Simulation of *Deepwater Horizon* oil plume reveals substrate specialization within a complex community of hydrocarbon degraders


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The *Deepwater Horizon* (DWH) accident released an estimated 4.1 million barrels of oil and 1010 mol of natural gas into the Gulf of Mexico, forming deep-sea plumes of dispersed oil droplets and dissolved gases that were largely degraded by bacteria. During the course of this 3-mo disaster a series of different bacterial taxa were enriched in succession within deep plumes, but the metabolic capabilities of the different populations that controlled degradation rates of crude oil components are poorly understood. We experimentally reproduced dispersed plumes of fine oil droplets in Gulf of Mexico seawater and successfully replicated the enrichment and succession of the principal oil-degrading bacteria observed during the DWH event. We recovered near-complete genomes, whose phylogeny matched those of the principal biodegrading taxa observed in the field, including the DWH Oceanospirillales (now identified as a *Bermanella* species), multiple species of *Colwellia*, *Cycloclasticus*, and other members of Gammaproteobacteria, Flavobacteria, and Rhodobacteria. Metabolic pathway analysis, combined with hydrocarbon compositional analysis and species abundance data, revealed substrate specialization that explained the successional pattern of oil-degrading bacteria. The fastest-growing bacteria used short-chain alkanes. The analyses also uncovered potential cooperative and competitive relationships, even among close relatives. We conclude that patterns of microbial succession following deep ocean hydrocarbon blowouts are predictable and primarily driven by the availability of liquid petroleum hydrocarbons rather than natural gases.

D *eepwater Horizon* drilling accident was the first major release of oil and natural gases in the deep ocean, and considerable uncertainty remains about the fate of vast amounts of hydrocarbons that never reached the surface. We simulated the deep-sea plumes of dispersed oil microdroplets and measured biodegradation of crude oil components. We successfully reproduced the successive blooms of diverse bacteria observed in the field and obtained near-complete genomes of all major hydrocarbon-degrading species, providing an assessment of the metabolic capabilities of the microbial community responsible for biodegradation. Our results show that rapidly degraded components of oil were consumed by bacteria with highly specialized degradation capabilities and that crude oil alone could explain the microbial dynamics observed in the field.

Significance

The *Deepwater Horizon* drilling accident was the first major release of oil and natural gases in the deep ocean, and considerable uncertainty remains about the fate of vast amounts of hydrocarbons that never reached the surface. We simulated the deep-sea plumes of dispersed oil microdroplets and measured biodegradation of crude oil components. We successfully reproduced the successive blooms of diverse bacteria observed in the field and obtained near-complete genomes of all major hydrocarbon-degrading species, providing an assessment of the metabolic capabilities of the microbial community responsible for biodegradation. Our results show that rapidly degraded components of oil were consumed by bacteria with highly specialized degradation capabilities and that crude oil alone could explain the microbial dynamics observed in the field.


The authors declare no conflict of interest.

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Data deposition: The whole genome shotgun project and the associated draft genomes have been deposited at the DNA Data Bank of Japan (DDBJ), European Nucleotide Archive, and GenBank (accession nos. MAAU00000000–MAAU00000000 and MAAW00000000–MAFW00000000). The version described in this paper is version MAAU00000000–MAAU00000000 and MAAW00000000–MAFW00000000. The raw reads have been deposited at DDBJ, the European Molecular Biology Laboratory, and GenBank (accession no. SRP975617). The project description and related metadata are accessible through BioProject PRJNA320927.

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promote microbes that are able to use dispersant for growth. Therefore, it was thought that the relative abundance of the natural hydrocarbon degraders, such as *Marinobacter*, was kept low (7). No study, to date, has been able to specify the relationship between hydrocarbon substrate availability and the metabolic capacities of the diverse group of organisms responsible for hydrocarbon degradation in the DWH plume.

Our goals in this study were to mimic the conditions present in the deep-sea oil plume at the time of the DWH oil release by producing highly dispersed (~10-μm) oil droplets in natural seawater, correlate the progression of oil degradation with shifts in the endemic microbial community by analyzing changes in hydrocarbon chemistry and bacterial populations over a 64-d time course, and recover high-quality draft genomes to determine the metabolic factors that drove the microbial community shifts throughout the oil biodegradation process.

