

## Genomics Tools to Understand Microbial Responses to Oiling in Coastal Ecosystems

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### Abstract

Microorganisms are central and cross-cutting to oil spill response strategies. Biodegradation mediated by indigenous microbial communities is the ultimate fate of the majority of petroleum (oil and gas) that enters the marine environment. Key ecosystem services provided by microbes, such as organic matter and nutrient cycling, may be adversely affected by oil contamination. The Deepwater Horizon (DWH) oil spill was the first large scale environmental disaster to which the methods of genomics were applied to determine microbial response to a major perturbation. Here we present a case study on coastal ecosystems to highlight the knowledge gained by application of genomics tools to interrogate mechanisms of petroleum hydrocarbon degradation and to elucidate impacts of oil exposure on ecosystem health and functioning.

At Pensacola Beach, results showed that oiling led to a large increase in the growth of indigenous microbes in the form of a series of bacterial blooms. Oil contamination strongly selected for microbial groups capable of hydrocarbon degradation. Oil was degraded and benthic microbial communities returned to near baseline levels approximately one year after oil came ashore. These results indicate that when small particles (< 1 cm) of weathered light oil are buried in the coastal zone, biodegradation by indigenous microbial communities is sufficient for the rapid mitigation of oil contamination after a major spill, whereas larger sand-oil-aggregates take longer to completely degrade because of their unfavorable surface to volume ratio. "Operation Deep Clean" removed these aggregates and enhanced biodegradation insofar as many larger oil aggregates were broken down into smaller ones thereby increasing the surface area available for microbial attack. For environmental managers, these results suggest that biodegradation 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 microbial access to hydrocarbons. While specialist microbial groups such as nitrifiers show promise as bioindicators of oil contamination in coastal ecosystems, more work is needed to further validate these biomarkers. Despite substantial progress, a predictive understanding of the fate and impacts of oil spills remains hampered by challenges in interpreting the in situ activity and ecosystem response of benthic microbial populations. To advance this understanding, a dedicated funding mechanism is needed to support fundamental research. A polyphasic approach is encouraged that employs metagenomics in the field along with cultivation and microcosm or mesocosm experiments 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.

Keywords: genomics, microbial, biodegradation, indicator, emergency response

## Introduction

Ubiquitous microorganisms play a key role in the functioning of nearly all ecosystems on Earth. For example, microbes are the ultimate decomposers in all natural ecosystems, acting to break down dead/ dying organic matter and recycle major nutrients for use by primary producers. By rapidly and adeptly reacting to changing conditions, microbes also act as “first-responders” to restore balance and stability to ecosystems. In particular, microbes are intimately involved in the response of ecosystems to accidental discharges of petroleum hydrocarbons. Similar to new organic matter produced by plants, fossil oil hydrocarbons act as a carbon source fueling the growth and metabolism of microbes. Thus, hydrocarbon-degrading bacteria are ubiquitous, diverse, and have adapted over millions of years to consume oil (Head et al., 2006; Yakimov et al., 2007). Moreover, microbial biodegradation is the ultimate fate of the majority of discharged oil that is released into the environment (Leahy and Colwell, 1990; Prince, 2010). Rates of biodegradation vary from hours to years and are determined by a complex interplay between hydrocarbon chemistry, the microbial food web, and ambient physical-chemical conditions (Kostka et al., 2020). A suite of environmental parameters limits the capacity and efficiency of biodegradation, with nutrient availability and oxygen supply being particularly important in marine ecosystems. Conversely, oil can be toxic to certain microbial groups resulting in adverse effects to ecosystem services provided by microbes (Urukawa et al., 2012).

Environmental microbiology in many ways has lagged behind studies of larger organisms over the past few centuries. Because of their small size, microbes evade easy observation, and in general, most cannot be cultured in the laboratory. A major revolution in the field occurred over the past 20 to 40 years with the development of molecular biology and next generation genetic sequencing approaches (Thompson et al., 2017). Thus, the Deepwater Horizon (DWH) incident was the first major environmental disaster for which genomics technologies had matured to such an extent that they could be deployed to quantify the microbial response over large spatial and temporal scales. The field of environmental genomics literally grew up in parallel with the DWH response over the past 10 years.

