

Microbial Genomics of the Global Ocean System*

Samantha B. Joye¹ and Joel E. Kostka²

¹ University of Georgia, Department of Marine Sciences, Athens GA 30602-3636;
e-mail: mjoye@uga.edu; ORCID: <https://orcid.org/0000-0003-1610-451X>

² Georgia Institute of Technology, School of Biological Sciences, Atlanta, GA
30332; e-mail: joel.kostka@biology.gatech.edu; ORCID: <https://orcid.org/0000-0003-3051-1330>

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Microbial Genomics of the Global Ocean System

Report on an American Academy of Microbiology (Academy), The American Geophysical Union (AGU), and The Gulf of Mexico Research Initiative (GoMRI) Colloquium held in 9 and 10 April 2019.

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*These authors contributed significantly to the development of the report

Colloquium Participants:

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Casey Hubert, Ph.D.
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University of Delaware

Kostas Kostantinidis, Ph.D.
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Elizabeth Kujawinski, Ph.D.
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Florida State University

**Annette Summers Engel,
Ph.D.**
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Knoxville

Karsten Zengler, Ph.D.
University of California San
Diego

Kelly McFarlin, Ph.D.
Exxon Mobil

Andreas Teske, Ph.D.
University of North Carolina

Beth Orcutt, Ph.D.
Bigelow Laboratory for
Ocean Sciences

Cameron Thrash, Ph.D.
University of Southern
California

Edward Overton, Ph.D.
Louisiana State University

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AGU is an international association of more than 60,000 advocates and experts in Earth and space science. Fundamental to our mission since our founding in 1919 is to live our values, which we do through our net zero energy building in Washington, D.C., and by making scientific discoveries and research accessible and engaging to all to help protect society and prepare global citizens for the challenges and opportunities ahead.

The Gulf of Mexico Research Initiative (GoMRI) is a 10-year (2010-2020) research program that was established through a \$500 million financial commitment by BP after the Deepwater Horizon oil spill. Led by a 20-member Research Board, GoMRI's goal is to improve society's ability to understand, respond to and mitigate the impacts of petroleum pollution and related stressors on the marine and coastal ecosystems, with an emphasis on conditions found in the Gulf of Mexico. Knowledge accrued will be applied to restoration and to improving the long-term environmental health of the Gulf of Mexico.

Finally, we'd like to give special thanks to Shannon Weiman, Ph.D., science writer, for her tireless effort, expertise and patience in developing this report.

(Outside cover)

The American Academy of Microbiology (Academy) is the honorific branch of the American Society for Microbiology (ASM), a nonprofit scientific society with more than 30,000 members. Fellows of the Academy have been elected by their peers in recognition of their outstanding contributions to the

microbial sciences. Through its colloquium program, the Academy draws on the expertise of these Fellows and other experts to address critical issues in the microbial sciences.

This report is based on the deliberations of experts who gathered for 2 days to discuss a series of questions developed by the Colloquium Steering Committee. All participants had the opportunity to provide feedback, and every effort has been made to ensure that the information is accurate and complete. The contents reflect the views of the participants and are not intended to reflect official positions of the Academy, ASM, AGU and GoMRI.

Microbial Genomics of the Global Ocean System

A Joint Colloquium of The American Academy of Microbiology (AAM), The American Geophysical Union (AGU), and The Gulf of Mexico Research Initiative (GoMRI) Held on 9 and 10 April 2019

Table of Contents

Part I. EXECUTIVE SUMMARY/ INTRODUCTION

Part II. OMICS TECHNOLOGIES ENABLE NEW BIOGEOCHEMICAL DISCOVERIES

1. Discovery of New Oil-Degrading Microbial Species, Genes, and Enzymes
2. Dynamics of Oil-Degrading Microbial Communities
3. Biogeochemical Processes

Part III. SIGNIFICANCE AND APPLICATIONS OF GoMRI RESEARCH

1. Informing the Oil Spill Response
2. Global versus Regional Lessons
3. Applications for Assessing Ecosystem Health in the Context of Environmental Disturbances
4. Establishing a Suite of Tools for Environmental Microbiology Research
5. Broad Lessons for Microbiology and Ocean Science

Part IV. THE FUTURE OF GENOMICS IN ECOLOGY

1. Biological Markers of Ecosystem Health
2. Predicting Environmental Impacts with Biomarkers and Models
3. Translating Omics into Function for Biogeochemical Models
4. Facilitating Progress through Open-Access Resources
5. Facilitating Advances through Multidisciplinary Collaboration

Part V. CONCLUSIONS

References

I. EXECUTIVE SUMMARY/ INTRODUCTION

Executive Summary

The year 2020 marks the 10th anniversary of the Deepwater Horizon (DWH) disaster. From April through July 2010, an estimated total of 4.9 million barrels of oil and 250,000 metric tonnes of natural gas were discharged into the Gulf of Mexico. Not only were eleven lives lost, but the tragedy also left a lasting impact on the Gulf's marine and coastal ecosystems and on the residents who depend on these habitats for their livelihood. After the oil spill, the Gulf of Mexico's microbial communities played a critical role in the cleanup, contributing core hydrocarbon bioremediation services. Despite its importance, marine hydrocarbon microbiology is a young field. Prior to the spill relatively little was known about marine hydrocarbon degraders.

Beginning in 2010, the development and application of genomics and bioinformatics tools enabled researchers – for the first time - to identify and examine individual microorganisms within their complex communities in unprecedented detail. Today, technical advances and new discoveries reveal a natural capacity of microbes in the Gulf of Mexico to catalyze bioremediation of petroleum hydrocarbons. This knowledge is critical to guide mitigation and restoration strategies that build on microbes' natural bioremediation capabilities without further disturbing sensitive ecosystems.

This report is based on the deliberations of experts who participated in the joint colloquium of the American Academy of Microbiology, ASM's honorific leadership group, the American Geophysical Union (AGU), and Gulf of Mexico Research Initiative (GoMRI) in April 2019. The report highlights new research tools, methodology, data resources, collaborations, and models that will advance basic and applied research to provide data-driven solutions to environmental challenges. The report is available at www.ASM.org/microbe_oceansystem.

Introduction

The year 2020 marks the 10th anniversary of the Deepwater Horizon (DWH) oil spill. Over the course of 86 days, from April through July 2010, an estimated total of 4.9 million barrels of oil and 250,000 metric tonnes of natural gas were released into the Gulf of Mexico. This unprecedented discharge contaminated vast areas of the open ocean, deep sea, and shoreline of the Gulf of Mexico and disrupted ecosystems throughout the Gulf region. The DWH spill represents one of the greatest environmental catastrophes of our generation.

The event was a terrible tragedy, not only because of the immediate loss of human lives, but also because of the lasting impact on Gulf marine and coastal ecosystems and on the residents who depend on these habitats. Given the magnitude of damage, the research community took on the responsibility of learning from this unprecedented environmental disaster to better understand its impacts and improve response measures. Gulf of Mexico Research Initiative (GoMRI) researchers from diverse fields assembled collaborative teams to study the spill and its effects on various ecosystem functions. The efforts to understand the basis of ecosystem health and the oil spill response will prepare future generations to better protect and restore these invaluable habitats in the event of future disasters by guiding disaster preparedness and response.

At the heart of all ecosystems are microbes that provide a foundation for all life by performing essential services like carbon and nutrient cycling, as well as other geochemical processes. Microbes are the Earth's first responders, and by rapidly and adeptly reacting and adapting to changing conditions, they restore balance and stability to the entire ecosystem. In the context of oil spills, microbes serve as emergency cleanup crews by feeding on hydrocarbons and contributing many other core biogeochemical remediation services. Despite its importance, marine hydrocarbon microbiology is a relatively young field, and relatively little was known about marine microbes, especially those in the deep ocean, that degrade hydrocarbons prior to the DWH spill due to a lack of molecular tools and funding to study them. The GoMRI program provided the necessary resources to significantly advance the understanding of marine hydrocarbon microbiology in ways that can now be leveraged to mitigate the impact of future environmental disasters.

The DWH spill coincided with the development and application of genomic and bioinformatics tools that, for the first time, enabled scientists to examine microbes and their complex communities in great and unprecedented detail. Technical advances also allowed for comprehensive analyses of microbes in their natural context directly from both oil-contaminated and uncontaminated water and sediment samples. With this array of transformational “omics” tools, scientists gained valuable insight into how microbes responded to the hydrocarbon infusion and restored ecosystem health. Many novel species, genes, metabolic pathways, and community dynamics that are instrumental to hydrocarbon degradation and other ecosystem functions were documented. These discoveries revealed a natural capacity of microbes in the Gulf of Mexico to catalyze the bioremediation of petroleum hydrocarbons. This knowledge is critical to guide mitigation and restoration strategies that build on microbes’ natural bioremediation capabilities without further disturbing sensitive ecosystems.

Thus, GoMRI researchers discovered many core ecological and evolutionary principles of the microbial response and ecosystem function that are widely translatable. These lessons form the foundation for understanding how microbes in various ecosystems around the globe respond to diverse environmental disturbances. From the Arctic to the equator and the deep sea to the shores, microbes adhere to these same principles to maintain and restore ecosystem balance. Such insights will help scientists better understand and prepare for future catastrophes, whether they be a tanker spill or the long-term disruptions of climate change.

Although much work is still needed to fully understand the microbially mediated biogeochemical processes that underpin ocean systems and their resilience, GoMRI research efforts have provided new research tools, methodology, data resources, and collaborations to enable future studies. With new strategies to employ from diverse fields and tools that span laboratory, environmental, and computational sciences, the GoMRI program established a new model for interdisciplinary research aimed at solving a real-world problem. This new model will pave the way for more powerful and impactful science, providing innovative and transformative strategies that simultaneously advance basic and applied research to provide data-driven solutions to environmental challenges.

Ultimately, one of the goals of GoMRI was to monitor and assess ecosystem health by analyzing microbial populations that are the stewards and sentinels of marine and coastal environments. With omics tools, scientists aim to take the pulse of the microbial community, thereby identifying disturbances, no matter how subtle, and guiding mitigation strategies. With these efforts, the ecosystems in the Gulf of Mexico, and those globally, can be protected and restored, when necessary, in the face of diverse environmental stressors.

Potential Figures for Executive Summary and/or other sections: (use only images the images below).





II. OMICS TECHNOLOGIES ENABLE NEW BIOGEOCHEMICAL DISCOVERIES

Hydrocarbons

Petroleum, derived from the Latin for “rock” (petra) and “oil” (oleum), describes the liquid, gaseous, and solid hydrocarbons that are formed within sediments when organic matter is exposed to extreme pressure and temperatures to undergo thermocatalytic alteration. Petroleum is an extremely complex mixture composed of thousands of individual hydrocarbon compounds. Generally speaking, all petroleum contains the same types of compounds, albeit in different relative proportions.

Petroleum can be separated into four fractions, as follows:

- **Saturates**- This fraction includes nonpolar compounds (linear, branched, and cyclic hydrocarbons, i.e., alkanes, paraffins, and naphthenes) as well as natural gases (methane, other alkanes up to C₆, and condensates that exist in the gas phase under reservoir conditions). This fraction also includes biomarkers (hopanes, steranes, and steroids) which serve as tracers of different source reservoirs.
- **Aromatic hydrocarbons (arenes)**- These compounds contain one or more benzene ring structures and can be monocyclic aromatic hydrocarbons (i.e., benzene, toluene, ethylbenzene, and xylene [BTEX]) or polycyclic aromatic hydrocarbons (PAHs; e.g, naphthalene and phenanthrene).
- **Resins**- This is a much more polar fraction of crude oil, and resins are miscible in hexane.
- **Asphaltenes**- This is also a more polar fraction of crude oil, and asphaltenes are soluble in dichloromethane but not hexane. Asphaltenes contain more nitrogen, oxygen, and sulfur than do other petroleum fractions and are more abundant in heavy oils, bitumen, and oil sands.

