Pulsed blooms and persistent oil-degrading bacterial populations in the water column during and after the Deepwater Horizon blowout

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Abstract. One of the defining features of the Deepwater Horizon oil spill was the rapid formation and persistence of a hydrocarbon plume in deep water. Here we use 16S rRNA gene clone libraries and pyrosequencing of 16S rRNA gene fragments to outline the temporal dynamics of the bacterial community in the water column near the Macondo wellhead. Our timeline starts with the pre-spill (March 2010) status of the water column bacterial community, continues through the bacterial enrichments dominating the hydrocarbon plume after the blowout (DWH *Oceanospirillales*, *Cycloclasticus*, *Colwellia*) in late May 2010), and leads towards post-spill bacterial communities with molecular signatures related to degradation of phytoplankton pulses (September and October 2010; July 2011) in the water column near the Macondo wellhead. We document a dramatic transition as the complex bacterial community before the oil spill was temporarily overwhelmed by a few specialized bacterial groups responding to the massive influx of hydrocarbons in May 2010. In September and October 2010, this bacterial bloom had been replaced by a diversified bacterial community which resembled its predecessor prior to the spill. Notably, the post-plume 16S rRNA gene clone libraries and pyrosequencing datasets illustrated the continued presence of oil-degrading bacteria in the water column near the Macondo wellhead which we posit to represent an inherent signature of hydrocarbon catabolic potential to the Gulf of Mexico. The pyroseqencing results detected and tracked minority bacterial populations that were not visible in the conventional 16S rRNA gene clone libraries and allowed us to identify natural reservoirs of the Deepwater Horizon *Oceanospirillales* within and outside of the Gulf of Mexico.

Keywords: Bacterial community / water column / hydrocarbon plume / *Cycloclasticus* / *Oceanospirillales* / pyrosequencing

1. Introduction

The explosion and sinking of the Deepwater Horizon platform discharged oil and gas into the Gulf of Mexico and generated massive and long-lasting perturbations in its ecosystem (Schrope 2011). One of the defining features of the Deepwater Horizon oil spill was the formation of a deepwater hydrocarbon-enriched plume during the multiphase ejection of gas and oil from the wellhead. The plume was positioned between approx. 1000 and 1300 m depth due to preferential entrainment of the soluble complex hydrocarbons within the deep, cold (5°C) water, and consisted mostly of light alkanes (C_1 to C_3), BTEX, submicrometer-size oil droplets (Ryerson et al. 2012, Reddy et al. 2012); it also entrained the dispersant compound dioctyl sodium sulfosuccinate (DOSS) (Kujawinski et al. 2011). The deep plume was detected initially in early May 2010 (Diercks et al. 2010b), and its gradual spread was monitored throughout the summer of 2010 (Hazen et al. 2010, Camilli et al. 2010, Kessler et al. 2011, Joye et al. 2011b) by tracking local oxygen depletion and C-DOM fluorescence maxima as proxies for the presence of hydrocarbons and microbial activity (Diercks et al. 2010b, Wade et al. 2011). However, tracking the evolving composition of the bacterial community in the oil-impacted water column, including the deep hydrocarbon plume, during 2010 was an extraordinary challenge.

Initially, changes of the microbial community in the water column were inferred from Phylochip® analyses of oil degrading communities (Hazen et al. 2010), or from models of methane, ethane and propane dynamics (Valentine et al. 2010, Kessler et al.

69 2011). These studies did not provide exact information on sampling times, water depths
70 and geographical positions for their molecular data. Additional 16S rRNA gene clone
71 library datasets were recently synthesized and published with precise sampling locations
72 and times, in order to coherently survey the changing bacterial community composition
73 over the lifetime of the deep hydrocarbon plume (Redmond and Valentine 2012). In late
74 May 2010, the plume-associated bacterial community was dominated by a specific cluster
75 within the *Oceanospirillales*, subsequently termed Deep Water Horizon (DWH)
76 *Oceanospirillales*, before changing in mid-June to a community where most clones
77 grouped with the genera *Cycloclasticus*, obligate degraders of aromatic hydrocarbons,
78 and *Colwellia*, known as a genus of psychrophilic marine heterotrophic generalists. By
79 early September, the bacterial community had diversified considerably and included
80 different *Alphaproteobacteria*, multiple lineages within the *Gammaproteobacteria*,
81 *Flavobacteria*, and several other phylum-level lineages such as the *Actinobacteria*,
82 *Planctomycetes*, *Chloroflexi*, and the SAR406 cluster (Redmond and Valentine 2012).
83 Here we extend the timeline of microbial oil spill response with molecular
84 analyses of samples from March 2010 to July 2011 (Table 1). By complementing clone
85 libraries of nearly full-length 16S rRNA genes with pyrosequencing surveys of shorter
86 16S rRNA gene fragments, we combine the taxonomic precision of full-length 16S rRNA
87 genes with the high-throughout resolution of bacterial community structure enabled by
88 pyrosequencing. Specifically, we extend previous molecular analyses in three ways. 1)
89 The pre-spill (March 10, 2010) water column bacterial community is compared to post-
90 spill communities (September 12 and October 18, 2010; July 3, 2011) near the Macondo
91 wellhead with 16S rRNA gene clone libraries. 2) A water column profile near the

Macondo wellhead with samples above, within and below the deep hydrocarbon plume during its *Oceanospirillales*-dominated phase (May 31, 2010) is analyzed with conventional 16S rRNA gene clone libraries and by 16S rRNA gene fragment pyrosequencing. 3) The water column profile is compared to surface water samples contaminated with weathered oil from early May 2010 (May 5, 2010), and post-plume water samples (September 12 and October 18, 2010) from near the wellhead and east of the wellhead, using pyrosequencing.

2. Materials and Methods

2.1 Sampling. Surface and water column samples were obtained during six research cruises (Table 1). The pre-spill sample (March 10, 2010) was obtained on RV *Pelican* by CTD cast at 800 m depth, ca. 10 nautical miles northwest of the Macondo wellhead (28°50.43 N, 88°30.29 W). The water column did not show any of oxygen or CDOM anomalies (Figure S1). From May 5 to 9, Oil spill surface water samples were collected via bucket sampling from the R/V *Pelican*, and kept at ca. 4°C during and after immediate transport to Chapel Hill. Surface water sampled ca. 0.5 nautical miles from the wellhead (28°44.175 N, 88°22.335 W, May 5, 2010) showed the strongest admixture of reddish-brown weathered oil sludge, and was used for DNA sequencing. These surface seawater samples are to the best of our knowledge the first samples collected on the earliest Rapid Response cruise to the Deepwater Horizon response zone (May 5 to 9, 2010; Diercks et al. 2010a). CTD surveys during the second cruise leg (May 10 to 16, 2010) provided the first evidence of the southwest-trending hydrocarbon plume in the

115 deep water column (Diercks et al., 2010b). About three weeks later, a water column
116 profile with four depths bracketing the deepwater plume was obtained by CTD approx.
117 4.7 nautical miles southwest of the wellhead (*R/V Walton Smith*, May 31, 2010;
118 28°41.686 N, 88°26.081 W). Water samples of approx. 500 ml were collected at 800,
119 1170, 1210, and 1320 m depth. Immediately after shipboard recovery, they were filtered
120 through 47 mm diameter and 0.22 µm poresize Anodisc filters; the filters were placed on
121 dry ice until DNA extraction in Chapel Hill**.** The 1170 m and the 1210 m samples of this
122 profile represent the deepwater hydrocarbon plume, as indicated by localized oxygen
123 depletion and increased water column fluorescence measured during the CTD cast
124 (Figure S2). On September 12, almost two months after the Macondo wellhead had been
125 capped on July 15, 2010, water column filter samples were collected again at the same
126 location (*R/V Pelican*; 28°41.713 N, 88°26.073 W) to evaluate the water column bacterial
127 community at 800 and 1210 m depth (Postplume I). CTD profiles no longer detected the
128 *in-situ* indicators (localized oxygen depletion coinciding with fluorescence maximum) of
129 the deep hydrocarbon plume (Figure S3), consistent with the deepwater circulation of the
130 Gulf of Mexico that moved the deep hydrocarbon plume in a southwesterly direction
131 already at the onset of the spill (Diercks et al. 2010b)**.** A negative control sample
132 (Postplume II) was obtained 37 nautical miles east of the wellhead (28°40.503 N,
133 87°39.250 W) at a depth of 1052 m (*R/V Cape Hatteras*, October 18, 2010). Due to the
134 predominantly west and southwest deepwater current pattern in this area, this sample was
135 unlikely to have been in contact with the Macondo wellhead and any residual
136 hydrocarbon leakage at this location. In July 2011, the water column near the Macondo
137 wellhead was sampled again (July 3; *R/V Endeavor*; 28°42.177 N, 88°21.240 W), to

138 initiate a multiannual survey of water column microbial community structure (Postplume
139 III). In the home laboratory, DNA extraction from filters (Teske et al. 2011), 16S rRNA
140 gene amplification with previously described 16S rRNA gene primers (Teske et al. 2002),
141 and clone library construction were performed using standard methods, detailed in the
142 supplementary information.

