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Rapid Degradation of *Deepwater Horizon* Spilled Oil by Indigenous Microbial Communities in Louisiana Saltmarsh Sediments

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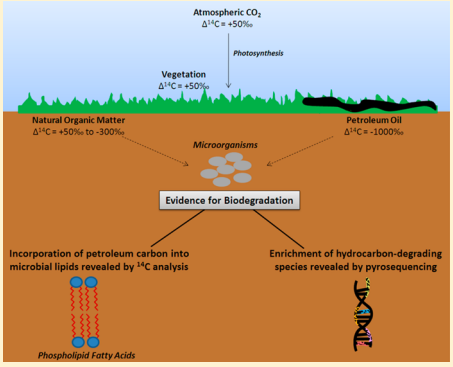
Supporting Information

ABSTRACT: The *Deepwater Horizon* oil spill led to the severe contamination of coastal environments in the Gulf of Mexico. A previous study detailed coastal saltmarsh erosion and recovery in a number of oil-impacted and nonimpacted reference sites in Barataria Bay, Louisiana over the first 18 months after the spill. Concentrations of alkanes and polyaromatic hydrocarbons (PAHs) at oil-impacted sites significantly decreased over this time period. Here, a combination of DNA, lipid, and isotopic approaches confirm that microbial biodegradation was contributing to the observed petroleum mass loss. Natural abundance ^{14}C analysis of microbial phospholipid fatty acids (PLFA) reveals that petroleum-derived carbon was a primary carbon source for microbial communities at impacted sites several months following oil intrusion when the highest concentrations of oil were present. Also at this time, microbial community analysis suggests that community structure of all three domains has shifted with the intrusion of oil. These results suggest that Gulf of Mexico marsh sediments have considerable biodegradation potential and that natural attenuation is playing a role in impacted sites.



INTRODUCTION

The April 2010 explosion on the *Deepwater Horizon* drilling platform resulted in the largest accidental marine oil spill in history. Over the course of three months, approximately 4.9 million barrels of light crude oil (MC252) were released into Gulf of Mexico waters leading to the contamination of hundreds of kilometers of shoreline.[1−4] Coastal ecosystems provide important ecological and economic services such as storm protection, carbon sequestration, and nurseries for fish and shellfish.[5−7] Salt marshes are particularly vulnerable to oil contamination due to low wave action and high organic matter content of sediments which lead to greater sorption of organic contaminants.[8] Previous studies have shown that spilled oils can persist for years to decades in salt marshes,[9−13] and media reports indicate that such persistence may be occurring in coastal salt marshes in the Gulf of Mexico.

Understanding the processes contributing to the removal and/or degradation of petroleum is an important step in assessing the ongoing impacts and the fate of the *Deepwater Horizon* spilled oil in this region. A range of physical and biological processes can lead to observed mass loss of petroleum hydrocarbons in coastal environments. While physical processes, such as shoreline erosion, volatilization, or water washing, may move hydrocarbons into other environmental compartments, these processes do not reduce the toxicity of these compounds. Biodegradation mediated by indigenous microbial communities is a key process by which petroleum hydrocarbons are mineralized and removed from contaminated environments.[3] Thus, microbial oil biodegradation is recognized as one of the most important methods for oil spill remediation, particularly in saltmarsh environments where physical cleanup activities can cause further damage to vegetation.[14,15]

Although microbial degradation of petroleum hydrocarbons has been well-studied, our ability to predict and discern this process by indigenous microbial communities under *in situ* conditions is limited. The majority of biodegradation studies have focused mainly on bacterial degradation of hydrocarbons under homogeneous enrichment conditions in laboratory

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settings.[16−22] However, biodegradation in the natural environment is highly variable and involves complex interactions between species and the geochemical properties of the surrounding environment. Environmental factors such as nutrients, temperature, salinity, and the presence of oxygen can significantly affect the occurrence and rate of biodegradation.[23,24] Furthermore, the presence and coexistence of species from all three microbial domains of life (bacteria, archaea, fungi) within the natural environment implies that multiple hydrocarbon-degrading groups may be contributing to biodegradation at oil-impacted sites. Knowledge of microbial community structure and the response of crucial hydrocarbon-degrading groups can provide insight into the dynamics and mechanisms controlling the growth and activity of microorganisms in contaminated environments.[25] However, few studies have attempted to investigate the response of all three microbial domains to petroleum contamination and to correlate these responses with geochemical indicators of biodegradation. To date, studies have observed enrichment of hydrocarbon-degrading bacteria and fungi in a number of marine environments following the *Deepwater Horizon* oil spill including the water column, beach sands, and marsh sediments.[26−32]

Beyond identifying the presence of hydrocarbon-degrading microbial groups, demonstrating biodegradation under *in situ* conditions is challenging. It is often difficult to confirm that mass loss of petroleum is a result of biodegradation rather than abiotic weathering processes due to the heterogeneous distributions of contaminants, high levels of oil sorption to organic matter and minerals, and dynamic conditions of coastal environments. Compound specific radiocarbon (^{14}C) analysis of microbial phospholipid fatty acids (PLFA) is a powerful tool for elucidating microbial carbon sources and confirming *in situ* biodegradation of petroleum in complex environmental systems.[12,33−37] This technique is based on the geologic age of petroleum hydrocarbons, which will have no detectable ^{14}C due to loss by radioactive decay ($\Delta^{14}C = -1000‰$). In contrast, sedimentary organic matter contains carbon with variable radiocarbon ages[38] including recently photosynthesized material with a $\Delta^{14}C$ value of approximately +50‰, consistent with a modern atmosphere source.[39] Microbial uptake and incorporation of petroleum carbon will reduce the ^{14}C content of microbial membrane lipids, such as PLFAs, relative to the surrounding natural organic matter.[12] In the case of salt marshes, a similar isotopic mass balance using stable carbon isotopes can also be used to confirm the occurrence of biodegradation.[40] Coastal marshes are dominated by grasses, specifically *Spartina sp.*, which have $\delta^{13}C$ values of −12 to −14‰ and sedimentary organic matter in these marshes have $\delta^{13}C$ values ranging −14 to −18‰ due to the contribution of other sources of organic matter.[41,42] In contrast, crude oils are more depleted in ^{13}C (MC252 oil, $\delta^{13}C = -27‰$). This isotopic difference was used to demonstrate that *Spartina*-derived organic matter, rather than petroleum hydrocarbons, was the primary source of carbon for microbial communities in other oil-impacted saltmarsh sediments.[12,43]

In a previous study, saltmarsh erosion and ecological impacts were detailed at seven different sites in Barataria Bay, Louisiana, which experienced some of the most extensive oil contamination due to the *Deepwater Horizon* oil spill.[44] Marsh sediments collected at 5, 11, and 18 months following oil intrusion showed significant decreases in hydrocarbon concentrations such that by 18 months (October 2011), oil-impacted sites had hydrocarbon concentrations that were comparable to those of nonimpacted reference sites. The study reported here focused on a subset of these sites with the goal of demonstrating that microbial biodegradation was contributing to the observed mass loss of petroleum in sediments. Further, the response of all three microbial domains (bacteria, archaea, and fungi) to severe petroleum contamination was assessed in order to observe shifts in community structure and identify taxonomic groups that may be potentially involved in biodegradation. To our knowledge, this is the first study to characterize all three microbial domains simultaneously in a petroleum-contaminated environment with the aim of elucidating microbial function and bioremediation outcomes.

MATERIALS AND METHODS

Study Site and Sample Collection. All sampling sites were located in *Spartina alterniflora*-dominated salt marshes in Barataria Bay, Louisiana. Samples were collected from two "impacted" sites and two "reference" sites (Figure S1, Table S1); from this point forward these sites will be referred to as impacted site 1, impacted site 2, reference site 1, and reference site 2. A detailed description of these sites can be found in Silliman et al.[44] Briefly, impacted sites had evident oil residues and oil-covered dead and decaying grass stems, whereas reference sites had no visible oil residues on substrates or marsh plants. Sediment samples were collected 3 m from the marsh platform edge at each site in October 2010, April 2011, and October 2011 (approximately 5, 11, and 18 months after initial oil intrusion). For each sediment sample, four cores (5 cm in depth) were collected from an area of 1 m^2 and were homogenized in the field to generate a representative sample for each location. Sediment samples were placed in precombusted glass jars and maintained in a cooler until reaching the laboratory, at which point they were stored at −80 °C.

Quantification of Hydrocarbons. Methods used to analyze sedimentary hydrocarbon compound concentrations in marsh sediments are detailed elsewhere.[44] Briefly, 0.05−2.5 g wet sediment samples were spiked with a range of deuterated PAH and alkane standards. The samples were extracted three times by accelerated solvent extraction (ASE) using a hexane and acetone mixture (50:50, v/v), concentrated, and then back-extracted three times into a 50:50 (v/v) mixture of aqueous sodium chloride and hexane to remove the remaining water. Samples were purified and separated into alkane and PAH compound classes using activated silica open-column chromatography. Alkanes and their associated 'unresolved complex mixture' (UCM) were analyzed by gas chromatography (Shimadzu GC-2010 coupled with an FID). The alkanes were quantified using alkane standards added just prior to GC injection, and the UCM was quantified assuming the average relative response factor for C16−C32 alkane standards. Polyaromatic hydrocarbons (PAHs) were identified and quantified by GC-mass spectrometry (Shimadzu GC-2010 coupled with a MS GCMS-QP2010S) using selected ion selective ion monitoring (SIMS).

Microbial DNA Analysis. Genomic DNA was extracted from a total of eight homogenized sediment samples collected 5 and 18 months after oil intrusion from impacted and reference sites using the PowerSoil DNA Isolation kit (MoBio Laboratories Inc., Carlsbad, CA) according to manufacturer's protocol. A total of six DNA extractions were carried out per homogenized sediment sample, and three extracts were pooled

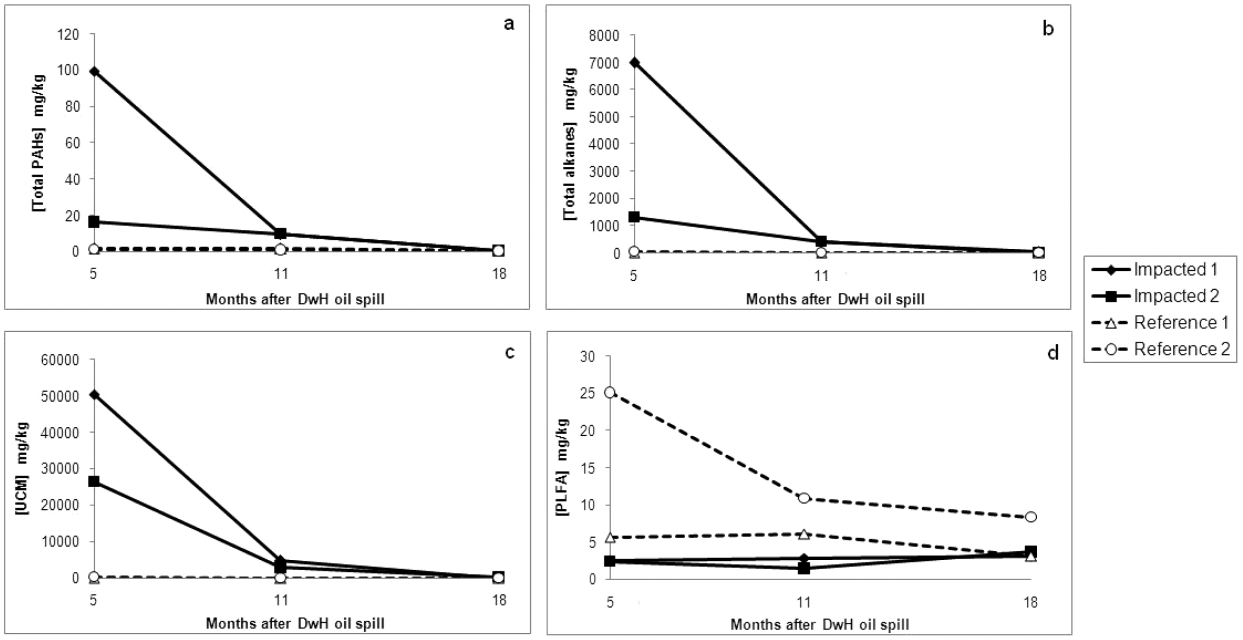

Figure 1. Sediment compound class concentrations at 5, 11, and 18 months after initial oil contact at two oil-impacted and two reference sites for (a) PAH, (b) alkane, (c) UCM, and (d) PLFA concentrations.

