amplification step to account for spurious amplification of possible contaminants. Bacterial 16S rRNA gene sequences were amplified from these WGA-amplified DNA extracts, including the negative extraction control, via Illumina MiSeq, generating 677,043 sequences that passed quality control from the 12 samples (Figure 8). The negative extraction control generated 296 sequences grouped into 15 operational taxonomic units (OTUs), while the environmental samples contained between 26,214–184,237 sequences that grouped into 4–14 OTUs per sample.

To account for possible amplification of known laboratory reagent or dust-borne contaminants, we used a conservative approach to classify the OTUs into categories of definite contaminants (closest BLASTn-based phylogeny to dust, soil, or skin microbial communities), possible contaminants (an environmentally relevant marine Oceanospirallales-related OTU that was also observed in the negative control), and not contaminants (closest phylogeny to environmentally relevant sequences that were not observed in the negative control). Following this approach, four OTUs were not contaminants, and these OTUs were observed in varying proportions in the weekly samples 5, 9, 13, and 21, with a maximum relative abundance of 6% of the total sequences (Figure 8). These OTUs grouped within the Gammaproteobacteria (one with 99% sequence similarity to unknown Alteromonadales from seawater and sediment, and the other with 100% sequence similarity to marine Pseudoalteromonas spp. with known hydrocarbon degradation ability; data not shown). The possible Oceanospirallales-related contaminant was observed in all sample sequence libraries, ranging from 0.001% to 98% of the sample sequences, including in the negative control (21% of sequences; Figure 8). The combined probable and definite sequence contaminants ranged from 2 to > 99% of all sequences (Figure 8). In summary, the BOSS sampling approach was not effective in addressing microbial community structure in the oiled experiment, due to strong overprinting by contaminant DNA sequences. Because of

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<th>FAMEs and carbon pools</th>
<th>Control sediment</th>
<th>Oiled sediment</th>
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<td>Rel. ab.</td>
<td>δ13C (%)</td>
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<td>Br-14:0</td>
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<td>19 other FAMEs C13–C21</td>
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aRelative abundance.
bNot determined.
cPhospholipid fatty acids.

Table 1: Relative concentrations and isotopic compositions of fatty acid methyl esters (FAMEs) and various carbon pools from oiled and unamended (control) sediment at the end of the MIMOSA experiment. DOI: https://doi.org/10.1525/elementa.129.t1
this outcome, a similar attempt was not made on samples from the control experiment.

3.6. Microbial community diversity and abundance in sediment from the MIMOSA incubation chambers

Purified environmental DNA extracts from the experimental chambers and background sediment had DNA concentrations ranging from 20 to 50 ng DNA μL⁻¹, which translates to 2–5 mg DNA g⁻¹ sediment (Table 2). Quantitative PCR analysis revealed bacterial 16S rRNA gene copy numbers of 0.8–4.0 × 10⁸ copies g⁻¹ sediment, with the highest concentrations in the oil-amendment experiments at the end of the deployment (Table 2). These gene copy numbers translate to roughly 2–10 × 10⁷ bacterial cells g⁻¹ sediment.

These DNA extracts were amenable to direct Illumina MiSeq sequencing of bacterial and archael 16S rRNA gene sequences, without prior whole genome

![Figure 8: Summary of 16S rRNA gene amplicon sequences from the MIMOSA experiment.](image)

**Figure 8:** Summary of 16S rRNA gene amplicon sequences from the MIMOSA experiment. Relative abundance of bacterial 16S rRNA gene sequences from the whole genome amplified-DNA extracted from the BOSS samples (pooled in two-week intervals, where “W1” equals weeks 1–2, etc.) of the oiled chamber as compared to a blank extraction control (“Neg”), as grouped by sequence category (potential to be a contaminant). The total number of quality controlled sequences (n) for each sample is shown in parentheses on the y-axis. Detailed description of the rationale for each sequence category is provided in the text. DOI: [https://doi.org/10.1525/elementa.129.f8](https://doi.org/10.1525/elementa.129.f8)

