Chapter 11
Toward a Predictive Understanding of the Benthic Microbial Community Response to Oiling on the Northern Gulf of Mexico Coast

Joel E. Kostka, Will A. Overholt, Luis M. Rodriguez-R, Markus Huettel, and Kostas Konstantinidis

Abstract Benthic ecosystems often act as a repository for oil contamination that washes ashore or is deposited onto sediments following a major oil spill. Sedimentary microorganisms mediate central ecosystem services on the coast, such as carbon and nutrient cycling, and these services may be adversely impacted by oil perturbation. Thus, during the response to the *Deepwater Horizon* (DWH) oil discharge in the Gulf of Mexico, considerable effort went into characterizing the response of benthic microbial communities to oil deposition on shorelines of the Northern Gulf where oil came ashore. Oil perturbation elicited a pronounced microbial response in coastal ecosystems, altering the abundance, diversity, and community composition of sedimentary microorganisms. Next-generation gene sequencing and metagenomic approaches, which were not available during previous large oil spills, have revolutionized the field of microbiology, providing new insights into the microbial response after the DWH discharge. This review centers on a case study of the fate of oil contamination in Pensacola Beach sands, which sheds light on the mechanisms

---

J. E. Kostka (✉)
Georgia Institute of Technology, Schools of Biological and Earth and Atmospheric Sciences,
Atlanta, GA, USA
e-mail: joel.kostka@biology.gatech.edu

W. A. Overholt
Georgia Institute of Technology, Schools of Biological and Earth and Atmospheric Sciences,
Atlanta, GA, USA

Friedrich Schiller University, Institute of Biodiversity, Jena, Germany

L. M. Rodriguez-R · K. Konstantinidis
Georgia Institute of Technology, School of Civil and Environmental Engineering,
Atlanta, GA, USA
e-mail: kostas.konstantinidis@gatech.edu

M. Huettel
Florida State University, Department of Earth, Ocean and Atmospheric Science,
Tallahassee, FL, USA
e-mail: mhuettel@fsu.edu

S. A. Murawski et al. (eds.), *Scenarios and Responses to Future Deep Oil Spills*,
https://doi.org/10.1007/978-3-030-12963-7_11

of microbially mediated hydrocarbon degradation and the impacts of oiling to ecosystem functions. Analysis of field and laboratory results is discussed along with the technological advances that made these observations possible. Metagenomics enabled the application of ecological theory, thereby building a stronger foundation for the effective prediction of baseline microbial community structure/function and response to oiling. Oil perturbation was shown to resemble a press ecosystem disturbance according to the disturbance-specialization hypothesis. Benthic microbial communities were shown to be resilient, maintained ecosystem functions, and recovered quickly after oil disturbance.

Keywords Sediment · Nearshore · Gulf of Mexico · Microorganisms · Petroleum hydrocarbons · Bacteria · Benthic · Deepwater Horizon

11.1 Introduction

The scale and impacts of the *Deepwater Horizon* (DWH) oil spill in the Gulf of Mexico (GoM) were unprecedented. The DWH disaster represents the largest accidental oil spill into the marine environment in history, discharging 4.1 million barrels of liquid hydrocarbons and 1.7×10^{11} g of natural gas into the deep ocean at ~1500 m water depth (McNutt et al. 2012). The emergency response to the DWH spill was also unique in that it incorporated the application of 7 million liters of chemical dispersants, mainly Corexit 9500 and 9527A, at the sea surface and at the discharging wellhead to prevent oil from reaching sensitive coastal ecosystems (Joye et al. 2016).

Approximately one-half of the oil discharged from the DWH blowout reached the ocean surface (Lubchenco et al. 2012), and an estimated 22,000 tons of weathered oil were transported to coastal ecosystems, where it contaminated approximately 1800 km of shoreline from East Texas to West Florida (Michel et al. 2013; Boufadel et al. 2014). Surfaced oil was either redeposited onto the seafloor of the coastal zone through the formation of marine snow (Passow et al. 2012; Brooks et al. 2015) or washed ashore (Michel et al. 2013). Oil was transported high onto the supratidal zone of beaches by waves and tides associated with storms (Michel et al. 2013), and a portion of the oil was deposited in the intertidal and subtidal zones. Because of the dynamic nature of coastal sediments, storms often resulted in the rapid burial of oil in the sediments. Submerged oil mats (SOMs), tens to hundreds of meters long and up to 20-cm thick, have been reported and likely still exist in the inner shelf of the Northern Gulf (Dalyander et al. 2014; Louchouarn et al. 2011; OSAT 2011). Surface residue balls (SRBs), typically 0.5–5 cm in diameter and containing 5–10% hydrocarbons by weight (Urbano et al. 2013) are washed up every day at northeastern GoM shores. Oil thus persists in submerged sublittoral sediments, tidally wetted intertidal sediments, and dry beach sands. Investigating and modeling the fate of buried oil are thus central for risk assessment associated with these hydrocarbons for environmental and human health, coastal food sources, and economic impacts.

Coastal benthic ecosystems are hotspots of biogeochemical activity that provide critical ecosystem services in the GoM (Huettel et al. 2014). These ecosystems drive the Gulf economy through tourism, provide a physical barrier protecting coastal municipalities from storms, and comprise an important habitat for commercially important fish and shellfish. Biodegradation mediated by indigenous microbial communities is the ultimate fate of the majority of oil hydrocarbon that enters the marine environment (Atlas 1995; Leahy and Colwell 1990; Prince 2010). Available oil biodegradation studies to date were primarily focused on the water column and/or deep-sea sediments, and less is known about the response or adaptation of microbial communities to oiling in nearshore or coastal ecosystems (see recent reviews by King et al. 2015; Joye et al. 2016).

Science is largely driven by technological improvements, and the scientific response to the DWH event illustrates this concept. At the time of the Exxon Valdez oil spill in 1989, environmental microbiology was a relatively nascent field, and the ability to determine the structure and functioning of microbial communities in nature was in its infancy. The DWH event was the first major oil spill that occurred after the advent of next-generation sequencing technologies. Prior to the DWH spill, relatively little baseline information was available from Gulf ecosystems. With newfound capabilities, microbiologists were able to effectively track the impacts of oil on microbial communities, and much progress has been made to determine the response of specific bacterial taxa to oil contamination in impacted Gulf ecosystems (see reviews of King et al. 2015 and Joye et al. 2016). However, beyond this descriptive understanding, very little quantitative information exists on the specific pathways and metabolites employed by microorganisms to break down oil and how the environmental conditions affect biodegradation rates. The majority of previous work on hydrocarbon-degrading bacterial communities in the marine environment has been conducted under enrichment conditions in laboratory microcosms, employing PCR (polymerase chain reaction) amplification and sequencing of SSU (small subunit) rRNA genes. A paucity of information exists on the impacts of oil contamination to the metabolic potential or functioning of indigenous bacterial communities under in situ or natural attenuation conditions. Addressing these knowledge gaps will lead to the identification of robust biomarkers of oil degradation and thereby, improvement in our ability to predict the fate of oil in nearshore ecosystems. Biomarkers that are representative of the different phases/stages in oil degradation, possibly including oil-sensitive organisms, can help site managers to monitor oil degradation and decide on the appropriate actions such as stimulating the biodegradation process (e.g., biostimulation, through manipulation of environmental conditions), with obvious benefits for restoring ecosystem functions and minimizing risk to human and animal health. To enable informed biostimulation of oil degradation, a thorough knowledge of the environmental controls of microbial decomposition processes is prerequisite.

In this chapter, the state of the field and overarching questions on the microbial response to oiling based on a case study conducted in shallow coastal sediments is reviewed. Due largely to their accessibility, coastal ecosystems provide an ideal natural laboratory to investigate the in situ effects of oil discharged from the DWH disaster. This review will center on advances to the field and lessons learned from these investigations.