DWH researchers benefitted from a wealth of microbial genomics data that enabled major discoveries in oil spill science. For the first time, scientists could answer the question of “who’s there?” for microbes in Gulf of Mexico ecosystems. The sequencing of all genes for all organisms present in a sample (metagenomics) enabled determination of microbial species present and an assessment of their metabolic potential. The sequencing of all active or expressed genes (metatranscriptomics), provided an opportunity to decipher the function or activity of carried out by those same microbial species. Bioinformatics tools, developed in parallel, offered the ability to elucidate the incredible diversity and complexity of microbial communities and data, separating out and unraveling the genetic repertoire or genomes of individual microbial species. Through a systems approach that integrates genomics along with a range of other disciplines (e.g. biogeochemistry, oceanography), researchers now have the ability to monitor and assess ecosystem health by analyzing microbial populations that are the stewards and represent bio-indicators of ecosystem functions and services. Scientists equipped with omics tools can monitor the pulse of the microbial community, thereby identifying disturbances and guiding mitigation strategies. Utilizing these new tools, the Gulf environment, and ecosystems globally, can be better protected and restored, when necessary, after environmental disasters. The objective of this paper is to review major discoveries that were

facilitated by the application of microbial genomics techniques during the DWH response under the auspices of the Gulf of Mexico Research Initiative (GoMRI). In particular, the focus is on a case study of coastal environments that reveals the feasibility and use of genomics technologies in the context of emergency response and preparedness.

## Results/ Discussion

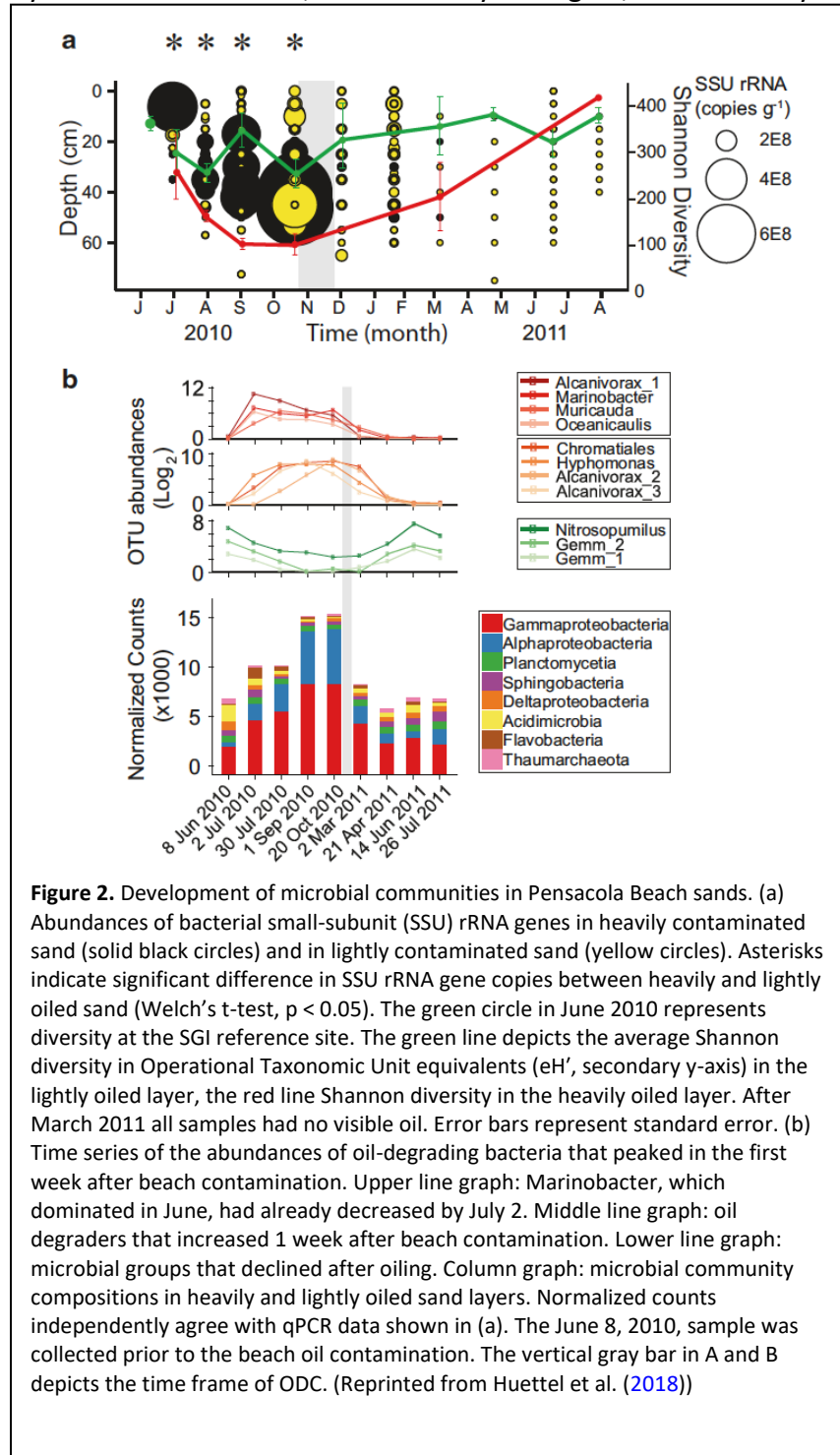
### Hydrocarbon-degrading bacteria and their response to oiling on beaches

