2016

Biodegradation of Highly Weathered Deepwater Horizon Oil

Joel T. Bostic
University of South Carolina

Biodegradation of Highly Weathered *Deepwater Horizon* Oil

by

Joel T. Bostic

Bachelor of Science
Western Carolina University, 2014

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Marine Science

College of Arts and Sciences

University of South Carolina

2016

Accepted by:

Lori Ziolkowski, Director of Thesis

Claudia Benitez-Nelson, Reader

Ron Benner, Reader

Paul Allen Miller, Vice Provost and Interim Dean of Graduate Studies

Acknowledgments

I would like to thank my advisor, Dr. Lori Ziolkowski. Dr. Z's guidance throughout the scientific process, patience to let my curiosity wander, and commitment to my success have been invaluable. Thank you to Dr. Chris Reddy for his insight and to Dr. Christoph Aeppli for oil fingerprinting and weathering analyses that greatly improve this project. Thank you to all the members of the Z-lab, past and present. Your friendship made the hundred of hours spent in the lab much more enjoyable. Last but not least I want to thank my wife, Emmely, and son, Remy. Remy, your smiles and giggly greetings when I get home are the highlights of my day. Emmely, thank you for your unwavering love and support, the past two years would not have been possible without you.

Abstract

Oil from the *Deepwater Horizon (DwH)* spill continues to be found on beaches five years post-spill. Understanding the role of microbes in removing *DwH* oil from sandy beaches is necessary to elucidate the fate of *DwH* oil in the coastal zone. Oiled sand patties and non-oiled sand were collected from affected beaches five years post-*DwH*. Biomarkers in oiled sand indicated that sand patties originated from the *DwH* spill, however the oil was heavily weathered and dominated by oxygenated hydrocarbons. Microbial communities, as measured by phospholipid fatty acids (PLFA), were present on oiled sand patties and distinct from microbes on non-oiled sand. Stable-carbon and radiocarbon measurements of PLFA show microbes on oiled sand patties were using different carbon sources than microbes on non-oiled sand. Radiocarbon measurements of bulk organic carbon and PLFA demonstrated that microbes on oiled sand patties were assimilating highly weathered *DwH* oil five years post-spill. These results are the first to show *in situ* biodegradation of highly weathered oil and have implications for removal of *DwH* oil continuing to wash ashore.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The *Deepwater Horizon (DwH)* oil spill was the largest accidental marine oil spill in history. An estimated 779 million liters of oil were released into the Gulf of Mexico beginning on April 20, 2010.[1] Large portions of spilled oil and gas were altered and removed through various physical, biological and mechanical processes. However, an estimated 10% of spilled oil formed a surface slick that oiled approximately 850 km of sandy beaches along the northern Gulf of Mexico coastline.[2,3] Oil deposited on beaches formed a variety of physical aggregates including tar balls, buried mats, and oiled sand patties.[4] While large aggregates of oil on coastlines were manually removed during initial cleanup efforts, oiled sand patties are still present today. One potential mechanism to remove remaining *DwH* oil is microbial degradation. However, little is known regarding microbial degradation of spilled oil on beach environments over extended times scales. Understanding the role of microbial degradation in oil's removal from coastal zones is critical to more accurately know the fate of *DwH* oil.

Oil released into the environment can be naturally altered and removed by a variety of physical, chemical, and biological processes, collectively referred to as weathering. One weathering process, microbial degradation, represents a major conduit for the removal of oil from the environment. Microbial degradation of oil is partially controlled by the chemical composition of spilled oil.[5] Spilled oil spans a wide range of chemical compositions, thus the spectrum of lability and recalcitrance for spilled oil varies. The chemical composition of crude oil is largely a function of precursor organic C

source (e.g. marine or terrestrial organic matter), surrounding lithology, age and the temperature and depth of formation.[6] Crude oil enriched in low molecular weight saturated compounds, such as saturated and branched alkanes, are readily degraded and considered labile. Conversely, crude oil enriched in high molecular weight polar compounds is not readily degraded and considered less labile.[7] Crude oil from the *DwH* spill was enriched in saturated and aromatic hydrocarbons[8] and was presumably initially labile. However, as the chemical composition of spilled oil is altered due to weathering processes, the lability or recalcitrance of spilled oil continually changes. *DwH* oil was chemically transformed in the environment, becoming dominated by oxygenated hydrocarbons.[8] To date, highly weathered, oxygenated hydrocarbon-rich *DwH* oil has been assumed to be recalcitrant, but this assumption has not yet been tested *in situ*.

Microbial degradation of *DwH* oil was detected in the Gulf of Mexico water column, salt marsh sediments, and beach sands up to 1 year post-spill.[9-12] In the water column, microbial degradation of *DwH* oil was demonstrated by increased cell abundance and decreased dissolved oxygen immediately after the spill.[9] In salt marsh sediments, microbes degraded *DwH* oil 5 months post-spill, but degradation ceased by 18 months post-spill.[10] In oiled beach sands, hydrocarbon-degrading microbes were identified 5 months post-spill using various genetic approaches,[11] but hydrocarbon-degraders were absent 1 year post-spill at the same beach.[12] While, hydrocarbon-degrading microbes have been found on oiled beach sands, it has yet to be established if those microbes are consuming DwH oil.

In situ degradation of oil can be detected using established organic geochemical methods. For example, elucidation of microbial carbon (C) sources is possible by

measuring carbon isotopes of microbial lipids. A commonly used class of microbial lipids in C source elucidation are Phospholipid fatty acids (PLFA).[10,13-20] PLFA are an integral component of cellular membranes and are thought to degrade days to weeks after cell death as the polar head group readily hydrolyzes.[21,22] Because PLFA readily degrade after cell death, they provide a snapshot of the viable microbial community at time of sampling. Carbon assimilated by microbes reflects the C isotope signature of the source, colloquially referred to as 'you are what you eat'.[23] C source elucidation, utilizing the 'you are what you eat' principle, has primarily been applied by measuring ^{13}C composition of microbial membranes.[19,24-26] But, when studying oiled environments, ^{13}C alone is not always sufficient to elucidate C sources utilized by microbes. Petroleum is formed from organic matter, thus modern organic matter and oil share similar ^{13}C compositions, complicating C source elucidation. Additionally, biological fractionation against heavier ^{13}C typically results in microbial lipids being depleted in ^{13}C to a given C source.[27-29] Overall, the similarity in ^{13}C composition of oil and modern organic matter coupled with biological fractionation makes it difficult to discern whether microbes are assimilating oil versus modern C. Thus, another method is needed to determine C sources used by microbes in oiled environments.

Radiocarbon measurements of microbial lipids provide greater resolution than ^{13}C for elucidating microbial C sources in oiled systems. Although oil originates from biological precursors, it forms on the order of millions of years. Oil has no measurable ^{14}C due to the short half-life of ^{14}C (~5730 years) relative to petroleum formation. Conversely, recently formed organic matter has measurable ^{14}C due to living organisms

continuously assimilating recently formed ^{14}C into biomass. Oil and modern organic matter are separated by a much larger range in ^{14}C (~1000‰) than ^{13}C (~0-10‰).[30,31] The greater range in ^{14}C allows for clearer interpretation between oil-derived and modern C sources used by microbes. Therefore, ^{14}C analysis of PLFA has proven to be an effective tool for tracking petroleum-derived C assimilation into microbial communities.[10,13-15,32]

The primary goals of this study were to establish the source of oil collected, measure chemical changes in oil collected relative to *DwH* source oil, and assess microbial degradation of oil. To address our goals, oiled sand patties and non-oiled sand were collected from three Gulf of Mexico beaches in July 2015, five years post-*DwH*. We show that collected sand patties are highly weathered *DwH* oil using established chromatographic methods. Additionally, we demonstrate that microbes are assimilating highly weathered *DwH* oil using compound-class isotope analysis of microbial biomarkers.

Chapter 2: Materials and Methods

Sample Location

Oiled sand patties and non-oiled sand were collected in July 2015 from Ship Island (SI), MS, Fort Morgan (FM), AL, and Fort Pickens (FP), FL (Figure 2.1). Samples were collected from both intertidal and supratidal locations. Intertidal (IT) regions are defined as the area between low and high tide while supratidal (ST) regions are the area between the high tide and dune line.[4] Samples were placed in pre-combusted glass containers, temporarily stored on ice for 1-3 days, then stored at -20 ºC until further analysis.

Oil Fingerprinting

Aliquots of oiled sand patties were extracted by shaking and centrifugation in 80:20 dichloromethane/methanol (DCM/MeOH).[8] Extracts were split and evaporated to a low volume under ultrahigh purity nitrogen (UHP N_2) before fingerprinting and weathering analyses. For oil fingerprinting analysis, biomarker ratios in oiled sand patties were compared to *DwH* source oil (NIST SRM 2779). Biomarker ratios were quantified by comprehensive two-dimensional gas chromatography (GC×GC) equipped with a flame ionization detector (FID) using established methods[33]. Briefly, 1 □L of extracted sample was injected into the GCxGC equipped with a Restek RXI-1 in the first dimension column (60 m, 0.25 mm i.d., 2.5 μm film thickness) and a SGE GPX in the second-dimension column (1.4 m, 0.1 mm i.d., 1 μm film thickness). Samples were

injected into the inlet held at 300 ºC in splitless mode. H_2 was the carrier gas at a flow rate of 1 mL min^{-1}. The first oven temperature program was held isothermal at 70 ºC from 10 min, before ramping at 2 ºC min^{-1} to 320 ºC (held for 15 min). The second oven was elevated 5 ºC but otherwise followed the temperature program of the first oven. The modulation period was 8 sec. Compounds were identified based on known elution order and mass spectra using authentic standards.[34,35] Names and chemical formulas of biomarker compounds are presented in Table S1.

