Microbial Community Composition, Functions, and Activities in the Gulf of Mexico 1 Year after the Deepwater Horizon Accident

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Several studies have assessed the effects of the released oil on microbes, either during or immediately after the Deepwater Horizon accident. However, little is known about the potential longer-term persistent effects on microbial communities and their functions. In this study, one water column station near the wellhead (3.78 km southwest of the wellhead), one water column reference station outside the affected area (37.77 km southeast of the wellhead), and deep-sea sediments near the wellhead (3.66 km southeast of the wellhead) were sampled 1 year after the capping of the well. In order to analyze microbial community composition, function, and activity, we used metagenomics, metatranscriptomics, and mineralization assays. Mineralization of hexadecane was significantly higher at the wellhead station at a depth of ~1,200 m than at the reference station. Community composition based on taxonomical or functional data showed that the samples taken at a depth of ~1,200 m were significantly more dissimilar between the stations than at other depths (surface, 100 m, 750 m, and >1,500 m). Both *Bacteria* and *Archaea* showed reduced activity at depths of ~1,200 m when the wellhead station was compared to the reference station, and their activity was significantly higher in surficial sediments than in 10-cm sediments. Surficial sediments also harbored significantly different active genera than did 5- and 10-cm sediments. For the remaining microbial parameters assessed, no significant differences could be observed between the wellhead and reference stations and between surface and 5- to 10-cm-deep sediments.

Following the explosion and sinking of the Deepwater Horizon (DWH) oil rig in the Gulf of Mexico (GOM), an estimated 3.26 to 4.9 million barrels of light crude oil were released at a depth of 1,544 m from 20 April to 15 July 2010, making it the largest and deepest offshore spill in U.S. history (1, 2). When including gaseous hydrocarbons, like methane, the total discharge was 40% higher than the above-mentioned estimate (3). During the spill, a deep-water oil plume was detected at depths of 1,000 to 1,200 m (4, 5), but this plume was no longer detectable after a few months (6), in agreement with the very high degradation rates observed in laboratory incubations (5). However, most microbiological research to date has focused on the effects of the oil spill with samples taken during the contamination event or shortly thereafter (5, 7–15), and only one study reported on the bacterial communities at plume depth 1 year after the spill (16). In view of the high degradation rates observed and slow mixing of deep water, it was suggested that oxygen depletion at plume depth might persist for several years (3, 17–19). The cause, extent, and duration of this oxygen depletion were subject to debate (8, 20, 21), and it is not clear how, and if, it would impact the microbial communities in the long term. Recent work also indicated that significant quantities of oil sank to the sea floor (22), potentially affecting microbial communities in the sediments.

The microbial characterization of the water column shortly after the beginning of the spill identified *Oceanospirillales* as a dominant group of hydrocarbon-degrading organisms, making up as much as 90% of the 16S rRNA gene clone libraries (5, 12, 13, 16, 23). Shortly after this, other *Gammaproteobacteria* affiliated with *Colwellia* and *Cycloclasticus* appeared, indicative of a succession from alkane to aromatic degrading bacteria (13, 15, 16, 23). In addition, other genera of bacteria (*Alteromonas*, *Halomonas*, and *Pseudoalteromonas*) were observed in the water column (5, 15). A recent DNA-stable isotope probing (SIP) study pro-

vided direct evidence that most of the above-mentioned taxa were in fact capable of degrading various hydrocarbons (24). Following the spill, after the flow of hydrocarbons had been arrested, methylotrophs, including known methane oxidizers, became dominant in the region of the plume (8). Microbial communities are at the base of several crucial biogeochemical processes in marine environments, including hydrocarbon degradation. Full ecosystem recovery is intimately linked to microbial community recovery. Microorganisms might also serve as highly sensitive bioindicators (25), as they have been shown to be sensitive to very low concentrations of pollutants, especially with regard to their transcriptome (26–28). For these reasons, microorganisms could be used as indicators of pollution and ecosystem recovery through the examination of their gene content and gene expression patterns.

Two approaches were used to determine the potential effects of the DWH blowout on microbial communities more than 1 year after the event: (i) comparison of two water column stations, one very close to the well and the second 38 km away, outside the

Received 14 May 2015 Accepted 14 June 2015

Accepted manuscript posted online 19 June 2015

Citation Yergeau E, Maynard C, Sanschagrin S, Champagne J, Juck D, Lee K, Greer CW. 2015. Microbial community composition, functions, and activities in the Gulf of Mexico 1 year after the Deepwater Horizon accident. *Appl Environ Microbiol* 81:5855–5866. doi:10.1128/AEM.01470-15.

Editor: J. E. Kostka

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AEM.01470-15>.

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FIG 1 Map of the sampling sites. The main image (data SIO, NOAA, U.S. Navy, NGA, GEBCO; image NOAA; image Landsat; data USGS) and the inset (data SIO, NOAA, U.S. Navy, NGA, GEBCO; image U.S. Geological Survey; image copyright 2012 Terrametrics; copyright 2012 Cnes/Spot Image) are from Google Earth.

plume area, and (ii) depth profile of deep-sea sediment cores taken in the proximity of the Macondo well. We used a shotgun metagenomic and metatranscriptomic approach and compared the microbial functions, community compositions, and activities of the different stations with depth.