143 ***2.2. Phylogenetic Analysis***. Near-complete 16S rRNA gene sequences were
144 analyzed using Sequencher (Gene Codes, Ann Arbor, MI) and compared to other
145 sequences via the Basic Local Alignment Search Tool (BLAST) of the National Center
146 for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/) (Altschul et al. 1990).
147 After construction of a general 16S rRNA alignment using the ARB phylogeny software
148 package (Ludwig et al. 2004) and the SILVA v95 database (Pruesse et al. 2007), separate
149 alignments for the *Gamma-* and *Alphaproteobacteria* were prepared with sequences for
150 related *Gammaproteobacteria* and *Alphaproteobacteria*. Sequences of well-characterized
151 pure cultures and described species were used for phylogenies whenever possible;
152 otherwise, molecular phylotypes with an informative literature history were selected to
153 anchor major phylogenetic branches of uncultured bacteria. Distance-based phylogenetic
154 trees were constructed and bootstrap checks (1000 reruns) of the tree topology were
155 performed using ARB's neighbor-joining function with Jukes-Cantor correction.
156 Sequences were deposited at NCBI Genbank with accession numbers JN015198 to
157 JN015212 and JX878917 to JX879086 (Table S1).

158 ***2.3. Pyrosequencing of partial 16S rRNA gene sequences.*** Highly variable
159 portions of 16S rRNA genes (*E.coli* positions 28 to 337) were amplified with five
160 barcoded bacterial 16S-targeted primer pairs (Table S2) to generate ca. 300 bp-long PCR

products. The PCR products were purified using MiniElute PCR Purification kit (QIAGEN) and stored in 1×TE buffer for pyrosequencing analysis using the Roche 454 GS LFX Titanium Sequencer in the Microbiome Core Facility at the University of North Carolina at Chapel Hill (www.med.unc.edu/microbiome). Raw data were trimmed and filtered using LUCY to remove poor quality reads (minimum PHRED score of 27.5) and those of less than 200 nt (Kunin et al. 2010). The 8 nt barcode was used to de-multiplex and assign reads to samples using QIIME (Caporaso et al. 2010). The reads were binned into operational taxonomic units (OTUs) at 97% sequence identity with UCLUST (Edgar, 2010) followed by selection of a representative sequence based on the most abundant unique read within each cluster. After initial phylum- and family-level identification using BLAST**,** the 300-bp fragments were imported and aligned into ARB, using the previously prepared full-length 16S rRNA gene alignments of the water column sequences, and related published sequences, as templates. In addition, the gamma- and alphaproteobacterial alignments were manually edited, and >90% of all pyrosequencing fragments could be assigned to genus- or family-level phylogenetic branches defined by 16S rRNA gene clone library sequences (Table S3). Sequence data were submitted to the European Nucleotide Archive Sequence Read Archive under the study accession number ERP002443.

3. Results and discussion

3.1. Bacterial community timeline. The timeline of bacterial community composition in the aftermath of the Deepwater Horizon blowout reveals a complex pattern of microbial

184 community succession within the oil and gas-impacted water column of the Gulf of
185 Mexico. The baseline for bacterial community composition in the Gulf of Mexico water
186 column on the eve of the Deepwater Horizon blowout is accessible thanks to a
187 serendipitous water sample, collected on March 10, 2010 at 800 m depth at the
188 Mississippi Canyon 118 Microbial Observatory, ca. 9 nautical miles northwest of the
189 Macondo wellhead (Table 1). The 16S rRNA clone library results indicated a water
190 column bacterial community where SAR11 and other *Alphaproteobacteri*a, the SAR 406
191 lineage, the deltaproteobacterial SAR324 lineage, and a complex gammaproteobacterial
192 assemblage of cultured and uncultured lineages, often within the families
193 *Oceanospirillale*s and *Alteromonadales*, constituted the dominant proportion (76%) of all
194 clones. Other phyla, such as *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, *Planctomycetes*,
195 *Verrucomicrobia*, *Gemmatimonadetes* and *Cyanobacteria* were also present (Figure 1).
196 This bacterial community matches, in phylum-level composition and in relative
197 abundance of the major community members, the open-ocean Atlantic and Pacific
198 bacterial communities from the same depth (800 m), as determined by single-cell genome
199 amplification and sequencing (Swan et al. 2011) (Figure S4). Thus, the pre-spill
200 deepwater column near the Macondo wellhead shared the microbial community of the
201 ultimate source reservoir of the Gulf of Mexico, the Atlantic Ocean.
202 In the course of the oil spill, this complex bacterial community was temporarily
203 overprinted by blooms of opportunistic bacteria that responded to the massive influx of
204 hydrocarbons. The pre-plume bacterial 16S rRNA gene clone library contrasted sharply
205 with the bacterial community composition of the oil-contaminated surface water sample
206 (May 5, 2010) and the hydrocarbon-enriched deepwater plume samples (May 31, 2010).

The 16S rRNA gene and pyrosequencing analyses of oil slick-contaminated surface water samples collected shortly after the beginning of the discharge (May 5-9 2010; RV *Pelican*) demonstrated rapid colonization of the surficial oil slick-seawater mixture by PAH-degrading bacteria of the genus *Cycloclasticus*, by oil-degrading members of the genera *Pseudoalteromonas*, *Alteromonas* and *Colwellia*, and by other heterotrophic bacterial groups (Figure 1). This microbial community formed extensive flocs of microbial exopolymeric substances (EPS), observed in the field as microbial flocs developed ubiquitously in the oil-contaminated surface waters in early May 2010 (Passow et al. 2012), and in the laboratory in roller table bottle incubations using fresh oil slick samples and Gulf of Mexico surface water (Ziervogel et al. 2012). Sinking flux of these oil slick-derived microbial EPS flocs exported the associated microbial communities into the deep Gulf of Mexico (Passow et al. 2012).

The clone libraries and pyrosequencing datasets from deep hydrocarbon plume samples (1170 and 1210 m depth) collected on May 31, 2010, were strongly dominated by members of the DWH *Oceanospirillales* cluster; *Cycloclasticus* and *Colwellia* were detected as the most substantial minority population in the pyrosequencing datasets (Figure 1; Table S3). The pyrosequencing datasets detected many bacterial groups in the plume layer that were not visible in the clone libraries, such as Deltaproteobacteria and the SAR406 lineage. The *Oceanospirillales*-dominated enrichment within the plume layer contrasted with the bacterial communities above and below the deep hydrocarbon plume (800 and 1320 m) that resembled the pre- and post-plume clone libraries by the presence – in variable proportions - of SAR11 and other *Alphaproteobacteria*, *Gammaproteobacteria*, and SAR406; these samples above and below the plume also

showed unusually high clone library representation of *Actinobacteria* (14% and 11%), *Planctomycetes* (8% in both depths), and uncultured *Deltaproteobacteria* (5% and 13%). Similar bacterial groups were recovered by pyrosequencing (Figure 1).

Based on sampling time and location, these water column samples are congruent with previous sampling surveys and bacterial community analyses of the well-documented deep hydrocarbon plume near the Macondo wellhead. Hazen et al. (2010) reported that uncultured members of the gammaproteobacterial order *Oceanospirillales* dominated 16S rRNA gene clone libraries in the deepwater plume between 1100 and 1220 m depth at the end of May 2010 (May 25 to June 2). Subsequent single-cell genome sequencing of two *Oceanospirillales* single cells revealed that they possessed genes involved in the degradation of n-alkanes and cycloalkanes (Mason et al. 2012). This genomic potential of the DWH *Oceanospirillales* is also consistent with the physiological capabilities of their close cultured relatives, *Thalassolituus oleivorans* (Yakimov et al. 2004) and *Oleispira antarctica* (Yakimov et al. 2003), which oxidize long-chain n-alkanes aerobically (Figure 2). Alkane oxidation remains to be checked in the cultured relatives *Bermanella marisrubri* (Pinhassi et al. 2009) and *Oceanoserpentilla haliotis* (Schlösser et al. 2008). Previous analyses show that the hydrocarbon plume had a strong enrichment effect on many heterotrophic genera of marine *Gammaproteobacteria*, whose 16S rRNA gene frequency had increased by 100 to 300% within the plume (Hazen et al. 2010); subsequent microarray-based phylochip analysis of DNA from hydrocarbon plume samples showed increased normalized signal intensity for functional genes involved in hydrocarbon degradation, especially alkane-1 monooxygenase among the alkane and cycloalkane-degrading genes, and a wide spectrum of dehydrogenases,

253 dioxygenases and decarboxylases involved in aromatic carboxylic acid degradation
254 (Hazen et al. 2010; Lu et al. 2012). Most likely, source populations for these genes
255 include cultured heterotrophs and hydrocarbon-degrading bacteria that were found in our
256 16S rRNA gene surveys either in plume or post-plume samples, such as *Marinobacter*,
257 *Alteromonas*, *Oleispira*, *Oceanobacter*, *Cycloclasticus*, and uncultured sister lineages of
258 the genera *Saccharophagus*, *Congregibacter* and *Fangia*.

