Microbial Responses to the *Deepwater Horizon* Oil Spill: From Coastal Wetlands to the Deep Sea

G.M. King,[1] J.E. Kostka,[2] T.C. Hazen,[3] and P.A. Sobecky[4]

[1]Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803; email: gkingme@gmail.com

[2]School of Biology and School of Earth and Atmospheric Science, Georgia Institute of Technology, Atlanta, Georgia 30332; email: joel.kostka@biology.gatech.edu

[3]Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996; email: tchazen@utk.edu

[4]Department of Biological Sciences, University of Alabama, Tuscaloosa, Alabama 35487; email: psobecky@as.ua.edu

Annu. Rev. Mar. Sci. 2015. 7:377–401

First published online as a Review in Advance on September 17, 2014

The *Annual Review of Marine Science* is online at marine.annualreviews.org

This article's doi: 10.1146/annurev-marine-010814-015543

Keywords

Gammaproteobacteria, hydrocarbon degradation, methane, bacterioplankton, metagenomics

Abstract

The *Deepwater Horizon* oil spill in the northern Gulf of Mexico represents the largest marine accidental oil spill in history. It is distinguished from past spills in that it occurred at the greatest depth (1,500 m), the amount of hydrocarbon gas (mostly methane) lost was equivalent to the mass of crude oil released, and dispersants were used for the first time in the deep sea in an attempt to remediate the spill. The spill is also unique in that it has been characterized with an unprecedented level of resolution using next-generation sequencing technologies, especially for the ubiquitous hydrocarbon-degrading microbial communities that appeared largely to consume the gases and to degrade a significant fraction of the petroleum. Results have shown an unexpectedly rapid response of deep-sea Gammaproteobacteria to oil and gas and documented a distinct succession correlated with the control of the oil flow and well shut-in. Similar successional events, also involving Gammaproteobacteria, have been observed in nearshore systems as well.

1. INTRODUCTION

nGoM: northern Gulf of Mexico

DWH: *Deepwater Horizon*

PAH: polycyclic aromatic hydrocarbon

On the night of April 20, 2010, a tragic series of events in the northern Gulf of Mexico (nGoM) led to a blowout of the Macondo oil well in Mississippi Canyon Block 252 (MC-252) (Norse & Amos 2010, Lubchenco et al. 2012). A gas explosion ensued that killed 11 members of the *Deepwater Horizon* (DWH) drilling crew and injured 17 others. The blowout released an uncontrolled, buoyant, pressurized, and heated stream of crude oil and hydrocarbon gases from a depth of approximately 1,500 m—the greatest depth at which a catastrophic well failure has occurred to date. Oil flow rates from the well have been estimated at up to 9,900 m^3 d^{-1} (62,000 barrels d^{-1}), with a total crude oil release of some 650,000–780,000 m^3 (4.1–4.9 million barrels) over an 87-day period (Crone & Tolstoy 2010, McNutt et al. 2011, Reddy et al. 2012). This spill exceeded by a factor of two or more all but the Ixtoc I oil spill, which occurred at a much shallower depth (50 m) in the southern Gulf of Mexico (the Bay of Campeche) and released approximately 30% less oil than the MC-252 well (Jernelov 2010).

Approximately $1.7–3.1 \times 10^8$ kg of natural gas (C_1–C_5, mostly methane) was also released during the discharge; this is comparable to the mass of liquid oil released and was a distinguishing feature of the spill. The MC-252 spill also differed from others in the extensive use of chemical dispersants: A total of 7,000 m^3 (approximately 2.1×10^6 gallons) of dispersants, largely Corexit EC9500A and EC9527A, was injected directly into the oil plume at the wellhead or sprayed onto surface slicks to enhance solubility and promote bacterial remediation (Lubchenco et al. 2012). Subsurface use of Corexit, which accounted for approximately 37% of the total use, had not been attempted previously, and its efficacy remains uncertain.

From the discharge source at 1,500 m, oil, gas, and dispersants rose through the water column, entraining seawater and undergoing a kinetic fractionation process that altered the plume's composition with increasing distance from the wellhead (Valentine et al. 2012). Droplets of oil and associated dispersants were found in several size classes. Neutrally buoyant droplets 10–60 μm in diameter formed an extensive, regional-scale plume at depths mostly between 1,000 and 1,200 m (Camilli et al. 2010, Valentine et al. 2012, Spier et al. 2013). However, at various times, transient currents were also observed to entrain oil and gas droplets at four other depths (Spier et al. 2013). The deep plume was characterized by elevated concentrations of polycyclic aromatic hydrocarbons (PAHs), intermediate-length alkanes, methane, and other gases. Relative to adjacent, unaffected water masses, the plume was also characterized by lower oxygen concentrations (or oxygen anomalies), altered bacterioplankton community composition, and elevated bacterial density and activity (see Section 6).

In addition, large, positively buoyant droplets (~300 μm in diameter) with higher proportions of PAHs and heavier hydrocarbons rose to the surface, where they formed thin but extensive surface slicks (Atlas & Hazen 2011). The distinct surface and subsurface environments selected for different microbial populations in response to the released oil, even though Gammaproteobacteria dominated both surface-slick and deepwater plumes. Not surprisingly, the dynamics and impacts of surface and deep oil differed significantly from each other.

In the plume, both hydrocarbon and bacterial dynamics were strongly determined by complex interactions among biological, chemical, and physical processes, the latter of which included advection and mixing as the plume moved laterally from the wellhead (Adcroft et al. 2010, Ryerson et al. 2011, Valentine et al. 2012). Plume movement was not unidirectional, however; rather, it was affected by deep eddies and currents (DeHaan & Sturges 2005) that resulted in what appeared to be at some times a southwestward motion and at other times a sinusoidal movement to the northeast. Model analyses, which were largely consistent with observations, suggested that the plume was advected away from the wellhead but also returned to the wellhead, where it accumulated additional

oil (Valentine et al. 2012). The circulation pattern of the plume and the reintroduction of oil had profound consequences for the dynamics of plume bacteria and for the degradation of at least some hydrocarbons (see Sections 3 and 4).

Although the amount of oil and gas entrained in subsurface plumes was substantial (approximately 2×10^8 kg), a considerable fraction of the total release (at least 50%) rose to the surface (Ryerson et al. 2012, Spier et al. 2013), including liquid oil and gas that were captured directly and stored (oil) or flared (gas). Once at the surface, the oil formed extensive slicks of varying thicknesses that affected an area of up to 180,000 km^2. The distribution of surface oil was determined by interactions between winds, currents, and eddies that reflected in part the dynamics of the Loop Current (Mezic et al. 2010). Contrary to initial expectations of a westward movement, partially weathered surface oil, including dispersants, moved to the northwest and northeast and washed ashore along the easternmost coast of Texas and much of coastal Louisiana (Mezic et al. 2010). Surface oil also contaminated beaches and wetlands from Mississippi eastward to the western Florida panhandle. Although extensive efforts were made to remove oiled beach sands and clean some wetlands, tar balls continue to appear on beaches, and oil persists in many wetlands both at the soil surface and belowground (Kelly 2013). In addition to the beached oil, an unknown but considerable amount of oil appears to have been buried in shallow nearshore sediments, where it remains susceptible to resuspension and subsequent beaching. (For a discussion of interactions between oil and bacteria in nearshore and wetland systems, see Sections 4 and 5.)

Of the oil released from the MC-252 well, relatively small percentages were collected by skimming or remediated by burning (approximately 3% and 5%, respectively; Lubchenco et al. 2012) (**Figure 1**). Approximately 17% was recovered directly from the wellhead, and 25% evaporated or dissolved (presumably degraded by bacteria). The remaining 50% comprised naturally and chemically dispersed oil phases (16% and 8%, respectively) and residual oil, which consisted of surface and subsurface sheen, tar balls, and oil that washed ashore or was buried. Some of this material (e.g., tar balls and tar mats) was and continues to be removed mechanically, but the fate of most of the residual oil depends on the dynamics and mechanisms of natural attenuation, which largely involves degradation by bacteria. The remainder of this review summarizes observations on bacterial hydrocarbon transformations measured to date and the prospects for future degradation.

2. PRESPILL BACTERIOPLANKTON COMMUNITIES IN THE GULF OF MEXICO

Before the DWH disaster, relatively little was known about nGoM bacterioplankton. Earlier studies had addressed their distribution, abundance, and secondary production but placed little emphasis on diversity. King et al. (2013) used a pyrosequencing-based analysis of 16S rRNA gene amplicons to assess community composition, diversity, and phylogenetic structure in samples taken at 17 nGoM stations approximately one month before the spill.

Principal component and nonmetric multidimensional scaling analyses showed that samples from depths of ≤100 m had similar bacterioplankton compositions, but these communities differed distinctly from those in deeper samples (**Figure 2*a***). Specifically, SAR11 Alphaproteobacteria and Bacteroidetes dominated communities at depths of ≤100 m, which were characterized by Alphaproteobacteria/Gammaproteobacteria ratios of >1.7 (**Figure 2*b***). At depths of >100 m, by contrast, Thaumarchaeota, Firmicutes, and Deltaproteobacteria were more abundant, and Alphaproteobacteria/Gammaproteobacteria ratios were <1 (**Figure 2*b***). The relatively low Alphaproteobacteria/Gammaproteobacteria ratios in deeper waters are notable because Gammaproteobacteria dominated the populations that responded to the spill (e.g., Hazen et al. 2010).

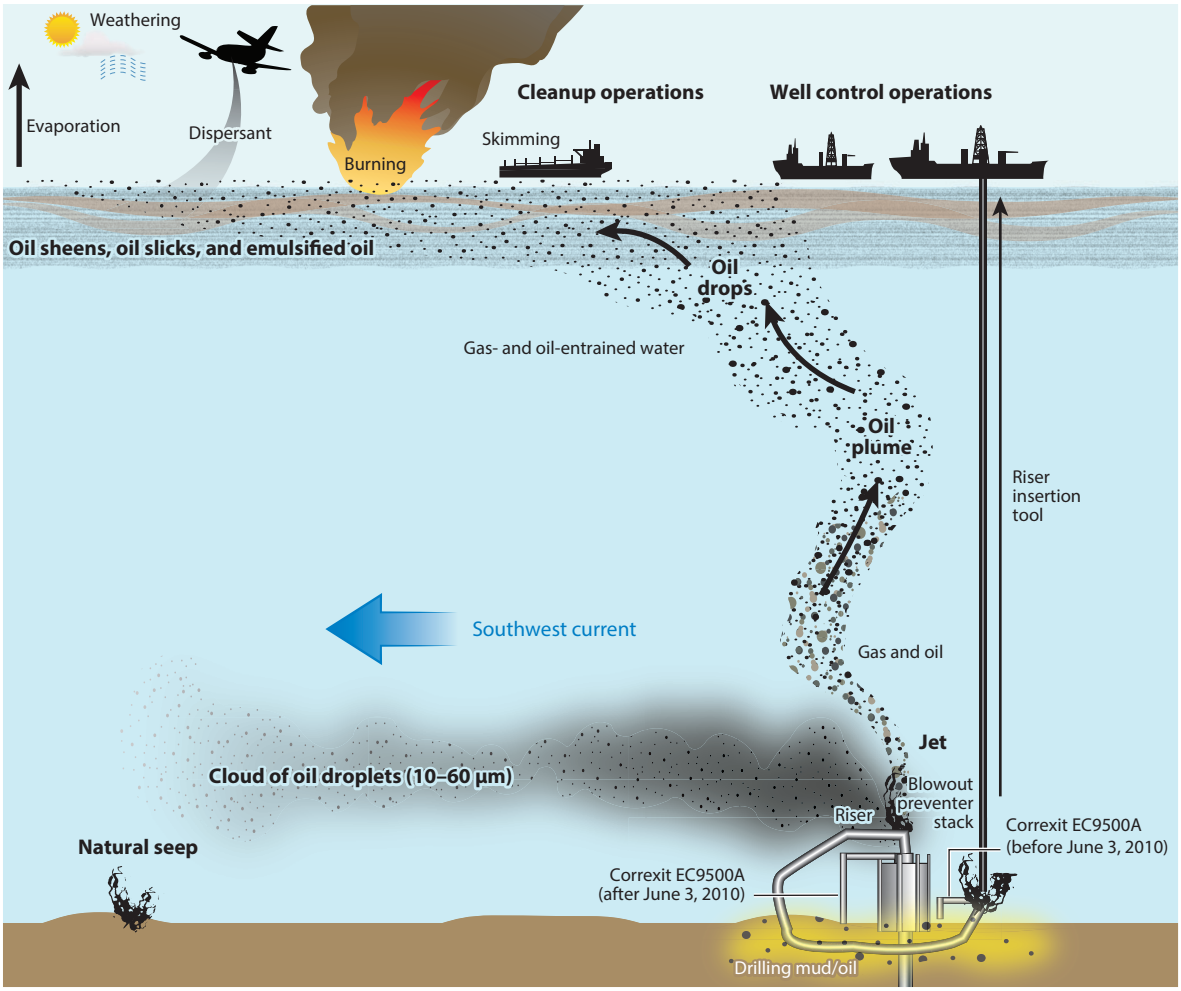

Figure 1

Schematic view of the *Deepwater Horizon* blowout, oil and gas distributions, and cleanup operations. Adapted from Atlas & Hazen (2011).

