Supplemental Material for

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

Authors
Beth N. Orcutt[a*], Laura L. Lapham[b], Jennifer Delaney[c], Neha Sarode[c], Kathleen S. Marshall[b], Kelly J. Whaley-Martin[d], Greg Slater[d], C. Geoff Wheat[e], Peter R. Girguis[c]

Author affiliations:
a: Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, United States
b: University of Maryland, Center for Environmental Science, Cambridge, United States
c: Harvard University, Cambridge, MA, United States
d: McMaster University, School of Geography & Earth Sciences, Hamilton, Ontario, Canada
e: College or Fisheries and Ocean Sciences, University of Alaska Fairbanks, Moss Landing, CA, United States
*borcutt@bigelow.org

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 other 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.

To assess microbial activity and diversity over time, sediment pore-fluids were continuously collected from the flow-through reactors and preserved for laboratory analysis using three different OsmoSampler systems (Figure 1 in main text). Each is based on autonomous, non-mechanical, non-powered osmotically-driven pumps (OsmoPumps; Jannasch et al., 2004) to "power" *in situ* experimental systems. These "pumps" are driven by 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. The pumps pull fluid into long coils of small (~ 1.2 mm inner diameter) continuously over time, as described below. Upon recovery, tubing is cut at distinct intervals and the fluid is extracted into desired sampling containers. Since the pumping rate is dependent upon temperature, and temperature did not vary appreciably during the deployment (4.5 ± 0.9ºC; Chris Martens and Howard Mendlovitz, University of North Carolina, personal communication), time stamps for discrete samples are determined by dividing the number of days that the sampler was deployed by the number of samples obtained from the fluid sample coil.

The three types of OsmoSamplers had different purposes. One OsmoSampler was configured to provide a continuous record of methane and sulfate concentrations (and methane stable carbon isotopic composition) to assess sulfate reduction and methane generation. This component is based on the Pore Fluid Array (PFA) for continuously collecting methane and porewater samples from multiple sediment depths in gas-tight tubing, as described previously (Lapham et al., 2008b, 2013). The second OsmoSampler was configured to sample porewater for determining dissolved major, minor and trace elements to examine redox conditions and the flux of potentially toxic and/or indicator elements of oil. Trace elements such as lithium, strontium, and boron can provide a measure of oil contamination (Williams et al., 2001), and vanadium and nickel are the most concentrated trace elements in crude oil (Al-Abdali et al., 1996). These and other trace elements in pore waters are potentially toxic (Carr et al., 1996) and are mobilized in certain redox conditions, which can change as microbial communities and organic matter diagenesis evolves. This sampling system is based on the "acid addition" OsmoSampler for preserving fluid samples with acid *in situ* for trace element analysis (Wheat et al., 2010, 2011). The third OsmoSampler in MIMOSA is designed to study changes in microbial community

composition and to determine which microbial groups are involved in hydrocarbon oxidation in the deep sea. To complete this task, environmental DNA is preserved *in situ* for laboratory phylogenetic analysis of taxonomic marker genes in microbial cells. This component is based on the Biological OSmoSampler (BOSS) for preserving environmental samples *in situ* for DNA & RNA analysis (Robidart et al., 2013). This sampling record is complemented by the parallel extraction of DNA from the sediment flow-through columns at the beginning and end of the deployment. Coupled together, the three OsmoSampler systems connected to the sediment flow-through reactors provide a long-term time series of chemical and community change in amended and non-amended sediment incubated at the seafloor in deep-water settings.