**Results**

**Microbial Community Structural Changes Correlated with Hydrocarbon Groups.** To observe the interaction between the microbial community and the consumption of hydrocarbons we simulated the deep-sea plume observed during the DWH incident in the laboratory. Natural seawater collected from Mississippi Canyon block MC-294 at 1,100- to 1,200-m depth was mixed to a final concentration of 2 ppm oil and 0.02 ppm Corexit EC9500A dispersant. MC-294 at 1,100- to 1,200-m depth was mixed to a final concentration of 2 ppm oil and 0.02 ppm Corexit EC9500A dispersant. Microdroplets were produced using a pressurized flow injection (PFI) droplet generator (15) to produce median-size oil droplets of 10-μm diameter using Macondo (MC252) oil (SI Methods). Oil droplets remained in suspension in replicate 2-L bottles for the 64 d of the experiment.

The chemical analysis in this deep-sea hydrocarbon plume simulation demonstrated that the biodegradation of linear alkane molecules began first, followed by biodegradation of one- to three-ring aromatics, followed by four- to six-ring polycyclic aromatic compounds; the half-lives were as follows: 6–13 carbon alkanes 6.22 d, 14–25 carbon alkanes 8.14 d, alkanes above 25 carbons 22.2 d, monoaromatics [benzene, toluene, ethylbenzene, and xylene (BTEX)] 17.8 d, two- to three-ring polycyclic aromatics 25.3 d, and four- to six-ring polycyclic aromatics >64 d (Fig. 1). Full results for the hydrocarbon analyses are provided in Dataset S1. The rates and sequence of hydrocarbon biodegradation are comparable to those observed in the deep-sea plume of the DWH oil release (3). Both the sequence and rates of hydrocarbon biodegradation are in overall agreement with observations of the decreases of hydrocarbon concentrations in the DWH oil plumes over time (3). Nutrient analysis showed no depletion of other nutrients (Dataset S2).

Analysis of 16S rRNA sequence data indicates that the microbial community changed concordant with the chemical changes in the residual oil. The starting relative abundance of all hydrocarbon-degrading bacteria in the laboratory was low, similar to 2010 field samples collected from uncontaminated waters. With the input of oil, the microbial response closely resembled successional patterns observed in 2010 during the DWH event (Fig. 2). Notably, in the laboratory simulation the DWH *Oceanospirillales* initially identified by Hazen et al. (4) and *Pseudomonas* taxa reached maximum relative abundance by 16S rRNA gene analysis at day 6, whereas *Colwellia* and *Cycloclasticus* peaked between days 9–18, and the orders Alteromonadales, Flavobacteriales, and Rhodobacterales peaked between days 36–64. This successional response was nearly identical to those seen in samples collected during the DWH event. In day-6 and day-9 samples, DWH *Oceanospirillales* and *Pseudomonas* reached peak dominance, analogous to the late-May, early-June samples from the DWH event that also contained the highest concentrations of linear alkanes (3). Between days 9 and 18, *Colwellia*, other related Alteromonadales, and *Cycloclasticus* emerged as dominant taxa as DWH *Oceanospirillales* and *Pseudomonas* receded, which was nearly identical to the successional pattern observed between early to mid June 2010 (3, 14). In late stages of the experiment (days 36 and 64), Flavobacteriales and Rhodobacterales became dominant as most Gammaproteobacteria populations declined, analogous to observations in advanced stages of degradation observed in field samples collected from the deep-sea plume during the DWH oil release (3, 5). Ordination analysis of the 16S rRNA-derived microbial community profile showed the largest shifts in microbial community structure between day 3 and day 6 and followed a sequential trajectory from day 6 to day 64 (Fig. S1). Replicated total cell counts started to increase between day 3 and day 6 and...
continued to increase until day 18, after which they remained around six times higher than initial counts until day 64 (Fig. S2). Control replicates with no added oil remained constant for the duration of the experiment, at $5 \times 10^5$ cells·mL$^{-1}$, with no overall changes in community composition (Figs. S1 and S2).

Based on the 16S rRNA information that identified time periods during which the microbial community underwent major shifts in composition, three samples from days 6, 18, and 64 were chosen for metagenomic sequencing and analysis. Through the reconstruction of the dominant genomes from the metagenome data we identified the same hydrocarbon-degrading organisms and successional pattern that was observed in the PhyloChip 16S rRNA analysis of both the laboratory and the 2010 DWH event samples (Figs. 2 and 3 and Fig. S3).