Coastal ecosystems often act as a repository for oil contamination that washes ashore or is deposited onto sediments following a major oil spill. Further, white sandy beaches, with their



close proximity to the shoreline, provide an opportunity to visualize and repeatedly sample contaminated zones and thus, represent an ideal natural laboratory for investigation of petroleum hydrocarbon degradation. Prior to 2010, the ecology and dynamics of microbes in beach sands remained understudied, with some studies suggesting that sands harbor relatively low microbial numbers and diversity (Huettel et al., 2014). GoMRI researchers showed with genomics tools that microbes in beach sands were just as abundant as in marine muds, and more diverse than marine bacterioplankton but not as diverse as the communities of marine muds. Abundant archaea were also detected (Kostka et al., 2011; Newton et al., 2013; Lamendella et al., 2014). Specifically, hydrocarbon-degrading microorganisms were shown to be ubiquitous and form part of the “rare biosphere”- found in low abundance when oil is not present (Kleindienst et al., 2015).

As result of the Deepwater Horizon (DWH) disaster, Pensacola municipal beach (PB) was exposed to repeated pulses of oil deposition during a one month period from June to July of 2010, with Total Petroleum Hydrocarbon (TPH) concentrations reaching up to ~2 kg per square meter of beach (Figure 1; Huettel et al., 2018). Stranded oil heated up and pooled over large areas of the beach surface, due to high ambient temperatures during the summer. Beaches are dynamic environments, and from July to August, oiled sand layers were buried by waves,

