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LETTERS

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The rise and fall of methanotrophy following a deepwater oil-well blowout

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The blowout of the Macondo oil well in the Gulf of Mexico in April 2010 injected up to 500,000 tonnes of natural gas, mainly methane, into the deep sea[1]. Most of the methane released was thought to have been consumed by marine microbes between July and August 2010[2,3]. Here, we report spatially extensive measurements of methane concentrations and oxidation rates in the nine months following the spill. We show that although gas-rich deepwater plumes were a short-lived feature, water column concentrations of methane remained above background levels throughout the rest of the year. Rates of microbial methane oxidation peaked in the deepwater plumes in May and early June, coincident with a rapid rise in the abundance of known and new methane-oxidizing microbes. At this time, rates of methane oxidation reached up to 5,900 nmol l $^{-1}$ d $^{-1}$—the highest rates documented in the global pelagic ocean before the blowout[4]. Rates of methane oxidation fell to less than 50 nmol l $^{-1}$ d $^{-1}$ in late June, and continued to decline throughout the remainder of the year. We suggest the precipitous drop in methane consumption in late June, despite the persistence of methane in the water column, underscores the important role that physiological and environmental factors play in constraining the activity of methane-oxidizing bacteria in the Gulf of Mexico.

A seafloor oil-well blowout and subsequent sinking of the Deepwater Horizon drilling rig in the Gulf of Mexico on 20 April 2010 initiated an unprecedented discharge of oil and gaseous hydrocarbons into the deep ocean. We conducted a nine-month sampling campaign (5 May–3 December 2010; Supplementary Fig. 1 and Supplementary Table) to document the fate and dynamics of methane in the system. Direct measurements of methane concentrations, microbially mediated aerobic methane-oxidation rates (for example, methanotrophy) and particulate methane monooxygenase gene abundance and diversity were made. Concentrations of inorganic nutrients and dissolved iron and copper were also quantified to explore the factors potentially regulating methanotrophic activity. Our detailed biogeochemical and molecular measurements acquired over a large geographic area ($>105,000$ km^2) suggest that, following the blowout, environmental or physiological factors ultimately limited methanotrophic abundance and activity, rather than availability of methane, oxygen, or higher hydrocarbon priming as previously reported[1,2,5]. The observed reduction in rates of methanotrophy after the end of June suggests that methanotrophs did not consume all of the discharged methane.

Time series sampling revealed clear spatiotemporal variations in dissolved alkane distribution. From May to early June, concentrations of dissolved alkanes, from methane to pentane[1,5], were enriched between 900 and 1,300 m water depth southwest of the wellhead (Fig. 1a); however, focused deepwater plumes were not apparent after that time (Supplementary Fig. 2). Previous studies reported these gas-rich, deepwater plumes from snapshot samples taken in May and June[1,5,6]; the time series data presented here show that the gases were subsequently dispersed (Supplementary Fig. 2), distributing Macondo methane with a distinct carbon isotopic signature of $-57.4‰ \pm 0.4‰$ ($n = 27$) throughout the water column by late June 2010. At that time, Macondo methane (1.7 μM; identified by its isotopic signature of $\delta^{13}C–CH_4$ of $-53.8 \pm 1.5‰$, $n = 5$; see further discussion in Supplementary Methods), was observed at a depth of 600 m at Mississippi Canyon MC118, a natural seep ten miles north of Macondo that releases thermogenic methane ($\delta^{13}C–CH_4$ of $-45‰$; ref. 7). By July, $C_2–C_5$ concentrations were below detection even though methane-enriched waters extended from 600 to 1,300 m (Supplementary Fig. 3). In August/September, methane concentrations remained elevated throughout the water column at sites north of the Macondo wellhead (for example, a 100 nM methane anomaly was observed at MC118 at 650 m in September 2010) but not to the south/southwest, where we observed low concentrations and low turnover rates of methane at plume depths (900–1,300 m), consistent with previously reported observations[2].

The observed redistribution of methane through the water column within two months may reflect diapycnal mixing amplified by ageostrophic internal waves whose presence is ensured by the eddy activity and bathymetric conditions typical of the region around the Macondo wellhead[8,9]. Rapid plume dispersion may have also resulted from the ascent of buoyant, probably oil-saturated hydrate flakes[1]. As C_2 and C_3 gases are preferentially concentrated into structure II gas hydrate[10] relative to their abundance in the venting gas, hydrate formation and dissociation could abiotically alter the relative abundance of alkanes in solution[1] thereby