The sediment flow-through reactors were designed to (1) retain oil and sediment; (2) allow seawater, sediment porewater, or microbial infiltration without loss of oil; and (3) allow continuous fluid sampling (Figure 1C in main text). Each reactor consisted of a polyvinyl chloride (PVC) column open at both ends, constructed from commercially available 1.5" pipe size PVC trap adapters and couplers (slip × slip) connected together with PVC glue. OsmoSampler tubing (described in more detail below) is attached through the center of the chamber using Delrin fittings threaded into hand-tapped ¼-28 threaded holes centered around 1/16" outer diameter holes for polyether ether ketone (PEEK) tubing. For the chemistry OsmoSamplers, the PEEK tubing end protruded 1.5 cm into the chamber, with the open end covered by 0.2 μm mesh polyethylsulfone filter material taken from commercial Rhizon® samplers (Rhizosphere Research Products, Netherlands), to filter out microorganisms from the sample. The PEEK tubing end for the biological OsmoSampler experiment terminated in a screen of 8-μm pore size nylon mesh (CellMicroSieve), to allow the collection of microbial cells without clogging with sediment. Each end of the PVC column was covered with the same nylon mesh to allow exchange of fluid and microorganisms between ambient fluids and the interior of the chamber but prevent the loss of particles. Importantly, this size of mesh also retains non-volatile fractions of crude oil inside the chamber. Laboratory investigations revealed that larger pore sizes (10, 15, 20 μm) of mesh would allow crude oil to leak out, while smaller sizes restricted fluid flow into the chamber from potential oil or sediment clogging (data not shown). The mesh was held in place using the fittings of the trap adapters. The internal volume of the PVC chambers was roughly 100 cm^3. For this deployment, one enrichment chamber was filled with sediment that had been previously collected from a natural oil seep in the Green Canyon lease block, i.e. GC600 (collected on cruise PE13-31 in June 2013 from the position 27^o 21.885N, 90^o 33.791W) and stored at 4ºC 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 4ºC until use.

In this experiment, fluid samples were continuously collected from the sediment flow-through reactors into three separate sampling lines: (1) 0.2 μm-filtered fluids were collected into copper tubing for methane and major ion analyses (presented as "CH_4" coils hereafter); (2) 0.2 μm-filtered fluids were fixed with dilute hydrochloric acid and collected into Teflon tubing for trace element analyses (presented as "ACID" coils hereafter); and (3) 8 μm-strained fluids were fixed with preservatives and collected into Teflon tubing for DNA preservation (presented as "BOSS" coils hereafter). For each sampling line, an OsmoPump with 8 membranes, which has a pumping

rate of ~ 1 ml d^{-1} at 20°C and ~ 0.5 ml d^{-1} at *in situ* temperatures of ~ 5°C (Jannasch et al., 2004), was connected to the 300 m-long coils of 1/16-inch outer diameter copper or 2-mm outer diameter FEP-Teflon tubing (~ 1.2 mm inner diameter) using plastic fittings (high pressure Valco© PEEK unions or Delrin® flangeless ¼-28 nuts and ferrules from IDEX®, respectively). The collection of OsmoPumps and sampling coils were housed within fiberglass boxes on the lander (Figure 1A in main text). The intakes for the sample coils were connected via 1/16-inch outer diameter PEEK tubing to a PVC probe tip that was placed in the seafloor at the desired sampling location (Figure 1B in main text). The PEEK tubing for the intakes was bundled into a 7 m long tether, enabling the probe tip to be placed away from the lander by a remotely operated vehicle (ROV). It is important to note that collected fluids transited through 7 m of gas-impermeable PEEK tubing before being preserved for analysis.

The sampling method for quantifying *in situ* methane was previously described (Lapham et al., 2008b). Briefly, a copper coil and all other gas-impermeable PEEK tubing is filled with deionized water prior to deployment. The intake end of tubing terminated in a piece of 0.15-mm-mesh filter material, to filter fluids *in situ* and to prevent microbial activity within the coil. A stainless steel high-pressure valve (Valco, 12 port maximum 5000 psi pressure valve, housed in an oil-filled PVC box) remains open to the sampling coil during deployment to allow sample to enter the copper coil. When the deployment is over, the valve is turned prior to recovery in order to isolate the sample in the copper coil and prevent the loss of sample from bubble formation and sample expansion during recovery. In this deployment, an ROV was required to push on a PVC rod connected to the valve (Figure 1A in main text) to close the system before recovery.

