Microbial community structure and methane-cycling activity of subsurface sediments at Mississippi Canyon 118 before the Deepwater Horizon disaster

Sarah Underwood[a], Laura Lapham[b], Andreas Teske[a], Karen G. Lloyd[a, c, d]

Submitted to Deep-Sea Research special issue edited by Samantha Joye

[a] Dept of Marine Sciences, University of North Carolina at Chapel Hill
[b] Chesapeake Biological Laboratory
[c] Current address: Dept of Microbiology, University of Tennessee at Knoxville
[d] Corresponding author, M409 Walters Life Sciences, Knoxville, TN 37996, klloyd@utk.edu, 865-974-3441

Abstract

The Deepwater Horizon disaster caused a shift in microbial communities in Gulf of Mexico seawater, but less is known about the baseline for microbial communities in the underlying sediments. We compared 16S rRNA and functional gene sequences deriving from DNA and RNA with geochemical profiles (sulfate and methane concentrations, $\delta^{13}C$ of methane and carbon dioxide, and chloride concentrations) of a sediment gravity core from the upper continental slope of the northwestern Gulf of Mexico (MC118) in 2008, 15 km from the spill site. The highest number of archaeal sequences were ANME-1 and ANME-2 archaea in the sulfate-reducing upper core segments (12 and 42 cmbsf), ANME-1 and *Methanomicrobiales* in the middle methanogenic depths (200 and 235 cmbsf), and ANME-1 at the deepest depths (309, 400, and 424 cmbsf). The presence of *mcrA* gene transcripts showed that members of the ANME-1 group are active throughout the core and transcribe the *mcrA* gene, a key gene of methanogenesis and anaerobic methane oxidation. The bacterial community consists mostly of members of the *Deltaproteobacteria*, *Chloroflexi*, *Cytophaga*, *Epsilonproteobacteria*, and the Japan Sea Group 1 throughout the core. The commonly detected genera of gammaproteobacterial hydrocarbon-degrading bacteria in the water column are not found in this sediment survey, indicating that the benthic sediment is an unlikely reservoir for these aerobes. However, the sediments contain members of the sulfate-reducing families *Desulfobulbaceae* and *Desulfobacteraceae*, some members of which degrade and completely oxidize aromatic hydrocarbons and alkanes, and the *Desulfobacterium anilini* lineage of obligately aromatics-degrading sulfate reducers. Thus, the benthic sediments are the most likely reservoir for the active deltaproteobacterial populations that were observed repeatedly after the Deepwater

37   Horizon spill in the fall of 2010.

38

39   Subject keywords: methane, methanogenesis, sulfate reduction, anoxic sediments, deepwater

40   horizon

41

42   **1.1 Introduction**

43   Hydrocarbon seeps on the continental slope of the northern Gulf of Mexico contain a

44   combination of methane and other light hydrocarbons resulting from thermal cracking of deeply

45   buried ancient organic matter, augmented by methane produced by the biological

46   remineralization of organic matter in shallower depths with cooler, microbially-compatible

47   temperatures (Sassen et al. 2004). In areas of active hydrocarbon seepage, upward flow of these

48   deeply-sourced fluids is met by sulfate-rich seawater diffusing into surficial sediments, where

49   sulfate is quickly consumed by microbial sulfate reduction with multiple carbon sources and

50   electron donors, including methane (Lloyd et al. 2006, 2010). In most cases, the rates of sulfate-

51   dependent anaerobic oxidation of methane (AOM) at hydrocarbon seeps in the Gulf of Mexico

52   and other locations are at least an order of magnitude lower than sulfate reduction rates with

53   other substrates that are available in-situ at the same sites (Bowles et al. 2011). In surficial Gulf

54   of Mexico cold seep sediments, the archaea and bacteria that drive sulfate-dependent AOM are

55   the methanogen-affiliated archaea ANME-1a/b, ANME2a/b, and ANME-2c co-occurring with

56   deltaproteobacterial sulfate-reducing bacteria; these AOM communities are consistently

57   detectable in surficial seep sediments in the Gulf of Mexico (Lloyd et al. 2006, 2010). However,

58   in addition to well-studied and highly active seep sites that are often associated with conspicuous

59   microbial mats and chemosynthetic invertebrate communities, many sediments of the slope of

60   the Gulf of Mexico are characterized by moderate or low seepage activity. Such sediments lack

61   the macrofaunal and microbial mat indicators of surficial seepage, and the sulfate-methane

62   transition zone where AOM can be sustained by overlapping methane and sulfate in the sediment

63   column is often found meters into the sediments (Lapham et al. 2008).

64   The role of ANME archaea in sediments with a deep (more than a few tens of cm below

65   the seafloor) methane-sulfate transition zone is uncertain, since ANME archaea are often not

66   detected by 16S rRNA and functional gene sequencing in deep sediments (Biddle et al. 2006 and

67   2011, Briggs et al. 2012, Inagaki et al. 2006, Sørensen and Teske 2006). Likewise, the detection

68   of other methanogen-affiliated archaea becomes challenging and spotty in deep subsurface

69   sediments (Briggs et al. 2012; Colwell et al. 2008; Inagaki et al. 2006; Lever 2013). In response

70   to these difficulties, some uncultured archaeal groups were speculatively linked with the

71   methane cycle (i.e., Sørensen and Teske 2006). However, plausible alternate candidates for

72   sulfate-dependent methane oxidation or methanogenesis in the deep subsurface have not been

73   identified or verified; the detection problems are currently viewed as sensitivity issues that

74   should be tackled by improved primer design (Lever 2008) and more sensitive DNA or cell

75   isolation techniques (Kallmeyer et al. 2008).

76   We addressed the detection and diversity of ANME and other methanogen-affiliated

77   archaea in subsurface sediments with a detailed study of an intermediate model system, a gravity

78   core from the vicinity (10m) of methane seeps at Mississippi Canyon lease block 118 (MC118)

79   with a methane-sulfate transition zone between ca. 1 and 1.5 m depth. The scale of this core

80   (4.55 m depth) places it into the intermediate subsurface interval between small push cores that

81   are recovered from active seafloor seeps by ROV, submersible or Multicorer (ca. 0.5 m length),

82   and deep subsurface ODP and IODP cores which retrieve spatially extended methane-sulfate

83   gradients and deep methane-sulfate transition zones from organic-rich continental margin

84   sediments on a scale of 50 m and up (for example, D'Hondt et al. 2004). One goal of this study

85   was to analyze the composition of methanogenic and methane-oxidizing microbial communities

86   in the intermediate subsurface, and to compare them to highly active surficial seeps and deep

87   subsurface sediment. Specifically, we identified the depth distribution of ANME archaea as well

88   as other potentially methane-cycling groups and sulfate-reducing groups by analyzing bacterial

89   and archaeal 16S rRNA genes and key functional genes (*dsrAB, mcrA*) of dissimilatory sulfate

90   reduction (Zverlov et al. 2005; Klein et al. 2001) and AOM and methanogenesis (Spring et al.

91   1995, Luton et al. 2002; Hallam et al. 2004), respectively. Since DNA can be well-preserved in

92   anoxic cold environments (Willerslev and Cooper 2005), we also analyzed archaeal and bacterial

93   16S rRNA transcripts, as well as *dsrAB* and *mcrA* mRNA transcripts that may be more indicative

94   of the active population.