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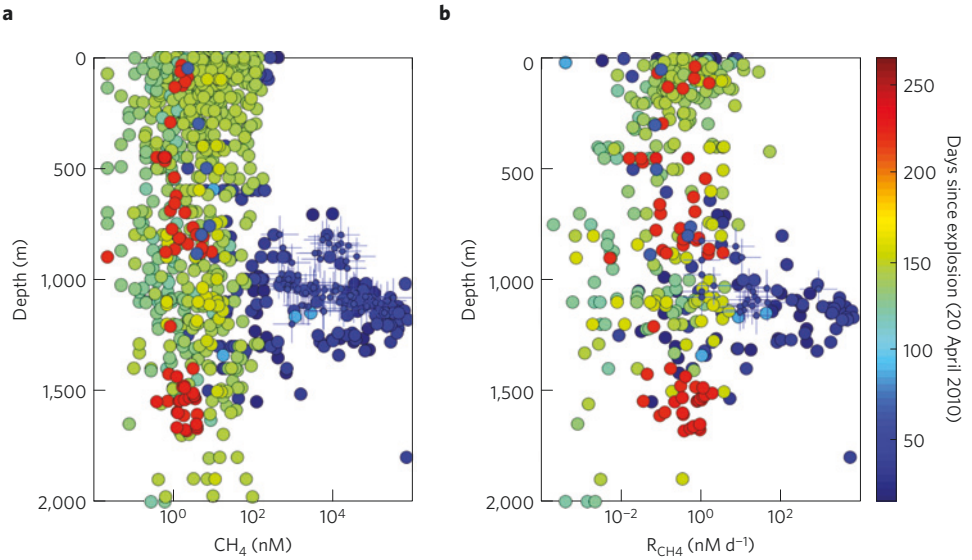

Figure 1 | Methane dynamics following the Deepwater Horizon discharge. a, Methane concentration data and **b**, methane-oxidation rates between 5 May and 2 December 2010. Data from ref. 5 are shown as crosses (colour coding is the same).

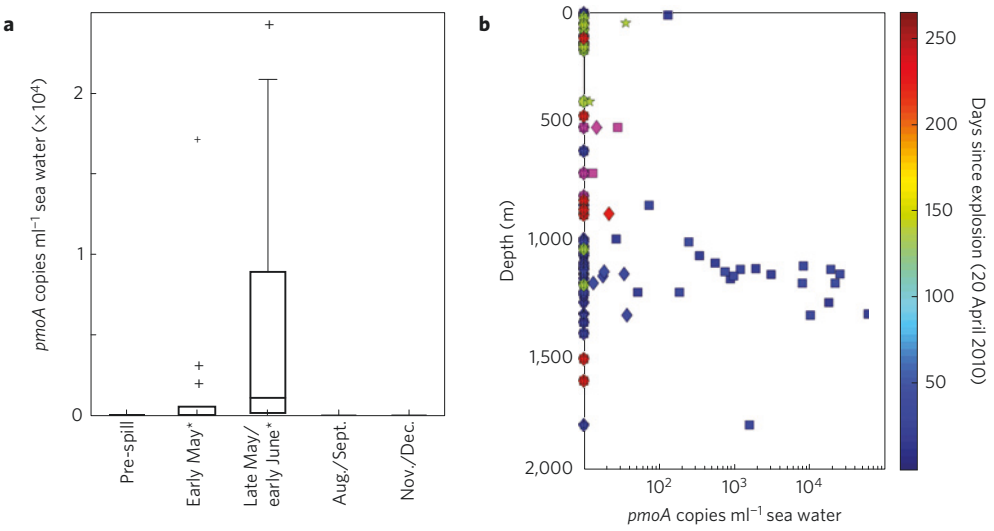

Figure 2 | Abundance of *pmoA* genes following the Deepwater Horizon discharge. a, Abundance of methanotrophic bacterial *pmoA* genes, data are binned by time period. **b**, Abundance of *pmoA* gene copies over time (same colour coding as Fig. 1; pre-spill samples are magenta). Stars, OPU1; diamonds, OPU3; squares, new phylotype. Data in the two time periods marked with an asterisk in **a** are different from those collected at other times with a statistical significance of $P < 0.05$. Plus signs denote extreme data outliers.

complicating the use of C_1/C_2 and C_1/C_3 ratios as indicators of microbial consumption[5].

The increase in methane concentration rapidly stimulated biological methane oxidation (methanotrophy), which later became constrained by physiological or environmental factors. In early May 2010, 11 days after the discharge began, methane-oxidation rates ranged from 0.014 to 502 nmol l^{-1} d^{-1} (hereafter nM d^{-1}) and were highest in the deepwater plumes (Fig. 1b) where the methane turnover time was ~400 days. Methane-oxidation rates increased through late May/early June, reaching maximal rates of 5,900 nM d^{-1} that, if sustained, lowered the methane pool turnover time to roughly two months. These high rates exceed those previously reported in the Gulf by four orders of magnitude[5,11], are the highest reported for the pelagic ocean[4,12] and are among the highest documented in aquatic ecosystems, including wetlands[13]. Methanotrophic activity dropped to tens to hundreds of nM d^{-1} in late June[5], despite methane concentrations well above background (tens of μM, with a maximum of 180 μM; Fig. 1) and continued to drop slowly between July and December to 3–5 nM d^{-1}. Though much lower than those observed in May, these rates were significantly above background rates, which ranged from 0.0001 to 0.1 nM d^{-1} (ref. 11).