11.2 Fate of Petroleum Hydrocarbons at Pensacola Municipal Beach

Oil washed ashore on Pensacola municipal beach (PB) in repeated pulses of deposition during a 1-month period from June to July of 2010, with total petroleum hydrocarbon (TPH) concentrations reaching up to ~2 kg per meter of beach (Huettel et al. 2018). Due to high ambient temperatures at this time of the year, stranded oil heated up and pooled over large swathes of the beach surface (Fig. 11.1). Beaches are dynamic environments, and over the course of the next few months, waves and storms acted to bury oiled sand layers deeper into the sediment column, with oiled sand grains and smaller oil particles mixed below the beach surface. By October 2010, the oiled sand layer had reached 70 cm below surface.

Responders of the Unified Area Command as well as stakeholders were concerned about the potential impacts of this buried oil to environmental and human health. Thus, BP initiated cleanup efforts termed “Operation Deep Clean” (ODP) designed to mechanically extract larger oil sand aggregates from beaches in Florida to Louisiana

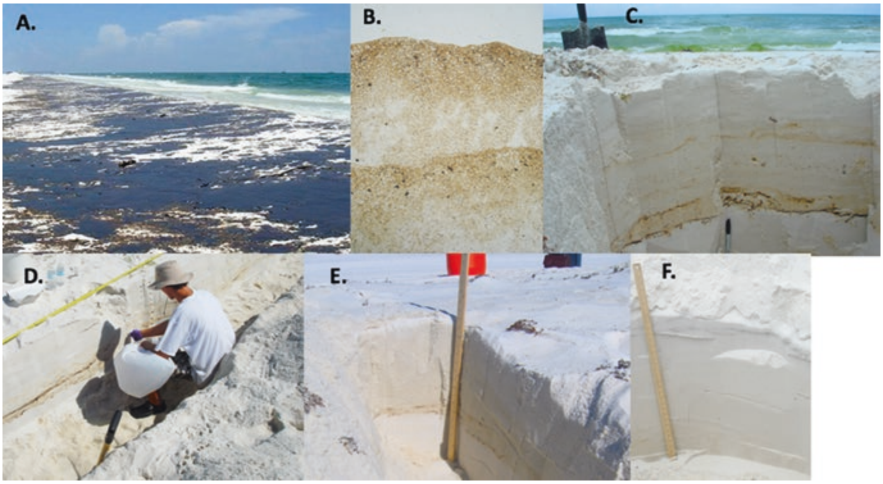

Fig. 11.1 DWH oil contamination at Pensacola Beach/Florida (30°19′32.08″N, 87°10′30.55″W). (**a**) The oil-contaminated beach on June 24, 2010 (image Florida DEP, public domain). (**b**) Oil film and small oil particles deposited on the beach surface by waves on July 1, 2010. The water containing oil drained through the permeable sand, leaving oil at the surface but also transporting small oil particles into the sediment. (**c**) Beach cross section on July 25, 2010, with a dark double layer produced by oil deposited during the nights of June 22 and June 23 at 45–50 cm depth. (**d**) Will Overholt sampling sediment on September 1, 2010, in a trench with a ~10-m long dark vein of buried oil. (**e**) Beach cross section on December 2, 2010, with a homogeneously brown-stained sand layer redeposited by ODC after removal and sifting. Some remains of the deep dark oil layers depicted in (**c**) were still present. (**f**) Beach cross section on April 21, 2011. No visible oil stains were left in the sediment. Gray layers near the surface are detrital material, not oil. (Reprinted from Huettel et al. (2018), Copyright (2018), with permission from Elsevier)

(Hayworth and Clement 2011). Using a machine dubbed the “Sand Shark,” oiled sands were excavated, pulverized, sifted, mixed with clean sands, and redeposited onto the beach. ODC cleaning operations were strictly physical, with no chemical or biological treatment, removing sand to a depth of ~45 cm and separating out particles >2 mm from the sand. Despite this massive cleanup, little quantitative information was collected to track the oil contamination, and therefore the processes controlling buried oil decomposition and decay rates remained largely unquantified. This cleanup activity illustrates a disconnect between the emergency and scientific responses to the DWH disaster.

The fact that the MC-252 discharge represented an unprecedented release of oil in deep water (1500-m water depth; Atlas and Hazen 2011) as well as concerns for understudied deep-sea habitats led to a surge of research on offshore systems surrounding the wellhead (King et al. 2015). By contrast, coastal response efforts somewhat lagged behind studies conducted in the deep sea (Hayworth and Clement 2011). The role of permeable sand microbial communities in mediating ecosystem services was studied, such as carbon and nutrient cycling, in the Northern Gulf for a number of years (Huettel et al. 2014). It was hypothesized that the burial of the oil would trigger blooms of aerobic microbial communities, including hydrocarbon-degrading microbial groups, and these bacterial blooms would rapidly degrade buried petroleum hydrocarbons due to the constant replenishment of oxygen by tidal pumping. Therefore, the objectives of this research were to (1) determine the rates and mechanisms of microbially mediated hydrocarbon degradation, (2) assess the impacts to critical ecosystem functions, and (3) develop microbial indicators for the extent of biodegradation. The assessment of the microbial response closely combined state-of-the-art biogeochemical and microbiological approaches along with leveraging previous research at Pensacola Beach. Field efforts were intense, including sampling at monthly intervals over a nearly 2-year period. This research also represented one of the first large-scale applications of next-generation sequencing technologies to the understanding of microbial community dynamics in Gulf sediments.

Oxygen is a master chemical variable limiting hydrocarbon biodegradation (Head et al. 2006; Prince 2010). Half-lives of petroleum hydrocarbons range from days to months under aerobic conditions, whereas these same compound classes persist for years under oxygen-depleted conditions. Warm, wet, and mostly well-aerated Gulf beach sands in many respects represent ideal environments for microbial growth and metabolism. Field observations supported these assumptions. In the summer and fall of 2010, sediment temperatures ranged from 30 to 40 °C. Most of the oil was stranded in the supratidal zone, where sands maintained a moisture level of 2–7%. In this moist but unsaturated environment, sands remained aerobic, although oxygen concentrations of 50% saturation were measured in layers exposed to heavy oiling (Huettel et al. 2018). Petroleum hydrocarbons (PHCs) were rapidly degraded in beach sediments, with half-lives of aliphatic and aromatic hydrocarbons reaching 25 and 22 days, respectively (Fig. 11.2). The majority of PHCs were depleted within the first 4 months after oil came ashore, and by March of 2011, concentrations were not significantly different from those of pristine reference sites.

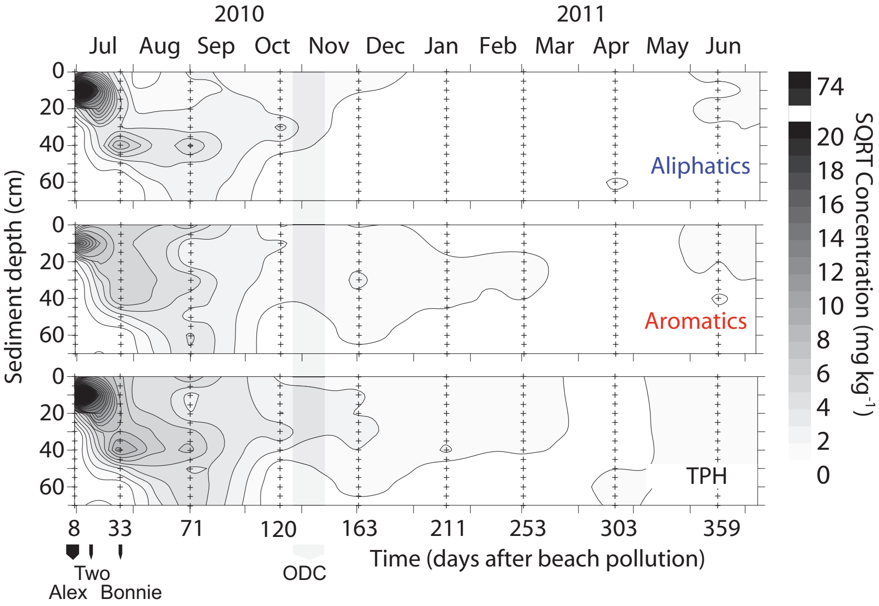

Fig. 11.2 Spatial and temporal distribution of buried oil concentrations in PB sand. Note that the concentrations were square root transformed to accommodate their full ranges. Small "+" symbols indicate sampling dates and depth intervals. The TPH panel depicts total petroleum hydrocarbon concentrations. Black arrows at the lower left (below numbered X-axis) indicate dates and durations of the tropical storms that affected Pensacola Beach. (Reprinted from Huettel et al. (2018), Copyright (2018), with permission from Elsevier)

11.3 Diagnosis of the Oil-Induced Bacterial Bloom Using Next-Generation Sequencing Methods

Prior to 2010, methodological limitations hampered the ability to characterize the dynamics of in situ bacterial communities responding to environmental perturbations such as the presence of oil contamination. The majority of previous work was conducted under enrichment conditions in laboratory microcosms, and microbial communities were investigated through cloning and sequencing of SSU rRNA genes. Little baseline information was available on microbial communities from sedimentary or planktonic environments in the Gulf.