MATERIALS AND METHODS

Sampling sites. A map of the sampling sites is provided as Fig. 1. The wellhead water column station (BM-57; lat 28.7051, long -88.4016) was located at a distance of 3.78 km southwest from the actual Deepwater Horizon wellhead and corresponded to the plume station BM-57 used by Hazen and colleagues (5). The reference water column station (A6; lat 28.6632, long -88.0095) was located 37.77 km southeast from the Deepwater Horizon wellhead; it was in the same “dome” area but was outside the plume area during the spill. A series of 6 deep-sea sediment cores were collected on 16 November 2011 during a second cruise. The cores were collected from the vicinity of the Deepwater Horizon wellhead (around lat 28.715011, long -88.358703 , 3.66 km southeast from the wellhead) at a depth of approximately 1,600 m.

Water and sediment sampling. Water samples were collected between 9 and 16 September 2011 using either a large bailing bucket (surface samples) or a CTD Niskin rosette equipped with 20-liter bottles. For each depth, three replicate water samples were taken. Samples were returned to the boat and immediately transferred to 4-liter carboys which had been previously rinsed with 70% ethanol and sterile distilled water. Sample filtration was started immediately after transfer using Millipore GSWP (0.22- μ m pore size, 47-mm diameter) filters and glass filter supports. Each 4-liter water sample was filtered on two filters, resulting in a total of six filters per depth. The filters were then transferred to ice and stored at -80°C . Between samples, the glass filter supports were rinsed with 70%

ethanol and sterile distilled water. For the shipping of filtered samples, coolers with dry ice were used and upon arrival at the lab, filters were stored at -80°C until nucleic acid extraction was performed. Water samples destined for mineralization analysis were collected from the same carboys as used for filtration. Samples were placed in sterile 50-ml Falcon tubes and stored at 4°C . Samples were shipped on ice and upon arrival at the lab were placed immediately at 4°C . Microcosms were started as soon as possible after arrival into the lab (within 24 h). Water for chemical analysis was also taken and kept at 4°C until processing.

Sediments were frozen onboard the sampling vessel. Samples were shipped and received frozen and stored at -20°C until processing. Sample processing was performed at -20°C based on a protocol modified from that of Juck et al. (29). In brief, an approximately 5-cm strip of the core sample plastic sleeve was cut and removed (from top to bottom of the core) and a “clean” area of the core (i.e., not contacted by the sample sleeve) was exposed using a sterile chisel. Once this flat clean area was exposed, a sterile 1.4-cm drill bit was used to slowly drill into the core sample, parallel to the core surface. The drilled core subsample was then transferred to a sterile 50-ml Falcon tube and stored at -80°C until extraction of nucleic acids was performed. Each core was sampled at 3 different depths: “0 cm” was from the surface of the sediment to 1.4 cm, “5 cm” was from approximately 4.3 to 5.7 cm from core surface, and “10 cm” was from approximately 9.3 to 10.7 cm from the sediment surface. From the remaining core samples, the material remaining at 0, 5, and 10 cm was sampled and used for hydrocarbon analysis as described below.

Water microcosm mineralization assays. Mineralization assays using microcosms were set up using 15 ml of seawater and ^{14}C -labeled (100,000 dpm) hexadecane (2.5 ppm) as the sole carbon source, with no amendments added. The sealed bottles containing seawater from all the different depths for both water column stations and the spiked substrate were all

incubated at 15°C (the range of sample temperatures at the time of collection was 4°C [bottom samples] to 30°C [surface samples]) with orbital shaking (140 rpm) and a microcosm KOH trap (1 ml of 1.0 M KOH in a test tube). Sampling was performed at 3, 7, 14, 22, 28, 35, 42, 49, 56, and 63 days. The amount of radioactive $^{14}\text{CO}_2$ produced due to the complete mineralization of the added carbon source (hexadecane) was determined by scintillation counting of the KOH solution recovered from the microcosm flasks and is presented as a percentage of $^{14}\text{CO}_2$ produced from the known quantity of carbon source added at time zero. During sampling of the KOH traps, atmospheric oxygen was introduced (through a 0.22- μm filter) into the microcosms to ensure sufficient aeration of the samples. The 700-m-depth sample from the reference station was also used as a sterile abiotic control by autoclaving for 20 min and cooling to room temperature before addition of the radioactive spike.

Hydrocarbon analyses. Water column samples were extracted for C_{10} to C_{50} chain-length alkane and polycyclic aromatic hydrocarbon (PAH) analyses using liquid-liquid extraction (U.S. EPA method 3510C [<http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3510c.pdf>]). Extracts of water were analyzed using high-resolution gas chromatography (GC) (Agilent 6890 GC) coupled to a mass selective detector (Agilent 5973N) (Wilmington, DE) operated in the selective ion monitoring mode (SIM) using the following GC (MDN-5S column, 30 m by 0.25 mm [inside diameter]; 0.25- μm film thickness; Supelco Canada) conditions: cool on-column injection with oven track mode (track 3°C higher than the oven temperature program), 80°C hold, 2-min ramp at 4°C/min to 280°C hold for 10 min. Deep-sea sediments were processed according to the method of King and Lee (30) and the GC-mass spectrometry (GC-MS) conditions outlined for the water extracts were applied to sediment extracts.