Characterized gammaproteobacterial methanotrophs prefer high methane concentrations and typically exhibit high maximal rates of activity[14]. Quantitative polymerase chain reaction (qPCR) targeting the *pmoA* gene of two cosmopolitan gammaproteobacterial marine methanotrophs[15] and a sequence-divergent *pmoA* identified during the time series revealed the response of these three putative methane-oxidizing lineages to the gas injection. Overall, *pmoA* gene abundance was substantially higher in May/June compared with prespill conditions (Fig. 2a), consistent with increases in methane-oxidation rate (Fig. 1b). The change in *pmoA* gene abundance was dominated by an ingrowth of a rare (tens of copies per millilitre in prespill samples), sequence-divergent *pmoA* variant whose abundance increased 200-fold by late May (Fig. 2b). By

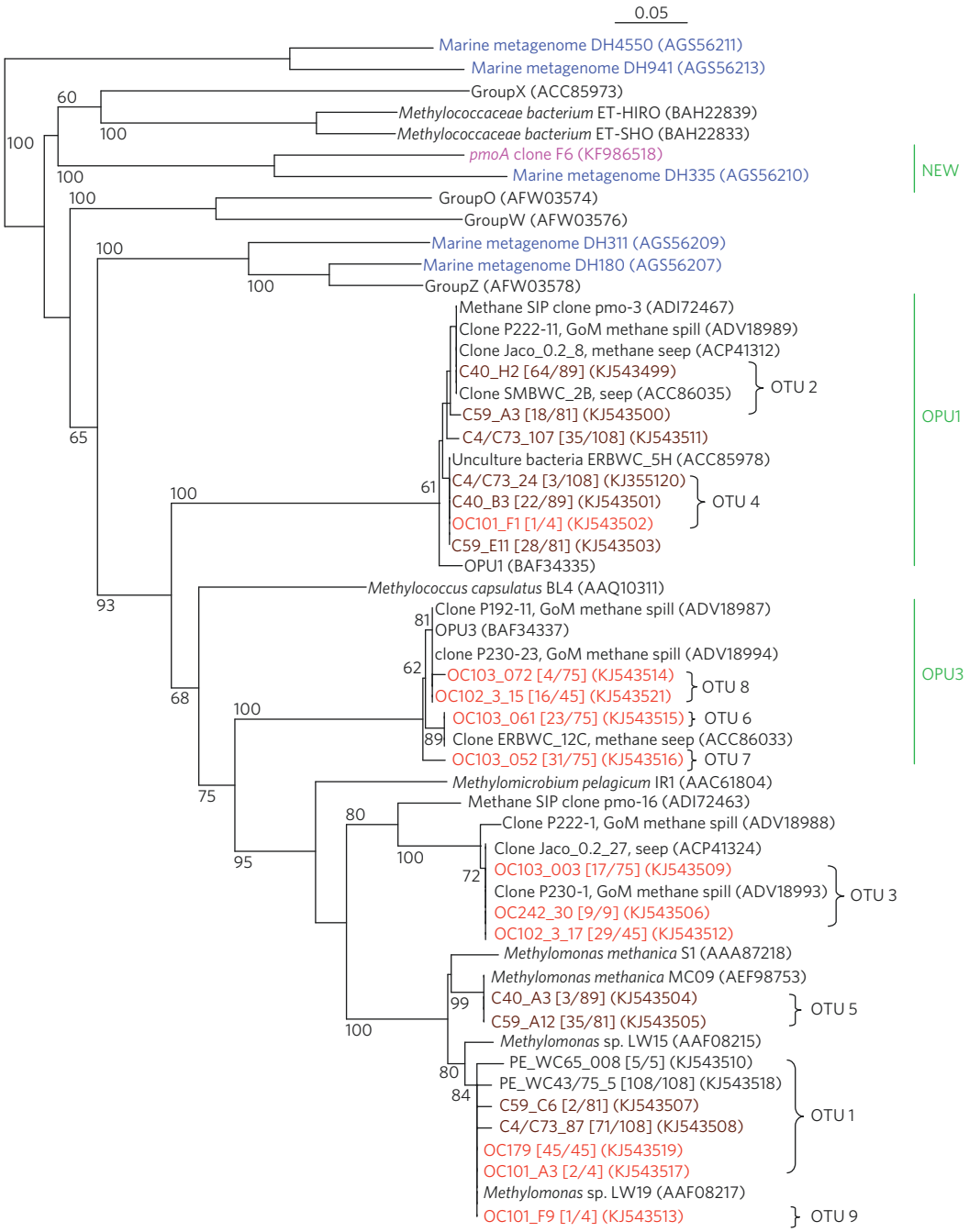

Figure 3 | Diversity of microbial *pmoA* genes between March and December 2010. Phylogenetic analysis of the inferred amino acid sequences encoded by the *pmoA* gene. The neighbour-joining tree was constructed using PHYLO_WIN. Sequences from this study are colour coded: black, RV *Pelican*, early May 2010; brown, RV *Walton Smith*, late May–early June 2010; red, RV *Oceanus*, August–September 2010. Known microbial species are denoted by italics. Green bars indicate groups targeted by the *pmoA* qPCR, including the novel *pmoA* sequence, KF986518 (magenta). Sequences in blue are from ref. 17 metatranscriptome. Numbers in brackets represent the number of clones represented by each sequence as a fraction of the total clones from each sample. Bootstrap values higher than 50% are indicated. Scale bar indicates 5% estimated substitutions.