Diagnosis of a large bacterial bloom in response to DWH oiling of PB sands and the subsequent succession of microbial populations were facilitated by next-generation sequencing technologies, mainly in terms of the scale and resolution of analysis. In a matter of months, hundreds of samples could be interrogated for abundance and community composition using PCR-based methods, and thousands of gene sequences per sample supported determination of taxonomic diversity. Herein, a description is provided on how these technological advances were employed in this PB case study.

Initial characterization after the spill supported previous work (Hunter et al. 2006; Mills et al. 2008; Gihring et al. 2009; Huettel et al. 2014) to demonstrate that permeable sands, which cover large portions of the shallow ocean globally, contain highly diverse bacterial communities that are predominated by members of the *Gammaproteobacteria* and *Alphaproteobacteria* (Kostka et al. 2011; Gobet et al. 2012). Microbial communities in Pensacola Beach sands were shown to be more diverse than marine bacterioplankton but not as diverse as the bacterial communities of marine muds. Once improved PCR primers were made available (Caporaso et al. 2010), abundant archaea were also observed in the PB sands (Huettel et al. 2018).

A series of field investigations demonstrated that buried oil at PB was degraded by a succession of hydrocarbon-degrading microbial populations (Kostka et al. 2011; Rodriguez-R et al. 2015; Huettel et al. 2018). Hydrocarbon degradation was concomitant over space and time with a large bacterial bloom (Fig. 11.3). Bacterial abundance increased by two to four orders of magnitude in buried oil layers in comparison to lightly oiled or unoiled sands, and moreover, the depth of the bacterial bloom moved deeper into the beach sand over time in parallel with the residual PHCs. RNA-based analyses as well as cultivation-based studies confirmed that predominant hydrocarbon-degrading bacterial groups were metabolically active in oiled sediment layers. Further, oiling exerted a strong selective pressure on benthic microbial communities, resulting in profound changes to taxonomic diversity and composition. Similar to bacterial abundance, taxonomic diversity declined in parallel with the concentration of PHCs in oiled sands and rebounded as hydrocarbons became depleted (Fig. 11.3). The distribution of microbial populations, soon after oil came ashore, reflected profound changes to community composition. In some cases, known hydrocarbon-degrading microbial groups such as *Marinobacter* comprised over 50% of the microbial community in heavily oiled sand layers (Huettel et al. 2018).

Oil perturbation selected for populations of winners and losers. Over a 1-year period, a succession of microbial populations, dominated by known aerobic hydrocarbon-degrading bacteria, was enriched and then declined concurrently with PHC concentrations. Microbial groups known to degrade aliphatic hydrocarbons, mostly members of the *Gammaproteobacteria* (*Alcanivorax*, *Marinobacter*), responded first to oil contamination and were then replaced after 3 months by populations of *Alphaproteobacteria* (*Hyphomonas*, *Parvibaculum*) capable of aromatic hydrocarbon decomposition (Fig. 11.3). After 1 year, a typical benthic microbial community had reestablished that showed little to no evidence of oil hydrocarbon degradation potential, but it differed significantly from the community present before the oil spill. Observations are generally corroborated by studies conducted at other Gulf beaches over the same period of time. In a study conducted at Elmer's Beach in Louisiana, Lamendella et al. (2014) found that microbial abundance increased by two orders of magnitude in oiled sands, with members of the *Alpha*- and *Gammaproteobacteria* dominating successional changes. Likewise, Newton et al. (2013) reported that members of the *Gammaproteobacteria* (*Alcanivorax*, *Alteromonas*, *Marinobacter*) exhibited the largest increase in relative abundance in oiled sands collected from beaches along the Mississippi, Alabama, and Florida coasts between June and November 2010.

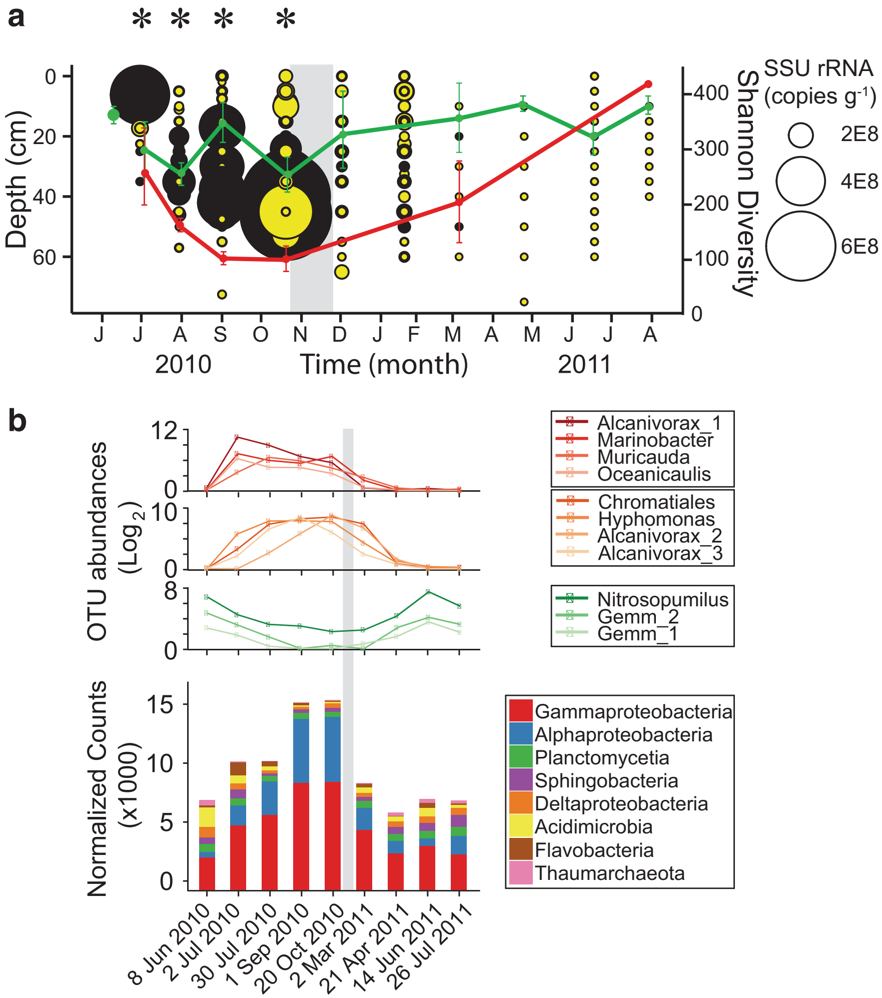

Fig. 11.3 Development of microbial communities in Pensacola Beach sands. (**a**) Abundances of bacterial small-subunit (SSU) rRNA genes in heavily contaminated sand (solid black circles) and in lightly contaminated sand (yellow circles). Asterisks indicate significant difference in SSU rRNA gene copies between heavily and lightly oiled sand (Welch's t-test, $p < 0.05$). The green circle in June 2010 represents diversity at the SGI reference site. The green line depicts the average Shannon diversity in OTU equivalents ($e^{H'}$, secondary y-axis) in the lightly oiled layer, and the red line represents Shannon diversity in the heavily oiled layer. After March 2011 all samples had no visible oil. Error bars represent standard error. (**b**) Time series of the abundances of oil-degrading bacteria that peaked in the first week after beach contamination. Upper line graph: *Marinobacter*, which dominated in June, had already decreased by July 2. Middle line graph: oil degraders that increased 1 week after beach contamination. Lower line graph: microbial groups that declined after oiling. Column graph: microbial community compositions in heavily and lightly oiled sand layers. Normalized counts independently agree with qPCR data shown in (**a**). The June 8, 2010, sample was collected prior to the beach oil contamination. The vertical gray bar in A and B depicts the time frame of ODC. (Reprinted from Huettel et al. (2018), Copyright (2018), with permission from Elsevier)

Clearly, the buried DWH oil enhanced microbial growth and metabolism in coastal sediments by acting as a carbon and energy source to heterotrophic bacteria. Although weathering during transport removed most short-chain (<14 C atoms) PHC from the oil before it was buried in the beach (Huettel et al. 2018), the oil from the Macondo well, a "light Louisiana Sweet Crude," was characterized by relatively high degradability (Reddy et al. 2012). Buried oil thus acted as a strong selective pressure on microbial communities and was rapidly degraded under close to ideal conditions, allowing recovery within 1 year.