Total DNA extraction from filters (seawater). From each water sample (2 stations $\times$ 5 depths $\times$ 3 replicates = 30 water samples), one filter was used and treated for DNA extraction, resulting in 30 DNA extracts. In the 50-ml Falcon tube containing the filter, 1.7 ml of Tris-EDTA (TE) (pH 8.0) buffer was added with 45 μl of 20% (wt/vol) sodium dodecyl sulfate (SDS) and 9 μl of 20-mg/ml proteinase K. The tube was incubated with gentle inversion at 37°C for 1 h. At the end of the incubation, 300 μl of 5 M NaCl was added in addition to 240 μl of a 10% (wt/vol) cetyltrimethylammonium bromide (CTAB) and 0.7 M NaCl solution. The tube was incubated at 65°C for 10 min. The total DNA was extracted with 1 volume of 24:1 chloroform-isoamyl alcohol. After centrifugation for 10 min at $3,000 \times g$, the upper phase was transferred and mixed with 1 volume of 25:24:1 phenol-chloroform-isoamyl alcohol. Following centrifugation at $16,000 \times g$ for 10 min at 4°C, the supernatant was precipitated by mixing 0.6 volume of isopropanol and 1/50 volume of glycogen (5 mg/ml), incubating 1 h at -80°C, and centrifuging at $12,000 \times g$ for 30 min at 4°C. The DNA pellets were washed using 1 ml of 80% (vol/vol) ice-cold ethanol and dried using a SpeedVac. The DNA was resuspended in 50 μl of nuclease-free water and treated with RNase If (NEB, Ipswich, MA) according to the manufacturer's instructions. After the reaction was complete and the enzyme was inactivated, the DNA was purified with the QIAEX II kit (Qiagen, Valencia, CA) and quantified using the PicoGreen assay (Invitrogen).

Total RNA extraction from filters (seawater). For RNA extraction, one replicate seawater sample was used (5 depths $\times$ 2 stations = 10 samples). All solutions were RNase free. In the 50-ml Falcon tube containing the filter, 1.6 ml of freshly prepared lysozyme (10 mg/ml in TE [pH 8.0]) and 80 μl of 20% SDS were added, and the tube was then incubated at 64°C for 5 min. At the end of the incubation, 176 μl of 3 M sodium acetate (pH 5.2) and 1.6 ml of prewarmed acid phenol was added to the lysate and incubated at 64°C for 6 min, with mixing every minute. The tube was transferred on ice for 2 min and then centrifuged at $16,000 \times g$ for 10 min at 4°C. The upper phase was transferred and mixed with 1.6 ml of chloroform before centrifugation at $16,000 \times g$ for 2 min at 4°C. The upper aqueous phase was transferred, mixed with 20 μl of glycogen (5 mg/ml), 160 μl of 3 M sodium acetate (pH 5.2), and 4 ml of 100% ice-cold ethanol, and incubated for 30 min on dry ice before centrifugation at $12,000 \times g$ for 30 min at 4°C. The pellet was washed with 1 ml of 80% ice-cold ethanol

and dried using a SpeedVac. The RNA was resuspended in 400 μl of nuclease-free water (Ambion, Life Technologies, Burlington, Ontario, Canada) and pooled in the same tube. The extracted total RNA was treated with Turbo DNase I (Ambion) and purified with an RNeasy Min-Elute Cleanup kit (Qiagen).

DNA and RNA extraction (deep-sea sediments). DNA and RNA were extracted simultaneously from 2 g of sediment using the MoBio RNA PowerSoil Total RNA Isolation kit with the RNA PowerSoil DNA Elution Accessory kit (MoBio Laboratories, Carlsbad, CA).

Metagenomic sequencing. Each DNA library was prepared for sequencing from 50 to 100 ng of DNA using the Ion Xpress Plus Fragment Library kit (Life Technologies) with the Ion Xpress Barcode Adapters 1 to 16 (Life Technologies), using the Ion Shear Plus reagents and a Pippin Prep instrument (SAGE Science, Beverly, MA) for size selection. Bar-coded libraries were pooled in an equimolar ratio three by three. A total of 3.50×10^7 molecules were used in an emulsion PCR using the Ion OneTouch 200 template kit (Life Technologies) and the OneTouch instrument (Life Technologies). The sequencing of the pooled libraries was performed using the Personal Genome Machine (PGM) system with the Ion Sequencing 200 kit and 316 chips (Life Technologies). Sequencing statistics are shown in Table S1 in the supplemental material.

Metatranscriptomic sequencing. In order to get enough RNA for library preparation, RNA samples were amplified using the MessageAmp II-Bacteria kit (Ambion) according to the manufacturer's protocol. The antisense RNA (aRNA) obtained was subjected to rRNA subtraction by following the procedure of Stewart et al. (31), with the exception that the T7 promoter was coupled to the forward primer instead of the reverse primer. After subtraction, a 227-bp control RNA transcribed from the pSPT18 vector (positions 2867 to 3104 and 1 to 70) was added in a 1:1,000 ratio (on a nanogram basis) to the total rRNA-subtracted RNA. This mixture was then reverse transcribed using the SuperScript III kit (Invitrogen, Life Technologies). Illumina libraries were prepared by following the protocol of Meyer and Kircher (32), with tags 1 to 34. The indexed libraries were pooled in an equimolar ratio and sent for eight lanes of Illumina HiSeq 2000 paired-end 2 $\times$ 100 bp sequencing at the McGill University and Genome Quebec Innovation Centre (Montreal, Canada). Sequencing statistics are shown in Table S2 in the supplemental material.