Extent of Oil Weathering

The relative proportion of saturated, aromatic, and oxygenated hydrocarbons in oiled sand patties and crude *DwH* oil (NIST SRM 2779) extracts were quantified using thin layer chromatography-flame ionization detection (TLC-FID) (Iatroscan MK-5, Iatron Laboratories, Tokyo, Japan).[36,37] Methods used are described in detail elsewhere.[8] Briefly, a small volume (1-5 μL) of solvent extracted (80/20 DCM/MeOH) material was placed on a silica gel sintered glass rod (Chromarod S V, Iatron Laboratories) and developed in hexane (26 min development time), toluene (12 min), and DCM/MeOH 97/3 (5 min), successively. Prior to the next development, rods were dried for 1 min at 500 mbar and 70 ºC, then analyzed with FID using a 30 sec scan time with flows of 2 L min^{-1} air and 160 mL min^{-1} H_2. Three distinct peaks were observed which have been defined in order of decreasing retention as: saturated, aromatic, and oxygenated hydrocarbons.

PLFA Extraction

PLFA were extracted using a modified Bligh and Dyer method.[38] Various masses of oiled sand patties and non-oiled sand (10-80 g) were extracted in a 1:2:0.8 DCM/MeOH/H_2O mixture. Extracts were filtered into separatory funnels and augmented with DCM and H_2O to form a 1:1:0.9 DCM/MeOH/H_2O mixture and separate organic and aqueous phases. The organic phase, or total lipid extract (TLE), was collected and evaporated to a low volume under UHP N_2. The TLE was loaded onto fully activated silica gel in glass columns and separated into fractions of increasing polarity by elution with DCM, acetone, and MeOH. PLFA were eluted in the MeOH fraction. PLFA were dried and converted to fatty acid methyl esters (FAME) via mild alkaline methanolysis. Converting PLFA to FAME is necessary for separation and quantification using gas chromatography.[39] PLFA-derived FAME were further purified using secondary silica gel chromatography. For secondary silica gel, samples were loaded onto fully activated silica gel and eluted with 4:1 hexane/DCM, DCM, and MeOH. Secondary silica gel was repeated for selected samples (denoted with a plus (+) sign) to remove oxygenated oil-derived compounds contained within the FAME fraction. FAME were identified and quantified using an Agilent 7890B/5977A GC-MS with a DB-5MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with helium as the carrier gas. The temperature program began at 50 ^oC, ramped 20 $^oC\ min^{-1}$ to 130, then ramped 4 $^oC\ min^{-1}$ and held isothermal at 160 ^oC (5 min), ramped up 8 $^oC\ min^{-1}$ to 300 (held for 5 min). FAME were identified using mass fragmentation patterns and retention times relative to commercially available standards (Supelco 37 Component FAME Mix). PLFA-derived FAME were quantified using external standards. Quantified PLFA-derived FAME are expressed normalized to

mass of sand used (PLFA μg g^{-1} sand) and relative abundance of PLFA in a given sample (mole percentage). Cell abundances were calculated using a conversion factor of 10^4 cell $pmol^{-1}$ PLFA.[25] Laboratory blanks were regularly analyzed to assess contamination.

PLFA nomenclature uses the following convention: C, denoting the C chain of PLFA, followed by subscripts, denoting the length and structure of the PLFA C chain. For example, $C_{16:2}$ represents a PLFA with a 16 C atom chain and two double bonds while C_{br15} represents a PLFA with a 15 C atom chain and a branched structure. Numbers in subscripts indicate C chain length and number of double bonds. Letters in subscripts following C indicate structure of C chain, such as cyclic (*cyc*) or branched (*br*) structures. PLFA were grouped into the following structural categories: saturated, monounsaturated, polyunsaturated, branched, cyclic, unknown PLFA, and other PLFA. Double bond and branch positions of C chains were not identified as this additional structural information was beyond the scope of this work. Additionally, acquisition of specific structural information requires repeated GC injections of each sample, which is not recommended for samples prior to ^{14}C analysis.

Statistical analysis of PLFA

Cluster analysis, a descriptive statistical technique, was used to assess differences between the relative distribution of PLFA in oiled and non-oiled sand, sampling locations, and beach setting (intertidal vs. supratidal). Specifically, hierarchical cluster analysis compared the mole percentage (mol%) of 110 distinct PLFA observed in eight samples. The average space between clusters was measured using the average Euclidean

distance. An average distance of 100 represents complete dissimilarity between samples or clusters while an average distance of 0 represents identical samples or clusters.

PLFA Carbon Isotope measurements

The entire fraction containing PLFA (ΣPLFA) was analyzed for ^{13}C and ^{14}C. Individual PLFA were not isolated due to the mass needed for ^{14}C measurements and due to the fact that individual PLFA extracted from oiled samples have shown very small variation in both ^{13}C and ^{14}C.[10,13,40] After GC-MS analysis, ΣPLFA extracts were dried under UHP N_2 in pre-combusted quartz tubes containing ~100 mg of copper oxide and silver wire. ΣPLFA in quartz tubes were evacuated on a vacuum line, then flame sealed and combusted at 900 ºC for 4 hours. Evolved CO_2 was purified from water and other combustion gases using cooling traps and quantified manometrically. The isotopic composition of bulk oiled sand patties and ΣPLFA were measured at the Center for Applied Isotope Studies at the University of Georgia. An aliquot of CO_2 was analyzed for ^{13}C on a dual inlet Finnigan MAT 252 Isotope Ratio Mass Spectrometer. The remaining CO_2 was reduced to graphite and analyzed for ^{14}C using accelerator mass spectrometry (AMS). ^{14}C values were normalized to $\delta^{13}C$ values of -25‰ to correct for isotope fractionation and presented as $\Delta^{14}C$ (‰) relative to the standard Oxalic Acid I (NBS SRM 4990).[41] $\Delta^{14}C$ is the deviation between sample and the standard Oxalic Acid I (NBS SRM 4990). Fossil derived C sources have no detectable ^{14}C ($\Delta^{14}C$ = -1000‰), while recently fixed atmospheric carbon is modern C ($\Delta^{14}C$ = +40‰). The isotopic values of ΣPLFA were corrected for the carbon added to PLFA during methanolysis. Corrections were made using mass balance by calculating the average PLFA chain length and

assuming C added from methanolysis was fossil derived (SI Text). Uncertainty of $\Delta^{14}C$ was ± 10‰ for bulk oiled sand patties and ± 20‰ for PLFA, while uncertainty of $\delta^{13}C$ was ± 0.5‰ or the variation among replicates, whichever was greater.

Correction of isotope values for oil influence

Highly weathered oil and PLFA share similar chemical properties. Due to their similar chemical nature, isolating PLFA from the oil matrix was impossible (SI Text). To correct for the isotope influence of oil in ΣPLFA fractions, isotope mass balance was used. The following isotope mass balance equations were used to constrain the isotope value of PLFA:

(1) $f_{PLFA}+f_{oil}=1$

(2) $\Delta^{14}C_{sample}=\Delta^{14}C_{\Sigma PLFA}* f_{PLFA}+\Delta^{14}C_{oil}* f_{oil}$

To constrain the isotope value of PLFA ($\Delta^{14}C_{\Sigma PLFA}$), the isotope value the sample analyzed ($\Delta^{14}C_{sample}$) and the relative mass contributions of oil (f_{oil}) and PLFA (f_{PLFA}) to the sample must be known. Equations 1 and 2 were also used to calculate $\delta^{13}C_{PLFA}$.

The mass contribution from oil (f_{oil}) to PLFA fractions was calculated two ways (Tables S6). First, the ratio of non-PLFA peak area to total peak area (PLFA + non-PLFA peak area) was calculated. Second, total petroleum hydrocarbons were quantified by integrating blank-corrected non-PLFA peaks using a response factor from *n*-alkane standards. $\Delta^{14}C_{sample}$ was the reported AMS value and $\Delta^{14}C_{oil}$ is assumed to be -1000‰. Error of $\Delta^{14}C_{\Sigma PLFA}$ was determined by adjusting f_{oil} by ± 0.1.

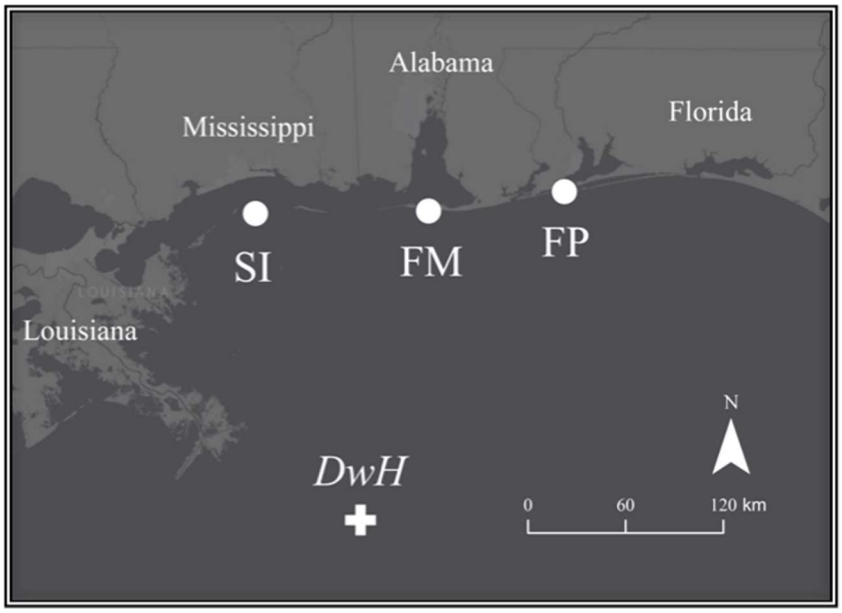

Figure 2.1: Oiled sand patty sample locations in relation to *DwH* drilling platform (SI=Ship Island, Mississippi; FM=Fort Morgan, Alabama; FP=Fort Pickens, Florida).