1. Discovery of New Oil-Degrading Microbial Species, Genes, and Enzymes

Before the genomics revolution, researchers could study only those microbes that could be grown in the laboratory, and these organisms represent a very limited number of species and genera that do not adequately capture the physiological diversity and capabilities present in nature. The majority of microbes are impossible or difficult to culture because they require partnerships with other microbes, trace nutrients, low oxygen tension, and/or other environmental conditions that may not be recognized or recreated effectively in the laboratory (Overmann et al., 2017). Therefore, in the past, researchers were essentially blind to many of the major players and underlying dynamics of microbial ecosystems.

Today's researchers wield a powerful toolbox of omics technologies and bioinformatic methods which allow them to probe the structure and function of microbial communities that form the foundation of marine ecosystems (Grossart et al., 2020). These approaches were instrumental in examining the impacts of the DWH spill, giving researchers the tools to comprehensively

examine microbial dynamics in real-world situations for the first time (Mason et al., 2014a; Mason et al. 2014b; Rodriguez-R et al., 2015). These advanced techniques and bioinformatics methods have generated discoveries and impacts far beyond what was previously possible (Karthikeyan et al. 2019).

Omics tools enable researchers to obtain a more complete picture of marine microbial communities by interpreting DNA, RNA, and other molecular information from environmental samples (Grossart et al., 2020). Much like reading a book about a plant or animal without having to rear it in captivity, omics information provides researchers with critical insights about an organism, such as what it does metabolically, its evolutionary history, and how it fits into its community and ecosystem.

Omics Toolbox:

Genomics-

High-throughput and deep-sequencing tools enable rapid and relatively inexpensive sequencing of entire microbial genomes. These gene sequences reveal clues as to a microbe's metabolic capabilities and ecosystem functions, like hydrocarbon degradation potential. These clues can then guide further investigation and laboratory studies to pin down specific metabolic activities and other gene functions.

Transcriptomics-

RNA sequencing shows which genes are expressed under specific conditions and circumstances, which gives a more accurate view of active microbial players, genetic programming, and functional responses. Differences or changes in these RNA profiles reveal how microbes respond to environmental disturbances, such as an oil spill.

Methylomics-

Analysis of DNA methylation patterns reveals how genes are regulated epigenetically to activate or shut down specific genes or genetic programs in response to environmental perturbations.

Proteomics-

Mass spectrometry analysis reveals the protein content of cells and posttranscriptional modifications that may alter protein activity. For example, phosphorylation is a major on/off switch for many proteins, which can dramatically affect their cellular functions.

Metabolomics-

Metabolomics involves a range of advanced analytical chemistry techniques, including nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry approaches such as Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), that provide metabolite fingerprints that elucidate metabolic currencies available to, produced by, and exchanged between microbes.

Meta-Omics-

Meta-omics is an integrated approach that incorporates all aspects of cell metabolism from genes to metabolites. This holistic approach reveals ecosystem functions accomplished by cooperative microbial communities, which are beyond the capabilities of a single community member. In addition, it provides scientists clues about the relative abundance, and therefore importance, of specific species, genes, etc. within the community.

Single-Cell Sequencing and Analysis-

With precise tools such as single-cell sequencing and analysis to examine individual microbial cells and species, researchers can attribute genes, transcripts, etc., and associated ecosystem functions to specific individuals within the microbial community. This has enabled researchers to identify key players in hydrocarbon degradation.

Systems Biology-

Systems biology is a holistic iterative approach that integrates environmental, laboratory, genetic, computational, and mathematical methods to assess biological community dynamics (Figure 1). Each technique described above provides unique layers of information that can be integrated with functional laboratory studies and bioinformatics to provide a detailed, multidimensional view of microbial communities and their function in the ecosystem.

Systems Biology approach

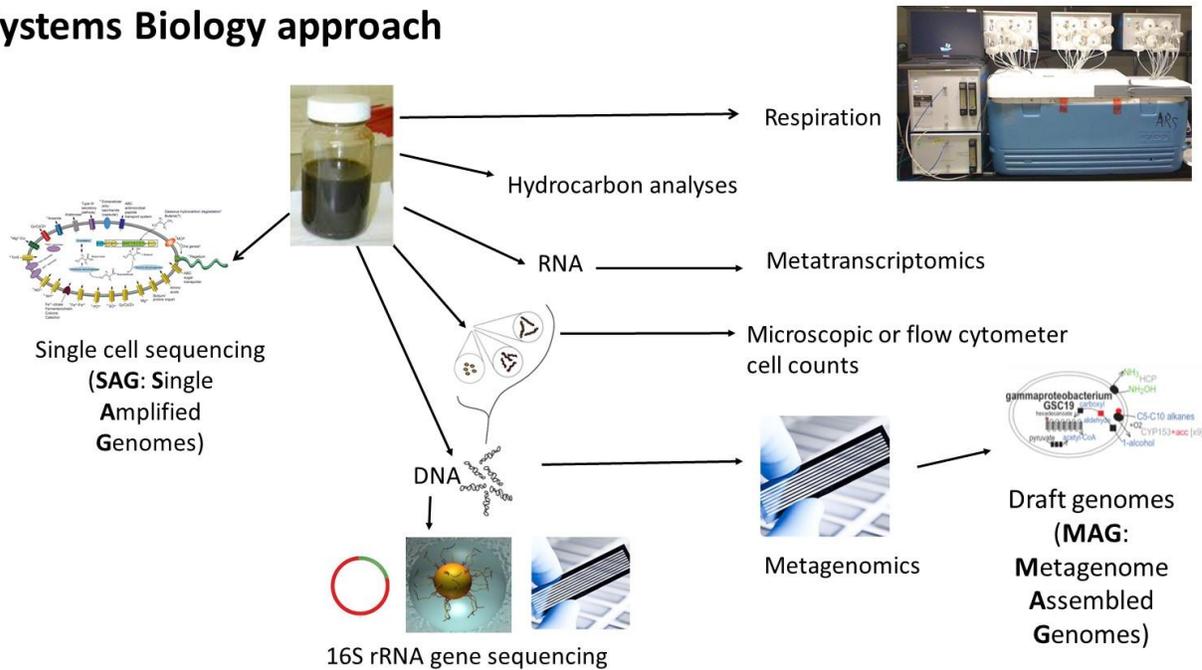


Figure 1. Recent omics-enabled techniques incorporated into a holistic iterative approach to assess biological community dynamics in the environment. (Image Credit: Janet Jansson, Pacific Northwest National Laboratory)

Meet the Microbes

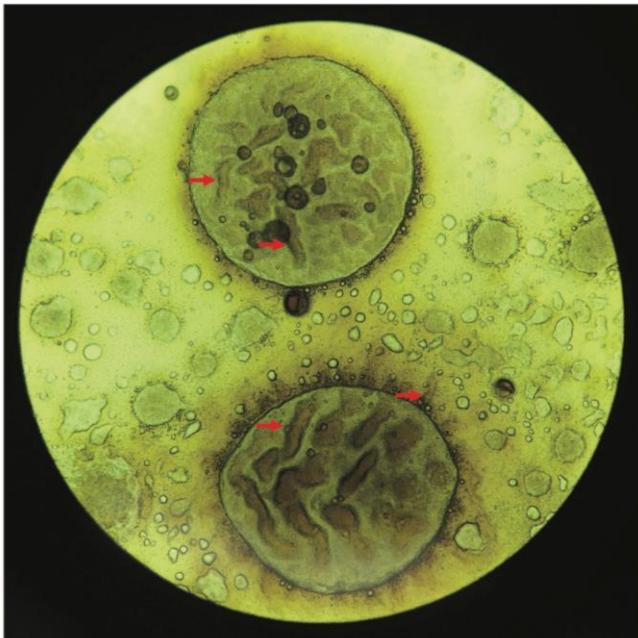


Figure 2. Light microscope image (10x) of oil droplet and associated microbial colonies (arrows) within and external to oil. A pure culture of “*Candidatus* Macondimonas diazotrophica,” a novel, nitrogen-fixing crude oil degrader taxon, was recovered from Florida beach sands contaminated with Macondo oil from the Deepwater Horizon discharge. Microbial colonies are shown growing on hexadecane (Image Credit: Smruthi Karthikeyan, Georgia Institute of Technology).

With omics tools, researchers have discovered many new microbial taxa that thrive in oil-contaminated marine environments and have described the unique genes and metabolic pathways they use for hydrocarbon degradation (Figure 2; Hazen et al., 2010; Kostka et al., 2011; Mason et al., 2014a; Crespo-Medina et al., 2014; Rodriguez-R. et al., 2015; Kleindienst et al., 2015; Yang et al., 2016; Karthikeyan et al., 2019). In addition, major known classes of microbes, like *Bacteroidetes*, have been found to harbor a previously unknown potential for hydrocarbon biodegradation (Liu and Liu, 2013). Omics snapshots of the entire community illuminate metabolic pathways and ecosystem functions accomplished by cooperative microbial communities that would be overlooked by examining only individual species or small subunit (ssu) rRNA gene sequence data (Mason et al., 2012). Microbes are cooperative organisms known for partitioning metabolic pathways across multiple community members. By mapping metatranscriptomic data onto metagenomes, researchers can assess these collective metabolic capabilities and attribute genes and functions to specific species (e.g., Mason et al., 2012).

Many major players and ecotypes have been discovered, but we have only scratched the surface in identifying microbes involved in hydrocarbon degradation (Gutierrez et al., 2018). There are vast worlds yet to uncover, and the search continues for those that remain hidden, with new additions to the community being discovered frequently.

Common Characteristics of Hydrocarbon Degraders

- Oil-degrading microbes are ubiquitous, being found around the world in low abundance when crude oil is not present. These important members of the “rare biosphere” harbor a metabolic potential of ecological significance, which can be deployed when hydrocarbons appear.

- When oil spills occur, these microbes are stimulated, increasing in abundance and expressing genes involved in hydrocarbon metabolism. They are able to sense and in many cases rapidly swim toward the source (Figure 3).
- With the ability to metabolize various hydrocarbon compounds as energy sources, they rapidly reproduce in the presence of oil to dominate the microbial ecosystem in contaminated waters and sediments.
- Many oil-degrading microbes possess multiple pathways for hydrocarbon degradation, and it is likely that a combination of environmental conditions and the nature of exposure (i.e., the type and amount of petroleum hydrocarbons) induce the expression of a given metabolism in specific instances.

These microbes are essential first responders in the event of environmental perturbations like oil spills, providing critical ecosystem cleanup and stabilization functions; their activity also regulates the fate and environmental distribution of hydrocarbons.

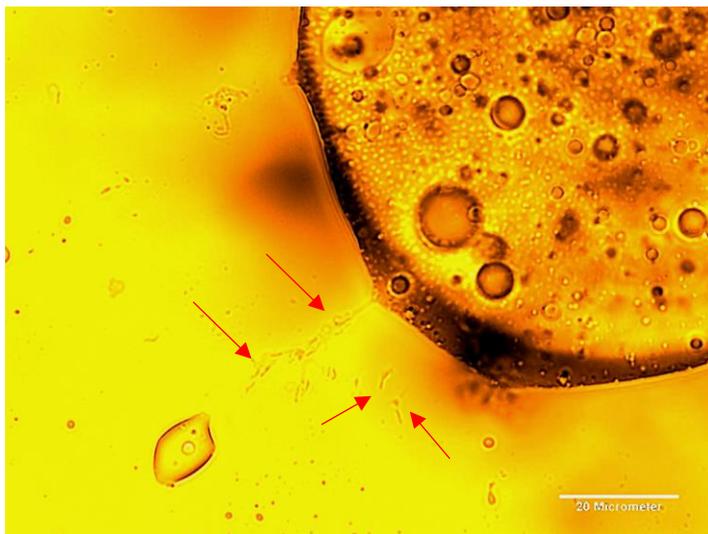


Figure 3. Microbes swimming toward an oil droplet. Arrows denote organisms. (Image Credit: Courtesy of Berkeley Synchrotron Infrared Structural BioImaging (BSISB) Program Archives, Lawrence Berkeley National Laboratory)

Alcanivorax spp.