Given the presence of numerous active hydrocarbon seeps in the nGoM and the magnitude of nGoM oil and gas production and spill potential, the composition and distribution of hydrocarbon oxidizers are of particular interest. Results from 16S rRNA gene sequence analyses revealed 11 known hydrocarbon-oxidizing genera, including *Alcanivorax*, *Cycloclasticus*, *Marinobacter*, *Oleiphilus*, *Oleispira*, *Pseudomonas*, and *Vibrio*, and 13 methanotrophic and methylotrophic genera, including *Methylobacter*, *Methylococcus*, *Methylocystis*, *Methylohalobius*, *Methylonatrum*, *Methylophaga*, *Methylophilus*, *Methylosinus*, and *Methylovorus* (King et al. 2013). Hazen et al. (2010) and Mason et al. (2012) reported that, of these genera, *Colwellia*, *Cycloclasticus*, and an Oceanospirillales taxon related to *Oleispira* were abundant members of the plume community, and Rivers et al. (2013) described evidence for the presence of *Colwellia*, *Cycloclasticus*, *Methylobacter*, and *Methylococcus* based on sequences from a plume transcriptome. Valentine et al. (2010) and Kessler et al. (2011) identified similar taxa in a study of methane and propane respiration within the plume. Gutierrez

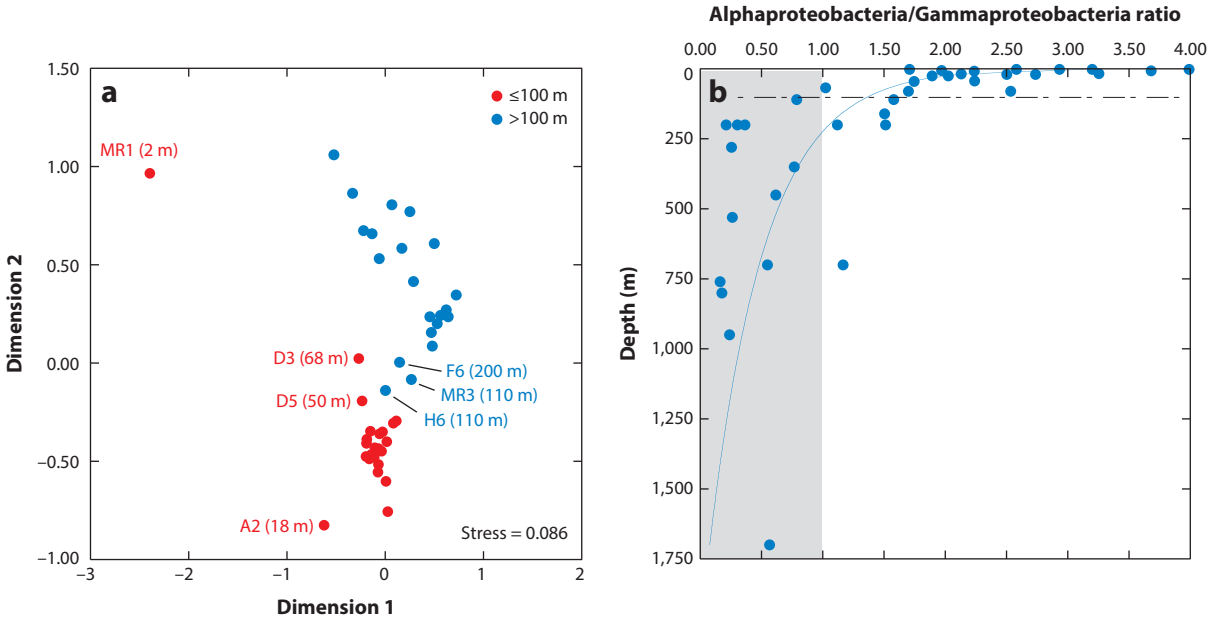

Figure 2

(*a*) Nonmetric multidimensional scaling analysis of northern Gulf of Mexico communities, indicating the structuring of composition by depth. (*b*) Alphaproteobacteria/Gammaproteobacteria ratios with depth. The shaded area indicates ratios less than 1. Adapted from King et al. (2013).

et al. (2013) documented additional taxa, including *Alcanivorax*, *Alteromonas*, *Halomonas*, *Marinobacter*, and *Pseudoalteromonas*, using stable isotope probing and cultivation-based approaches to analyze surface-slick and deepwater plume samples.

Colwellia was unique among the genera identified in the plume in that it was not observed in samples obtained before the spill (King et al. 2013), possibly because its prespill abundances were too low to detect. Regardless, *Colwellia* species responded rapidly to the spill, whereas *Alcanivorax* species—which were observed throughout the nGoM (King et al. 2013, Smith et al. 2013) and which have been regularly associated with hydrocarbon pollution (Harayama et al. 2004, Head et al. 2006)—were not readily detected in plume samples (e.g., Hazen et al. 2010, Mason et al. 2012, Gutierrez et al. 2013, Rivers et al. 2013). This suggests that, although the nGoM harbors a wide range of hydrocarbonoclastic taxa capable of responding to hydrocarbon inputs, the responses of specific groups might vary substantially across time and space.

Smith et al. (2013) provided additional insights into the potential for alkane degradation by analyzing partial sequences for *alkB*, one of the structural genes for alkane hydroxylase. The authors sequenced 401 *alkB* gene clones amplified from genomic extracts obtained from the samples described by King et al. (2013). In contrast to the distinct depth stratification observed using 16S rRNA gene sequences, principal component analyses of alkB operational protein units (OPUs) indicated that community composition did not vary consistently with depth or other major physical-chemical variables. Of 22 distinct OPUs, one was ubiquitous and accounted for 57% of all sequences. This OPU clustered with alkB sequences from known hydrocarbon oxidizers (e.g., *Alcanivorax* and *Marinobacter*) but did not appear to be represented in plume metagenomes or metatranscriptomes (Mason et al. 2012, Rivers et al. 2013). Likewise, none of the less abundant

OPUs appeared to be represented in the plume community that responded to the spill. Thus, results from *alkB* and 16S rRNA gene sequence analyses indicate that *Alcanivorax* occurs widely within the Gulf of Mexico but might not compete well with other taxa for deepwater hydrocarbons. Variables that affect the hydrocarbon utilization by specific populations and that determine responses to spills remain uncertain.

3. DEGRADATION OF METHANE AND OTHER LIGHT HYDROCARBONS

C_1–C_5 hydrocarbons constituted approximately 24% of the DWH spill by mass, with methane accounting for approximately 60% of the light hydrocarbons (Reddy et al. 2012). Concentration profiles indicted that most of the light hydrocarbons that entered the water column remained at depths of >800 m (Kessler et al. 2011), which explained the low sea–air methane fluxes reported by Yvon-Lewis et al. (2011) from analyses during June 2010.

Several lines of evidence indicated that methanotrophic bacteria substantially oxidized plume methane. Methane concentrations were high and methanotrophic population densities and activities were low in the plume and near the wellhead during the early phases of the spill (e.g., in June 2010) (Hazen et al. 2010, Joye et al. 2011, Kessler et al. 2011), consistent with limited methane oxidation. Although metagenomic and metatranscriptomic data revealed some methanotrophic populations in the plume in late May and early June (Hazen et al. 2010, Rivers et al. 2013), methanotroph abundance appeared to have increased substantially later in the summer (Kessler et al. 2011). Methanotroph blooms characterized by members of the Methylococcaceae expressing particulate methane monooxygenase genes were accompanied by a return of methane concentrations to near-ambient levels, with the decline in methane mirrored in and largely accounting for the plume oxygen anomaly (Kessler et al. 2011). There were initial concerns about long-term and deleterious effects of large methane releases in the deep sea (Joye et al. 2011), but a community of methanotrophs responded robustly and consumed the methane relatively rapidly, with no known adverse impacts.

Other gases (e.g., ethane and propane) were also oxidized rapidly in the plume. Valentine et al. (2010) documented the consumption of both during early stages of microbial responses. They suggested that ethane, propane, and possibly butane supported some of the dominant plume populations, including *Colwellia* and taxa related to *Cycloclasticus*, and that respiration of ethane and propane accounted for up to 70% of the observed June 2010 plume oxygen anomalies. In addition, results from ex situ incubations and stable isotope probing showed that *Colwellia*, and possibly the Oceanospirillales observed in the plume, likely used both ethane and propane (Redmond & Valentine 2012).

These observations were consistent with the results of metagenomic and metatranscriptomic analyses, which showed significant expression of alkane monooxygenase genes that are related most closely to homologs of the particulate methane monooxygenase genes coding for ethane and propane monooxygenases (Mason et al. 2012, Rivers et al. 2013). Rivers et al. (2013) used phylogenetic analyses of particulate monooxygenase sequences to infer putative functions. The sequences clustered into four clades, one of which included particulate methane monooxygenase gene sequences found in methanotrophic Gammaproteobacteria; this is consistent with results from 16S rRNA gene sequence analyses, which showed enrichments of this group (Hazen et al. 2010, Valentine et al. 2010). Two other clades, which accounted for 42% of the monooxygenase reads, contained sequences related most closely to genes coding for ethane and propane monooxygenases, whereas a fourth clade, which accounted for most of the monooxygenase

reads (50%), contained sequences for which no function could be inferred. The fact that these sequences were relatively highly expressed suggests a role in alkane degradation that should be assessed further.

OTU: operational taxonomic unit

4. HYDROCARBON IMPACTS AT MESOPELAGIC DEPTHS OFFSHORE

Owing to the unique nature of the MC-252 oil spill, considerable attention has been focused on the offshore water column; detailed analyses have been performed of hydrocarbon and dispersant degradation, bacterioplankton community composition and activity, trophic interactions, and connections between the water column and sediment. One somewhat surprising finding from these observations was the robust response of the cold, deepwater bacterial community, which appeared to use hydrocarbons with little or no lag and with no nutrient additions, thereby partially attenuating the contamination (see below for details). Rapid responses were also observed in offshore surface waters, even though they were considered phosphorus limited (Edwards et al. 2011, Ziervogel et al. 2012). In this sense, the MC-252 spill was very different from the *Exxon Valdez* spill, degradation of which appeared to be hindered by the cold conditions in Prince William Sound, Alaska, and for which a variety of bioremediation efforts were attempted (Prince 2010, Atlas & Hazen 2011).