Bioinformatics. Metagenomic sequences were submitted to MG-RAST, where they were dereplicated using the method of Gomez-Alvarez et al. (33) and trimmed using the dynamic trimming method of Cox et al. (34) in a way that each individual sequence would contain a maximum of 5 bases below a Phred score of 15. Within MG-RAST, significant matches were defined as having 60% sequence identity over at least 15 amino acids (aa) or 50 bp and with an E value below 10^{-5} . Metagenomic data were used as relative abundance by dividing the abundance of sequences for a particular organism or gene by the total number of sequence retrieved from the sample. Metatranscriptomic data resulted in 544 files (34 samples $\times$ 2 reads $\times$ 8 lanes). Data from the different lanes were pooled, and the resulting 68 files were filtered using a custom-made Perl script, as follows. Paired-end reads were processed in parallel. Reads were first trimmed at the first occurrence of a low-quality base (Phred score below 20) or when the adapter sequence was encountered. Following this step, sequences of less than 75 bp were removed from further analyses. If only one of the paired reads was filtered out, then the remaining read was also removed. The filtered reads were then submitted to MG-RAST 3.0 (35), where mate-paired reads were joined using the fastq-join utility. Mate-paired reads that did not overlap were kept for downstream analyses. Within MG-RAST, significant matches were defined as having 60% sequence identity over at least 15 aa or 50 bp and with an E value below 10^{-5} . The number of sequences related to the pSPT18 vector in the filtered metatranscriptomic data sets was obtained by BLAST using an E value cutoff of 10^{-25} ; this number was used to normalize the number of transcripts using the method of Moran et al. (36).

Statistical analyses. All statistical analyses were carried out in R (v2.13.2; The R foundation for statistical computing, Vienna, Austria).

FIG 2 C_{10} to C_{16} and C_{17} to C_{35} alkane and polycyclic aromatic hydrocarbon (PAH) and methylated PAH concentrations for the wellhead and reference station water column samples and for the deep-sea sediments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Note that PAH and methylated PAH were not detected in any of the deep-sea sediment samples.

Normal distribution of the data was tested using the “shapiro.test” function. If necessary, data were then transformed using log or square root transformations. Analysis of variance (ANOVA) was performed using the “aov” function, while *post hoc* Tukey honestly significant difference (HSD) tests were carried out using the “TukeyHSD” function. If these transformations failed to normalize the data, a nonparametric Kruskal-Wallis test was carried out in lieu of ANOVA (function “kruskal.test”). Correlation analyses were carried out based on Spearman correlation using the “cor” function. Bray-Curtis dissimilarities were calculated using the “vegdist” function of the “vegan” library.

Sequence data accession numbers. Raw sequence reads were submitted to the NCBI Sequence Read Archive (SRA) under BioProject accession number [PRJNA288120](#). Annotated metagenomes (MG) and metatranscriptomes (MT) are available in MG-RAST under accession numbers 4494020.3 to 4494048.3 and 4494917.3 (MG, water, project 1012), 4500695.3 to 4500711.3 (MG, sediments, project 1891), 4508873.3 to 4508882.3 (MT, water, project 2834), and 4508988.3 to 4509004.3 (MT, sediments, project 2866).

RESULTS

The goal of this study was to observe the effects of the DWH spill approximately 1 year after the successful capping of the well. In order to do this, water column samples from a reference station that was outside the spill area were compared to water column samples taken at similar depths at a station that was directly in the spill area. In addition, deep-sea sediment cores were taken in the direct vicinity of the well, and the surface, 5-cm, and 10-cm sediment layers were compared.

Chemical analyses and mineralization assays. The chemical analyses of the water and sediments revealed very low concentrations of alkanes mostly in the range of nanograms per liter of water or nanograms per gram of sediment range (Fig. 2). At these concentrations, near the detection limit, variation between replicates was quite high, and the only significant difference between the

reference and affected water column was between the surface water samples (t test: $t = 3.53$, $P < 0.05$), where the reference station water column samples contained significantly more alkanes (Fig. 2). The hydrocarbon measurements in the deep-sea sediments were only carried out on one of the samples, so differences could not be tested for significance. However, the 0-cm-depth sediments showed higher concentrations of alkanes (Fig. 2). For all samples (water and sediments), the majority of the alkanes detected had chains longer than C_{20} (Fig. 2). Polycyclic aromatic hydrocarbons (PAH) and methylated PAHs were below detection limits in most water samples and in all sediment samples (Fig. 2). When detected in water samples, PAHs were mostly related to phenanthrene or one of its methylated forms. For hexadecane mineralization assays, there was a significant difference between the 1,174-m sample of the wellhead water column station and the 1,284-m sample of the reference water column station (t test: $t = 12.63$, $P < 0.001$), with the wellhead sample showing significantly higher mineralization after 63 days of incubation (Fig. 3).