For ion concentration analysis, samples are preserved with acid *in situ*, lowering the pH to < 2 to avoid precipitation of metals during sample storage and arrest microbial activity, as described in detail elsewhere (Wheat et al., 2011). This pH lowering is accomplished by slowly injecting dilute acid into the sample stream via a PEEK tee fitting connecting the PEEK tether sample line with the Teflon sampling coil (Figure 1D in main text). The dilute acid (0.23 M trace metal-free HCl) is stored in a separate Teflon tubing coil that is connected to the outflow of a 2-membrane OsmoPump (pumps at a quarter the rate of the sample intake), which effectively pushes the dilute acid into the sample stream at the tee fitting. An additional Teflon tubing coil is attached to the inflow of the slower OsmoPump, to collect a reference fluid sample that is not fixed with acid. All Teflon tubing for this sample set is cleaned with 10% concentrated hydrochloric acid and rinsed with copious amounts of deionized water (18.2-MΩ resistance quality). For comparison to the acid OsmoSamplers collecting fluids from the sediment flow-through chambers, an additional acid OsmoSampler sampled ambient bottom water.

To determine the composition of *in situ* microbial communities with time, DNA samples are preserved in a similar manner as described above for the trace metal samples (Figure 1D in main text), with the exception that (1) the fixative is a cocktail of 15 μg ml^{-1} saturated $HgCl_2$ solution in concentrated RNALater® and (2) the Teflon coils are pre-cleaned with dilute bleach before filling with deionized water, as described elsewhere (Wheat et al., 2011; Robidart et al., 2013).

2.2. Benthic lander design, deployments, and recoveries

Briefly, landers are essentially a structural frame that supports specialized instruments for delivery to and recovery from the seafloor. Landers are designed to be released from a surface ship and land in a desired location, and then to potentially be serviced by an ROV to collect desired samples or position instruments on the seafloor. After a set period of time, the lander is retrieved by releasing an attached weight – typically through a burn-wire, mechanical, or acoustic release – that allows the device to float back to the surface. Similar to designs presented elsewhere (Tengberg et al., 1995; Bowen et al., 2000), landers used in this study had a vertical rectangular prism shape consisting of a 5-foot-wide x 5-foot-long x 7-foot tall aluminum scaffold that houses the scientific components (Figure 1A in main text). Connecting nuts and bolts were fabricated from Type 316 stainless steel that was isolated from the aluminum frame using nylon bushings. A 5-foot-wide x 5-foot-long x 1-inch-thick slotted fiberglass platform was mounted 1-foot above the lander frame bottom. Twelve 17" outer diameter glass spheres in plastic "hard hat" housings (Teledyne Benthos Inc.), plus one additional glass sphere housing an acoustic transponder (Teledyne Benthos TR-6001), provided at least 300 kg of positive buoyancy. A central aluminum post provided support to hold a sacrificial lead weight (400 lbs) underneath the platform, held in place with a "burn wire" release mechanism controlled by the acoustic transponder. Ten sacrificial zinc anodes (2.2 kg each) were mounted on the mainframe to minimize galvanic corrosion. A red-light strobe beacon (Novatech) and acoustic tracking transponder (LinkQuest TrackLink 5000) were also mounted near the top of the lander frame to aid in locating the lander during recovery operations. Recovery operations were aided by loops of aluminum positioned around the frame for attaching ropes. Landers used in this study were constructed at the Mississippi Marine Resources Institute (MMRI) machine shop. The final weights for landers with scientific instrumentation are typically ~ 550 kg in air, requiring ship cranes and winches with a lifting capacity and snap loads well beyond that weight. The size of the landers used in this study was a function of all of the scientific payload for deployment, including MIMOSA.

Placement of the landers used in this study was guided through the use of a video camera release system named I-SPIDER (Lowe et al., 2013). The lander was held under the I-SPIDER and lowered to within 10 meters of the seafloor. Live video was used to identify an optimal site for deployment, and then a signal was sent to an acoustic release, which uncoupled the lander from the I-SPIDER. The lander remained in place on the seafloor for the length of the deployment (Figure 1A, 1B in main text), and was recovered by either sending an acoustic release command to the burn wire acoustic transponder (for autonomous recovery using a shipboard deck box, Teledyne Benthos) or by pulling on a mechanical release (for recovery enabled by ROV), triggering the release of the weight and subsequent ascent of the lander under the positive buoyancy provided by the flotation. The ascending lander was tracked by sending signals to the acoustic transponder with a shipboard deck box. Upon surfacing, a smaller boat was deployed from the research vessel to connect a lift line to the lift bracket on the top of the lander, or a grappling hook was used to attach a lift line as the ship passes by the lander. With the line attached, the lander was lifted out of the water using the ship's crane or A-frame.