4.1. Hydrocarbon Effects on Bacterial Community Structure Within the Deep-Sea Plume

Hazen et al. (2010) showed that bacterial densities in deep-sea plume samples collected between May 25 and June 2, 2010 (5.5×10^4 cells cm^{-3}), were elevated relative to those in adjacent nonplume samples (2.7×10^4 cells cm^{-3}). Sixteen members of the Gammaproteobacteria were significantly enriched in the plume samples, one of which constituted >90% of 16S rRNA gene sequences recovered from plume DNA extracts (Hazen et al. 2010, Mason et al. 2012). Clone library analysis revealed that the closest cultured relatives of this operational taxonomic unit (OTU) were *Spongiispira norvegica* (95% similarity) and *Oceaniserpentilla haliotidis* (94% similarity), both of which are members of the Oceanospirillales. This same OTU accounted for <3% of sequences in nonplume samples and samples taken in the same area at similar depths one month before the spill (Mason et al. 2012, Dubinsky et al. 2013, King et al. 2013). Many of the other abundant plume sample OTUs were closely related to psychrophilic hydrocarbon degraders (Hazen et al. 2010), which is consistent with in situ temperatures (4–5°C).

Metagenomic and metatranscriptomic analyses revealed both functional potential and community structure (Hazen et al. 2010), in that a single Oceanospirillales OTU was documented as the dominant community member between May 26 and June 3 (Mason et al. 2012), with Oceanospirillales sequences accounting for >60% of all plume metagenomic reads and 49–69% of all plume transcripts. In addition, Rivers et al. (2013) found that 95% of the 16S rRNA gene sequences in their samples were from only four families: Colwelliaceae, Methylococcaceae, Oceanospirillaceae, and Piscirickettsiaceae. Their metatranscriptomic results also indicated that Alteromonadales, Deltaproteobacteria, Pseudomonadales, and SAR86 were active but more limited members of the plume. Results from 16S rRNA gene sequences were consistent with transcript annotations, which revealed relatively few active plume community members; most of the transcripts binned to only six reference genomes. The metatranscriptomic analysis showed that, even though *Colwellia* species were only minor constituents of the 16S rRNA gene libraries from samples collected at this time, they were present and active in the plume, with Alteromonadales comprising 8–11% of recovered transcripts (Mason et al. 2012).

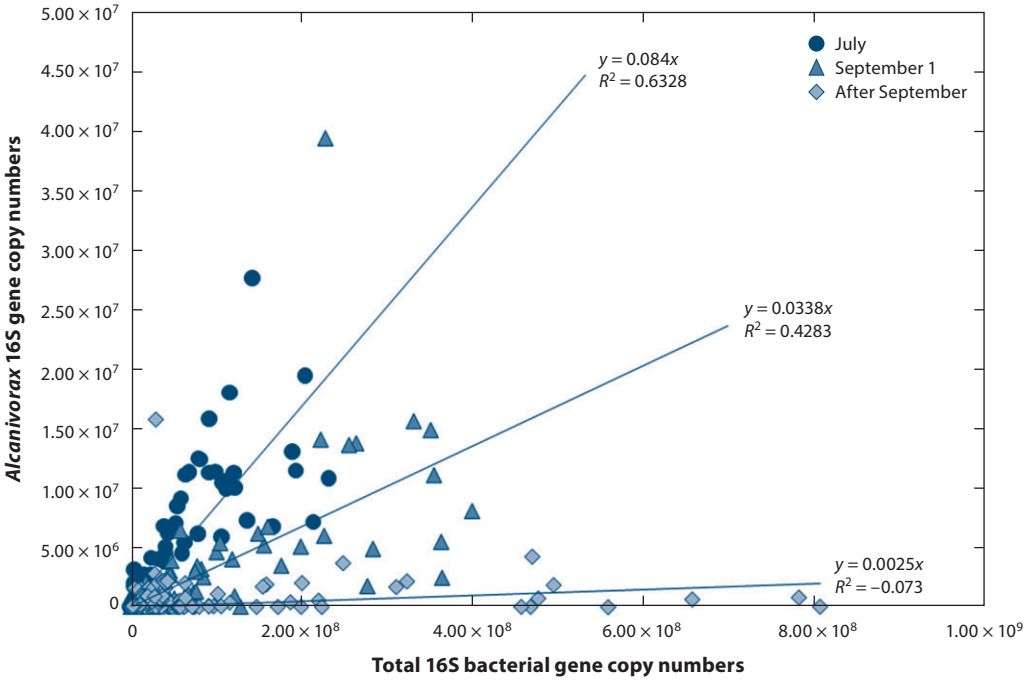

Figure 3

Alcanivorax succession over time plotted as abundance of *Alcanivorax* 16S rRNA gene sequence copy numbers versus total 16S rRNA gene sequence copy numbers for samples collected in summer and fall 2010. Adapted from Kostka et al. (2011).

Samples collected later during the course of the spill (June 2010) revealed a succession in community membership (**Figure 3**), with *Colwellia* (Alteromonadales) and *Cycloclasticus* (Thiotrichales) emerging as the dominant genera (Redmond & Valentine 2012). *Colwellia* also increased in abundance during ex situ enrichments of deep-sea water with crude oil at 4°C (Redmond & Valentine 2012). Stable isotope probing revealed that *Colwellia* consumed propane and ethane, whereas *Cycloclasticus* likely consumed benzene-toluene-ethylbenzene-xylene (BTEX) compounds. Results from a later sampling (September 2010) showed additional shifts in plume community composition, with high abundances of methylotrophic bacteria and low abundances of Oceanospirillales, *Colwellia*, and *Cycloclasticus* (Kessler et al. 2011). Although the bacterial community in the plume changed rather dramatically over time and space, the archaeal community remained fairly constant (Redmond & Valentine 2012), even though archaea account for a significant fraction of deep nGoM bacterioplankton (King et al. 2013). This is consistent with the fact that, although some evidence indicates that archaea degrade hydrocarbons (e.g., Al-Mailem et al. 2013), the majority of previous work suggests that many archaeal taxa are either unaffected or adversely affected by oil spills (e.g., Roling et al. 2004, Urakawa et al. 2012).

BTEX: benzene-toluene-ethylbenzene-xylene

To explain microbial community succession in the plume, Valentine et al. (2012) developed a simulation model that combined horizontal circulation and mixing in the deep Gulf of Mexico with hydrocarbon inputs, which fed bacterial metabolism and growth. The model output suggested that as bacteria were exposed to oil, hydrocarbon-degrading taxa bloomed until their preferential substrate was depleted, at which point they declined in abundance and were replaced by bacteria growing on different substrates. The model also indicated that deepwater circulation near the

MC-252 well resulted in repeated exposures to fresh oil for given parcels of water. Repeated exposure led to increased degradation rates because the standing stock of hydrocarbon degraders was elevated owing to prior exposure.

Changes in the composition and quantity of hydrocarbons over time and space undoubtedly contributed to community succession (Dubinsky et al. 2013). Community structure was correlated with three distinct spill phases: uncontrolled oil flow (April 25–June 4), partial hydrocarbon capture (June 5–July 14), and well shut-in (July 15 onward). During the period of uncontrolled flow, Oceanospirillales and *Pseudomonas* dominated the plume community (Hazen et al. 2010, Mason et al. 2012, Dubinsky et al. 2013). Redmond & Valentine (2012) and Dubinsky et al. (2013) found that, during the period of partial hydrocarbon capture, the community shifted to dominance by *Colwellia*, *Cycloclasticus*, *Pseudoalteromonas*, *Methylomonas*, and *Thalassomonas*. After well shut-in, Flavobacteria (*Tenacibaculum* and *Polaribacter*), Alteromonadaceae, and Rhodobacteraceae dominated the community at plume depths in the persistent dissolved oxygen anomaly (Dubinsky et al. 2013). These groups have been reported to degrade high-molecular-weight organics and dissolved organic matter (DeLong et al. 1993, Kirchman 2002, McCarren et al. 2010, Teeling et al. 2012). Thus, many of the populations that proliferated after well shut-in most likely scavenged organic matter and cell biomass from the decaying hydrocarbon degrader bloom.

4.2. Functional Responses of Deepwater Plume Microbial Communities to Hydrocarbons

Microarray- and sequencing-based approaches revealed dramatic increases in the presence and expression of genes related to hydrocarbon metabolism within the plume (Lu et al. 2012, Mason et al. 2012, Rivers et al. 2013). Microarrays showed that gene diversity was greater within the plume than outside of it and that microbial community functional structure differed significantly between plume and nonplume samples (Lu et al. 2012). Microarray data also showed that 1,600 genes involved in hydrocarbon metabolism, including genes for alkane and PAH degradation, occurred at higher abundances within the plume. In addition, microarray analyses revealed strong shifts in biogeochemical cycling genes, including assimilatory nitrogen reduction, ammonia assimilation, and sulfate reduction (*dsrA/B* and *AprA*) genes that were enriched in plume samples. A large number of cytochrome *c* genes were also observed, suggesting that hydrocarbon-coupled metal reduction may have occurred in the deep-sea plume. Finally, genes encoding proteins involved in organic phosphorus release [exopolyphosphatase (ppx) for inorganic polyphosphate degradation and phytase for phytate degradation] were stimulated in the plume, which might have been a response to phosphorus limitation.

Metagenomic data for the plume during the latter part of the uncontrolled flow period revealed all of the genes required for *n*-alkane degradation and a nearly complete cyclohexane degradation pathway (Mason et al. 2012). Alkane 1-monooxygenase genes (*alkB*) were also identified throughout the water column in samples taken before the spill (Smith et al. 2013) and by microarray analyses of plume samples (Lu et al. 2012). In addition, metatranscriptomic analyses showed that *alkB* sequences accounted for a modest fraction ($>10^{-4}$) of gene transcripts overall (Rivers et al. 2013). Although these genes have been assumed to reflect alkane degradation, some uncertainty exists about their function and substrate specificity (Smith et al. 2013).

Genes for PAH degradation were also enriched within the plume. However, they accounted for a much smaller relative abundance than genes for alkane degradation. This is consistent with the composition of the oil, which contained <2% PAHs (Reddy et al. 2012). These results also suggest that PAHs were not preferentially degraded relative to alkanes with small to intermediate chain lengths.

Gene transcripts mapping to genes of the dominant Gammaproteobacteria that responded to the spill were enriched by up to two orders of magnitude in the plume relative to samples taken outside of it (Rivers et al. 2013). However, expression analyses based on comparisons of gene and gene transcript abundance indicated that a large number of taxa within the plume were unaffected by the presence of oil, and only a small percentage (5%) appeared to have been inhibited (Rivers et al. 2013). These findings suggested that much of the natural background community existed within the plume at their prebloom levels and that only a few responded positively or negatively.

To better understand the characteristics of the Oceanospirillales that dominated plume samples early during the spill (Hazen et al. 2010), genome sequences were obtained from single cells with >95% 16S rRNA gene similarity to the dominant OTU observed by Mason et al. (2012) and 99% similarity to the Oceanospirillales clone observed by Redmond & Valentine (2012). Based on 16S rRNA gene sequences, the closest cultured representatives to these single cells were *Oleispira antarctica* (Yakimov et al. 2003, Kube et al. 2013) and *Thalassolituus oleivorans* (97% for both). The single-cell genomes encoded cyclohexane degradation and alkane degradation genes similar to those found in metagenomes and metatranscriptomes from plume samples. The single-cell genomes also contained genes for chemotaxis and nutrient acquisition, which suggested that some hydrocarbon degraders sense and swim toward oil droplets. Results from synchrotron radiation-based Fourier-transform infrared spectromicroscopy provided additional support for this possibility (Mason et al. 2012).