**Table 2:** Summary of DNA concentration, abundance of bacterial 16S rRNA genes (as measured by quantitative PCR), and abundance of bacterial and archael 16S rRNA gene sequences after quality control (QC) filtering of amplicon libraries. DOI: [https://doi.org/10.1525/elementa.129.t2](https://doi.org/10.1525/elementa.129.t2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>DNA (mg g⁻¹ sed)</th>
<th>Bacterial 16S rRNA gene copies (× 10⁸ g⁻¹ sed)</th>
<th>Bacterial QC seqs.</th>
<th>Archaeal QC seqs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background sediment, begin</td>
<td>3.8</td>
<td>1.9 ± 0.6</td>
<td>23,051</td>
<td>34,273</td>
</tr>
<tr>
<td>Background sediment, end</td>
<td>1.9</td>
<td>1.0 ± 0.02</td>
<td>15,474</td>
<td>55,419</td>
</tr>
<tr>
<td>Oil chamber, begin</td>
<td>2.9</td>
<td>1.5 ± 0.2</td>
<td>12,160</td>
<td>38,671</td>
</tr>
<tr>
<td>Oil chamber top, end</td>
<td>5.1</td>
<td>3.5 ± 2.4</td>
<td>13,870</td>
<td>28,020</td>
</tr>
<tr>
<td>Oil chamber bottom, end</td>
<td>4.8</td>
<td>4.0 ± 0.3</td>
<td>25,381</td>
<td>20,364</td>
</tr>
<tr>
<td>Control chamber, begin</td>
<td>1.6</td>
<td>0.8 ± 0.1</td>
<td>10,745</td>
<td>38,246</td>
</tr>
<tr>
<td>Control chamber top, end</td>
<td>3.1</td>
<td>2.6 ± 0.4</td>
<td>9,894</td>
<td>32,238</td>
</tr>
<tr>
<td>Control chamber bottom, end</td>
<td>2.5</td>
<td>2.1 ± 0.2</td>
<td>21,485</td>
<td>33,986</td>
</tr>
</tbody>
</table>
amplification, generating 9,894–55,419 sequences per sample after quality control (Table 2). Overall, the microbial community composition across samples was highly similar (Figure 9), with the majority of bacterial sequences grouping within three taxonomic groups, the Epsilonproteobacteria (35 ± 13% of reads), Chloroflexi (24 ± 11%), and Deltaproteobacteria (9 ± 3%), and the majority of archaeal sequences grouping within three clades, the ANME-1 (55 ± 21%), Methanomicrobia (22 ± 16%), and Thermoplasmata (19 ± 8%). Within the Epsilonproteobacteria, two Helicobacter-related OTUs were dominant: one most closely related to *Sulfurimonas* spp., and the other to an unknown genus. The *Sulfurimonas*-related OTU was relatively more abundant in the incubation chambers at the end of the experiment as compared to the beginning or background sediment samples, although there was no difference between the oiled or the unamended treatments (Figure 9). Within the Chloroflexi, the majority of sequences grouped within the Anaerolineae (64–93% of Chloroflexi sequences) and Dehalococcoidetes (7–36%) clades (data not shown), as is common for organic-rich anaerobic sediment. The Deltaproteobacteria sequences had relatively high diversity across the Bdellovibrionales, Desulfarculales, Desulfobacterales, Desulfurellales, Desulfuromonadales, Myxococcales, and Syntrophobacterales (data not shown). Although not very abundant, Gammaproteobacteria sequences (4 ± 2% of bacteria sequences) were present in all samples. The majority of these sequences grouped within the Thiocritichales (data not shown) that comprise the clades of giant sulfur-oxidizers commonly found in Gulf of Mexico sediment. Only a minor portion of the Gammaproteobacteria sequences were phylogenetically related to groups that have been associated with aerobic hydrocarbon degradation in Gulf of Mexico seawater, such as *Alcanivorax, Colwellia, Cycloclasticus, Marinobacter, Neptunibacter* and *Oceanospirallales*.