August/September the median *pmoA* gene abundance was similar to background levels (Fig. 2).

The peak in abundance of the *pmoA* genes (Fig. 2) in May and early June corresponded to maximal methane-oxidation rates (Fig. 1b), indicating that the methanotrophy response to methane injection occurred on a time scale of days to weeks rather than months as suggested previously[2,3,5]. The measured increase in activity requires an increase in cell specific rates[16] and/or ingrowth of a subpopulation with radically different kinetics. An ingrowth of new methanotrophs is supported by the observed increase in sequence-divergent *pmoA* sequences during May and June (Fig. 2) and by the abundance of transcripts with homology to this new *pmoA* sequence in the transcriptome of samples collected contemporaneously with our samples during May/June[17].

Clone library analysis targeting canonical *pmoA* sequences sheds additional light on the dynamics of methanotroph response to the Macondo blowout. Phylogenetic analysis of the inferred amino acid sequence of the *pmoA* gene from selected samples (Fig. 3 and Supplementary Table 2) indicated that sequences similar to the *pmoA* gene from several *Methylomonas* spp. were present

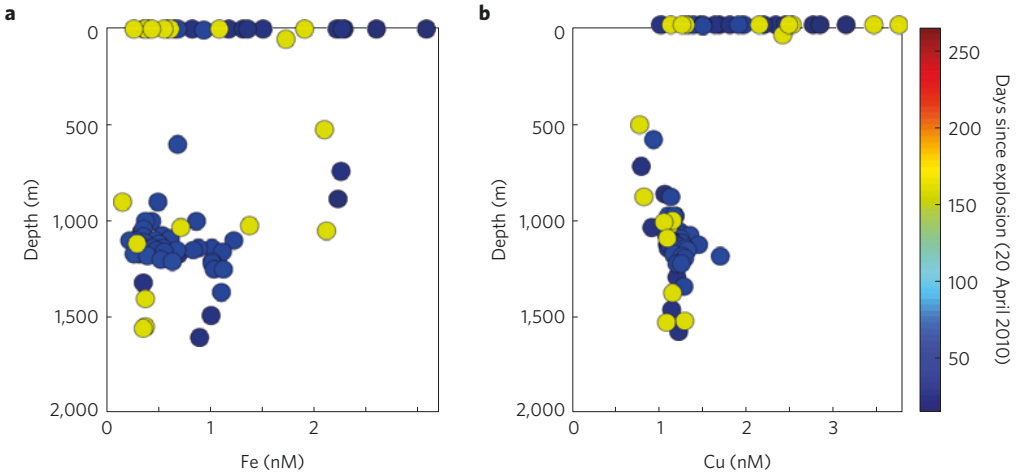

Figure 4 | Concentrations of dissolved trace metals required by methanotrophs. a, Water column concentration of dissolved iron and **b**, copper in samples collected in May, June and October 2010.

throughout the sampling campaign. However, sequences similar to *pmoA* genes associated with natural marine hydrocarbon seeps off the coast of Costa Rica (P.L.T. and V.J.O., unpublished observations) and off the coast of California[18], and to sequences identified months after the spill started[2] were detected in samples from May/June and August/September (Fig. 3). Transcripts related to these new *pmoA* sequences were identified in May/June samples[17] parallel to our rate and molecular samples. Together, these data show that the activity and response of both canonical and phylogenetically divergent methanotrophs to the methane injection was essentially instantaneous. However, the specific metabolic capabilities of the new phylotype is not known; it could be capable of oxidizing methane and higher alkanes (for example, propane), which, in light of the high abundance of C_1–C_5 alkanes, could provide it with a metabolic advantage.

The sudden decrease in methanotrophic activity during June/July, in the presence of sufficient primary substrates—O_2 and CH_4—suggests physiological or environmental limitations such as kinetic selection of the methanotroph population, trophic interactions or mortality (for example, viral lysis, selective grazing pressure), nutrient limitation, or trace metal limitation. The observed increase of predominantly gammaproteobacterial (Type I) methanotrophs, as documented by the *pmoA* libraries (Fig. 3) and metatranscriptomic analysis[17], in response to the extraordinarily high methane concentration in pelagic ocean waters agrees with previous results[14] from soils, where gammaproteobacterial (Type I) methanotrophs with a low affinity for methane and high maximal oxidation rates responded rapidly to methane amendment. Following the blowout, the highly active population persisted only briefly, as evidenced by the transient increase in sequence-divergent *pmoA* gene abundances during the first six weeks of the discharge (Fig. 2). As the gas-rich plumes dispersed, we surmise that methane concentrations dropped to levels below those accessible to these new methanotrophs, resulting in a notable decrease in methane-oxidation rates and, subsequently, a decrease in *pmoA* gene abundance.