General patterns in community composition and function.

Bray-Curtis dissimilarities were calculated between water column sample pairs taken at similar depths based on genus relative abundance (Genus-DNA), genus-related mRNA abundance (Genus-RNA), MG-RAST “functions” relative abundance (Functions-DNA) and “functions”-related mRNA abundance (Functions-RNA) (Fig. 4a). For the Genus-DNA data, the Bray-Curtis dissimilarities were significantly higher for the 1,174- to 1,284-m sample pairs than for any other sample pairs from comparable depths (one-way ANOVA: $F = 11.759$, $P < 0.001$) (Fig. 4a), suggesting more dissimilar communities between the stations in water samples taken at this depth. In contrast, for the Functions-DNA, no significant differences between the average dissimilarity for water samples taken at similar depths for the two stations were detected, indicat-

FIG 3 Hexadecane mineralization for the wellhead and reference station water column samples. At day 63, different letters indicate significant differences ($P < 0.05$) in Tukey HSD *post hoc* test.

ing that similarity in the functional potential did not vary with depth. For the RNA data, only one water sample per depth was analyzed, so significance could not be tested. However, the Bray-Curtis dissimilarities were higher for the 1,174- to 1,284-m sample pair for both Genus and Functions (Fig. 4a), indicating that at this depth, there are relatively more differences in active organisms and gene expression between the two water column stations.

For the deep-sea sediments, we compared the different depths among and between each other. The only significant differences observed were for the Genus-RNA data sets, where the Bray-Curtis dissimilarities within the surface layers of the six sediment cores were higher than the distance within the 5- or 10-cm-deep layers (one-way ANOVA: $F = 12.48$, $P < 0.001$), suggesting a higher heterogeneity in the active community composition in the surface sediments. Furthermore, when comparing the Bray-Curtis dissimilarities between the different sediment layers, the dissimilarities were significantly higher when the surface layer was involved (one-way ANOVA: $F = 12.25$, $P < 0.001$) (Fig. 4b), indicating that the active microbial community at the surface of the sediment is different from the one in the deeper layers.

Microbial community composition and activity. As expected, *Cyanobacteria* were dominant in surface water samples from the wellhead and the reference column stations, while deeper-water samples were dominated by *Proteobacteria*, mostly from the *Gammaproteobacteria* subclass (Fig. 5). The *Thaumarchaeota* showed a particular pattern, having very high relative abundance at a depth of 1,174 m (nearly 40%) for the wellhead water column station and at a depth of 700 m (over 30%) for the reference water column station (Fig. 5). When comparing the two water column stations, it was found that *Thaumarchaeota* were significantly more abundant at the wellhead water column station for the 1,174- to 1,284-m depths (t test: $t = 5.3021$, $P < 0.05$). The majority of the *Thaumarchaeota* sequences were related to the *Nitrosopumilus* genus. At the phylum level, the deep-sea sediments appeared relatively homogenous, with small increases in *Deltaproteobacteria* and small decreases in *Alphaproteobacteria* with increasing depth below the seafloor (Fig. 5).

The *Oceanospirillales* have been previously reported as being dominant in the water column during the DWH oil spill. *Oceanospirillales* were not very abundant in our samples, forming less than 3% of all DNA reads in the water column samples and less than 2% of all DNA reads in the deep-sea sediment samples.

Oceanospirillales were significantly more abundant at the reference water column station at depths of 700 to 850 m, but that did not strongly affect their activity. However, the activity in the water column at depths of 1,174 to 1,284 m was 2 orders of magnitude lower at the wellhead water column station. In the deep-sea sediments, *Oceanospirillales* were significantly more active ($F = 4.31$, $P < 0.05$) and abundant ($F = 7.93$, $P < 0.05$) in the surface sediments than in the 10-cm-deep sediments, with the 5-cm-deep sediments showing intermediate values (DNA average relative abundances of 1.70% in the surface sediments, 0.62% at 5 cm, and 0.37% at 10 cm). The obligate hydrocarbonoclastic bacteria (OHCB) *Alcanivorax* and *Marinobacter* did not show any significant differences between the two water column stations at the DNA level (DNA average relative abundances of 0.16% and 0.24%, respectively) but were less active at plume depth (1,174 to 1,284 m) in the wellhead water column station than at the reference water column station. For the sediments, no significant differences were observed at the DNA level (DNA average relative abundances of 0.28% for *Alcanivorax* and 0.022% for *Marinobacter*), but at the RNA level, both *Marinobacter* ($F = 7.69$, $P < 0.05$) and *Alcanivorax* ($F = 9.84$, $P < 0.01$) showed higher activity in the surface sediments. *Colwellia* did not show any significant differences between the two water column stations or the different sediment depths at the DNA level (DNA average relative abundances of 0.087% for the water column samples and 0.13% in the sediments). *Colwellia* was significantly more active in the surface sediments than in the 5- and 10-cm-deep sediments ($F = 9.13$, $P < 0.05$) and showed maximum activity in the deepest-water column samples for both stations. Methanotroph DNA and RNA were detected at all depths for both water column stations and for the sediments, with a dominance of type I methanotrophs from the *Methylococcaceae* family (DNA average relative abundances of 0.17% for *Methylococcaceae* and 0.0078% for *Methylocystaceae* in the water column samples and 0.65% for *Methylococcaceae* and 0.00033% for *Methylocystaceae* in the sediment samples). The only statistically significant effect was a higher activity of *Methylococcaceae* in the surface sediments ($F = 7.83$, $P < 0.05$). Methanotrophs were also 1 order of magnitude more active at the wellhead water column station, as determined by comparing the deepest-water samples to each other (1,574 to 2,174 m).