together resulting in two replicate DNA extracts per sediment sample. A total of 16 DNA extracts (8 sediment samples in replicate) were sent to Molecular Research LP (Lubbock, TX, USA) for tag-encoded GS FLX Titanium amplicon pyrosequencing (Roche 454). The 16S rDNA (rDNA) was targeted using primers 27F-530R for bacteria and Arch344F-Arch915R for archaea.[45,46] The fungal internal transcribed spacer (ITS) region of rDNA was targeted using primers ITS1F-ITS4R.[47−49] 16S and ITS sequences were quality trimmed using a custom sequence preprocessing pipeline. 16S sequences were classified using a local installation of the Ribosomal Database Project 16S classifier v. 2.5 (http://pyro.cme.msu.edu/) and MEGAN. ITS sequences were further processed using the Fungal ITS Extractor and classified using BLAST+MEGAN. Details of microbial DNA analysis including primer sequences and bioinformatic analyses can be found in the Supporting Information.

Microbial PLFA Analysis. For each sediment sample, 40 to 100 g of sediment was extracted using a modified Bligh and Dyer method,[50] as per Slater et al., 2005. Briefly, lipids were separated into nonpolar, neutral, and polar fractions using silica gel chromatography. The polar fraction, which contained phospholipids, was subjected to mild alkaline methanolysis, according to standard methods,[50] in order to convert phospholipids into to fatty acid methyl esters (FAMEs). This mild alkaline methanolysis step is designed to hydrolyze and methylate only the esterified fatty acids, leaving other bonds such as amides and ether linkages intact.[50,51] Since ester bonds hydrolyze fairly rapidly[52] and are not expected to be significant components of natural organic matter,[53−55] compounds such as humic acids remain intact. Thus, this reaction produces primarily FAMEs derived from phospholipid fatty acids (PLFA). In order to further reduce the possibility of other carbon compounds being present in this fraction, the FAMEs were then further purified via silica gel chromatography designed to elute only a limited polarity range and thus to remove any other contributions to this fraction. The FAME fraction was then reanalyzed by GC/MS to ensure that it contained only FAMEs. This fraction is representative of the microbial PLFA in the sample. However, in recognition of the fact that we cannot directly demonstrate the absence of compounds that would not be detected by GC/MS, we have termed this fraction the 'PLFA-rich' fraction. Details of phospholipid extraction, purification, and identification are described in the Supporting Information.

Compound Specific Stable Carbon and Radiocarbon Analysis. Stable carbon and radiocarbon isotopes were measured for PLFA-rich fractions and total organic carbon pools in sediments collected 5 and 18 months after initial oil contact.

The PLFA-rich fraction was measured as a bulk sample for $\delta^{13}C$ and $\Delta^{14}C$ analyses since there was insufficient sample to analyze individual PLFAs. However, bulk PLFA analysis still allows for accurate assessment of microbial carbon sources since there is little variation between the isotopic signature of PLFA compounds from the same site.[12,34,36] Further, as described above and in Ahad et al., 2010 and Mahmoudi et al., 2013, several steps were taken to ensure that the PLFA-rich fraction was free of any contaminants and/or background lipids. Process blanks which were exposed to the same solvents and procedures as sediment samples were found to be free of contaminants. In addition, PLFA-rich fractions were evaporated to a small volume in order to assess the presence of color due any potential humic contamination and were found to be clear. Lastly, $\delta^{13}C$ analysis of individual PLFAs was carried out for a number of samples in order to confirm consistency between individual PLFAs ($\delta^{13}C_{16:0,}$ $\delta^{13}C_{18:1}$) and the bulk PLFA-rich fraction ($\delta^{13}C_{PLFA\text{-}rich}$). The PLFAs 16:0 and 18:1 were dominant across all sediment samples, and, although they are not associated with a particular microbial group, they are known to be present in almost all microorganisms.[56] In all cases, $\delta^{13}C$ values for the bulk PLFA-rich fraction and individual PLFAs, 16:0 and 18:1, were found to be within 0−2‰ (within error) (Table S5). This agreement further

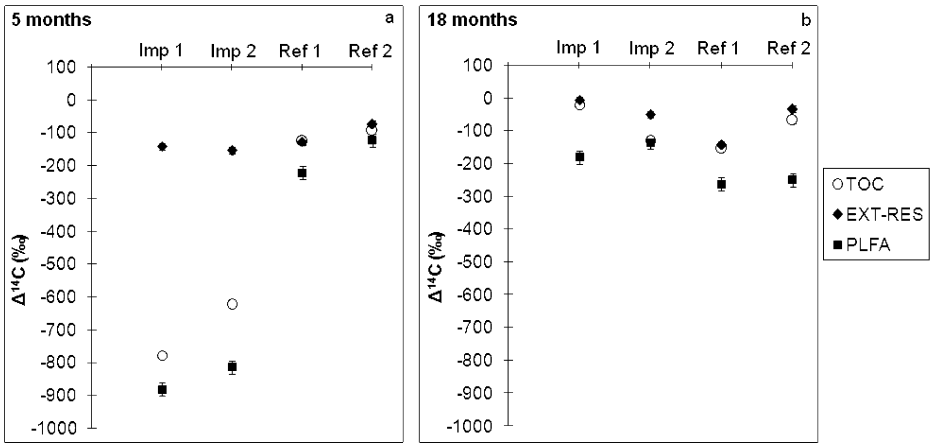

Figure 2. Radiocarbon ($\Delta^{14}C$) values for TOC, EXT-RES, and PLFA-rich fractions for oil-impacted and reference sites (a) 5 months after oil intrusion (October 2010) and (b) 18 months after oil intrusion (October 2011). Error bars represent accuracy and reproducibility of the analyses.

supports that the PLFA-rich fraction is dominated, if not exclusively, derived from microbial PLFA.

Bulk TOC-$\delta^{13}C$ and of TOC-$\Delta^{14}C$ of each sample was determined after decarbonating oven-dried sediment (50 °C for 48 h) using HCl. In addition, $\delta^{13}C$ and $\Delta^{14}C$ of residual sediment remaining following solvent extraction, defined as solvent-extracted residues (EXT-RES), was also determined. Based on White et al.,[57] this EXT-RES was taken to represent the natural organic matter since any petroleum hydrocarbons are removed during extraction. Details of the total lipid extraction procedure used can be found in the Supporting Information.

Stable and radiocarbon isotope signatures of the PLFA-rich fraction, TOC, and EXT-RES were measured at the Centre for Applied Isotope Studies at University of Georgia. Samples were combusted to carbon dioxide (CO_2), and approximately 10% of the CO_2 was reserved for $\delta^{13}C$ analysis which was performed on a dual inlet Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS). The remaining CO_2 was converted to graphite for $\Delta^{14}C$ analysis by accelerator mass spectrometry (AMS). Radiocarbon values were normalized to a $\delta^{13}C$ of −25‰ and expressed in $\Delta^{14}C$ notation as permil (‰) relative to deviation from the ^{14}C Standard Reference Material 4990B oxalic acid.[58] This normalization removes the effects of isotopic fractionation, thereby allowing for interpretation of $\Delta^{14}C$ as a direct tracer of microbial carbon sources.[12] In this context, petroleum will have a $\Delta^{14}C$ value of −1000‰ since it contains no detectable ^{14}C, whereas carbon derived from recently photosynthesized materials will have values of approximately +50‰. The accuracy and reproducibility for $\Delta^{14}C$ analysis was ±10‰ for TOC and EXT-RES, ±20‰ for PLFA-rich fraction, and ±0.5‰ for $\delta^{13}C$ analysis. These errors include the accuracy and precision of the instrument as well as the limitations of the preparation method.[59,60]

RESULTS

Hydrocarbons. Five months after the spill the impacted sites had UCM concentrations of 26,465 to 50,380 mg/kg, total alkane concentrations of 1303 to 6987 mg/kg, and PAH concentrations of 16.2 to 99.4 mg/kg (Figure 1). These concentrations were 100 times higher than those of the reference sites, which had UCM concentrations of 18 to 280 mg/kg, total alkane concentrations of 17 to 52 mg/kg, and total PAH concentrations of 1.1 to 1.5 mg/kg. Following the 5 month time point, UCM, alkane, and PAH concentrations at impacted sites rapidly decreased, and, by 11 months, concentrations had been reduced by 80−90%. By 18 months, PAH, alkane, and UCM concentrations at impacted sites were almost equivalent to those at reference sites.

Microbial PLFAs. Microbial PLFA concentrations were greater at reference sites at 5 and 11 months after oil intrusion compared to those of impacted sites (Figure 1, Table S2). However, by 18 months, PLFA concentrations at impacted sites were comparable to those at reference site 1. PLFA concentrations at reference site 2 decreased over time, whereas PLFA concentrations at the other three sites remained relatively consistent or increased slightly. Using an average conversion factor of 4×10^4 cells $pmol^{-1}$ of PLFA,[61] these PLFA concentrations correspond to cell densities of 4.6×10^8 to 3.6×10^9 cells g^{-1} at the reference sites and 2.1×10^8 to 5.3×10^8 cells g^{-1} at the impacted sites (Table S2), which are within the range of previously reported cell densities for salt marshes and contaminated environments.[61,12,34] The PLFA distribution at all sites was dominated by monounsaturated and *n*-saturated PLFAs, as expected for surface sediments (Table S3).[62] Consistent with previous findings,[12,35,37] there was no relationship between PLFA distributions and hydrocarbon concentrations in sediments (Table S2).

Compound-Specific Radiocarbon. Impacted sites sediments had dramatically lower $\Delta^{14}C_{TOC}$ values 5 months after oiling relative to reference sites (by at least 500‰) due to the presence of highly depleted petroleum carbon ($\Delta^{14}C$ = −1000‰) (Figure 2). By 18 months, $\Delta^{14}C_{TOC}$ values at impacted and reference sites were comparable, consistent with the similar hydrocarbon concentrations found across sites at this later time point. There was less variation in $\Delta^{14}C_{EXT\text{-}RES}$ values across sites; this is expected since EXT-RES represents naturally occurring organic matter and does not include the contribution of petroleum carbon.[57] Reference sites had similar $\Delta^{14}C_{EXT\text{-}RES}$ and $\Delta^{14}C_{TOC}$ values (within error) confirming the minimal presence of petroleum at these sites. Temporal variations in $\Delta^{14}C_{EXT\text{-}RES}$ and $\Delta^{14}C_{TOC}$ values were observed across all sites over the 18 months of this study; however, these variations were fairly small (~20−30‰) and may be due to natural variability in the age of organic matter in this system.