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Oceanospirillales*, Family: *Alcanivoracaceae*, Genus: *Alcanivorax*
- **Location:** Ubiquitous in marine ecosystems in seawater and sediment from the coastal zone to the deep sea.
- **Oil Degradation Capabilities:** Known to degrade linear and branched alkanes and their derivatives, with carbon chain lengths between 9 and 32, as well as a few other carbon substrates such as fatty acids or pyruvate. Enzymes for alkane metabolism, alkane hydroxylases, and P450 cytochromes are induced upon oil exposure. *Alcanivorax* spp. produce their own biosurfactants and form stable emulsions with oil compounds.
- **Motility:** Some strains are motile, containing flagella, while others are not.
- **Prevalence:** This genus is the best studied and among the most often detected in oil-contaminated ecosystems. *Alcanivorax* spp. are rare in uncontaminated seawaters but in the presence of hydrocarbon compounds quickly become enriched and dominate the microbial community. These microbes are early responders and among the first to provide a rapid response to hydrocarbons in the deep sea.

***Colwellia* spp.**

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Alteromonadales*, Family: *Colwelliaceae*, Genus: *Colwellia*
- **Location:** Marine waters and sediments.
- **Oil Degradation Capabilities:** Alkane gases (including ethane, propane, and benzene) and polycyclic aromatic hydrocarbon (PAH) degradation. These microbes congregate around oil in the water column, forming large particles that fall like snow, referred to as marine oil snow (MOS). MOS particles degrade and trap hydrocarbon compounds, pulling them down to the seafloor.
- **Motility:** Possess genes for chemotaxis and flagellum-based motility that allow quick movement toward hydrocarbons.
- **Prevalence:** *Colwellia* species are found around the world, but those at the DWH spill site are thought to have specialized adaptation for degrading hydrocarbons at low

temperature. As such, they are known to respond to petroleum hydrocarbon contamination in surface seawaters of the Arctic.

***Cycloclasticus* spp.**

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Thiotrichales*, Family: *Piscirickettsiaceae*, Genus: *Cycloclasticus*
- **Location:** Marine waters and sediments.
- **Oil Degradation Capabilities:** Well known for aerobic PAH degradation but recently associated with aerobic ethane, propane, and butane oxidation as well, suggesting an unappreciated role in aerobic alkane (including methane) degradation of these microbes.
- **Motility:** Exhibit chemotaxis and motility via a polar flagellum.
- **Prevalence:** *Cycloclasticus* spp. are found frequently in surface and deep waters and in sediment, illustrating their wide temperature tolerance. Metabolic plasticity may promote its wide distribution.

Marinobacter hydrocarbonoclasticus

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Alteromonadales*, Family: *Alteromonadaceae*, Genus: *Marinobacter*
- **Location:** Ubiquitous in seawater and marine sediments.
- **Oil Degradation Capabilities:** Oxidizes long-chain (noncyclic) alkanes and aromatics, producing biofilms that aid in hydrocarbon oxidation. This organism can use either oxygen or nitrate/nitrite as an electron acceptor, which provides substantial metabolic flexibility.
- **Motility:** A single polar flagellum is used to propel the microbe toward hydrocarbon sources.
- **Prevalence:** *Marinobacter* spp. are ubiquitous in the ocean and some of the most metabolically complex species known, possessing an extremely broad suite of capabilities that enable them to survive almost anywhere. Dubbed a “biogeochemical opportunitroph,” this species is jack-of-all-trades that can tolerate a range of conditions,

including high salinity, and is an important player not only in hydrocarbon cycling, but also biogeochemical cycling in general.

***Oceanospirillaceae* spp.**

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Oceanospirillales*, Family: *Oceanospirillaceae*
- **Location:** Deep-water oil plume and proximal to wellhead (discharge point).
- **Oil Degradation Capabilities:** Straight-chain, normal (n) alkane, cycloalkane, and cyclohexane degradation genes are induced upon oil exposure. Possess other genes for degrading more resistant compounds such as benzene, toluene, ethylbenzene, xylenes, and polycyclic aromatic hydrocarbons, but these genes are expressed only at low levels or not at all. These metabolic pathways may be induced under different environmental conditions, such as in the presence of other types of petroleum hydrocarbons of crude oil.
- **Motility:** With genes for chemotaxis and flagellum-driven motility, these microbes can move quickly toward sources of hydrocarbon compounds.
- **Prevalence:** Rare in uncontaminated seawater but in the presence of hydrocarbon compounds quickly become enriched and dominate the microbial community. These microbes are early responders and the first to provide rapid response to hydrocarbons in the deep sea.

“*Candidatus* *Macondimonas diazotrophica*”

- **Classification:** Domain: *Bacteria*, Phylum: *Proteobacteria*, Class: *Gammaproteobacteria*, Order: *Chromatiales*, Family: *Woeseiaceae*, Genus: “*Candidatus* *Macondimonas*”
- **Location:** Beaches and coastal sediments where hydrocarbon compounds washed ashore.
- **Oil Degradation Capabilities:** Capable of degrading a range of alkanes. Genome indicates the potential for methanotrophy (*pmoA* and other genes).
- **Motility:** Genes for flagella and chemotaxis suggest that this species also moves toward hydrocarbon sources.
- **Prevalence:** Undetectable in uncontaminated sediments but accounted for 30% of the microbial community in contaminated sediments during mid- to late-phase hydrocarbon

degradation, falling back to low abundance once the habitat has recovered and is cleared of hydrocarbons. This species appears to be a dominant responder to oil spills on shorelines around the globe (Figure 4). Key to its success is its nitrogen fixation capability, which likely allows it to survive and thrive in nitrogen-limited petroleum hydrocarbon-contaminated ecosystems.

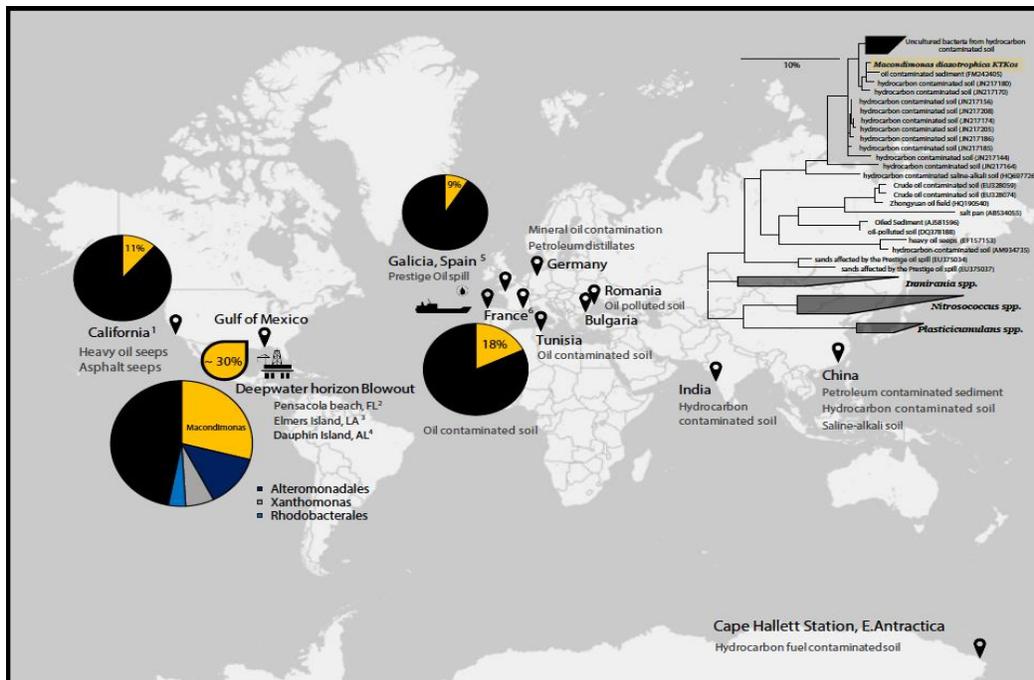


Figure 4. Global distribution of “*Ca. Macondimonas diazotrophica*” (an organism isolated as a direct result of GoMRI support) in oiled coastal sediments based on SSU rRNA gene libraries (Karthikeyan et al., 2019).

Oil-Degrading Enzymes and Pathways

The advent of deep-sequencing technologies enabled full-genome sequencing of individual microbial species comprising complex marine microbial communities, yielding the discovery of novel taxa, genes, and pathways for degrading hydrocarbon compounds of oil. Although these genomic techniques provide important details about individual microbial species, researchers can take a more holistic view to examine the collection of metabolic machinery present in an entire community with metagenomics. Metagenomes can be assembled from shotgun sequencing of complex microbial samples, and with this information, scientists can ascertain the prevalence

and, therefore, importance of certain hydrocarbon degradation genes and pathways (Figure 5). By integrating studies of individual species and holistic communities, researchers paint a more complete picture of microbial ecosystem function and response to environmental disturbances like the DWH spill.

Using these tools, GoMRI researchers discovered that:

- Many metabolic genes for aerobic and anaerobic hydrocarbon degradation were prevalent in microbial communities within the deep-sea oil plume, suggesting significant potential for natural bioremediation. Many of these genes were novel and previously undescribed and have now been characterized and demonstrated to carry out hydrocarbon degradation.
- In oil-contaminated sediments, microbial communities rely heavily on alkane monooxygenases to degrade aliphatic and aromatic compounds. In contrast, PAHs are more resistant to degradation by these indigenous microbial communities, and their persistence could have a negative impact on the sediment ecosystem.
- Genes for hydrocarbon degradation, such as *alkB*, are unusually common in uncontaminated samples from the Gulf of Mexico compared to many other regions of the world, suggesting that this microbial ecosystem is primed for oil bioremediation. This priming may be due to the natural, persistent oil seepage that occurs throughout the Gulf system and/or through natural (i.e., cyanobacterial) production of short-chain alkanes, as well as spillage from oil and gas production operations.
- Altogether, the composition, structure, and function of marine microbial communities were dramatically altered by the DWH spill. These responses highlight the natural capacity of indigenous microbiota for oil remediation, providing an essential ecosystem service in the Gulf of Mexico.

Functional Shifts in Response to Oil

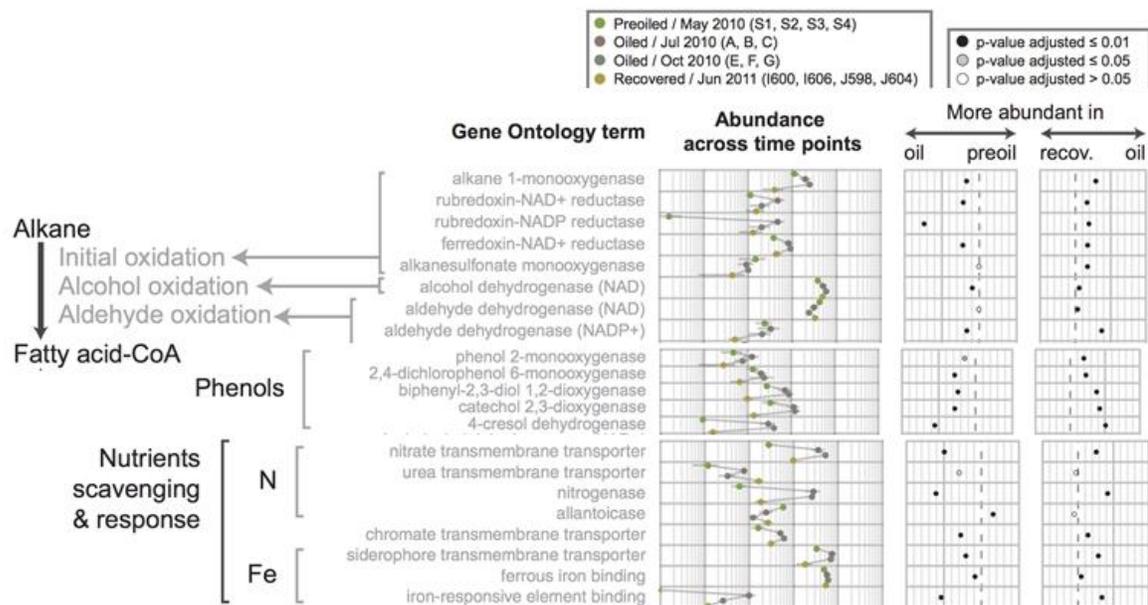


Figure 5. Microbial community functional shifts in coastal sediments in response to oil from the Deepwater Horizon discharge. Genes of selected molecular functions related to hydrocarbon degradation, nutrient scavenging and response, photosynthesis, and some housekeeping genes are listed (left) along with the mean genome equivalents per group of samples (middle) and the log₂ of pre-oil/oiled and oiled/recovered (recov.) fold changes (right). Gene abundance was assessed as the average genome equivalents (mean copies per bacterial or archaeal cell) at each sampling period. The log₂ fold change was estimated as the log₂ of the ratio of normalized counts between pre-oiled samples (S1 to S4) and oiled samples (A to G) and between oiled samples and recovered samples (I600, I606, J598, and J604). P values were estimated using a negative binomial test. Interesting patterns were the succession of genes related to easily degradable, light-hydrocarbon fractions, followed by genes specializing in PAHs and complex aromatics and an increase in nitrogen fixation genes that denote nitrogen limitation for the microbial communities during crude oil biodegradation (Rodriguez-R et al., 2015). CoA, coenzyme A. (Rodriguez-R et al., 2015).