95   This sediment core was obtained two years before the Deepwater Horizon oil spill from

96   the northwestern margin of Woolsey Mound at MC118, approx. 15 km northwest of the

97   Macondo wellhead. Woolsey Mound is a transient, thermogenic, fault-controlled gas hydrate

98   system characterized by abundant gas hydrate/carbonate outcrops, bubble plumes, clam shells

99   and microbial mats (Macelloni et al. 2013, Lloyd et al. 2010). Yet, information on the

100   sedimentary microbial community below 15 cmbsf is absent. This study therefore opens up a

101 second scientific goal, to provide a sample of the sedimentary microbial community composition
102 of the upper continental slope before the Deepwater Horizon blowout. Previously, microbial
103 community analyses in the deep Gulf of Mexico have focused on brine lakes and hydrocarbon
104 seeps (Joye et al. 2009), *Beggiatoa* mats at seep sites (Mills et al. 2004), highly active cold seep
105 sediments (Lloyd et al. 2006, 2010; Bowles et al. 2011), and methane hydrate deposits (Lanoil et
106 al. 2001; Mills et al. 2003). These case studies of extreme habitats do not provide a suitable
107 baseline for the wide range of benthic sediments in the Gulf of Mexico that have been affected
108 by the Macondo blowout. The broad spectrum of all bacterial and archaeal phylotypes from this
109 core will contribute to a general baseline for microbial community structure in moderately active
110 upper continental slope sediments in the Gulf of Mexico.

111 Since the continental slope of the Gulf of Mexico experiences substantial natural oil
112 seepage (Kennicutt et al. 1988) and is likely to constitute a natural reservoir of oil-degrading
113 microbial populations, we finally address a third scientific goal which examines whether the
114 deep sedimentary community could possibly provide a source of microbial populations that are
115 primed to metabolize hydrocarbon contamination from the spill. To do this, the abundant
116 sequence datasets from the Gulf of Mexico after the Deepwater Horizon blowout (Redmond and
117 Valentine 2012, Yang et al. 2014, Arnosti et al., in review) were mined for comparative
118 identification of possible hydrocarbon-degrading microorganisms.

119

120 **2.1 Materials and Methods**

121

122 *Core collection and pore-water chemistry*. Gravity core 0408-06 was taken by R/V *Pelican* on
123 April 28, 2008, at 12:58pm, Latitude 28°51'27.8996" N, Longitude 88°29'40.3390" W, at 879 m
124 water depth to the west of the Northwest Crater (Figure 1), a region with active seepage and
125 microbial mats (Lloyd et al. 2010). Upon retrieval of the core on deck, it was cut into 1.5 m
126 sections, split lengthwise into two halves and immediately sub-sampled for methane, porewater
127 ions, dissolved inorganic carbon, and molecular biology. Horizontal gas cracks in the sediment
128 column introduced empty spaces between different core sections that were removed from the
129 reported depths for all core analyses. For methane measurements, 6 mL sediment was added to a
130 serum vial containing 1 mL 1M KOH and immediately crimp sealed with a butyl stopper and
131 shaken to stop biological activity. Methane concentrations were determined from the headspace
132 of sediment aliquots at Florida State University using a Shimadzu mini-2 gas chromatograph

equipped with a flame ionization detector. Methane and dissolved inorganic carbon (DIC) isotopes were measured by directly injecting microvolumes of headspace aliquots into a continuous flow Hewlett-Packard 5890 GC equipped with a 6 m Poroplot Q column at 35°C and a Finnigan Mat Delta S (Bremen, Germany). Isotope ratios were reported using the standard "del" notation, $\delta^{13}C$ (‰) = $(R_{(sample)}/R_{(PDB\ standard)} - 1)*1000$, where R is the ratio of the heavy to light isotope (^{13}C:^{12}C). For porewater analysis, plastic 15 ml tubes were filled completely with sediment and centrifuged. The resulting porewater was filtered at 0.2 μm, acidified with 10% HCl, and frozen at -20°C on the ship. An unacidified portion was also frozen for chloride concentrations. Sulfate and chloride concentrations were measured at Florida State University by diluting samples 1:1000 with the eluent buffer and directly injecting into a 2010i Dionex ion chromatograph (Sunnyvale, CA). For molecular biology, ~20 mL samples were placed into sterile 50 mL plastic tubes and immediately frozen in liquid nitrogen, but had to be transferred to -20ºC in the ship's freezer before the end of the cruise. They were returned to the lab packed in a large quantity of dry ice and stored at -80°C. Due to the constrains of shipboard sampling and sample demand for other analyses, molecular biology samples were not evenly spaced throughout the sediment column but turned out to represent two samples from the sulfate-rich upper sediment, and five samples from the methane-rich deep sediment layers.

DNA Extraction. Total genomic DNA was extracted using the PowerSoil DNA kit according to the manufacturers specifications (MoBio Laboratories, Inc., Carlsbad, CA). For each extraction, 0.25 g of sediment were used. A blank extraction procedure was performed in parallel and was used as a negative blank in PCR amplifications.

RNA Extraction. Total genomic RNA was extracted using the TCA RNA prep described in McIlroy et al., 2008. A blank extraction procedure was performed in parallel. For each extraction, 3-5 g of sediment, 2.6 g of 0.10 mm beads, 0.65 g of 0.45-0.50 mm beads, 16 mL 4.5 M trichloracetic acid, 1.25 mL 1 M Tris-HCl, 0.75 mL 0.5 M EDTA, 2.5 mL 10% N-lauryl sarcosinate, 2.75 mL diethylpyrocarbonate-treated water, 0.25 g Polyvinylpyrrolidone, 0.25 mL 1 M Dithiotheitol, and 0.4 mL Antifoam[TM] were added to an autoclaved bead beating canister with a total volume of 25 mL. The canisters were put in a mechanical bead beater (B. Braun Biotech International, Meisungen, Germany), shaken for 40 seconds, stopped, then shaken for another 40 seconds. After settling for a few minutes, the supernatant was transferred to a sterile 50 mL plastic tube, centrifuged for 10 minutes at 4ºC at 2500 rpm. The supernatant was then transferred again and 0.6 volume of isopropanol was added. The supernatant was allowed to sit

5

165 for 2-4 hours at -20ºC to precipitate. The tubes were then centrifuged for 1 hour at 4ºC at 3400

166 rpm. The supernatant was removed and 1.5 mL 70% ethanol was added and the tubes were

167 centrifuged for 15 minutes at 4ºC at 3400 rpm. The ethanol was added again and the tubes were

168 centrifuged under the same conditions. The ethanol was poured off and the pellet allowed to air

169 dry for 3-5 hours.   The pellet containing RNA was then resuspended in 100 μL

170 diethylpyrocarbonate-treated water. To 100 μL suspended nucleic acid, 2 μL TURBO DNase and

171 11 μL 10 X TURBO DNase Buffer were added (Ambion Inc., Austin, TX). The mixture was

172 incubated for 30 minutes at 37ºC. 2 μL DNase Inactivation Reagent were then added and flicked

173 to mix for 2 minutes. The mixture was centrifuged and the supernatant was removed and saved

174 for amplification.

175   *PCR conditions.* For each DNA extraction, a PCR and reverse transcription-PCR (RT-

176 PCR) amplification was attempted for DNA or RNA dilutions of 1:1, 1:10, 1:100, 1:1000, a

177 water blank and a DNA positive control, as well as the blank extraction control in a BioRad

178 iCycler Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA). A sample without reverse

179 transcriptase was included to check the RT-PCR samples for DNA contamination.   DNA

180 extractions were amplified using primers A8F and A1492R for archaeal, and B8F and B1492R

181 for bacterial 16S rRNA genes (Table 1). Each PCR mixture contained 2.5 μL TaKaRa FB1 10X

182 Buffer, 2.0 μL TaKaRa 2.5 mM dNTP mixture, 1 μL 10 mg/mL Bovine Serum Albumin, 0.25 μL

183 TaKaRa SpeedSTAR Hot Start Polymerase (TaKaRa Bio, Inc., Otsu, Japan), 2.0 μL A8f or B8f

184 primer (10 μM), 2.0 μL A1492r or B1492r primer (10 μM), 1 μL extracted DNA and sterile

185 water to a total volume of 25 μL. One denaturation cycle at 94ºC for 2 minutes was followed by

186 30 cycles consisting of 15 seconds denaturation at 98ºC, 15 seconds annealing at 58ºC, and 20

187 seconds elongation at 72ºC. A final elongation step at 72ºC for 10 minutes was followed by

188 storage of the PCR products at -20ºC.