The $\Delta^{14}C_{PLFA\text{-}rich}$ values at impacted sites 5 months after oiling ($\Delta^{14}C_{PLFA}$ = −815 and −882‰) were highly depleted in ^{14}C relative to TOC and by up to 740‰ from EXT-RES. These

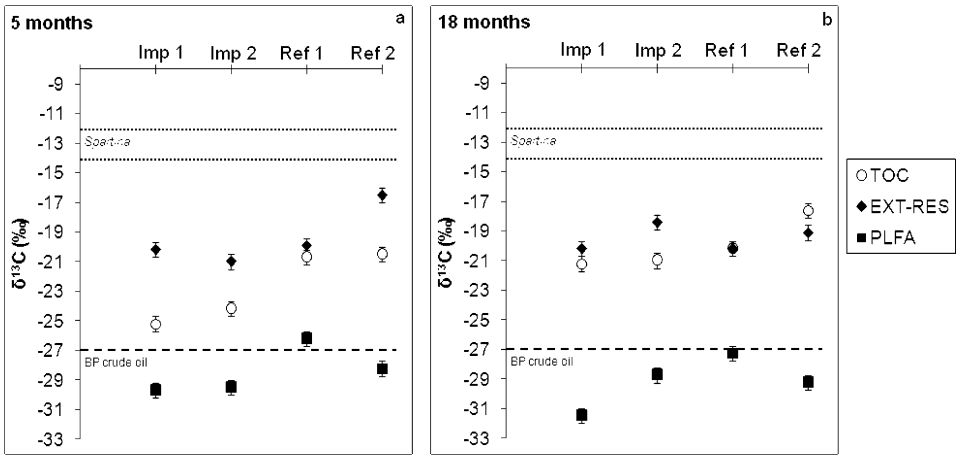

Figure 3. Stable carbon ($\delta^{13}C$) values for TOC, EXT-RES, and PLFA-rich fractions for oil-impacted and reference sites (a) 5 months after oil intrusion (October 2010) and (b) 18 months after oil intrusion (October 2011). Error bars represent the accuracy and reproducibility of the analyses.

depleted $\Delta^{14}C_{PLFA}$ values are consistent with previous studies in which microbial degradation of petroleum was observed.[33,35,36] By 18 months, $\Delta^{14}C_{PLFA\text{-}rich}$ values at impacted sites ($\Delta^{14}C_{PLFA}$ = −181 and −136‰) were considerably less depleted relative to TOC and EXT-RES and comparable to $\Delta^{14}C_{PLFA\text{-}rich}$ values at reference sites.

Compound-Specific Carbon Stable Isotopes. The $\delta^{13}C_{TOC}$ of the impacted site sediments 5 months following oil intrusion were 4−5‰ lower compared to reference sites, consistent with the presence of ^{13}C-depleted petroleum carbon (Figure 3, BP crude oil; $\delta^{13}C$ = −27‰). By 18 months, $\delta^{13}C_{TOC}$ values at impacted sites were comparable to that of references sites, consistent with minimal hydrocarbon concentrations measured at this later time point. The observed sediment $\delta^{13}C_{TOC}$ values (∼20−21‰) were slightly more depleted than the expected values for *Spartina*-dominated marsh systems (∼14−18‰).[41,42] However, Silliman et al.,[44] detailed marsh vegetation at these sites and found *Spartina* sp. to be the dominant marsh plant. There is potential for input of organic matter from other carbon sources such as C_3 vegetation via the Mississippi-Atchafalaya river system[63] which may account for more depleted $\delta^{13}C_{TOC}$ values. Likewise, Wang et al.,[64] found sediment $\delta^{13}C_{TOC}$ values of −19‰ in *Spartina*-dominated marshes due to the contribution of organic matter from other sources. In contrast, the $\delta^{13}C_{EXT\text{-}RES}$ values did not show inputs of other carbon sources and were comparable to the expectation for *Spartina*-dominated environments.[12]

The $\delta^{13}C_{PLFA\text{-}rich}$ values of impacted sites ranged from −28.7 to −31.5‰, while the reference sites ranged from −26.2 to −29.2‰. Unlike for $\Delta^{14}C$, the $\delta^{13}C_{PLFA\text{-}rich}$ values at impacted sites were indistinguishable from reference sites, and there was little temporal variation (less than 2‰) in $\delta^{13}C_{PLFA\text{-}rich}$ values.

Microbial Community Structure. Pyrosequencing-based analysis of 16S rDNA and fungal ITS amplicons recovered a total of 66,129 bacterial 16S, 85,645 archaeal 16S, and 99,013 fungal ITS raw pyrotag sequences. Following stringent quality control, a total of 49,433 bacterial 16S, 79,213 archaeal 16S, and 95,893 fungal ITS reads were retained for community analysis. Sequence clustering based on 97% sequence similarity yielded a total of 5,916 bacterial, 4,664 archaeal, and 948 fungal operational taxonomic units (OTUs).

The dominant bacterial phylum across all sites was *Proteobacteria* (65.4% of classified OTUs on average), although *Bacteroidetes* (7.3%), *Firmicutes* (4.3%), and *Acidobacteria* (3.3%) were also present in all samples. Among the *Proteobacteria*, the predominant classes were the *Alphaproteobacteria* (36.0%), *Gammaproteobacteria* (19.3%), and *Deltaproteobacteria* (15.5%) (Figure 4a). *Alphaproteobacteria* were the dominant taxa at impacted sites at 5 months, whereas *Gammaproteobacteria* and *Bacilli* were dominant at reference sites. By 18 months, relative abundances of *Alphaproteobacteria* decreased at impacted sites such that the relative abundance of dominant taxa across sites became similar. Order-level identification of *Alphaproteobacteria* showed a slightly higher relative abundance of *Sphingomonadales* and *Rhodobacterales* at impacted sites at 5 months compared to other sites and time points (Figure S2). Nonmetric multidimensional scaling (NMDS) analysis of the bacterial community based on the Chi Squared distance between sites (summarized to the order rank) showed that 5 month-impacted sites clustered together, whereas 18 month-impacted sites were clustered more closely with the reference sites (Figure S3a, 2D stress: 0.14, r^2 = 0.87). Fitting of hydrocarbon concentrations to the NMDS ordination revealed a significant relationship ($p < 0.05$) between the observed pattern of taxonomic clustering of impacted sites at 5 months with alkane, PAH, and UCM concentrations.

The archaeal pyrosequencing data revealed the presence of two phyla across all sites and time points, *Crenarchaeota* and *Euryarchaeota* (55.1% and 44.8%, average classified OTUs, respectively), and three dominant classes, *Thermoprotei* (71.4%), *Methanomicrobia* (26.6%) (Figure 4b). Impacted sites had higher relative abundance of *Methanomicrobia* and lower relative abundance of *Thermoprotei* compared to reference sites. NMDS analysis of the archaeal community based on the Chi Squared distance between sites (summarized to the order rank) showed distinct clustering of impacted sites of both time points (Figure S3b, 2D stress: 0.09, r^2 = 0.99). However, there was no significant correlation ($p > 0.05$) between the observed pattern of taxonomic clustering and total alkane, PAH, or UCM concentrations. Thus, the archaeal communities at impacted sites were more taxonomically similar to each other than to reference sites, regardless of the concentrations of petroleum.

The fungal communities across all sites were dominated by the phylum *Ascomycota* (56.1% of classified OTUs on average), though *Basidomycota* (25.2%) and *Chytridiomycota* (12.0%)

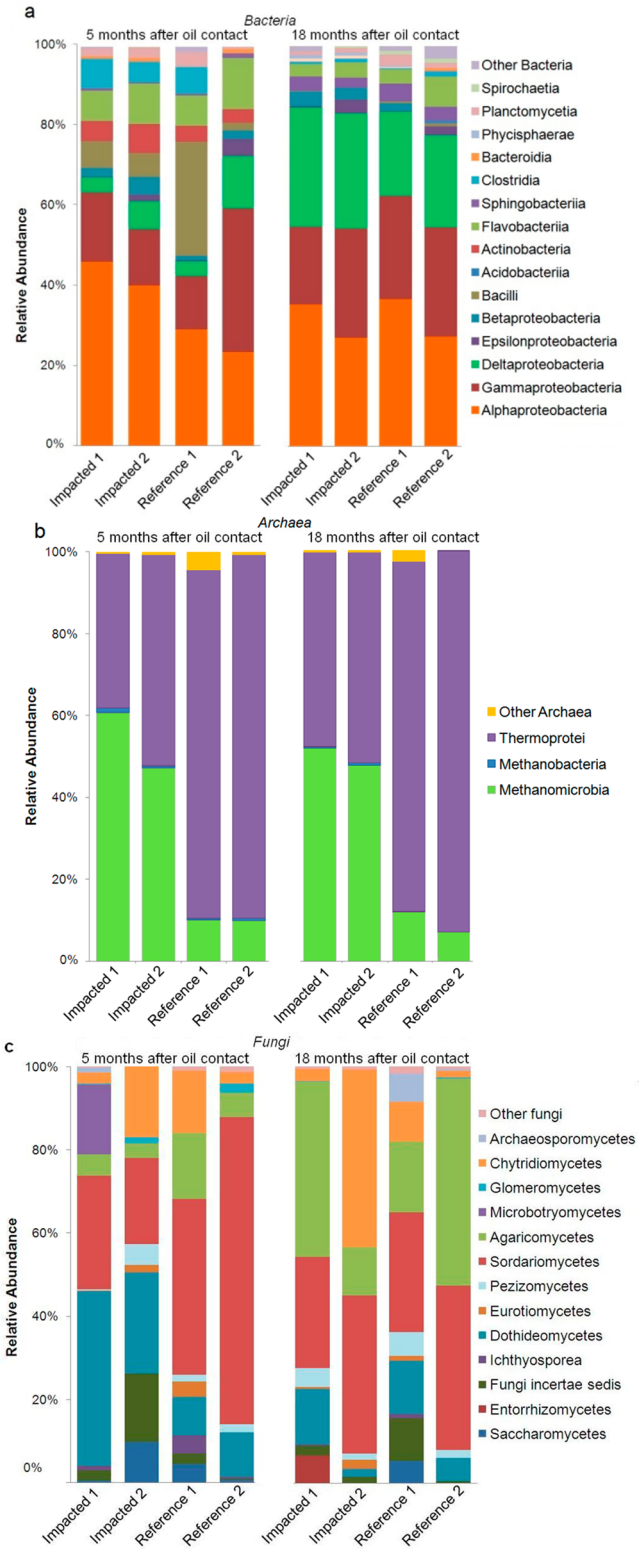

Figure 4. Relative frequency of dominant (a) bacterial, (b) archaeal, and (c) fungal OTUs (summarized to class rank) in sediments collected from two oil-impacted and two reference sites 5 and 18 months after oil contact.

were also prevalent. The most abundant fungal class was *Sordariomycetes* (32.5%) followed by *Agaricomycetes* (21.0%) and *Dothideomycetes* (18.1%) (Figure 4c). Five months after oil intrusion, impacted sites were dominated by *Dothideomycetes*, while reference sites were dominated by *Sordariomycetes*. By 18 months, the relative abundance of *Dothideomycetes* decreased at impacted sites such that both impacted and reference sites were dominated by *Sordariomycetes* and *Agaricomycetes* as well as *Chytridiomycetes,* in one case. NMDS analysis of fungal communities based on the Chi Squared distance between sites (summarized to the class rank) show minimal clustering of impacted sites at either time point (Figure S3c, 2D stress: 0.17, $r^2 = 0.78$). Fitting of hydrocarbon concentrations to the NMDS ordination found a significant correlation ($p > 0.05$) between the observed pattern of fungal taxonomic clustering for impacted site 1 at 5 months and total alkane, PAH, or UCM concentrations.