2. Dynamics of Oil-Degrading Microbial Communities

Microbial Partnerships

A microbial community is much more than the sum of its parts; it is an array of cooperative interactions that range from mutualistic symbioses to simpler commensal or synergistic metabolic exchanges. Microbial partnerships are critical to microbial community functions and processes, including hydrocarbon degradation.

Ecosystem processes are driven by certain groups of microorganisms. In some cases, scientists have identified key contributing taxa, like certain members of the family *Oceanospirillaceae*, in hydrocarbon metabolism. However, we are far from knowing all the contributors and interactions involved in hydrocarbon cycling in the ocean. Metagenomics allows researchers to identify associations and partnerships, pinpoint contributing taxa and species, and attribute specific roles to individual contributors. GoMRI researchers have uncovered many new microbial partnerships in oil degradation, as follows:

- Genes involved in oil degradation pathways may not be possessed by a single microbe but may be dispersed throughout the community. In some cases, different community members specialize in specific metabolic steps and shuttle metabolites between them, thereby creating collaborative networks of microbes that together accomplish the oil degradation process. In other cases, certain species specialize in breaking down by-products generated by other species. Together, these microbial networks accomplish far more, with greater efficiency, than any single species.
- Microbes within a community share hydrocarbon degradation genes with each other via lateral gene transfer, mediated by mobile genetic elements, e.g., plasmids, transposable elements, and bacteriophages. Mobile genetic material expands the hydrocarbon degradation potential of the entire community. It can also generate new catabolic pathways for hydrocarbon degradation as different genes get shuffled around, yielding novel combinations of degradation enzymes and capabilities in new hosts.

Community Dynamics

Scientists can now analyze the prevalence of specific genes and DNA sequences with bioinformatics tools in order to determine the proportions of different species within a microbial community. A comparison of data between sites and over time reveals how microbial communities respond to perturbations, such as an oil spill, or other environmental disturbance.

GoMRI researchers found that the DWH spill caused major shifts in microbial ecosystem structure and function.

rare biosphere- organisms that are present in low abundance across ecosystems, which increase in prevalence to dominate the microbial community when conditions change to promote a “bloom.”

bloom- an overgrowth of a particular group of microorganisms in response to a change in carbon or other resource availability

- Oil-degrading microbes were typically found to be present in very small numbers in uncontaminated Gulf environments but had a significant growth advantage upon influx of oil and weathered oil residues. With the unique ability to utilize hydrocarbons as a food source, these rare biosphere species rapidly outgrew their neighbors during the DWH spill, ultimately accounting for up to 90% of the community (Kleindienst et al., 2015; Karthikeyan et al., 2019). Most of these responsive populations could not be documented by culture methods. Thus, omics methods revealed keystone species that should be targeted for future cultivation efforts. Populations of ammonia oxidizers and other autotrophs and heterotrophs that typically dominate healthy systems declined in abundance after the DWH spill (Newell et al., 2014; Huettel et al., 2018). While coastal nitrifiers returned after the oil dissipated in offshore pelagic waters, populations remained altered for at least a year (Newell et al., 2014; Huettel et al., 2018).
- Over time, the bloom bacteria consumed hydrocarbons, sometimes transforming them into metabolic by-products that were more difficult to break down. After depleting available nutrients, bloom species died off, while other organisms grew and took over the degradation of newly produced compounds. These shifts in microbial community structure and ecosystem function were driven by the pulse of hydrocarbons into the environment, modulated to some degree by the availability of nutrients and other resources. Multiple hydrocarbon-degrading microbial species functioned concurrently throughout the spill, and dominant species shifted substantially over time, depending on the abundance of different hydrocarbon fractions and other environmental factors.

Microbial succession was observed in both deep-sea and beach sand environments, each with unique species and dynamics:

Deep-Sea Plume- Methane, and potentially propane, butane, and ethane to some degree, fueled the early microbial response (Figure 6; Crespo-Medina et al., 2014; Joye et al., 2011; Valentine et al., 2010). Immediately after the spill, when n-alkanes and cycloalkanes were more abundant, *Oceanospirillaceae* and *Pseudomonas* spp. were dominant in clone libraries (Figure 7; Hazen et al., 2010). These were later supplanted mainly by *Colwellia* spp. and, to a lesser degree, *Cycloclasticus* and *Pseudoalteromonas* spp., which peaked when gaseous and simple aromatic hydrocarbons became abundant (Dubinsky et al., 2013; Kleindienst et al., 2015). With metagenomics, scientists confirmed that the different hydrocarbon degradation genes and pathways of each of these microbial species corresponded with abundances of different substrates at different times within the plume (Mason et al., 2012; Redmond and Valentine, 2012). Methane was a major driver, coinciding with the emergence of *Methylomonas* species with methane oxidation capabilities (Crespo-Medina et al., 2014). These studies underscore the critical importance of timing in sampling and drawing conclusions, as with the fluctuation of major hydrocarbon components and microbial degraders over time, conclusions based on a particular time point cannot be generalized (Crespo-Medina et al., 2014).

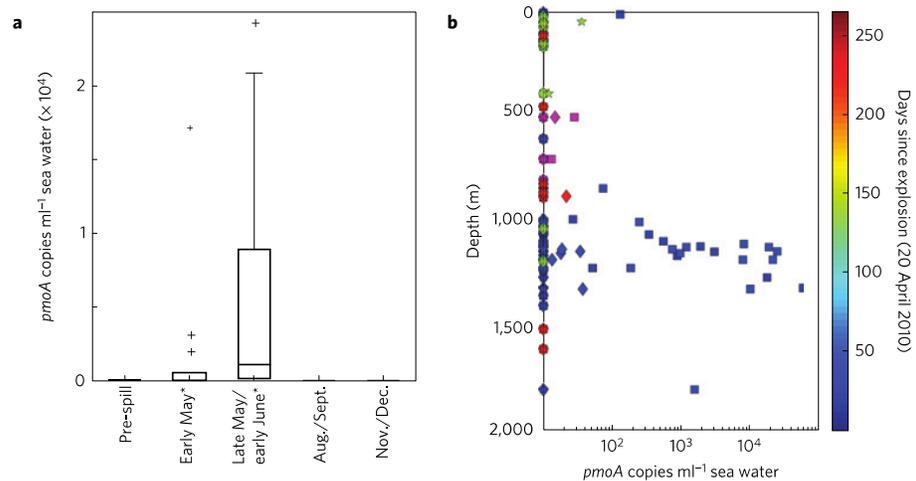


Figure 2 | Abundance of *pmoA* genes following the Deepwater Horizon discharge. a. Abundance of methanotrophic bacterial *pmoA* genes, data are binned by time period. **b.** Abundance of *pmoA* gene copies over time (same colour coding as Fig. 1; pre-spill samples are magenta). Stars, OPU1; diamonds, OPU3; squares, new phylotype. Data in the two time periods marked with an asterisk in **a** are different from those collected at other times with a statistical significance of $P < 0.05$. Plus signs denote extreme data outliers.

Figure 6. Abundance of *pmoA* genes following the Deepwater Horizon discharge. (a)

Abundance of methanotrophic bacterial *pmoA* genes. Data are binned by time period. (b) Abundance of *pmoA* gene copies over time (pre-spill samples are magenta). Stars, OPU1; diamonds, OPU3; squares, new phenotype. Data in the two time periods marked with an asterisk in panel a are different from those collected at other times, with a statistical significance of a P value of <0.05. Plus signs denote extreme data outliers. (Crespo-Medina et al., 2014).

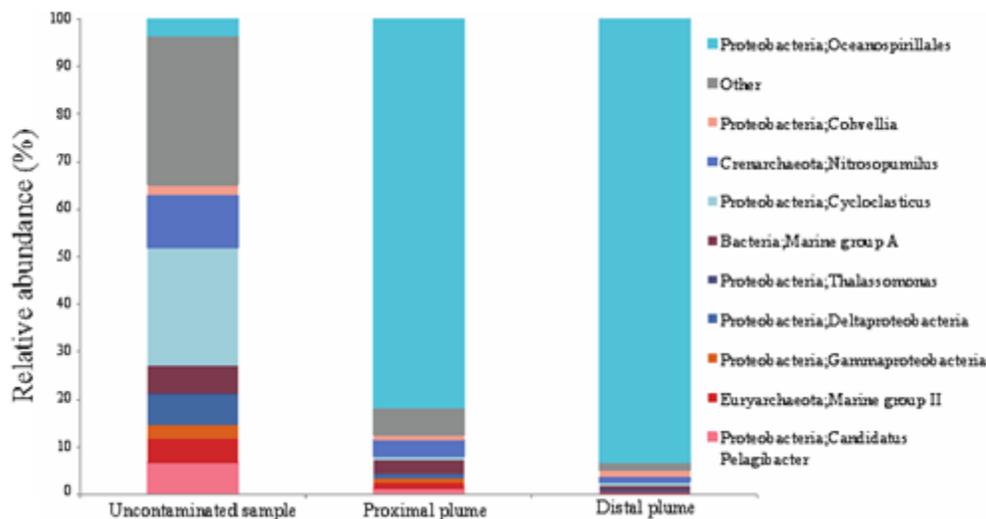


Figure 7. Microbial community structure and function change drastically in response to hydrocarbons. Community structure, function, and succession patterns varied depending on the location and time of sampling (Mason et al., 2012).

Beach Sands- Oil contamination caused a bloom of indigenous microbes capable of hydrocarbon degradation, resulting in a drastic decline in taxonomic diversity (Kostka et al., 2011).

Metagenomic time-series studies revealed a succession of microbial populations that paralleled the chemical evolution of petroleum hydrocarbons (Rodriguez-R et al., 2015). Early responders were from microbial groups known to degrade aliphatic hydrocarbons, mostly members of the *Gammaproteobacteria* (*Alcanivorax* and *Marinobacter* spp.). These were replaced after 3 months by populations of *Alphaproteobacteria* (*Hyphomonas* and *Parvibaculum* spp.) capable of aromatic hydrocarbon decomposition. This shift coincided with the disappearance of alkanes and persistence of aromatics in field samples (Huettel et al., 2018). Nearly all of the oil on Pensacola Beach was degraded after approximately 1 year, and a typical beach community was

reestablished, showing minimal evidence of oil hydrocarbon degradation potential, with nearly all of the known hydrocarbon degraders along with hydrocarbon degradation genes returning to low or undetectable levels (Rodriguez-R et al., 2015).



(Images Credit: Markus Huettel, Huettel et al., 2018)

Salt Marshes- Knowledge of salt marsh microbial community structure and composition was limited before the DWH oil spill. During and after the spill, microbial diversity in aerobic and anaerobic sediments decreased as weathered oil and oil residues accumulated over time (Atlas et al., 2015). Dominant microbial groups were capable of degrading hydrocarbon compounds, such as alkanes and aromatics. In particular, studies of anoxic sediments reported the enrichment of sulfate-reducing bacteria, primarily *Desulfococcus* spp., in parallel with oil contamination.