11.4 Using Metagenomics to Determine the Functional Response to Oil Contamination

Although findings from next-generation sequencing of rRNA gene amplicons provided important insights on the benthic microbial community response to oiling, the pathways/controls of hydrocarbon degradation and specific impacts to ecosystem function remained mostly unknown. The use of metagenomic techniques catalyzed more fundamental discoveries in these areas.

Researchers generated what remains as the largest metagenomic time series available from the DWH response. This time series encompassed 16 samples, with at least triplicate metagenomes from 4 time points, and over 450 million gene sequences (Rodriguez-R et al. 2015). More importantly, the time course covered from pre-oiling to recovered microbial communities. Overall, metagenomic results supported observations based on next-generation sequencing of SSU rRNA genes (Fig. 11.4). Metagenomes from PB sands were more diverse than bacterioplankton and less diverse than fine-grained soils and sediments. The same abundant groups of known hydrocarbon-degrading bacteria were detected in metagenomes from oiled sands, and shifts in taxonomic diversity closely paralleled observations from studies of SSU rRNA genes. Novel observations were revealed in the functional diversity and metabolic potential of benthic microbial communities.

The pulsed inputs of weathered Macondo oil on PB can be considered as a press disturbance of the ecosystem. The ecological principles controlling the response of biological communities to disturbance were mainly studied previously in plants and animals, and the role of ecological theory in microbial ecology is in its infancy (Prosser et al. 2007; Prosser 2012). Rodriguez-R et al. (2015) posited that the response of PB sand microbial communities to oiling could be predicted by the specialization-disturbance hypothesis following previous observations in plant communities of other ecosystems (Vazquez and Simberloff 2002). This was surprising in that it was contrary to the expectations from most microbiology enrichment experiments. The hypothesis states that the majority of the microbial community is selected based on the ability to survive under disturbed conditions and the response to disturbance correlates negatively with the level of functional specialization. The result is that generalist microbial populations with traits selected by the disturbance, largely hydrocarbon-degrading bacteria in this case,

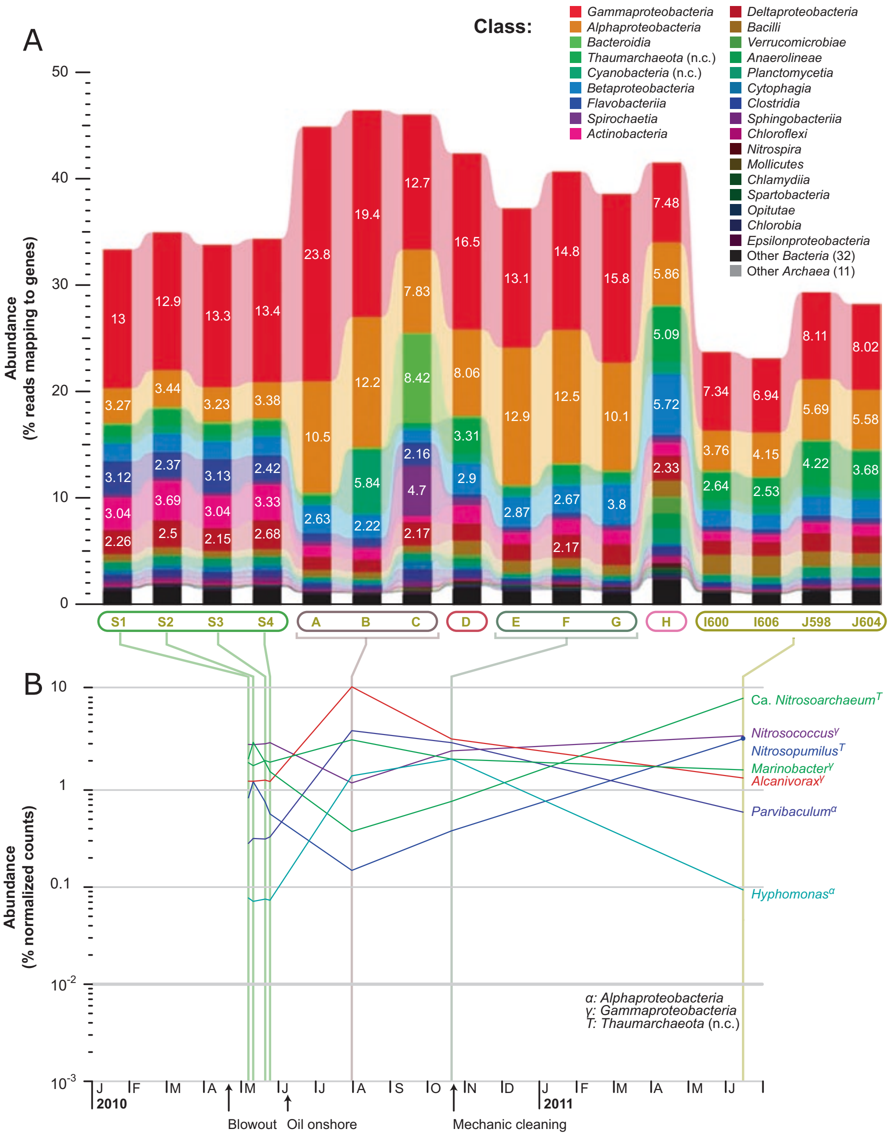

Fig. 11.4 Taxonomic shifts in the microbial community in response to oil. The distribution of metagenomic reads in (**a**) classes displayed for taxa that recruited more than 10% and 2% of the total reads, respectively (white numbers). (**b**) Genera with abundance above 0.1% and significantly different between pre-spill and oiled or between oiled and recovered samples (P-value adjusted, $p < 0.01$) are also displayed. The minimum and maximum abundance of each genus is indicated with open and filled circles, respectively, and the class is indicated with superscripts. (Reprinted by permission from Springer Nature: Rodriguez-R et al. (2015))

become highly abundant. Conversely, specialist taxa, adapted to relatively narrow niches, are selected against. Field observations from our metagenome time series supported the disturbance-specialization hypothesis. In contrast to taxonomic diversity, communities exhibited an increase in functional diversity in oiled samples with respect to pre-oiled samples and a reduction in functional diversity in recovered samples in comparison to oiled samples. It was concluded that specialists adapted to oligotrophic conditions, mainly chemolithoautotrophic nitrifiers, were outcompeted upon oil deposition. Sandy sediments are nutrient poor and would favor functional specialization in their pristine condition. Comprised of tens of thousands of different carbon compounds, oil input would likely stimulate fast-growing copiotrophic generalists during early succession, while toxic compounds would likely select against specialists. Indeed, fast-growing hydrocarbon-degraders (*Pseudomonas*, *Marinobacter*, *Parvibaculum*, *Hyphomonas*) dominated, and generation times calculated from metagenomic data were lower in oiled communities, whereas putative chemolithoautotrophic archaea (*Nitrosopumilus*, *Cenarchaeum*), presumably adapted to carbon-limited conditions, predominated in the pre-oil and recovered communities. Selection against nitrifying taxa suggests that critical ecosystem services provided by the microbial nitrogen cycle may be disrupted by oiling. These observations warrant further investigation under controlled conditions in the laboratory where the functioning of microbial communities can be better constrained.