DISCUSSION

Presence of Petroleum Oil in Marsh Sediments. An isotopic mass balance approach was used to estimate the fraction of TOC made up of petroleum carbon (f_{petro}) in each sample:

$$\Delta^{14}C_{TOC} = f_{EXT\text{-}RES}(\Delta^{14}C_{EXT\text{-}RES}) + f_{petro}(\Delta^{14}C_{petro}) \quad (1)$$

where $\Delta^{14}C_{petro}$ was assumed to be −1000‰, and $\Delta^{14}C_{EXT\text{-}RES}$ was assumed to be natural sediment organic matter containing no petroleum contribution and assuming $f_{EXT\text{-}RES} + f_{petro} = 1$. Using this approach, petroleum was estimated to make up 56% and 74% of the carbon in marsh sediments at impacted sites 1 and 2, respectively, 5 months after oil intrusion and 1% and 9% of the carbon 18 months after oil intrusion (Table S6). These contributions were consistent with measured PAH, alkane, and UCM concentrations measured at these sites. Similarly, using an isotopic mass balance as per eq 1 but using $\delta^{13}C$ values, petroleum was estimated to make up 54% and 74% of the carbon at impacted sites 1 and 2, respectively, 5 months after oil intrusion. However, there was no concurrent increase in microbial biomass at this time point as shown by total PLFA concentrations, indicating that microbial community growth was limited by other factors. This suggests that removal of these limiting factors may represent a means to stimulate biodegradation particularly in environments where it is occurring slowly.

Microbial Incorporation of *Deepwater Horizon* Oil in Marsh Sediments. The highly depleted $\Delta^{14}C_{PLFA\text{-}rich}$ values observed at impacted sites 5 months after oil intrusion demonstrate that *in situ* biodegradation by the indigenous microbial communities was contributing to the loss of petroleum at these sites. Using an isotopic mass balance similar to eq 1, it was estimated that 86% and 78% of the carbon in microbial PLFAs was derived from petroleum at impacted sites 1 and 2, respectively, at this time. This high proportion of petroleum carbon present in PLFA, which are produced by both bacteria and fungi, suggest that petroleum hydrocarbons were the primary microbial carbon source at impacted sites at this time through direct degradation by hydrocarbon-degrading microbes and/or recycling of microbial organic compounds to nonhydrocarbon-degrading microbes.

By 18 months, $\Delta^{14}C_{PLFA\text{-}rich}$ values were significantly less depleted indicating that the relative contribution of petroleum carbon to microbial PLFAs decreased at the impacted sites. $\Delta^{14}C_{PLFA\text{-}rich}$ values at impacted sites at 18 months were depleted relative to the TOC and EXT-RES values by up to 174‰. With one exception, $\Delta^{14}C_{PLFA\text{-}rich}$ values at reference sites at all time points were similarly depleted by approximately 100 to 200‰ with respect to TOC and EXT-RES values

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(Figure 2, Table S4). This depletion in PLFA-rich fraction relative to TOC and EXT-RES implies that there was some ^{14}C depleted carbon source being metabolized by these microbial communities and incorporated into PLFA. Assuming a fossil carbon source such as petroleum, the input of highly depleted carbon ($\Delta^{14}C = -1000‰$) to microbial PLFAs was estimated to be 9−18% at impacted sites at 18 months and 5 to 22% at reference sites. Given that the Gulf of Mexico experiences frequent inputs of petroleum hydrocarbons via natural hydrocarbon seeps as well as smaller scale spills, the presence of hydrocarbons in sediments may be a common occurrence. Indeed, hydrocarbon concentrations and estimates of petroleum carbon in TOC even at some of our reference sites were nonzero (Table S6); this may represent a potential source of highly depleted carbon to microbial PLFAs in sediments with no visible oil. However, the highly depleted $\Delta^{14}C_{PLFA\text{-}rich}$ values observed at impacted sites at 5 months coupled to the decrease in hydrocarbon concentrations demonstrate that biodegradation was contributing to mass loss of petroleum in these sediments. It is important to note that additional processes such as abiotic oxidation and export via sediment erosion were also likely contributing to the overall loss of petroleum in these sediments.[44]

The relatively large offset between the $\delta^{13}C$ of the natural organic matter, represented by EXT-RES, and petroleum make this one of the most favorable systems for applying $\delta^{13}C$ analysis as a tool for differentiating microbial carbon sources.[12] Despite the fact that petroleum presence indicated by $\delta^{13}C_{TOC}$ values was comparable to that obtained using $\Delta^{14}C$ analysis, microbial incorporation of petroleum carbon could not be resolved based on $\delta^{13}C_{PLFA\text{-}rich}$ values. The $\delta^{13}C_{PLFA\text{-}rich}$ values at impacted sites were expected to be more depleted relative to reference sites at 5 months due to the utilization and incorporation of $\delta^{13}C$-depleted petroleum carbon into microbial lipids. However, no such depletion was observed at 5 months, and $\delta^{13}C_{PLFA\text{-}rich}$ values at impacted and reference sites differed by only 1 to 3‰. At 18 months, $\delta^{13}C_{PLFA\text{-}rich}$ values between impacted and reference sites varied by 2 to 4‰, and $\delta^{13}C_{PLFA\text{-}rich}$ at all sites were more depleted than TOC and EXT-RES (by 7 to 12‰), greater than the expected 4 to 6‰ fractionation between heterotrophic microbes and their carbon source[65] (Table S7). The lack of resolvable $\delta^{13}C$ signal of petroleum incorporation into PLFA may be due to variation in fractionations during lipid synthesis as depletions of 3 to 14‰ between microbial lipids and carbon sources have been previously observed,[66−68] particularly in anaerobic systems. Further, the production of highly depleted methane ($\delta^{13}C$ = −30 to −60‰) by methanogens and subsequent utilization of this carbon by methanotrophs may also confound the use of $\delta^{13}C$ in this system. It is also possible that in the microbial community is utilizing more depleted C_3-derived organic matter that may be deposited into sediments via the Mississippi-Atachafalyla river system. These results demonstrate that natural abundance $\Delta^{14}C$ analysis can overcome these factors and resolve *in situ* biodegradation of petroleum in saltmarsh environments.

Impact of Petroleum Oil on Microbial Community Structure. Biodegradation of petroleum involves complex interactions between microbes and the geochemical properties of the surrounding environment.[23] Consequently, assessing the degradation of hydrocarbons in the natural environment is challenging because these interactions are difficult to replicate within a laboratory setting. Studies investigating the *in situ* response of microbial communities to released MC252 oil are providing valuable information regarding the biodegradation potential of indigenous microbial communities in the Gulf of Mexico.[26,27,29−32,69,70] Our study is the first to demonstrate direct utilization and incorporation of this oil into microbial biomass in the first few months following the spill and subsequent removal of this oil from sediments by 18 months.

Although previous studies have detected dramatic shifts in bacterial community structure in response to the presence of petroleum, we observed a much less dramatic shift in the relative abundance of dominant bacterial taxa between impacted and reference sites in this study. However, we did observe a higher relative abundance of bacterial groups, *Rhodobacterales* and *Sphingomonadales*, in impacted sediments 5 months after oil intrusion. As the hydrocarbon concentrations decreased, the taxonomic composition of impacted and reference sites became more similar by 18 months after oil intrusion. This is consistent with previous observations in the literature which suggest that members of *Sphingomonadales* are dominant PAH degraders in soils and sediments,[71,72] based on their frequent occurrence in contaminated sites and their known metabolic capability. Recent metagenomic analyses from Elmer's Island, Louisiana show enrichment of *Rhodobacterales* in oil-impacted samples.[73] Likewise, Kostka et al.[27] found a significant community shift toward a number of bacterial groups, including *Rhodobacterales*, in oil-contaminated beach sands in Florida. Although we observed an apparent shift in the microbial community structure, no concomitant increase in microbial biomass in oil-impacted sediments, as measured by total PLFA concentrations, was observed. This is consistent with previous work that investigated surface waters near the *Deepwater Horizon* site and found microbial biomass to be comparable inside and outside the oil slick.[70]

Archaea have been detected in many petroleum-impacted environments including aquifers,[74−76] soils,[77,78] and petroleum reservoirs.[79,80] However, the effect of petroleum on archaea and their role in hydrocarbon degradation is not well understood. Redmond and Valentine[32] found that archaeal community compositions were similar in well oxygenated oil plume and nonplume samples over a period of five months, even while the samples contained different bacterial communities, suggesting that presence of petroleum does not have a large impact on the archaeal communities. In this study, the archaeal communities at impacted sites were found to be taxonomically more similar to one another than to that of the reference sites even when petroleum concentrations in the sediments were minimal. The greater abundance of *Methanomicrobia* at impacted sites may have resulted from anoxic conditions induced by the aerobic degradation of petroleum or because the impacted sites happened to be more similar sedimentary environments, even prior to the oiling event. Nevertheless, methanogenic archaea such as *Methanomicrobia* have been found to be prevalent in saltmarsh sediments.[81−83] Due to our limited knowledge of the metabolic capability of archaea, it is difficult to discern whether the composition of the archaeal community at impacted sites resulted from their role in petroleum degradation. Future studies that further characterize these archaeal communities, including radiocarbon analysis of archaea-specific lipids, may provide sufficient context to address their contribution to biodegradation.

One might expect fungi to play an important role in the breakdown of the higher molecular weight components of petroleum due to their production of nonspecific phenol

oxidase enzymes such as laccase and peroxidase.[84,85] The higher relative frequency of OTUs belonging to *Dothideomycetes* detected at impacted sites 5 months after oil intrusion suggests that this group may be playing a role in biodegradation. Similarly, Bik et al. [30] found postspill beach sediments impacted by MC252 oil to be dominated by the ascomycete genera *Cladosporium* and *Alternaria*, both of which belong to the class *Dothideomycetes*. Species belonging to this class have been shown to degrade hydrocarbons and thrive in polluted environments.[86,87] In addition, some species within this class (*Dothideomycetes*) have increased activity of lignin-degrading enzymes such as laccases that are capable of degrading PAHs.[88−90] While many studies of marine fungi focus on taxonomic novelty and phylogenetic diversity, it is recognized that this is an understudied field.[91] Additional functional studies of marine fungi, including radiocarbon studies tracing carbon sources into unique fungal biomarkers, are needed to elucidate the role that fungi may play in the breakdown of petroleum in natural settings.

The highly depleted $\Delta^{14}C_{PLFA\text{-}rich}$ values directly show that *in situ* biodegradation by the indigenous microbial communities contributed to degradation and thus remediation of MC-252 oil in the severely oiled marsh sediments of Barataria Bay. Furthermore, microbial community analysis of bacterial communities points to the groups, *Sphingomonadales* and *Rhodobacterales*, as being potential degraders at these sites. Our limited understanding regarding the role and function of archaea and fungi in contaminated environments makes it difficult to assess whether these groups may be playing a role in petroleum biodegradation. The findings of this study suggest that Gulf of Mexico coastal systems have considerable biodegradation potential and that natural attenuation may be feasible remediation strategy in this region.