In this study, one benthic lander equipped with MIMOSA (Figure 1A, 1D in main text) was deployed on 11 October 2013 during cruise PE14-09 of the RV *Pelican* (Louisiana University Marine Consortium) at a known natural oil seep in the GC600 lease block (Figure 2 in main text). The lander was deployed using the I-SPIDER camera guided release system, and then the

experiment mounted on the probe tip was placed on the seafloor using the Station Service Device ROV from MMRI (Figure 1B in main text). The lander remained in position for 149 days, until 9 March 2014, at which time it was recovered during RV *Pelican* cruise PE14-14 with the aid of the ROV *Global Explorer MK3* (Deep Sea Systems International, Oceaneering International). The ROV closed the valve on the CH_4 sampling line prior to pulling the mechanical release and dropping the lead weights. These actions enabled lander ascent that occurred at roughly 40 m min^{-1}.

Upon recovery of the lander on deck, the MIMOSA sampling boxes were immediately disconnected and carried into the lab for further processing. Over the course of two hours, sample coils were disconnected from the pumps 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 immediately 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 copper tubing. Prior to disconnection from the closed valve, copper coils were crimped on both ends, enabling the copper tubing to hold up to 138 bar (determined through previous testing). The procedure for measuring *in situ* methane, sulfate and chloride concentrations in fluids stored in copper coil fluids was slightly modified from previous work (Lapham et al., 2008a). In a shore-based laboratory, each copper coil was unraveled from the most recent sample end and sampled at 2.5 m increments, corresponding to a time resolution of ~ 4 days. A 2-m-long section was crimped off for methane concentration analysis followed by a 0.5-m-long section for sulfate and chloride concentrations. For the 0.5-m-long section, the tubing was rolled through a Mini Rolling Mill (Central Machinery, model 4832) to flatten the tubing, building up enough internal pressure to break the crimp seal and express the liquid into a 2 mL centrifuge tube with o-ring seal. The samples were acidified 1:10 [vol:vol] with 0.1M phosphoric acid to drive off sulfide. An aliquot (40 μL) of each sub-sample was then diluted to 5.5 mL with 18.2-MΩ deionized water for sulfate and chloride analysis using an ion chromatograph equipped with anion exchange columns and conductivity detector (Dionex ICS-1000) according to the manufacturer protocols. Certified seawater standard (IAPSO) was used for both sulfate and chloride calibration curves. Sulfate and chloride concentrations were also measured from the acid-fixed samples as described below; results from both sample collection methods are consistent (data not shown).

For methane analyses, concentrations within one of the 2-m-long sections were below the detection limit of 40 nM. Thus, three serial 2-m-long sections were pooled within one sample in order to analyze methane concentrations and stable isotopes. Each 2-m-long section was expressed in a similar manner to the sulfate/chloride samples, taking care to minimize exposure to air and loss of pressure. This was accomplished by adapting a plastic tube with luer lock fitting and a 22-gauge needle to one end of the copper tubing. As the other end of the tubing was rolled into the Rolling Mill, the needle was placed into a helium-flushed 20 ml glass serum vial

with butyl rubber stopper, breaking the crimp seal and expressing the fluid into the glass vial. This was repeated on each of the three 2-m-sections into the same vial. Blanks were collected with degassed water within copper tubing sections to assess possible levels of contamination. Gas concentrations were determined by gas chromatography with flame-ionization detection (SRI, 8610C) according to standard protocols and compared to certified standards (Airgas). Where methane concentrations were high enough, the sample vials were sent to Florida State University for stable carbon isotope analysis using a Thermo Delta V isotope ratio mass spectrometer.