189   *McrA* genes were amplified using the primers ANME-1f, ANME-1r, mcrIRDf and

190 mcrIRDf in each PCR reaction (Table 1). Each PCR contained all four of these primers mixed

191 together, using the same reaction mixture as stated above. Touchdown PCR was performed using

192 the following procedure. A denaturation cycle of 94°C for 2 minutes was followed by 20 cycles,

193 each one consisting of 5 seconds denaturation at 95°C, 15 seconds of annealing at 60°C (touch

194 down, decreasing 0.5 degrees per cycle), and 20 seconds elongation at 72°C. Thirty additional

195 PCR cycles were run at constant annealing temperature, and consisted of 5 seconds denaturation

196 at 95°C, 15 seconds annealing at 55°C, and 20 seconds elongation at 72°C, before concluding

197 with a final 2 minutes at 72°C. *McrA* genes were also amplified using only the mcrIRDf and

198 mcrIRDr primers. *DsrAB* genes were amplified using the same protocol, except the primers

199 Dsr1f and Dsr1r were used (Table 1).

200   RNA was reverse transcribed and amplified using the same primers as were used for

201 DNA amplification (Table 1). Each RT-PCR mixture contained 12.5 µL 2x One Step RNA PCR

202 Buffer, 2 µL of each primer (10 µM), 0.5 µL Rnase Inhibitor, 0.5 µL TaKaRa Ex Taq HS, 0.5 µL

203 Reverse Transcriptase XL (AMV), 1 µL sample and sterile water to a total volume of 25 µL. The

204 same temperature cycles were followed as described above except that a Reverse Transcriptase

205 step of 42°C for 15 minutes was added to the beginning. The same protocol was followed for

206 *mcrA* and *dsrAB* genes except that a reverse transcription step was added to the touchdown PCR

207 protocol described above. All PCR products were stained with ethidium bromide dye and

208 viewed on an agarose gel.

209   *Cloning and sequencing.* Gel purification was performed for PCR and RT-PCR products

210 with the following method. Approximately 24 µL of PCR product with 5 µL of blue/orange

211 loading dye (Promega) and 2 µl 50x SYBR green dye was run in a 1.8% TAE low melting point

212 agarose gel for 40 minutes at 100 V in 1x TAE buffer. The correct band was extracted on a Dark

213 Reader Transilluminator (Clare Chemical Research, Inc., Dolores, CO). The extracted band was

214 cleaned using the Ultra Clean Gel Spin DNA Purification kit according to the manufacturer's

215 specifications with two exceptions (MoBio Laboratories, Inc., Carlsbad, CA). At step 15, the

216 spin filter contains ethanol, and was centrifuged for 5 minutes instead of 30 seconds to ensure

217 ethanol removal. At step 17, 30 µL of Elution Buffer was added instead of 50 µL to obtain a

218 more concentrated product.

219   PCR products were ligated into a PCR 2.1 TOPO Cloning Vector and transformed into

220 chemically competent TOP-10 E. coli cells using the TOPO TA Cloning Kit according to the

221 manufacturer's protocol with one exception (Invitrogen, Inc., Carlsbad, CA). The vector was

222 allowed to incubate with the PCR product and salt solution for 30 minutes at room temperature

223 instead of 5 minutes. The One Shot Chemical Transformation procedure was followed according

224 to the manufacturer's protocol (Invitrogen, Inc., Carlsbad, CA). 25 µL and 100 µL of

225 transformed cells were then plated onto two minimal media agar plates containing bromo-chloro-

226 indolyl-galactopyranoside (X-GAL) and Kanamycin for blue/white screening. After 24 hours of

227 incubation at 37ºC, white colonies were picked. The colonies were re-plated after another 24

228 hours for sequencing (GENEWIZ, Inc., South Plainfield, NJ) using M13 (-20) forward primer

7

229   and M13 (-20) reverse primer.

230   Sequences were edited using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI).

231   Operational taxonomic units (OTUs) were determined using MOTHUR (Schloss et al. 2009).

232   OTUs where all members had multiple ambiguous sites at the overlap between forward and

233   reverse reads were removed due to low quality. Chimeras were identified using Pintail,

234   Greengenes (DeSantis et al. 2006) and also by Blasting 5' and 3' ends separately to check for

235   agreement. Bacterial and archaeal 16S genes and cDNA were aligned using Arb (www.arb-

236   home.de), *mcrA* genes were aligned using MUSCLE (Edgar 2004), and *dsrAB* genes were

237   aligned to an Arb database of *dsrAB* genes (Loy et el. 2009). Full-length representatives of each

238   16S OTU were included in the phylogenetic trees. Out of 1741 total clones sequences, 1097 were

239   of high enough quality to be included in the analysis. All sequences shown in trees were

240   deposited at Genbank with accession numbers (being submitted currently, numbers will be added

241   before publication).

242

243   **3.0 Results and Discussion**

244   *3.1 Geochemistry.* Authigenic carbonate was observed in the core, indicating anaerobic

245   microbial activity. The methane and sulfate concentration profiles also indicate a microbially

246   active core (Figure 2). Below a depth of about 80 cm, methane concentrations increase and vary

247   between 500 and almost 2000 μM; the noise in the data is most likely a consequence of methane

248   oversaturation, gas cavity formation in the sediment within the core liner, and subsequent

249   outgassing from the core during shipboard processing. The accumulation of methane below 80

250   cmbsf (cm below seafloor) coincides with the depletion of sulfate porewater concentrations at ca.

251   130 cmbsf, indicating sulfate-dependent microbial oxidation of methane (Figure 2). This

252   methane-sulfate interface of core 0408-06 is located at substantially greater depth than in cores

253   from the active seep area ("Northwest Crater") east to the sampling site which is a hot spot

254   marked by seafloor microbial mats, and by a narrow zone of coexisting dissolved methane and

255   porewater sulfate in the upper 5 to 10 centimeters of surficial sediment (Lloyd et al. 2010). A

256   persistent porewater sulfate background of ca. 0.2 to 0.5 mM extending throughout the length of

257   core 0408-06 is most likely explained by reoxidation of porewater sulfide due to air exposure

258   during shipboard subsampling and processing, regardless of precautions such as acidification to

259   convert all sulfide to H_2S for purging with N_2, and working quickly to avoid prolonged oxygen

260   exposure.