Community dissimilarity (beta diversity) between samples (n = 8) was visualized using Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance (Figure 10). There was a clear separation of samples clustered by treatment type (i.e., oil-amendment versus unamended control). Fitting of experimental factors indicated that there was a strong influence of treatment type on clustering (r² = 0.49, p < 0.05). Time of sample collection (beginning vs. end) also appeared to have a moderate influence (r² = 0.26, p < 0.1) on clustering. The two background sediment samples were less similar compared to the other samples, likely reflecting spatial variability being more pronounced than variability associated with change over time, or that the “beginning” sediment had a starting microbial community already influenced by storage conditions.

![Figure 9: Summary of 16S rRNA gene amplicon sequences from the MIMOSA sediment chambers.](https://doi.org/10.1525/elementa.129.f9) Relative abundance of 16S rRNA gene sequences for all Bacteria (left), for the majority of Epsilonproteobacteria (middle), and for Archaea (right) in DNA extracts from sediment from the oiled chamber (OC) and control chamber (CC) at the beginning and end of the MIMOSA experiment, as well as from background (Bck) sediment collected from the MIMOSA deployment site. Samples at the end of the experiment derive from the top of the chamber exposed to seawater (top), and the bottom of the chamber in surficial sediment (bot). Left: Phylum-level bacterial groups of sequences, with the exception of phylum Proteobacteria shown by class. Middle: Family-level groups (operational taxonomic units) of the Helicobacteraceae comprising the majority of Epsilonproteobacteria sequences, with one most closely related to *Sulfurimonas* and the other of unknown genus affiliation. Right: Class-level archaeal groups of sequences. DOI: https://doi.org/10.1525/elementa.129.f9
4. Discussion
4.1. Sulfate reduction and methanogenesis spurred by oil addition

Sulfate and methane patterns during the experiment clearly show the influence of oil amendment in elevating sulfate reduction and methanogenesis (Figure 3). Reaction transport modeling indicates that microbial sulfate reduction increased during the experiment in response to the oil input (Figure 5). Both linear and exponential sulfate reduction reaction rates could simulate the observed profiles. When scaling the volumetric sulfate reduction rates predicted from the model (600–800 nmol cm\(^{-3}\) sediment d\(^{-1}\)) to the area of the reaction chamber, the calculated areal potential sulfate reduction rates of roughly 700 mmol m\(^{-2}\) d\(^{-1}\) are among the highest measured for naturally oiled sediment on the seafloor (Figure 6).

These potential sulfate reduction rates are generally higher than those reported for ex situ studies (Bowles et al., 2011). The elevated rates may reflect (1) a replenishment of electron acceptors over time via the advective pumping of the OsmoSamplers; (2) the effect of conducting the experiments at in situ temperature and pressure instead of under laboratory conditions (which are not comparable to in situ conditions unless done under high pressure to increase gas solubility); and/or (3) stimulation of the resident sediment microbial community with relatively fresh (i.e., unweathered) crude oil carbon. Hence, these potential rates may be overestimates of actual in situ rates of sediment sulfate reduction fueled by oil exposure; however, they provide the first benthic assessment of the capacity for sediment microbial communities to respond to oil inputs. Despite advection in these experiments continuously supplying sulfate and other electron acceptors, declines in the sulfate concentration during the experiment indicate that this electron acceptor would become limiting within a year’s time as compared to the oil electron donor carbon source. This limitation would occur sooner under in situ conditions, where sulfate is diffusion-limited and not resupplied by advection, suggesting that bioturbation and physical mixing processes are important for providing electron donors for oil degradation in the environment. If benthic faunal communities are negatively impacted by the presence of oil, however, the potential for biological irrigation of oiled sediment will be reduced.

The profiles of methane concentration and stable carbon isotopic composition, which revealed an increase in methanogenesis towards the end of the experiment, may also signify a contribution to oil degradation. The measured rate of methane production, however, is roughly six orders of magnitude lower than the rates of sulfate reduction.