Additional factors may have contributed to the decline in methane-oxidation rates and prevented other components of the methanotroph population from increasing their activity during later phases of the event. Though not documented in the ocean, selective grazing of methanotrophs by protists in soils[19] and zooplankton in lakes[20] can regulate their abundance and activity. Similarly, viral infection and targeted mortality are known to affect microbial community composition[21] and—in combination with grazing—may have impacted methanotroph abundance and activity[21] and contributed to the observed rapid decrease in abundance and oxidation rates. It is less likely that the availability of the main nutrients, nitrogen and phosphorus, which were present in sufficiently high concentrations (Supplementary Fig. 4), limited methanotrophic activity or the recovery of methanotrophs after the observed drop in rates. Particulate methane monooxygenase, the enzyme encoded by *pmo*, has a high demand for both copper and iron[22–24]. Dissolved (<0.45 μm) iron and copper (Fig. 4) concentrations during early May were in the low nM range. Within the deepwater plume, Fe (0.4–1 nM) and Cu (1.1 nM) concentrations were comparable to values observed in the open Atlantic[25]. By late May/early June, dissolved Fe concentrations exhibited a strong minimum in the deepwater plume (Fig. 4) and were low enough to limit methanotroph activity at high (μM) methane concentration[26,27].

The factors that regulate the fate of hydrocarbons in the ocean owing to sudden, large discharge events are poorly constrained at the microbial level. The response and regulation of microbial activity and microbial community succession, and the impacts of microbial activity at the ecosystem level, including generation of oxygen-depleted waters[2,28] or microbially mediated carbon transfer within the marine food web, is limited. Our data underscore the key roles of vertical mixing[8,9] and gas hydrate dynamics[1] in driving methane distributions following the Macondo blowout. Patterns of methane abundance thus generated unexpected patterns of microbial activity and distributions. The efficient, and faster than previously reported[2,3,5], response of methanotrophs to the Macondo-derived methane injection revealed a surprising capability of the pelagic oceanic microbial community. Rapid microbial community shifts and metabolic flexibility has been observed previously in soils[14], permafrost[29] and nearshore pelagic[3], microbial communities, but not yet in the deep pelagic ocean. Most surprising, though, was the inability of the methanotroph community to maintain the high levels of activity achieved in late May/early June, because maintaining those observed levels of activity for less than two months would have efficiently consumed the entirety of Macondo-derived methane. Instead, the activity of methanotrophs crashed in late June, which limited consumption of the introduced methane and highlights the complex feedbacks between physics, geochemistry and microbiology that shape and regulate microbial community responses to large-scale perturbations in the open ocean. Such complexities limit our ability to constrain the ultimate fate of Macondo methane in the Gulf ecosystem.

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Author contributions

S.B.J., M.C-M., K.S.H., A-R.D., V.L.A., J.P.C., J.P.M., A.M.W., R.M.W.A., A.M.S., D.J.J., A.V. and T.A.V. collected the samples and/or carried out geochemical and microbial activity rate assays; M.C-M., L.M.N, P.T., J.J.B. and V.J.O. conducted the methanotroph gene analyses; S.B.J., C.D.M., M.C-M., P.T. and A.B. contributed to data reduction and analyses; S.B.J. led development of the manuscript; C.D.M., M.C-M., P.L.T. and V.J.O. made significant contributions and critical feedback was provided by the other co-authors.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.B.J.

Competing financial interests

The authors declare no competing financial interests.

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SUPPLEMENTARY INFORMATION

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The rise and fall of methanotrophy following a deepwater oil-well blowout

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Methods

Study Sites:

A total of 1128 water column samples were collected during ten research cruises to the Gulf of Mexico spanning March (the background sampling cruise) through December 2010, on board of the R/V Pelican: 1st Mar. 2010 – 7 Mar. 2010, 5th May 2010 – 15 May 2010 and 20th June 2010 – 26 June 2010; R/V F.G. Walton Smith: 25th May 2010 – 6 June 2010; R/V Nancy Foster: 30 June 2010 – 18 July 2010; R/V Oceanus and R/V Cape Hatteras: 21st Aug. 2010 – 16 Sept. 2010; R/V Arctic Sunrise: 20th Sept. 2010 – 28 Sept 2010; and R/V Atlantis: 8th Nov. 2010 – 3rd Dec. 2010 (See On-Line Supplementary Table). The pre-spill samples were collected from above a fracture zone at a natural hydrocarbon seep, Mississippi Canyon block 118, in March 2010, roughly a month before the Macondo Blowout began. It is important to note that the pre-spill data does not reflect the microbial abundance of an inactive site that lacks natural seepage inputs rather the data reflects a site impacted by low rates of natural seepage.

Sample collection:

A CTD-Niskin rosette system was used to obtain hydrographic profiles throughout the water column. Niskin bottles were triggered at specific depths and upon return to the surface, each bottle was individual samples were collected for dissolved gas

concentration determination, rates of aerobic methane oxidation, nutrient and metal concentration determination, and microbial molecular analysis (see below).