8

261   The δ^{13}C data for methane and DIC provide further evidence for anaerobic methane
262   oxidation at the sulfate-methane transition zone since at this depth upwardly mobile methane
263   grows progressively more ^{13}C-enriched, from the preferential oxidation of ^{12}CH$_4$ over ^{13}CH$_4$.
264   After sulfate is depleted, methane becomes progressively more ^{13}C-enriched downcore as
265   methanogenic substrates, such as DIC, have heavier δ^{13}C values. A constant background of ca.
266   500 mM chloride (data not shown) suggests that there is no direct intrusion of the subsurface
267   brines that have seismic studies identified as the main drivers of subsurface advection at MC118
268   (Macelloni et al., 2012).
269
270   *3.2 Microbial community analyses.* The archaeal and bacterial 16S rRNA gene clone libraries
271   revealed compositional patterns in relation to sulfate and methane porewater gradients and
272   geochemical zonation of the core. Phylotypes of the ANME-1 archaea were found throughout the
273   core, whereas the phylogenetically and physiologically distinct ANME-2 and ANME-3 archaea
274   (Knittel and Boetius 2009) were detected only in the sulfate-rich upper sediment layers (Figure
275   3A, Figure S1). Phylotypes related to cultured methanogens (mostly *Methanomicrobiales* and
276   some *Methanosarcinales*) were found only in the sulfate-depleted and methane-rich deeper
277   sediment layers at 200 and 235 cmbsf (Figure 3). Based on the physiology of their cultured
278   relatives, these phylotypes could represent either autotrophic, hydrogenotrophic methanogens
279   (*Methanomicrobiales*) or methylotrophic or acetoclastic methanogens (*Methanosarcinales*)
280   (Whitman et al. 2006). Representatives of the uncultured Marine Benthic Groups B and D
281   (Vetriani et al. 1999) were found mostly in the methane-rich sediment layers but also occurred in
282   smaller clone numbers in the surficial sediments. Thus, the archaeal community composition
283   falls into two modes - the sulfate-rich surface layer and the methane-rich deep sediment -
284   distinguished by different methane-cycling archaea, although they both have ANME-1 archaea.
285   When *mcrA* genes were amplified with a mixture of primers specific for ANME-1 *mcrA* genes
286   and other methanogen-affiliated archaea *mcrA* genes, only ANME-1 was amplified at all seven
287   depths that were sampled, down to 424 cmbsf (Figure 3A, Figure S2). When the primers biased
288   against ANME-1 were used alone, other methanogen-affiliated archaea were amplified:
289   *Methanomicrobiales* at 12 cmbsf, and ANME-2 and group e at 235 cmbsf. The ANME-1 archaea
290   were not just present but also active; transcripts of *mcrA* genes throughout the sediment core
291   yielded consistent ANME-1 phylotypes (Figure 3A, Figure S2).

292   The bacterial 16S rRNA gene clone libraries contained a greater number of phylum- and
293   subphylum-level lineages than the archaea; most clones were members of the
294   *Deltaproteobacteria*, *Chloroflexi*, *Bacteriodetes*, Japan Sea Group I, and unnamed bacterial
295   groups (Figures 3B, S3, and S4). The consistent detection of *Desulfobacteraceae* and
296   *Desulfobulbaceae* – highly diversified groups of sulfate-reducing and sulfur-disproportionating
297   bacteria – in sulfate-replete sediments suggested the presence of these anaerobic metabolisms.
298   Most of our *Desulfobacteraceae* and *Desulfobulbaceae* clones fall into the previously defined
299   SRB-1 and SRB-2 clusters that were found dominant at cold seep sites (Knittel et al. 2003). The
300   *Desulfobacteraceae* completely remineralize a wide range of carbon substrates, including
301   aromatics, to CO_2. More specifically, the SRB-1 cluster within the *Desulfobacteraceae* contains
302   a subcluster (SRB-1b) that are likely to either be members of the ANME-2/SRB consortia or
303   perform alkane degradation (Schreiber et al. 2010). The members of the *Desulfobulbaceae*
304   oxidize low molecular weight substrates incompletely and leave acetate as the principal end
305   product. They also include cultured representatives that specialize in the disproportionation of
306   elemental sulfur (genus *Desulfocapsa*), or retain this ability in addition to sulfate reduction
307   (genus *Desulforhopalus*) (Faurschou and Teske 1996). SRB-2 is synonymous with the Eel-2
308   cluster, a group of uncultured sulfate reducers found in hydrocarbon seep sites and strongly
309   reducing settings (Orphan et al. 2001). A newly described elemental sulfur-disproportionating
310   thermophile, *Dissulfuribacter thermophilus*, is the closest cultured representative of the SRB-2
311   cluster (Slobodkin et al. 2013). It is possible that, like *D. thermophilus*, the uncultured SRB-2 are
312   chemolithoautotrophs who disproportionate sulfur to sulfide and sulfate. Two clones represented
313   the *Desulfobacterium anilini* cluster, a group of specialists available in pure culture that
314   completely oxidize substituted aromatics and polyaromatic compounds (Galushko et al. 1999;
315   Harms et al. 1999; Kniemeyer et al. 2003; Schnell and Schink 1991). RNA-based bacterial 16S
316   sequences of the *Desulfobulbaceae* (Seep-SRB2) were identified within the sulfate-replete zone,
317   indicating likely activity of this group (Figure 3B and S3). These sulfate-reducing lineages were
318   absent in the sulfate-depleted, methanogenic depths of the core.
319   The diversity of sulfate-reducing bacteria was further explored with *dsrAB* gene
320   sequencing (Figure 3B, and S5). In contrast to the *Desulfobacteraceae* and *Desulfobulbaceae*
321   that dominated the 16S rRNA gene survey, the *dsrAB* survey yielded, almost exclusively,
322   phylotypes within the *Desulfobacterium anilini* group, a cluster of obligately aromatics-
323   degrading sulfate-reducing bacteria in the *Desulfobacteraceae* isolated repeatedly from marine

324   sediments (Teske 2010). Previous analyses of Gulf of Mexico seep sediments have also yielded

325   *dsrAB* genes from this group (Lloyd et al. 2006; Lloyd et al. 2010). A few *dsrAB* phylotypes

326   representing *Syntrophobacteraceae* were also recovered. The *dsrAB* phylotypes were only found

327   in the two upper sulfate-rich sediment samples, and not in the methane-rich, sulfate-depleted

328   sediment layers where *dsrAB* amplification was unsuccessful (Table S1).

329   After the *Deltaproteobacteria*, phylotypes of the *Chloroflexi* were found in high clone

330   abundance at MC118 (Figures 3B and S3). Following the previously proposed subdivision of the

331   *Chloroflexi* into four subphyla (Hugenholtz et al. 1998), most phylotypes from MC118 fall into

332   subdivisions I and II. Subdivision I has been populated over time with cultured genera and

333   species of heterotrophic, anaerobic filamentous bacteria, and was renamed as the proposed class

334   "Anaerolineae" (Yamada et al. 2006). Subdivision II contains the cultured dehalogenating

335   anaerobe *Dehalococcoides ethenogenes* and related dehalogenating isolates (Moe et al. 2009), as

336   well as phylotypes from shallow and deep marine sediments and the terrestrial subsurface.

337   Another group of bacterial phylotypes detected in the MC118 subsurface sediments were

338   members of the phylum *Planctomycetes*, mostly composed of uncultured phylotypes within the

339   *Phycisphaeraceae*, a family proposed on the basis of a phylogenetically distinct, heterotrophic

340   and facultatively anaerobic isolate, *Phycisphaera mikurensis*, from a marine alga (Fukunaga et

341   al. 2009). Anammox bacteria were not detected. *Bacteroidetes*, *Gammaproteobacteria*, and the

342   JS-1 group were the most frequently detected phylum-level lineages from a total of about 20

343   mutually exclusive bacterial lineages in the MC118 core (Figure S3 and S4).

344

345   ***3.3 Methodological issues and RNA-based community analysis.*** No *dsrAB* transcripts were

346   detected from any depth, although nested amplification was performed. Bacterial 16S rRNA

347   transcripts amplified at all depths except 12 cmbsf (Table S1). Archaeal 16S rRNA transcripts

348   did not amplify at any depth, but this is not likely to indicate that archaea are inactive because

349   *mcrA* transcripts were amplified from the same RNA aliquots at depths of 12 and 235 cmbsf

350   (Table S1). The lack of archaeal 16S rRNA amplification was most likely due to our failure to

351   denature rRNA molecules before the reverse transcription step (Sambrook and Russell 2001). It

352   is possible that the reverse archaeal 16S rRNA primers must access a section of the ribosomal

353   rRNA that is unavailable in its native conformation.