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 estimated 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 diameter). Another controlled parameter includes the rate at which bottom seawater is pulled into the chamber, which is equivalent to the pumping speed of the four OsmoSampler systems connected to the chamber (i.e., roughly 1.4 ml d^{-1}). Several assumptions were invoked. First, we assume that bottom seawater flow into the chamber occurs only within the upper half of the chamber, even though the chamber is open on both ends, as flow will find the easiest path, and 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 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 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 (85ºC 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 were below the detection limit with the Qubit® dsDNA HS Assay Kit and Fluorometer (Thermo Fisher Scientific). Environmental DNA was then amplified 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'-GTNTTACNGCGGCKGCTG]) and Archaea (assay a.9: primers Arch519wF [5'-CAGCMGCCGCGGTAA] and Arch1017R [5'-GGCCATGCACCWCCTCTC]) as described elsewhere (Hand et al., 2011).

The oiled and unamended experiment sediment chamber microbial communities 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. Crude 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). Cleaned DNA extracts were sent to Research and Testing Laboratory for Illumina MiSeq sequencing using the same conditions described above.

Raw reads were processed according to a standard RTL company pipeline for sequence quality trimming and merging using PEAR Illumina paired-end read merger (Zhang, 2014) (as per 10/26/2012 version of pipeline). The merged reads were then analyzed using Quantitative Insights Into Microbial Ecology (QIIME v1.9) pipeline (Caparaso et al., 2010b). Briefly, the reads were first screened for chimeras and then clustered into operational taxonomic units, utilizing both *de novo* and reference based (Greengenes 13_8 release methods (DeSantis et al., 2006), with USEARCH and Uclust respectively (Edgar, 2010). Representative set of sequences from operational taxonomic units (OTUs) were aligned to reference sequences using PyNAST (Caparaso et al., 2010a) and FastTree (Price et al., 2010) was used to construct the phylogenetic tree. Samples were rarefied to match the depth of the lowest covered dataset (Bacteria: 7,800; Archaea: 19,000). The QIIME generated OTU abundance table was used for ordination and environmental factor significance analyses using functions from R package Vegan (Dixon, 2003). Envfit function (with 999 permutations) was used to identify environmental factors influencing community difference in NMDS ordination space.

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. qPCR was performed on an iCycler with iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad) using Quanta PerfeCTa SYBR Green master mix (VWR). Two reactions were run for each sample, using 10× and 100× diluted DNA extract, to assess for possible inhibitors. Bacterial 16S rRNA

genes were amplified using the Bac8Fmod (5'-AGA GTT TGAT YMT GGC TCA G; Juretschko et al., 1998) and Bac338Rabc (5'-GCW GCC WCC CGT AGG WGT; Daims et al., 1999) primer combination. qPCR protocols consisted of 1 × 1 min denaturation at 95°C, followed by 40 cycles of (a) 5s denaturation at 95°C, (b) 15s annealing at 55°C, (c) 15s elongation at 68°C, (d) 5s fluorescence acquisition, and were followed by melting curve analyses. Standards were prepared from plasmids with 16S rRNA gene copies at known concentrations. Estimates of bacterial cell density were made from the gene copy numbers assuming an average of 4 gene copies per cell based on the information available in the *rrnDB* database version 4.4.4 (Stoddard et al., 2015).

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 chromatography-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 Rxi-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.

2.6. 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 gas chromatography/mass spectrometry (GC-MS). Roughly 10 grams of sediment from each sample type was extracted twice overnight at room temperature using a modified Bligh & Dyer method with 1:2:0.8 dichloromethane:methanol:phosphate buffer (Bligh and Dyer, 1959). Phospholipids were isolated from all other compounds in the total lipid extract through silica-gel chromatography, trans-esterified through methanolysis using previously isotopically characterized methanol, and eluted through a secondary silica-gal clean-up stage. Phospholipid fatty acid (PLFA) identifications and relative concentrations were determined via GC-MS on an Agilent 6890N GC (30 m × 0.32 mm DB-5 MS column, 0.25 µm film thickness) coupled to a 5973 quadrupole mass spectrometer. Operational GC-MS conditions included an initial hold for 1 minute at 40°C ramped to 130°C at 20°C min^{-1} to 160°C at 4°C min^{-1} and finally to 300°C at 8°C min^{-1}, monitoring for the mass range 50-450 m/z. The limit of quantification on the GC-MS was 1 mg L^{-1} based on the lowest concentration of alkane standard used in the calibration curves.