Figure 10: Ordination of MIMOSA sediment bacterial 16S rRNA genes. Non-metric multidimensional scaling plot of the relationships between bacterial 16S rRNA gene sequences from the sediment DNA extracts, as shown in Figure 9. Top and Bottom refer to the position of the chambers relative to seawater exposure and surficial sediments, respectively, at the end of the experiment. DOI: https://doi.org/10.1525/elementa.129.f10
(nM production rates for methane versus mM declines in sulfate; Figure 3), suggesting only very sluggish oil degradation rates under methanogenic conditions relative to sulfate reducing conditions when sulfate is still available. It may be possible that methanogenesis rates associated with oil degradation would increase upon sulfate depletion, due to reduced competition between methanogens and sulfate reducers. Notably, methanogenesis appears to begin when sulfate concentrations are still relatively high (10 mM), suggesting that methanogens and sulfate reducers are utilizing different non-competitive substrates, as sulfate reducers are known to outcompete methanogens for reduced substrates when sulfate concentrations are higher than 0.1 mM (Hoehler et al., 1994).

4.2. Potential oil degradation rates and limitations
Assuming oil degradation is directly linked to organoclastic sulfate reduction, wherein one molecule of sulfate is reduced to two molecules of CO₂ produced from organic matter, then potential oil degradation rates of over 1 μmol CO₂ produced cm⁻³ sediment d⁻¹ (0.4 μmol CO₂ produced g⁻¹ sediment d⁻¹) were observed by the end of the deployment (i.e., multiplying potential sulfate reduction rates in Figure 5 by 2). Roughly 7 mmol of CO₂ would have been produced over the course of the entire experiment (i.e., the sum of the daily sulfate consumed from the linear and exponential models, multiplied by 2). These predicted potential rates of CO₂ production from oil degradation are lower by roughly an order of magnitude than rates measured in oxic and sunny oiled beach sand enrichments incubated with 1–50 mg oil g⁻¹ sediment (Singh et al., 2014). The lower rates in our study likely reflect the difference in energy availability under oxic versus anoxic and light versus dark conditions, as well as differences in physical forcing (e.g., temperature, pressure, advection).

In the oil-amendment experiment, roughly 17 g of crude oil were added, which is equivalent to about 14.3 g carbon (or 1.2 moles, assuming that carbon is roughly 84% of crude oil; Valentine et al., 2014). Thus, approximately 0.6% of the oil was degraded during the 5-month experiment (assuming the direct link between sulfate reduction and hydrocarbon degradation), which explains why the hydrocarbon profile at the beginning and end of the experiment appears unchanged (Figure 7). Although the amount of oil amendment in this experiment was relatively high (17 g of oil was mixed with 81 cm⁻³ of sediment, resulting in an oiling level of 84 mg oil g⁻¹ sediment at the beginning of the experiment) in comparison to other recent sediment microcosm experiments (e.g., Singh et al., 2014), it is within the range of observed values following the Deepwater Horizon event. Oil contamination levels after the Deepwater Horizon event were reported up to 510 mg oil per gram sediment in Gulf of Mexico coastal marsh environments (Lin and Mendelssohn, 2012), and up to 221 mg oil per gram sediment (dry) in surficial sediment around the Macondo well-head (extrapolated from hopane concentrations assuming 58 μg hopane per gram oil; Valentine et al., 2014). Thus, these findings from the initial deployment of the MIMOSA observatory suggest that the crude oil that sank to the seafloor following the Deepwater Horizon event – estimated to be 2–14% of the nearly 5 million barrels of oil spilled (Valentine et al., 2014; Chanton et al., 2015) – will be similarly limited by electron acceptor availability for degradation. Bioturbation and other physical forcing, such as bottom currents, will be important for providing sufficient oxidants to degrade the oil.