354   In samples where RNA was available, the results generally supported the communities
355   seen using DNA analysis. At 12 cm and 235 cm, *mcrA* RNA sequences were all from the ANME-
356   1 group, the same as the *mcrA* DNA sequences.

357

358   **4.0 Conclusions**

359

360   ***4.1 Comparing core 0408-06 to other deep sediment cores***. The MC118 sediment core 0408-06
361   provides an example where ANME-1 archaea remain detectable into the subsurface at 4 m depth,
362   using conventional 16S rRNA gene PCR and cloning as well as *mcrA* transcript sequencing. The
363   presence of ANME-1 and other methanogen-affiliated archaea at MC118 stands in contrast to
364   cloning and sequencing surveys of deep marine subsurface sediment with sulfate-methane
365   transition zones below 30 m sediment depth where methanogen-affiliated phylotypes have been
366   elusive (Inagaki et al 2006, Sørensen and Teske 2006, Biddle et al. 2006). Subsurface *mcrA* gene
367   signatures of *Methanobrevibacter sp.* (Parkes et al. 2005) have been questioned as possible
368   contamination issues (discussed in detail by Lever 2013). It is likely that core 0408-06, with a
369   ~1.5 m depth sulfate-methane transition zone, has a more active methane cycle than deep
370   subsurface cores, and therefore its methane cycling communities are dense enough to be detected
371   by PCR methods, aided by improved *mcrA* primers with better coverage (Lever 2008).
372   Following the empirical categorization of cored sediments at MC118, the core 0408-06 samples
373   would fall into the "high microbial activity" category based on methane-sulfate transition depth
374   and methane porewater concentrations (Lapham et al. 2008). The combination of geochemical
375   indicators and consistent molecular detection of methane-cycling microorganisms indicates that
376   core 0408-06 is more similar to active seepage sediments than to continental margin sediment
377   without seepage component.

378

379   ***4.2 Physiological potential of ANME-1 archaea***. Previous molecular and geochemical studies of
380   marine and estuarine sediments had provided evidence that the ANME-1 archaea were able to
381   persist in methane-rich, sulfate-depleted sediments, and grow as methanogenic archaea if
382   required (Lloyd et al. 2011). The possibility of multiple roles and capabilities for this frequently
383   detected archaeal lineage in the marine methane cycle is intriguing. Here, ANME-1 archaea
384   remain prominently detectable throughout the sediment column, and are transcriptionally active
385   in both the sulfate-rich surface sediments and the methane-rich deeper sediments. The high

12

386   concentrations of porewater methane, the $\delta^{13}C$ signature of methanogenesis, the presence of

387   diverse *Methanomicrobiales* and *Methanosarcinales*, and the absence of key genes of sulfate

388   reduction in the deep sediment indicates that this layer is indeed the methanogenic zone where

389   sulfate reducers are excluded and ANME-1 archaea would have to adapt their metabolic mode

390   accordingly.

391

392   ***4.3 Sulfate-reducing bacteria as oil spill responders***. The bacterial baseline of the sediments

393   that bear the impact of the Deepwater Horizon-derived fallout shows evidence for priming of

394   hydrocarbon-degrading potential. The sulfate-reducing bacterial community in the gravity core

395   samples from MC118 is comprised of members of the *Desulfobacteraceae* (evidenced by 16S

396   rRNA and *dsrAB* genes), the *Desulfobulbaceae* (16S rRNA genes) and the *Desulfobacterium*

397   *anilini* lineage (the majority of *dsrAB* genes and a single 16S rRNA gene clone). Although the

398   transcription of 16S rRNA from these groups indicates viability, they may not have been very

399   active since their *dsrAB* gene transcripts were below detection limit. These sulfate-reducing

400   families and clades include numerous bacterial strains and species that respond to hydrocarbon

401   availability by complete oxidation of alkanes and aromatics with sulfate as the electron acceptor

402   (Teske 2010). It has been shown that the oil-derived fallout on the seafloor near the Macondo

403   wellhead stimulated the sulfate-reducing community in the surficial sediments and catalyzed *in-*

404   *situ* enrichment of sulfate-reducing bacteria in fall 2010. For example, the *Desulfobacterales*

405   proportion of metagenomes from sediment collected in September and October 2010 near the

406   wellhead increased in relation to distant control sites (Kimes et al. 2013). Within an extensive

407   monitoring timeline from May 2010 to July 2011, clone library detection of members of the

408   *Desulfobacteraceae* and *Desulfobulbaceae* peaked in mid-October 2010 for sediments collected

409   near the wellhead (Yang et al. 2014). Inferences that connect our pre-2010 sediment phylotypes

410   of *Desulfobacteraceae* and *Desulfobulbaceae* from the MC118 gravity core with the 2010 oil

411   spill responders have to be qualified since hydrocarbon-degrading capabilities occur only

412   scattered throughout these two families. Yet, the cultured members of the *Desulfobacterium*

413   *anilini* lineage are so far exclusively specialized in sulfate-reducing aromatics degradation, and

414   therefore represent the strongest case for an inducible sedimentary reservoir of specifically

415   hydrocarbon-degrading sulfate reducers. Salt wedge-induced upward sediment advection,

416   subsurface brine flow, and – on a smaller scale – tidal pumping and bioturbation can facilitate the

417   exchange between the subsurface microbial reservoir and the sediment-water interface.

418

4.4 Outlook: Beyond the Deltaproteobacteria. These considerations on microbial hydrocarbon-degrading function have focused on the deltaproteobacterial proportion of bacteria in the MC118 sediments. However, it is important to consider the full taxonomic range of benthic microorganisms where relevant functionalities could be hiding in plain sight. Degradation of complex biopolymers and cleavage of strong covalent bonds (C-halogen or C-C) is a recurring theme among the major microbial lineages in the MC118 sediment samples. Members of the *Chloroflexi* are active in the production of polysaccharide-degrading exoenzymes (Kragelund et al. 2007), in the fermentation of carbohydrate polymers (Yamada et al. 2006), and in the reductive dehalogenation of halogenated compounds (Moe et al. 2009). Members of the *Planctomycetes* and *Bacteroidetes* stand out by their genomic potential for carbohydrate polymer utilization and also by cell attachment and capture of polysaccharides (Martinez-Garcia et al. 2012). Lineages within the uncultured benthic archaea MCG and MBG-D likely catalyze the hydrolysis and fermentative degradation of proteins and amino acids (Lloyd et al. 2013). Syntrophic associations of hydrogenotrophic methanogens and bacteria (members of the deltaproteobacterial family *Syntrophobacteraceae*) are capable of complete methanogenic remineralization of n-alkanes in a process of anaerobic microbial alkane cracking at low temperatures (Zengler et al. 1999). The sediments of the Gulf of Mexico and their resident microbial populations have been exposed to hydrocarbon seepage for millions of years, and the wide range of uncultured phyla in this survey strongly cautions against closing the book on their anaerobic hydrocarbon degradation capabilities.

450

Figure 1. A) Location of MC118 Northern Gulf of Mexico (reprinted with permission from Ma-
celloni et al. 2013). B) Close-up swath bathymetric image of MC118 with position of core 0408-
06 in the vicinity of the Northwest crater.

Figure 2. Geochemical measurements of A) SO_4^{2-} and CH_4 concentrations, B) $\delta^{13}C$ of CH_4 and
CO_2. Horizontal lines in panel A show the depths that were sampled for molecular biology.