Weathered oil can remain in the anoxic sediments for decades, but marshes are dynamic ecosystems, where hurricanes and tropical storms redistribute weathered oil. Upon redistribution, hydrocarbon substrates are re-exposed to aerobic hydrocarbon degraders, driving growth. Consequently, marsh sediment microbial communities maintained a high relative abundance of hydrocarbon degraders relative to nondegraders and did not return to pre-spill compositions. These lingering shifts in microbial community diversity and function have the potential to impact the overall marsh ecosystem, particularly marsh vegetation and fishery health.

Seafloor Environments- Biodegradation occurs much more slowly in the absence of oxygen, and microbial communities in seafloor environments, which tend to be anoxic, received comparatively little attention during studies of the response to the DWH spill (Lui et al., 2012). Anaerobic microorganisms that degrade hydrocarbons remain understudied relative to their aerobic counterparts (Meckenstock et al., 2016). However, GoMRI researchers provided some evidence of anaerobic hydrocarbon degradation at the seafloor and in salt marsh sediments, even

though these environments remained largely anoxic, but for the most part, oil persists in marshes and along the deep seafloor (Liu et al., 2012; Chanton et al., 2015).

Studies of sediments collected in relatively close proximity to the DWH wellhead within 1 to 2 years of the discharge showed evidence of enhanced anaerobic oil metabolism (Mason et al., 2014). Genes for anaerobic respiration (denitrification and sulfate reduction) were enriched in parallel with genes for anaerobic hydrocarbon degradation (*assA* and *bssA*), and members of the *Deltaproteobacteria* associated with these processes increased in relative abundance. Other studies of sediments collected at the continental shelf off the coast of Louisiana provided some of the first evidence for anaerobic PAH degradation pathways, though these sediments were not affected by the Deepwater Horizon spill (Shin et al., 2019a). Although it remains difficult to attribute the metabolism of specific hydrocarbons to specific microbes, novel consortia that were observed include:

- Hexadecane-degrading microbes closely related to the *Desulfobacteraceae* that are known alkane degraders
- Phenanthrene-degrading microbes were found to be most closely related to *Desulfatiglans* spp.
 - PAH degradation was likely mediated by novel genera or families of sulfate-reducing bacteria along with their fermentative syntrophic partners.
 - Candidate genes linked to the degradation of aromatic hydrocarbons were detected and are available for future study.

3. Biogeochemical Processes

Microbes play fundamental roles in basic biogeochemical cycles of marine and coastal ecosystems, including carbon, nitrogen, and phosphorus cycling. These cycles are interconnected with each other and also with hydrocarbon degradation. Omics analysis of these pathways reveals how these ecosystem functions were impacted by the DWH spill.

Nitrogen Fixation

Oil-degrading microbes require nitrogen to grow, an element in limited supply in marine environments. Although nitrogen fertilizers are often added to accelerate microbial growth and

biodegradation, as observed during the *Exxon Valdez* spill in Prince William Sound, AK (Pritchard et al., 1992), microbes can also generate their own nitrogen through biochemical fixation of dinitrogen gas (Foght, 2010). Nitrogen fixation pathways are well known in soil microbes that support crop growth and in oceanic microbes such as *Trichodesmium* cyanobacteria (Bergman et al., 2013), but nitrogen fixation by oil degraders in response to hydrocarbon exposure is a new discovery.

Researchers studying the DWH spill discovered that nitrogen fixation is a key ecosystem function of microbial communities in response to oil (Rodriguez-R. et al., 2015; Fernandez-Carrera et al., 2016). In the offshore and coastal ocean ecosystems where nitrogen is limiting, nitrogen fixation genes increase in abundance in response to oil contamination (Scott et al., 2014; Rodriguez-R et al., 2015; Gaby et al., 2018; Shin et al., 2019b). These genes coincide with the presence of hydrocarbon degradation pathways and dissipate once oil and associated hydrocarbon compounds have disappeared (Shin et al., 2019b).

Fixing nitrogen “imparts” a strong selective advantage for rare species in the presence of oil, allowing them to utilize hydrocarbon energy sources without restriction. Indeed, species with the metabolic capabilities to fix nitrogen and degrade hydrocarbons are uncommon but thrive in oil-contaminated environments. These include the keystone oil-degrading organisms such as “*Candidatus* Macondimonas diazotrophica,” explaining its niche dominance (Karthikeyan et al., 2019).

Various other genes that are relevant to carbon, nitrogen, phosphorus, sulfur, and iron cycling were also enriched in microbial populations found within oil-contaminated ecosystems (Rodriguez-R. et al., 2015), including the deep-sea plume (Mason et al., 2012). Their roles in facilitating hydrocarbon degradation and ecosystem functions require further study and likely fluctuate over time with changing conditions. Understanding these geochemical dependencies could inform fertilization strategies to enhance the biodegradation of specific compounds.

For example, in the DWH plume, nitrogen and phosphorus became limiting at first, and later, trace metal availability limited specific enzymatic functions such that certain methylotrophs

could no longer oxidize methanol due to the depletion of required lanthanide cofactors (Shiller et al., 2017). Supplying these essential nutrients could enhance natural biodegradation.

Marine oil snow- oil entrained in biosurfactants or transparent exopolymer (TEP) substances, which sinks to the seafloor, thereby removing hydrocarbons from the water column.

Hydrocarbon-degrading microbes flock to sources of oil and assemble large communities around oil droplets. These oil degraders (and in some cases phytoplankton) generate TEP substances that provide a framework for the formation of marine oil snow (MOS), which entraps oil in a macroscopic assemblage of carbohydrates and biomass (Figure 8; Passow et al., 2012; Ziervogel et al., 2012). MOS particles appear like snow in the water column and sink downwards toward the seafloor due to their large size and density (Daly et al., 2016; Passow, 2016). This was an important fate of oil after the DWH spill, removing significant amounts of hydrocarbons from the water column and transporting them to the seafloor (Vonk et al., 2015).

The DWH spill triggered the formation of this MOS in unprecedented quantities, leading researchers to thoroughly examine this microbially mediated biogeochemical process for the first time (Joye et al., 2014). They found that MOS has a major impact on the fate of spilled oil through the following two major mechanisms (Figure 8):

Degradation- MOS particles are hot spots for oil degradation, with high levels of lipase activity (Gutierrez et al., 2018). MOS contains microbial communities that are distinct from the surrounding water column and specialized for breaking down oil. In particular, *Colwellia* and *Marinobacter* spp. (as well as other *Alteromonas* spp.) are prevalent in these particles, with the unique capacity to rapidly degrade oil in cold, deep marine environments (Gutierrez et al., 2018).

Deposition- MOS particles sink rapidly, eventually depositing on the seafloor within approximately 10 days (Passow, 2016). This acts as a pump, removing large amounts of oil from the water column and transferring it to the seabed, serving as a natural decontamination process for the water column and a pollution source for the benthos.

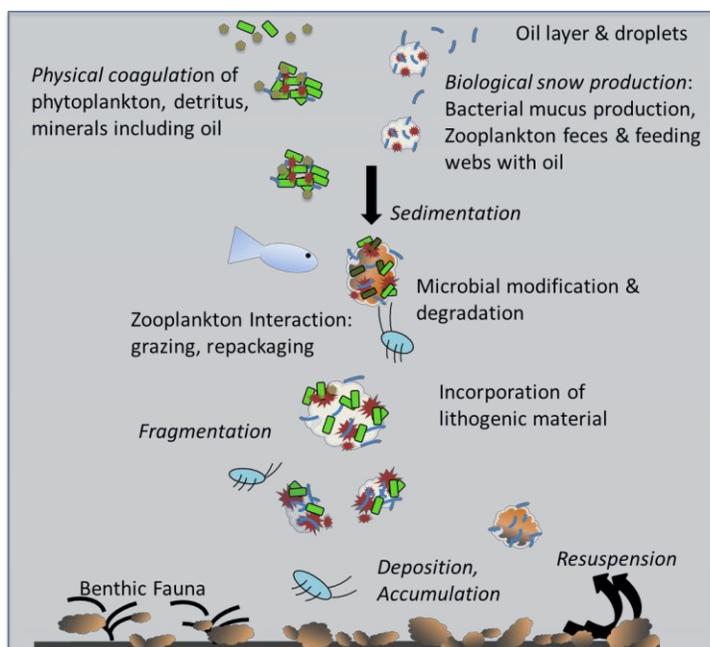


Figure 8. The formation of marine oil snow (MOS), oil entrained in biosurfactants or transparent exopolymer (TEP) substances, interacting with biotic and abiotic particles, which sinks to the seafloor, thereby removing hydrocarbons from the water column. (Image Credit: Uta Passow, University of California, Santa Barbara).

MOS formation has been documented during previous oil spills, such as the *Tsesis* (Baltic Sea in 1977) (Johansson et al., 1980) and *Ixtoc-I* (Mexico in 1979) (Patton et al., 1981) spills. However, whether MOS formation would be triggered by more refined oils like diesel or gasoline or by specific hydrocarbon fractions like aliphatic or aromatic hydrocarbons is unknown. Although physical and chemical processes that mediate MOS formation are unknown, researchers have pinpointed some environmental factors that can induce aggregation, as follows:

- Detritus
- Feces
- Minerals
- Diatoms and other microalgae
- Microbial cells (bacteria)
- Polymers excreted by microbial cells and diatoms (i.e., extracellular polysaccharides and other dissolved compounds)

- Chemical dispersants

Is MOS Good or Bad?

MOS benefits the aquatic environment by removing hydrocarbons from the water column through both microbial degradation and deposition on the seafloor. Therefore, triggering its formation during the event of a spill might mitigate impacts on aquatic ecosystems. This could be accomplished by adding “seeding” agents, like clay minerals, to favor MOS formation. More research is required to determine which concentrations and conditions would optimally trigger MOS under various circumstances.

However, MOS may have negative consequences at the seafloor where it is ultimately deposited. MOS particles can effectively seal off the sediment surface, and although the oil concentration in the majority of MOS is quite low, the initial depositional event may suffocate fauna, and the enriched organic content in these layers may stimulate respiration in the benthos, with further negative consequences for filter-feeding fauna. The impact of MOS deposition to sediment is unclear, and more research is needed to understand its consequences in the water column and benthos.



Under the light microscope, these *Alteromonas* sp. strain TK46(2)-formed marine oil snow (MOS) particles stained with Alcian Blue (Image Credit: Tony Gutierrez; Gutierrez, et al. 2018).

III. SIGNIFICANCE AND APPLICATIONS OF GoMRI RESEARCH

Efforts spent characterizing the response of microbial communities in the Gulf of Mexico to the DWH oil spill generated knowledge with far-reaching impacts. Scientific and technological advances derived from this work lay the foundation for research across broad scientific disciplines, and far beyond this region of the world, to improve strategies for the prediction and mitigation of ecosystem perturbations.

1. Informing the Oil Spill Response

With the knowledge and tools gained from GoMRI studies of marine microbial ecosystem responses to the DWH spill, scientists can now play key roles in informing future oil spill response plans. In the past, some emergency response efforts consisted of trial and error, gambling on success with minimal information or data to back the strategy. Now, for the first time, scientific evidence and tools are available that enable a data-driven approach for oil spill response and mitigation. These insights will improve both the success and impact of future emergency responses.