■ ASSOCIATED CONTENT

Supporting Information

Figures S1−S3, Tables S1−S7, additional text, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Camilli, R.; Reddy, C. M.; Yoerger, D. R.; Van Mooy, B. A. S.; Jakuba, M. V.; Kinsey, J. C.; McIntyre, C. P.; Sylva, S. P.; Maloney, J. V. Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* **2010**, *330*, 201−204.

(2) Crone, T. J.; Tolstoy, M. Magnitude of the 2010 Gulf of Mexico oil leak. *Science* **2010**, *330*, 634−634.

(3) Atlas, R. M.; Hazen, T. C. Oil biodegradation and bioremediation: A tale of the two worst spills in US history. *Environ. Sci. Technol.* **2011**, *45*, 6709−6715.

(4) Thibodeaux, L. J.; Valsaraj, K. T.; John, V. T.; Papadopoulos, K. D.; Pratt, L. R.; Pesika, N. S. Marine oil fate: Knowledge gaps, basic research, and development needs; A perspective based on the Deepwater Horizon spill. *Environ. Eng. Sci.* **2011**, *28*, 87−93.

(5) Mitsch, W. J.; Gosselink, J. G. The value of wetlands: importance of scale and landscape setting. *Ecol. Econ.* **2000**, *35*, 25−33.

(6) Engle, V. D. Estimating the provision of ecosystem services by Gulf of Mexico coastal wetlands. *Wetlands* **2011**, *31*, 179−193.

(7) Deegan, L. A.; Johnson, D. S.; Warren, R. S.; Peterson, B. J.; Fleeger, J. W.; Fagherazzi, S.; Wollheim, W. M. Coastal eutrophication as a driver of salt marsh loss. *Nature* **2012**, *490*, 388−392.

(8) Venosa, A. D.; Lee, K.; Suidan, M. T.; Garcia-Blanco, S.; Cobanli, S.; Moteleb, M.; Haines, J. R.; Tremblay, G.; Hazelwood, M. Bioremediation and biorestoration of a crube oil-contaminated freshwater wetland on the St. Lawrence River. *Biorem. J.* **2002**, *6*, 261−281.

(9) Reddy, C. M.; Eglinton, T. I.; Hounshell, A.; White, H. K.; Xu, L.; Gaines, R. B.; Frysinger, G. S. The West Falmouth oil spill after thirty years: The persistence of petroleum hydrocarbons in marsh sediments. *Environ. Sci. Technol.* **2002**, *36*, 4754−4760.

(10) Peacock, E. E.; Hampson, G. R.; Nelson, R. K.; Xu, L.; Frysinger, G. S.; Gaines, R. B.; Farrington, J. W.; Tripp, B. W.; Reddy, C. M. The 1974 spill of the Bouchard 65 oil barge: Petroleum hydrocarbons persist in Winsor Cove salt marsh sediments. *Mar. Pollut. Bull.* **2007**, *54*, 214−225.

(11) Peacock, E. E.; Nelson, R. K.; Solow, A. R.; Warren, J. D.; Baker, J. L.; Reddy, C. M. The West Falmouth Oil Spill: ∼100 kg of oil found to persist decades later. *Environ. Foren.* **2005**, *6*, 273−281.

(12) Slater, G. F.; White, H. K.; Eglinton, T. I.; Reddy, C. M. Determination of microbial carbon sources in petroleum contaminated sediments using molecular ^{14}C analysis. *Environ. Sci. Technol.* **2005**, *39*, 2552−2558.

(13) Oudot, J.; Chaillan, F. Pyrolysis of asphaltenes and biomarkers for the fingerprinting of the Amoco-Cadiz oil spill after 23 years. *C. R. Chim.* **2010**, *13*, 548−552.

(14) Hoff, R. Z. *Responding to Oil Spills in Coastal Marshes: The Fine Line Between Help and Hindrance*; Hazardous Material Response and Assessment Division, National Oceanic and Atmospheric Administration: 1995.

(15) Pezeshki, S. R.; Hester, M. W.; Lin, Q.; Nyman, J. A. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review. *Environ. Pollut.* **2000**, *108*, 129−139.

(16) Carman, K. R.; Means, J. C.; Pomarico, S. C. Response of sedimentary bacteria in a Louisiana salt marsh to contamination by diesel fuel. *Aquat. Microb. Ecol.* **1996**, *10*, 231−241.

(17) Coates, J. D.; Woodward, J.; Allen, J.; Philp, P.; Lovley, D. R. Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. *Appl. Environ. Microbiol.* **1997**, *63*, 3589−3593.

(18) Cui, Z.; Lai, Q.; Dong, C.; Shao, Z. Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge. *Environ. Microbiol.* **2008**, *10*, 2138−2149.

(19) Teramoto, M.; Suzuki, M.; Okazaki, F.; Hatmanti, A.; Harayama, S. Oceanobacter-related bacteria are important for the degradation of petroleum aliphatic hydrocarbons in the tropical marine environment. *Microbiology* **2009**, *155*, 3362−3370.

(20) Jung, S. W.; Park, J. S.; Kown, O. Y.; Kang, J.-H.; Shim, W. J.; Kim, Y.-O. Effects of crude oil on marine microbial communities in short term outdoor microcosms. *J. Microbiol.* **2010**, *48*, 594−600.

(21) Lekunberri, I.; Calvo-Díaz, A.; Teira, E.; Morán, X. A. G.; Peters, F.; Nieto-Cid, M.; Espinoza-González, O.; Teixeira, I. G.; Gasol, J. M. Changes in bacterial activity and community composition caused by exposure to a simulated oil spill in microcosm and mesocosm experiments. *Aquat. Microb. Ecol.* **2010**, *59*, 169−183.
(22) Dash, H. R.; Mangwani, N.; Chakraborty, J.; Kumari, S.; Das, S. Marine bacteria: potential candidates for enhanced bioremediation. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 561−571.
(23) Atlas, R. M. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **1981**, *45*, 180.
(24) Leahy, J. G.; Colwell, R. R. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* **1990**, *54*, 305−315.
(25) Head, I. M.; Jones, D. M.; Röling, W. F. M. Marine microorganisms make a meal of oil. *Nat. Rev. Microbiol.* **2006**, *4*, 173−182.
(26) Hazen, T. C.; Dubinsky, E. A.; DeSantis, T. Z.; Andersen, G. L.; Piceno, Y. M.; Singh, N.; Jansson, J. K.; Probst, A.; Borglin, S. E.; Fortney, J. L. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* **2010**, *330*, 204−208.
(27) Kostka, J. E.; Prakash, O.; Overholt, W. A.; Green, S. J.; Freyer, G.; Canion, A.; Delgardio, J.; Norton, N.; Hazen, T. C.; Huettel, M. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Appl. Environ. Microbiol.* **2011**, *77*, 7962−7974.
(28) Lu, Z.; Deng, Y.; Van Nostrand, J. D.; He, Z.; Voordeckers, J.; Zhou, A.; Lee, Y.-J.; Mason, O. U.; Dubinsky, E. A.; Chavarria, K. L. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. *ISME J.* **2011**, *6*, 451−460.
(29) Beazley, M. J.; Martinez, R. J.; Rajan, S.; Powell, J.; Piceno, Y. M.; Tom, L. M.; Andersen, G. L.; Hazen, T. C.; Van Nostrand, J. D.; Zhou, J. Microbial community analysis of a coastal salt Marsh affected by the deepwater horizon oil spill. *PloS One* **2012**, *7*, e41305.
(30) Bik, H. M.; Halanych, K. M.; Sharma, J.; Thomas, W. K. Dramatic shifts in Benthic microbial eukaryote communities following the deepwater horizon oil spill. *PLoS One* **2012**, *7*, e38550.
(31) Mason, O. U.; Hazen, T. C.; Borglin, S.; Chain, P. S. G.; Dubinsky, E. A.; Fortney, J. L.; Han, J.; Holman, H.-Y. N.; Hultman, J.; Lamendella, R. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *ISME J.* **2012**, *6*, 1715−1727.
(32) Redmond, M. C.; Valentine, D. L. Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci.* **2012**, *109*, 20292−20297.
(33) Slater, G. F.; Nelson, R. K.; Kile, B. M.; Reddy, C. M. Intrinsic bacterial biodegradation of petroleum contamination demonstrated in situ using natural abundance, molecular-level ^{14}C analysis. *Org. Geochem.* **2006**, *37*, 981−989.
(34) Wakeham, S. G.; McNichol, A. P.; Kostka, J. E.; Pease, T. K. Natural-abundance radiocarbon as a tracer of assimilation of petroleum carbon by bacteria in salt marsh sediments. *Geochim. Cosmochim. Acta* **2006**, *70*, 1761−1771.
(35) Ahad, J. M. E.; Burns, L.; Mancini, S.; Slater, G. F. Assessing microbial uptake of petroleum hydrocarbons in groundwater systems using natural abundance radiocarbon. *Environ. Sci. Technol.* **2010**, *44*, 5092−5097.
(36) Cowie, B. R.; Greenberg, B. M.; Slater, G. F. Determination of microbial carbon sources and cycling during remediation of petroleum hydrocarbon impacted soil using natural abundance 14C analysis of PLFA. *Environ. Sci. Technol.* **2010**, *44*, 2322−2327.
(37) Mahmoudi, N.; Fulthorpe, R. R.; Burns, L.; Mancini, S.; Slater, G. F. Assessing microbial carbon sources and potential PAH degradation using natural abundance14C analysis. *Environ. Pollut.* **2013**, *175*, 125−130.
(38) Trumbore, S. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecol. Appl.* **2000**, *10*, 399−411.
(39) Czimczik, C. I.; Welker, J. M. Radiocarbon content of CO2 respired from high arctic tundra in Northwest Greenland. *Arct. Antarct. Alp. Res.* **2010**, *42*, 342−350.
(40) Boschker, H. T. S.; Middelburg, J. J. Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiol. Ecol.* **2002**, *40*, 85−95.
(41) Chmura, G. L.; Aharon, P.; Socki, R. A.; Abernethy, R. An inventory of 13C abundances in coastal wetlands of Louisiana, USA: vegetation and sediments. *Oecologia* **1987**, *74*, 264−271.
(42) Natter, M.; Keevan, J.; Wang, Y.; Keimowitz, A. R.; Okeke, B. C.; Son, A.; Lee, M.-K. Level and degradation of Deepwater Horizon spilled oil in coastal marsh sediments and pore-water. *Environ. Sci. Technol.* **2012**, *46*, 5744−5755.
(43) Pearson, A.; Kraunz, K. S.; Sessions, A. L.; Dekas, A. E.; Leavitt, W. D.; Edwards, K. J. Quantifying microbial utilization of petroleum hydrocarbons in salt marsh sediments by using the 13C content of bacterial rRNA. *Appl. Environ. Microbiol.* **2008**, *74*, 1157−1166.
(44) Silliman, B. R.; van de Koppel, J.; McCoy, M. W.; Diller, J.; Kasozi, G. N.; Earl, K.; Adams, P. N.; Zimmerman, A. R. Degradation and resilience in Louisiana salt marshes after the BP-Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci.* **2012**, *109*, 11234−11239.
(45) Lane, D. J. 16S/23S rRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*; Stackebrandt, E., Goodfellow, M., Eds.; Wiley: New York, 1991; pp 115−175.
(46) Raskin, L.; Stromley, J. M.; Rittmann, B. E.; Stahl, D. A. Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl. Environ. Microbiol.* **1994**, *60*, 1232−1240.
(47) White, T. J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **1990**, *18*, 315−322.
(48) Gardes, M.; Bruns, T. D. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113−118.
(49) Manter, D. K.; Vivanco, J. M. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. *J. Microbiol. Methods* **2007**, *71*, 7−14.
(50) White, D. C.; Davis, W. M.; Nickels, J. S.; King, J. D.; Bobbie, R. J. Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia* **1979**, *40*, 51−62.
(51) Guckert, J. B.; Ringelberg, D. B.; White, D. C.; Hanson, R. S.; Bratina, B. J. Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the Proteobacteria. *J. Gen. Microbiol.* **1991**, *137*, 2631−2641.
(52) Luddy, F. E.; Barford, R. A.; Riemenschneider, R. W. Direct conversion of lipid components to their fatty acid methyl esters. *J. Am. Oil Chem. Soc.* **1960**, *37*, 447−451.
(53) Chen, J.; Gu, B.; LeBoeuf, E. J.; Pan, H.; Dai, S. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. *Chemosphere* **2002**, *48*, 59−68.
(54) Kögel-Knabner, I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* **2002**, *34*, 139−162.
(55) Lützow, M. V.; Kögel-Knabner, I.; Ekschmitt, K.; Matzner, E.; Guggenberger, G.; Marschner, B.; Flessa, H. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions−a review. *Eur. J. Soil Sci.* **2006**, *57*, 426−445.
(56) *Microbial lipids*; Ratledge, C., Wilkinson, S. G., Eds.; Academic Press: London, 1988; Vol. *1*.
(57) White, H. K.; Reddy, C. M.; Eglinton, T. I. Isotopic constraints on the fate of petroleum residues sequestered in salt marsh sediments. *Environ. Sci. Technol.* **2005**, *39*, 2545−2551.
(58) Stuiver, M.; Polach, H. A. Discussion: Reporting of ^{14}C data. *Radiocarbon* **1977**, *19*, 355−363.
(59) Cherkinsky, A.; Ravi Prasad, G. V.; Dvoracek, D. AMS measurement of samples smaller than 300 μg at Center for Applied Isotope Studies, University of Georgia. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2013**, *294*, 87−90.
(60) Culp, R.; Noakes, J.; Cherkinsky, A.; Ravi Prasad, G. V.; Dvoracek, D. A decade of AMS at the University of Georgia. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2013**, *294*, 46−49.