4.3. Dispersant Effects

To better understand dispersant effects on oil biodegradation in the Gulf of Mexico and to determine the potential for dispersant degradation, Baelum et al. (2012) assessed enriched microbial communities in water samples amended with high concentrations of oil and dispersants (100 ppm oil and 60 ppm Corexit EC9500A). Uncontaminated water from 1,100 m was treated with a combination of oil, Corexit EC9500A, and iron and incubated at 5°C. Oil degradation was measured as losses of total petroleum hydrocarbons. Twenty-five percent of the dissolved oil was degraded in the oil-only microcosms in the first 5 days, but not much more oil degradation was observed in the following 16 days. By contrast, 60% of the oil was degraded in samples with oil plus dispersant after 21 days. This demonstrated that even at very high concentrations, dispersants likely did not inhibit oil biodegradation in the deep waters of the Gulf of Mexico. Although this study reported only total petroleum hydrocarbon loss, the authors attributed changes predominantly to *n*-alkane degradation.

The fate of dispersants in the nGoM remains a concern because persistent concentrations of dispersant components were found at plume depths after dispersant application had ceased (Oper. Sci. Advis. Team 2010, Kujawinski et al. 2011, Chakraborty et al. 2012). Corexit EC9500A comprises three major components: hydrocarbons (50%), glycols (40%), and the anionic surfactant dioctyl sodium sulfosuccinate (DOSS) (10%). Baelum et al. (2012) showed that the hydrocarbon fraction of Corexit EC9500A degrades rapidly in microcosms with nGoM water from 1,100 m, whereas glycols and DOSS are metabolized very slowly. By contrast, glycols were degraded rapidly by sediment collected near the MC-252 wellhead (Mason et al. 2014). Baelum et al. (2012) also observed the formation at later time points of large flocs in oil- and oil-plus-dispersant-containing microcosms. These flocs were composed of degraded hydrocarbons and macromolecules of microbial origin, such as proteins and extracellular polymeric substances. *Colwellia* constituted more than 70% of the floc community, and Methylococcaceae constituted an additional 16%.

Campo et al. (2013) compared the degradation of dispersed oil in surface water at 25°C with that in water from 1,240 m at 5°C. They used a lower dispersant-to-oil ratio than Baelum et al.

(2012) (1:25 compared with 1:1.67). Campo et al. (2013) found that >99% of the DOSS was degraded after 8–14 days at 25°C, but at lower temperatures (5°C), there was a 28-day lag before degradation was observed. In the absence of crude oil, 98% of DOSS was degraded at 5°C after 42 days; in the presence of crude oil, only 61% of the DOSS was degraded at 5°C after 42 days. Campo et al. (2013) also observed that alkane degradation rates for South Louisiana crude oil were much faster at 25°C than at 5°C, and that Corexit accelerated degradation at 25°C. However, 7% of the alkanes remained after day 42 of the 5°C incubations, regardless of the presence of Corexit.

By contrast, PAH degradation occurred only after 42 days at 25°C with or without Corexit EC9500A, but began after 14 days at 5°C for dispersed crude oil. Campo et al. (2013) concluded that the difference in behavior between alkanes and PAHs at cold temperatures was due to the solubility of the PAHs and the physical state of the alkanes. These studies indicated that oil biodegradation in the Gulf of Mexico was not inhibited by dispersants and that Corexit EC9500A can increase the extent of oil degradation under some circumstances for some hydrocarbon classes. Furthermore, many of the components of Corexit EC9500A appeared to be relatively labile depending on conditions (i.e., temperature and water column or sediment).

4.4. Hydrocarbon Effects on Deep-Sea Sediment Microbial Communities

Oil from the MC-252 well affected microbial communities in deep-sea sediments (Kimes et al. 2013, Mason et al. 2014). Results from metagenomic analyses revealed increases in the functional repertoires of contaminated sediments relative to those of adjacent sediments. Both hydrocarbon and inorganic nitrogen concentrations were important determinants of community structure for surface sediments, in which the dominant organisms were closely related to uncultured Gammaproteobacteria and *Colwellia* OTUs present in the deepwater plume (Mason et al. 2014). Increases in *Colwellia* could have been due to their very broad substrate range, which includes gaseous hydrocarbons (Redmond & Valentine 2012) and PAHs (Gutierrez et al. 2013).

Genes involved in cycloalkane, aromatic hydrocarbon, and BTEX degradation were significantly enriched in metagenomes from contaminated cores, which included a complete pathway for cyclohexane degradation related most closely to the pathway described from *Brachymonas petroleovorans*, an isolate from a petroleum refinery (Mason et al. 2014). Microcosm incubations with ^{14}C-labeled model substrates also showed that propylene glycol (a component of dispersants) was mineralized rapidly by sediment communities (Mason et al. 2014), whereas alkane degradation was faster than toluene and phenanthrene degradation. Thus, at least some components of dispersed oil appeared to be relatively labile in deep-sea sediments.

Metagenomic analyses also indicated that pathways involved in nitrogen cycling were significantly altered in contaminated samples (Mason et al. 2014). Using both metagenomic and geochemical data, Mason et al. (2014) proposed that nitrogen fixation, together with mineralization, might have resulted in higher ammonium concentrations in oil-impacted sediments, and that nitrate might have been consumed via denitrification. Evidence supporting the latter conclusion was drawn from metagenomic annotations of the major genes for denitrification, which might have been the dominant process for nitrogen loss.

Genes and metabolites involved in anaerobic hydrocarbon degradation were identified in a metagenomic study of cores collected near the MC-252 wellhead (Kimes et al. 2013). Many of the reads from these metagenomes were related to sequences from known anaerobic hydrocarbon degraders, e.g., *Desulfatibacillum alkenivorans* AK-01. Thus, sulfate-reducing, hydrocarbon-degrading Deltaproteobacteria (Kniemeyer et al. 2007, Jaekel et al. 2013) likely responded to hydrocarbon inputs, with consequences for both local nitrogen and sulfur cycling (Kimes et al. 2013).

5. HYDROCARBON IMPACTS AND MICROBIAL METABOLISM IN THE NEARSHORE WATER COLUMN, SEDIMENTS, AND BEACHES

PB: Pensacola Beach

During the MC-252 discharge, concern for understudied deep-sea habitats led to a surge of research on offshore systems surrounding the wellhead. By contrast, coastal response efforts centered on economically valuable beaches and ecologically valuable salt marshes (Hayworth et al. 2011, Oper. Sci. Advis. Team 2011, Wang & Roberts 2013). MC-252 oil had a pronounced impact on indigenous, benthic microbial communities in intertidal and inland ecosystems, but much less information is available on the responses of planktonic and subtidal microbial communities on the continental shelf (<200-m water depth).

Although microbial responses to oiling in beach ecosystems were documented extensively prior to 2010, these studies were limited by methodological constraints. The DWH spill is the first major event in which next-generation sequencing approaches have been applied to illustrate with high resolution the dramatic changes in the abundance, structure, and metabolic potential of oiled beach and sediment microbial communities (Kostka et al. 2011, Bik et al. 2012, Newton et al. 2013, Lamendella et al. 2014).

A time-series study conducted at municipal Pensacola Beach (PB), Florida, where total petroleum hydrocarbons reached 11,000 mg kg^{-1}, revealed a bloom of bacteria during the first four months after oil came ashore, with the microbial abundance in oiled sands exceeding that in clean sands by one to four orders of magnitude (Kostka et al. 2011). Geochemical evidence confirmed that bacterial degradation of weathered oil took place concurrently with the formation of the bloom (Aeppli et al. 2012, Ruddy et al. 2014). As observed in planktonic ecosystems offshore (Hazen et al. 2010), Gammaproteobacteria—including many bacterial groups known to degrade hydrocarbons—were enriched in oiled beach sands. The abundance of the known hydrocarbonoclastic groups *Alcanivorax* and *Marinobacter* increased dramatically in response to oil contamination, and a comparison of ribosome abundance in RNA extracts indicated that these groups were active within the bacterial community (**Figure 4**).

Oil acts as a strong selective force on the ecology of indigenous microbial communities in marine ecosystems (Head et al. 2006). For example, it has been hypothesized that nutrient availability

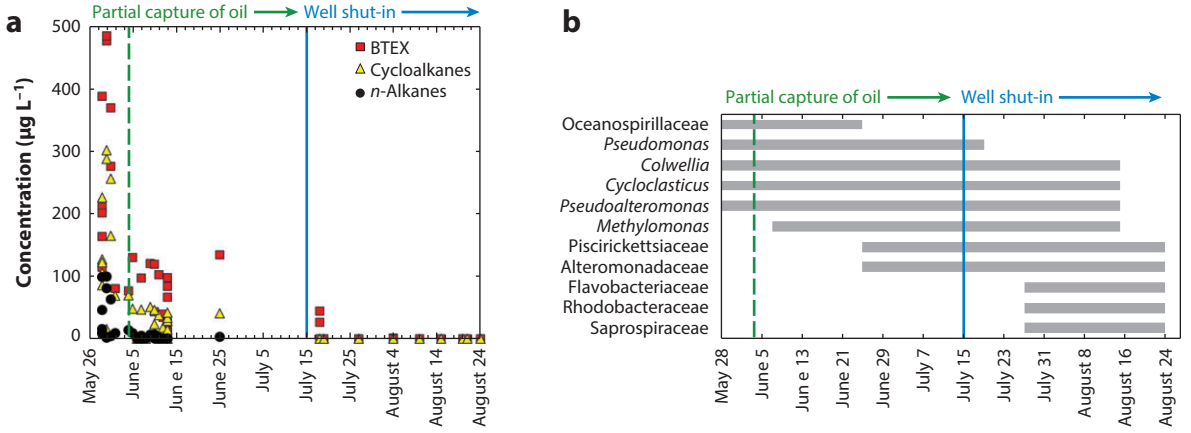

Figure 4

(*a*) Plume hydrocarbon composition [sums of benzene-toluene-ethylbenzene-xylene (BTEX), cycloalkanes, and *n*-alkanes] as a function of sampling date after the initiation of oil discharge. (*b*) Changes in dominant members of plume bacterioplankton communities as a function of sampling date after the initiation of oil discharge. Adapted from Dubinsky et al. (2013).

controls selection for different strains or ecotypes of *Alcanivorax*, which have been proposed to outcompete other bacteria for hydrocarbons by utilizing both branched- and straight-chain alkane compounds (Head et al. 2006). Analyses of PB sands and sediment have supported the hypothesis that alkane degradation is a species-specific trait, because two distinct *Alcanivorax* phylotypes were observed during the early stages of hydrocarbon degradation. Sequence evidence has also pointed to a succession of microbial populations related to the utilization of different hydrocarbon compound classes. Whereas the Oceanospirillales were dominant in sequence libraries within the first few months after oil came ashore, a shift toward the Alphaproteobacteria (Rhodobacteraceae) and gram-positive (*Bacillus*) groups took place in subsequent months. Thus, microbial community succession occurred in both deep nGoM water column and nearshore systems (see also Section 4). Gammaproteobacteria were prominent in early successional stages in all systems, but the mix of taxa varied considerably. In particular, *Alcanivorax* species were significant members of nearshore communities but minor members of the plume community, suggesting that *Alcanivorax* might be more competitive in benthic rather than planktonic systems.

Studies have examined both the taxonomic and functional diversity of microbial communities in a time series of 12 metagenomes constructed from oiled and clean sands sampled at PB in 2010 and 2011. The succession of microbial populations recorded by 16S rRNA gene amplicon sequencing (Kostka et al. 2011) was in general supported by the metagenome results (Rodriguez-R. et al. 2014). For example, taxonomic diversity largely decreased in oiled sands and then rebounded one year after oil came ashore. An increase in the metabolic potential for petroleum hydrocarbon catabolism and nutrient acquisition was also detected in the metagenomes of oiled sands as a large and statistically significant increase in the relative abundances of genes associated with these pathways. In contrast to the results for taxonomic diversity, the metagenomes revealed an increase in functional diversity in response to disturbance by MC-252 oil, consistent with observations in deep-sea sediments (Mason et al. 2012). Moreover, a functional transition was observed from generalist populations (within four months after oil came ashore) to specialists (a year later, when oil was undetectable), thereby supporting the specialization-disturbance hypothesis (Rodriguez-R. et al. 2014).