Data-Driven Spill Response

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 be instrumental in guiding spill response efforts before, during, and after a spill to evaluate ecosystem responses and recovery. These tools give scientists the means to:

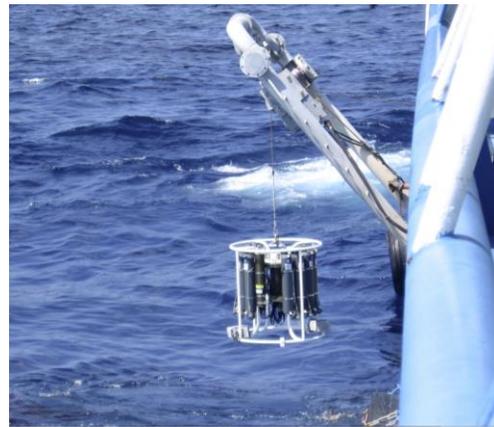
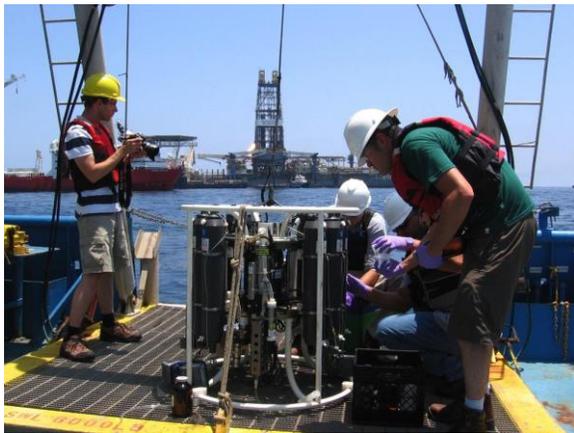
- Document the “normal” or “baseline” state of the ecosystem prior to a crisis, from which changes and impacts can be identified
- Plan intelligent mitigation strategies aimed at restoring these native microbial communities, otherwise known as microbiomes, and their ecosystem functions
- Guide decision-making during a crisis based on ecosystem and environmental measurements in real time
- Monitor mitigation and restoration efforts after a crisis to evaluate their success, thereby informing changes in course of action for current and/or future spills

With these omics tools, scientists can help first responders determine:

- Potential environmental impacts of a spill and risks posed to the environment
- Actions that should be taken, and on what time frames, to minimize risks and damage?
Which actions should be prioritized, and in which locations?
- Whether response actions are working as predicted or alternative plans should be implemented now or in the future?

Given the new availability of omics tools, microbiological analysis and expertise should be incorporated into the spill response as quickly as possible and throughout the life of the spill to evaluate ecosystem responses and recovery. Specifically, scientists can inform first responders by examining:

- ***Oil-Degrading Species***- The presence or absence of oil degraders can reveal the natural bioremediation potential of the community and advise whether and how their metabolic capabilities could be augmented.
- ***Metabolism, Metabolites, and Limiting Nutrients***- Observations on the metabolic capabilities of the community (i.e., nitrogen fixers and ammonium oxidizers) along with the presence of metabolites and major nutrients (i.e., nitrogen versus phosphorus) allow scientists to predict hydrocarbon degradation potential and the environmental parameters likely to limit activity (Edwards et al., 2011, Fernandez-Carrera et al., 2016.). This information can guide strategies to alter these limitations.
- ***Natural Dispersants***- The presence or absence of microbes that produce exopolymeric substances that emulsify oil and act like dispersants might indicate to scientists and first responders whether man-made dispersants (e.g., Corexit) are necessary (Ziervogel et al., 2019). Scientists can help develop strategies to promote the growth of these species or to design such species (using genetic engineering to introduce genes coding for surfactant production into selected species), thereby providing responders with natural, biodegradable, and nontoxic surfactants as an alternative to chemical dispersants. Meanwhile, genomic studies can inform the development of nontoxic or biologically inert dispersants with fewer environmental concerns than with chemical dispersants currently employed (Bacosa et al., 2018).



Scientists collect water samples to determine the response of the microbial ecosystem to the DWH spill. (Images Credit: Gulf of Mexico Research Initiative)

Transferable Lessons

Many of the principal conclusions drawn from microbial studies of the DWH spill by GoMRI researchers can be applied to future oil spills or other disasters in marine environments, as follows:

- Hydrocarbon-degrading microorganisms are ubiquitous and occupy the rare biosphere across the world's oceans. Usually present in low numbers, oil-degrading species can rapidly reproduce, going from low abundance or even undetectable levels to a substantial and metabolically active community when exposed to oil (Kleindienst et al., 2015a). However, growth rates and response times will vary with environmental (physical, chemical, and biological) parameters (Huettel et al., 2018; Sun and Kostka, 2019).
- Marine oil snow (MOS) can play an important role in transporting oil and its components to the seafloor, with positive and negative consequences (Passow, 2016). Chemical dispersants may accelerate this process (Suja et al., 2017; Suja et al., 2019).
- The effects of dispersants on microbial communities currently are debated, with many expressing concerns about negative influence (Kleindienst et al., 2015; Tremblay et al., 2017). At high concentrations, dispersants are toxic to many corals and other metazoans. Dispersants also promote MOS deposition (Suja et al., 2019), which can injure delicate

organisms and kill their larvae. Dispersants can also interfere with oil biodegradation, since microbes may consume labile components of the dispersant preferentially instead of the oil (Kleindienst et al., 2015).

Variable Influences

GoMRI scientists have established that the response of a microbial community to an oil spill will vary depending on the following:

- ***Environmental Conditions-*** Hydrocarbon degradation is a respiration process, and like all biological processes, it is limited by temperature, the supply of oxygen and major nutrients, and other environmental parameters (Head et al., 2006; Redmond and Valentine, 2012; Rodriguez-R. et al., 2015; Fernandez-Carrera et al., 2016; Sun and Kostka, 2019). For example, petroleum hydrocarbons are generally rich in carbon and poor in major nutrients (e.g. nitrogen and phosphorus; Edwards et al., 2011). Thus, oil acts as a major input of carbon for the microbial food web, and depending on the ecosystem, nutrients can rapidly become limiting and impact the responses of hydrocarbon degraders along with other microbes that play a key role in ecosystem functioning.
- ***Microbial Community Composition-*** The species composition and metabolic state of a microbial community at the onset of a spill can direct different trajectories of response, hence driving different outcomes. For example, if the ecosystem is compromised by environmental stress, e.g., nutrient (nitrogen or phosphorus) limitation or water temperature fluctuating with the seasons, it may be more sensitive to a spill (Sun and Kostka, 2019). Genomics can provide information about the resident microbial populations, pinpointing sensitive ecosystems needing protection, e.g., by limiting drilling or tanker traffic. If sensitive systems are known, then they can receive more attention in the event of a spill. Then, mitigation and remediation efforts can be taken to restore ecosystem health.

GoMRI research has identified major microbial players that degrade specific hydrocarbon compounds, providing a starting point for investigating microbial communities that can degrade different types of oil. Although many microbial species and genes have yet to be

discovered, methods and technology for identifying and characterizing these novel microbial species have been developed by GoMRI scientists. Some of the oil-specific players and their metabolic responses will be further studied by analysis of field samples and laboratory mesocosm studies.

- ***Oil Composition-*** Oil comes in many unique compositions; crude (light or heavy) versus refined petroleum derivatives contain different types and proportions of hydrocarbon compounds, i.e., various alkanes, PAHs, BETX, etc., that have different rates of natural and microbial degradation. For a given incident, the composition of hydrocarbons will select for microbial species that possess the metabolic capability to degrade those oil components. In addition, physical and chemical factors in the environment can alter the composition of oil over time. For example, sunlight oxidizes oil, producing new hydrocarbon intermediates that are more bioavailable to microorganisms (Bacosa et al., 2015; Shin et al., 2019b). Some species will thrive as new hydrocarbon substrates become available, whereas others may die off from UV toxicity, exposure to free radicals, or accumulation of toxic concentrations of degradation or weathering products. The state of weathering can also affect the propensity of the oil to induce MOS formation.
- ***Spill Dynamics-*** The rate of discharge and the scale of a spill will affect the physical form of the oil released, along with nutrient and hydrocarbon availability over time scales and within geographic areas. Sudden versus gradual release of hydrocarbons into the environment will drive different ecosystem responses (Socolofsky et al., 2015).

2. Global versus Regional Lessons

Whether lessons learned from GoMRI research are applicable to other geographical regions will depend on physical, chemical, and biological properties of the region, as well as the scientific research (molecular, ecological, etc.) undertaken.

Global Commonalities

Many ecological and evolutionary conclusions drawn from the study of microbial communities present in the Gulf of Mexico, and how these communities responded to the DWH oil spill, are applicable to other regions:

- Oil-degrading species, as well as their genetic and metabolic potential, discovered by GoMRI scientists have been documented to occur in oil-contaminated waters and coastal regions around the world. These microbial first responders are ubiquitous, providing essential ecosystem cleanup services globally.
- Useful information about microbial processes, responses to disturbance, and community succession related to ecosystem function are also geographically transferable. These principles, described in Omics Technologies Enable New Biogeochemical Discoveries, underlie microbial community composition and response universally across diverse environments and contexts.
- Research methods derived from GoMRI research provide a globally useful foundation for studying marine microbial ecosystems and elucidating their role in responding to environmental perturbation (see Establishing a Suite of Tools for Environmental Microbiology Research).

In summary, GoMRI research provides an interdisciplinary, genome-enabled, road map informing scientists and government responders on directions for preparation and response to future oil spills and/or environmental disasters, wherever they may occur. Natural seeps in the Gulf of Mexico occur because hydrocarbons from deep reservoirs flow to the surface along fault networks. These discharged hydrocarbons prime waters, creating exposure regimes that favor oil-degrading microorganisms (Figure 9).

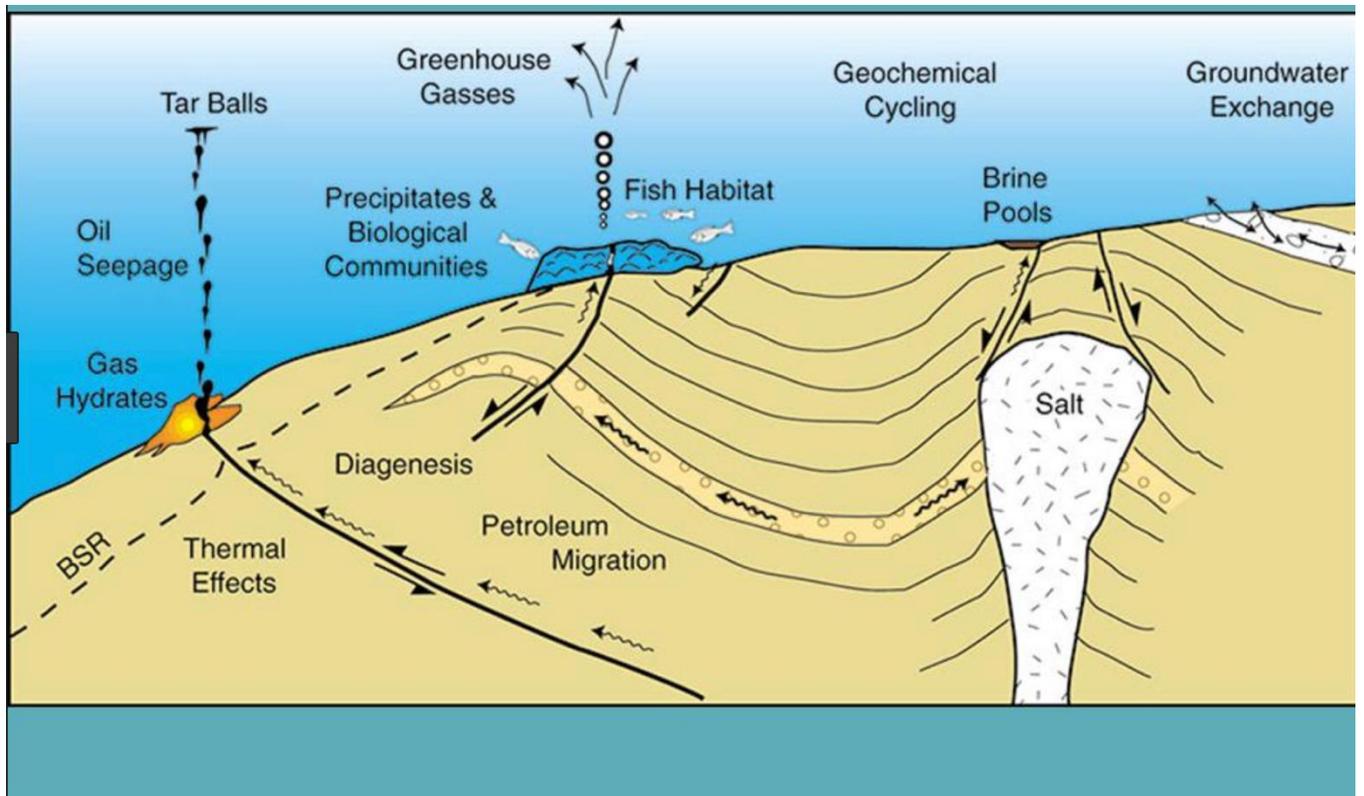


Figure 9. Geologic underpinning of natural hydrocarbon seeps at the seafloor in the Gulf of Mexico. (Moore, 1999).