(61) Green, C. T.; Scow, K. M. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. *Hydrogeol. J.* **2000**, *8*, 126−141.
(62) Zelles, L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fert. Soils* **1999**, *29*, 111−129.
(63) Waterson, E. J.; Canuel, E. A. Sources of sedimentary organic matter in the Mississippi River and adjacent Gulf of Mexico as revealed by lipid biomarker and $\delta^{13}C_{TOC}$ analyses. *Org. Geochem.* **2008**, *39*, 422−439.
(64) Wang, X. C.; Chen, R. F.; Berry, A. Sources and preservation of organic matter in Plum Island salt marsh sediments (MA, USA): long-chain n-alkanes and stable carbon isotope compositions. *Estuarine, Coastal Shelf Sci.* **2003**, *58*, 917−928.
(65) Hayes, J. M. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Rev. Mineral. Geochem.* **2001**, *43*, 225−277.
(66) Teece, M. A.; Fogel, M. L.; Dollhopf, M. E.; Nealson, K. H. Isotopic fractionation associated with biosynthesis of fatty acids by a marine bacterium under oxic and anoxic conditions. *Org. Geochem.* **1999**, *30*, 1571−1579.
(67) Cifuentes, L. A.; Salata, G. G. Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments. *Org. Geochem.* **2001**, *32*, 613−621.
(68) Londry, K. L.; Jahnke, L. L.; Des Marais, D. J. Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **2004**, *70*, 745−751.
(69) Valentine, D. L.; Kessler, J. D.; Redmond, M. C.; Mendes, S. D.; Heintz, M. B.; Farwell, C.; Hu, L.; Kinnaman, F. S.; Yvon-Lewis, S.; Du, M. Propane respiration jump-starts microbial response to a deep oil spill. *Science* **2010**, *330*, 208−211.
(70) Edwards, B. R.; Reddy, C. M.; Camilli, R.; Carmichael, C. A.; Longnecker, K.; Van Mooy, B. A. S. Rapid microbial respiration of oil from the Deepwater Horizon spill in offshore surface waters of the Gulf of Mexico. *Environ. Res. Lett.* **2011**, *6*, 035301.
(71) Leys, N. M. E. J.; Ryngaert, A.; Bastiaens, L.; Verstraete, W.; Top, E. M.; Springael, D. Occurrence and phylogenetic diversity of Sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons. *Appl. Environ. Microbiol.* **2004**, *70*, 1944−1955.
(72) Alonso-Gutiérrez, J.; Figueras, A.; Albaigés, J.; Jiménez, N.; Viñas, M.; Solanas, A. M.; Novoa, B. Bacterial communities from shoreline environments (Costa da Morte, Northwestern Spain) affected by the Prestige oil spill. *Appl. Environ. Microbiol.* **2009**, *75*, 3407−3418.
(73) Chakraborty, R.; Borglin, S. E.; Dubinsky, E. A.; Andersen, G. L.; Hazen, T. C. Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. *Front. Microbiol.* **2012**, *3*, 357.
(74) Dojka, M. A.; Hugenholtz, P.; Haack, S. K.; Pace, N. R. Microbial diversity in a hydrocarbon-and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. *Appl. Environ. Microbiol.* **1998**, *64*, 3869−3877.
(75) Haack, S. K.; Fogarty, L. R.; West, T. G.; Alm, E. W.; McGuire, J. T.; Long, D. T.; Hyndman, D. W.; Forney, L. J. Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. *Environ. Microbiol.* **2004**, *6*, 438−448.
(76) Kleikemper, J.; Pombo, S. A.; Schroth, M. H.; Sigler, W. V.; Pesaro, M.; Zeyer, J. Activity and diversity of methanogens in a petroleum hydrocarbon-contaminated aquifer. *Appl. Environ. Microbiol.* **2005**, *71*, 149−158.
(77) Liu, R.; Zhang, Y.; Ding, R.; Li, D.; Gao, Y.; Yang, M. Comparison of archaeal and bacterial community structures in heavily oil-contaminated and pristine soils. *J. Biosci. Bioeng.* **2009**, *108*, 400−407.
(78) Wang, X.; Han, Z.; Bai, Z.; Tang, J.; Ma, A.; He, J.; Zhuang, G. Archaeal community structure along a gradient of petroleum contamination in saline-alkali soil. *J. Environ. Sci.* **2011**, *23*, 1858−1864.
(79) Magot, M.; Ollivier, B.; Patel, B. K. C. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek* **2000**, *77*, 103−116.
(80) Jones, D. M.; Head, I. M.; Gray, N. D.; Adams, J. J.; Rowan, A. K.; Aitken, C. M.; Bennett, B.; Huang, H.; Brown, A.; Bowler, B. F. J. Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature* **2007**, *451*, 176−180.
(81) Oremland, R. S.; Marsh, L. M.; Polcin, S. Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. *Nature* **1982**, *296*, 143−145.
(82) Franklin, M. J.; Wiebe, W. J.; Whitman, W. B. Populations of methanogenic bacteria in a Georgia salt marsh. *Appl. Environ. Microbiol.* **1988**, *54*, 1151−1157.
(83) Munson, M. A.; Nedwell, D. B.; Embley, T. M. Phylogenetic diversity of Archaea in sediment samples from a coastal salt marsh. *Appl. Environ. Microbiol.* **1997**, *63*, 4729−4733.
(84) Peng, R. H.; Xiong, A. S.; Xue, Y.; Fu, X. Y.; Gao, F.; Zhao, W.; Tian, Y. S.; Yao, Q. H. Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiol. Rev.* **2008**, *32*, 927−955.
(85) Qasemian, L.; Billette, C.; Guiral, D.; Alazard, E.; Moinard, M.; Farnet, A.-M. Halotolerant laccases from Chaetomium sp., Xylogone sphaerospora and Coprinopsis sp. isolated from a Mediterranean coastal area. *Fungal Biol.* **2012**, *116*, 1090−1098.
(86) Colfone, L.; Walker, J. D.; Cooney, J. J. Utilization of hydrocarbons by Cladosporium resinae. *J. Gen. Microbiol.* **1973**, *76*, 243−246.
(87) Okpokwasili, G. C.; Okorie, B. B. Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. *Tribol. Int.* **1988**, *21*, 215−220.
(88) Clemente, A. R.; Anazawa, T. A.; Durrant, L. R. Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. *Braz. J. Microbiol.* **2001**, *32*, 255−261.
(89) Alcalde, M.; Bulter, T.; Arnold, F. H. Colorimetric assays for biodegradation of polycyclic aromatic hydrocarbons by fungal laccases. *J. Biomol. Screen.* **2002**, *7*, 547−553.
(90) Atalla, M. M.; Zeinab, H. K.; Eman, R. H.; Amani, A.; Abeer, A. Screening of some marine-derived fungal isolates for lignin degrading enzymes (LDEs) production. *Agric. Biol. J. North Am.* **2010**, *1*, 591−9.
(91) Richards, T. A.; Jones, M. D. M.; Leonard, G.; Bass, D. Marine fungi: their ecology and molecular diversity. *Annu. Rev. Mar. Sci.* **2012**, *4*, 495−522.

Supporting Information

Rapid degradation of *Deepwater Horizon* spilled oil by indigenous microbial communities in Louisiana salt marsh sediments

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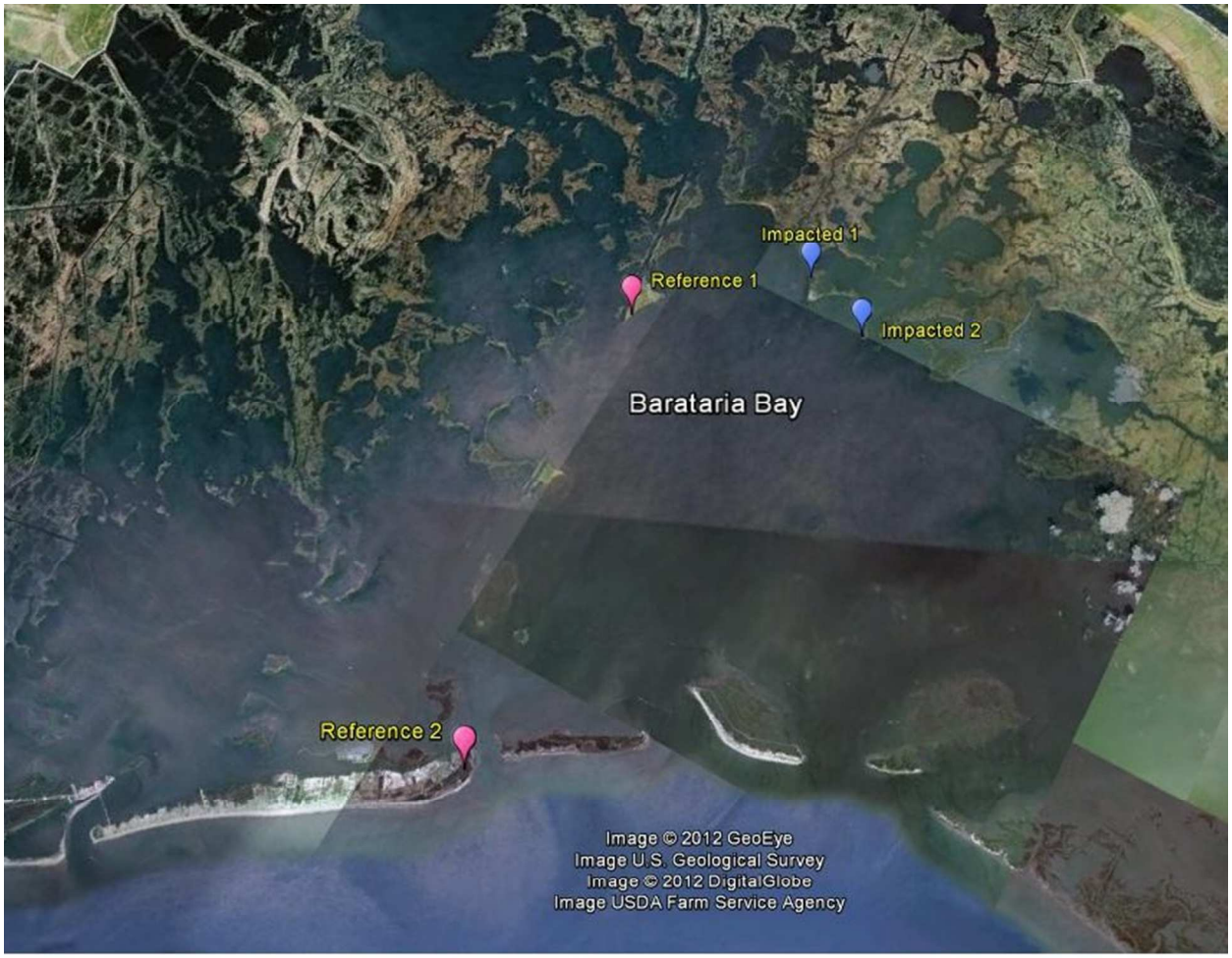