The Initial Stage of Low-Molecular-Weight Alkane Biodegradation Was Dominated by a Novel Bermanella Species. The initial stage of degradation, primarily resulting in biodegradation of low-middle-weight linear $n$-alkanes starting by day 6, was dominated by a single novel Bermanella species with its contigs representing almost 33.5% relative abundance of all of the assembled sequences (Fig. 3 and Fig. S3) of the day-6 sample. Other prominent taxa in the day-6 assembly included Oleispira (0.44%), Marinomonas (<1%), and Pseudoalteromonas (<1%). By subsampling (random sampling reads to achieve various coverage levels) of the day-6 dataset we recovered a near-complete draft genome of Bermanella sp. (2.55 Mb, all bacterial single-copy genes and 51 out of 55 ribosomal sequences were recovered). The 16S rRNA gene (1,532 bp) was 99% identical to the full-length DWH Oceanospirillales 16S rRNA sequences previously identified by Hazen et al. (4). The closest sequenced relative is Bermanella marisrubri RED65 (17) (National Center for Biotechnology Information reference sequence: NZ_AAQH00000000.1) (Fig. 3), with 16S rRNA sequence identity at 94%. Thus, we have taxonomically resolved the culture-resistant and numerically abundant organism found in the initial stage of the DWH spill to the genus level. This novel Bermanella genome rapidly decreased in relative abundance to 1.3% and 0.02% of the bacterial community in day-18 and day-64 samples, respectively (Fig. S3).

![Phylogeny of Gammaproteobacteria genomes reconstructed from metagenome data, based on concatenated ribosome protein sequences, using the maximum likelihood algorithm RAxML. Genome assembly of Cyclolocisticus sp. Phe_8 by Dombrowski et al. (37) was not included in our tree, because it was highly incomplete. Length of bar represents relative abundance of resolved genomes for each time point.](image-url)
We searched for the presence of potential hydrocarbon-utilization genes in both genome-resolved contigs as well as in assembled sequences that were not binned. Genes involved in three different alkane degradation reactions were detected, including alkane monooxygenase (alkB) and COP15 alkane hydroxylase (EC 1.14.15.1) that degrade medium-chain (C5–C20) alkanes and hydrocarbon monoxygenase (18) for short-chain (C2–C5) alkane oxidation. Of these, only the abundance of alkB changed considerably between early and later phases and closely correlated with the shifts of the Bermanella genomes. AlkB was highly abundant by day 6 and decreased significantly afterward as the concentrations of linear alkanes diminished (Fig. S4). The alkB gene was the only hydrocarbon degradation gene identified in the nearly complete Bermanella genome (Table S1). Calculating the inferred in situ replication rates with the iRep (19) rate algorithm and assuming that the Bermanella was the fastest-growing organism in the early and also in the intermediate stage of the simulation experiment (Fig. S3).

### Colwellia, Cycloclasticus, and Single-Ring Aromatic Hydrocarbon Degradation Are Highly Abundant in the Intermediate Stage.

The day-18 metagenome sequence sample was dominated by members of the genera Colwellia and Cycloclasticus (Fig. S3). Bermanella that dominated the day-6 samples was present (1.3%), as were Oleispira (0.74%), Arcobacter (2.2%), and Marinomonas (0.5%). We detected at least three different genomes of Colwellia, two of which were nearly complete (Fig. 3), and recovered one nearly complete genome of Cycloclasticus with the highest coverage at day 18. Another Cycloclasticus genome (Cycloclasticus sp. 44_32_T64) was detected, but at much lower relative abundance compared with this highly abundant one, and with an increase in relative concentration at day 64 (Fig. S5).

The abundances of aromatic degradation genes peaked at day 18, both in ratio to alkane degradation genes and in relative abundance (Fig. S4). This is consistent with the conclusion from chemical data and the community analysis based on both genome sequences and 16S rRNA genes and provides evidence that there was a shift from alkane to aromatic utilization by the dominant members of the microbial community (Figs. 1, 2, and 3 and Fig. S5). We resolved a number of genomes from the day-18 sample with the genetic potential to biodegrade single-ring aromatics, including those genomes with the highest relative abundance. For instance, nearly complete genome sequences for the two most abundant Colwellia isolates were identified in the day-18 sample. One is closely related to the sequenced genome Colwellia psychrotolerans 54H, whereas the other Colwellia is phylogenetically distinct at the species level (Fig. 3). Resolved genomes of Marinomonas, Oleispira, and an additional genome with the Osedax symbiont as its nearest neighbor contained cyclohexanone monoxygenase and mandelate racemase genes with the potential to degrade single-ring aromatic hydrocarbons.