Regional Differences

Oil-degrading microbes are globally distributed, but regional variables can significantly influence microbial response to oil spills and the environmental impact. For example, a deepwater blowout off the coast of Brazil would have different consequences than would a tanker spill in Arctic surface waters. Even the same type of oil spilled can have varied consequences in geographically different regions. Differences in physical and chemical conditions will select for microbial community composition and properties prior to a spill and, therefore, drive different responses relative to hydrocarbon biodegradation, as follows:

- Salinity, nutrient availability (including N, P, Fe, Cu, and rare earth elements), sunlight, oxygen, and temperature will select for certain species and metabolic pathways in

different regions. Salinity may be important in the context of oil tanker travel in the open ocean compared to an oil spill in an estuary, coastal port, or freshwater lake.

- Hydrostatic pressure affects degradation, with rates varying according to ocean depth through impacts on the supply rate and dilution of hydrocarbons as well as through physiological effects on the oil-degrading microbes themselves. Hydrostatic pressure can also influence the nature of the discharge from a well blowout, a particularly important factor when considering the behavior of live oil at depth and the biodegradation of oil in deepwater plumes.
- Water current and stratification of the water column, including flux between deep and surface layers, can influence both oil and microbe dispersal and thereby affect oil degradation rates.
- Water flow can impact biodegradation dramatically in different coastal environments. For example, wave-washed beach sand versus a salt marsh (static environment).
- Sea ice influences oil dispersal in the Arctic and microbial community assembly and dynamics.
- MOS formation and deposition on the seafloor can differ as a function of many of these parameters and the presence of particulate organic matter.

A majority of the hydrocarbons from the DWH spill remained in the deepwater plume and were thus more confined within the water column, although the final fate of the plume material remains unknown. Had the spill occurred elsewhere in the Gulf of Mexico, or during a different season with different physics or a strong hurricane, the oil would have been distributed differently, with the potential for a worse outcome.

In addition, the DWH spill provided a unique opportunity to study many of these environmental factors, because the spill spanned a wide variety of open-water and coastal ecosystems with large variations in physical and chemical conditions. However, much work is left to be done to quantify the impacts of these variables on microbial response and oil degradation. To do so, environmental parameters can be manipulated in laboratory mesocosm experiments, where microbial responses are measured in a controlled environment. Such studies will inform

numerical models, which can be deployed from the Arctic to the Amazon, to predict ecosystem response based on environmental and genomics measurements.

The Arctic

Oil spills are a universal concern but are particularly threatening in Arctic regions where climate change is already wreaking havoc. Major drilling activities further threaten these delicate marine and coastal ecosystems. It is therefore critical to understand the environmental risks and develop mitigation strategies before a major spill occurs in these sensitive areas. For example, the Canadian government is actively engaged with the GoMRI community to build on GoMRI discoveries, methodology, and technology developments in their efforts to prepare for a spill. In fact, GoMRI research is particularly pertinent to this region, as preliminary work has revealed similar microbial responses and community succession in Arctic marine environments. However, environmental factors specific to Arctic ecosystems, notably sea ice and extremely low temperatures, can alter the degradation properties of both oil and microbial communities, which may have unique impacts on the potential for oil biodegradation.

Is the Gulf of Mexico Uniquely Primed for Oil Degradation?

Some evidence suggests that microbial communities in the Gulf of Mexico may be uniquely primed for rapid response to oil spills, due to the prevalence of natural hydrocarbon seeps and ongoing oil drilling operations that constantly leak oil into the Gulf. Background levels of hydrocarbons appear to sustain higher proportions of oil-degrading microbes in the microbial community, and these communities are ready for action in the event of a large spill. However, this may also be true of many other regions around the world with natural seepage or hydrothermal venting, including the Gulf of California, Mexico, Bay of Bengal, Black Sea, and the Arctic. Open waters or ports devoid of natural hydrocarbon sources may be primed for oil response because of industrial and human activities, since shipping lanes inevitably introduce hydrocarbons into the water. Last, phytoplankton produce hydrocarbons, such as alkanes, which could prime microbes for hydrocarbon degradation through persistent exposure at low levels.

3. Applications for Assessing Ecosystem Health in the Context of Environmental Disturbances

GoMRI discoveries and methodology are widely applicable beyond the context of oil spills. By establishing basic principles of microbial ecosystem function and response, and the techniques to study them, GoMRI scientists have laid the groundwork to understand diverse marine and terrestrial ecosystems in the face of various natural and man-made disasters, from chemical spills to algal blooms.

These methodologies will be indispensable in studying gradual and long-term stressors like climate change, the impacts of which are unconstrained. Climate change poses increasingly urgent threats to Earth's ecosystems, and microbes form the foundation of them all.

Understanding microbial ecosystem dynamics and function in response to climate perturbations is essential to predict long-term impact and prepare mitigation and restoration plans in the event of another oil spill or other large disturbances.

Understanding the impact of any environmental disturbance on microbial communities and their ecosystem function requires information on community composition and activities under normal circumstances, i.e., the natural baseline. Establishing this foundational information is essential for revealing recognizing changes. Therefore, characterizing microbial communities prior to a disaster is essential for documenting the native microbial community and the ecosystem services it provides. From this baseline, scientists can then:

- Assess the condition of the microbial community (i.e., the microbiome) over time
- Examine community structure and ecosystem functional changes during disturbance
- Develop mitigation and restoration strategies based on microbiome profiles
- Determine the state of ecosystem recovery after a disturbance

Unfortunately, baseline microbiome profiles are woefully lacking. The absence of baseline data was a significant impediment to documenting the effects of the DWH spill. Hurricane forecasting stations provided physical oceanographic baselines that could be used for the surface ocean during the DWH, but such information was absent for deep water. Similarly, coastal surveys provided baselines on animal and plant communities prior to the DWH spill, but no microbial genomic baseline data were available. Documenting indigenous microbial communities in

diverse marine and terrestrial ecosystems around the world now is critical to prepare for future disasters beyond the context of oil spills.

Defining “Baselines”

Baseline is not a fixed target but the range of variation around the mean in a natural environment.

GoMRI researchers learned many lessons about how to define microbial baselines while attempting to establish such information retroactively in Gulf of Mexico ecosystems:

- Microbial communities undergo seasonal, monthly, and even daily fluctuations as water temperatures and nutrient availability shift, i.e., summertime hypoxia, so establishing baselines across a range of time scales is important to assess what is “normal” at a particular time of year at sites across an ecosystem. Variation may occur even more quickly in shallow coastal systems, which are more dynamic, requiring baseline measurements to be assessed at even shorter time scales.
- Microbial communities also exhibit interannual variation, much like weather patterns that may be very wet or warm some years but still within normal variation. Thus, microbial communities should be sampled over long time scales (many years or decades) from the same location, as frequently as possible to establish a range of normal variation, from which an outlier can be easily identified as a significant or problematic change.

Because natural temporal and spatial variation in microbial communities is poorly understood, evaluating the effects of environmental perturbations and/or resilience of the community is difficult. Moreover, studies of ocean microbial populations have revealed decade-scale variability, underscoring the need for a long-term commitment and strategy to fully understand ocean microbiomes.

Establishing the baselines of microbial communities necessary to provide critical information about ecosystem health will require sampling from diverse environments, from deep water benthic regions to coral reefs, beach sands, marshes, and associated terrestrial ecosystems (Magnuson, 1990; Magnuson, 1995). These ecosystems each likely have a unique microbial baseline. Partnership with the oil industry, which has significant economic (\$234 billion in total

revenue per year) and infrastructure resources, could provide the necessary support to carry out the sampling and profiling efforts needed for the capture of baselines in the Gulf of Mexico. Indeed, GoMRI itself has forged this model as a productive approach. The tourism industry and glider lines could also provide some of these solutions to establishing long-term Global Oceans Observing System (GOOS) stations in the Gulf and other potentially vulnerable regions like the Arctic.

Microbial Indicators of Ecosystem Health and Disturbance

GoMRI researchers identified various microbial indicators of ecosystem disturbance in the context of the DWH spill which will help scientists assess ecosystem health across various contexts.

- ***Drop in Diversity-*** A healthy microbial ecosystem is composed of a wide variety of species that provide diverse ecosystem functions and maintain the stability of the ecosystem. Environmental disturbances like the DWH spill disrupt this balance, favoring a limited number of species with the ability to adapt to new conditions. These few species thrive over the others, which is reflected in low overall species diversity. A community with low diversity may be more sensitive and less resilient to further disturbances. The same phenomenon is true of human microbiomes, where low diversity in gut microbial communities is indicative of various diseases.

Thus, low microbial diversity can be a red flag signaling an ecosystem disruption across a wide variety of contexts, including ocean health.

- ***Overgrowth of Rare Species/Genera-*** In undisturbed conditions, the rare biosphere contains microbes across many different taxa that are each present in very small numbers and may not even be detectable. Despite their low numbers, these community members are very important, particularly during environmental disturbances. They harbor unique genes and functional capabilities that may be dormant under normal conditions, but set these species up for success when environmental conditions change. For example, during the DWH spill, rare species with the genetic potential to utilize hydrocarbon energy sources and/or fix nitrogen were poised to take advantage of new energy sources. Under

novel conditions, these core species outcompete their neighbors and grow to comprise one-third or more of the entire microbial community.

The presence of a single species or genus in very high numbers indicates a recent ecosystem disruption, even if the original composition of the community is unknown.

- ***Novel genes, pathways, and ecosystem functions***- As microbes respond to environmental change, responsive organisms may express different genes and metabolic pathways to adapt to new sources of energy (hydrocarbons), nutrients, physical-chemical limitations, and other factors. These changes may reflect the overgrowth of previously undiscovered species with unique genes and metabolic capabilities, or the stimulation of adaptive pathways within the existing community. Many genetic and molecular changes are revealed through metagenomic, metatranscriptomic, and metaproteomic analyses and reveal novel and adaptive ecosystem functions.

The emergence of different and novel genes, metabolic pathways, and ecosystem functions within microbial communities reflects adaptation to environmental disturbances.

The emergence of these genetic and metabolic indicators not only informs scientists of ecosystem disruption, but it may also provide critical information about how the environment has changed, such as the presence of new nutrient sources or toxins. This information can guide mitigation efforts to restore ecosystem health. For example, overgrowth of particular species or genera known for providing certain ecosystem functions can inform scientists which geochemical cycles have been disrupted. A community dominated by a species known for nitrogen fixation may signal that there is a nitrogen deficiency, suggesting that nitrogen fertilizers may accelerate recovery.

Similarly, the emergence of new genes, metabolic pathways, and ecosystem functions can point to the presence of new nutrient sources, novel toxins, or other environmental changes. With this knowledge, scientists can determine what steps should be taken to restore ecosystem health.

Meanwhile, low-diversity communities may be more sensitive and less resilient to further insults and therefore require more effort to protect and restore. Furthermore, previously stressed communities may respond differently to perturbation, leading to unexpected outcomes.

At this point, scientists are only beginning to understand how microbial indicators translate into information about ecosystem function and disruption. The relationship between community function and diversity, on taxonomic and genetic levels, is complex, with inherent redundancies and complex dynamics that are not fully understood. In addition, scientists are continually discovering new organisms and attributing new functions to microbes. This very active area of research promises to yield critical insights in coming years, but many mysteries remain at this time. As such, microbial indicators provide clues for further investigation into ecosystem disruptions but in most cases have limited interpretative value and cannot currently provide definitive answers.