Given the observations that oiling selected for generalist heterotrophs, the dominance of *Alcanivorax* in oiled sediments throughout these studies is somewhat puzzling. *Alcanivorax* is considered as an obligate hydrocarbonoclastic organism with a narrow range of carbon substrate utilization and therefore does not fit the definition of a generalist. Some evidence indicates that the utilization of PHCs is a strain-specific trait (Head et al. 2006). Perhaps, the *Alcanivorax* genus contains generalists that have not yet been cultivated or the definition of obligate hydrocarbonclastic bacteria requires revision (see below for further discussion). For example, Barbato et al. (2016) reported the capacity of *Alcanivorax* strains to utilize carbon sources alternative to hydrocarbons, which allows them to permanently colonize pristine marine environments. According to these authors, the differential transcriptomic responses to hydrocarbons in the different *Alcanivorax* strains make them a functionally redundant and plastic reservoir of oil-degrading players, able to activate in the case of oil-spill occurrence, sustaining natural attenuation and biostimulation-based cleanup processes.

Our metagenomic time series further revealed the pathways and controls of PHC degradation. Communities of oiled sands contained a statistically significant, higher abundance of gene annotations associated with hydrocarbon degradation in comparison to pre-oiled and recovered communities (Rodriguez-R et al. 2015; Fig. 11.5). Concurrent with the transition from alkane-degrading *Gammaproteobacteria* to *Alphaproteobacteria* capable of utilizing more recalcitrant aromatics, the relative abundance of genes associated with aliphatics degradation peaked in July and dropped from July to October 2010. Conversely, the abundance of genes associated

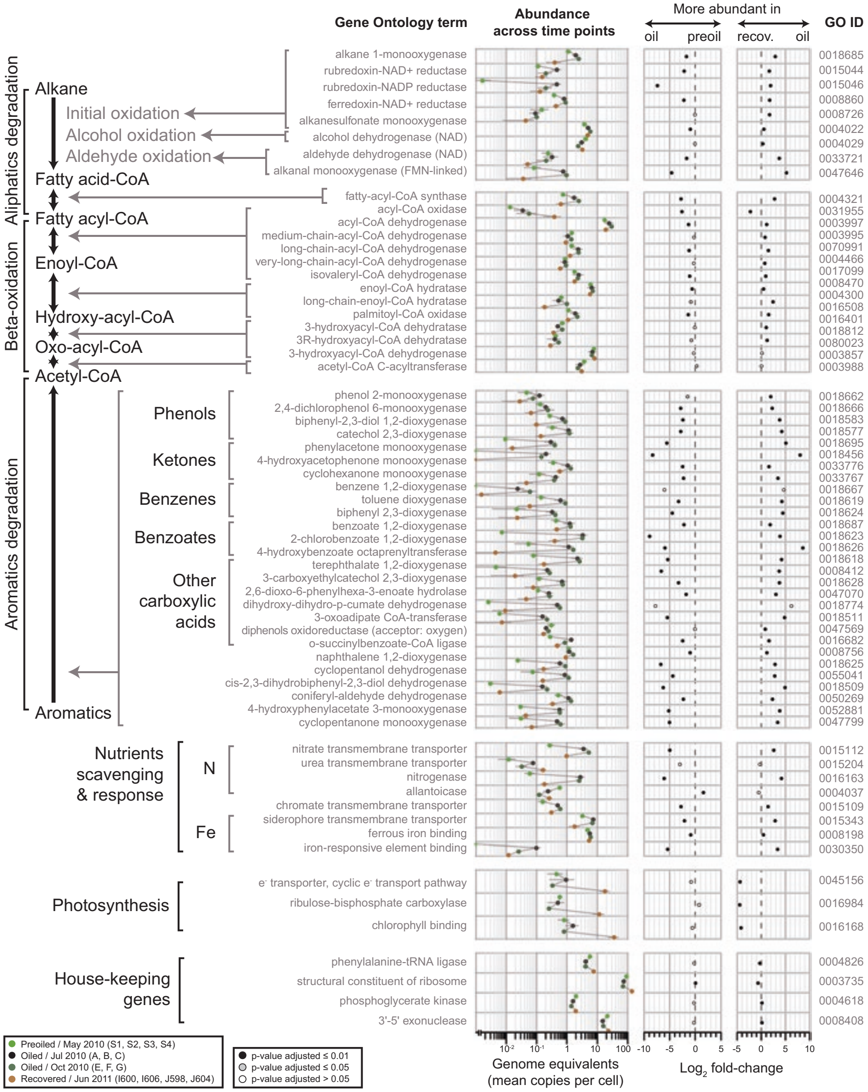

Fig. 11.5 Microbial community functional shifts in response to oil. Selected molecular functions related to hydrocarbon degradation, nutrient scavenging and response, photosynthesis, and some housekeeping genes are listed (left) along with the mean genome equivalents per group of samples (middle) and the log2 of pre-oil/oiled and oiled/recovered fold changes (right). The rightmost column indicates the GO ID of the terms. The abundance was assessed as average genome equivalents (mean copies per bacterial/archaeal cell) on each sampling time (downwards; see legend). The triangles indicate values below the plotted range. The log2-fold-change was estimated as the log2 of the ratio of normalized counts between pre-oiled samples (S1, S2, S3, S4) and oiled samples (A, B, C, E, F, G) and between oiled samples and recovered samples (I600, I606, J598, J604). P-values were estimated using a negative binomial test. (Reprinted by permission from Springer Nature: Rodriguez-R et al. (2015))

with aromatics degradation was maintained or increased from July to October. Genes associated with nutrient acquisition, such as genes encoding nitrogenase that catalyzes the reduction of atmospheric dinitrogen to ammonium, showed elevated abundance in oiled samples. These observations are corroborated by previous studies that indicate oil-contaminated environments are nutrient-limited (Joye et al. 2016). Moreover, the genomes of these key populations have been recovered, and the corresponding sequences are publicly available as part of NCBI's SRA database. These genomes are expected to greatly facilitate future studies such as the design of PCR assays to study in situ gene expression and activity. The great majority of these abundant populations during oiling were shown to represent novel species, if not genera, compared to the already described taxa (Rodriguez-R et al. 2015), indicating that several more mysteries remain to be solved in the not-so-distant future.

A key knowledge gap in oil spill science is the definition of baselines in biological communities. At PB, both SSU rRNA gene amplicon and metagenomic data indicated that after 1 year, microbial communities recovered to a state that resembled the pre-oil condition (Rodriguez-R et al. 2015; Huettel et al. 2018). However, significant differences were observed between the pre-oiled and recovered communities. Taxonomic diversity remained elevated in recovered communities, above that of the pre-oiled community, while functional diversity was lower in the recovered compared to the pre-oiled. Why and how are these recovered communities different? Recovered communities contained higher relative abundances of eukaryotic and archaeal taxa in comparison to pre-oiled and oiled communities. Such differences could be due to unrecognized long-term effects of disturbance, such as the emergence of new taxa, and stochastic environmental events such as changes in nutrient or carbon input. However, they may also be linked to patterns in seasonality and reflect the dynamic nature of beaches rather than long-term impacts. This is an open question that is actively being pursued as a legacy DWH issue. Future work such as laboratory incubations with beach sands that simulate well the in situ environment while circumventing the stochastic fluctuations are expected to offer new insights into the latter issues.

Another open question in the field is whether oil-contaminated environments are "primed" for hydrocarbon degradation. In other words, it has been hypothesized that microbial populations have evolved and adapted to degrade petroleum compounds in environments where these have been introduced on a regular basis, such as in natural hydrocarbon seeps in the Gulf. An alternative hypothesis states that since oil is a natural product and natural organic matter from recently dead plants/animals contains similar compounds (Lea-Smith et al. 2015), microbial populations that degrade petroleum hydrocarbons are ubiquitous in marine and terrestrial ecosystems. To investigate the evolutionary history of hydrocarbon degradation, genes encoding AlkB (alkane hydrolase) were characterized as a marker for alkane degradation. A large diversity of *alkB* genes was observed in PB sands that did not appear to be constrained by oiling. Alkane degradation was mediated by a large latent diversity of alkane-degraders that were already present in the sand prior to oiling, perhaps living off of residual oil or natural organic matter.