FIGURE S1. Location of sediment samples collected from impacted and reference salt marshes. Impacted sites were located in the northeast corner of Barataria Bay on St. Mary Island which received heavy oil coverage due to prevailing winds and currents after the *Deepwater Horizon* spill. Reference site 1 was located in the northwest corner of the bay, east of Hackberry Bay. Reference site 2 was located on the south side of the bay in Grand Isle State Park (Silliman et al., 2012).

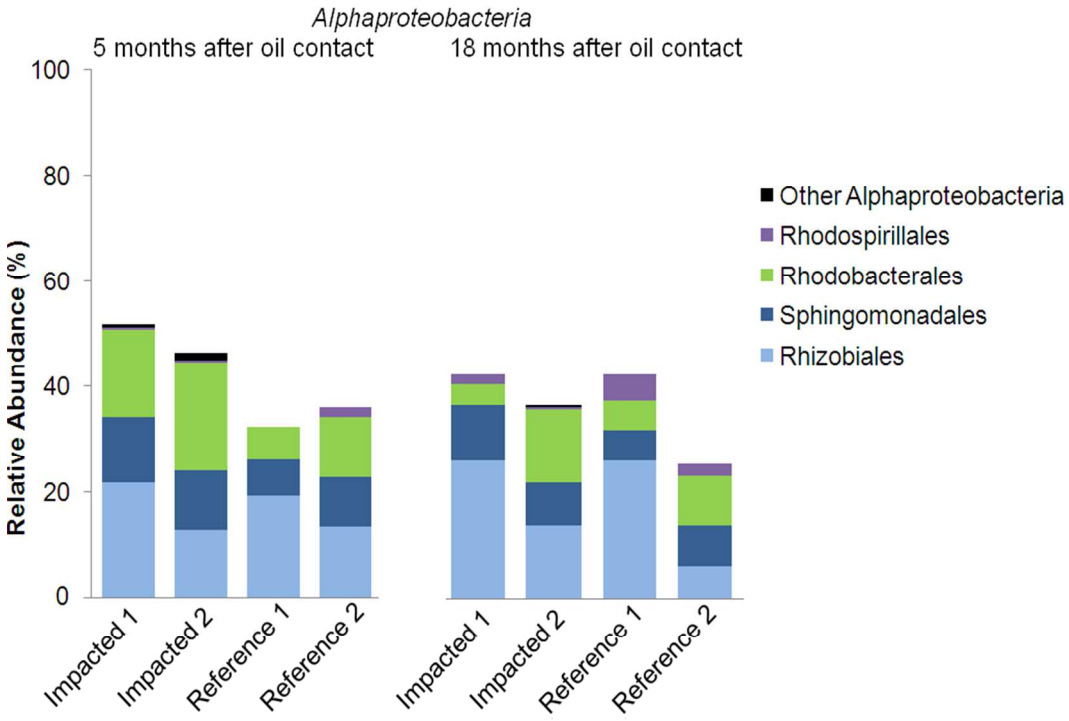

FIGURE S2. Relative frequency of OTUs classified to bacterial orders within the *Alphaproteobacteria* class at impacted and reference sites in Barataria Bay at 5 and 18 months after oil contact.

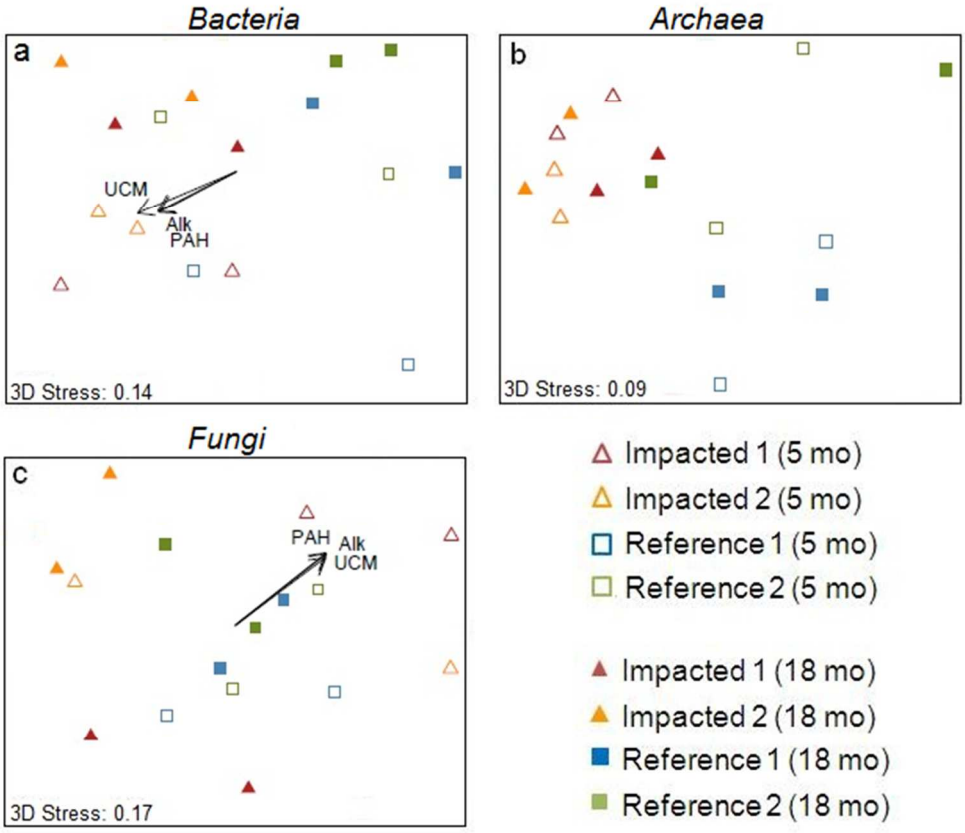

FIGURE S3. Non-metric multi-dimensional scaling (NMDS) ordination of (a) archaeal (b) bacterial and (c) fungal communities in sediments at 5 months (open symbols) and 18 months (closed symbols) after oil intrusion. Two data points shown for each sediment sample represent environmental replicates. Distance matrices were generated using Chi squared distances and is based on the relative frequency of OTU sequences taxonomically assigned using RDP + MEGAN (archaea and bacteria) or BLAST + MEGAN (fungi). Only statistically significant fitted ($p < 0.05$) hydrocarbon concentrations (alkanes, PAHs, UCM) are shown as vectors (arrows).

TABLE S1. Coordinates of impacted and reference sites sampled in Barataria Bay, Louisiana

Sites	Latitude	Longitude
Impacted 1	29º26’819”N	89º56’264”W
Impacted 2	29º26’196”N	89º54’681”W
Reference 1	29º24’535”N	89º59’239”W
Reference 2	29º13’402”N	90º00’443”W

Adapted from Silliman et al., 2012

Table S2. Sediment PAHs, alkane, UCM and PLFA concentrations (mg/kg) at 5, 11 and 18 months after initial oil contact at two impacted and two reference sites

Sites	Months following oil intrusion	Date of Sample Collection	TOC (%)	Total PAHs (mg/kg)	Total alkanes (mg/kg)	UCM (mg/kg)	Total PLFA (mg/kg)	Cells g^{-1}*
Impacted 1	5	Oct 2010	38.6	99.4	6987	50380	2.5	3.6E+08
Impacted 2	5	Oct 2010	14.7	16.2	1303	26465	2.4	3.3E+08
Reference 1	5	Oct 2010	4.4	1.5	17	18	5.7	8.2E+08
Reference 2	5	Oct 2010	3.9	1.1	52	280	25.1	3.6E+09
Impacted 1	11	Apr 2011	10.1	9.5	413	4673	2.9	4.1E+08
Impacted 2	11	Apr 2011	8.6	10.0	415	2875	1.4	2.1E+08
Reference 1	11	Apr 2011	5.1	1.5	19	51	6.2	9E+08
Reference 2	11	Apr 2011	5.1	1.0	25	41	10.8	1.6E+09
Impacted 1	18	Oct 2011	5.7	0.7	29	42	3.1	4.6E+08
Impacted 2	18	Oct 2011	6.9	0.8	19	282	3.7	5.3E+08
Reference 1	18	Oct 2011	8.2	0.4	20	43	3.2	4.6E+08
Reference 2	18	Oct 2011	5.3	0.4	14	24	8.4	1.2E+09

*cells per gram estimate was estimated from PLFA concentrations

TABLE S3. Distribution of PLFA classes expressed as mole percentage of the total

Sites	Months following oil intrusion	Monounsaturated FA	n-Saturated FA	Terminally branched saturated FA[1]	Mid branched saturated FA	Cyclopropyl FA[2]	Polyunsaturated FA
Impacted 1	5	28	49	8	16	0	0
Impacted 2	5	26	42	12	17	4	0
Reference 1	5	39	32	12	8	8	2
Reference 2	5	24	32	14	19	7	4
Impacted 1	11	29	28	14	14	12	3
Impacted 2	11	34	28	27	0	11	0
Reference 1	11	28	29	17	15	5	7
Reference 2	11	22	23	15	33	7	1
Impacted 1	18	21	33	16	21	7	1
Impacted 2	18	28	33	15	15	5	3
Reference 1	18	19	38	16	20	3	4
Reference 2	18	24	31	18	16	8	4

[1]iso- and anteiso- PLFA
[2]cyc 17:0 and cyc 19:0

TABLE S4. $\delta^{13}C$ and $\Delta^{14}C$ signatures of PLFA-rich fraction, Total Organic Carbon (TOC), Extracted-Residue (EXT-RES) at impacted and reference sites following oil intrusion