259 The detection of *Cycloclasticus* and *Colwellia* spp. in our pyrosequencing surveys
260 of the plume samples (May 31, 2010) is consistent with the previously published clone
261 library detection of these genera in plume samples from May 26 to June 5 (Redmond and
262 Valentine 2012), and shows that these two oil-degrading genera co-occurred with DWH
263 *Oceanospirillales* in the deep plume (Figure 1). In plume samples collected two weeks
264 later (June 13 to 16, 2010), 16S rRNA gene phylotypes of the genera *Cycloclasticus* and
265 *Colwellia* predominated (Redmond and Valentine 2012); these genera were discussed as
266 bacterial catalysts of the dominant oxygen-consuming process, ethane and propane
267 oxidation, in the deep-water plume (Valentine et al. 2010). This interpretation contrasts
268 with the known substrate spectrum of *Cycloclasticus*, a genus described originally as
269 aerobic degraders of polycyclic aromatic hydrocarbons (Dyksterhouse et al. 1995).
270 *Cycloclasticus* remains recognized as an obligate degrader of these compounds (Yakimov
271 et al. 2005); several *Cycloclasticus* strains were previously isolated from Gulf of Mexico
272 sediments by enrichment with PAH substrates (Geiselbrecht et al. 1998). Thus, a likely
273 role for *Cycloclasticus* is the degradation of BTEX compounds in the plume. The
274 moderately psychrophilic genus *Colwellia*, consistently present in plume- and post-plume
275 samples (Figure 2), was selectively enriched on crude oil at 4ºC (Redmond and Valentine

276 2012) and was capable of oil degradation at in-situ temperatures of 5ºC (Bælum et al.
277 2012), consistent with the in-situ temperature of the deep Gulf of Mexico water column.
278 Viewed in context, the bacterial community in the deep plume apparently changed within
279 two weeks from being dominated by DWH *Oceanospirillales* in late May to becoming
280 dominated by *Colwellia* and *Cycloclasticus* in mid-June (Valentine et al. 2010, Redmond
281 and Valentine 2012).

282 By mid-September 2010, oxygen depletion signals, CDOM fluorescence and
283 DOSS concentrations showed that the slowly decaying deep hydrocarbon plume drifted
284 in a generally west-southwesterly direction away from the Macondo wellhead area
285 (Kessler et al. 2011; Kujawinski et al. 2011); this is consistent with our CTD profile of
286 the water column near the Macondo wellhead, recorded on Sept 12 2010, that lacks
287 hydrocarbon plume signatures (Figure S3). The post-plume 16S rRNA gene clone
288 libraries and pyrosequencing surveys of September and October 2010, and the 16S rRNA
289 gene clone library of July 2011 shared dominant bacterial groups with the clone library of
290 March 2010, indicating a partial recovery towards the pre-spill bacterial community. The
291 SAR11 *Alphaproteobacteria*, the SAR406 lineage, the deltaproteobacterial lineage
292 SAR324, and a complex assemblage of *Gammaproteobacteria* dominated the clone
293 libraries and accounted together for 81 to 88% of all post-plume clones. The
294 *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria*, *Chloroflexi* and
295 *Gemmatimonadetes* accounted for smaller proportions or remained undetected in some
296 samples (Figure 1).

297 Not only dominant phylum-level lineages, but also specific pelagic alpha- and
298 gammaproteobacterial lineages, reappeared in post-spill clone libraries: the SAR11

299 subclusters (Field et al. 1997; Figure S5); the Arctic96BD-19 group of sulfur-oxidizing
300 heterotrophs (Marshall and Morris 2013) that is prevalent in stratified, oxygen-depleted
301 conditions (Walsh et al. 2009); the uncultured AGG47 cluster associated with marine
302 snow (DeLong et al. 1993); the uncultured North Sea ZD0417 cluster (Stevens and Ulloa
303 2008), and the uncultured SAR156 lineage (Mullins et al. 1995) (Figure 3). The widely
304 distributed SUP05 lineage, a presumable sulfur oxidizer typical of oxygen-depleted water
305 columns (Walsh et al. 2009; Canfield et al. 2010), was found during and after the plume
306 stage.
307
308 ***3.2. Pyrosequencing results for surface oil slick and plume-impacted water column.***
309 The pyrosequencing results for the weathered oil mixture at the surface from May 5,
310 2010, and the water column samples of May 31, 2010 were broadly consistent with the
311 16S rRNA gene clone libraries for the same samples (Figure 1), but in addition revealed
312 bacterial populations that had remained undetected in the clone libraries (Table S3). In
313 the surface sample, pyrosequencing representation for *Cycloclasticus* (>93%),
314 *Alteromonas* (1.45%) and *Pseudoalteromonas* (1.2%) resembled the clone library results,
315 whereas *Colwellia* and *Halomonas* were detected in smaller proportions (Table S1). In
316 contrast, the alkane-degrading DWH *Oceanospirillales* accounted for near 90 and 70% of
317 the pyrosequencing reads in the two deep plume samples of late May 2010 (Table S3).
318 The DWH *Oceanospirillales* pyrosequencing reads were congruent with full-
319 length 16S rRNA gene clones of DWH *Oceanospirillales* from the Gulf of Mexico
320 (Redmond and Valentine 2012) and from the Atlantic Ocean offshore North Carolina
321 (D'Ambrosio 2011), and formed at least three distinct phylogenetic clusters (Figure 4).

322 The pyrosequencing survey also validated a diverse community of hydrocarbon-
323 degrading bacteria in the plume profile that went largely undetected in the clone libraries
324 (Table S3): The PAH-degrading genus *Cycloclasticus* remained variably detectable
325 throughout the water column. Psychrophilic heterotrophs of the genus *Colwellia* (the only
326 group detected in the plume clone libraries besides the DWH *Oceanospririllales*)
327 accounted for approx. 1 to 3 % of the pyrosequencing reads within the plume. The
328 alkane-degrading genera *Oleiphilus* and *Oleispira* were found in low abundances below
329 and within the plume. The pyrosequencing representation of the uncultured
330 gammaproteobacterial groups (AGG47, Arctic96BD19, SUP05, ZD0417, SAR156)
331 above and below the plume was strongly reduced within the plume (Table S3). A similar
332 trend was observed for *Alphaproteobacteria*. While SAR11 bacteria accounted for a tenth
333 of the pyrosequencing fragments above and below the plume, their representation
334 decreased within the plume (Figure 1). In general, pyrosequencing analysis indicated a
335 functionally and phylogenetically diversified alpha- and gammaproteobacterial
336 community in the hydrocarbon plume; pre-spill populations of uncultured bacteria and
337 oil-degrading bacteria remained detectable against the dominant plume populations of
338 DWH *Oceanospirillales*. This result is compatible with a complex functional gene
339 repertoire of plume microbial communities sampled at the same time (Lu et al. 2012).
340
341 ***3.3. Pyrosequencing results for post-plume water column***. The pyrosequencing results
342 for the post-plume water column samples of September 12, 2010, and October 18, 2010,
343 were broadly consistent with the corresponding 16S rRNA gene clone libraries (Figure 1),
344 but revealed additional bacterial populations that had not been observed in the clone

345 libraries (Table S3). The DWH *Oceanospirillales* that had disappeared from the clone
346 libraries remained detectable at low levels in the pyrosequencing dataset (up to 0.2% at
347 1200 m, Sept. 12 sample). Interestingly, the post-plume pyrosequencing datasets showed
348 that oil-degrading bacteria persisted in the water column near the Macondo wellhead,
349 although the deep hydrocarbon plume had been drifting in a southwesterly direction, and
350 was no longer detectable in the wellhead region as indicated by CTD profiling in
351 September 2010 (Figure S3). Bacterial alkane degraders (*Alcanivorax*, *Oleiphilus*,
352 *Marinobacter*) remained detectable in low proportions (<1%), and the PAH oxidizer
353 *Cycloclasticus* and relatives of gammaproteobacterial methylotrophs accounted for near
354 5 % of pyrosequencing reads in the 1210 m sample (Table S3). These results suggest
355 local sources that re-inject reservoir populations of these bacteria into the water column,
356 either from small-scale accidental leakage or natural hydrocarbon seepage (Joye et al.
357 2011b).

358 Most pyrosequencing fragments from the post-plume water column do not
359 represent specialized oil degraders; these pyrosequencing results resemble (and extend)
360 the diversified 16S rRNA gene clone library results for the same samples. Within the
361 *Gammaproteobacteria*, the cultured genera *Oceanobacterium*, *Oceanobacter*,
362 *Oceanospirillum*, *Alteromonas*, *Pseudoalteromonas*, *Halomonas*, *Idiomarina*,
363 *Marinimicrobium*, *Congregibacter*, were complemented by uncultured water column
364 lineages (two different AGG47 clusters; Delong et al. 1993; Arctic96BD19 and SUP05,
365 Walsh et al. 2009; SAR156, Mullins et al. 1995; a ZD0417-related group, Stevens and
366 Ulloa 2008). Within the *Alphaproteobacteria,* relatives of the genera *Oceanibaculum* and
367 *Roseobacter*, of the *Rhizobiales*, *Rhodoplanes*, *Rhodospirillales*, *Sphingomonadales*,

several uncultured clusters, and the SAR11 lineage (the latter in the 10 to 25% range) were found in all post-plume samples (Table S3). The *Deltaproteobacteria* (dominated by SAR324) and the SAR406 lineage accounted for ca. 10 to 25% of the pyrosequencing dataset, similar to their representation in the 16S rRNA clone libraries (Figure 1). A wide range of phylum-level lineages, the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadetes*, *Planctomycetes*, and *Verrucomicrobia* accounted for approx. 0.5% to 5% of the pyrosequencing reads (Table S3), and appeared to a limited extent in the corresponding clone libraries (Figure 1). Other phylum-level lineages (Candidate Division OD1, *Epsilonproteobacteria*, *Lentisphaerae*) were barely detected in the pyrosequencing dataset, and were not observed in the clone libraries (Table S3).