Studies conducted at other beaches over the same period in 2010 generally corroborated observations from PB. Lamendella et al. (2014) investigated the impacts of oil contamination on microbial communities at Elmer's Beach in Louisiana. They observed successional changes in the microbial populations in parallel with partial degradation of the oil present and reported elevated abundances of Alpha- and Gammaproteobacteria. Metatranscriptome results revealed that PAH, *n*-alkane, and toluene degradation genes were expressed in oiled sediments (Lamendella et al. 2014). Whereas *Alcanivorax* dominated in oiled sands at PB, *Marinobacter* dominated over the active hydrocarbon-degrading microbial communities at Elmer's Beach. In addition, Newton et al. (2013) searched for microbial indicators of ecosystem disturbance in microbial communities at seven beaches from Mississippi to Florida. Although the presence of oil appeared to be outweighed by the influence of other selective forces on community patterns, the relative abundances of known hydrocarbon degraders (e.g., *Alcanivorax*, *Alteromonas*, and *Marinobacter*) increased in oiled sands, consistent with the results of other studies.

The warm, shallow waters of the nGoM shelf represent a favorable environment for bacterial growth, as evinced by the fact that many of the predominant microbial groups detected in oiled sands by gene sequencing could be cultivated in the laboratory. Isolates were obtained from a variety of hydrocarbon-oxidizing genera (*Alcanivorax*, *Acinetobacter*, *Bacillus*, *Marinobacter*, and *Pseudomonas*), and the cultivation of multiple strains of the same species (*Alcanivorax dieselolei*, *Marinobacter hydrocarbonoclasticus*, and *Marinobacter vinifirmus*) will allow for further testing of the role of genotype in biodegradation (Hamdan & Fulmer 2011, Kostka et al. 2011). Initial physiological testing of oil-degrading strains indicated niche specialization in carbon and major

nutrient metabolism (Kostka et al. 2011). Whereas *Acinetobacter*, *Marinobacter*, and *Vibrio* strains all utilized a fairly broad range of carbon substrates, the substrate range of *Alcanivorax* was relatively limited, corroborating previous work (Yakimov et al. 2007).

Overholt et al. (2013) further explored the relationship between genotype and metabolic potential by sequencing the genomes of 10 bacterial isolates from oiled PB sands. Based on comparisons of genome assemblies and numbers of hydrocarbon degradation genes in strains of the same genus (*Alcanivorax* or *Marinobacter*), they proposed strain-specific differences in hydrocarbon degradation potential. In addition, they described the genome of the first known *Labrenzia* (Alphaproteobacteria) isolate capable of growth on crude oil. Multiple studies of oil-contaminated marine environments have detected environmental sequences with high sequence identity to *Labrenzia*, and closely related isolates have been shown to degrade hydrocarbons in the laboratory (Lai et al. 2010, Kostka et al. 2011, Al-Awadhi et al. 2012, Liu & Liu 2013). Collectively, these results advance understanding of how native microbial communities respond to crude oil perturbation in beach ecosystems and provide biomarkers for the chemical evolution of oil hydrocarbons during degradation and weathering.

Clearly, hydrocarbons act as a strong selective force on nearshore sedimentary microbial communities in the nGoM. However, in addition to serving as a carbon and energy source, some components of crude oil and dispersants used during response efforts might adversely affect the ecosystem services provided by specific microbial groups. Hamdan & Fulmer (2011) enriched for hydrocarbon-degrading bacteria in oiled sands collected from the Louisiana shoreline closest to the DWH wellhead, using a minimal medium supplemented with hexadecane and the dispersant Corexit EC9500A. Somewhat surprisingly, Corexit was toxic to hydrocarbon-degrading bacteria in a dose-dependent manner. For example, pure cultures of *Marinobacter* were particularly sensitive, showing a nearly 100% reduction in viability as a result of exposure to concentrations likely to be encountered during the DWH spill. In addition, Urakawa et al. (2012) and Radniecki et al. (2013) reported that oil hydrocarbons and Corexit EC9500A inhibit nitrifying bacteria and archaea. However, it should be noted that these studies used levels of Corexit that greatly exceed concentrations that were detected in seawater during DWH response efforts (Kujawinski et al. 2011). Further studies are needed to determine whether toxic effects observed in the laboratory can be extrapolated to responses in situ.

Nearly all previous work on the microbial response to the DWH spill has targeted bacteria and archaea. However, microbial eukaryotes, specifically fungi, are known to degrade hydrocarbons (Head et al. 2006), and fungal communities appeared to thrive at the expense of metazoans in response to oil contamination from the MC-252 discharge (Bik et al. 2012). Bik et al. (2012) also observed a dramatic change in the community structure of microbial eukaryotes in oiled sediments collected from Louisiana and Alabama beaches from May to September 2010.

Relatively few studies have explored the fate of crude oil–derived hydrocarbons or the impacts of the MC-252 discharge on coastal nGoM planktonic ecosystems. Bianchi et al. (2011) examined bacterioplankton in Barataria Bay, Louisiana, in July and September 2010. Although some Barataria Bay salt marshes had been oiled, the authors found no evidence for MC-252 oil in the water column during the sampling periods and no evidence for bacterioplankton community responses to oil; instead, the bacterioplankton community structure appeared to have been determined largely by the massive freshwater diversion through the bay that had been undertaken in an unsuccessful attempt to limit oiling. Likewise, in spite of predictions in the scientific community and news media that MC-252 oil might lead to blooms of pathogenic *Vibrio parahaemolyticus*, no evidence for such blooms was observed, and Smith et al. (2011) showed that coastal isolates had no capacity to grow using PAHs or oil. By contrast, Tao et al. (2011) showed that pathogenic *Vibrio vulnificus* was substantially enriched in tar balls.

A single study of MC-252 oil impacts on bacterioplankton community structure has been conducted on two surface water and two oil mousse samples collected in the nearshore nGoM off Louisiana (Liu & Liu 2013). As observed in other oiled nGoM environments, Gammaproteobacteria and Alphaproteobacteria (including *Alcanivorax*, *Alteromonas*, and *Marinobacter*) dominated the bacterial communities. Consistent with the PB time series, a high relative abundance of *Rhodovulum* and *Stappia/Labrenzia* (Rhodobacteraceae) was associated with the more weathered oil components of surface mousse, implicating these genera in the degradation of higher-molecular-weight aliphatic and aromatic compounds. In a study of seawater mesocosms, Ortmann et al. (2012) showed that dispersants employed during DWH response efforts short-circuited the planktonic microbial food web. Amendment of dispersant or dispersed oil resulted in an increase in the biomass of heterotrophic prokaryotes but a significant inhibition of ciliates, suggesting reductions in grazing and in the transfer of carbon to higher trophic levels. However, on the continental shelf, where most nGoM elemental biogeochemical cycling occurs, specific impacts of the DWH spill on the ecology (abundance, distribution, structure, and activity) of in situ planktonic communities remain largely unknown.

By contrast, using Microtox and QwikLite assays, Paul et al. (2013) showed that seawater samples collected in the northeastern Gulf of Mexico in August 2010 were toxic to indicator bacteria and phytoplankton. These assays also indicated that the degree of toxicity was correlated with total petroleum hydrocarbon concentrations. However, Prince & Parkerton (2014) commented that, in the Paul et al. (2013) study, the reported toxicity and mutagenicity in field samples were limited in extent, and the study provided little evidence of toxicity gradients or causality. Furthermore, Prince & Parkerton (2014) pointed out that particulates in the unfiltered water samples tested by Paul et al. (2013) could have confounding effects on bioluminescence attenuation, which is the basis for toxicity detection.

Isotope geochemistry has provided further evidence for the impacts of petroleum hydrocarbons on nGoM planktonic food webs. Carbon isotope ratios in seawater and particulate organic matter indicate the extent to which petrocarbon enters the food web, because petroleum hydrocarbons are depleted in ^{13}C and ^{14}C (radiocarbon) relative to recent photosynthetically derived carbon. In a study conducted in the nGoM near Mobile Bay, Alabama, Graham et al. (2010) determined the stable carbon isotopic composition in filtered samples of small suspended particles (1–200 μm in diameter) and larger particles (i.e, mesozooplankton, 0.2–2 mm in diameter). Carbon isotope depletion was coincident with the arrival of surface oil slicks to the nGoM, whereas terrestrial carbon sources, which also show a depleted carbon isotope signal, were ruled out.

^{14}C provides an even more sensitive indication of oil incorporation into planktonic food webs because crude oil is essentially ^{14}C free. Chanton et al. (2012) observed that ^{14}C was much more depleted relative to dissolved inorganic carbon in samples from the same site visited by Graham et al. (2010), and there was a linear correlation between the ^{14}C and ^{13}C content of particulates. Furthermore, using a carbon isotope mass balance approach, Chanton et al. (2012) concluded that more methane than petroleum was incorporated into the planktonic food web, suggesting that carbon from methanotrophic bacteria enters the food web through the microbial loop.

6. HYDROCARBON IMPACTS AND MICROBIAL METABOLISM IN COASTAL WETLANDS

The coastlines of the nGoM encompass approximately 15.6 million hectares of wetland habitats that provide critical ecosystem services (Corn & Copeland 2010), including support for a large fraction of US seafood production (Engle 2011, Gulf Coast Ecosyst. Restor. Task Force 2011). Thus, the impacts of oiling remain a major concern. Unlike the more uniform conditions of

shelf and mesopelagic waters, coastal wetlands experience highly variable physical and chemical conditions that alter biogeochemical patterns, primarily through changes in oxidation-reduction regimes (Mitsch et al. 2009). Hydrologic regimes also affect oxygen availability, nutrient levels, salinity, pH, and temperature, which in turn affect patterns and rates of microbial metabolism and hydrocarbon degradation. In this context, it is notable that a large fraction of wetland soil is anoxic, because hydrocarbon degradation proceeds at a much faster rate under oxic conditions than it does under anoxic conditions.

Although various forms of oil (e.g., mousse and tar balls) heavily affected numerous nGoM marshes, surprisingly little is known about microbial responses to the impact in these areas. We consider here the effects of oiling on saline, brackish, and freshwater marshes of the nGoM dominated by *Spartina alterniflora*, *Juncus roemarianus*, *Avicennia germinans*, *Phragmites australis*, and *Sagittaria lancifolia* (Beazley et al. 2012, Mendelssohn et al. 2012, Natter et al. 2012, Tao & Yu 2013).

The DWH spill affected nGoM wetlands from Louisiana to Florida, with most of the impacts occurring in Louisiana (60.6% of the total; oiled shorelines in Florida, Mississippi, and Alabama accounted for 16.1%, 14.6%, and 8.7% of the total, respectively). Approximately 690 km of marsh shoreline was oiled, with more than 280 km (41%) receiving heavy or moderate oiling (Michel et al. 2013). In contrast to the *Exxon Valdez* and Ixtoc I spills in other regions, oil arriving in nGoM wetlands was extensively weathered by a combination of physical, chemical, and biological processes (Beazley et al. 2012, Mendelssohn et al. 2012, Reddy et al. 2012, Anderson 2013). Weathered oil, mousse, tar mats, and tar balls reported at multiple locations in Alabama, Florida, Louisiana, and Mississippi were characterized by increases in heavier (less volatile) oil fractions (Beazley et al. 2012, Natter et al. 2012). For example, tar mats and tar balls collected from Point aux Pins, Alabama, and Bay Jimmy, Louisiana, contained increased relative abundances of long-chain hydrocarbons (elevated C_{17}–C_{30} and elevated C_{23}–C_{35}, respectively) (Beazley et al. 2012, Natter et al. 2012). They were also enriched in asphaltenes, resins, and complex PAHs. For example, oil mousse collected from Marsh Point, Mississippi, contained increased abundances of C_{14}–C_{38} alkanes and PAHs dominated by the four-ring chrysene. The ratio of phenanthrene to chrysene (0.1) indicated that physical, chemical, and biological processes degraded the lighter components of the crude oil.