Chapter 3: Results

Oil Fingerprinting

Ratios of recalcitrant biomarker compounds in oiled sand patties are consistent with *DwH* oil (Table 3.1). Biomarker compounds analyzed for fingerprinting included hopanoids such as 17α(H),21β(H)-hopane (H) and 17α(H),21β(H)-30-norhopane (NH) (Table S1). Biomarker ratios from oiled sand are within ±0.1 of *DwH* oil, with one exception. The ratio of 17α(H),21β(H)-22R-tetrakishomohopane/ 17α(H),21β(H)-22S-tetrakishomohopane (4HH(R)/4HH(S)) deviates more than ±0.1 relative to *DwH* oil.

Oil Weathering

Oil in sand patties was chemically altered from wellhead release to sample collection (Figure 3.1). The chemical composition of *DwH* crude oil was dominated by saturated (50%) and aromatic (34%) hydrocarbons, with a lesser abundance of oxygenated hydrocarbons (16%). Conversely, the chemical composition of oiled sand patties was dominated by oxygenated hydrocarbons (60%), with a reduced proportion of saturated (29%) and aromatic (11%) hydrocarbons. All oiled sand patties were composed of >50% oxygenated hydrocarbons, despite some variability in the composition of oiled sand patty samples (Table S2). An increase in oxygenated hydrocarbons is consistent with previous measurements of *DwH* oil in the marine and coastal environment.[8]

PLFA type and distribution

PLFA concentration and cell abundance are presented in Table 3.2. PLFA concentrations ranged from 5.4-10.4 μg PLFA g^{-1} in oiled samples and 4.3-6.7 μg PLFA g^{-1} in non-oiled sand. The highest PFLA concentrations were found in oiled IT sand. PLFA concentrations were used to calculate cell abundances.[25] Cell abundances ranged from 6.4-13.0×10^8 cells g^{-1} in oiled sand and 4.5-7.0×10^8 cells g^{-1} in non-oiled sand. Calculated cell abundances are similar to previous studies of microbial communities using a fossil C source.[32]

The relative distribution of PLFA are shown in Figure 3.2. Clear differences in PLFA distribution exist between oiled and non-oiled samples. Branched and unknown PLFA were more abundant in oiled samples, 33 mol%, than non-oiled samples, 10 mol%. Mono- and poly-unsaturated PLFA were more abundant in non-oiled samples, 57 mol%, than oiled samples, 27 mol%. The grouping "Other PLFA" was chosen to classify a branched and unsaturated PLFA and this group only appeared in non-oiled sand samples. The distribution of PLFA in oiled sand patties varied with where in the tidal zone oiled sand patties were collected. Oiled sand patties from IT settings had a greater abundance of saturated and unsaturated PLFA while oiled sand patties from ST settings had a greater abundance of unknown PLFA. Both oiled sand and non-oiled sand samples had similar fractions of saturated PLFA. The most abundant PLFA from all samples were 16:0, 16:1, 18:1, or 18:2; all of which are ubiquitous PLFA appearing in both algae and bacteria (Table S2).[20] Oiled samples also contained non-PLFA peaks, which were not present in non-oiled samples or method blanks. Non-PLFA peaks were likely highly oxygenated oil-derived compounds formed during oil weathering.[8] Oxygenated hydrocarbons are

chemically similar to PLFA, thus complete separation of PLFA and oxygenated hydrocarbons proved challenging.

Cluster analysis of PLFA is presented in Figure 3.3. The greatest distance between clusters, ~30, is between clusters containing all oiled samples and all non-oiled samples. The large distance between oiled and non-oiled clusters suggests possible differences in microbial composition in oiled and non-oiled sand. The second greatest distance between clusters, ~15, is between clusters containing all oiled IT and all oiled ST samples. The distance between oiled IT and oiled ST clusters suggest microbial communities differ more between intertidal setting (ST or IT) than sample location (SI, FM, or FP).

PLFA isotope data

Stable isotope data (^{13}C)

The ^{13}C composition of both bulk oiled sand patties and the ΣPLFA from oiled and non-oiled sand are shown in Figure 3.4. Bulk oiled sand patties had an average $\delta^{13}C$ value of -26.8 ± 0.5‰ (n=4), consistent with both *DwH* oil (-27.3 ±0.3‰)[42] and terrestrial organic matter from C_3 vegetation (-25 to -29‰).[30] ΣPLFA in oiled sand patties had an average $\delta^{13}C$ of -27.3 ± 0.5‰ (n=6), which is within the uncertainty range of the bulk oiled sand patties. Non-oiled sand had an average $\delta^{13}C_{\Sigma PLFA}$ of -22.1 ±0.7‰ (n=2), which is significantly different than the oiled sand.

Radiocarbon isotope data (^{14}C)

The ^{14}C composition of bulk oiled sand patties and ΣPLFA from oiled and non-oiled sand are shown in Figure 3.5. Bulk oiled sand patties had an average $\Delta^{14}C$ value of -982 ± 20‰ (n=4). This value is consistent with the $\Delta^{14}C$ of oil (-1000‰), which indicates the primary source of carbon (C) in oiled sand patties was fossil in origin. ΣPLFA in oiled sand patties had an average corrected $\Delta^{14}C$ value of -921 ±41‰ (n=6), indicating microbes are assimilating a ^{14}C-free source of C. Microbial lipids in non-oiled sand had an average $\Delta^{14}C_{\Sigma PLFA}$ value of 35 ±20‰ (n=2), indicating microbes in non-oiled sand are assimilating a modern C source.

Table 3.1: Biomarker ratios of source oil (*DwH*) and oiled sand patties collected. Full biomarker compound names are presented in Table S1.

Biomarkers	***DwH***	**SI ST**	**FM ST**	**FM IT**	**FP IT**
Ts/Tm	1.6	1.5	1.4	1.4	1.4
Ts/H	0.25	0.25	0.25	0.25	0.28
NH/H	0.51	0.54	0.56	0.58	0.58
M/H	0.10	0.10	0.11	0.11	0.11
M/NM	1.7	1.8	1.7	1.6	1.6
HH(R)/HH(S)	0.72	0.70	0.72	0.74	0.72
2HH(S)/H	0.35	0.35	0.37	0.38	0.36
4HH(R)/4HH(S)	0.84	0.73	0.69	0.57	0.66
4HH(R+S)/H	0.26	0.28	0.25	0.29	0.28

Table 3.2: PLFA concentrations and cell abundances from oiled sand patties and non-oiled sand. A conversion factor of $2x10^4$ cells $pmol^{-1}$ PLFA[25] was used to calculate cell abundance. SI=Ship Island, FM=Fort Morgan, FP=Fort Pickens, ST=Supratidal, IT=Intertidal, + denotes samples with additional purification step.

Oiled Samples	**µg PLFA g^{-1} sand**	**Cells g^{-1} (x 10^8)**
SI ST	5.85	6.5
SI ST+	7.56	8.3
FM ST	5.4	6.4
FM ST+	6.95	8.3
FM IT+	10.4	13.0
FP IT	7.6	10.1
Non-Oiled Samples		
FM IT	6.7	7.0
FP IT	4.3	4.5

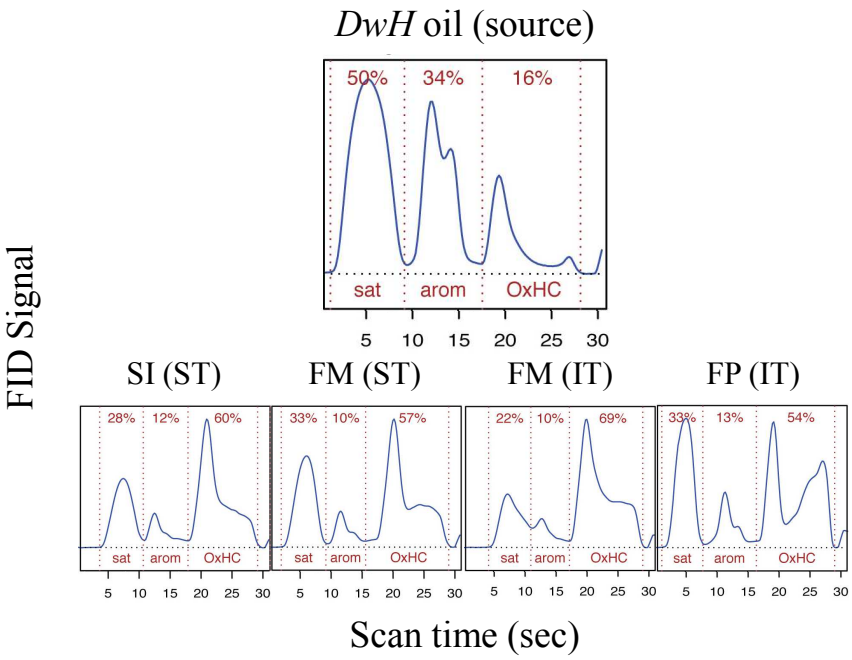

Figure 3.1: TLC-FID traces comparing source (*DwH* oil) to oiled sand patties collected 5 years later. Saturated and aromatic hydrocarbons have been removed due to various weathering processes while oxygenated hydrocarbons have increased in the five years post-spill.