3.4. Contrasting interpretations of the post-plume bacterial community. The interpretation of bacterial communities in the water column of late summer 2010 remains contested. Bacterial 16S rRNA gene clone libraries from post-plume water column samples (Sept. 7 to 17, 2010) detected diverse *Alpha-* and *Gammaproteobacteria*, *Flavobacteria*, *Chloroflexi*, and *Planctomycetales* (Kessler et al. 2011); the *Gammaproteobacteria* included *Cycloclasticus*, members of the *Oceanospirillales* (not the DWH group), and members of the *Methylophilaceae*, *Methylococcaceae* and the genus *Methylophaga*. The latter three lineages constitute a phylogenetic assemblage of C_1-oxidizing marine bacteria; this assemblage was regarded as evidence for bacterial methane oxidation as the dominant hydrocarbon-degrading process in the water column during the decay of the deep plume (Kessler et al. 2011), although re-examination of the

391 clone libraries and comparison with substrate spectra of cultured C_1-oxidizing bacteria
392 suggested that methylotrophy was at least as likely (Joye et al., 2011b). The phylogenetic
393 analysis of these clones and their closest matches reported here shows that they are not
394 representatives of cultured methylotrophic and methanotrophic genera. Instead, they form
395 two separate sister lineages to the methylo- and methanotrophic genera *Methylobacterium*,
396 *Methylosarcina*, *Methylobacter*, *Methylomonas*, and *Methylosphaera*, and to the
397 separately branching, obligately methylotrophic genus *Methylophaga* (Figure 3). If these
398 uncultured bacteria represent methylotrophs or methanotrophs, they would constitute new
399 genera with potentially novel physiological properties. Assuming that these uncultured
400 lineages represent C_1-oxidizing bacteria, the sampling campaign appears to have caught
401 the last stages of a methanotrophic bacterial bloom that pushed the methane
402 concentrations to below typical Gulf of Mexico ambient levels at the time of sampling in
403 September 2010 (Kessler et al. 2011). However, alternative interpretations are possible.
404 Transcriptomics studies that explored the impact of high molecular weight dissolved
405 organic matter on microbial community structure and activity showed a selective
406 enrichment of marine heterotrophs within the *Gamma-* and *Alphaproteobacteria*
407 (*Alteromonas*, *Thalassobius*) and gammaproteobacterial methylotrophs (*Methylophaga*)
408 after a short incubation time (27h) under DOM-amendment (McCarren et al. 2010).
409 These strains could be enriched in consequence of a DOM-degrading heterotrophic
410 cascade that releases naturally abundant methylated sugars from DOM, and leads to the
411 frequently observed high abundance of methylotrophic bacteria in clone libraries from
412 DOM-rich coastal waters (McCarren et al. 2010). In this interpretation, the combined
413 presence of DOM-degrading methylotrophic and heterotrophic *Gammaproteobacteria*

414 and *Alphaproteobacteria* marks the microbial degradation of a DOM pulse; this
415 explanation is consistent with dissolved oxygen and fluorescence anomalies and the lack
416 of detectable methane at the sampling stations that yielded this bacterial signature
417 (Kessler et al. 2011). The methylotroph-related clones disappeared from the October
418 2010 clone library, but reappeared in July 2011 (Figures 1, 3). Methylotroph-related
419 sequences remained detectable among the pyrosequencing reads in September and
420 October 2010 (Table S3). Their continued occurrence near the Macondo wellhead and in
421 other widely dispersed marine habitats (for a high-arctic example see Teske et al. 2011)
422 may not be specifically linked to methanotrophy or methylotrophy sustained by fossil
423 hydrocarbons; seasonal phytoplankton blooms provide an alternative explanation that
424 requires systematic investigation.
425
426 ***3.5. Natural Reservoirs of DWH Oceanospirillales***. The rapid enrichment of specific
427 bacterial types associated with the deep hydrocarbon plume indicates the existence of
428 easily accessible natural reservoirs or seed populations of these bacteria in the Gulf of
429 Mexico. Identifying their natural reservoir is of particular interest toward a more
430 complete understanding of their ecology and adaptability to a massive and prolonged
431 input of oil. The DWH *Oceanospirillales*, for example, lacked closely related
432 representatives in Genbank when first reported (Hazen et al., 2010). The closest relatives
433 in GenBank (EU050833) were a clone from Arctic marine sediments (Tian et al. 2009)
434 and cultured sister groups within the *Gammaproteobacteria*, including the hydrocarbon
435 degraders *Oleispira* and *Thalassolituus*, and the genera *Bermanella*, *Spongispira* and
436 *Oceanoserpentilla* (Hazen et al. 2010). While our pre- and post-plume 16S rRNA gene

437 clone libraries did not contain any full-length DWH *Oceanospirillales* clones, the DWH
438 *Oceanospirillales* were detected by pyrosequencing in the post-plume samples
439 (September and October 2010), indicating a low-level background population and
440 reservoir of these bacteria in the Gulf of Mexico water column.

441 Unexpectedly, members of the DWH *Oceanospirillales* were found in bacterial
442 16S rRNA gene and rRNA transcript libraries from the Atlantic shelf break offshore
443 North Carolina, sampled on December 4th, 2009 (D'Ambrosio, 2011), at a depth of 146 m
444 in a distinct water mass known as the Subtropical Underwater (SUW) layer and
445 distinguished by high salinity and warm temperature (Cléroux et al. 2009). They
446 constituted a substantial proportion (around 20% to 25%) of all clone libraries from the
447 SUW sample, regardless of whether these were derived from 16S rRNA genes or 16S
448 rRNA transcripts of the particle-associated or free-living fraction (D'Ambrosio 2011).
449 The North Carolina *Oceanospirillales* 16S rRNA genes fell into the same phylogenetic
450 clusters as the *Oceanospirillales* 16S rRNA genes and pyrosequencing fragments from
451 the DWH oil spill (Figure 4). Since the North Carolina *Oceanospirillales* were sampled
452 in December 2009, they do not originate from the DWH oil spill; yet they are members of
453 the DHW *Oceanospirillales* cluster by phylogenetic affiliation. The conspicuous
454 enrichment of DWH *Oceanospirillales* in the Subtropical Underwater layer might be the
455 consequence of natural hydrocarbon seepage and hydrocarbon enrichment in this water
456 layer in the southwest North Atlantic (Harvey et al. 1979; Requejo and Boehm, 1985).
457 This North Atlantic population of DWH *Oceanospirillales* could be in constant exchange
458 with the Gulf of Mexico, and might represent a parent population. More generally, the
459 North Atlantic and the Gulf of Mexico occurrences of this microbial group could be the

460 result of localized enrichments from a widely distributed low-abundance seed population.
461
462 ***4. Conclusions.*** Pyrosequencing and clone library analyses of PCR-amplified 16S rRNA
463 genes and gene fragments have revealed strong microbial community stratification in the
464 deep-plume water column, dominated by abundant populations of alkane-oxidizing DWH
465 *Oceanospirillales* and aromatics-degrading *Cycloclasticus* spp. After the Macondo
466 wellhead was capped and the source for the deep plume extinguished, the pre-spill
467 pelagic microbial community re-established itself near the vicinity of the Macondo
468 wellhead. However, even after the deep hydrocarbon plume was no longer detectable in
469 the wellhead area in September and October 2010, small populations of oil-degrading
470 *Gammaproteobacteria* and of the DWH *Oceanospirillales* remained detectable by
471 pyrosequencing, indicating persistent and widely occurring seed populations in the water
472 column that respond quickly to natural or anthropogenic hydrocarbon pulses.
473
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481 Impacts of Oil and Gas Inputs to the Gulf (ECOGIG)" administered by the University of
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492 **References**

493

494 Altschul, S.F., et al. 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

495

496 Bælum, J., et al. 2012. Deep-sea bacteria enriched by oil and dispersant from the
497 Deepwater Horizon Spill. Environ Microbiol 14, 2405-2416.

498

499 Camilli, R., et al. 2010. Tracking Hydrocarbon Plume Transport and Biodegradation at
500 Deepwater Horizon. Science 330, 201-204.

501

502 Canfield, D.E., et al. 2010. A cryptic sulfur cycle in oxygen-minimum-zone waters off
503 the Chilean coast. Science 330, 1375-1378.

504

505 Caporaso, J.G., et al. 2010. QIIME allows analysis of high-throughput community
506 sequencing data. Nature Methods 7, 335-336.

507

508 Cléroux, C., et al. 2009. Evidence for calcification depth change of *Globorotalia*
509 *truncatulinoides* between deglaciation and Holocene in the Western Atlantic Ocean.
510 Marine Micropaleontology 73, 57-61.

511

512 D'Ambrosio, L. 2011. Bacterial community composition and extracellular enzymatic
513 function in marine waters. Masters Sci. thesis, Dept. of Marine Sciences, UNC Chapel
514 Hill.

515

516 DeLong, E.F., Franks, D.G., Alldredge, A.L. 1993. Phylogenetic diversity of aggregate
517 vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38, 924–934.

518

519 Diercks, A-R, et al. 2010a. NIUST – Deepwater Horizon Oil Spill Response Cruise.
520 (100528-051). Paper at Oceans 2010 MTS/IEEE conference. Sept. 20-23, Seattle, WA.