FIG 4 Average Bray-Curtis dissimilarities based on genus-level community composition or expression patterns or MG-RAST function-level community composition or expression patterns calculated for water samples taken at similar depths at the reference and wellhead stations (a) and replicate sediments taken at similar depths and sediments taken at different depths (b).

The activity of *Archaea*, as measured by the abundance of related mRNA, was slightly higher in the wellhead water column samples, with the exception of the 1,174- to 1,284-m water samples, where *Archaea* were less active at the wellhead water

column station. A similar pattern emerged for bacterial activity, with the exception that the differences were larger and *Bacteria* were more active at the reference water column station for the surface samples. For the sediments, *Archaea* were significantly

FIG 5 Community composition at the phylum or class level for the different stations and sediment sampled based on the taxonomic affiliation of bacterial metagenomic (DNA) reads in MG-RAST.

more active in the surface layer ($F = 40.29$, $P < 0.001$), while *Bacteria* were significantly less active in the surface layer ($F = 9.94$, $P < 0.01$). These patterns at the domain level were not significant in the metagenomic data sets for both water column and sediment samples.

Microbial hydrocarbon degradation-related functions. For both sediments and water samples, no significant differences were found for the relative abundance of aerobic (alkane monooxygenase and ring-opening dioxygenase) and anaerobic (acetyl coenzyme A [acetyl-CoA] acetyltransferase and benzoyl-CoA reductase) key hydrocarbon degradation genes based on the DNA data set. For gene expression based on RNA sequencing, some interesting trends emerged (Fig. 6). For instance, the alkane monooxygenase gene was not expressed in the surface waters (0 to 100 m) of the reference water column station, while it was not strongly expressed in the deeper waters of the wellhead water column station (1,174 to 1,574 m) (Fig. 6a). In contrast, the ring-opening dioxygenases were more expressed at the wellhead water column station for depths greater than 700 m, sometimes by several orders of magnitude (Fig. 6a). The two anaerobic genes were less expressed at the wellhead water column station at depths of 1,174 to 1,284 m, but the benzoyl-CoA reductase gene was more expressed at the wellhead water column station for depths of 750 to 800 m (Fig. 6a). For the sediments, the only significant difference was in the expression of alkane monooxygenase, which was significantly more expressed in the top layer of the sediments ($F = 9.71$, $P < 0.01$) (Fig. 6b).

Starvation-related functions. The expression of gene categories related to nutrient deficiency (e.g., siderophore production, carbon starvation, and nutrient transporters) was examined. When comparing the two water column stations, it was found that siderophore genes were more expressed at the wellhead water column station at 1,174 to 1,284 m, while they were more expressed at the reference water column station at the surface (Fig. 7a). Carbon starvation genes were not expressed at the surface and at depths of 100 m and 2,174 m for the reference water column station, while these genes were expressed throughout the depth profile for the wellhead water column station, except for the surface samples (Fig. 7a). The highest expression of carbon starvation-related genes was observed at a depth of 850 m for the wellhead water column station (Fig. 7a). Ammonium transporters were expressed similarly at most of the depths of the two water column stations, with the exception of the surface and the 1,174- to 1,284-m depths, where the expression was higher at the reference water column station (Fig. 7a). In contrast, the expression of genes related to phosphate starvation was higher for the reference water column station at almost all depths, except the 700- to 850-m depth (Fig. 7a). In the sediments, most of the gene categories were less expressed in the surface of the sediment cores, except for the phosphate starvation genes, which were significantly less expressed in the 10-cm-deep sediments ($F = 4.59$, $P < 0.05$) (Fig. 7b). The only other significant trend was for ammonium transporters that were significantly less expressed in the surface sediments ($F = 9.17$, $P < 0.05$) (Fig. 7b).

FIG 6 Normalized expression of key aerobic and anaerobic hydrocarbon-degradation genes for the different stations (a) and sediments sampled (b) based on the taxonomic affiliation of bacterial metagenomic (DNA) and metatranscriptomic (RNA) reads in MG-RAST. In panel a, no replicates were available, so significance could not be tested. In panel b, different letters indicate significant differences ($P < 0.05$) in Tukey HSD *post hoc* test.

FIG 7 Normalized expression of genes related to nutrient depletion for the different stations (a) and sediments sampled (b) based on the taxonomic affiliation of bacterial metagenomic (DNA) and metatranscriptomic (RNA) reads in MG-RAST. In panel a, no replicates were available, so significance could not be tested. In panel b, different letters indicate significant differences ($P < 0.05$) in Tukey HSD *post hoc* test.

DISCUSSION

In the present study, we looked simultaneously at the metatranscriptome and metagenome of water samples taken at an offshore station strongly affected by the DWH spill and at another offshore station that was unaffected by the spill, approximately 1 year after the capping of the well. We also analyzed deep-sea sediments from the surface to 10 cm deep adjacent to the well.