A dominant genome recovered at day 18 is represented by Cycloclasticus_46_83_sub15_T18. This genome does not have as many varieties of hydrocarbon degradation genes (Table S1) as the known Cycloclasticus polymycic aromatic hydrocarbon (PAH) degraders, PS-1 (20), FY7M (21), 78ME (22), or P1 (23), although it does have hydrocarbon monoxygenase, which can degrade low-molecular-weight hydrocarbons (18), and 2-polypropenyl phenyl hydroxylase, ring-hydroxylating dioxygenase, which involves degradation of aromatics substrates.

### Genomes Capable of Degrading Polycyclic Aromatics Were Observed During Late-Stage Hydrocarbon Degradation.

At day 64 we detected the increase of Rhodobacterales and Flavobacteriales (Figs. 2 and 3 and Fig. S3). There was also transition within Oceanospirillales (Fig. 3). On day 6, the dominant genome within Oceanospirillales was Bermanella, and then by day 18 switched to Marinomonas, Oleispira antarctica (represented by a partial genome, not included in Fig. 3), and an organism related to an Osedax symbiont bacterium Rs2 (24). Finally, only the relative to the Osedax symbiont bacterium was observed within Oceanospirillales on day 64.

This genome possessed enzymes that are involved in the degradation of short-chain alkanes, BTEX, aromatic carboxylic acid, cyclohexane, and other aromatics (Table S1). Aromatic hydrocarbon degradation genes were also found in genomes of Bacteriovorax marinus, Thalassotalea sp., Sulfitobacter sp., Hydrocarboniphaga effuse, and many other partial genomes belonging to Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria.

### Discussion

This study used a laboratory-based method to recreate the conditions that were present in the deep ocean in the Gulf of Mexico and recapitulate the hydrocarbon degradation pattern and microbial community succession observed in the 2010 DWH event and partially observed during an incomplete time course in an earlier laboratory simulation (15). The combination of cold temperature, lack of light, bioavailable oil in the form of 10-μm droplets, dispersant, and the indigenous microbial community allowed us to successfully resolve high-quality genomes and their functional capacities for all key microbes implicated throughout the entire DWH plume oil degradation time course. It also allowed us to monitor the biodegradation of low-molecular-weight aliphatic and aromatic hydrocarbons including benzene, toluene, and xylene.

In this simulation we were able to enrich the dominant hydrocarbon-degrading organism that was detected in the initial stage of the DWH plume and reconstructed it as a nearly complete genome. This genome is most closely related to Bermanella marisrubri strain RED65 from the Red Sea (17). Given only 94% homology over the entire 16S rRNA gene sequence, we propose a new species within this genus with the name “Candidatus Bermanella maconodprimits” to reflect the region from within the Gulf of Mexico where this uncultured organism was obtained. This novel Bermanella species seems to have the fastest replicating genome at both days 6 and 18 by measuring assembled sequence from high-quality draft genomes with the iRep algorithm. This highlights not only the fast acquisition of carbon by this organism but also provides evidence that the organism is very successful in the occupied niche likely defined by rapid degradation of short chain alkanes. It is interesting that the replication rate of this Bermanella was still high at day 18 when Cycloclasticus and Colwellia emerged as the dominant members of the microbial community.