521

522 Diercks A-R, et al. 2010b. Characterization of subsurface polycyclic aromatic hydro-
523 carbons at the Deepwater Horizon Wellhead Site. Geophys Res Lett 37, doi:10.1029/
524 2010GL045046

525

526 Dyksterhouse, S.E., et al. 1995 *Cycloclasticus pugetii* gen. nov., sp. nov., an aromatic
527 hydrocarbon-degrading bacterium from marine sediments. Int. J. Syst. Bacteriol. 45, 116-
528 123.

529

530 Edgar, R.C. 2010. Search and clustering orders of magnitude faster than BLAST.
531 Bioinformatics 26, 2460 -2461.

532

533 Field, K.G., et al. 1997. Diversity and depth-specific distribution of SAR11 cluster rRNA
534 gene from marine planktonic bacteria. Appl. Environ. Microbiol. 63, 63-70.

535

536 Geiselbrecht, A.D., et al. 1998. Isolation of marine polycyclic aromatic hydrocarbon
537 (PAH)-degrading *Cycloclasticus* strains from the Gulf of Mexico and comparison of their

538 PAH degradation ability with that of Puget Sound *Cycloclasticus* strains. Appl. Environ.
539 Microbiol. 64, 4703-4710.
540
541 Harvey, G., et al. 1979. Observation of a subsurface oil-rich layer in the open ocean.
542 Science 205, 999-1001.
543
544 Hazen, T.C., et al. 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria.
545 Science 330, 204-208.
546
547 Joye, S.B., et al. 2011a. Magnitude and oxidation potential of hydrocarbon gases released
548 from the BP oil well blowout. Nature Geoscience 4,160-164.
549
550 Joye, S.B., et al. 2011b. Technical comment on "A persistent oxygen anomaly reveals the
551 fate of spilled methane in the deep Gulf of Mexico". Science 322, 1033-1034.
552
553 Kessler, J.D., et al. 2011. A persistent oxygen anomaly reveals the fate of spilled methane
554 in the deep Gulf of Mexico. Science 331, 312-315.
555
556 Kujawinski, E.B., et al. 2011. Fate of Dispersants Associated with the Deepwater
557 Horizon Oil Spill. Environ. Sci. Technol. 45, 1298-1306.
558
559 Kunin, V., et al. 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to
560 artificial inflation of diversity estimates. Environ Microbiol 12, 118-123.

561

562 Lu, Z.M., et al. 2012. Microbial gene functions enriched in the Deepwater Horizon deep-
563 sea oil plume. The ISME J. 6, 451-460.

564

565 Ludwig, W., et al. 2004. ARB: a software environment for sequence data. Nucleic Acids
566 Res. 32, 1363-1371.

567

568 Marshall, K.T., Morris, R.M. 2013. Isolation of an aerobic sulfur oxidizer from the
569 SUP05/ Arctic96BD-19 clade. The ISME J. 7, 452-455.

570

571 Mason, O.U., et al. 2012. Metagenome, metatranscriptome and single-cell sequencing
572 reveal microbial response to Deepwater Horizon oil spill. The ISME J. 6**,** 1715-1727.

573

574 McCarren, J., et al. 2010. Microbial community transcriptomes reveal microbes and
575 metabolic pathways associated with dissolved organic matter turnover in the sea. Proc.
576 Natl. Acad. Sci. USA 107, 16420-16427.

577

578 Mullins, T.D., et al. 1995 Genetic comparisons reveal the same unknown bacterial
579 lineages in Atlantic and Pacific bacterioplankton communities. Limnol Oceanogr 40,
580 148-158.

581

582 Passow, U., et al. 2012. Marine snow formation in the aftermath of the deepwater
583 Horizon oil spill in the Gulf of Mexico. Environ. Res. Lett. 7, 035301. doi:10.1088/1748-

9326/7/3/035301

Pinhassi, J., et al. 2009. *Bermanella marisrubri* gen. nov. sp. nov., a genome-sequenced gammaproteobacterium from the Red Sea. Int. J. Syst. Evol. Microbiol. 59, 373-377.

Pruesse, E., et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188-7196.

Redmond, M.C., Valentine, D.L. 2012. Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. Proc. Natl. Acad. Sci. USA 109, 20292-20297.

Schrope, M. 2011. Deep wounds. Nature 472, 152-154.

Stevens, H., Ulloa, O. 2008. Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol 10, 1244-1259.

Reddy, C.M., et al. 2012. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc. Natl. Acad. Sci. USA 109, 20229-20234.

Requejo, A., Boehm, P. 1985. Characterization of hydrocarbons in a subsurface oil-rich

607 layer in the Sargasso Sea. Mar. Environ. Res. 17, 45-64.
608
609 Ryerson, T.B., et al. 2012. Chemical data quantify Deepwater Horizon hydrocarbon flow
610 rate and environmental distribution. Proc. Natl. Acad. Sci. USA 109, 20246-20259.
611
612 Schlösser, A, et al. 2008. *Oceaniserpentilla haliotis* gen. nov., sp. nov., a marine
613 bacterium isolated from haemolymph serum of blacklip abalone. Int. J. Syst. Evol.
614 Microbiol. 58, 2122-2125.
615
616 Swan, B.K., et al. 2011. Potential for chemolithoautotrophy among ubiquitous bacteria
617 lineages in the dark ocean. Science 333, 1296-1300.
618
619 Teske, A., et al. 2002. Microbial Diversity in Hydrothermal Sediments in the Guaymas
620 Basin: Evidence for Anaerobic Methanotrophic Communities. Appl. Environ. Microbiol.
621 68, 1994-2007.
622
623 Teske, A., et al. 2011. Microbial community composition and function in permanently
624 cold seawater and sediments from an Arctic fjord of Svalbard. Appl. Environ. Microbiol.
625 77, 2008-2018.
626
627 Tian, F., et al. 2009. Bacterial, archaeal and eukaryotic diversity in Arctic sediment as
628 revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. Polar Biology 32,
629 93-103.

630

631 Valentine, D.L., et al. 2010. Propane respiration jump-starts microbial response to a deep
632 oil spill. Science 330, 208-211.

633

634 Wade, T.L., et al. 2011. Analyses of water samples from the Deepwater Horizon Oil spill:
635 Documentation of the Subsurface Plume. Pp 77 – 82. Monitoring and Modeling the
636 Deepwater Horizon Oil Spill: A Record-Breaking Enterprise. Geophysical Monograph
637 Series 195. Copyright 2011, American Geophysical Union. 10.1029/2011GM001103

638

639 Walsh, D.A., et al. 2009. Metagenome of a versatile chemolithoautotroph from
640 expanding oceanic dead zones. Science 326, 578-582.

641

642 Yakimov, M.M., et al. 2003. *Oleispira antarctica* gen. nov., sp. nov., a novel hydro-
643 carbonoclastic marine bacterium isolated from Antarctic coastal sea water. Int. J. Syst.
644 Evol. Microbiol. 53, 779-785.

645

646 Yakimov, M.M., et al. 2004. *Thalassolituus oleivorans* gen. nov., sp. nov., a novel
647 marine bacterium that obligately utilizes hydrocarbons. Int. J. Syst. Evol. Microbiol. 54,
648 141-148.

649

650 Ziervogel, K., et al. 2012. Microbial Activities and Dissolved Organic Matter Dynamics
651 in Oil-Contaminated Surface Seawater from the Deepwater Horizon Oil Spill Site. PLoS
652 One **7**(4), e34816.

653

654 **Figure legends**

655

656 **Figure 1**. Pie charts of phylum- and subphylum composition of bacterial 16S rRNA gene
657 clone libraries and bacterial 300-bp pyrosequencing fragments from the Gulf of Mexico
658 water column near the Macondo wellhead. Within the *Gammaproteobacteria*, the genus
659 *Cycloclasticus*, the methylotroph-affiliated lineages, the DWH *Oceanospirillales* , and
660 other *Oceanospirillales* and *Alteromonadales* are highlighted at family- or genus-level
661 resolution. A) Surface water sample collected on May 5, 2010; hydrocarbon plume water
662 column samples near Macondo wellhead from 800, 1170, 1210, and 1320 m depth
663 collected on May 31, 2010. B) Pre-plume March 2010 sample from 800 m depth near
664 MC118; water column samples from 800 and 1210 m depth near Macondo wellhead,
665 collected September 12, 2010; water column samples from October 18, 2010, and July 3,
666 2011. The upper pie charts in A) and B) show 16S rRNA gene clone library composition,
667 the lower pie charts show the corresponding pyrosequencing results.