Water. A large part of our data set showed no significant differences between the water of the reference and wellhead stations. No trace of the massive amount of oil released during the spill could be found, with most hydrocarbon concentrations being near or below the detection limit and not significantly different between the water of the wellhead and reference stations. *Oceanospirillales* relative abundance was also not different between the water column stations, after being reported as largely dominant during the spill (5, 12). No differences were observed between the water of the wellhead and reference stations for the relative abundance and expression of hydrocarbon degradation genes and the presence of obligate hydrocarbonoclastic bacteria (OHCB). Kessler et al. (8) hypothesized that methanotrophs were no longer active in September 2010 even though they were detected in high numbers, because methane concentrations and oxidation rates became very low. In this study, we found methanotrophs to be present and active at all depths for the two water column stations, as previously reported for nonplume samples (14) and in postspill samples (16). Since methanotrophs can only use methane or methanol as their carbon source, their presence and activity at all depths in the water column is indirectly indicative of the presence of either methane or methanol. The amount of gas emitted during the spill comprised 40% (500,000 tons) of the total hydrocarbon discharge (3) but was reported to be degraded very rapidly (8). Another study recently suggested that the genus *Colwellia* was responsible for the majority of ethane and propane oxidation during the Deepwater Horizon spill (13). In our study, this genus had a very low relative abundance but was active in several water column and sediment samples, with relatively more transcripts in the deepest samples and in the sediments. Similarly, several hydrocarbonoclastic bacteria were present in GOM water 1 month before (37) and up to 1 year after (16) the DWH accident. These and our results suggest that in the water of the Gulf of Mexico, although hydrocarbon concentrations are typically very low, there are permanent microbiological activities related to the degradation of hydrocarbons. These activities are probably carried out by a minority of the microbial community (as hydrocarbon degraders had very low relative abundance), but upon feeding with fresh hydrocarbon substrate (as in the microcosm experiments), this minority could be rapidly stimulated. This continuous background hydrocarbon degradation activity probably explains the very rapid disappearance of the hydrocarbon released during the DWH spill (5). This continuous hydrocarbon degradation activity also results in the maintenance of the genetic potential for the degradation of hydrocarbons in the indigenous microbial communities. The natural seepage of hydrocarbons from the GOM seafloor was estimated to amount to 4×10^{10} to 10×10^{10} g per year (38), which results in constant exposure of the microbial community to hydrocarbons, and since this is essentially a continuous process, residual hydrocarbons may remain very low even though hydrocarbon degradation activity is quite high.

Several intriguing differences were observed between the water of the reference and wellhead stations at depths where a dissolved

hydrocarbon plume was detected during the spill (~1,200 m). For instance, there were significantly higher mineralization rates in the water of the wellhead column station, indicating a residual higher potential for alkane degradation. However, no significant differences were observed for hydrocarbon-degrading genes in the metagenomic data sets, suggesting that the potential for hydrocarbon degradation is not necessarily related to the presence of specific functional genes. Rather, this potential might be related to the identity of the microorganisms that harbor these genes, as the water microbial communities were significantly more dissimilar at depths around 1,200 m, suggesting that unique microbial communities were present in the water of the wellhead station at depths where the plume was found. Indeed, some hydrocarbon-degrading organisms have higher metabolic rates, and their presence in the water samples could have increased mineralization rates in the microcosm experiments. We can only hypothesize on the reasons behind these differences: presence of natural seeps, low mixing rates in the deeper water, or some kind of persistent anomaly at plume depth.

Another difference between the wellhead and the reference water column stations was that *Thaumarchaeota* were significantly more abundant at the wellhead at ~1,200 m. All organisms of this lineage thus far identified are chemolithoautotrophic ammonia oxidizers, and *Thaumarchaeota* sequences made up more than 40% of the metagenomic reads at the wellhead water column station, from which the majority were related to *Nitrosopumilus*. *Archaea* were previously reported to dominate the mesopelagic zone (39). This high abundance was, however, very surprising in view of the sensitivity of *Nitrosopumilus* and other ammonia oxidizers to Macondo crude oil (25). *Nitrosopumilus* was even proposed as a bioindicator to map future spills (25). In contrast, Rivers et al. (14) reported that *Nitrosopumilus* was similarly active in plume and nonplume samples during the DWH spill. *Nitrosopumilus maritimus* was reported to be dominant in the suboxic zone of the Baltic Sea (40), and the lower oxygen concentrations observed in some areas after the spill (up to 30 to 50% oxygen depletion [3]) could have favored *Nitrosopumilus*. Although some studies predicted this localized oxygen depletion to persist for months to years because of slow water mixing rates at depth (3, 8, 17–19), the causes, extent, and duration of this depletion have been debated (20, 21). Modeling efforts suggested that physical dynamics would result in the absence of an extensive and persistent oxygen depletion in the deep plume horizon (41), and recent studies of the microbial dynamics suggested that the oxygen anomaly observed at plume depth after the spill could be due to consumption of organic matter from dead organisms in the plume (23).