Fatty acid methyl esters (FAMEs) were identified using two FAME Mix reference standard mixes (Bacterial Acid Methyl Esters CP Mix, Supelco Inc. and 37 Comp Fatty-Acid Methyl Ester Mix, Supelco Inc.), mass fragmentation patterns and relative retention times. FAMEs were quantified with alkane standards C_{14} (methyl myristate, $C_{15}H_{30}O_2$, MW 242 g/mol, ≥ 99%, GC), C_{16} (methyl palmitate, $C_{17}H_{34}O_2$, MW 270 g mol^{-1}, ≥ 99 % capillary GC; Sigma Aldrich), C_{18}

(methyl stearate, $C_{19}H_{38}O_2$, MW 326 g mol^{-1}, 99% capillary GC; Sigma Aldrich) and C_{20} (methyl arachidate, $C_{21}H_{42}O_2$, MW 326 g mol^{-1}, 99% GC; Sigma Aldrich). All GC-MS data were processed using the Enhanced MSD ChemStation Software (Agilent Technologies). Bacterial cell abundance estimates in the sediments were calculated using a conversion factor of 2×10^4 cells $pmol^{-1}$ PLFA (Green and Scow, 2000).

Stable carbon isotope ratios of individual FAMEs were analyzed with an Agilent 6890 GC (30 m × 0.32 mm DB-5 MS column, 0.25 μm film thickness) coupled to a Thermo Delta Plus XP isotope ratio mass spectrometer via a Conflo III interface (GC-IRMS). The GC temperature protocol was 50°C for 1 min; 10°C min^{-1} to 150°C; 1.5°C min^{-1} to 180°C for 20 min; 10°C min^{-1} to 280°C; 15°C min^{-1} to 320°C for 15 min. $\delta^{13}C$ values were standardized against the Vienna Pee Dee Belemnite (VPDB) standard values and all GC-IRMS data was processed using the Enhanced MSD ChemStation Software (Agilent Technologies). Triplicate injections were made of each sample and standard deviation between the values for each of the FAMEs of triplicate injections were all < 0.7 ‰.

Prior to submission for radiocarbon analysis, individual PLFA compounds were collected using preparative fraction collector-gas chromatography (PC-GC) on a Gerstel PC and an Agilent 6890N GC (60 m × 0.53 mm DB-5 MS column, 0.5 μm film thickness). PLFA extracts and sediments (total organic carbon and extracted residue) were sent to National Ocean Sciences Accelerated Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institution (Maine, Massachusetts) for radiocarbon analysis through AMS. Samples were analyzed using standard operating procedures for NOSAMS (www.whoi.edu/nosams/page.do?pid=40135) using Oxalic Acid II and VPDB standards. An error ± 20 ‰ was assumed for all PLFA $\Delta^{14}C$ values and is an appropriate estimate of error for micro-scale $\Delta^{14}C$ measurements (Pearson et al., 1998)

References

Al-Abdali F, Massoud MS, Al-Ghadban AN. 1996. Bottom sediments of the Arabian Gulf-III. Trace metal contents as indicators of pollution and implications for the effect and fate of the Kuwait oil slick. *Environmental Pollution* **93**(3): 285-301.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**(8): 911-917.

Bowen MF, Bernard PJ, Gleason DE, Whitcomb LL. 2000. Elevators - Autonomous transporters for deep sea benthic sample recovery. Woods Hole, MA: Woods Hole Oceanographic Institution.

Caparaso J, Bittinger K, Bushman F, DeSantis TZ, Andersen GL, et al. 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266-267.

Caparaso J, Kuczynski J, Stombaugh J, Bittinger K, Bushman F, et al. 2010b. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**(5): 335-336.

Carr RS, Chapman DC, Presley BJ, Biedenbach JM, Robertson L, et al. 1996. Sediment porewater toxicity assessment studeies in the vicinity of offshore oil and gas production platforms in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Science* **53**: 2618-2628.

Daims H, Bruhl A, Amann R, Schleifer K-H, Wagner M. 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evalution of a more comprehensive probe set. *Systematic Applied Microbiology* **22**: 434-444.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* **75**: 5069-5072.