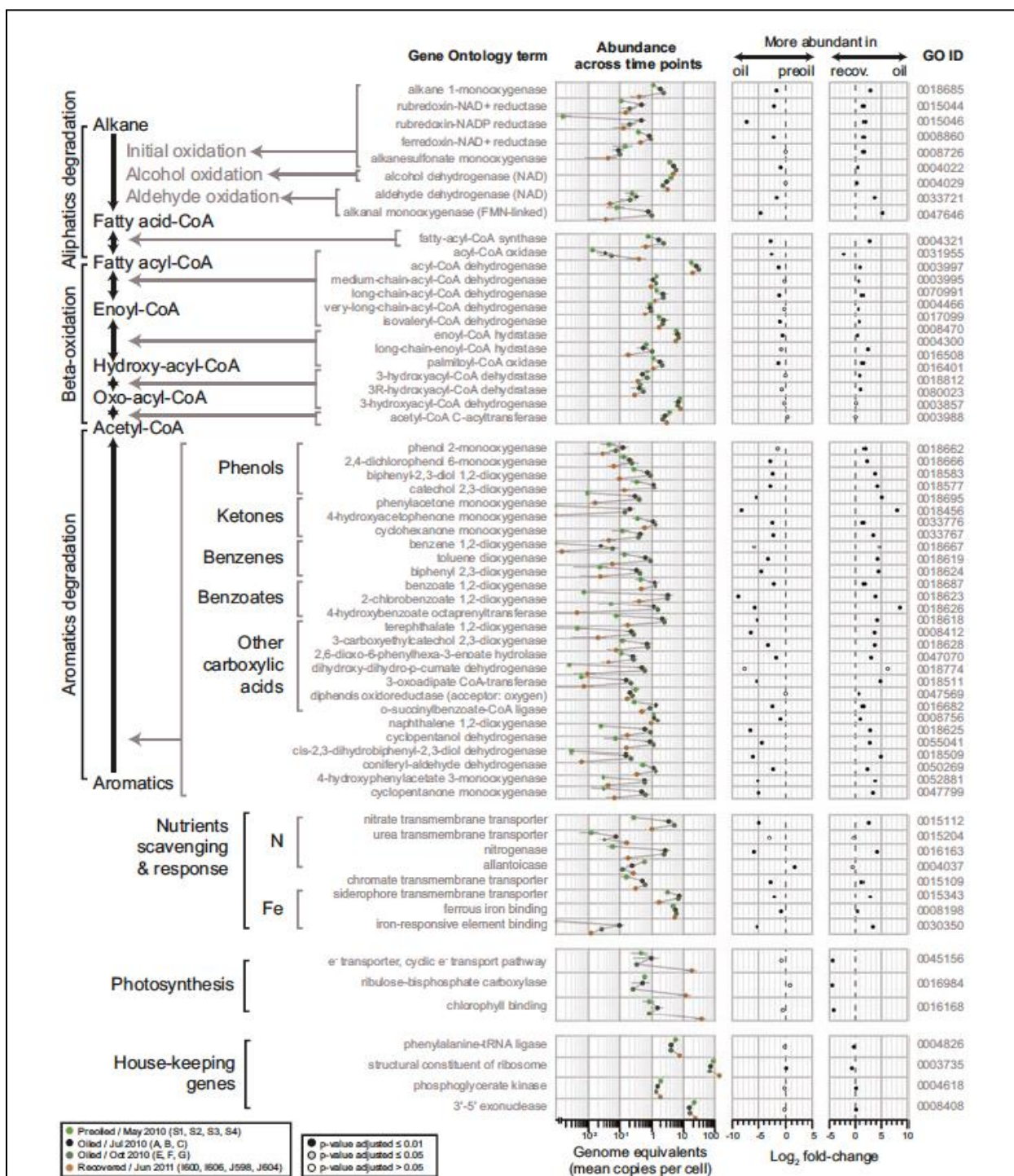


storms and human activities deeper into the sediment column. By October, 2010, the oiled sand layer had reached 70 cm below surface.

PB was the most intensively studied beach site examined by microbiologists after the DWH discharge. The beach sands appeared to represent an ideal environment for the growth of oil-degraders. Many of the microbial strains that were detected by genomics methods could also be cultivated in the laboratory, thereby facilitating the elucidation of microbial functions. A series of field investigations demonstrated that buried oil initiated profound changes to the abundance and diversity of microbes in PB beach sands, leading to a succession of hydrocarbon-degrading microbial populations (Figure 2; Kostka et al., 2011; Rodriguez-R. et al., 2015; Huettel et al., 2018). A large bloom was observed as bacterial abundance increased by two to four orders of magnitude in

buried oil layers in comparison to lightly oiled or unoiled sands, and the bloom moved deeper into the beach sand over time in parallel with the residual petroleum hydrocarbons (PHCs).

In response to oiling, taxonomic diversity declined in parallel with the concentration of PHCs and rebounded as hydrocarbons became depleted. Known hydrocarbon-degrading bacteria outcompeted other microbial groups and showed large increases in relative abundance.



**Figure 3.** Representative results from the metagenomic time series generated from field-collected Pensacola Beach sands, encompassing 16 samples, with at least triplicate metagenomes from 4 time points, and over 450 million gene sequences. 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 log<sub>2</sub> 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 log<sub>2</sub>-fold-change was estimated as the log<sub>2</sub> 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 from Rodriguez-R et al. (2015))

Dominant hydrocarbon-degrading microbial groups such as members of the Gammaproteobacteria (*Alcanivorax*, *Marinobacter*) were shown to comprise up to 50 % of the microbial community in heavily-oiled sand layers (Huettel et al., 2018). Representatives of these abundant bacterial groups were able to be cultivated from oiled sediment layers and shown to be metabolically-active using RNA-based analyses (Kostka et al., 2011).