Figure 3. Bar plot of A) archaeal 16S rRNA genes as well as *mcrA* genes and gene transcripts,
and B) bacterial 16S rRNA genes and gene transcripts as well as *dsrAB* genes. Numbers to the
right of bar plots signifiy the number of clones included in the bar plot. Table S1 lists depths with
negative PCR or RT-PCR attempts.

460

461

462

463

464

465

466

467

468

469

470

471

472

473

474

475

476

477

478

479

480

481

482

483

484

485

486

487

488

489

490

491 Table 1. Primers used in PCR and RT-PCR amplification

Primer	Target cDNA	Predicted target group	Sequence (5' to 3')	Annealing temp (°C)	Ref
A8f	16S	Archaea	TCC GGT TGA TCC TGC C	58	Teske 2002
A1492r	16S	Archaea	GGC TAC CTT GTT ACG ACT T	58	Teske 2002
B8f	16S	Bacteria	AGR GTT TGA TCC TGG CTC AG	58	Teske 2002
B1492r	16S	Bacteria	CGG CTA CCT TGT TAC GAC TT	58	Teske 2002
mcrIRDf	*mcrA*	Methanogens/ANME	TWYGACCARATMTGGYT	60	Lever 2008
McrIRDr	*mcrA*	Methanogens/ANME	ACRTTCATBGCRTARTT	60	Lever 2008
ANME-1f	*mcrA*	ANME-1	GACCAGTTGTGGTTCGGAAC	60	Lever 2008
ANME-1r	*mcrA*	ANME-1	ATCTCGAATGGCATTCCCTC	60	Lever 2008
Dsr1f	*dsrA*	SRB	ACS CAY TGG AAG CAC G	54	Wagner 1998
Dsr4r	*dsrB*	SRB	GTG TAG CAG TTA CCG CA	54	Wagner 1998

492

493

References

495

Arnosti, C., K. Ziervogel, T. Yang, and A. Teske. 2014. Oil-derived marine aggregates – hot spots of polysaccharide degradation by specialized bacterial communities. Deep-Sea Research II, in review.

16

499

500 Biddle, J.F., J.S. Lipp, M.A. Lever, K.G. Lloyd, K.B. Sørensen, R. Anderson, H.F. Fredricks, M.

501   Elvert, T.J. Kelly, D.P. Schrag, M. L. Sogin, J. E. Brenchley, A. Teske, C.H. House, and

502   K.-U. Hinrichs. 2006. Heterotrophic Archaea dominate sedimentary subsurface

503   ecosystems off Peru. *Proc. Natl. Acad. Sci. USA* 103:3846-3851.

504

505 Biddle, J.F., J.R. White, A.P. Teske, C.H. House. 2011. Metagenomics of the subsurface Brazos-

506   Trinity Basin (IODP site 1320): comparison with other sediment and pyrosequenced

507   metagenomes. *ISME Journal* 5: 1038-1047.

508

509 Bowles, M.W., V.A. Samarkin, K.M. Bowles, and S.B. Joye. 2011. Weak coupling between sul-

510   fate reduction and the anaerobic oxidation of methane in methane-rich seafloor sediments

511   during ex-situ incubation. *Geochim. Cosmochim. Acta* 75:500-519.

512

513 Briggs, B.R., F. Inagaki, Y. Morono, T. Futagami, C. Huguet, A. Rosell-Mele, T.D. Lorenson,

514   F.S. Colwell. 2012. Bacterial dominance in subseafloor sediments characterized by me-

515   thane hydrates. *FEMS Microbiol. Ecol.* 81: 88-98.

516

517 Colwell, F.S., S. Boyd, M.E. Delwiche, D.W. Reed, T.J. Phelps, and D.T. Newby. 2008. Esti-

518   mates of biogenic methane production rates in deep marine sediments at Hydrate Ridge,

519   Cascadia Margin. *Appl. Environ. Microbiol.* 74: 3444-3452.

520

521 DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., *et al*. 2006.

522   *Greengenes*, a chimera-checked 16S rRNA gene database and workbench compatible

523   with ARB. *Appl. Environ. Microbiol.* 72: 5069–5072.

524

525 D'Hondt, S., B.B. Jørgensen, D.J. Miller, A. Batzke, R. Blake, B.A. Cragg. et al. 2004. Distribu-

526   tions of microbial activities in deep subseafloor sediments. *Science* 306:2216-2221.

527

528 Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high

529   throughput. *Nuc. Acids Res.* 32: 1792-1797.

530

531   Faurschou, M. I., and A. Teske. 1996. *Desulforhopalus vacuolatus gen. nov., sp. nov.,* a new

532   moderately psychrophilic sulfate-reducing bacterium with gas vacuoles isolated from a

533   temperate estuary. *Arch*. *Microbiol*. 166: S. 160–168.

534

535   Fukunaga, Y., M. Kurahashi, Y. Sakiyama, M. Ohuchi, A. Yokota, S. Harayama. 2009. *Phycis-*

536   *phaera mikurensis*, gen. nov., sp. nov., isolated from a marine alga, and proposal of *Phy-*

537   *cispaeraceae* fam.nov., *Phycisphaerales* ord. nov., and *Phycisphaerae* classis nov. in the

538   phylum *Planctomycetes*. *J. Gen. Appl. Microbiol.* 55:267-275

539

540   Galushko, A., D. Minz, B. Schink, and F. Widdel. 1999. Anaerobic degradation of naphthalene

541   by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ.*

542   *Microbiol.* 1:415–420.

543

544   Hallam, S. J., N. Putnam, C. M. Preston, J. C. Detter, D. Rokhsar, P. Richardson, and E. F.

545   DeLong. 2004. Reverse methanogenesis: testing the hypothesis with environmental

546   genomics. *Science* 305:1457-1462.

547

548   Harms, G., K. Zengler, R. Rabus, F. Aeckersberg, D. Minz, R. Rossello-Mora, and F. Widdel.

549   1999. Anaerobic oxidation of o-xylene, m-xylene, and homologous alkylbenzenes by

550   new types of sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 65:999–1004.

551

552   Hugenholtz, P., B.M. Goebel, and N.R. Pace. 1998. Impact of culture-independent studies on the

553   emerging phylogenetic view of bacterial diversity. *J. Bacteriol*. 180:4765-4774.

554

555   Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M.A. Lever, A. Lauer, M. Suzuki, K. Takai, M.

556   Delwiche, F.S. Colwell, K.H. Nealson, K. Horikoshi, S.L. D'Hondt, and B.B. Jørgensen.

557   2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing

558   deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. USA*

559   103:2815-2820.

560

561   Joye, S.B., V.A. Samarkin, B. N. Orcutt, I, R. MacDonald, K.-U. Hinrichs, M. Elvert, A. Teske,

562   K. G. Lloyd, M. A. Lever, J. P. Montoya, and C. D. Meile. 2009. Metabolic variability in

563   seafloor brines revealed by carbon and sulphur dynamics. *Nature Geoscience* 2:349-354.

564

565   Kallmeyer, J., D. C. Smith, A. J. Spivack, S. D'Hondt. 2008. New cell extraction procedure

566   applied to deep subsurface sediments. *Limnol. Oceanogr. Methods* 6:236-245.

567

568   Kennicutt II, M.C., J.M. Brooks, and G.J. Denoux. 1988. Leakage of deep, reservoired petroleum

569   to the near surface of the Gulf of Mexico continental slope. *Mar. Chem.* 24:39 – 59.