11.5 Linking Advances in Metagenomics to Cultivation

Metagenomics approaches have revolutionized the field of microbiology; nevertheless, these methods remain new and they should be employed as a part of a polyphasic approach. To completely verify the metabolism or function of a microorganism, it is necessary to bring that organism into pure culture. This case study demonstrates the need to employ a combination of cultivation-dependent approaches alongside cultivation-independent molecular techniques to fully characterize the microbial response to the input of petroleum hydrocarbons. Pure cultures were obtained for many of the predominant hydrocarbon-degrading genera which were detected in abundance with next-generation sequencing approaches in field samples (Kostka et al. 2011). Overholt et al. (2013) then sequenced the genomes of ten representative strains to facilitate their use as model organisms for further physiological studies in the laboratory. In parallel, Eren et al. (2015) leveraged our metagenomic time series to test a new bioinformatics platform, named Anvi'o, designed to analyze and visualize metagenomic data. Whereas previously published analysis involved assembly of metagenomic sequences without binning (Rodriguez-R et al. 2015), the Anvi'o platform was used to bin the sequences and construct genomes for individual microbial populations. This is now referred to as metagenome-assembled genomes (MAGs). Anvi'o detected a total of 56 MAGs from 33 genera, including a fungus and a cyanobacterium in PB sands. At a broader resolution, the taxonomy of the MAGs largely agreed with the results presented in Rodriguez-R et al. (2015). However, Eren et al. (2015) concluded that model pure cultures such as *Alcanivorax* sp. P2S70, though present in abundance, represented a minority of the hydrocarbon-degraders responding to oil input at PB. Rather, the genomes of uncharacterized members of the *Gammaproteobacteria* and *Alphaproteobacteria* showed the highest relative abundance in oil-impacted sediments. The results imply that characterization targeted to the sequencing of single taxonomic marker genes, such as SSU rRNA, is insufficient for the accurate determination of microbial community dynamics at high resolution. The fact that uncharacterized organisms are dominant indicates that there is much to learn about the physiology of microbial responders to oil spills. The conclusion that many of the important oil-degraders had been cultivated from PB beach turned out to be only partially correct. This calls for more cultivation-based studies directed by information on the putative metabolism of uncharacterized taxa gleaned from MAGs. In addition, whole genomes should be used, and further advances in bioinformatics are needed to fully investigate the metabolic potential of complex microbial communities that respond to oil.

Alcanivorax is arguably the best studied of hydrocarbon-degrading taxa, and the 15 described species of this group are considered as obligate hydrocarbonoclasts, with a narrow range of carbon substrate utilization (Yakimov et al. 2007). While there have been no detailed comparative genomics studies on this group, *Alcanivorax borkumensis* has been extensively studied as a model organism for the genus due to its cosmopolitan distribution, putative ecological role in oil-degrading consortia, and the ability for researchers to manipulate its genetic system (Schneiker et al. 2006;

Shao and Wang 2013; Yakimov et al. 2007). *A. borkumensis* is characterized by a highly restrictive growth profile and contains the features of oligotrophic bacteria with a large number of nutrient transporters that allow a rapid response to hydrocarbons under nutrient poor conditions (Schneiker et al. 2006; Yakimov et al. 2007). *A. borkumensis* grows on a diverse range of aliphatic hydrocarbons including n-alkanes (max n-C_{32}), long-chain isoprenoids, phytane, pristine, and alkyl aromatic-hydrocarbons (Yakimov et al. 2007). Members in this genus have a large number of alkane degradation systems under complex metabolic regulation associated with chemotaxis as well large changes in central metabolic networks (Sabirova et al. 2006, 2011; Schneiker et al. 2006; van Beilen and Funhoff 2007; Wang and Shao 2014; Barbato et al. 2016).

It is not well known how interacting *Alcanivorax* populations respond to hydrocarbons within an impacted community, and these case studies document multiple distinct groups. Of these, multiple investigations pointed to *Alcanivorax* sp. P2S70 as the most abundant characterized strain in oiled PB beach sands, and strain P2S70 showed the highest potential for oil degradation in pure culture studies (Overholt et al. 2016). Initial physiological characterization revealed contrasts between strains isolated from PB sands impacted by the DWH spill, suggesting niche specialization in carbon and major nutrient metabolism (Kostka et al. 2011). Another isolate from oiled PB sands, *Alcanivorax* strain PN3, showed a much lower potential for hydrocarbon degradation in culture. Detailed phylogenetic analysis of full-length rRNA genes shows that strain PN3 shares high sequence identity with *Alcanivorax dieselolei*, whereas P2S70 is more related to *A. nanhaiticus* (Fig. 11.6). Comparison of whole genomes using conserved single copy genes generally supports the interpretation based on SSU rRNA genes, although P2S70 is more closely related to the *A. borkumensis* genomes (Fig. 11.6). Strain PN3 is confirmed to be highly similar to the *A. dieseloli* group, sharing an average nucleic acid identity (ANI) of >99% to *A. xenomutans* and *A. dieseloli* KS-293 genomes as well as an ANI of 93% with *A. dieseloli B5* (the type strain). Moreover, genome sequencing revealed substantial variation in the assembly and number of hydrocarbon degradation genes between *Alcanivorax* strains, suggesting strain-specific differences in metabolism and hydrocarbon degradation potential (Overholt et al. 2013). P2S70 exhibited similar genome streamlining as seen in the *A. borkumensis* genomes with around 3000 genes, while PN3 and all *A. dieselolei* strains have 1 Mb larger genomes with over 4000 genes. Field observations corroborate these genotypic and phenotypic results from the laboratory to indicate that hydrocarbon degradation is a strain-specific trait (Head et al. 2006; Kostka et al. 2011; Huettel et al. 2018). At least three different dominant *Alcanivorax* OTUs were detected in our PB time series with contrasting ecological response strategies. One OTU, similar to P2S70, showed a maximum in abundance during the first few months after oil came ashore when lower molecular weight aliphatic compounds were degraded, whereas other *Alcanivorax* OTUs peaked in abundance later in the time course, concurrent with the depletion of more recalcitrant classes of hydrocarbon compounds. A similar pattern was evident in the Eren et al.'s (2015) study where two MAGs (P2S70 and one similar to *A. jadensis*) responded immediately following oiling and one followed later. However, follow-up