Methane concentration:

Samples for dissolved alkane quantification were collected as soon as the CTD rosette was secured on deck, as described previously by Joye et al.[31] with some modifications between cruises. Concentration of C_1 to C_5 alkanes were determined using headspace extraction (May through

or a modified sonication/vacuum extraction technique (August through December)[32], followed by gas chromatography. A sub-sample (0.25 to 1 mL) gas sample was injected into a gas chromatograph (model 8610C, SRI, California) equipped with a flame ionization detector[31]. A temperature ramp was employed to elute C_{3+} alkanes. Concentrations were calculated by comparison to a certified mixed alkane standard (C_1 to C_5, including both *n*- and *i*- C_4) (Scott Specialty Gases®).

Aerobic methane oxidation rates:

Water column aerobic methane oxidation rates were measured using a tritiated (^{3}H) CH_4 radiotracer technique[33,34] . Reactions were done in triplicates in gas-tight glass vials. A 100 µl aliquot of the C^3H_4 tracer solution was injected into each replicate yielding a tracer activity of 2 kBq. Killed controls were achieved by treating a sample with 3.7 % formaldehyde, to arrest microbial activity, prior to tracer addition. The samples were incubated at *in situ* temperature for 48 to 72 hours; linearity of activity was confirmed by time series. Reactions were terminated by adding 20% (vol:vol) of pure ethanol to each

vial. Labeled C^3H_4 was removed by purging the sample with hydrated air for at least 25 minutes. Scintillation cocktail (ScintiSafe Gel®) was then added to an aliquot of the sample and ^3H_2O produced was quantified using a Beckman 6500 liquid scintillation counter.

Methane stable carbon isotopic signature:

Water samples for methane stable carbon isotopic ($\delta^{13}C$) analyses (May and July) were collected into 125 mL serum vials. A headspace sub-sample from each vial was injected into a Finnegan Mat Delta V Isotope Ratio Mass Spectrometer coupled to a Hewlet Packard gas chromatograph with a poraplot capillary column. Samples were cryo-focused as described in Chanton and Liptay (2000)[35], and reported relative to Vienna Pee Dee Belemnite (VPDB) standard.

Nutrients/Biogeochemistry:

Fixation and analysis of water samples for quantifying dissolved ammonium (NH_4^+), inorganic carbon (DIC or HCO_3^-), nitrate plus nitrite (NO_x^-), and phosphate (PO_4^{3-}) followed previously described methods[36]. Samples for dissolved trace elements were collected using trace element cleaned, rosette-mounted, teflon-coated, external spring Niskin and Go-Flo bottles. Samples were cleanly syringe-filtered[37]. Metals were isolated from a small volume (3 mL) of sample using Mg-induced co-precipitation with added enriched ^{57}Fe and ^{65}Cu[38] and then analyzed using isotope-dilution by sector-field inductively coupled plasma-mass spectrometry.

DNA extraction:

Water samples for DNA extraction were filtered through a 0.22 µm Sterivex filter (Millipore) and stored frozen until extraction. The filter was extracted using the Ultra Clean Soil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA) following the manufacturer's instructions with few modifications. Two samples used for *pmoA* clone libraries (see section below) were extracted using different phenol:chloroform DNA extractions protcols; sample C40-6 was extracted following Vetriani *et al*. (1999)[39] while sample C59-9 was extracted according to Adams et al. (2013)[40]. Similar results from *pmoA* clone libraries were obtained, regardless of the extraction method used, see Fig. 3.

qPCR amplification:

Amplification of Type I relatives OPU1 and OPU3 was achieved with primer set putative_16S_OPU1F (5'-CAATGCCGCGTGTGTGAA-3') and putative_16S_OPU1R (5'-CCTCTCTTCCCCCGACTGAA-3') for OPU1, and primer set putative_OPU3F (5'-AGCACTTTCAATTGGGAGGAAA-3') and putative_OPU3R (5' GCCGGTGCTTCTTCTAAAGGT-3') for OPU3[41]. The unusual *pmoA* sequences were originally detected using monooxygenenase intergenic spacer analysis (hereafter, MISA)[42] and full length *pmoA* sequences were recovered subsequently following the strategy reported in Tavormina 2013[42]. The MISA assay was validated on cultured strains as reported in Tavormina et al.[42]. qPCR primers were developed from the full length sequence using Primer3 software http://frodo.wi.mit.edu/primer3/.