570

571   Kimes, N.E., A.V. Callaghan, D.F. Aktas, W.L. Smith, J. Sunner, B.T. Golding. M. Drozdowska,

572   T.C. Hazen, J.M. Suflita, and P.J. Morris. 2013. Metagenomic analysis and metabolite

573   profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Hori-

574   zon oil spill. *Front. Microbiol.* 4:50

575

576   Klein, M., M. Friedrich, A.J. Roger, P. Hugenholtz, S. Fishbain, et al. 2001. Multiple lateral

577   transfers of dissimilatory sulfite reductase genes between major lineages of sulfate-

578   reducing prokaryotes. *J. Bacteriol.* 183:6028-6035.

579

580   Kniemeyer, O., T. Fischer, H. Wilkes, F.O. Glöckner, and F. Widdel. 2003. Anaerobic degrada-

581   tion of ethylbenzene by a new type of marine sulfate-reducing bacterium. *Appl. Environ.*

582   *Microbiol.* 69:760–768.

583

584   Knittel, K., A. Boetius, A. Lemke, H. Eilers, K. Lochte, O. Pfannkuche, P. Linke, and R. Amann.

585   2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sedi-

586   ments above gas hydrate (Cascadia Margin, OR). *Geomicrobiol. J.* 20:269–294.

587

588   Knittel, K. and A. Boetius 2009. Anaerobic oxidation of methane: Progress with an unknown

589   process. *Annu. Rev. Microbiol*. 63:311-334.

590

591   Kragelund, C., C. Levantesi, A. Borger, K. Thelen, D. Eikelboom, V. Tandoi, et al. 2007. Identi-

592   ty, abundance and ecophysiology of filamontous *Chloroflexi* species present in activated

593   sludge treatment plants. *FEMS Microbiol. Ecol*. 59:671-682.

594

595 Lanoil, B.D., R. Sassen, M.T. La Duc, S.T. Sweet, and K.H. Nealson. 2001. Bacteria and archaea
596   physically associated with Gulf of Mexico gas hydrates. *Appl. Environ. Microbiol*.
597   67:5143-5153.

598

599 Lapham, L. L., J. P. Chanton, C. S. Martens, K. Sleeper, and J. R. Woolsey. 2008. Microbial
600   activity in surficial sediments overlying acoustic wipeout zones at a Gulf of Mexico cold
601   seep. *Geochem. Geophys. Geosyst*., 9. Q06001, http://dx.doi.org/10.1029/
602   2008GC001944.

603 Lever M.A., 2008. Anaerobic carbon cycling pathways in the deep subseafloor investigated via
604   functional genes, chemical gradients, stable carbon isotopes, and thermodynamic
605   calculations. Chapel Hill, NC: University of North Carolina.

606

607 Lever, M.A. 2013. Functional gene surveys from ocean drilling expeditions – a review and
608   perspective. *FEMS Microbiol. Ecol*. 84:1-23.

609

610 Lloyd, K.G., L. Lapham, and A. Teske. 2006. An anaerobic methane-oxidizing community of
611   ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Appl. Environ. Microbiol*.
612   72:7218-7230.

613

614 Lloyd, K.G., D. Albert, J.F. Biddle, L. Chanton, O. Pizarro, and A. Teske. 2010. Spatial structure
615   and activity of sedimentary microbial communities underlying a *Beggiatoa* spp. mat in a
616   Gulf of Mexico hydrocarbon seep. *PLoS ONE* 5(1): e8738.
617   doi:10.1371/journal.pone.0008738.

618

619 Lloyd, K. G., M. Alperin, and A. Teske. 2011. Environmental evidence for net methane
620   production and oxidation in putative Anaerobic MEthanotrophic (ANME) archaea.
621   *Environ. Microbiol*. 13:2548-2564.

622

623 Lloyd, K.G., L. Schreiber, D.G. Petersen, K.U. Kjeldsen, M.A. Lever, A.D. Steen, R.
624   Stepanauskas, M. Richter, S. Kleindienst, S. Lenk, A. Schramm, and B.B. Jørgensen.
625   2013. Predominant archaea in marine sediments degrade detrital proteins. *Nature*
626   496:215-218.

627

628 Loy, A., S. Duller, C. Baranyl, M. Mussman, J. Ott, O. Beja, et al. 2009. Reverse dissimilatory

629   sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes.

630   *Environ. Microbiol*. 11:289-299.

631

632 Luton, P. E., J. M. Wayne, R. J. Sharp, and P. W. Riley. 2002. The *mcrA* gene as an alternative to

633   16S rRNA in the phylogenetic analysis of methanogen populations in landfill.

634   *Microbiology* 148:3521–3530.

635

636 Martinez-Garcia, M., D.M. Brazel, B.K. Swan, C. Arnosti, P.S.G. Chain, et al. 2012. Capturing

637   single cell genomes of active polysaccharide degraders: an unexpected contribution of

638   *Verrucomicrobia*. PLoS One 7(4) 335314; doe:10.1371/journal.pone.0035314

639

640 Macelloni, L., A. Simonetti, J. H. Knapp, C. C. Knapp, C. B. Lutken, and L. L. Lapham. 2012.

641   Multiple resolution seismic imaging of a shallow hydrocarbon plumbing system, Woolsey

642   Mound, Northern Gulf of Mexico. *Mar. Petrol. Geol*. 38: 128-142.

643

644 Macelloni, L., C.A. Brunner, S. Caruso, C.B. Lutken, M. D'Emidio, and L.L. Lapham. 2013.

645   Spatial distribution of seafloor geo-biological and geochemical processes as proxies of

646   fluid flow regime and evolution of a carbonate/hydrates mound in the northern Gulf of

647   Mexico. *Deep-Sea Res. I* 74: 25-38

648

649 McIlroy, S., K. Porter, R. J. Seviour, and D. Tillett. 2008. Method for Simultaneous Isolation of

650   Microbial RNA and DNA from problematic populations. *Appl. Environ. Microbiol*.

651   74:6806-6807.

652

653 Mills, H.J., R.J. Martinez, S. Story, and P.A. Sobecky. 2004. Identification of members of the

654   metabolically active microbial populations associated with *Beggiatoa* species mat

655   communities from Gulf of Mexico cold-seep sediments. *Appl. Environ. Microbiol*.

656   70:5447-5458.

657

658 Mills, H.J., C. Hodges, K. Wilson, I.R. MacDonald, and P.A. Sobecky. 2003. Microbial diversity

659   in sediments associated with surface-breaching gas hydrate mounds in the Gulf of

660   Mexico. *FEMS Microbiol. Ecol*. 46:39-52.

661

662 Moe, W.M., J. Yan, M.F. Nobre, M.S. da Costa, and F.A. Rainey. 2009. *Dehalogenimonas*

663   *lykanthroporepellens* gen. nov., sp. nov. a reductively dehalogenating bacterium isolated

664   from chlorinated solvent-contaminated groundwater. *J. Int. Syst. Evol. Microbiol.*

665   59:2692-2697.

666

667 Orcutt, B., A. Boetius, M. Elvert, V. Samarkin, and S.B. Joye. 2005. Molecular biogeochemistry

668   of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of

669   Mexico cold seeps. *Geochim. Cosmochim. Acta*. 69: 4267-4281.

670

671 Orphan, V. J., K.-U. Hinrichs, W. Ussler III, C. K. Paull, L. T. Taylor, S. P. Sylva, et al. 2001.

672   Comparative Analysis of Methane-Oxidizing Archaea and Sulfate-reducing Bacteria in

673   Anoxic Marine Sediments. *Appl. Environ. Microbiol.* 67:1922-1934.

674

675 Redmond, M.C., and D.L. Valentine. 2012. Natural gas and temperature structured a microbial

676   community response to the Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci. USA*

677   109:20292-20297.