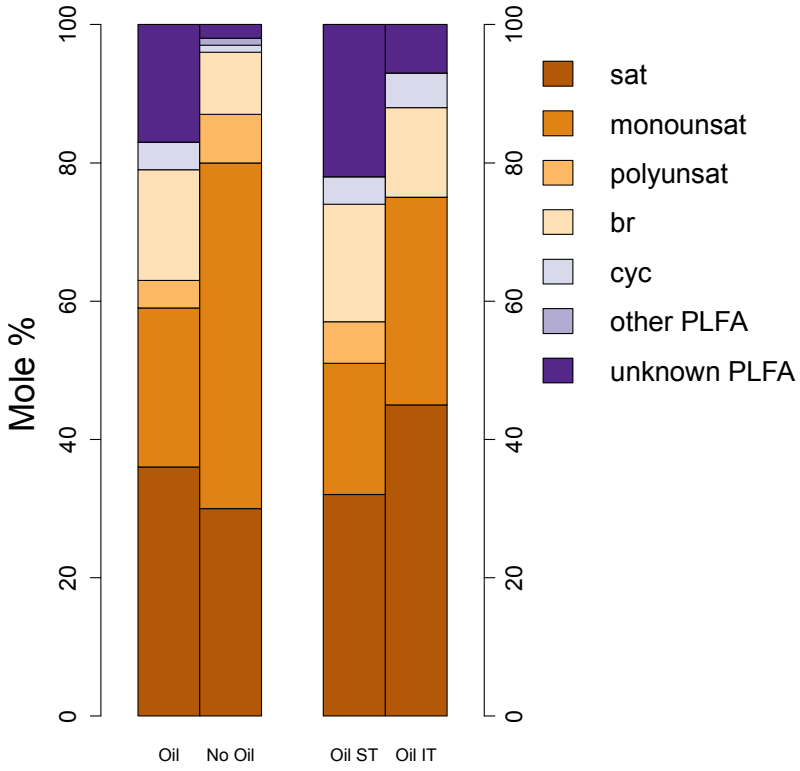

Figure 3.2: Mol% of PLFA structure between oiled and non-oiled sand and between oiled sand patties collected in intertidal (IT) relative to supratidal (ST) settings.

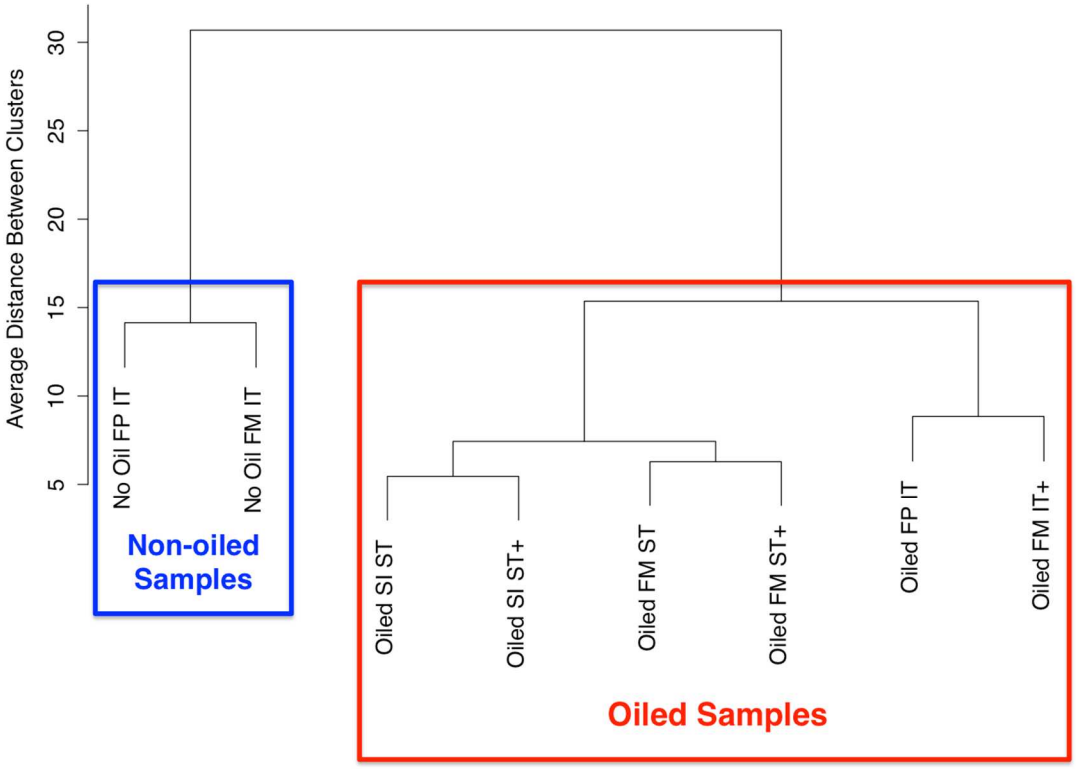

Figure 3.3: Dendrogram from hierarchical cluster analysis of 110 different PLFA from 8 samples. PLFA from oiled samples (red box) show greatest similarity, clustering together while non-oiled PLFA (blue box) form separate clusters.

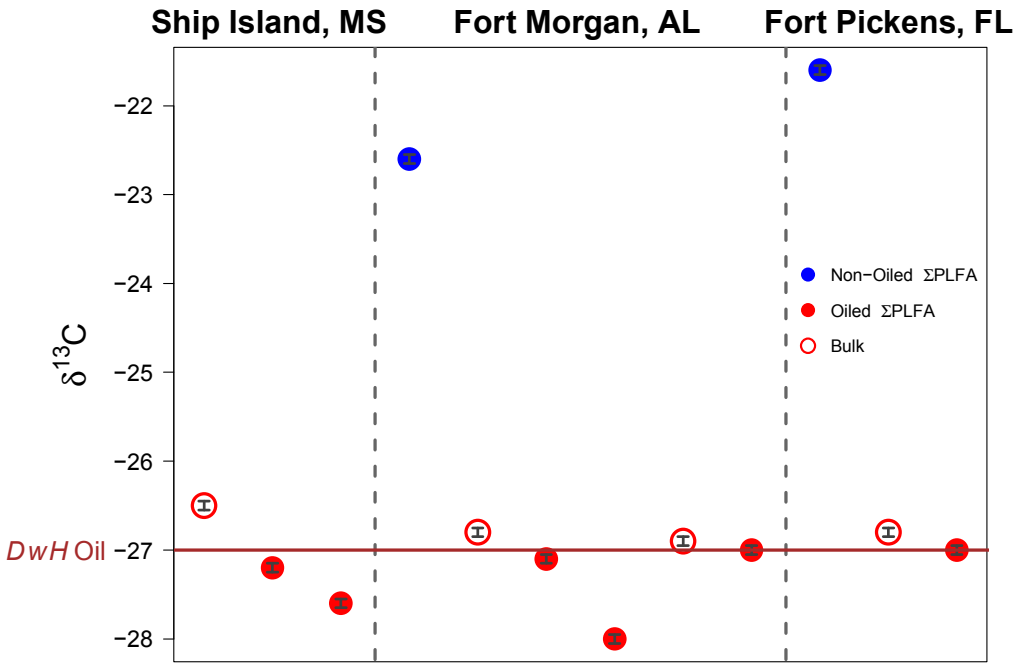

Figure 3.4: $\delta^{13}C$ of bulk oiled sand (unfilled red circles), non-oiled ΣPLFA (filled blue circles), and oiled ΣPLFA (filled red circles). Bulk oiled samples and ΣPLFA from oiled sand were consistent with *DwH* $\delta^{13}C$ (-27‰).[42] Error bars (±0.1‰) are smaller than data points.

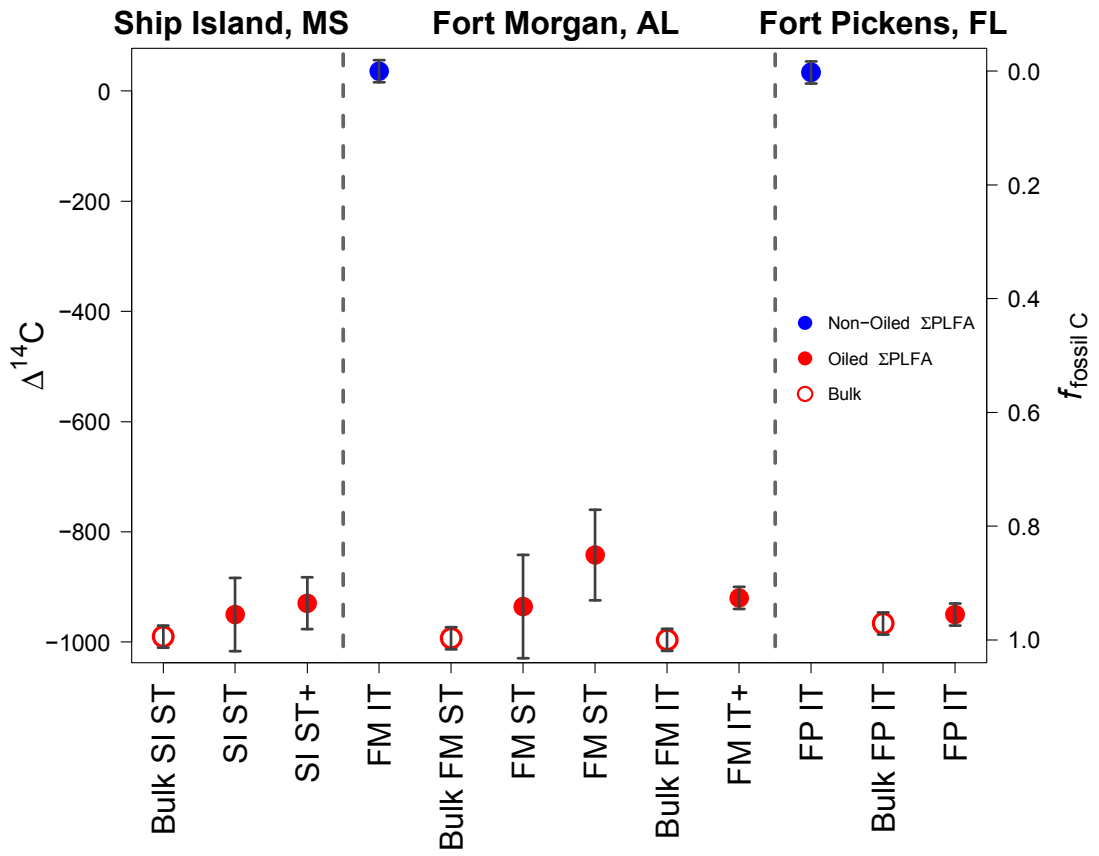

Figure 3.5: $\Delta^{14}C$ and $f_{fossil\ C}$ for bulk oiled sand patties (unfilled red circles), ΣPLFA from oiled sand (filled red circles), and ΣPLFA from non-oiled sand (filled blue circles). ΣPLFA $\Delta^{14}C$ indicates microbes present on oiled sand patties are assimilating a fossil source of C while microbes on non-oiled sand are assimilating a modern source of C.