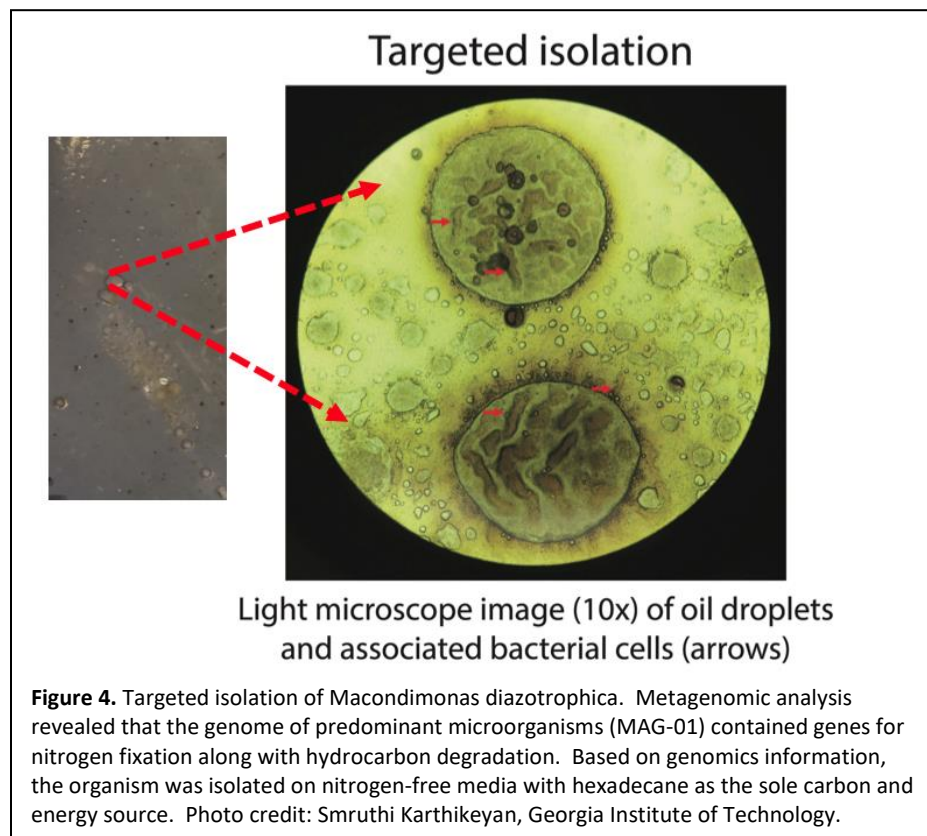
### **New Genes and Microbial Species**

The largest metagenomic time series available from the DWH response was generated at PB, encompassing 16 samples, with at least triplicate metagenomes from 4 time points, and over 450 million gene sequences (Figure 3; Rodriguez-R. et al., 2015). In contrast to most studies supported by GoMRI, pre-oiled samples were obtained from collaborators for establishment of baseline conditions. This robust metagenomic dataset enabled the discovery of new genes, metabolic pathways, and microbial species.

Prior to the DWH event, little baseline metagenomic data was available for native microbial communities in the Gulf of Mexico. The lack of a comprehensive database that integrates existing genomic/metagenomic data from oiled environments with physicochemical parameters known to regulate the fate of PHCs limited data analysis and interpretations. GoMRI researchers addressed this knowledge gap by creating the Genome Repository of Oiled Systems (GROS; Karthikeyan et al., 2020; <http://microbial-genomes.org/projects/24>). GROS is a curated, comprehensive, and searchable database that documents microbial populations in oiled ecosystems on a global scale, along with underlying physicochemical data, geocoded via GIS to reveal geographic distribution patterns of the populations (Karthikeyan et al., 2019). Using state-of-the-art bioinformatics approaches, approximately 2000 microbial genomes were assembled from the database, revealing strong ecological niche specialization within habitats e.g., specialization to coastal sediments vs. water-column vs. deep-sea sediments. Over 95 % of the recovered genomes represented novel and uncultured species underscoring the limited representation of cultured organisms from oil-enriched ecosystems. GROS should greatly facilitate future work toward a more predictive understanding of the microbial taxa and their activities that control the fate of oil discharged into natural ecosystems as well as in oil reservoirs. The creation and validation of this database would not have been possible without the extensive investments by GoMRI into the field and laboratory studies of oil contamination at Pensacola Beach.