678

679 Reeburgh, W. S. 1996. ''SOFT SPOTs'' in the Global Methane Budget, 342 pp., Kluwer Acad.,

680   Andover, U. K.

681

682 Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring

683   Harbor Laboratory Press, Cold Spring Harbor, NY.

684

685 Sassen, R., H.H. Roberts, R. Carney, A.V. Milkov, D.A. DeFreitas, B. Lanoil, and C. Zhang.

686   2004. Free hydrocarbon gas, gas hydrate, and authigenic minerals in chemosynthetic

687   communities of the northern Gulf of Mexico continental slope: relation to microbial

688   processes. *Chem. Geol*. 205:195-217.

689

690 Schnell, S., and B. Schink. 1991. Anaerobic aniline degradation via reductive deamination of 4-

691   aminobenzoyl-CoA in *Desulfobacterium anilini*. *Arch. Microbiol*. 155:183-190.

692

693   Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, *et al*. 2009. In-

694   troducing mothur: open-source, platform-independent, community-supported software for

695   describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75:7537–

696   7541.

697

698   Schreiber, L., T. Holler, K. Knittel, A. Meyerdierks, and R. Amann. 2010. Identification of the

699   dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2

700   clade. *Environ. Microbiol.* 12:2327-2340.

701

702   Slobodkin, A.I., A.-L. Reysenbach, G.B. Slobodkina, T.V. Kolganova, N.A. Kostrikina, and E.A.

703   Bonch-Osmolovskaya. 2013. *Dissulfuribacter thermophilus* gen. nov., sp. nov., a

704   thermophilic, autotrophic, sulfur-disproportionating, deeply branching

705   deltaproteobacterium from a deep-sea hydrothermal vent. *Int. J. Syst. Evol. Microbiol.*

706   63:1967-1971.

707

708   Springer, E., M. S. Sachs, C. R. Woese, and D. R. Boone. 1995. Partial gene sequences for the A

709   subunit of methyl-coenzyme M reductase (*mcrA*) as a phylogenetic tool for the family

710   *Methanosarcinaceae*. *Int. J. Syst. Bacteriol*. 45:554–559.

711

712   Sørensen, K.B., and A. Teske. 2006. Stratified communities of active archaea in deep marine

713   subsurface sediments. *Appl. Environ. Microbiol.* 72:4596-4603.

714

715   Teske, A., K.-U. Hinrichs, V. Edgcomb, A. de Vera Gomez, D. Kysela, S. P. Sylva, M. L. Sogin,

716   and H. W. Jannasch. 2002. Microbial diversity in hydrothermal sediments in the Guaymas

717   Basin: Evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol*.

718   68:1994-2007.

719

720   Teske, A. 2010. Sulfate-reducing and methanogenic hydrocarbon-oxidizing microbial

721   communities in the marine environment. Part 21: Microbial Communities based on

722   hydrocarbons, oils and fats: Natural habitats. Pp. 2203-2223. Handbook of Hydrocarbon

723   and Lipid Microbiology, Edited by Kenneth Timmis. Springer, DOI 10.1007/978-3-540-
724   77587-4_160

725

726   Vetriani, C., H.W. Jannasch, B.J. MacGregor, D.A. Stahl, and A.-L. Reysenbach. 1999.
727   Population structure and phylogenetic characterization of marine benthic archaea in deep-
728   sea sediments. *Appl. Environ. Microbiol.* 65:4375–4384.

729

730   Wagner, M., A.J. Roger, J.L. Flax, G.A. Brusseau, and D.A. Stahl. 1998. Phylogeny of
731   dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J.*
732   *Bacteriol.* 180:2975-2982.

733

734   Whitman, W.B., T.L. Bowen, and D.R. Boone. 2006. The Methanogenic Bacteria, in *The*
735   *Prokaryotes, 3rd edition.*, Eds M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and
736   E. Stackebrandt (New York, Springer), 3:165-207.

737

738   Willerslev E, and A. Cooper. 2005. Ancient DNA. *Proceedings of the Royal Society B*. 272:3–16.

739

740   Yamada, T., Y. Sekiguchi, S. Hanada, H. Imachi, A. Ohashi, H. Harada, and Y. Kamagata. 2006.
741   *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov, sp. nov., and
742   *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of
743   the new classes *Anaerolinea* classis nov., and *Caldilineae* classis nov., in the bacterial
744   phylum *Chloroflexi*. Int. J. Syst. Evol. Microbiol. 56:1331-1340.

745

746   Yang, T., L. M. Nigro, T. Gutierrez, L. D'Ambrosio, S. B. Joye, R. Highsmith, A. Teske. 2014.
747   Pulsed blooms and persistent oil-degrading bacterial populations in the water column
748   during and after the Deepwater Horizon blowout. Deep-Sea Res. II, early online.
749   http://dx.doi.org/10.1016/j.dsr2.2014.01.014

750

751   Yang. T. K. Speare, S. Joye, and A. Teske. 2014. Bacterial community dynamics in oil-polluted
752   seafloor sediment (May 2010-July 2011). Poster Presentation at the 2014 Gulf of Mexico
753   Oilspill and Ecosystem Science Conference, January 27-30, Mobile, AL.

754

755   Zengler, K., H. H. Richnow, R. Rosselló-Mora, W. Michaelis, F. Widdel. 1999. Methane

756   formation from long-chain alkanes by anaerobic microorganisms. Nature. 401:266-269.

757

758   Zverlov, V., M. Klein, S. Lücker, M.W. Friedrich, J. Kellermann, D.A. Stahl, A. Loy, and M.

759   Wagner. 2005. Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited. *J.*

760   *Bacteriol.* 187:2203-2208.

A

B

A

SO$_4^{2-}$ (mM)

B

δ^{13}C (‰)

A

Percentage of clones

Sediment depth

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

12 cm
- 16S DNA — 36
- mcrA DNA — 88
- mcrA RNA — 73
- mcrA DNA* — 75

42 cm
- 16S DNA — 114
- mcrA DNA — 54

200 cm
- 16S DNA — 22
- mcrA DNA — 85

235 cm
- 16S DNA — 23
- mcrA DNA — 19
- mcrA RNA — 37
- mcrA DNA* — 82

309 cm
- mcrA DNA — 71

400 cm
- 16S DNA — 32
- mcrA DNA — 4

424 cm
- mcrA DNA — 33

Archaeal 16S DNA
- ANME-1b
- ANME-1a
- ANME-2a
- ANME-2c
- ANME-3
- Methanomicrobiales
- TMEG
- MBGD
- MBGB

mcrA DNA and RNA
- mcrA group a/b (ANME-1)
- group c/d (ANME-2abc)
- Methanomicrobiales
- group e

*Amplified with primer set biased against ANME-1

B

Percentage of clones

Sediment depth

12 cm
- 16S DNA — 14
- dsrAB DNA — 27

42 cm
- 16S DNA — 39
- 16S RNA — 10
- dsrAB DNA — 40

200 cm
- 16S DNA — 22

235 cm
- 16S DNA — 18

400 cm
- 16S DNA — 28
- 16S RNA — 15

424 cm
- 16S DNA — 36

Bacterial 16S DNA and RNA
- Chloroflexi subphyla I, II, IV
- Deltaproteobacteria Seep-SRB1
- Deltaproteobacteria Seep-SRB2
- D. anilini
- Desulfobulbaceae
- Epsilonproteobacteria (Sulfurimonas and Sulfurivorum)
- Bacteriodetes
- Planctomycetes
- JS1
- Thermotogales
- Unnamed groups
- Gammaproteobacteria
- Alphaproteobacteria
- Firmicutes
- SAR40
- BRC1
- Fibrobacterales
- WS3
- GN04
- OP8
- TA06
- OP3
- Caldithrix

dsrAB DNA
- Other Desulfobacteraceae
- D. anilini group
- Syntrophobacteraceae