Chapter 4: Discussion and Conclusion

Oiled sand patties collected, as indicated by their chemical signatures, are likely from the *DwH* spill. In the five-plus years since the spill, weathering processes have transformed the chemical composition of the oil. These chemical transformations include both the removal of labile components and the formation of oxygenated hydrocarbons. The depleted radiocarbon signature of microbial lipids in oiled sand patties demonstrates that microbes are assimilating oil into their biomass. Microbial assimilation of oil indicates ongoing degradation of *DwH* oil. All together, these results provide evidence that microbes are able to degrade highly weathered *DwH* oil.

Who is there?

The distribution of microbial lipids suggests different communities exist in oiled and non-oiled sand. Differences in the microbial community composition can be determined by comparing both specific PLFA and general PLFA structures.[43] Specific PLFA structures are classified using both C bond configurations (e.g. *sat* or *br*) and C chain length (e.g. C_{16} or C_{18}). General PLFA structures are classified using only C bond configurations. Broad differences in microbial communities are evident by the distribution of general PLFA structures. Microbes in oiled sand had a higher proportion of branched and cyclic PLFA while microbes in non-oiled sand had a higher proportion of unsaturated PLFA (Figure 3.2). More specifically, non-oiled sand contained an algal-specific PLFA, $C_{20:5}$ [18,39], that was not present in oiled sand (Table S3). Further,

polyunsaturated microbial lipids, which can indicate algae, were not present in oiled IT sand.[18,39] The lack of algal-specific lipids in oiled IT sand is somewhat surprising for samples collected in the IT zone, as algae are expected to be pervasive in the IT zone. Unknown PLFA were more abundant in oiled sand patties than non-oiled sand. Unknown PLFA were most abundant around low to medium molecular weight lipids, primarily eluting between $C_{8:0}$ and $C_{15:0}$ (Table S3). Unknown PLFA peaks were classified as PLFA using characteristic masses of PLFA-derived FAME as measured by GC-MS. Characteristic masses of FAME used include m/z 74, the McLafferty rearrangement ion,[44] m/z 87, and m/z 143.[45] Additionally, differences in microbial communities between oiled and non-oiled sand were illustrated with cluster analysis (Figure 3.3). Cluster analysis also revealed smaller differences between microbes in oiled IT and ST sand. All samples had high proportions of ubiquitous, non-specific PLFA $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$, which are representative of the entire microbial community.[20] Oiled sand contained a slightly greater concentration of microbial lipids and therefore cells (Table 3.2), suggesting oiled sand patties were able to support a greater population of microbes than non-oiled sand. Heterotrophic specific PLFA (e.g. $C_{15:0}$, C_{br15}, $C_{17:0}$)[11,13,46], were present in both oiled and non-oiled sand, indicating heterotrophy was likely occurring in all samples extracted. Overall, the distribution of microbial lipids shows algal-specific microbes in non-oiled sand and a heterotrophic microbial presence in both oiled and non-oiled sand.

Microbes are eating fossil C

Radiocarbon values of microbial lipids indicated that microbes in oiled sand were assimilating different C sources than microbes in non-oiled sand. Bulk organic C of oiled sand patties had radiocarbon values that ranged from -966 to -996‰ (n=6), consistent with previous ^{14}C measurements of oiled sand patties.[8] Therefore, in oiled sand patties, C sources available for heterotrophic use (bulk organic C) were primarily fossil C.For all ^{14}C-measured samples, we calculated the fraction of fossil C ($f_{\text{fossil C}}$) using end-member values of oil C ($\Delta^{14}C$= -1000‰) and modern C ($\Delta^{14}C$= ~40‰). The $f_{\text{fossil C}}$ for oiled sand patties ranged from 0.97-1, suggesting that nearly all organic C in oiled sand patties is devoid of ^{14}C and likely oil-derived. Microbial lipids in oiled sand patties were all highly depleted in ^{14}C ($\Delta^{14}C_{\Sigma PLFA}$= -842‰ to -950‰). For microbial lipids to be this depleted in ^{14}C, two explanations are possible. First, microbial lipids on oiled sand patties were relicts and $\Delta^{14}C$ reflects the age of lipids. However, PLFA, the microbial lipids measured, hydrolyze days to weeks after cell death.[21,22] Thus, microbial lipids were not likely to be relict features. Second, microbes assimilated fossil C from oiled sand patties into lipids. Microbial lipids of heterotrophic microbes have C isotope values that reflect the C source assimilated, e.g. 'you are what you eat'.[23] Microbial assimilation of fossil C from oiled sand patties is the likely explanation. Using the same end-member values of oil and modern C above, the $f_{\text{fossil C}}$ for microbial lipids from oiled sand ranged from 0.85-0.95. In samples that underwent additional purification to remove oil-derived C (indicated by + in figures), microbial lipids still contained a high fraction of fossil C ($f_{\text{fossil C}}$ = 0.85-0.93). In contrast, microbial lipids from non-oiled sand contained modern values of ^{14}C ($\Delta^{14}C_{\Sigma PLFA}$ = 34-36‰), which corresponds to negligible assimilation of fossil C ($f_{\text{fossil C}}$ of

0.0-0.1). Therefore, radiocarbon measurements clearly show that microbes in oiled sand patties are assimilating primarily deplete C (e.g. oil), while microbes in non-oiled sand are using modern C.

Stable carbon isotopes of microbial lipids suggest that microbes in oiled sand use different C sources than microbes in non-oiled sands, providing additional support to ^{14}C measurements of microbial lipids. Microbial lipids from oiled sand had $\delta^{13}C$ values depleted by ~5‰ relative to non-oiled sand (Figure 3.4). This ~5‰ depletion in $\delta^{13}C$ suggests microbes on oiled sand patties are assimilating a separate C source from microbes on non-oiled sand. The observed differences in $\delta^{13}C$ are notable, even though $\delta^{13}C$ has a smaller dynamic range than $\Delta^{14}C$, as they suggest different carbon sources assimilated by microbes. The $\delta^{13}C$ values of oiled sand patties (-26.8 ±0.5 ‰, n = 4) were similar to terrestrial derived organic matter (-25 to -29‰)[30] and *DwH* oil (-27.3 ±0.3‰).[42] Considering both $\delta^{13}C$ and $\Delta^{14}C$ values of microbial lipids, oiled sand patties likely reflect an oil-derived source rather than relatively modern terrestrial organic matter. Biological fractionation typically results in lipids depleted in ^{13}C relative to the C source, with the magnitude of fractionation related to metabolic pathways.[29] Autotrophic microbes fixing CO_2 are likely less depleted in ^{13}C than heterotrophic microbes assimilating highly ^{13}C-depleted oil. Microbial lipids on non-oiled sand were less depleted in ^{13}C, suggesting that an autotrophic microbial processes dominate over heterotrophic processes. Additionally, an autotrophic presence on non-oiled sand was supported by the presence of algal specific PLFA found only in non-oiled sand. Biological fractionation requires cautious interpretation of ^{13}C data when attempting to apportion C sources. Microbes on oiled sand patties and non-oiled sand using different C

sources is supported by differences in both $\delta^{13}C_{\Sigma PLFA}$ and $\Delta^{14}C_{\Sigma PLFA}$. Isotopic analyses of microbial lipids clearly demonstrated that microbes on oiled sand patties were consuming oil, however further analysis was required to determine the source of the oil.

What is source of oil?

Similar biomarker ratios between *DwH* oil and oiled sand patties strongly suggest that oiled sand patties originated from the *DwH* spill (Table 3.1). Ratios of recalcitrant biomarker compounds are used to establish sources of oil spills. Biomarkers commonly used include: triaromatic steroids, steranes, and hopanoids.[33] Ratios of hopanoids were measured, which are considered recalcitrant to degradation.[33,47,48] Additionally, the recalcitrance of hopanoids with less than 32 C atoms has been demonstrated over year-plus time scales with *DwH* oil.[33] Biomarker ratios of hopanoids in oiled sand patties are remarkably similar to *DwH* oil. The similarity of hopanoid ratios between oiled sand patties and *DwH* oil indicate hopanoids are recalcitrant for five-plus years in marine and beach environments. The average ratios of hopanoids between *DwH* oil and other crude oils from the Gulf of Mexico deviate by approximately 29%.[33] This large deviation between various oils in the Gulf of Mexico further supports oiled sand patties originated from *DwH*. Ratios of hopanoids with greater than 32 C atoms (homohopanes 4HH(R) and 4HH(S)) deviate between *DwH* oil and oiled sand patties (Table 3.1, Table S1). Preferential degradation of higher molecular weight homohopanes has been demonstrated previously and is attributed to biodegradation.[49,50]

Extent of oil weathering

Oil from sand patties was dominated by oxygenated hydrocarbons (Figure 3.1). Oxygenated hydrocarbons were created post-*DwH* spill by photo-oxidation and biodegradation.[8,51] Oxygenated hydrocarbons (or similar high boiling point, polar compounds) are assumed to be recalcitrant to further biodegradation,[8,52-54] with one incubation study suggesting otherwise.[55] However, our results show microbial degradation of oxygenated hydrocarbon-rich oiled sand patties. Microbial degradation of oiled sand patties suggests highly weathered oil is not recalcitrant.