Advances in bioinformatics along with our extensive metagenomic dataset enabled the isolation and characterization of a novel microbe that represents a group of previously uncharacterized, petroleum hydrocarbon degraders (Karthikeyan et al., 2019). Gene sequence data showed that an uncharacterized group within the commonly detected Gammaproteobacteria increased from undetectable levels in the clean/pre spill samples to 30 % of the community in oiled samples, returning to undetectable levels in the recovered sands. From the metagenomes, the genome of this highly abundant group (MAG-01) was then assembled, revealing a roadmap of its metabolic potential, including the potential for nitrogen fixation along with alkane degradation. With guidance from the roadmap, the organism was isolated from field samples on nitrogen-free media with hexadecane as the sole carbon and energy source (Figure 4), and sequencing confirmed that the isolate genome (KTK-01) encodes functional nitrogen fixation and hydrocarbon degradation genes together with putative genes for biosurfactant production. An analysis of publically available gene sequence datasets

revealed a remarkable distribution of nearly identical sequences to KTK-01 in oil-contaminated sediments of coastal ecosystems across the globe, often comprising ~30% of the total community, and virtually absent in pristine sediments or seawater. Comparisons to available genomes, the metabolic roadmaps of other microbes, revealed that this isolate represents a novel genus, for which GOMRI researchers proposed the provisional name “*Candidatus* *Macondimonas diazotrophica* gen. nov., sp. nov. “, after the Macondo oil discharged during the DWH catastrophe. *Macondimonas* appears to play a key role in the response of coastal ecosystems to oil spills around the world and represents a promising model organism for investigating the mechanisms controlling oil biodegradation.



### Biomarkers of Ecosystem Disturbance

The ultimate goal of oil spill microbiology is to translate genomic findings into actionable information to help scientists and environmental managers monitor and restore ecosystem health in the face of natural or man-made disasters (Joye and Kostka, 2020). Genomics can uncover underlying functional deficiencies, or additions, that impact the overall health of

the entire ecosystem. Through examination of the genetic roadmap of microbial communities, researchers can ‘take the pulse’ of the ecosystem and diagnose functioning that is out of the ordinary. Genomic indicators serve as biological markers (biomarkers) to direct scientists to imbalances and guide mitigation strategies, much like blood tests can point doctors towards disease diagnosis and treatment options. During and after the DWH discharge, microbial genomics demonstrated the potential for development of effective genetic proxies or biomarkers for recording oil inputs, exposure regimes and hydrocarbon degradation. Ecosystem disturbances were identified by imbalances in community diversity, overgrowth of certain species, and emergence of novel genes, metabolic pathways, and functions. For example at PB, Ca. *Macondimonas diazotrophica* was shown to dominate microbial communities in oiled beach sands and a large increase in the abundance of nitrogen fixation genes signified nutrient limitation and perturbations to the nitrogen cycle brought on by oiling (Gaby et al., 2018; Karthikeyan et al., 2019; Shin et al., 2019). The abundance of genes for



specific hydrocarbon degradation pathways was directly correlated to the concentration of the corresponding class of hydrocarbon compounds such as alkanes and polycyclic aromatics (Figure 3; Rodriguez-R et al., 2015). Further, a decline in chemolithoautotrophic nitrifying Archaea in oiled sediments followed by the rebound of these microbes in recovered sands provided evidence for ecosystem recovery. Thus, new tools and approaches developed by GoMRI researchers during the DWH response show proof-of-principle for deployment as part of the emergency response and restoration toolkit. Scientists envision a future where omics measurements will enable assessment of environmental risks, identification of ecosystem deficits, selection of appropriate action plans for mitigation, and the monitoring of ecosystem recovery.

### **Implications for preparedness response and restoration**

GoMRI programs catalyzed transformative discoveries during the response to the DWH discharge. Genomics research characterizing the response of microbial communities in Gulf ecosystems to the DWH oil spill generated knowledge that will pay dividends for years to come. With knowledge and tools gained from the emergence of metagenomics approaches, scientists now can respond more effectively to future environmental disasters, informing practitioners in preparedness, response, and restoration. For the first time, metagenomic tools are available that enable a data-driven approach for oil spill response and mitigation. With advanced genomic tools, microbiologists can now quickly and inexpensively analyze field samples to provide essential information about microbial ecosystem functions and responses during an oil spill. These tools, along with expertise from scientists, can guide spill response efforts before, during and after a spill, to evaluate ecosystem responses and recovery.

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