668

669 **Figure 2.** Phylogeny of *Gammaproteobacteria* (*Oceanospirillales* and *Alteromonadales*
670 including DWH *Oceanospirillales*) in the Gulf of Mexico water column near the
671 Macondo wellhead, based on near-full length 16S rRNA genes. Clones from the pre-spill
672 water column sample (March 10, 2010) are labeled "Prespill"; clones from surface oil
673 slicks (May 5, 2010) are labeled "Surfaceoil"; clones from plume water column samples
674 (May 31, 2010) are labeled "Plumeprofile". Clones from September 12 and October 18,
675 2010, and from July 3, 2011, are labeled Postplume I, II and III, respectively. The clone

676 designations are followed by sampling depth in meters, and a 3-digit clone ID (Table S1).
677 The scale bar corresponds to 10 % sequence distance.
678
679 **Figure 3.** Phylogeny of *Gammaproteobacteria* (Uncultured lineages, *Cycloclasticus* and
680 methaneotrophs/methylotrophs) in the Gulf of Mexico water column near the Macondo
681 wellhead, based on near-full length 16S rRNA genes. Clones from the pre-spill water
682 column sample (March 10, 2010) are labeled "Prespill"; clones from surface oil slicks
683 (May 5, 2010) are labeled "Surfaceoil"; clones from plume water column samples (May
684 31, 2010) are labeled "Plumeprofile". Clones from September 12 and October 18, 2010,