4.3. Minimal impacts on sediment microbial community structure from oil exposure
Many prior studies of oxic environments have documented changes in microbial community structure in response to oil exposure (e.g., as reviewed in Leahy and Colwell, 1990). Similarly, in response to the Deepwater Horizon event, several studies have documented changes in the microbial communities in the oxic water column, such as the stimulation of indigenous Gammaproteobacteria including the hydrocarbon degraders Colwellia, Cycloclasticus, and Oceanospirillales (Hazen et al., 2010; Redmond and Valentine, 2012; Kleindienst et al., 2015a, 2015b). Likewise, in coastal beach sands impacted by the oil spill (3,1–4,500 mg oil kg⁻¹ sand), aerobic hydrocarbon degraders such as Acinetobacter, Alcanivorax, Hyphomonas, Marinobacter, Parvibaculum, and Pseudomonas were shown to vary over time in response to oil exposure (Kostka et al., 2011; Rodríguez-R et al., 2015). Recent oil enrichment experiments with deep water from the Gulf of Mexico also documented the enhanced response of Colwellia, Cycloclasticus, and Marinobacter within a few weeks of oil exposure (Kleindienst et al., 2015b).

By contrast, anoxic sediment from this study did not exhibit marked changes in microbial community structure (based on 16S rRNA gene sequence abundance in DNA extracts) between oiled and unamended treatments, nor from the beginning to the end of the five-month deployment (Figure 9). In all cases, Epsilonproteobacteria and Chloroflexi dominated the bacterial communities, and ANME-1 type archaea dominated the archaeal communities. These groups are not known to be involved in anaerobic hydrocarbon degradation. Most known anaerobic hydrocarbon degraders from marine sediment are either sulfate-reducing bacteria from the Deltaproteobacteria (Ruerter et al., 1994; Kniekemeyer et al., 2007; Musat et al., 2008) or nitrate reducers from the Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria (Heider et al., 1999; Alain et al., 2012). These phylogenetic classes were present in relatively low and unchanging abundance in this study (Figure 9).

This lack of change in microbial community structure as observed in the DNA sequence libraries is supported by the similar lack in change in structure of the dominant fatty acids between treatments, and by the lack of difference in fatty acid carbon content (Table 1). Thus, it is unclear which microbial groups in the current study were responsible for the significant increase in sulfate reduction, assumed to be linked to any degradation of the crude oil added. Examining the RNA fraction for differential expression of 16S rRNA genes could help to resolve whether or not different microbial groups respond to oil exposure in anoxic sediment, and should be considered in future studies.
4.4. Oil impacts on sediment pore fluid element composition

The MIMOSA experimental approach collects pore fluids that are exposed to transport (e.g., advection and diffusion) and reaction processes; an assessment of ion behavior can provide clues as to the balance and interaction of these processes. For example, concentrations of boron and lithium are extremely elevated in oil field brines (Williams et al., 2001; Drusel and Rose, 2010). In our experiment, their concentrations were higher than in seawater at the start of the oiled experiment (Figure 4). With time, these ion concentrations decreased as initial fluids in the experiments were diluted with bottom seawater due to OsmoSampler-induced advection into the chambers. By the end of the experiment, both ions had reached bottom seawater values. This result does not preclude these ions from having reacted within the experimental matrix (e.g., secondary clay formation and ion exchange); however, the extent of such potential reactions would be limited, such that the resulting reactive flux would only be a small fraction of the advective flux from seawater transport through the experiment.

By contrast, reactive fluxes outpace or equal advective fluxes for many reactions that are connected to microbial sulfate reduction. One example can be seen in the calcium concentrations in the time series (Figure 4), with calcium concentrations mirroring assumed changes in alkalinity and carbonate (as calcium plays a role in maintaining carbonate equilibrium). Due to the high gas permeability of Teflon tubing allowing CO₂ exchange, carbonate could not be measured in this experiment. Oils are typically higher in alkalinity than seawater, and as such the oiled treatment starts off with a lower calcium concentration, reflecting calcium carbonate equilibrium. Before the onset of sulfate reduction, as the chamber is being flushed with seawater of lower alkalinity, the alkalinity decreases and the concentration of calcium rises to maintain carbonate equilibrium. When sulfate reduction increases, corresponding increases in alkalinity outpace the advective flux of low alkalinity seawater into the chamber. This increase in alkalinity results in lowering of calcium concentrations to maintain equilibrium with carbonate. The uniform rise in the calcium concentration in the unamended treatment is likely the result of seawater-mineral exchange, which will increase the calcium concentration and lower the alkalinity if the system is in equilibrium with carbonate, or is possibly a product from the dissolution of carbonate if the initial solution was undersaturated with respect to carbonate.