Laboratory experimental studies by other groups have not replicated the suspension of dispersed oil droplet conditions observed in deep-water plumes, which may explain why these studies have been unable to enrich the early responding Bermanella and recreate the succession of bacteria observed in the field. For example, Kleindienst et al. (7) observed an initial enrichment of Colwellia and Marinobacter rather than Bermanella in their experiments, but they provided only water-soluble fractions of crude oil as substrate and did not include insoluble microdroplets to test the effects of dispersants on hydrocarbon-degradation rates and microbial community responses. Unlike Marinobacter that can efficiently degrade a variety of soluble PAHs in addition to aliphatics, the metabolic capabilities of Bermanella are constrained to poorly soluble aliphatics. Studies that underrepresent insoluble fractions incompletely represent the physical and chemical conditions of the crude oil that was encountered by the deep-ocean microbial community, and conclusions about the overall effectiveness of chemical dispersants that are drawn from such studies are incomplete. If chemical dispersants enhanced the formation of crude oil microdroplets that fed the large bloom of Bermanella then they may have had an overall positive effect on the degradation of the poorly soluble oil fractions.
In uncovering the genetic potential of the 18 draft genomes identified in this study to degrade hydrocarbons we see a more complex and dynamic community-level response to the sudden influx of oil in the deep ocean than was previously determined by 16S rRNA gene sequence analysis. The results of the genomes of the oil-degrading bacteria identified to hydrocarbon-degrading organisms for which there is current genetic or physiological information is, for the majority, distant. Among the diverse set of hydrocarbon degraders enriched upon the addition of the MC252 oil, it was unexpected that we would find multiple members within a genus, each with distinct complement of hydrocarbon degradation genes. We found that several genomes from novel taxa possessed fewer hydrocarbon degradation genes and seemed to be present in equal or higher abundance relative to their better-studied counterparts. For instance, we recovered three high-quality genomes within the genus Cycloclasticus. Cycloclasticus_44_32_T64, with 11 identified aromatic degradation genes, was closely related to the known PAH degrader Cycloclasticus P1, PY97M, 78ME, or P1 (Fig. 3) (22, 23, 25, 26), which is generally used as a reference organism for studying hydrocarbon degradation in the ocean. However, although the more distantly related Cycloclasticus_46_83_sub15_T18 and Cycloclasticus_46_120_T64 each possessed only three genes capable of degrading aromatic substrates, they were present in higher relative abundance at both days 18 and 64. This trend was also noted in the novel Bemanella, which was the dominant organism identified early in the DWH oil spill. This fast-replicating genome possessed only a single alkB gene capable of hydrocarbon degradation. Although there are alkB genes in the genomes of other hydrocarbon degraders, considering the dominance of Bemanella genome in the day-6 sample we concluded the Bemanella alkB was the major contributor in response to linear alkane biodegradation. We speculate that specialization of hydrocarbon substrate provided a substantial advantage due to the given hydrocarbon composition and thus generalists (capable of degrading a wide range of the MC252 oil components) are likely not as responsive in the event of a large environmental release with a sudden influx of high concentrations of hydrocarbons.

One major difference between our laboratory experiment and the DWH oil plume was the lack of input of natural gas. Methane was the greatest single hydrocarbon species emitted during the oil release, whereas ethane and propane were considered to be the most microbially accessible. No study, to date, has been able to create the concentrations and pressure that would have existed in the deep plume to definitively identify any natural gas-degrading microorganism that would have been present in the spill. However, in our study the nearly identical structure and succession pattern of the microbial community observed in the DWH oil plume strongly suggests that the microbial community structure was primarily a consequence of crude oil rather than these natural gases. Modeling efforts have shown that separation of the gas and oil plumes were sent to Battelle and processed by liquid-liquid extraction with methylene chloride for total petroleum hydrocarbons and saturated hydrocarbons by gas chromatography–flame ionization detector using a modification of SW-846 Method 8015. Parent and alkylated PAHs, decains, and the recalibrant bio- marker 30αij-hopane (28) were analyzed by gas chromatography/mass spectrometry in selected ion monitoring using modifications of SW-846 Method 8270. Samples for volatile organic compounds were analyzed by purge-and-trap GC/MS (Battelle SOP 5-245, a modification of SW-846 Method 8260).

Analytical methods for hydrocarbons fulfilled the requirements described in the National Oceanic and Atmospheric Administration’s Mississippi Canyon 251 (Deepwater Horizon) Natural Resource Damage Assessment Analytical Quality Assurance Plan Version 3.0, 2011. Although individual analysis may have additional quality assurance requirements, batch quality assurance/quality control included at least the analysis of laboratory blanks, fortified blanks, sample duplicates, and standard reference materials (National Institute of Standards and Technology SRM 2779) as needed.

DNA Extraction. Samples (800–1,400 mL) were filtered through sterile filter units of 47-mm-diameter polyethylenesulfone membranes with 0.22-μm pore size (MO BIO Laboratories, Inc.). Eight sample times (days 0, 3, 6, 9, 12, 18, 36, and 64) were analyzed on G3 PhyloChip for 16S rRNA gene-based community analysis. Two replicate oil-treated samples and one unamended control were analyzed for each time point extracted from control and added oil treatments. Three oil-treated samples (days 6, 18, and 64) were selected for metagenome sequencing. These samples represented roughly the three phases of the succession (Fig. S1). For each sample, the same extracted genomic DNA was used for both PhyloChip and metagenomic studies. Genomic DNA was extracted from filters using a modified Miller method (29) described in detail in Supporting Information.