Weathered oil remnants are considered more toxic than unweathered emulsions, and they persist for much longer (i.e., decades) owing to their degradation resistance. Increased toxicity can be attributed in part to the presence in tar balls of environmentally persistent free radicals. For example, Kiruri et al. (2013) reported a known asphaltene radical species and a newly discovered radical species (Kiruri et al. 2013) in Gulf Coast tar balls. These observations are important because in aqueous systems, environmentally persistent free radicals generate potent reactive oxygen species that can cause damage to a wide range of eukaryotic cellular systems. The impacts on bacteria and hydrocarbon degradation are uncertain, however.

The responses of wetland microbial communities to oil contamination depend on numerous factors, including the physical state of the oil (i.e., weathered), temperature, salinity, nutrient and oxygen availability, and presence of hydrocarbonoclastic microbes (Atlas 1991, Venosa & Zhu 2003, Head et al. 2006, DeLaune & Wright 2011). However, what distinguishes coastal wetlands from other aquatic systems is the presence of vegetation, sediment deposition, and recurring inundation by saline and sometimes fresh water, all of which can collectively contribute to either enhanced oil degradation or subsurface oil sequestration (Lin & Mendelssohn 1998, Hester & Mendelssohn 2000, Beazley et al. 2012).

Previous field and mesocosm studies have determined that the effects of nutrient addition on heavy crude oil biodegradation in salt marshes depend on the type of nutrient used, the

temperature, and the inundation frequency of the site (Wright et al. 1997, Jackson & Pardue 1999). Wright et al. (1997) demonstrated that an average of 56% of oil hydrocarbons were degraded during winter (17–30°C), whereas 72% were degraded in comparable treatments in summer (27–42°C). The increased degradation in summer was naturally attributed to increased microbial metabolism (Leahy & Colwell 1990), but increases in temperature also reduce crude oil viscosity, which can enhance bioavailability and degradation rates. Nonetheless, even in the most active remediation treatments, 30% of the oil remained (Wright et al. 1997). In a more recent study, Tate et al. (2011) showed that nutrient additions had little effect on crude oil degradation in a Louisiana marsh, largely because the background pore water ammonium concentrations were high enough to preclude nitrogen limitation.

Previous studies have also shown that oil can persist for decades in wetlands (Oudot & Chaillan 2010), and results from a recent study by Natter et al. (2012) indicated that elevated levels of organic carbon in soils and pore water are likely to persist in heavily oiled Louisiana marshes. Although high levels of oil contamination were observed in Louisiana salt marsh sediments, oiling was generally restricted to the periphery of the marsh (within 10 m of the shoreline) (Silliman et al. 2012). Lighter hydrocarbons were rapidly degraded by microbes in marsh sediments, whereas heavier fractions persisted (Natter et al. 2012). Natural-abundance ^{14}C analysis of microbial phospholipid fatty acids provided direct evidence for biodegradation and the incorporation of petrocarbon into microbial biomass at impacted sites several months after oil intrusion, when the highest concentrations of oil were present (Mahmoudi et al. 2013). Although relatively minor shifts in overall bacterial community composition occurred in sediments from oiled sites in comparison with reference sites, higher relative abundances of bacterial groups from known hydrocarbon-degrading taxa (Rhodobacterales and Sphingomonadales) were found in impacted sediments (Mahmoudi et al. 2013). Natter et al. (2012) also noted that elevated total organic carbon and dissolved organic carbon were associated with elevated sulfate-reducing bacteria numbers and higher pore water sulfide concentrations, indicating that dissimilatory sulfate reduction likely was coupled directly or indirectly to hydrocarbon degradation.

Hydrocarbon degradation has long been known to occur more rapidly under oxic conditions than it does under anoxic conditions (Ward et al. 1980, Atlas 1981), even though some alternative terminal electron acceptors (e.g., nitrate and sulfate) support the degradation of some hydrocarbons (Kniemeyer et al. 2007, Widdel et al. 2010, Zedelius et al. 2011). The rhizospheres of marsh vegetation, including *Spartina alterniflora*, *Spartina patens*, *Juncus maritimus*, and *Juncus roemarianus* (Lin & Mendelssohn 2009, Ribeiro et al. 2011, Lin & Mendelssohn 2012), can enhance oil degradation by increasing soil aeration and releasing exudates that act as primers (Beazley et al. 2012, Natter et al. 2012, Mahmoudi et al. 2013). Macrofaunal burrows, particularly those of fiddler crabs, can also increase aeration, enhance hydrocarbon biodegradation, and alter bacterial community composition and structure (Gribsholt et al. 2003, Stauffert et al. 2013).

However, at depths below the rooting zone, anoxic conditions prevail, and hydrocarbon turnover depends almost entirely on anaerobic processes. Microcosm studies have shown that mixed electron acceptors, including sulfate and nitrate, significantly enhance anaerobic biodegradation (Boopathy et al. 2012), and Natter et al. (2012) reported moderate increases in sulfate-reducing bacteria numbers in oiled marsh sediment samples obtained from Louisiana. These results are consistent with the fact that numerous sulfate-reducing bacteria degrade hydrocarbons (Bose et al. 2013).

Soil surface samples from wetlands affected by the DWH spill have consistently shown increased relative abundances of bacteria that degrade lighter crude oil components (Beazley et al. 2012, Natter et al. 2012, Mahmoudi et al. 2013). Beazley et al. (2012) reported increases in the relative abundances of hydrocarbon-degrading Proteobacteria, Actinobacteria, and Bacteroidetes

when oil concentrations were highest in Point aux Pins, Alabama, during June and July 2010, shortly after the period of moderate oiling in May and June. In addition, analyses of salt marsh soils indicated that Gammaproteobacteria abundance increased as oil reached field sites in June and that Actinomycetaceae, Dietziaceae, Rhizobiaceae, Xanthobacteraceae, Nocardioidaceae, Erythrobacteraceae, and Aeromonadaceae increased in relative abundance in June and July. Mortazavi et al. (2013) further reported that alkane and PAH degraders such as *Hydrocarboniphaga*, *Pseudomonas*, and *Pseudoxanthomonas* dominated in oil-amended intertidal sandy sediments collected from Dauphin Island, Alabama, and Horel et al. (2012) reported significant increases in hydrocarbon biodegradation rates following the addition of organic matter to these sediments.

Collectively, these studies show that coastal marshes in Alabama harbored indigenous hydrocarbon-degrading populations that contributed to crude oil degradation and natural attenuation. Similarly, Mahmoudi et al. (2013) observed biodegradation in oil-impacted marsh soils from Barataria Bay, Louisiana, as well as increases in the relative abundances of hydrocarbon-degrading Rhodobacterales and Sphingomonadales. Mahmoudi et al. (2013) also showed that the class Dothideomycetes dominated fungal communities in impacted marshes. Previous reports have shown that members of Dothideomycetes are often enriched in polluted environments and are capable of hydrocarbon degradation. For example, Bik et al. (2012) detected two Dothideomycetes genera, *Cladosporium* and *Alternaria*, in beach sediments impacted by MC-252 oil, indicating an important and little-investigated role of fungal populations in natural attenuation in nGoM coastal marshes.

SUMMARY POINTS

1. Based on high-resolution next-generation sequencing technologies, extensive analyses of northern Gulf of Mexico (nGoM) bacterial communities support the hypothesis that known hydrocarbon-degrading taxa, especially Gammaproteobacteria, are ubiquitous and that their response to oil is largely determined by physical-chemical conditions in the surrounding environment. However, the rapid and robust response of nGoM microbial communities to oil might reflect adaptations to the extensive hydrocarbon seepage that occurs there, raising the possibility that the observed responses might be somewhat unusual and not necessarily applicable to other ocean basins.
2. Evidence from the nGoM indicates that, although oil acts as a selective force to stimulate hydrocarbon-degrading bacteria and generalist heterotrophs, ecosystem services may be adversely affected through the toxic effects of oil and dispersants on specific microbial groups.
3. Substantial and rapid changes occurred in nearshore to deep-sea microbial communities affected by oil from the Macondo well in Mississippi Canyon Block 252. In general, populations of Gammaproteobacteria formed blooms, the composition of which shifted over time and space in an ecological succession as the oil discharge rate was reduced and in response to physical-chemical conditions and the evolution of oil composition.
4. Hydrocarbon degraders in the deep-sea plume were dominated by members of the Oceanospirillales (Gammaproteobacteria) that were related most closely to *Oleispira* and by members of *Colwellia*, *Cycloclasticus*, and several methanotrophs. By contrast, members of the ubiquitous hydrocarbon-degrading *Alcanivorax* genus (also Gammaproteobacteria) appeared to be more abundant in coastal systems and in sea surface oil.

5. Dissolved propane might have provided a readily metabolized substrate that fueled blooms of the early hydrocarbon-degrading populations, in essence acting as a primer for biodegradation.
6. Dissolved methane was consumed almost entirely by methanotrophs within the water column, thus limiting exchange across the air-sea interface. Methane and oil consumption collectively accounted for a relatively small oxygen anomaly that dissipated over time.
7. Corexit (a dispersant) likely enhanced oil degradation, and its components were degraded as well. However, Corexit may have affected carbon flow pathways in the water column owing to the inhibition of protozoan grazers, and it might also have inhibited some biogeochemically significant bacterial populations.
8. Hydrocarbon-degrading fungi increased in abundance in oiled salt marshes and beach sands, suggesting that they may contribute significantly to hydrocarbon turnover in some systems. However, the potential roles of fungi in oil remediation are largely unknown.

FUTURE ISSUES

1. To what extent do naturally occurring hydrocarbon inputs potentiate the response of benthic and planktonic microbial communities to marine oil spills?
2. How do the impacts of dispersants on microbial communities and biogeochemical processes vary among systems (e.g., water column versus sediment, salt marshes versus deep sea) and with temperature and nutrient availability?
3. What determines the specific composition of microbial communities that respond to hydrocarbon inputs? To what extent do community compositions reflect stochastic as opposed to deterministic processes?
4. To what extent do low-molecular-mass hydrocarbon gases (C_1–C_5) serve as primers for higher-molecular-mass alkane and polycyclic aromatic hydrocarbon degradation? How does the ratio of hydrocarbon gases to crude oil affect the rates and patterns of crude oil degradation?
5. What determines the rates and patterns of hydrocarbon degradation in mousses and emulsions? Are these forms of oil more or less resistant to decomposition, and to what extent do populations of hydrocarbon degraders within them differ from those in oiled water column or sediment systems?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We collectively acknowledge support from our multiple funders: the National Science Foundation Rapid Response Research (RAPID) program, the Gulf of Mexico Research Initiative (GoMRI),

LSU-GoMRI, and the Department of Energy. We are grateful for the support of our many colleagues, research associates, postdoctoral associates, and graduate and undergraduate students who have participated in numerous field campaigns, conducted laboratory analyses, and contributed substantially to data analysis and interpretation. We also acknowledge the many men and women who work in the Gulf of Mexico's oil and gas fields, their families, and those who lost their lives or were injured during the DWH explosions and sinking.