685 and from July 3, 2011, are labeled Postplume I, II and III, respectively. The clone
686 designations are followed by sampling depth in meters, and a 3-digit clone ID (Table S1).
687 The scale bar corresponds to 10 % sequence distance.
688
689 **Figure 4**. Phylogeny of DWH *Oceanospirillales* based on 300 bp pyrosequencing
690 fragments and corresponding sections of 16S rRNA gene clones, showing the
691 phylogenetic fine structure of this cluster. The phylogeny was obtained with an alignment
692 mask that excluded all sequence regions except *E.coli* 16S rRNA gene positions 28-337,
693 equivalent to the pyrosequencing fragment. The tree was rooted with the
694 gammaproteobacterial North Sea clone ZD0417 (AJ400353). The number of occurrence
695 for each type of pyrosequencing fragment and 16S rRNA gene clone in the different
696 samples is listed in brackets in the following order: Surface sample; Plume profile at 800
697 m; Plume profile at 1170 m; Plume profile at 1210 m; Plume profile at 1320 m;
698 Postplume I at 800 m; Postplume I at1210 m; Postplume-II at 1050 m.

699 The scale bar corresponds to 2 % sequence distance.

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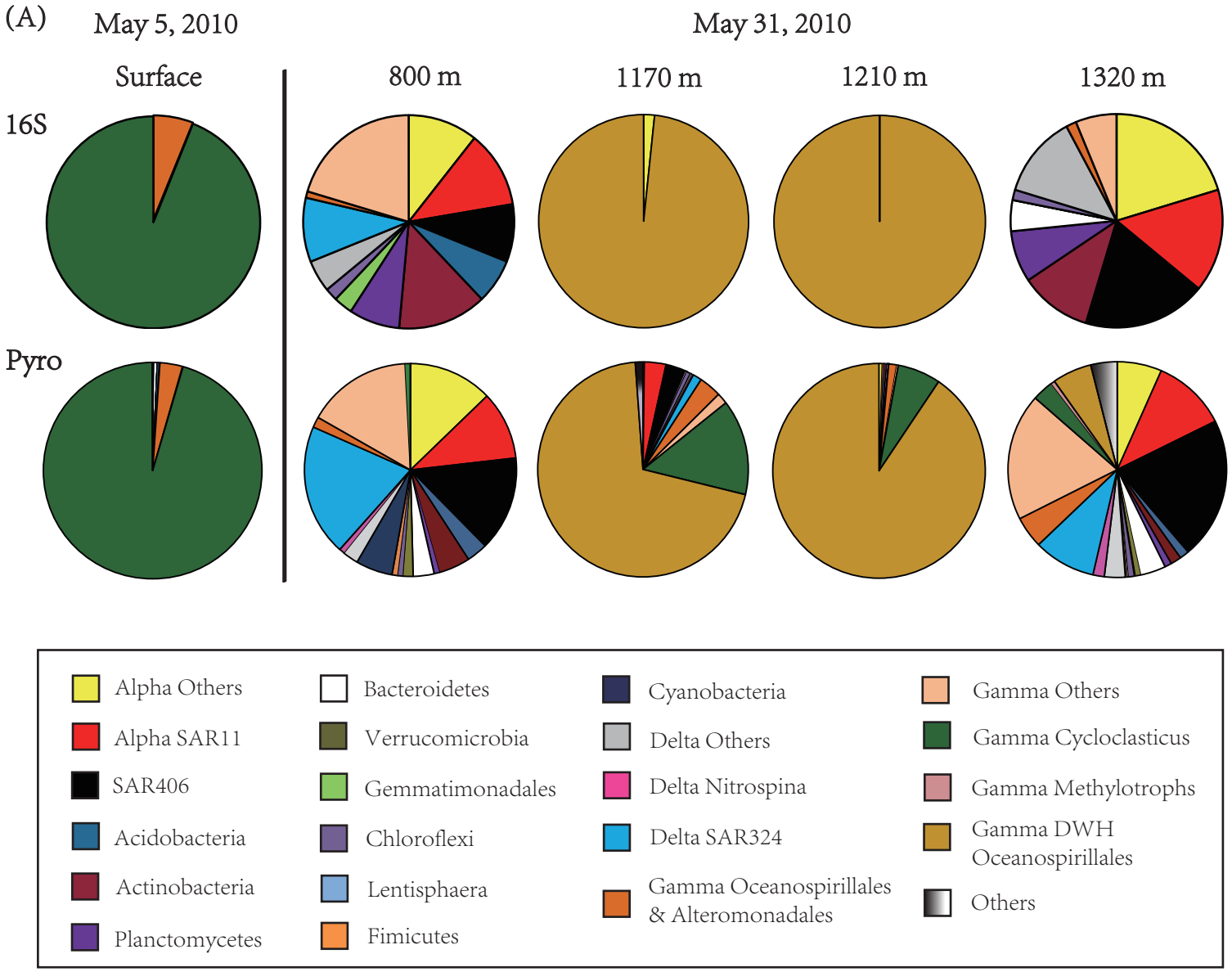
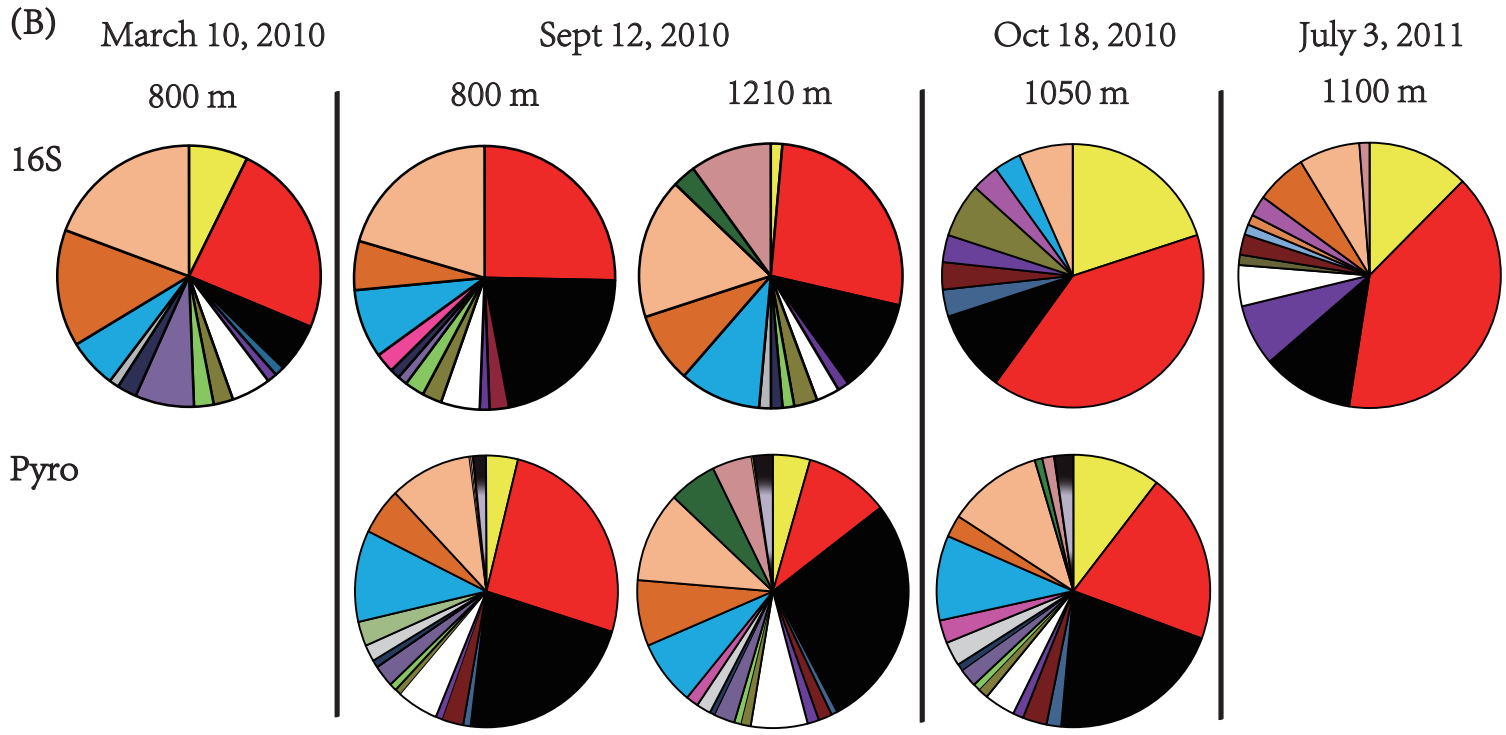
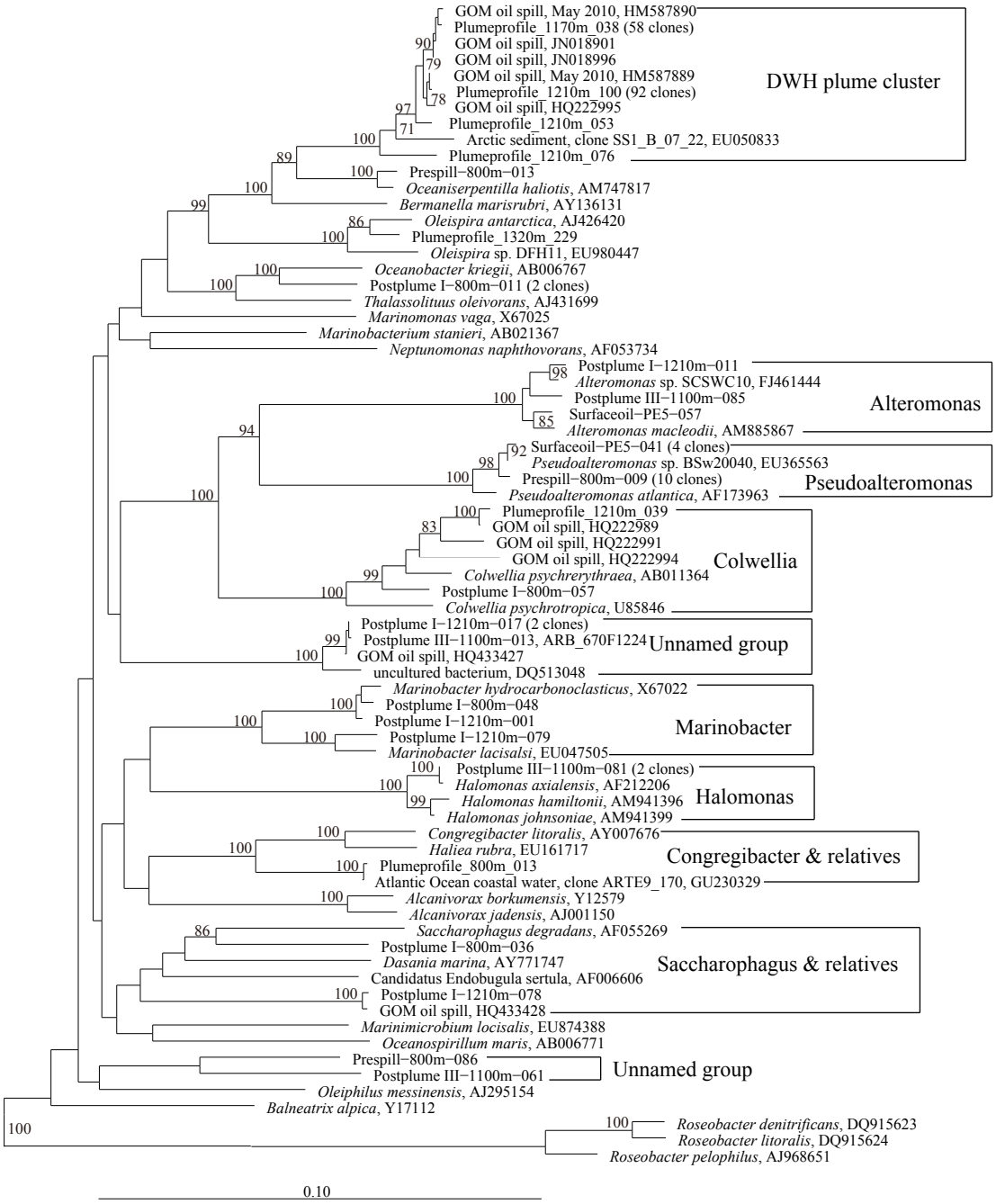
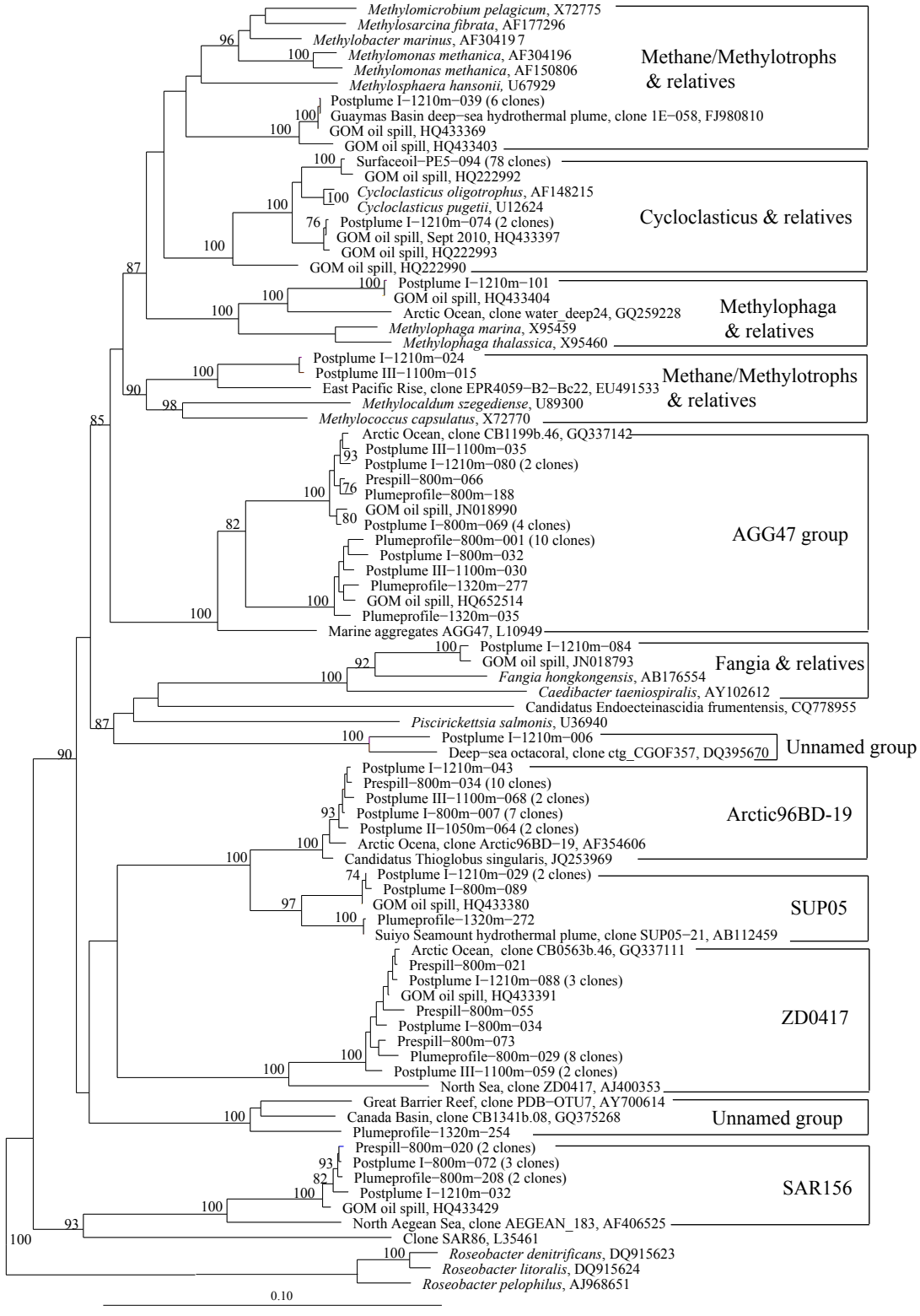