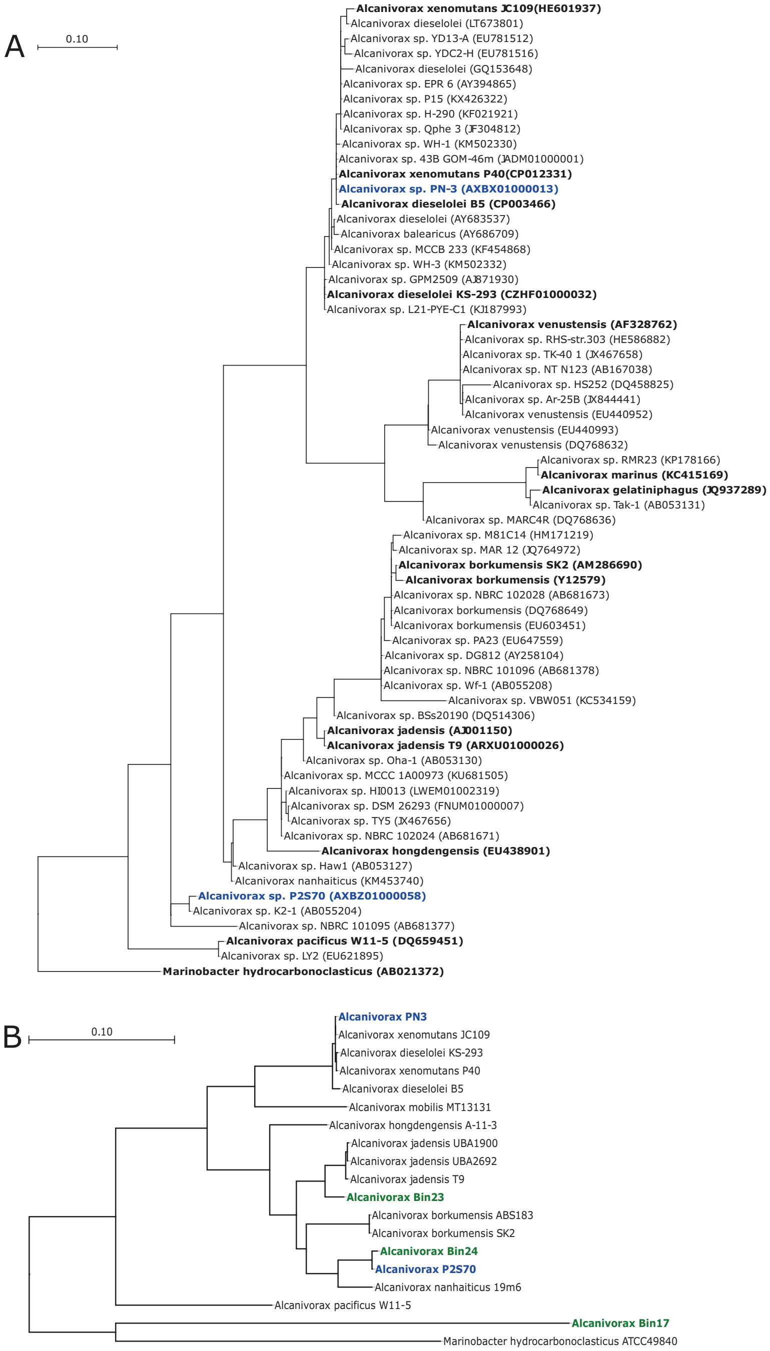

Fig. 11.6 Phylogenetic analysis of the characterized strains of the *Alcanivorax* genus. Isolates from Pensacola Beach are highlighted in blue, and type strains as well as strains with a whole genome available are bolded. (**a**) Full length, bootstrapped, maximum likelihood SSU rRNA gene tree generated from SILVA aligned sequences with the bacterial weighting mask available in ARB. (**b**) Whole genome tree generated according to the approach of Parks et al. (2018) from a concatenated alignment of single copy reference genes used by the Genome Taxonomy Database (Parks et al. 2018). The *Alcanivorax* subtree displayed is congruent with a whole genome tree generated from all orthologous genes (845), as well as a UPGMA dendrogram from average amino acid identities (AAI) across all genomes (data not depicted)

phylogenetic analysis suggests that the late responder (Bin17) is not with the *Alcanivorax* group and is instead affiliated with the order *Cellvibrionales* (Fig. 11.6, MiGA taxonomic assignment not shown). It is concluded that the *Alcanivorax* group exhibits a much larger genotypic and phenotypic plasticity than was previously perceived. *Alcanivorax* strains appear to exploit a larger range of niches, including the ability to metabolize more recalcitrant aromatic or aliphatic compounds along with alkanes.

11.6 Conclusions and Guidance for Future Emergency Response Efforts

Technological advances have enabled tremendous progress in the ability to identify the predominant microbial populations and their succession in response to oil perturbation. The emergence of metagenomics approaches has greatly facilitated the interpretation of the metabolic potential of microbial responders. The research community has a much better idea of "who's there," while it remains difficult to interpret "what they are doing there" and perhaps more importantly, "how fast and under what environmental conditions." At Pensacola Beach, results showed that oiling led to a large increase in the growth and metabolism of indigenous microbes in the form of a bacterial bloom. Oil contamination strongly selected for microbial groups capable of hydrocarbon degradation, leading to a drastic decline in taxonomic diversity, as has been observed in other oiled ecosystems. A succession of microbial populations was observed to parallel the chemical evolution of petroleum hydrocarbons. Oil was degraded, and benthic microbial communities returned to near-baseline levels, with little evidence of hydrocarbon-degrading bacteria, approximately 1 year after oil came ashore. Close coupling of biogeochemistry, next-generation sequencing, and metagenomics approaches provided evidence in support of the disturbance-specialization hypothesis. Surprisingly, the functional diversity of microbial communities increased during active degradation in oiled sand layers, indicating a selection for generalist copiotrophs and against specialist oligotrophs such as nitrifying prokaryotes. These observations comprise promising steps toward using ecological principles to predict the microbial response to major perturbations such as oil spills.

This case study indicates that benthic microbial communities contain a high potential for petroleum hydrocarbon biodegradation. Moreover, these results indicate that even when small oil particles (<1 cm) are buried in the coastal zone, biodegradation by indigenous microbial communities is sufficient for the rapid mitigation of oil contamination after a major spill, in the presence of sufficient levels of oxygen and nutrients. Larger sand-oil aggregates take longer to completely degrade because of their unfavorable surface to volume ratio. "Operation Deep Cleaning" removed these aggregates but also involved biostimulation insofar as many larger oil aggregates were broken down into smaller ones thereby increasing the surface area available for microbial degradation. For environmental managers, these results suggest that petroleum hydrocarbon degradation in beach sands is relatively rapid because oxygen can easily penetrate to the buried oil, and resources may be better placed elsewhere in environments where degradation is limited by oxygen availability or physical access to hydrocarbons. Other remediation strategies such as bioaugmentation, which involves seeding the natural microbial communities with hydrocarbon-degrading bacteria, are unlikely to facilitate a more rapid biodegradation (AAM 2011). Further, specialist microbial groups such as chemolithoautotrophic nitrifiers show promise as microbial bioindicators of oil contamination in coastal marine ecosystems. However, more work is needed to further validate these biomarkers, especially under different environmental conditions and fluctuations.

Despite excellent progress, a predictive understanding remains hampered by challenges in interpreting the in situ activity and ecosystem response of benthic microbial populations. A polyphasic approach is encouraged that employs metagenomics in the field along with cultivation and microcosm or mesocosm experiments in the laboratory to further elucidate impacts to ecosystem function and the in situ metabolism of microbial communities that mediate hydrocarbon degradation. In particular, more information is needed about which microbes mediate degradation of individual classes of hydrocarbon carbon compounds and how oil impacts important ecosystem services provided by microbes, such as organic matter mineralization and nutrient cycling. Interpretation of microbial community dynamics based on SSU rRNA gene sequences alone, which was commonly done during the DWH response, may lead to conclusions that are not sufficiently supported or even incorrect and thus insufficient for predictive modeling. Next-generation sequencing of taxonomic genes is great for initial “range finding” of the microbial community response over large scales. However, amplicon-based studies should be supplemented by metagenomics and comparisons of metagenome-assembled genomes wherever possible. Hypotheses from field observations should be tested by experiments conducted under controlled conditions in the laboratory. Further, research during future disasters would be greatly facilitated by improved coordination between the emergency responders directing mitigation efforts and scientists investigating the success of those efforts.

Funding Information This research was made possible by grants from The Gulf of Mexico Research Initiative through its consortia: The Center for the Integrated Modeling and Analysis of the Gulf Ecosystem (C-IMAGE), Deep Sea to Coast Connectivity in the Eastern Gulf of Mexico (Deep-C). This research was also supported by grants from the National Science Foundation (to MH, JEK, WAO), the Florida Institute of Oceanography (to MH and JEK), and the Northern Gulf Institute (to MH and JEK).