Metagenomic Sequencing Library Preparation. DNA was quantified by Qubit fluorometer (Invitrogen) and 200 ng were sheared using a Covaris instrument. The sheared DNA was cleaned, end-repaired, and size-selected using a TruSeq Nano DNA kit (Illumina) targeting fragments around 300 bp. The size and quality of the DNA was checked via Bioanalyzer using a High Sensitivity DNA kit (Agilent Technologies). The size-selected DNA was further processed for library construction according to the manufacturer’s instructions. The final libraries (peak at ~440 bp) were assessed via Bio-analyzer using a DNA 7500 kit and sent to Yale Center for Genomic Analysis for 2-bp paired-end sequencing on Illumina HiSeq 2500. The three libraries were pooled and run on a single lane according to the standard protocol performed at the Yale Center for Genomic Analysis.

PhyloChip Analysis. The 16S rRNA gene was amplified using bacterial primers 27F/1492R and archaeal primers 46F/1492R (30), with annealing temperatures from 50 to 56 °C. Bacterial PCR product (500 ng) and archaeal PCR product (25 ng) were hybridized to each array following previously described
Metagenome Assembly, Binning, and Annotation. Metagenomic reads quality assessment, reads trimming, contig assembly, and annotation followed the general methods described previously (31). The pipeline is described in Supporting Information. Assembled genome fragments were assigned to draft genomes of origin (binned) with a combination of online binning tools (kgbase.berkeley.edu) and binning software, Maxbin 2.0 (32) and Metabat (33). Subassemblies were obtained by assembling subset of reads (reads were randomly sampled to obtain a 1/10th to 1/80th of total reads) using IDBA-UD. This technique was applied specifically to samples T6 and T18, to obtain near-complete genomes of Bacteriovorax sp., Colwellia, Cyclolasticus, and Acrobacter. The genome bins were refined based on tetranucleotide frequency information analyzed using an emergent self-organizing map (34), with refinement of some bins using organism abundance pattern data. Contig abundance was calculated from reads mapping using Bowtie2 (35).

Contig abundance was calculated from reads mapping using Bowtie2 (35).

22. Messina E, et al. (2016) Genome sequence of obligate marine polycyclic aromatic hydrocarbon (PAH) degrader Acrobacter sp. MAAU00000000. The version described in this paper is version MAAU00000000-MAAU00000000 and MAAW00000000-MABF00000000. The raw reads have been deposited at DDBJ, the European Nucleotide Archive, and GenBank under the accession nos. MAAA00000000-MAAA00000000 and MAAW00000000-MABF00000000.


Dissolved oxygen was for 5 min at 4 °C. Approximately 480 μg were analyzed using SERC SOP-004 by × g μg Ex Taq buffer (Takara Bio Inc.), 0.025 units/μL of soluble iron was conducted at University of Miami using a high-precision spectrophotometer. The dispersion generator was adjusted to produce median-size oil droplets of 10-μm diameter using Macondo (MC252) oil, the same oil involved in the DWH oil spill. Although empirical data were not readily collected during the DWH incident, all of the available evidence suggests that droplet sizes less than 100 μm were needed for the plumes to remain buoyant at depth (1, 2). Previous work did show that 10-μm droplets of MC252 oil were stable under the operational parameters required for the biodeterioration experiment (15).

To form the MC252 10-μm oil droplets, Macondo oil and dispersant were mixed with seawater at the final concentration of 2 ppm oil and 0.02 ppm Corexit EC9500A dispersant (Nalco). The ratio of oil to dispersant replicated concentrations observed in deep-water plumes (1, 15). All samples were incubated in 2-L bottles at 4 °C in the dark at a rotation speed of 0.75 rpm on a rotation carousel system (15) that maintained the droplets in suspension and prevented the formation of slicks. Unfiltered Gulf of Mexico seawater samples that were not amended with microdroplets of oil and dispersant were also included in the experiment as controls.

Five replicate samples for each dispersion treatment were destructively sampled at each sampling point at 0, 3, 6, 9, 12, 18, 36, and 64 d of incubation. One unfiltered control and one sterile blank were also killed at each sampling time. For each sampling date, three oil-amended bottles were analyzed for hydrocarbon degradation experiment (15).

Dissolved Oxygen and Nutrient Analysis. Dissolved oxygen was measured in every sacrificial bottle as well as in a sentinel bottle kept in the rotating carousels by using a YSI 5100 dissolved oxygen meter system equipped with a stirring YSI 5010 BOD probe. Oil droplets were measured using Beckman Coulter Multisizer 4 particle counter equipped with a 100-μm aperture calibrated with 10-μm nominal size latex beads. All nutrient samples were analyzed at the Southeast Environmental Research Center at Florida International University. N+ and NO− were analyzed using SERC SOP-004 by a modification from EPA 355.2 (D). NH4+ was analyzed using a modification from EPA 350.1(D). Soluble reactive phosphorous was analyzed using EPA 365.1(D). Ultratrace determination of soluble iron was conducted at University of Miami using a high-resolution, isotope dilution inductively coupled plasma MS method after coprecipitation with magnesium hydroxide (39).