On linearized, CsCl-purified plasmid templates, these primer pairs demonstrate >95% amplification efficiency (as measured over 5 orders of magnitude template concentrations). Cross reactivity between target plasmid templates is <1%, however these primers may amplify related sequences in complex environmental samples. Quantitative PCR reactions were performed in a StepOne Real Time PCR System (Applied Biosystem), in a reaction consisting of 12.5µl of Power SYBR® Green PCR Master Mix, 1µl of template DNA (1-6 ng/µl), 0.625µl of BSA (10 ng/µl), 1.25µl of each primer (0.5µM final concentration), and water to a final volume of 25µl. qPCR programs consisted in a 95ºC initial denaturation for 10 min, followed by 40 cycles of 95ºC for 15 sec and a one step annealing/extension of 1 min at 57ºC for Type I methanotrophs 16S rRNA gene, as well as for OPU1 and OPU3, or 1 min at 60ºC in the case of Type II methanotrophs. The limit of detection for the qPCR assays was estimated to be 30 copies per reaction for Type I, 50 copies per reaction for Type II, 200 copies per reaction for OPU1, and 56 copies per reaction for OPU3. All the results were standardized to the volume (per mL) of seawater the DNA was extracted from.

Enumeration of *pmoA* phylotypes was performed in an ABI 7300 real time PCR system with the following conditions. The OPU3 *pmoA* phylotype was performed using primers, Taqman probe, and conditions as previously reported[42]. Primers and Taqman probe specific to the OPU1 *pmoA* phylotype (primers *pmoA*_OPU1qPCR_242f: 5'-TTACCCCGATCATGCTGGTT-3' and *pmoA*_OPU1qPCR_312r: 5'-GATTCTGAAGTGTTCCCAAACGA-3'. Probe: 5'-TTCCCAGCCGCTGTTCAGGCA-3') and primers specific to the novel *pmoA* phylotype (primers *pmoA*_spill_f: 5'-

AAACTATGAGTTTAACTGCTG-3' and *pmoA*_spill_r: 5'-TCAAAATTCCGCACAATCTTT-3') were developed using Primer Express v 2.0. OPU1 enumeration was performed using cycling conditions as reported previously for the OPU3 *pmoA* phylotype. To enumerate the novel phylotype, a SYBR assay (Power SYBR green, Applied Biosystems) was developed. 1µl each of template DNA, forward and reverse primer (0.5µM final concentration), and water were added to a final volume of 20µl. qPCR was performed with a 95ºC denaturation for 10 min, followed by 40 cycles of 95ºC (15") and an extension at 58ºC (1'). A final dissociation curve was included. For all functional gene assays, CsCl-purified linearized plasmid DNA was used as a standard. Primers displayed >95% amplification efficiency as measured over five orders magnitude and cross reactivity between target templates was below detection. Limit of detection for *pmoA* qPCR assays was 10 copies per reaction. The results were standardized to the mL of seawater.

Statistics:

A Wilcoxon Test was performed using Kaleidagraph (version 4.1) to determine the significance of the changes observed in qPCR results over the time.

Clone libraries, sequencing, and phylogenetic analysis:

pmoA gene fragments were amplified by polymerase chain reaction (PCR) from DNA obtained form eleven samples representing different time points and locations during our sampling campaign (Supplementary Table S2) using primer set wcpmoA189f and wcpmoA661r[43]. The PCR protocol consisted of an initial denaturation of 3 min at 94ºC,

followed by 28-40 cycles of 1 min at 94 ºC, 30s at 56 ºC, and 45s at 72ºC, ending with a final extension of 5 min at 72 ºC. The PCR master mix consisted of of 1-20 ng/µl DNA and either 1X GoTaq Flexi Reaction Buffer, 1.25 u of GoTaq Flexi DNA Polymerase (Promega Corporation, Madison, WI), 2.5 mM $MgCl_2$, 0.25 mg/ml BSA, 100 µM dNTP mix, 1µM of each primer, and ddH_2O to a final volume of 25 µl; or OmniTaq DNA polymerase (0.5 µL), 1X Omni Taq reaction buffer, 100 µM dNTP mix, 1µM of each primer and ddH_2O to a final volume of 25 µl.

Amplified *pmoA* gene fragments were gel-purified using the QIAGEN Qiaquick gel extraction kit (Qiagen, Santa Clarita, CA, USA), and cloned into a pCR4-TOPO plasmid vector (Invitrogen, Inc., Carlsbad, CA, USA) at a 1:3 vector to insert ratio. The ligation products were transformed into *E. coli* One Shot Top10 competent cells (Invitrogen, Inc., Carlsbad, CA, USA). Ampicillin resistant clones were selected and grown up overnight at 37 ºC in freezing medium (LB + 100 µg/ml Amp + 10% glycerol). One mL of the clones was spun down and cell pellets were sent to Laragen, Inc (Culver City, CA) for Sanger sequencing. Sequences were also screened for closest relatives using the blastn application of the NCBI database. Sequences were aligned using Clustal X version 2.1 and manually adjusted using Seaview[44]. Operational taxonomic units (OTUs) were defined as sequences sharing more than 97% similarity and were calculated using MOTHUR[45].

Sequences were translated using the online tool EMBOSS Transeq (http://www.ebi.ac.uk/emboss/transeq/). The amino acid sequences were aligned with Clustal X version 2.1 and manually adjusted using Seaview[44]. Phylogenetic distances were calculated using the Observed Divergence matrix, and the neighbor joining method

was used to evaluate tree topologies. Phylo_win version 2.0 was utilized to plot tree topologies[44] whose robustness was tested by bootstrap analysis (1000 resamplings).