LITERATURE CITED

Adcroft A, Hallberg R, Dunne JP, Samuels BL, Galt JA, et al. 2010. Simulations of underwater plumes of dissolved oil in the Gulf of Mexico. *Geophys. Res. Lett.* 37:L18605

Aeppli C, Carmichael CA, Nelson RK, Lemkau KL, Graham WM, et al. 2012. Oil weathering after the *Deepwater Horizon* disaster led to the formation of oxygenated residues. *Environ. Sci. Technol.* 4616:8799–807

Al-Awadhi H, Al-Mailem DM, Dashti N, Khanafer M, Radwan SS. 2012. Indigenous hydrocarbon-utilizing bacterioflora in oil-polluted habitats in Kuwait, two decades after the greatest man-made oil spill. *Arch. Microbiol.* 194:689–705

Al-Mailem DM, Eliyas M, Radwan SS. 2013. Bioremediation of oily hypersaline soil and water via potassium and magnesium amendment. *Can. J. Microbiol.* 59:837–44

Anderson CJ. 2013. Degradation and composition of polycyclic aromatic hydrocarbons (PAHs) following oil exposure in experimental salt marshes. *Water Air Soil Pollut.* 224:1608

Atlas RM. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* 45:180–209

Atlas RM. 1991. Microbial hydrocarbon degradation-bioremediation of oil spills. *J. Chem. Technol. Biotechnol.* 52:149–56

Atlas RM, Hazen TC. 2011. Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history. *Environ. Sci. Technol.* 45:6709–15

Baelum J, Borglin S, Chakraborty R, Fortney JL, Lamendella R, et al. 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environ. Microbiol.* 14:2405–16

Beazley MJ, Martinez RJ, Rajan S, Powell J, Piceno YM, et al. 2012. Microbial community analysis of a coastal salt marsh affected by the *Deepwater Horizon* oil spill. *PLoS ONE* 7:e41305

Bianchi TS, Cook RL, Perdue EM, Kolic PE, Green N, et al. 2011. Impacts of diverted freshwater on dissolved organic matter and microbial communities in Barataria Bay, Louisiana, USA. *Mar. Environ. Res.* 72:248–57

Bik HM, Halanych KM, Sharma J, Thomas WK. 2012. Dramatic shifts in benthic microbial eukaryote communities following the Deepwater Horizon oil spill. *PLoS ONE* 76:e38550

Boopathy R, Shields S, Nunna S. 2012. Biodegradation of crude oil from the BP oil spill in the marsh sediments of southeast Louisiana, USA. *Appl. Biochem. Biotechnol.* 167:1560–68

Bose A, Rogers DR, Adams MM, Joye SB, Girguis PR. 2013. Geomicrobiological linkages between short-chain alkane consumption and sulfate reduction rates in seep sediments. *Front. Microbiol.* 4:386

Camilli R, Reddy CM, Yoerger DR, Van Mooy BAS, Jakuba MV, et al. 2010. Tracking hydrocarbon plume transport and biodegradation at Deepwater Horizon. *Science* 330:201–4

Campo P, Venosa AD, Suidan MT. 2013. Biodegradability of Corexit 9500 and dispersed south Louisiana crude oil at 5 and 25 °C. *Environ. Sci. Technol.* 47:1960–67

Chakraborty R, Borglin SE, Dubinsky EA, Andersen GL, Hazen TC. 2012. Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico. *Front. Microbiol.* 3:357

Chanton JP, Cherrier J, Wilson RM, Sarkodee-Adoo J, Bosman S, et al. 2012. Radiocarbon evidence that carbon from the Deepwater Horizon spill entered the planktonic food web of the Gulf of Mexico. *Environ. Res. Lett.* 7:045303

Corn ML, Copeland C. 2010. *Deepwater Horizon oil spill: coastal wetland and wildlife impacts and response*. CRS Rep. Congr. R41311, Congr. Res. Serv., Washington, DC

Crone TJ, Tolstoy M. 2010. Magnitude of the 2010 Gulf of Mexico oil leak. *Science* 330:634

DeHaan CJ, Sturges W. 2005. Deep cyclonic circulation in the Gulf of Mexico. *J. Phys. Oceanogr.* 35:1801–12

DeLaune RD, Wright AL. 2011. Projected impact of Deepwater Horizon oil spill on U.S. Gulf Coast wetlands. *Soil Sci. Soc. Am. J.* 75:1602–12

DeLong EF, Franks DG, Alldredge AL. 1993. Phylogenetic diversity of aggregate-attached versus free-living marine bacterial assemblages. *Limnol. Oceanogr.* 38:924–34

Dubinsky EA, Conrad ME, Chakraborty R, Bill M, Borglin SE, et al. 2013. Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environ. Sci. Technol.* 47:10860–67

Provides an analysis of changes in microbial community composition and function over time resulting from changes in the composition of oil within the plume.

Edwards BR, Reddy CM, Camilli R, Carmichael CA, Longnecker K, Van Mooy BAS. 2011. Rapid microbial respiration of oil from the *Deepwater Horizon* spill in offshore surface waters of the Gulf of Mexico. *Environ. Res. Lett.* 6:035301

Engle VD. 2011. Estimating the provision of ecosystem services by Gulf of Mexico coastal wetlands. *Wetlands* 31:179–93

Graham WM, Condon RH, Carmichael RH, D'Ambra I, Patterson HK, et al. 2010. Oil carbon entered the coastal planktonic food web during the Deepwater Horizon oil spill. *Environ. Res. Lett.* 5:045301

Gribsholt B, Kostka JM, Kristensen E. 2003. Impact of fiddler crabs and plant roots on sediment biogeochemistry in a Georgia saltmarsh. *Mar. Ecol. Prog. Ser.* 259:237–51

Gulf Coast Ecosyst. Restor. Task Force. 2011. *Gulf of Mexico regional ecosystem restoration strategy.* Rep., Gulf Coast Ecosyst. Restor. Task Force. **http://www.epa.gov/gulfcoasttaskforce/pdfs/GulfCoastReport_Full_12-04_508-1.pdf**

Gutierrez T, Singleton DR, Berry D, Yang T, Aitken MD, Teske A. 2013. Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP. *ISME J.* 7:2091–104

Hamdan LJ, Fulmer PA. 2011. Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. *Aquat. Microb. Ecol.* 63:101–9

Provides an initial metagenomics-based analysis of bacterial responses to oil in the plume and documents novel Oceanospirillales as major members of the hydrocarbon-degrading communities.

Harayama S, Kasai Y, Hara A. 2004. Microbial communities in oil-contaminated seawater. *Curr. Opin. Biotechnol.* 15:205–14

Hayworth JS, Clement TP. 2011. BP's Operation Deep Clean—could dilution be the solution to beach pollution? *Environ. Sci. Technol.* 45:4201–2

Hayworth JS, Clement TP, Valentine JF. 2011. Deepwater Horizon oil spill impacts on Alabama beaches. *Hydrol. Earth Syst. Sci.* 15:3639–49

Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, et al. 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* 330:204–8

Head IM, Jones DM, Roling WFM. 2006. Marine microorganisms make a meal of oil. *Nat. Rev. Microbiol.* 4:173–82

Provides the most recent definitive review of the ecology of aerobic hydrocarbon-degrading microorganisms in marine environments.

Hester MW, Mendelssohn IA. 2000. Long-term recovery of a Louisiana brackish marsh plant community from oil spill impact: vegetation response and mitigating effects of marsh surface elevation. *Mar. Environ. Res.* 49:233–54

Horel A, Mortazavi B, Sobecky PA. 2012. Responses of microbial community from northern Gulf of Mexico sandy sediments following exposure to Deepwater Horizon crude oil. *Environ. Toxicol. Chem.* 315:1004–11

Jackson WA, Pardue JH. 1999. Potential for intrinsic and enhanced crude oil biodegradation in Louisiana's freshwater marshes. *Wetlands* 19:28–34

Jaekel U, Musat N, Adam B, Kuypers M, Grundmann O, Musat F. 2013. Anaerobic degradation of propane and butane by sulfate-reducing bacteria enriched from marine hydrocarbon cold seeps. *ISME J.* 7:885–95

Jernelov A. 2010. The threats from oil spills: now, then, and in the future. *Ambio* 39: 353–66

Joye SB, MacDonald IR, Leifer I, Asper V. 2011. Magnitude and oxidation potential of hydrocarbon gases released from the BP oil well blowout. *Nat. Geosci.* 4:160–64

Kelly J. 2013. *Oily gunk found on Louisiana shore surges three years after BP spill.* Thomson Reuters, Oct. 19. **http://uk.reuters.com/article/2013/10/18/uk-bp-spill-idUKBRE99H0RM20131018**

Kessler JD, Valentine DL, Redmond MC, Du M, Chan EW, et al. 2011. A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. *Science* 331:312–15

Presents an analysis of the fate of methane released into the nGoM and its impact on dissolved oxygen at depth.

Kimes NE, Callaghan AV, Aktas DF, Sunner J, Golding BT, et al. 2013. Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. *Front. Microbiol.* 4:50

King GM, Smith CB, Tolar B, Hollibaugh JT. 2013. Analysis of composition and structure of coastal to mesopelagic bacterioplankton communities in the northern Gulf of Mexico. *Front. Microbiol.* 3:438

Provides the first detailed analysis of nGoM bacterioplankton structure across the shelf and to depths of 1,700 m using samples obtained approximately one month prior to the oil discharge.

Kirchman DL. 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* 39:91–100

Kiruri LW, Dellinger B, Lomnicki S. 2013. Tar balls from Deep Water Horizon oil spill: environmentally persistent free radicals (EPFR) formation during crude weathering. *Environ. Sci. Technol.* 479:4220–26

Kniemeyer O, Musat F, Sievert SM, Knittel K, Wilkes H, et al. 2007. Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. *Nature* 449:898–901

Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, et al. 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Appl. Environ. Microbiol.* 77:7962–74

Provides evidence from next-generation sequencing, cultivation, and biogeochemistry indicating that indigenous microbial communities are drastically affected by oil hydrocarbons in beach sands impacted by the MC-252 oil discharge.

Kube M, Chernikova TN, Al-Ramahi Y, Beloqui A, Lopez-Cortez N, et al. 2013. Genome sequence and functional genomic analysis of the oil-degrading bacterium *Oleispira antarctica*. *Nat. Commun.* 4:2156

Kujawinski EB, Kido Soule MC, Valentine DL, Boysen AK, Longnecker K, Redmond MC. 2011. Fate of dispersants associated with the Deepwater Horizon oil spill. *Environ. Sci. Technol.* 45:1298–306

Lai Q, Qiao N, Wu C, Sun F, Yuan J, Shao Z. 2010. *Stappia indica* sp. nov., isolated from deep seawater of the Indian Ocean. *Int. J. Syst. Evol. Microbiol.* 60:733–36

Lamendella R, Strutt S, Borglin SE, Chakraborty R, Tass N, et al. 2014. Assessment of the Deepwater Horizon oil spill impact on Gulf Coast microbial communities. *Front. Microbiol.* 5:130

Leahy JG, Colwell RR. 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* 54:305–15

Lin Q, Mendelssohn IA. 1998. The combined effects of phytoremediation and biostimulation in enhancing habitat restoration and oil degradation of petroleum contaminated wetlands. *Ecol. Eng.* 10:263–74

Lin Q, Mendelssohn IA. 2009. Potential restoration and phytoremediation with *Juncus roemerianus* for diesel-contaminated coastal wetlands. *Ecol. Eng.* 33:85–91

Lin Q, Mendelssohn IA. 2012. Impacts and recovery of the *Deepwater Horizon* oil spill on vegetation structure and function of coastal salt marshes in the northern Gulf of Mexico. *Environ. Sci. Technol.* 46 7:3737–43

Liu Z, Liu J. 2013. Evaluating bacterial community structures in oil collected from the sea surface and sediment in the northern Gulf of Mexico after the *Deepwater Horizon* oil spill. *MicrobiologyOpen* 2:492–504

Lu Z, Deng Y, Van Nostrand JD, He Z, Voordeckers J, et al. 2012. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. *ISME J.* 6:451–60

Lubchenco J, McNutt MK, Dreyfus G, Murawski SA, Kennedy DM, et al. 2012. Science in support of the *Deepwater Horizon* response. *Proc. Natl. Acad. Sci. USA* 109:20212–21

Mahmoudi N, Porter TM, Zimmerman A, Fulthorpe R, Kasozi GN, et al. 2013. Rapid degradation of *Deepwater Horizon* spilled oil by indigenous microbial communities in Louisiana salt marsh sediments. *Environ. Sci. Technol.* 47:13303–12

Mason OU, Hazen TC, Borglin S, Chain PS, Dubinsky EA, et al. 2012. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *ISME J.* 6:1715–27

Documents both metagenomic and metatranscriptomic responses of microbial communities in the plume and provides insights from single-cell sequencing of the dominant members.