Barium and manganese also are linked to sulfate reduction: barium concentrations increase in response to barite saturation (Monnin et al., 1999), resulting from the removal of sulfate by sulfate reduction; and manganese data are a good indicator of redox state and thus sensitive to degree of sulfate reduction. In our oiled experiment, manganese concentrations likely decreased from initial conditions associated with redox conditions at the beginning of the experiment to sub-micromolar levels (Figure 4) due to reaction with carbonate and/or sulfides produced during sulfate reduction. In the unamended experiment, the observed increase in manganese concentrations with time is consistent with the removal of dissolved oxygen from bottom seawater and reduced conditions. However, the fluid within the unamended experiment was less reduced, given the lack of change in sulfate concentration. Molybdenum is also a redox sensitive element, likely scavenged by sulfide generated during sulfate reduction in the oiled experiment and partially removed with silicate minerals or oxides in the unamended experiment (Emerson and Huested, 1991; Canfield et al., 1992; Erickson and Helz, 2000). Collectively, these ion behaviors provide clues about reactions that occurred within the reactors on the seafloor, beyond the indications of activity surmised from sulfate and methane behavior.

The motivation for using the acid OsmoSamplers was to measure trace elements, especially those directly linked to oil. Vanadium and nickel are the most concentrated trace elements in crude oil, and degradation of only a small amount of crude oil could have a large impact on the dissolved concentration of these elements (Al-Abdali et al., 1996). However, no clear trends were observed in the nickel data, and there is only a hint of an increase in vanadium concentrations by the end of the oiled experiment (data not shown). Other trace elements showed no distinct trends between the two experimental conditions.

4.5. Suitability of this experimental approach for future sediment microbial activity studies

Results of this first MIMOSA study show the ability of benthic enrichment experiments conducted in situ to provide information on potential rates of microbial activity, limitations on activity, and involvement of the microbial community in reaction processes. Namely, the MIMOSA experiment demonstrated that crude oil exposure significantly stimulated sulfate reduction and methanogenesis (Figures 3, 5), with potential rates of activity that are among the highest ever measured (Figure 6), and that sulfate availability may ultimately limit oil degradation rates in marine sediment.

While the MIMOSA experiment enables experimentation on the seafloor under in situ conditions, including low temperature and high pressure that are critical for influencing gas solubility, there are some caveats to the approach that limit directly translating the results of the experiments to the in situ environment. For example, the advection through the reaction chambers caused by the withdrawal of pore fluids over time by the OsmoSamplers may generate conditions atypical of benthic sediment, although faunal communities also irrigate sediment to varying degrees. Elevated advection would cause higher replenishment of electron donors over time, possibly stimulating higher rates of activity than would occur under in situ conditions. Stimulated rates, however, can be useful for determining what factors may limit activity, such as sulfate availability. Stimulation can help set upper bounds on in situ reaction rates, which are important, for example, to constraining the rate of oil degradation and the longevity of oil exposure in the benthic environment.

The ability to conduct enrichment experiments on the seafloor under environmentally relevant conditions does
require a commitment of resources, particularly ship time and lander equipment. This commitment is modest, however, in comparison to more typical ex situ experiments to determine potential rates of activity in deep-sea sediment (e.g., Joye et al., 2004; Orcutt et al., 2005; Orcutt et al., 2010b), which also require ship time and investment in custom-built high-pressure equipment (and likely also shipboard radioisotope facilities). Hence, cost should be a minor factor in deciding if the MIMOSA approach is warranted for future benthic experiments. In contrast, the use of osmotic pumps as microbial sampling systems has much promise for improving our ability to conduct higher-resolution studies in the deep sea, as these pumps do not require electrical power and can sample for years (Jannasch et al., 2004; Wheat et al., 2011).