Supplementary Discussion

Evidence that Macondo Methane was Dispersed through the Water Column after June 2010:

The methane present in the water column at MC118 in June 2010 was distinct from the thermogenic methane that typifies the MC118 site (Bowles et al. 2011, Geochimica et Cosmochimica Acta). The mid-water methane concentration measured at MC118 in June 2010 had a $d^{13}C\text{-}CH_4$ of -53.8 ± 1.5 ‰ (n=5) and was as depleted as -55 ‰, which is significantly lighter than the average $d^{13}C\text{-}CH_4$ signature of methane at MC118 (-45‰) and outside the bounds of the range noted by the reviewer (-48 to -52 ‰). We have made nearly 100 measurements of methane concentration and isotopic composition in the water column above MC118 since 2010. Typically, methane concentrations are maximal near the seafloor and decrease with distance above the bottom. The concentrations measured in June 2010 were low except for the anomalous high concentration zone present in the mid-water; so we conclude that this methane was Macondo in origin.

Furthermore, other stations north of MC252, and east/northeast from MC118 also exhibited methane concentration >100 nM after July 2010. For example, we observed a methane concentration of 109 nM at 750 m at Cape Hatteras Station 68 (29.04, -88.12). This station is located 23 nm from MC252, which lead us to conclude that deepwater plume methane was dispersed upwards through the water column in a broad fashion.

185 Other examples of stations east/northeast of MC252 that exhibited methane
186 concentrations >100 nM are presented in a table on the following page.
187

Site/ Station	Date	Lat	Long	Distance to MC252 (nautical miles)	Heading from MC252 (degrees)	Depth (m)	Methane (nM)
CH065	11 Sept 2010	28.71	-87.93	24	94	309	138
CH066	12 Sept 2010	28.88	-87.93	26	70	719	132
CH066	12 Sept 2010	28.88	-87.93	26	70	567	157.
CH066	12 Sept 2010	28.88	-87.93	26	70	444	125
CH067	12 Sept 2010	29.04	-87.93	30	52	51	143
CH068	12 Sept 2010	29.04	-88.12	23	37	750	109
CH076	13 Sept 2010	29.24	-88.01	35	33	68	174
CH077	13 Sept 2010	29.23	-87.82	42	44	212	125
CH077	13 Sept 2010	29.23	-87.82	42	44	186	141

Data Availability:

All of the data presented in this paper are available electronically through the Gulf of Mexico Research Initiative's Data Warehouse (https://data.gulfresearchinitiative.org/).

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Captions for Supplementary Figures

Supplementary Fig. 1. **Map showing the study sites over space and time**. The sampling sites as a function of time since the Deepwater Horizon exploded are shown (colour code denotes time). The star denotes the location of the Macondo wellhead.

Supplementary Fig. 2. **Average methane concentration over 50-day periods**. Maximum methane concentrations over time, binned in 200m depth and 50 day intervals (colour coding is the same as Fig. 1) illustrating dispersion of the deep-water plume over time.

Supplementary Fig. 3. **Dissolved alkane concentrations in the water column during May and June 2010**. Concentration of C_2-C_5 alkanes (time colouring extends to day 50 only as concentrations were below detection after that time; colour coding same as Figs. 1.

Supplementary Fig. 4. **Dissolved nutrient concentrations in the water column over the 10-month time-course.** Concentration of ammonium, nitrate+nitrite, and phosphate over depth (all concentrations are in μM) for the various study sites are shown.

Supplementary Table 1.

Date	Research Vessel	Site/ Station	Lat	Long	ID	Depth (m)	Extraction Method	Remarks
12-May-10	Pelican	Site_39	28.70	-88.39	WC45	1265	MoBio Kit	Combined DNA
14-May-10		Site_33A	28.72	-88.42	WC75	1220	MoBio Kit	
14-May-10	Pelican	Site_34A	28.71	-88.39	WC65	1020	MoBio Kit	Individual sample
26-May-10	Walton Smith	WS2	28.73	-88.41	C4-9	1120	MoBio Kit	Combined DNA
4-Jun-10		WS6	28.74	-88.38	C73-3	1180	MoBio Kit	
30-May-10	Walton Smith	WS36	28.71	-88.41	C40-6	1220	Vetriani *et at.*, 1999	Individual sample
1-Jun-10	Walton Smith	WS53	28.73	-88.38	C59-3	1170	Adams *et al.,* 2013	Individual sample
9-Sep-10	Oceanus	022.01	29.00	-88.80	179	162	MoBio Kit	Individual sample
30-Aug-10	Oceanus	014.02	27.37	-90.56	101	1222	MoBio Kit	Individual sample
30-Aug-10	Oceanus	014.02	27.37	-90.56	102	1000	MoBio Kit	Individual sample
30-Aug-10	Oceanus	014.02	27.37	-90.56	103	700	MoBio Kit	Individual sample
13-Sep-10	Oceanus	027.11	28.55	-88.32	242	1289	MoBio Kit	Individual sample