DNA Extraction. Half of each filter was cut into pieces and placed into a Lysing Matrix E tube (MP Biomedicals). Miller phosphate buffer (300 μL), Miller SDS buffer (300 μL), and phenol:chloroform:isoamyl alcohol (25:24:1) (600 μL) were added, and the tubes were bead-beat at 5.5 m/s for 45 s in a FastPrep instrument (MP Biomedicals). The tubes were centrifuged at 10,000 × g for 5 min at 4 °C. Approximately 560 μL of supernatant was transferred into a new tube and an equal volume of chloroform was added. The tubes were gently inverted for 5 s and centrifuged at 10,000 × g for 5 min at 4 °C. Approximately 480 μL of supernatant was transferred into a new tube and two volumes of Solution C4 (MO BIO) was added and mixed by inversion. The rest of the purification procedures followed the instructions in the MO BIO PowerSoil DNA Isolation Kit. Samples were eluted in 60 μL of Solution C6 and stored at −20 °C.

PhyloChip Analysis. Bacterial PCR product (500 ng) and archaeal PCR product (25 ng) were hybridized to each array following previously described procedures (4). Briefly, PCR product and a custom spike mix containing amplicons of known concentration were combined, fragmented to 50–200 bp using DNase I (Invitrogen), biotin-labeled, and hybridized overnight at 48 °C and 60 rpm. The arrays were washed, stained, and scanned as previously described. Data from the resulting .CEL files were processed through PhyCA using the same bacterial Stage1 and Stage2 cutoffs as previously described (30).

Metagenome Assembly, Binning, and Annotation. Adapter sequences were removed using Cutadapt (https://www.usadellab.org/cms/index.php?page=ctadapt) (1.21). Sequences were trimmed for quality using Trimmomatic (www.usadellab.org/cms/index.php?page=trimmomatic) with the following parameters: leading 3, trailing 3, sliding window of 4, quality score ≥25, minimum read length of 60 bases.). The trimmed reads were assembled using IDBA-UD (40). Contigs of >1 kb were retained for further studies. The relative abundance of the contigs was defined as the sum of reads mapped to contigs of interest divided by the sum of reads mapped to all metagenome contigs (at least 1 kbp). Gene predictions were made using Meta-Prodigal (41) and functional predictions were made using a standard annotation pipeline, including amino acid similarity searches (42) against UniRef (43) and KEGG (44, 45). Functional annotations were searched across the dataset in ggKbase (ggbkbase.berkeley.edu) to predict the metabolic repertoire of specific organisms.

Construction of Phylogenetic Tree. The phylogenetic tree is based on concatenated alignments of the amino acid sequences of 16 ribosomal proteins (ribosomal protein L2, S3, L3, L4, L5, L6P-L9E, L11, L16-L10E, S8, L14, L18, L22, L24, S10, S19, and S17),
which were identified using BLASTP as described previously (36). We created alignments of the single proteins using Muscle (46). Subsequently, we trimmed ends manually, concatenated the single alignments, and removed columns with more than 95% gaps. We calculated the phylogenetic tree using the maximum likelihood algorithm RAxML (47) with 100 bootstraps on the CIPRES webserver (www.phylo.org). This approach yields a higher-resolution tree than is obtained from a single gene, such as the widely used 16S rRNA gene. The chosen ribosomal proteins generally locate in a small genomic region in synteny, which will reduce binning errors.

**Total Reads Profiling.** The alkane monooxygenase gene (*alkB*) was used as a representative for alkane degradation. Enzymes for aromatic compound degradation were based on the degradation profiles demonstrated by chemical data (Fig. 1), including aromatic ring monooxygenase, aromatic ring-hydroxylating dioxygenase, aromatic ring hydroxylase, benzene dioxygenase, benzene monooxygenase, cyclohexanol dehydrogenase, cyclohexanone-1,2-monooxygenase, ethylbenzene dehydrogenase, benzene dioxygenase, toluene monooxygenase, mandelate dehydrogenase, naphthalene-1,2-dioxygenase, and salicylate hydroxylase. All reads passed trimming were searched against the enzyme databases using BLASTX. The reads that produced alignments of greater than or equal to 90% identity with over 45 aa were searched against the nr database. If an alignment against nr had an identity of over 45 aa, and the nr hit was a different enzyme, the read was removed from the total number of hits against the enzyme.