Sites	Months following oil intrusion	$\Delta^{14}C_{PLFA\text{-}rich}$ ± 20‰	$\Delta^{14}C_{TOC}$ ± 10‰	$\Delta^{14}C_{EXT\text{-}RES}$ ± 10‰	$\delta^{13}C_{PLFA\text{-}rich}$ ± 0.5‰	$\delta^{13}C_{TOC}$ ± 0.5‰	$\delta^{13}C_{EXT\text{-}RES}$ ± 0.5‰
Impacted 1	5	-882	-778	-142	-29.7	-25.2	-20.2
Impacted 2	5	-815	-625	-155	-29.5	-24.2	-21
Reference 1	5	-222	-127	-127	-26.2	-20.7	-19.9
Reference 2	5	-123	-94	-73	-28.2	-20.5	-16.5
Impacted 1	18	-181	-21	-7	-31.5	-21.2	-20.2
Impacted 2	18	-136	-132	-52	-28.7	-21	-18.4
Reference 1	18	-263	-156	-144	-27.2	-20.2	-20.2
Reference 2	18	-250	-69	-34	-29.2	-17.6	-19.1

TABLE S5. Comparison between $\delta^{13}C$ measurements of individual PLFAs (16:0 and 18:1) and bulk PLFA-rich fraction at two impacted and one reference site following oil intrusion

Sites	Months following oil intrusion	$\delta^{13}C_{PLFA\text{-}rich}$ ± 0.5‰	$\delta^{13}C_{16:0}$ ± 0.5	$\delta^{13}C_{18:1}$ ± 0.5	16:0 Mole percentage of total	18:1 Mole percentage of total
Impacted 1	5	-29.7	-28.0	-27.2	21	19
Impacted 2	5	-29.5	-27.2	-27.6	17	19
Reference 1	5	-26.2	-28.6	-24.9	18	19
Impacted 1	18	-31.5	-33.0	--	18	9
Impacted 2	18	-28.7	-30.3	-27.2	21	13
Reference 1	18	-27.2	-26.3	-24.5	18	5

-- could not be measured due to insufficient mass

TABLE S6. Estimated fraction of TOC made up of petroleum carbon based on isotopic mass balance using $\Delta^{14}C$ values

Sites	Months following oil intrusion	Date of Sample Collection	TOC (%)	Fraction of TOC made up of petroleum carbon (%)*
Impacted 1	5	Oct 2010	38.6	74
Impacted 2	5	Oct 2010	14.7	56
Reference 1	5	Oct 2010	4.4	0
Reference 2	5	Oct 2010	3.9	2
Impacted 1	18	Oct 2011	5.7	1
Impacted 2	18	Oct 2011	6.9	8
Reference 1	18	Oct 2011	8.2	1
Reference 2	18	Oct 2011	5.3	4

*as per eq (1)

TABLE S7. $\delta^{13}C$-depletion of PLFA-rich fraction relative to TOC, EXT-RES and BP crude oil

Months following oil intrusion	Sites	TOC	EXT-RES	BP crude oil (-27.2‰)
5	Impacted	4-6‰	8-10‰	2-3‰
5	Reference	5-8‰	6-12‰	0-1‰
18	Impacted	7-11‰	10-12‰	1-5‰
18	Reference	7-12‰	7-10‰	0-2‰

Total Lipid Extraction

$\Delta^{14}C$ of EXT-RES (residue remaining after solvent extraction) was used as a proxy for natural organic matter based on White et al., (2005), Ahad et al., (2010) and Mahmoudi et al., (2013). Petroleum hydrocarbons and solvent extractable organic materials were removed by carrying out a total lipid extraction (TLE). This was done by extracting approximately 5g of oven-dried sediment using 1:1 hexane:acetone along with a microwave accelerated reaction system (MARS, CEM Corporation). Subsequently, organic compounds extracted by solvent (referred to as TLEs) were filtered using burned glass fiber filters to remove sediment particles (GF/G, Whatman) and treated with activated copper to remove elemental sulfur. Residual sediment collected by filters (defined as EXT-RES) was decarbonated, analyzed for total organic carbon (% TOC) and sent for ^{14}C analysis.

PLFA extraction and analysis

Approximately 40 to 100g of freeze-dried sediment was extracted using a modified Bligh and Dyer method (White et al., 1979) as per Slater et al., (2005). Using 2:1 methanol/DCM, sediments were extracted and the resulting sediment/solvent mixture was centrifuged in solvent-rinsed centrifuge tubes (10 min, 2000 rpm). Following centrifugation, samples were filtered into separatory funnels using 0.45μm pre-combusted glass fiber filters (GF/G, Whatman). Nanopure water was added to separatory funnels in order to separate aqueous and organic phases. Subsequently, the organic phase was collected and separated into three fractions by gravity column chromatography using fully activated silica (precombusted at 450°C for 8 h) and dicholormethane (DCM), acetone and methanol to elute non-polar, neutral and polar fractions, respectively. The polar fraction which contained phospholipids was evaporated to to dryness under a stream of nitrogen gas and reacted to fatty acids methyl esters (FAMEs) via mild alkaline methanolysis reaction. Subsequently, FAMEs were further purified using a secondary silica gel step (hexane/DCM 4:1, DCM, methanol) and eluted in dichloromethane. Identification and quantification of FAMEs utilized an Agilent 6890 gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer (equipped with a 30 m x 0.25 mm DB-5 MS column). The temperature program for the GC oven was 40 ºC for 1 min, ramp to 130 at 20 ºC/min, to 160 at 4 ºC/min and then to 300 at 8 ºC/min, with a final hold time of 5 min. Lastly, FAMEs were identified using a bacterial reference standard (Bacterial Acid Methyl Esters CP, Mix, Matreya Inc), mass-fragmentation patterns and retention times and quantified using external calibration standards (which contained FAMEs of various chain length).

Microbial DNA and Bioinformatics Analysis

16S rRNA gene amplicons for bacteria were created using primers 27F-530R (27F: 5'-AGRGTTTGATCMTGGCTCAG-3'; 530R: 5'-CCGCNGCNGCTGGCAC-3') and Arch344F-Arch915R for archaea (Arch344F: 5'-ACGGGGYGCAGCAGGCGCGA-3'; Arch915R: 5'GTGCTCCCCCGCCAATTCCT-3') (Lane, 1991; Raskin et al., 1994). Bacterial and archaeal 16S rRNA gene amplicons were analyzed using a semi-automated custom pipeline as follows: Reads were sorted by barcode, zero mismatches allowed, and the barcodes were removed. Reads were checked for the presence of the forward primer, one mismatch allowed. Reads were quality trimmed using SeqTrim (Falgueras et al., 2010) using a sliding window size of 10bp, windows were discarded if the average Phred quality score was less than 20, and trimmed sequences were discarded if they were less than 80 bp in length. Trimmed sequences were dereplicated, 'denoised' by clustering at 99% sequence similarity, sorted by decreasing OTU frequency, clustered by 97% sequence similarity, then singletons were removed using USEARCH v 6.0.307 (Edgar, 2010 Bioinformatics 26: 2460-2461). A single representative 'centroid' sequence per operational taxonomic unit (OTU) was classified using a local installation of the Ribosomal Database Project classifier v. 2.5. Classified OTUs were imported into MEGAN using the following lowest common ancestor settings: min support=1, min score = 80 (i.e. 80% bootstrap support), top percent = 100, win score = 100, minimum complexity = 0.44. MEGAN uses a lowest common ancestor (LCA) algorithm to parse through taxonomic reports from the RDP classifier or BLAST to make assignments to variable taxonomic ranks by summarizing the taxonomic lineages associated with these hits.

Fungal ITS amplicons were created using primers ITS1F-ITS4 (ITS1F: 5'CTTGGTCATTTAGAGGAAGTAA; ITS4: 5'CCTCCGCTTATTGATATGC-3') (White et al., 1990; Gardes and Bruns, 1993). ITS amplicons were processed using the custom pipeline described above, except that after sequences were sorted by barcode, they were further processed using the Fungal ITS Extractor. The extractor was used to target fungal ITS sequences with the adjacent small and large subunit RNA gene sequences removed (Nilsson et al., 2010). Trimmed reads were clustered as described above. A single representative centroid sequences per OTU was subjected to BLAST 2.2.26+ (Altschul et al., 1997) searches using the blastn (megablast) algorithm against a local copy of the GenBank nucleotide database [April 2013] with an e-value cutoff of 1e-10. BLAST results were imported into MEGAN 4.70.4 (Huson et al., 2011) for automated taxonomic assignments. The parameters we used to define the best set of BLAST hits used by the LCA parser are as follows: minimum support = 1, minimum score = 50, top percent = 1, win score = 0.0, minimum complexity filter = 0.44. Since taxonomic assignments based on the ITS1 region only (70% of the raw data) resulted in 75% of sequences with no BLAST hits or no taxonomic assignment (data not shown), we focused our analyses on the sequences covering the ITS1-5.8S-ITS2 region (40% of the raw data) where nearly all the sequences were taxonomically assigned by MEGAN. To avoid the error associated with over-assigning our partial ITS sequences, we summarized taxonomic assignments to the class rank.

Distance matrices for the archaeal (order), bacterial (order), and fungal (class) community samples were created in MEGAN using normalized OTU counts (normalized to 100,000 OTUs per sample) and the Chi-squared distance metric. Non-metric

multidimensional scaling (NMDS) plots were calculated using the ecodist package in [R] using the default parameters with 3 dimensions and 1000 iterations (Goslee and Urban, 2007). Environmental variables were fitted to the ordinations using 1000 permutations.

References

Ahad, J. M. E., Burns, L., Mancini, S., Slater, G. F. (2010). Assessing Microbial Uptake of Petroleum Hydrocarbons in Groundwater Systems Using Natural Abundance Radiocarbon. *Environmental Science & Technology, 44*(13), 5092-5097.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation fo protein database search programs. *Nucleic Acids Research*, *25*(17), 3389-3402.

Falgueras, J., Lara, A.J., Fernandez-Pozo, N., Canton, F.R., Perez-Trabado, G., Claros, M.G. (2010) SeqTrim: a high-throughput pipeline for processing any type of sequence reads. *BMC Bioinformatics*,11: 38.

Gardes M., Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2, 113-118.

Goslee, S.C., Urban, D.L. (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, *22*(7), 1-19.

Huson, DH, Mitra, S, Weber, N, Ruscheweyh, H, and Schuster, SC (2011). Integrative analysis of environmental sequences using MEGAN4. *Genome Research*, *21*, 1552-1560.

Lane, D. J. (1991). 16S/23S rRNA Sequencing. In E. Stackebrandt, M. Goodfellow (Eds.), *Nucleic Acid Techniques in Bacterial Systematics* (pp. 115–175). New York: Wiley.

Mahmoudi, N., Fulthorpe, R. R., Burns, L., Mancini, S., Slater, G. F. (2013). Assessing microbial carbon sources and potential PAH degradation using natural abundance14C analysis. *Environmental Pollution, 175*, 125-130.

Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., Abarenkov, K. (2010) An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. *Fungal Ecology*, *3*, 284-287.

Raskin, L., Stromley, J. M., Rittmann, B. E., Stahl, D. A. (1994). Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Applied and Environmental Microbiology, 60*(4), 1232-1240.

Slater, G. F., White, H. K., Eglinton, T. I., Reddy, C. M. (2005). Determination of microbial carbon sources in petroleum contaminated sediments using molecular 14C analysis. *Environmental Science & Technology, 39*(8), 2552-2558.

Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology, 73*(16), 5261-5267.

White, T.J., Bruns, T., Lee, S., Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., eds. *PCR protocols*. San Diego, CA, USA: Academic Press, 315-322.

White, H. K., Reddy, C. M., Eglinton, T. I. (2005). Isotopic constraints on the fate of petroleum residues sequestered in salt marsh sediments. *Environmental Science & Technology, 39*(8), 2545-2551.