One drawback of the MIMOSA approach as initially undertaken concerns the inability of the biological OsmoSamplers to effectively document sediment microbial community structure over time. Only a low proportion of sediment-hosted microbes would have resided in the sampled fluid fraction (as opposed to attached to sediment particles), and the fixatives used in this BOSS system may have poorly preserved nucleic acids. Previous studies that used the osmotic pumps for biological sampling (Robidart et al., 2013) deployed these systems at two diffuse hydrothermal vents, where microbial densities in fluids were likely one to two orders of magnitude greater than in the fluids of our sediment-based experiments, contributing to the successful detection of changes in microbial community composition and abundance, as well as in the metaproteome. In the current study, these technologies were far less effective, and community structure changes were not detected. The efficacy of RNALater™ in nucleic acid preservation (at the seafloor conditions of our experiment) is greatly reduced over time (as described (Robidart et al., 2013), so the preserved biomass would have been at an even lower concentration in our experiments. Finally, the approach used here employed filtering of the fixed samples in the laboratory to capture cells prior to DNA extraction; if cells had ruptured during preservation due to the high salinity of RNALater™ solution, the DNA would not have been retained on the filter. Future MIMOSA deployments will include new preservation reagents that have, in preliminary studies, been more effective at preserving DNA. Moreover, future MIMOSA deployments will be configured to capture more fluid per unit time, to help offset the limitations imposed by working with lower cell densities. Finally, another improvement to the MIMOSA approach would be to use freshly collected sediment from the same location as the experiment deployment, to limit bias from sample storage and transplant.

5. Conclusions

The multi-month deployment of the MIMOSA experiment system (Figure 1) at a natural oil seep in the Gulf of Mexico (Figure 2) demonstrated the ability of native sediment microbial communities to respond to a significant input of crude oil, which spurred elevated sulfate reduction and methanogenesis (Figures 3, 5) and slightly higher microbial densities as reflected by extracted DNA concentrations (Table 2) without significant changes in microbial community structure (Figures 9, 10) or oil composition (Figure 7). The potential in situ rates of sulfate reduction are some of the highest reported for seafloor sediment environments impacted by oil (Figure 6). However, based on this five-month MIMOSA experiment, electron acceptor availability may become limiting in heavily oiled deep-sea environments (Figure 3), ultimately limiting degradation of the crude oil input (Figure 7). This first demonstration of MIMOSA reveals the potential for such observational research to fill gaps in understanding seafloor microbial processes under in situ conditions, especially for ephemeral and stochastic events such as oil spills. This first deployment also reveals ways to improve in the approach and enable better tracking of microbial community composition and function over time.

Data accessibility statement

All dissolved element data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (DOI: https://doi.org/10.7266/N7M043FK). 16S rRNA sequence data are archived at the NCBI’s Short Read Archive (BioSample accession numbers SAMN05853380-87) and at GRIIDC (DOI: https://doi.org/10.7266/N7Bk19DV).

Supplemental File

The supplemental file for this article can be found as follows:

- Text S1. Microbial response to oil enrichment in Gulf of Mexico sediment measured using a novel long-term benthic lander system. DOI: https://doi.org/10.1525/elementa.129.s1

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Competing interests

The authors have no competing interests to declare.

Author contributions

LLL and BNO designed the experiment with input from PRG and CGW. KSM and BNO deployed and recovered the experiment with assistance from LLL. LLL and KSM generated and analyzed gas chemistry; JD, PRG, BNO, and NS generated and analyzed DNA sequences; KJWM and GS generated and analyzed fatty acid isoproteins; and CGW generated and analyzed dissolved ions. LLL and BNO wrote the paper with input from all coauthors.

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