References

American Academy of Microbiology (2011) FAQ: microbes & oil spills. http://www.asmscience.org/content/report/faq?order=date

Atlas RM (1995) Petroleum biodegradation and oil spill bioremediation. Mar Pollut Bull 31:178–182

Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history. Environ Sci Technol 45(16):6709–6715. https://doi.org/10.1021/es2013227

Barbato M, Scoma A, Mapelli F, De Smet R, Banat IM, Daffonchio D, Boon N, Borin S (2016) Hydrocarbonoclastic alcanivorax isolates exhibit different physiological and expression responses to n-dodecane. Front Microbiol 7:2056

Boufadel MC, Abdollahi-Nasab A, Geng X, Galt J, Torlapati J (2014) Simulation of the landfall of the Deepwater Horizon oil on the shorelines of the Gulf of Mexico. Environ Sci Technol 48(16):9496–9505

Brooks GR, Larson RA, Schwing PT, Romero I, Moore C, Reichart GJ, Jilbert T, Chanton JP, Hastings DW, Overholt WA, Marks KP, Kostka JE, Holmes CW, Hollander D (2015) Sedimentation pulse in the NE Gulf of Mexico following the 2010 DWH blowout. PLoS One 10(7):e0132341

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267

Dalyander PS, Long JW, Plant NG, Thompson DM (2014) Assessing mobility and redistribution patterns of sand and oil agglomerates in the surf zone. Mar Pollut Bull 80(1–2):200–209

Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO (2015) Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ 3:e1319

Gihring TM, Humphrys M, Mills HJ, Huette M, Kostka JE (2009) Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. Limnol Oceanogr 54(4):1073–1083

Gobet A, Böer SI, Huse SM, Van Beusekom JE, Quince C, Sogin ML, Boetius A, Ramette A (2012) Diversity and dynamics of rare and of resident bacterial populations in coastal sands. ISME J 6(3):542

Hayworth JS, Clement TP (2011) BP's operation deep clean-could dilution be the solution to beach pollution? Environ Sci Technol 45:4201–4202

Head IM, Jones DM, Roling WFM (2006) Marine microorganisms make a meal of oil. Nat Rev Microbiol 4:173–182

Huettel M, Berg P, Kostka JE (2014) Benthic exchange and biogeochemical cycling in permeable sediments. Annu Rev Mar Sci 6(1):23–51

Huettel M, Overholt WA, Kostka JE, Hagan C, Kaba J, Wells WB, Dudley S (2018) Degradation of Deepwater Horizon oil buried in a Florida beach influenced by tidal pumping. Mar Pollut Bull 126:488–500

Hunter EM, Mills HJ, Kostka JE (2006) Microbial community diversity associated with carbon and nitrogen cycling in permeable shelf sediments. Appl Environ Microbiol 72(9):5689–5701

Joye SB, Kleindienst S, Gilbert JA, Handley KM, Weisenhorn P, Overholt WA, Kostka JE (2016) Responses of microbial communities to hydrocarbon exposures. Oceanography 29(3):136–149

King GM, Kostka JE, Hazen TC, Sobecky PA (2015) Microbial responses to the Deepwater Horizon oil spill: from coastal wetlands to the deep sea. Annu Rev Mar Sci 7:377–401

Kostka JE, Prakash O, Overholt WA, Green S, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M (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: AEM 77:7962–7974. https://doi.org/10.1128/AEM.05402-11

Lamendella R, Strutt S, Borglin SE, Chakraborty R, Tas N, Mason OU, Hultman J, Prestat E, Hazen TC, Jansson JK (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 54:305–315

Lea-Smith DJ, Biller SJ, Davey MP, Cotton CA, Sepulveda BMP, Turchyn AV, Scanlan DJ, Smith AG, Chisholm SW, Howe CJ (2015) Contribution of cyanobacterial alkane production to the ocean hydrocarbon cycle. Proc Natl Acad Sci 112(44):13591–13596

Louchouarn P, Yeager KM, Brunner CA, Briggs K, Guo L, Asper V, Coney N, Fortner C, Prouhet J, Schindler KJ, Martin KM, Zhou Z, Loeffler J, Jung A, Cruz V (2011) Deepwater Horizon: coastal ocean to Marsh margin sediment impacts. Abstracts of Papers of the American Chemical Society 241

Lubchenco J, McNutt MK, Dreyfus G, Murawski SA, Kennedy DM, Anastas PT, Chu S, Hunter T (2012) Science in support of the Deepwater Horizon response. Proc Natl Acad Sci 109(50):20212–20221

McNutt MK, Chu S, Lubchenco J, Hunter T, Dreyfus G, Murawski SA, Kennedy DM (2012) Applications of science and engineering to quantify and control the Deepwater Horizon oil spill. Proc Natl Acad Sci 109(50):20222–20228

Michel J, Owens EH, Zengel S, Graham A, Nixon Z, Allard T, Holton W, Reimer PD, Lamarche A, White M, Rutherford N (2013) Extent and degree of shoreline oiling: Deepwater Horizon oil spill, Gulf of Mexico, USA. PloS One 8(6):e65087

Mills HJ, Hunter E, Humphrys M, Kerkhof L, McGuinness L, Huettel M, Kostka JE (2008) Characterization of nitrifying, denitrifying, and overall bacterial communities in permeable marine sediments of the northeastern Gulf of Mexico. Appl Environ Microbiol 74(14):4440–4453

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 8:e74265

OSAT (2011) Summary report for fate and effects of remnant oil in the beach environment, Operational Science Advisory Team (OSAT-2). Team, Gulf Coast Incident Management Team. In: OSAT reports. USCG

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(6):e01015–e01013. https://doi.org/10.1128/genomeA.01015-13

Overholt WA, Marks KP, Romero IC, Hollander DJ, Snell TW, Kostka JE (2016) Hydrocarbon-degrading bacteria exhibit a species-specific response to dispersed oil while moderating ecotoxicity. Appl Environ Microbiol 82(2):518–527

Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004

Passow U, Ziervogel K, Asper V, Diercks A (2012) Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environ Res Lett 7(3):035301

Prince RC (2010) Bioremediation of marine oil spills. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin Heidelberg, pp 2618–2626

Prosser JI (2012) Ecosystem processes and interactions in a morass of diversity. FEMS Microbiol Ecol 81(3):507–519

Prosser JI, Bohannan BJ, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, Green JL, Green LE, Killham K, Lennon JJ, Osborn AM (2007) The role of ecological theory in microbial ecology. Nat Rev Microbiol 5(5):384

Reddy CM, Arey JS, Seewald JS, Sylva SP, Lemkau KL, Nelson RK, Carmichael CA, McIntyre CP, Fenwick J, Ventura GT, Van Mooy B (2012) Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc Natl Acad Sci 109(50):20229–20234

Rodriguez-R LM, Overholt WA, Hagan C, Huettel M, Kostka JE, Konstantinidis KT (2015) Microbial community successional patterns in beach sands impacted by the Deepwater Horizon oil spill. ISME J 9:1928–1940

Sabirova JS, Ferrer M, Regenhardt D, Timmis KN, Golyshin PN (2006) Proteomic insights into metabolic adaptations in Alcanivorax borkumensis induced by alkane utilization. J Bacteriol 188(11):3763–3773. https://doi.org/10.1128/JB.00072-06

Sabirova JS, Becker A, Lünsdorf H, Nicaud J-M, Timmis KN, Golyshin PN (2011) Transcriptional profiling of the marine oil-degrading bacterium Alcanivorax borkumensis during growth on n-alkanes. FEMS Microbiol Lett 319(2):160–168. https://doi.org/10.1111/j.1574-6968.2011.02279.x

Schneiker S, dos Santos VAM, Bartels D, Bekel T, Brecht M, Buhrmester J, Chernikova TN, Denaro R, Ferrer M, Gertler C, Goesmann A, Golyshina OV, Kaminski F, Khachane AM, Lang S, Linke B, McHardy AC, Meyer F, Mechitaylo T, Pühler A, Regenhardt D, Rupp O, Sabirova JS, Selbitschka W, Yakimov MM, Timmis KN, Jorhölter FJ, Weidner S, Kaiser O, Golyshin PN (2006) Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium Alcanivorax borkumensis. Nat Biotechnol 24(8):997–1004. https://doi.org/10.1038/nbt1232

Shao Z, Wang W (2013) Enzymes and genes involved in aerobic alkane degradation. Front Microbiol 4:116

Urbano M, Elango V, Pardue JH (2013) Biogeochemical characterization of MC252 oil:sand aggregates on a coastal headland beach. Mar Pollut Bull 77:183–191

van Beilen JB, Funhoff EG (2007) Alkane hydroxylases involved in microbial alkane degradation. Appl Microbiol Biotechnol 74(1):13–21. https://doi.org/10.1007/s00253-006-0748-0

Vázquez DP, Simberloff D (2002) Ecological specialization and susceptibility to disturbance: conjectures and refutations. Am Nat 159:606–623

Wang W, Shao Z (2014) The long-chain alkane metabolism network of Alcanivorax dieselolei. Nat Commun 5:5755

Yakimov MM, Timmis KN, Golyshin PN (2007) Obligate oil-degrading marine bacteria. Curr Opin Biotechnol 18:257–266