Dixon P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* **14**(6): 927-930.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**(19): 2460-2461.

Green CT, Scow KM. 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. *Hydrogeology Journal* **8**(1): 126-141.

Hand S, Dowd SE, Garcia-Mazcorro J, Steiner J, Suchodolski J. 2011. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. *FEMS Microbiology Ecology* **76**: 301-310.

Jannasch HW, Wheat CG, Plant JN, Kastner M, Stakes DS. 2004. Continuous chemical monitoring with osmotically pumped water samplers: OsmoSampler design and applications. *Limnology and Oceanography: Methods* **2**: 102-113.

Juretschko S, Timmermann G, Schmid M, Schleifer K-H, Pommerening-Röser A, et al. 1998. Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: Nitrosococcus mobilis and Nitrospira-like Bacteria as dominant populations. *Applied and Environmental Microbiology* **64**: 3042-3051.

Lapham L, Wilson R, Riedel M, Paull CK, Holmes E. 2013. Temporal variability of *in situ* methane concentrations in gas hydrate-bearing sediments near Bullseye Vent, Northern Cascadia Margin. *Geochemistry Geophysics Geosystems* **14**(7): 2445-2459.

Lapham LL, Chanton JP, Martens CS, Higley PD, Jannasch HW, et al. 2008a. Measuring temporal variability in pore-fluid chemistry to assess gas hydrate stability: Development of a continuous pore-fluid array. *Environ Sci Technol* **42**(19): 7368-7373. doi:10.1021/es801195m.

Lapham LL, Chanton JP, Martens CS, Higley PD, Jannasch HW, et al. 2008b. Measuring temporal variability in pore-fluid chemistry to assess gas hydrate stability: Development of a continuous Pore-Fluid Array. *Environmental Science & Technology* **42**: 7368-7373.

Lowe PM, Woolsey M, Jarnigan R, Lutken CB, Noakes B, et al. 2013. Development of I-SPIDER: a towed platform for video survey and instrument placement. I3 MTS/IEEE; San Deigo.

Pearson A, McNichol A, Schneider RJ, Von Reden KF, Zheng Y. 1998. Microscale AMS ^{14}C measurement at NOSAMS. *Radiocarbon* **40**(1): 61-75.

Price M, Dehal PS, Arkin AP. 2010. FastTree 2-Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **5**(3): e9490. doi:10.1371/journal.pone.0009490.

Robidart J, Callister SJ, Song P, Nicora CD, Wheat CG, et al. 2013. Characterizing microbial community and geochemical dyamics at hydrothermal vents using osmotically driven continuous fluid samplers. *Environmental Science & Technology* **47**(9): 4399-4407. doi:10.1021/es3037302.

Stoddard SF, Smith BJ, Hein R, Roller BRK, Schmidt TM. 2015. *rrn*DB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. *Nucleic Acids Research* **43**: D593-8. doi:10.1093/nar/gku1201.

Tengberg A, DeBovee F, Hall P, Berelson W, Chadwick D, et al. 1995. Benthic chamber and profiling landers in oceanography - a review of design, technical solutions and functioning. *Progress in Oceanography* **35**: 253-294.

Wheat CG, Jannasch HW, Fisher AT, Becker K, Sharkey J, et al. 2010. Subseafloor seawater-basalt-microbe reactions: Continuous sampling of borehole fluids in a ridge flank environment. *Geochemistry Geophysics Geosystems* **11**: Q07011. doi:10.1029/2010GC00305.

Wheat CG, Jannasch HW, Kastner M, Hulme S, Cowen J, et al. 2011. Fluid sampling from oceanic borehole observatories: design and methods for CORK activities (1990-2010), in

488 Fisher AT, Tsuji T, Petronotis K, Expedition327Scientists eds., *Proc IODP, 327*. Tokyo:
489 Integrated Ocean Drilling Program Management International, Inc.

490 Williams LB, Hervig RL, Hutcheon I. 2001. Boron isotope geochemistry during diagenesis. Part
491 II. Applications to organic-rich sediments. *Geochim Cosmochim Acta* **65**(11): 1783-1794.

492 Zhang J. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*
493 **30**(5): 614-620.
494