**SI Results**

In assaying for potential methanotrophs in the microbial community of the laboratory simulation we found that the relative abundance of *Methylophaga* was highest at the intermediate to late stage of hydrocarbon degradation (Fig. 3 and Fig. S3). We found a key enzyme of C1 metabolism (formate dehydrogenase, EC 1.2.1.2) in *Methylophaga* and members of Rhodobacterales genomes. Additionally, methanol oxidation capability (methanol dehydrogenase) was found in genomes of *Methylphaga* and members of Flavobacterales. We identified genes having three subunits and high amino acid homology (>90–100%) to hydrocarbon monooxygenase (18). Besides two copies in unbinned contigs from the day 18 sample, this hydrocarbon monooxygenase gene was only found in the two *Cycloclasticus* genomes and three Gamaproteobacteria genomes (Table S1). We did not find genes with high homology to methane monooxygenases in any assembled sequence.
Fig. S1. Microbial community changes during 64 d based on community 16S rRNA gene structure. Nonmetric multidimensional scaling (NMDS) ordination with the Bray–Curtis distance metric was performed in Primer-7 using Bray–Curtis similarity (stress = 0.04). Closed symbols are oil-treatment samples and are labeled by time point. Open symbols are control (no oil) samples.

Fig. S2. Microbial growth measured by direct cell counts with DAPI staining and microscopy.
Fig. S3. In situ replication rate measures, raw coverage (used for iRep calculations), and relative abundance measure (normalized raw coverage) for the different organisms detected in the samples from days T6, T18, and T64. Only genomes of high quality were used for iRep calculations (Methods). The cutoffs for iRep were a raw coverage >5, minimum number of windows = 0.98, $r^2 = 0.9$, maximum number of fragments/ Mbps = 175, and GC correction minimum $r^2 = 0.0$.

<table>
<thead>
<tr>
<th>Organism</th>
<th>in situ rate</th>
<th>raw coverage</th>
<th>relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T6</td>
<td>T18</td>
<td>T64</td>
</tr>
<tr>
<td>Bacterioides sp. 47_1443_vc880_T6</td>
<td>29.24%</td>
<td>26.4%</td>
<td>30.8%</td>
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<tr>
<td>Arrobacter sp. 32_11_vc1005_T18</td>
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<td>2.5 ± 2.8</td>
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<tr>
<td>Cytophagales sp. 46_88_vc854_T18</td>
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<tr>
<td>&quot;Ruminococcus sp. 46_46_vc350_T18</td>
<td>0.0 ± 0.0</td>
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<tr>
<td>Methylocapri, sp. 43_13_T18</td>
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<td>0.0 ± 0.0</td>
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<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<td>Caldocubicoccus psychrophilus 36_32_vc03_T18</td>
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Fig. S4. Percentage of reads assigned to alkane degradation gene alk8, or aromatic hydrocarbon degradation genes, including aromatic ring hydroxylases, dioxygenases and monoxygenases, benzene dioxygenase, benzene monoxygenase, cyclohexanol dehydrogenase, cyclohexanone 1,2-monoxygenase, ethylbenzene dehydrogenase, hydrobenzene dioxygenase, hydrotoluene monoxygenase, mandelate dehydrogenase, naphthalene 1,2-dioxygenase salicylate hydroxylase, toluene dioxygenase, and muconate cycloisomerase.

n/a, as requirement for iRep were not met; n/d not determined due to genome quality.
Phylogeny of near-complete genomes based on concatenated ribosome protein sequences (ribosomal protein L2, S3, L4, L5, L6P-L9E, L15, L16-L18, S8, L14, L18, L22, L24, S10, S19, and S17). The maximum likelihood algorithm RAxML (47) was used for constructing the phylogenetic tree.
<table>
<thead>
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<th>Genomes</th>
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<th>Potential degradation pathway</th>
<th>Genome completeness*</th>
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<td>BTEX, proline</td>
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<td>Genomes</td>
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Genomes were named in taxonomy_number1_number2_sampleID. Number 1 was the percent of genome completeness content. Number 2 was the coverage. Sample ID T6, T18, and T64 denote samples of days 6, 18, and 64, respectively. BSCG, bacterial single-copy gene; LMW, low molecular weight; RP, ribosomal protein.

*Ribosomal and bacterial single-copy genes (55 and 51 were used to assess genome completeness, respectively) detected in the draft genome.
Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)