Microbial activities in the plume could also have resulted in localized nutrient depletion. Nutrient limitation was previously reported during surface oil degradation at an offshore station during the DWH oil spill, as alkaline phosphatase activity increased, indicative of phosphate limitation. Bacterial respiration also increased when hydrocarbons were present but without a concomitant increase in bacterial biomass, which only increased upon nutrient addition (42). *Nitrosopumilus* is especially efficient at low nutrient concentrations, which might explain its predominance in the plume depth wellhead samples. Genes involved in iron limitation were overexpressed at plume depth (1,174 m) at the wellhead station, further suggesting nutrient limitation at these depths. Another study revealed that the draft genome sequence of a dominant member of *Oceanospirillales* contained many genes related to

the uptake of various nutrients, including ammonium, phosphate, and iron, all of which were also found in the metagenomes and expressed in the plume metatranscriptome (12). Some of the differences observed between the reference and wellhead stations at plume depth could have been related to a persistent nutrient depletion. However, during the spill, nitrogen and phosphorus did not appear to be limiting based on measured values at plume depth (14), and other factors might explain the differences observed in microbial communities between the affected and unaffected water columns at the depths where the oil plume was found.

Sediments. The sedimentation rate in the Gulf of Mexico was reported to be 0.09 cm/year at a station located 70.91 km away from the wellhead station, at similar depths (1,849 m) (43). Other stations in the same area and at similar depths also showed very similar sedimentation rates. Bioturbation was reported to be responsible for the active mixing that occurred in the top 2 to 3 cm, and macrofaunal density was significantly correlated with organic carbon inventory (43). This probably resulted in an increased heterogeneity for the surficial sediments, supported by the higher dissimilarity observed for the genus activity between the six replicate surface sediments compared to those in 5- and 10-cm-deep sediments.

In both the DNA and RNA data sets, the microbial communities in the surface layer sediments were very often significantly different from the 10-cm layer and sometimes from the 5-cm layer. This is consistent with the very sharp redox gradient in deep-sea sediments, with microbial communities in surface sediments being under aerobic conditions and those in deeper sediments under anaerobic conditions. Surficial sediments in our study were codominated by the generally aerobic *Alphaproteobacteria* and *Gammaproteobacteria*, consistent with previous reports on the bacterial communities of sediments taken less than 6 km away from the wellhead 1 year after the DWH spill (44). In contrast, directly after the spill, the relative abundance of *Deltaproteobacteria* anaerobic hydrocarbon degraders and associated functional genes was higher in surficial sediments closest to the blowout site (9). In our case, *Deltaproteobacteria* were not very abundant in the surface layers but increased in abundance with depth, in line with the expected decrease in sediment oxygen concentration with depth. Similarly, the genes related to oxygen-dependent enzymes, like the alkane monooxygenase, were significantly more expressed at the surface of the sediments than in deeper sediment. Hydrocarbon degradation genes were reported to be more abundant in highly contaminated surficial sediments shortly after the spill (45).

The alkane concentrations in our surficial sediments were similar to the ones measured by Mason et al. (45) in sediments taken less than 5 km from the wellhead in October 2010 (4,619 ng g⁻¹ in this study, versus average of 7,304 ng g⁻¹ for Mason et al.), suggesting very slow degradation, if any, in the >1 year between the samplings. In contrast, PAHs were undetected in our surficial sediments, compared to an average of 1,895 ng g⁻¹ with only one sample showing concentrations below the detection limit in the study of Mason et al. (45). Taken together, these results suggest that PAHs were probably degraded more rapidly in the surficial sediments near the DWH wellhead than the alkanes, even though surficial sediments showed higher mineralization rates for dodecane (C₁₂) than for phenanthrene or toluene at 5°C (45). The bulk of the alkanes detected in our surficial sediments had chains longer than C₂₃ (82%; 3,765 ng g⁻¹ out of 4,619 ng g⁻¹), which are generally more recalcitrant to microbial degradation.

Conclusions. Most of the data indicated no significant differences between the wellhead water column station and the refer-

ence station. This included some hydrocarbonoclastic activity, probably related to the presence of hydrocarbons from natural seeps. Hydrocarbon concentrations in the water column were very low, in the range of nanograms per liter, indicating that very little, if any, hydrocarbon from the DWH spill remained in the water column on the date and at the location sampled, in agreement with studies that reported a very rapid degradation of hydrocarbons from the spill (5). However, a few microbial indicators showed significant differences between the reference and wellhead stations at the depth where the hydrocarbon plume was detected (~1,200 m), most likely related to a persistent nutrient or oxygen limitation, or a legacy effect, rather than to the presence of residual hydrocarbons from the spill. As for the sediments, several significant differences were observed between surficial and deeper sediments, probably related to differences in geochemical conditions (mainly oxygen availability). Surficial sediments collected near the wellhead contained alkane concentrations similar to those measured a few months after the spill, probably because of the higher recalcitrance of the long-chain alkanes detected.

ACKNOWLEDGMENTS

Suzanne Labelle, Claude Masson, and Danielle Ouellette from the NRC and Thomas King from DFO are thanked for their excellent technical support in sample preparation and analyses. We are thankful to Arden Ahnell and Marie BenKinney for insightful comments and support throughout this study.

This study was supported by British Petroleum, but they participated neither in the preparation of the manuscript nor in the analysis or interpretation of the results.

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