Implications

Our measurements are the first to demonstrate *in situ* microbial degradation of highly weathered oil over extended time scales. *In situ* microbial degradation of oil has been documented immediately and up to one year following spills,[10,15] but ceased or was severely limited after one year.[10,14,13] However, previous studies of *in-situ* oil biodegradation were primarily conducted in salt marsh sediments, which are typically anoxic with abundant sources of natural organic matter. Thus, salt marsh sediments represent a near opposite environment from oxic, organic matter poor beach sands. Additionally, our results are unique due to the high proportion of oil-derived C ($f_{\text{fossil C}}$ >0.8) comprising microbial lipids. The high fraction of fossil C comprising microbial lipids is likely due to *DwH* oil representing the primary C source available on beach sands. Microbial degradation of *DwH* oil five-plus years after the spill implies that weathering processes have not made oil less amenable to degradation.

Microbial degradation of highly weathered *DwH* oil has implications for oil continuing to wash ashore. Oiled sand patties continuing to be collected on affected beaches are likely from offshore submerged oil mats.[56] As submerged oil mats are reworked by coastal processes, oil deposition on beaches is likely to continue for several years into the future.[57] Our results demonstrate that microbial degradation is a viable removal mechanism for oil continuing to be deposited on Gulf Coast beaches.

Microbes on Gulf Coast beaches are degrading *DwH* oil, but it is unclear who is degrading oil and how quickly oil is degraded. Future research should aim to constrain what microbial populations are degrading oil. Microbes capable of hydrocarbon degradation (*Gamma- and Alphaproteobacteria*) increased in abundance on affected beaches in the months post-spill.[12] No genetic-based studies have been conducted on oiled sand patties beyond one year post-*DwH*. Discovery of microbes capable of degrading highly weathered oil has implications for potential bioremediation of future oil spills. Additionally, compound-specific ^{14}C analyses of microbial lipids can provide insight into specific lipids that are depleted in ^{14}C and thus associated with oil degradation.[11,14,15] If specific ^{14}C-depleted lipids are known, inferences can be made regarding responsible oil-degrading microbes. Lastly, establishing degradation rates of remaining *DwH* oil on sandy beaches will allow for better estimates of when affected beaches will return to pre-*DwH* conditions.

Our results show microbes on Gulf Coast beaches are degrading oil, demonstrated by ^{14}C analysis of microbial lipids. Oil being degraded is most likely from the *DwH* spill and is heavily weathered after 5+ years in the environment. Microbial degradation of heavily weathered oil represents a loss term for *DwH* oil continuing to wash ashore Gulf

Coast beaches. Our results, showing microbial degradation of *DwH* oil, further elucidate the fate of *DwH* oil in coastal environments.

References

(1) Reddy, C. M.; Arey, J. S.; Seewald, J. S.; Sylva, S. P.; Lemkau, K. L.; Nelson, R. K.; Carmichael, C. A.; McIntyre, C. P.; Fenwick, J.; Ventura, G. T.; et al. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (50), 20229–20234.

(2) Ryerson, T. B.; Aikin, K. C.; Angevine, W. M.; Atlas, E. L.; Blake, D. R.; Brock, C. A.; Fehsenfeld, F. C.; Gao, R. S.; De Gouw, J. A.; Fahey, D. W.; et al. Atmospheric emissions from the deepwater Horizon spill constrain air-water partitioning, hydrocarbon fate, and leak rate. *Geophys. Res. Lett.* **2011**, *38* (7), 6–11.

(3) Michel, J.; Owens, E. H.; Zengel, S.; Graham, A.; Nixon, Z.; Allard, T.; Holton, W.; Reimer, P. D.; Lamarche, A.; White, M.; et al. Extent and Degree of Shoreline Oiling: Deepwater Horizon Oil Spill, Gulf of Mexico, USA. *PLoS One* **2013**, *8* (6), 1–9.

(4) Operational Science Advisory Team (OSAT). *Gulf Coast Indicent Manag. Team* **2011**, 1–36.

(5) Atlas, R. M. M. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **1981**, *45* (1), 180–209.

(6) Tissot, B. P.; Welte, D. H. *Petroleum Formation and Occurrence*; 1984; Vol. 66.

(7) Leahy, J. G.; Colwell, R. R. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* **1990**, *54* (3), 305–315.

(8) Aeppli, C.; Carmichael, C. A.; Nelson, R. K.; Lemkau, K. L.; Graham, W. M.; Redmond, M. C.; Valentine, D. L.; Reddy, C. M. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. *Environ. Sci. Technol.* **2012**, *46* (16), 8799–8807.

(9) 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.; et al. Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria. *Science (80-.).* **2010**, *330* (6001), 204–208.

(10) Mahmoudi, N.; Porter, T. M.; Zimmerman, A. R.; Fulthorpe, R. R.; Kasozi, G. N.; Silliman, B. R.; Slater, G. F. Rapid degradation of deepwater horizon spilled oil by indigenous microbial communities in louisiana saltmarsh sediments. *Environ. Sci. Technol.* **2013**, *47* (23), 13303–13312.

(11) 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* (22), 7962–7974.

(12) Rodriguez-R, L. M.; Overholt, W. A.; Hagan, C.; Huettel, M.; Kostka, J. E.; Konstantinidis, K. T. Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill. *ISME J.* **2015**, 1–13.

(13) 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* (7), 1761–1771.

(14) 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* (8), 2552–2558.

(15) 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 14C analysis. *Org. Geochem.* **2006**, *37* (9), 981–989.

(16) Mahmoudi, N.; Fulthorpe, R. R.; Burns, L.; Mancini, S.; Slater, G. F. Assessing microbial carbon sources and potential PAH degradation using natural abundance 14C analysis. *Environ. Pollut.* **2013**, *175*, 125–130.

(17) 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* (13), 5092–5097.

(18) Ahad, J. M. E.; Pakdel, H. Direct evaluation of in situ biodegradation in athabasca oil sands tailings ponds using natural abundance radiocarbon. *Environ. Sci. Technol.* **2013**, *47* (18), 10214–10222.

(19) Boschker, H. T. S.; de Brouwer, J. F. C.; Cappenberg, T. E. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. *Limnol. Oceanogr.* **1999**, *44* (2), 309–319.

(20) Boschker, H. T. S.; Kromkamp, J. C.; Middelburg, J. J. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. *Limnol. Oceanogr.* **2005**, *50* (1), 70–80.

(21) 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* (1), 51–62.

(22) Harvey, H. R.; Fallon, R. D.; Patton, J. S. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. *Geochim. Cosmochim. Acta* **1986**, *50* (5), 795–804.

(23) Boschker, H. T. S.; Middelburg, J. J. Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiol. Ecol.* **2002**, *40* (2), 85–95.

(24) Londry, K. L.; Jahnke, L. L.; Marais, D. J. D. Stable carbon isotope ratios of lipid biomarkers of sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **2004**, *70* (2), 745–751.

(25) Green, C. T.; Scow, K. M. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. *Hydrogeol. J.* **2000**, *8* (1), 126–141.

(26) Petsch, S. T.; Edwards, K. J.; Eglinton, T. I. Abundance, distribution and $\delta^{13}C$ analysis of microbial phospholipid-derived fatty acids in a black shale weathering profile. *Org. Geochem.* **2003**, *34* (6), 731–743.

(27) DeNiro, M. J.; Epstein, S. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science (80-.).* **1977**, *197* (4300), 261–263.

(28) Blair, N.; Leu, a; Muñoz, E.; Olsen, J.; Kwong, E.; Des Marais, D. Carbon isotopic fractionation in heterotrophic microbial metabolism. *Appl. Environ. Microbiol.* **1985**, *50* (4), 996–1001.

(29) Hayes, J. M. Factionation of the Isotopes of Carbon and Hydrogen in Biosynthetic Processes. *Rev. Mineral. Geochemistry* **2001**, *43* (March), 225–277.

(30) O'Leary, M. Carbon isotopes in photosynthesis. *Bioscience* **1988**, *38* (May), 328–336.

(31) Baskaran, M. Handbook of Environmental Isotope Geochemistry. *Advances in Isotope Geochemistry*. 2011, p 951.

(32) Petsch, S. T.; Eglington, T. I.; Edwards, K. J. 14C-dead living biomass: evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science* **2001**, *292* (5519), 1127–1131.

(33) Aeppli, C.; Nelson, R. K.; Radovi??, J. R.; Carmichael, C. A.; Valentine, D. L.; Reddy, C. M. Recalcitrance and degradation of petroleum biomarkers upon abiotic and biotic natural weathering of Deepwater Horizon oil. *Environ. Sci. Technol.* **2014**, *48* (12), 6726–6734.

(34) Gaines, R.B., Frysinger, G.S., Reddy, C.M., Nelson, R. K. Oil Spill Source Identification by Comprehensive Two-Dimensional Gas Chromatography (GCxGC). In *Oil Spill Forensics - Fingerprinting and Source Identification*; Academic Press, 2007; pp 169–206.

(35) Eiserbeck, C.; Nelson, R. K.; Grice, K.; Curiale, J.; Reddy, C. M. Comparison of GC-MS, GC-MRM-MS, and GC??GC to characterise higher plant biomarkers in Tertiary oils and rock extracts. *Geochim. Cosmochim. Acta* **2012**, *87*, 299–322.