Mason OU, Scott NM, Gonzalez A, Robbins-Pianka A, Bælum J, et al. 2014. Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *ISME J.* 8:1464–75

McCarren J, Becker JW, Repeta DJ, Shi YM, Young CR, et al. 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc. Natl. Acad. Sci. USA* 107:16420–27

McNutt MK, Camilli R, Crone TJ, Guthrie GD, Hsieh PA, et al. 2011. Review of flow rate estimates of the *Deepwater Horizon* oil spill. *Proc. Natl. Acad. Sci. USA* 109:20260–67

Mendelssohn IA, Andersen GL, Baltz DM, Caffey RH, Carman KR, et al. 2012. Oil impacts on coastal wetlands: implications for the Mississippi River Delta ecosystem after the *Deepwater Horizon* oil spill. *BioScience* 626:562–74

Mezic I, Loire S, Fonoberov VA, Hogan P. 2010. A new mixing diagnostic and Gulf oil spill movement. *Science* 330:486–89

Michel J, Owens EH, Zengel S, Graham A, Nixon Z, et al. 2013. Extent and degree of shoreline oiling: *Deepwater Horizon* oil spill, Gulf of Mexico, USA. *PLoS ONE* 86:e65087

Mitsch WJ, Gosselink JG, Anderson CJ, Zhang L. 2009. *Wetland Ecosystems*. Hoboken, NJ: Wiley & Sons

Mortazavi B, Horel A, Beazley MJ, Sobecky PA. 2013. Intrinsic rates of petroleum hydrocarbon biodegradation in Gulf of Mexico intertidal sandy sediments and its enhancement by organic substrates. *J. Hazard. Mat.* 244:537–44

Natter M, Keevan J, Wang Y, Keimowitz AR, Okeke BC, et al. 2012. Level and degradation of Deepwater Horizon spilled oil in coastal marsh sediments and pore-water. *Environ. Sci. Technol.* 4611:5744–55

Newton RJ, Huse SM, Morrison HG, Peake CS, Sogin ML, McLellan SL. 2013. Shifts in the microbial community composition of Gulf Coast beaches following beach oiling. *PLoS ONE* 89:e74265

Norse E, Amos J. 2010. Impacts, perception, and policy implications of the Deepwater Horizon oil and gas disaster. *Environ. Law Rep.* 11:11058–73

Oper. Sci. Advis. Team. 2010. *Summary report for sub-sea and sub-surface oil and dispersant detection: sampling and monitoring.* Rep., US Coast Guard, US Dep. Homel. Secur., Washington, DC

Oper. Sci. Advis. Team. 2011. *Summary report for fate and effects of remnant oil in the beach environment.* Rep., US Coast Guard, US Dep. Homel. Secur., Washington, DC

Ortmann J, Anders NS, Gong L, Moss AG, Condon RH. 2012. Dispersed oil disrupts microbial pathways in pelagic food webs. *PLoS ONE* 7:e2548

Oudot J, Chaillan F. 2010. Pyrolysis of asphaltenes and biomarkers for the fingerprinting of the Amoco Cadiz oil spill after 23 years. *C. R. Chim.* 13:548–52

Overholt WA, Green SJ, Marks KP, Venkatraman R, Prakash O, Kostka JE. 2013. Draft genome sequences for oil-degrading bacterial strains from beach sands impacted by the Deepwater Horizon oil spill. *Genome Announc.* 1:e01015–13

Paul JH, Hollander D, Coble P, Daly KL, Murasko S, et al. 2013. Toxicity and mutagenicity of Gulf of Mexico waters during and after the Deepwater Horizon oil spill. *Environ. Sci. Technol.* 47:9651–59

Prince RC. 2010. Bioremediation of marine oil spills. In *Handbook of Hydrocarbon and Lipid Microbiology*, ed. KN Timmis, pp. 2617–30. Berlin: Springer

Prince RC, Parkerton TF. 2014. Comment on "Toxicity and mutagenicity of gulf of Mexico waters during and after the Deepwater Horizon oil spill." *Environ. Sci. Technol.* 48:3591–92

Radniecki TS, Schneider MC, Semprini L. 2013. The influence of Corexit 9500A and weathering on Alaska North Slope crude oil toxicity to the ammonia-oxidizing bacterium, *Nitrosomonas europea*. *Mar. Pollut. Bull.* 68:64–70

Reddy CM, Arey JS, Seewald JS, Sylva SP, Lemkau KL, et al. 2012. Composition and fate of gas and oil released to the water column during the *Deepwater Horizon* oil spill. *Proc. Natl. Acad. Sci. USA* 10950:20229–34

Redmond MC, Valentine DL. 2012. Natural gas and temperature structured a microbial community response to the *Deepwater Horizon* oil spill. *Proc. Natl. Acad. Sci. USA* 109:20292–97

Ribeiro H, Mucha AP, Almeida CM, Bordalo AA. 2011. Hydrocarbon degradation potential of salt marsh plant-microorganism associations. *Biodegradation* 22:729–39

Rivers AR, Sharma S, Tringe SG, Martin J, Joye SB, Moran MA. 2013. Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill. *ISME J.* 7:2315–29

Rodriguez-R LM, Overholt W, Huettel M, Kostka JE, Konstantinidis KT. 2014. Succession in the indigenous intertidal sand microbial communities of Florida in response to the Deepwater Horizon oil spill. *ISME J.* In review

Roling WF, de Brito Couto IR, Swannell RP, Head IM. 2004. Response of archaeal communities in beach sediments to spilled oil and bioremediation. *Appl. Environ. Microbiol.* 70:2614–20

Provides a comprehensive analysis of the composition of the oil and gas that were released and the evolution of their composition during transport.

Ruddy BM, Huettel M, Kostka JE, Lobodin VV, Bythell BJ, et al. 2014. Targeted petroleomics: analytical investigation of Macondo well oil oxidation products from Pensacola Beach. *Energy Fuels* 28:4043–50

Ryerson TB, Aikin KC, Angevine WM, Atlas EL, Blake DR, et al. 2011. Atmospheric emissions from the Deepwater Horizon spill constrain air-water partitioning, hydrocarbon fate, and leak rate. *Geophys. Res. Lett.* 38:L07803

Ryerson TB, Camilli R, Kessler JD, Kujawinski EB, Reddy CM, et al. 2012. Chemical data quantify *Deepwater Horizon* hydrocarbon flow rate and environmental distribution. *Proc. Natl. Acad. Sci. USA* 109:20246–53

Silliman BR, van de Koppel J, McCoy MW, Diller J, Kasozi GN, et al. 2012. Degradation and resilience in Louisiana salt marshes after the BP–*Deepwater Horizon* oil spill. *Proc. Natl. Acad. Sci. USA* 10928:11234–39

Smith CB, Johnson CN, King GM. 2011. Assessment of polyaromatic hydrocarbon degradation by potentially pathogenic environmental *Vibrio parahaemolyticus* isolates from coastal Louisiana, USA. *Mar. Pollut. Bull.* 64:138–43

Smith CB, Tolar BB, Hollibaugh JT, King GM. 2013. Alkane hydroxylase gene (*alkB*) phylotype composition and diversity in northern Gulf of Mexico bacterioplankton. *Front. Microbiol.* 4:370

Spier C, Stringfellow WT, Hazen TC, Conrad M. 2013. Distribution of hydrocarbons released during the 2010 MC252 oil spill in deep offshore waters. *Environ. Pollut.* 173:224–30

Stauffert M, Cravo-Laureau C, Jézéquel R, Barantal S, Cuny P, et al. 2013. Impact of oil on bacterial community structure in bioturbated sediments. *PLoS ONE* 86:e65347

Tao R, Yu K. 2013. Nitrate addition has minimal effect on anaerobic biodegradation of benzene in coastal saline salt, brackish and freshwater marsh sediments. *Wetlands* 33:759–67

Tao Z, Bullard S, Arias C. 2011. High numbers of *Vibrio vulnificus* in tar balls collected from oiled areas of the north-central Gulf of Mexico following the 2010 BP *Deepwater Horizon* oil spill. *EcoHealth* 8:507–11

Tate PT, Shin WS, Pardue JH, Jackson WA. 2011. Bioremediation of an experimental oil spill in a coastal Louisiana salt marsh. *Water Air Soil Pollut.* 223:1115–23

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, et al. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* 336:608–11

Urakawa H, Garcia JC, Barreto PD, Molina GA, Barreto JC. 2012. A sensitive crude oil bioassay indicates that oil spills potentially induce a change of major nitrifying prokaryotes from the Archaea to the Bacteria. *Environ. Pollut.* 164:42–45

Valentine DL, Kessler JD, Redmond MC, Mendes SD, Heintz MB, et al. 2010. Propane respiration jumpstarts microbial response to a deep oil spill. *Science* 330:208–11

Valentine DL, Mezić I, Maćešić S, Črnjarić-Žic N, Ivić S, et al. 2012. Dynamic autoinoculation and the microbial ecology of deep water hydrocarbon irruption. *Proc. Natl. Acad. Sci. USA* 109:20286–91

Venosa AD, Zhu X. 2003. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. *Spill Sci. Technol. Bull.* 8:163–78

Wang P, Roberts TM. 2013. Distribution of surficial and buried oil contaminants across sandy beaches along NW Florida and Alabama coasts following the Deepwater Horizon oil spill in 2010. *J. Coast. Res.* 304:144–55

Ward DM, Atlas RM, Boehm PD, Calder JA. 1980. Microbial biodegradation and chemical evolution of oil from the Amoco Spill. *Ambio* 9:277–83

Widdel F, Knittel K, Galushko A. 2010. Anaerobic hydrocarbon-degrading microorganisms: an overview. In *Handbook of Hydrocarbon and Lipid Microbiology*, ed. KN Timmis, pp. 1998–2021. Berlin: Springer

Wright AL, Weaver RW, Webb JW. 1997. Oil bioremediation in salt marsh mesocosms as influenced by N and P fertilization, flooding, and season. *Water Air Soil Pollut.* 95:179–91

Yakimov MM, Giuliano L, Gentile G, Crisafi E, Chernikova TN, et al. 2003. *Oleispira antarctica* gen. nov., sp nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *Int. J. Syst. Evol. Microbiol.* 53:779–85

Yakimov MM, Timmis KN, Golyshin PN. 2007. Obligate oil-degrading marine bacteria. *Curr. Opin. Biotechnol.* 18:257–66

Provides results from a model analysis that uses deep circulation patterns to determine the response patterns of hydrocarbon degraders to multiple exposures to oil.

Yvon-Lewis SA, Hu L, Kessler J. 2011. Methane flux to the atmosphere from the Deepwater Horizon oil disaster. *Geophys. Res. Lett.* 38:L01602

Zedelius J, Rabus R, Grundmann O, Werner I, Brodkorb D, et al. 2011. Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. *Environ. Microbiol. Rep.* 31:125–35

Ziervogel K, McKay L, Rhodes B, Osburn CL, Dickson-Brown J, et al. 2012. Microbial activities and dissolved organic matter dynamics in oil-contaminated surface seawater from the Deepwater Horizon oil spill site. *PLoS ONE* 7:e34816

Annual Review of
Marine Science

Volume 7, 2015

Contents

Errata

An online log of corrections to *Annual Review of Marine Science* articles may be found at http://www.annualreviews.org/errata/marine