(A)
May 5, 2010
May 31, 2010
Surface
800 m
1170 m
1210 m
1320 m
16S
Pyro
Alpha Others
Alpha SAR11
SAR406
Acidobacteria
Actinobacteria
Planctomycetes
Bacteroidetes
Verrucomicrobia
Gemmatimonadales
Chloroflexi
Lentisphaera
Fimicutes
Cyanobacteria
Delta Others
Delta Nitrospina
Delta SAR324
Gamma Oceanospirillales & Alteromonadales
Gamma Others
Gamma Cycloclasticus
Gamma Methylotrophs
Gamma DWH Oceanospirillales
Others


(B)
March 10, 2010
Sept 12, 2010
Oct 18, 2010
July 3, 2011
800 m
800 m
1210 m
1050 m
1100 m
16S
Pyro


GOM oil spill, May 2010, HM587890
Plumeprofile_1170m_038 (58 clones)
GOM oil spill, JN018901
GOM oil spill, JN018996
GOM oil spill, May 2010, HM587889
Plumeprofile_1210m_100 (92 clones)
GOM oil spill, HQ222995
Plumeprofile_1210m_053
Arctic sediment, clone SS1_B_07_22, EU050833
Plumeprofile_1210m_076
DWH plume cluster
Prespill−800m−013
Oceaniserpentilla haliotis, AM747817
Bermanella marisrubri, AY136131
Oleispira antarctica, AJ426420
Plumeprofile_1320m_229
Oleispira sp. DFH11, EU980447
Oceanobacter kriegii, AB006767
Postplume I−800m−011 (2 clones)
Thalassolituus oleivorans, AJ431699
Marinomonas vaga, X67025
Marinobacterium stanieri, AB021367
Neptunomonas naphthovorans, AF053734
Postplume I−1210m−011
Alteromonas sp. SCSWC10, FJ461444
Postplume III−1100m−085
Surfaceoil−PE5−057
Alteromonas macleodii, AM885867
Alteromonas
Surfaceoil−PE5−041 (4 clones)
Pseudoalteromonas sp. BSw20040, EU365563
Prespill−800m−009 (10 clones)
Pseudoalteromonas atlantica, AF173963
Pseudoalteromonas
Plumeprofile_1210m_039
GOM oil spill, HQ222989
GOM oil spill, HQ222991
GOM oil spill, HQ222994
Colwellia psychrerythraea, AB011364
Postplume I−800m−057
Colwellia psychrotropica, U85846
Colwellia
Postplume I−1210m−017 (2 clones)
Postplume III−1100m−013, ARB_670F1224
GOM oil spill, HQ433427
uncultured bacterium, DQ513048
Unnamed group
Marinobacter hydrocarbonoclasticus, X67022
Postplume I−800m−048
Postplume I−1210m−001
Postplume I−1210m−079
Marinobacter lacisalsi, EU047505
Marinobacter
Postplume III−1100m−081 (2 clones)
Halomonas axialensis, AF212206
Halomonas hamiltonii, AM941396
Halomonas johnsoniae, AM941399
Halomonas
Congregibacter litoralis, AY007676
Haliea rubra, EU161717
Plumeprofile_800m_013
Atlantic Ocean coastal water, clone ARTE9_170, GU230329
Congregibacter & relatives
Alcanivorax borkumensis, Y12579
Alcanivorax jadensis, AJ001150
Saccharophagus degradans, AF055269
Postplume I−800m−036
Dasania marina, AY771747
Candidatus Endobugula sertula, AF006606
Postplume I−1210m−078
GOM oil spill, HQ433428
Saccharophagus & relatives
Marinimicrobium locisalis, EU874388
Oceanospirillum maris, AB006771
Prespill−800m−086
Postplume III−1100m−061
Unnamed group
Oleiphilus messinensis, AJ295154
Balneatrix alpica, Y17112
Roseobacter denitrificans, DQ915623
Roseobacter litoralis, DQ915624
Roseobacter pelophilus, AJ968651
0.10


Methylomicrobium pelagicum, X72775
Methylosarcina fibrata, AF177296
Methylobacter marinus, AF304197
Methylomonas methanica, AF304196
Methylomonas methanica, AF150806
Methylosphaera hansonii, U67929
Postplume I−1210m−039 (6 clones)
Guaymas Basin deep−sea hydrothermal plume, clone 1E−058, FJ980810
GOM oil spill, HQ433369
GOM oil spill, HQ433403
Methane/Methylotrophs & relatives
Surfaceoil−PE5−094 (78 clones)
GOM oil spill, HQ222992
Cycloclasticus oligotrophus, AF148215
Cycloclasticus pugetii, U12624
Postplume I−1210m−074 (2 clones)
GOM oil spill, Sept 2010, HQ433397
GOM oil spill, HQ222993
GOM oil spill, HQ222990
Cycloclasticus & relatives
Postplume I−1210m−101
GOM oil spill, HQ433404
Arctic Ocean, clone water_deep24, GQ259228
Methylophaga marina, X95459
Methylophaga thalassica, X95460
Methylophaga & relatives
Postplume I−1210m−024
Postplume III−1100m−015
East Pacific Rise, clone EPR4059−B2−Bc22, EU491533
Methylocaldum szegediense, U89300
Methylococcus capsulatus, X72770
Methane/Methylotrophs & relatives
Arctic Ocean, clone CB1199b.46, GQ337142
Postplume III−1100m−035
Postplume I−1210m−080 (2 clones)
Prespill−800m−066
Plumeprofile−800m−188
GOM oil spill, JN018990
Postplume I−800m−069 (4 clones)
Plumeprofile−800m−001 (10 clones)
Postplume I−800m−032
Postplume III−1100m−030
Plumeprofile−1320m−277
GOM oil spill, HQ652514
Plumeprofile−1320m−035
Marine aggregates AGG47, L10949
AGG47 group
Postplume I−1210m−084
GOM oil spill, JN018793
Fangia hongkongensis, AB176554
Caedibacter taeniospiralis, AY102612
Candidatus Endoecteinascidia frumentensis, CQ778955
Fangia & relatives
Piscirickettsia salmonis, U36940
Postplume I−1210m−006
Deep−sea octacoral, clone ctg_CGOF357, DQ395670
Unnamed group
Postplume I−1210m−043
Prespill−800m−034 (10 clones)
Postplume III−1100m−068 (2 clones)
Postplume I−800m−007 (7 clones)
Postplume II−1050m−064 (2 clones)
Arctic Ocena, clone Arctic96BD−19, AF354606
Candidatus Thioglobus singularis, JQ253969
Arctic96BD-19
Postplume I−1210m−029 (2 clones)
Postplume I−800m−089
GOM oil spill, HQ433380
Plumeprofile−1320m−272
Suiyo Seamount hydrothermal plume, clone SUP05−21, AB112459
SUP05
Arctic Ocean, clone CB0563b.46, GQ337111
Prespill−800m−021
Postplume I−1210m−088 (3 clones)
GOM oil spill, HQ433391
Prespill−800m−055
Postplume I−800m−034
Prespill−800m−073
Plumeprofile−800m−029 (8 clones)
Postplume III−1100m−059 (2 clones)
North Sea, clone ZD0417, AJ400353
ZD0417
Great Barrier Reef, clone PDB−OTU7, AY700614
Canada Basin, clone CB1341b.08, GQ375268
Plumeprofile−1320m−254
Unnamed group
Prespill−800m−020 (2 clones)
Postplume I−800m−072 (3 clones)
Plumeprofile−800m−208 (2 clones)
Postplume I−1210m−032
GOM oil spill, HQ433429
North Aegean Sea, clone AEGEAN_183, AF406525
Clone SAR86, L35461
SAR156
Roseobacter denitrificans, DQ915623
Roseobacter litoralis, DQ915624
Roseobacter pelophilus, AJ968651
0.10

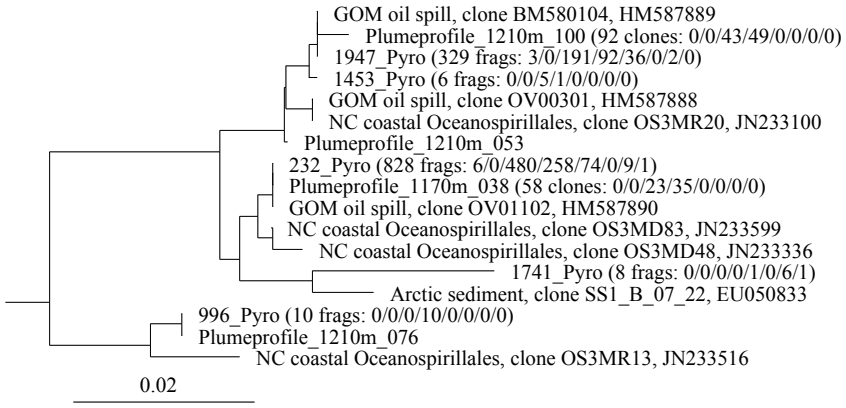

GOM oil spill, clone BM580104, HM587889
Plumeprofile_1210m_100 (92 clones: 0/0/43/49/0/0/0/0)
1947_Pyro (329 frags: 3/0/191/92/36/0/2/0)
1453_Pyro (6 frags: 0/0/5/1/0/0/0/0)
GOM oil spill, clone OV00301, HM587888
NC coastal Oceanospirillales, clone OS3MR20, JN233100
Plumeprofile_1210m_053
232_Pyro (828 frags: 6/0/480/258/74/0/9/1)
Plumeprofile_1170m_038 (58 clones: 0/0/23/35/0/0/0/0)
GOM oil spill, clone OV01102, HM587890
NC coastal Oceanospirillales, clone OS3MD83, JN233599
NC coastal Oceanospirillales, clone OS3MD48, JN233336
1741_Pyro (8 frags: 0/0/0/0/1/0/6/1)
Arctic sediment, clone SS1_B_07_22, EU050833
996_Pyro (10 frags: 0/0/0/10/0/0/0/0)
Plumeprofile_1210m_076
NC coastal Oceanospirillales, clone OS3MR13, JN233516
0.02

Table 1: Samples collected on multiple research cruises near the Macondo wellhead with dates, water depths, and geographical coordinates.

Sample names with cruise-specific sampling codes in parentheses	Ship	Date	Depth (m)	Latitude (N)	Longitude (W)
Prespill-800m	RV *Pelican*	March 10, 2010	800	28°50.43	88°30.29
SurfaceOil-PE5	RV *Pelican*	May 5, 2010	0	28°44.175	88°22.335
Plumeprofile-800m (B11)	RV *Walton Smith*	May 31, 2010	800	28°41.686	88°26.081
Plumeprofile-1170m (B6)	RV *Walton Smith*	May 31, 2010	1170	28°41.686	88°26.081
Plumeprofile-1210m (B3)	RV *Walton Smith*	May 31, 2010	1210	28°41.686	88°26.081
Plumeprofile-1320m (B1)	RV *Walton Smith*	May 31, 2010	1320	28°41.686	88°26.081
Postplume I-800m (C4B8)	RV *Pelican*	Sept 12, 2010	800	28°41.713	88°26.073
Postplume I-1210 m (C4B4)	RV *Pelican*	Sept 12, 2010	1210	28°41.713	88°26.073
Postplume II (GIP22)	RV *Cape Hatteras*	Oct 18, 2010	1052	28°40.503	87°39.250
Postplume III (E002)	RV *Endeavor*	July 3, 2011	1100	28°42.177	88°21.240