(36) Garrett, R. M.; Pickering, I. J.; Haith, C. E.; Prince, R. C. Photooxidation of crude oils. *Environ. Sci. Technol.* **1998**, *32* (23), 3719–3723.

(37) Sharma, B. K.; Sarowha, S. L. S.; Bhagat, S. D.; Tiwari, R. K.; Gupta, S. K.; Venkataramani, P. S. Hydrocarbon group-type analysis of petroleum heavy fractions using the TLC-FID technique. *Fresenius' J. Anal. Chem.* **1998**, *360* (5), 539–544.

(38) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37* (8), 911–917.

(39) Guckert, J. B.; Antworth, C. P.; Nichols, P. D.; White, D. C. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol. Lett.* **1985**, *31* (3), 147–158.

(40) 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 abundance14C analysis of PLFA. *Environ. Sci. Technol.* **2010**, *44* (7), 2322–2327.

(41) Stuvier, M.; Polach, H. a. Reporting of 14C Data. *Radiocarbon* **1977**, *19* (3), 355–363.

(42) Graham, W. M.; Condon, R. H.; Carmichael, R. H.; D'Ambra, I.; Patterson, H. K.; Linn, L. J.; Hernandez Jr, F. J. Oil carbon entered the coastal planktonic food web during the Deepwater Horizon oil spill. *Environ. Res. Lett.* **2010**, *5* (4), 045301.

(43) Zelles, L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. *Biology and Fertility of Soils*. 1999, pp 111–129.

(44) McLafferty, F. W. Mass Spectrometric Analysis. Molecular Rearrangements. *Anal. Chem.* **1959**, *31* (1), 82–87.

(45) Bobbie, R. J.; White, D. C. Characterization of benthic microbial community structure by high-resolution gas chromatography of Fatty Acid methyl esters. *Appl. Environ. Microbiol.* **1980**, *39* (6), 1212–1222.

(46) Hanson, J. R.; Macalady, J. L.; Harris, D.; Scow, K. M. Linking toluene degradation with specific microbial populations in soil. *Appl. Environ. Microbiol.* **1999**, *65* (12), 5403–5408.

(47) Peters, K. E.; Walters, C. C.; Moldowan, J. M. The Biomarker Guide. *Biomark. Guid. Biomarkers Isot. Environ. Hum. Hist.* **2007**, *I*.

(48) Wenger, L.; Davis, C.; Isaksen, G. Multiple Controls on Petroleum Biodegradation and Impact on Oil Quality. *SPE Reserv. Eval. Eng.* **2002**, *5* (5).

(49) Munoz, D.; Guiliano, M.; Doumenq, P.; Jacquot, F.; Scherrer, P.; Mille, G. Long term evolution of petroleum biomarkers in mangrove soil (Guadeloupe). *Mar. Pollut. Bull.* **1997**, *34* (11), 868–874.

(50) Wang, Z.; Fingas, M.; Owens, E. H.; Sigouin, L.; Brown, C. E. Long-term fate and persistence of the spilled Metula oil in a marine salt marsh environment: Degradation of petroleum biomarkers. *J. Chromatogr. A* **2001**, *926* (2), 275–290.

(51) Hall, G. J.; Frysinger, G. S.; Aeppli, C.; Carmichael, C. A.; Gros, J.; Lemkau, K. L.; Nelson, R. K.; Reddy, C. M. Oxygenated weathering products of Deepwater Horizon oil come from surprising precursors. *Mar. Pollut. Bull.* **2013**, *75* (1-2), 140–149.

(52) Fusey, P., Oudot, J. Relative influence of physical removal and biodegradation in the depuration of petroleum-contaminated seashore sediments. *Mar. Pollut. Bull.* **1984**, *15* (4), 136–141.

(53) Jobson, a; Cook, F. D.; Westlake, D. W. Microbial utilization of crude oil. *Appl. Microbiol.* **1972**, *23* (6), 1082–1089.

(54) Walker, J. D.; Colwell, R. R.; Petrakis, L. Biodegradation rates of components of petroleum. *Can. J. Microbiol.* **1976**, *22* (8), 1209–1213.

(55) Dutta, T. K.; Harayama, S. Fate of crude oil by the combination of photooxidation and biodegradation. *Environ. Sci. Technol.* **2000**, *34* (8), 1500–1505.

(56) Sparks, T.; Guard, U. S. C. OPERATIONAL SCIENCE ADVISORY TEAM (OSAT-3) Unified Command Investigation of Recurring Residual Oil in Discrete Shoreline Areas in the Eastern Area of Responsibility Prepared for Deepwater Horizon MC252 Contents. **2013**, No. October.

(57) Hayworth, J. S.; Prabakhar Clement, T.; John, G. F.; Yin, F. Fate of Deepwater Horizon oil in Alabama's beach system: Understanding physical evolution processes based on observational data. *Mar. Pollut. Bull.* **2015**, *90* (1-2), 95–105.

Appendix A: Supporting Information

Table A.1: Abbreviations, names, and chemical formulas of biomarkers used for oil fingerprinting analysis.

Abbreviation	**Name**	**Chemical Formula**
Ts	18α(H)-22,29,30-trisnorneohopane	$C_{27}H_{46}$
Tm	17α(H)-22,29,30-trisnorhopane	$C_{27}H_{46}$
H	17α(H),21β(H)-hopane	$C_{30}H_{52}$
NH	17α(H),21β(H)-30-norhopane	$C_{29}H_{50}$
M	17β(H),21α(H)-hopane; moretane	$C_{30}H_{52}$
NM	17β(H),21α(H)-30-norhopane; normoretane	$C_{29}H_{50}$
HH (S/R)	17α(H),21β(H)-22S/R-homohopane	$C_{31}H_{54}$
2HH (S/R)	17α(H),21β(H)-22S/R-bishomohopane	$C_{32}H_{56}$
4HH (S/R)	17α(H),21β(H)-22S/R-tetrakishomohopane	$C_{34}H_{60}$

Table A.2: Relative abundance of saturated, aromatic, and oxygenated hydrocarbons in oiled sand patties from Ship Island (SI), Fort Morgan (FM), and Fort Pickens (FP).

	Saturated Hydrocarbons	**Aromatic Hydrocarbons**	**Oxygenated Hydrocarbons**
SI ST 1	28%	12%	60%
SI ST 2	37%	10%	53%
FM ST	33%	10%	57%
FM IT	22%	10%	69%
FP IT 1	21%	11%	68%
FP IT 2	33%	13%	54%
Average	27%	11%	60%
SD	6.7	1.3	6.9

Table A.3: Mol% of all PLFA >0.5% detected in samples. Sat=saturated, monounsat=monounsaturated, polyunsat=polyunsaturated, br=branched, cyc=cyclic, otherFAME=branched and monounsaturated.

PLFA	Structure	Oiled SI ST	Oiled SI ST+	Oiled FM ST	Oiled FM ST+	Oiled FM IT+	Oiled FP IT	No Oil FM IT	No Oil FP IT
unknown	unknown	-	-	1.0	-	2.6	-	-	-
$C_{7:0}$	sat	-	-	1.0	-	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
$C_{8:0}$	sat	0.7	0.7	1.5	0.7	2.7	3.3	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
$C_{9:0}$	sat	0.8	0.7	1.4	0.8	2.3	2.7	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
$C_{10:0}$	sat	0.8	0.7	1.3	0.8	2.2	2.6	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
unknown	unknown	0.5	-	0.8	0.5	-	-	-	-
unknown	unknown	0.5	-	0.9	-	1.8	-	-	-
unknown	unknown	0.5	-	0.8	0.5	-	-	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
unknown	unknown	-	-	0.8	0.5	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-

$C_{11:0}$	sat	0.9	0.9	1.3	0.9	1.9	2.4	-	-
unknown	unknown	0.6	0.5	0.9	0.6	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	-	-	0.8	-	-	-	-	-
unknown	unknown	-	-	0.9	-	-	-	-	-
unknown	unknown	0.6	-	0.9	0.5	-	-	-	-
unknown	unknown	0.5	-	-	-	-	-	-	-
unknown	unknown	0.6	-	0.8	0.5	-	-	-	-
unknown	unknown	-	-	-	0.5	-	-	-	-
$C_{12:0}$	sat	1.0	1.0	1.4	1.1	1.8	2.4	-	-
unknown	unknown	0.5	-	-	0.5	-	-	-	-
unknown	unknown	0.5	-	-	-	-	-	-	-
unknown	unknown	0.6	0.5	0.8	0.5	-	-	-	-
unknown	unknown	0.5	-	-	0.5	-	-	-	-
unknown	unknown	0.5	0.5	0.8	0.6	-	-	-	-
unknown	unknown	0.6	0.5	0.8	0.5	-	-	-	-
unknown	unknown	0.6	0.5	0.8	0.5	-	-	-	-
unknown	unknown	0.6	0.5	0.9	0.6	-	-	-	-
unknown	unknown	-	-	0.8	0.6	-	-	-	-
unknown	unknown	0.6	0.6	-	-	-	-	-	-
unknown	unknown	0.5	-	-	-	-	-	-	-
$C_{13:0}$	sat	1.0	1.0	1.3	1.0	1.6	2.0	-	-
unknown	unknown	0.6	0.5	-	0.6	-	-	-	-
C_{br13}	br	0.7	0.6	0.8	0.7	-	-	-	-
unknown	unknown	0.6	0.5	-	0.6	-	-	-	-
unknown	unknown	0.6	0.6	-	-	-	-	-	-

unknown	unknown	0.6	0.5	0.8	0.6	-	-	-	-
unknown	unknown	0.7	0.6	1.0	0.7	1.5	1.7	-	0.5
unknown	unknown	0.6	0.5	-	0.6	-	-	-	-
unknown	unknown	0.6	-	-	-	-	-	-	-
$C_{14:0}$	sat	1.6	1.9	2.7	2.3	3.1	4.7	3.5	3.0
unknown	unknown	-	-	0.8	-	-	-	-	-
unknown	unknown	0.6	0.7	-	0.7	-	-	-	-
C_{br14}	br	0.8	0.8	0.9	0.8	-	-	-	-
unknown	unknown	0.7	0.6	0.8	0.7	-	-	-	-
C_{br14}	br	1.2	1.4	2.2	1.9	2.7	3.6	1.9	2.1
C_{br14}	br	1.1	0.9	1.4	1.2	2.4	2.6	1.0	1.1
$C_{15:1}$	monounsat	-	-	-	-	-	-	0.9	1.0
unknown	unknown	-	0.5	-	-	-	-	-	-
unknown	unknown	0.7	0.6	-	0.6	-	-	-	-
$C_{15:0}$	sat	1.4	2.0	1.9	1.9	3.1	3.7	2.8	2.1
C_{br15}	br	-	-	-	-	-	-	2.2	-
C_{br15}	br	1.0	1.0	1.0	0.9	-	-	-	-
unknown	unknown	0.8	0.8	0.8	0.7	-	-	-	-
C_{br15}	br	0.8	0.7	0.9	0.7	1.3	-	-	-
C_{br15}	br	1.7	1.5	1.1	0.9	1.5	-	-	1.3
C_{br15}	br	-	-	-	-	-	1.8	-	-
C_{br15}	br	-	0.7	0.9	0.7	1.4	-	-	-
$C_{16:1}$	monounsat	-	-	-	-	-	-	1.3	1.6
$C_{16:1}$	monounsat	2.1	2.0	2.3	2.1	4.2	3.4	33.7	22.2
unknown	unknown	-	-	0.9	0.8	3.6	3.3	-	1.6
$C_{16:1}$	monounsat	-	-	-	1.6	2.4	2.1	1.5	1.5

$C_{16:1}$	monounsat	-	-	-	-	-	-	1.8	1.3
$C_{16:0}$	sat	4.8	6.3	6.3	6.4	10.8	16.7	18.1	21.8
$C_{br16:1}$	otherFAME	-	-	-	-	-	-	-	1.4
C_{br16}	br	0.9	0.9	0.9	0.9	1.5	-	1.4	1.8
unknown	unknown	-	-	-	0.7	-	-	0.8	-
unknown	unknown	1.2	0.8	-	-	-	-	0.7	-
C_{br16}	br	1.1	1.2	1.0	0.9	1.4	-	0.7	1.0
C_{br16}	br	1.4	1.4	1.1	0.9	1.4	1.7	0.6	0.7
$C_{17:1}$	monounsat	2.5	3.1	2.3	2.0	3.2	2.4	3.0	2.4
$C_{17:1}$	monounsat	2.5	2.1	2.3	1.9	2.8	1.8	0.6	0.8
$C_{17:0}$	sat	2.0	2.5	2.0	2.3	3.9	4.6	0.9	1.2
unknown	unknown	-	-	0.8	0.8	-	-	-	-
C_{br17}	br	1.1	1.1	0.9	0.9	1.2	-	-	-
$C_{18:3}$	polyunsat	-	-	-	-	-	-	0.9	-
$C_{18:4}$	polyunsat	-	-	-	-	-	-	1.1	1.2
unknown	unknown	-	-	-	0.7	-	-	-	-
$C_{18:2}$	polyunsat	5.5	9.5	3.9	3.9	-	-	1.3	1.8
$C_{18:1}$	monounsat	4.3	5.6	3.8	3.3	2.9	2.0	3.1	4.2
$C_{18:1}$	monounsat	5.8	5.1	4.2	4.4	9.4	9.0	5.0	10.9
$C_{18:1}$	monounsat	-	-	-	2.1	3.2	2.7	-	-
$C_{18:0}$	sat	2.7	3.0	2.7	3.0	3.1	4.5	0.9	1.6
C_{cyc18}	cyc	1.2	1.1	-	1.1	1.4	1.9	-	-
C_{br18}	br	1.7	1.9	1.0	0.9	-	-	-	0.5
unknown	unknown	2.2	1.9	1.5	1.1	-	-	-	-
$C_{19:1}$	monounsat	-	-	-	2.3	4.1	3.7	-	-

C_{cyc18}	cyc	3.6	3.0	2.3	2.1	2.9	4.3	-	1.0
C_{br18}	br	2.2	2.3	1.6	1.8	1.4	-	-	-
C_{cyc19}	cyc	-	-	-	-	-	-	0.7	0.9
$C_{20:5}$	polyunsat	-	-	-	-	-	-	4.1	2.8
$C_{20:3}$	polyunsat	-	-	-	-	-	-	0.7	-
$C_{20:0}$	sat	2.3	2.5	1.5	1.7	1.1	-	-	0.5
$C_{21:0}$	sat	1.9	2.0	1.3	1.4	-	0.9	-	-
unknown	unknown	1.5	-	-	-	-	-	-	-
$C_{22:0}$	sat	1.9	2.1	1.2	1.3	-	0.7	-	0.5
$C_{23:0}$	sat	1.6	0.9	1.1	1.2	-	0.6	-	-
$C_{24:0}$	sat	1.4	1.7	1.1	1.2	-	-	-	0.6
$C_{25:0}$	sat	-	-	-	1.0	-	-	-	-

Table A.4: PLFA structure, as mol%, of microbes in oiled and non-oiled sand.

	Oiled						Non-oiled	
Structure	**SI ST**	**SI ST+**	**FM ST**	**FM ST+**	**FM IT+**	**FP IT**	**FM IT**	**FP IT**
sat	30%	33%	31%	33%	38%	52%	27%	32%
monounsat	19%	20%	15%	22%	32%	27%	53%	47%
polyunsat	6%	11%	4%	4%	0%	0%	9%	6%
br	17%	18%	16%	16%	16%	10%	8%	9%
cyc	5%	5%	2%	4%	4%	6%	1%	2%
otherFAME	0%	0%	0%	0%	0%	0%	0%	1%
unknown	23%	14%	32%	20%	9%	5%	2%	2%

Correction of isotope values for methanolysis

The methanolysis step of the PLFA extraction cleaves the polar head group of the molecule and adds a methyl group, adding an additional C atom, which was assumed to be ^{14}C-free ($\Delta^{14}C$= -1000‰). To correct for the isotopic value of the added C the following formula was used:

$$\Delta^{14}C_{PLFA} = [(\Delta^{14}C_{measured} * n) + (\Delta^{14}C_{MeOH} * 1)]/(n+1)$$

Where n= # of C atoms in PLFA, $\Delta^{14}C_{MeOH}$= ^{14}C content of MeOH (assumed to = -1000‰).

Oil derived C in PLFA fractions

Oil derived C remained in microbial lipid fractions despite additional purification steps. Highly weathered *DwH* oil shares chemical properties to microbial lipids, making complete isolation of microbial lipids nearly impossible. An additional purification step was used on select samples (denoted with +) to attempt to isolate microbial lipids. Most samples that underwent additional purification were replicates, which allowed for quantification of oil-derived C removed by comparing the PLFA extracts with those that underwent additional purification. Total organic C in PLFA fractions was measured using both manometry and GC-MS. While the additional purification step removed total organic C, as measured by manometry, the total organic C measured by GC-MS was not reduced (Table S5). Removal of total organic C revealed by manometry indicates the additional purification step removes C that was not amenable to detection via GC-MS. Total organic C removed, as measured by manometry, likely reflects loss of *DwH* oil derived C, as highly weathered *DwH* oil is not amenable to GC-MS analysis.[8] Therefore,

more accurate measurements of total organic C using manometry indicate large quantities of oil-derived C were removed in samples with an additional purification step (Table S6).

Table A.5 PLFA C and Total C measured using GC-MS and total C measured using manometry. Samples denoted with + underwent additional purification step. Oiled samples without additional clean up step show high total C measured by manometry. This is consistent with highly weathered *DwH* oil not being amenable to GC.[8]

Oiled Samples	**GC-MS PLFA C (µg)**	**GC-MS Total C (µg)**	**Manometry Total C (µg)**
Oiled SI ST	217.9	416.7	1067.0
Oiled SI ST+	303.3	475.7	400.0
Oiled FM ST	166.8	284.5	1159.0
Oiled FM ST+	231.8	293.4	117.0
Oiled FM IT+	126.8	132.0	104.0
Oiled FP IT	116.7	125.4	311.8
Non-oiled samples			
No Oil FM IT	523.3		284.0
No Oil FP IT	316.8		160.0

Table A.6 Calculations of f_{oil} using two methods. Method 1 calculates f_{oil} using the ratio of non-PLFA peak area to total peak area as measured by GC-MS. Method 2 calculates f_{oil} using the ratio of TPH to total C as measured by GC-MS.

Sample	**PLFA PA**	**Total PA**	**UCM PA**	***f* oil Method 1**	**TPH (ug C)**	**PLFA (ug C)**	***f* oil Method 2**
Oiled SI ST	8.89E+08	4.48E+09	3.60E+09	0.80	198.73	217.92	0.48
Oiled SI ST+	1.54E+09	4.61E+09	3.07E+09	0.67	172.45	303.27	0.36
Oiled FM ST	5.86E+08	3.09E+09	2.50E+09	0.81	117.66	166.82	0.41
Oiled FM ST+	7.50E+08	1.83E+09	1.08E+09	0.59	61.60	231.81	0.21
Oiled FM IT+	1.87E+09	1.96E+09	9.24E+07	0.05	5.21	126.80	0.04
Oiled FP IT	4.13E+08	5.67E+08	1.54E+08	0.27	8.67	116.68	0.07