4. Establishing a Suite of Tools for Environmental Microbiology Research

GoMRI researchers developed and optimized genetics, bioinformatics, and experimental methods to advance the knowledge of microbial ecosystem functions in the context of the DWH spill. These approaches represent the cutting edge of microbial genomics and systems biology and can be broadly applied to diverse fields of genetics, microbiology, biogeochemistry, and ecology to advance science well beyond marine microbiology. These tools and strategies provide a powerful means to integrate these diverse fields and promise to yield a wealth of novel insights and directions for science in general.

Although this suite of tools is in the early stages of development, it lays the groundwork for future discovery. In establishing these tools, GoMRI researchers also exposed many challenges and learned lessons about how to overcome them successfully. These lessons will inform further development of technologies and methodologies that build on this foundation. These novel approaches have already illuminated many new avenues of investigation and hypotheses to pursue in future studies.

Field Study Design

GoMRI researchers have devoted a great deal of time, money, and effort to develop and optimize research protocols for field sample collection and data analysis. These established protocols can now be easily deployed in the event of another spill, or they can be adapted for studying microbial responses to other environmental disturbances across diverse ecosystems. Although such protocols will require adaptation for specific circumstances of the incident and location at hand, GoMRI researchers have determined important spatiotemporal dynamics and environmental variables that may help define:

- When and where to collect field samples throughout the course of an event; which requires broad knowledge of ocean systems, particularly the physical context
- What environmental conditions to record (i.e., water temperature, salinity, oxygenation, pressure, nutrients, etc.)
- What microbial data to examine (i.e., metagenomics, transcriptomics, etc.)
- Strategies for data analysis and integration

GoMRI researchers also had to develop and implement new, more portable, and powerful tools to collect and analyze samples more easily in the field. Examples of *in situ* research tools that advanced various aspects of marine science include:

- Mapping technology, such as multibeam echo sounding, sonar swath beam mapping, and remote sensing, to identify areas of hydrocarbon seepage
- Sophisticated gliders with enhanced chemical sensors, such as underwater mass spectrometers
- *In situ* biological sampling systems powered by osmotic pumps

Laboratory Methods

GoMRI researchers established experimental methods to study marine microbes in controlled laboratory systems. The DWH oil spill provided a unique opportunity to develop and improve laboratory designs to better reflect environmental circumstances. These include techniques to:

- Leverage genomic information to isolate and culture novel microbes of interest
- Coculture microbes culture to investigate cooperative partnerships/antagonistic relationships

- Generate model systems that recreate environmental conditions (i.e., microcosm and mesocosm systems) to verify and calibrate field observations
- Omics-directed biochemical assays to examine and confirm predicted ecosystem functions

Genomics and Bioinformatics Tools

The DWH spill coincided with the advent of advanced genetic sequencing technologies, which for the first time enabled high-throughput and cost-effective genomic analysis in a natural context. In comparison, when the *Exxon Valdez* spill occurred, these sequencing technologies were in their infancy and not able to generate sufficient data to promote broad knowledge, leaving scientists without the means to examine the integral roles of microbes in the oil spill response.

To examine microbial processes in the DWH spill, GoMRI researchers incorporated and advanced novel genomics technologies to tease apart the complex microbial communities from marine and coastal ecosystems. Cutting-edge approaches generated vast amounts of new omics data that span across population, ecosystem, and environmental contexts. The complexity of these new data sets required GoMRI researchers to develop innovative bioinformatics strategies to analyze and integrate results. Their innovations include advanced tools to:

- Construct full genomes of individual species within complex communities with novel single-cell and metagenomic assembly methods
- Tease apart metabolic genes and pathways of interest and attribute them to individual species within microbial partnerships and communities
- Integrate multi-omics approaches to provide a complete picture of microbial ecosystem functions and dynamics
- Describe microbial community composition and structure as a whole, as well as changes therein
- Utilize meta-omics data to make predictions and guide functional studies
- Combine meta-metabolomics with other omics technologies

The above-mentioned innovations yielded many novel discoveries that can be applied to study microbial ecosystems and functions in diverse contexts, from soil microbiology to human medicine.

Human Infrastructure Advances and Shared Resources

The GoMRI research effort required diverse scientific fields to come together to study and solve a real-world problem. Biogeochemists, ocean scientists, microbiologists, geneticists, computer scientists, and many others learned to work together in a directed manner to address concerns from the molecular to system scale. In doing so, they established new strategies for collaboration to simultaneously address basic and applied research concerns in the context of an environmental catastrophe. GoMRI scientists pioneered infrastructure and strategies for:

- Coordination of efficient emergency response teams and tasks
- Multidisciplinary consortium-based research on a very large scale
- Development of open-access marine databases and resources like GRIIDC (<https://data.gulfresearchinitiative.org/>), funded by GoMRI. Tara Oceans, funded by the Tara Oceans Foundation, is another such resource, although it was not developed by GoMRI researchers (<https://oceans.taraexpeditions.org/en/m/science/>).
- New funding models for industry support of independent basic and applied research

These forward-thinking strategies for multidisciplinary collaboration will be instrumental in responding to future oil spills and other catastrophic events, as well as for investigating large-scale research questions to prepare for any event with global impact.

5. Broad Lessons for Microbiology and Ocean Science

The DWH spill provided an opportunity to investigate many new aspects of marine microbiology and its intersection with other fields of microbiology and ocean sciences. In forging this research frontier, GoMRI researchers made discoveries that advanced broad fields and moved diverse fields in new directions. Some examples are presented below.

Microbial Ecology

Genomic analysis of microbes directly from field samples enabled the discovery of new species and strains of archaea, bacteria, and viruses, many of which provide essential biogeochemical functions in the ocean ecosystem. In addition, GoMRI studies have expanded our understanding of microbial partnerships and communities that can be extended to other contexts by researchers in related fields.

Ocean Sciences

In examining the biogeochemical cycles and ecosystem functions of microbes, GoMRI researchers have advanced the field significantly over the last decade:

- GoMRI research highlighted the importance of marine oil snow in biogeochemical cycling, with many transferable lessons regarding its formation and fate in other contexts.
- Knowledge of coupled biogeochemical cycles (e.g., carbon, sulfur, oxygen, and nitrogen) in the oceans has been expanded, and the realization was made that the Gulf of Mexico is highly interconnected relative to other bodies of water.

IV. THE FUTURE OF GENOMICS IN ECOLOGY

Although there is still much to learn about how microbial ecosystems respond to environmental disturbance, ultimately, the goal is to translate genomic findings into actionable information to help scientists monitor and restore ecosystem health in the face of climate change and natural or man-made disasters. For example, how might microbiologists help fisheries or oyster farms recover after an oil spill or hurricane? Microbiome information can reveal underlying functional deficits, or gains, that impact the overall health of the entire ecosystem. Scientists can use these insights to direct and fine tune strategies to correct these problems and support ecosystem health.

1. Biological Markers of Ecosystem Health

By examining the genes, metabolic pathways, and organisms present in microbial communities, researchers can “take the pulse” of the ecosystem and identify atypical functioning. These indicators serve as biological markers (biomarkers) to alert scientists to disruptions and guide mitigation strategies, much like how blood tests can point doctors toward disease diagnosis and treatment options.

Ecosystem health should be assessed using multiple parameters and not be limited to one or two measurements. Monitoring diverse parameters will clue scientists in earlier to disturbances and provide more information to pinpoint causes and mitigate consequences. Each biomarker provides one piece of the puzzle that together forms a complete picture of ecosystem health. For example, genomic information can reveal unique insights into hydrocarbon degradation, toxin accumulation, hypoxic conditions, or other environmental disturbances. Meanwhile, metatranscriptomic data can signal an immediate ecosystem response, whereas meta-community-level restructuring may suggest more long-term consequences.

Biomarkers might be used in various ways to assess and support ecosystem health:

- ***Predictive Biomarkers***- As first responders, disruptions in microbial communities are often visible before ecological consequences or impacts are apparent. Microbes can serve as the “canary in the coal mine” to warn of impending consequences of a disturbance. This can help scientists act earlier to better prevent or mitigate potential damages caused by natural and man-made disasters in the oceans, as well as other ecosystems.
- ***Diagnostic Biomarkers***- A disrupted, and possibly unhealthy, ecosystem can be identified by reduction in community diversity, overgrowth of specific species, and the emergence of novel genes, metabolic pathways, and ecosystem function. In the future, specific indicator species, genes, etc. might be used to “diagnose” specific disturbances, which could signal to scientists what has gone awry within an ecosystem.
- ***Therapeutic Biomarkers***- Indicator species, genes, etc. might also point scientists toward solutions by providing information about how the environment has changed and, therefore, ecosystems might be restored. In addition to informing mitigation and restoration strategies, these indicators can dictate action plans during a crisis event.

To define the necessary array of biomarkers for assessing healthy and diseased states of microbiomes in marine ecosystems, researchers suggest drawing from the Human Microbiome Project, which has already forged this frontier in the context of human medicine.

Microbiome- the collection of all microbes within a community or ecosystem

Biological indicators are an essential component of the environmental emergency response toolkit, and GoMRI researchers envision a future where critical omics measurements will inform scientists and first responders in real time, at the site of an oil spill or other disturbance, to enable improved and timely:

- Assessment of environmental risks and predict consequences
- Identification of ecosystem deficits that might be augmented with specific microbial species (such as oil degraders)
- Selection of appropriate action plans to mitigate damage and accelerate recovery
- Prioritization of sites for emergency response based on ecosystem status
- Monitoring of ecosystem recovery

Scientists need robust, portable (i.e., hand-held devices), cost-effective, and integrative analytic instruments designed for the rigors of field research to facilitate decisions and guide actions on-site in real time. The Oxford Nanopore MinION and related sequencers, which are the size of a universal serial bus (USB) stick and easy to use in the field or at sea, represent a significant advance for rapid, high throughput gene sequencing. Codified protocols and procedures for extracting marker molecules (from DNA to protein), as well as community standards with respect to bioinformatics pipelines, would further expedite progress and generate an internally consistent suite of baseline data.

2. Predicting Environmental Impacts with Biomarkers and Models

A primary goal of GoMRI researchers is to utilize omics biomarkers to predict the impact of environmental disturbances like oil spills. With knowledge of microbial community structure and function, it is possible to develop biogeochemical models to answer relevant questions like:

- When will oil be removed from the system?
- What toxic by-products will result, and how much will accumulate?
- What biogeochemical processes, such as nutrient cycles, will be altered?
- How long will it take the ecosystem to recover?
- How can responders intervene to speed ecosystem recovery?
- How will specific interventions ameliorate the impacts, and how soon?

Models can also provide important details and quantitative information about biogeochemical processes that can inform scientists about factors most influential in controlling damage under specific conditions. By targeting these factors, scientists can tailor appropriate mitigation strategies, rather than deploying a one-size-fits-all strategy that may or may not work.

To inform oil spill mitigation strategies with omics-enabled biogeochemical models, the following questions should be addressed:

- Should oil spills be seeded with probiotic microbes with hydrocarbon degradation capabilities? If so, which ones and in what quantity?
- Should dispersants and/or fertilizers be added? If so, in what quantity? How will such interventions impact ability of the indigenous microbes to degrade oil?
- When will the ecosystem recover if an intervention is or is not employed? What environmental impact might be avoided with interventions? What are the potential unintended consequences of intervention?
- Will leveraging natural microbial processes be more successful than alternative approaches such as burning or vacuuming the oil?

Establishing Biogeochemical Models

There is no single comprehensive model that encompasses the full complexity of biogeochemical processes. Too many interacting biological, chemical, and physical variables must be considered to generate a model with accuracy and tailored to a specific purpose, at this time. Further, few to no models capture the complexity or diversity of microbial species present.

Current models are generally designed to fulfill a specific task, e.g., predict oil degradation rates or microbial growth rates under various conditions. Individual models cover different spatial and temporal scales, range from the single-cell to the community level, and examine individual metabolic pathways to ecosystem function. Each requires unique input, such as water temperature, nitrogen concentration, hydrocarbon concentration, and prevalence of specific microbial species, genes, and enzymes. Physical and microbiological parameters are combined to provide a qualitative or quantitative description of biogeochemical processes to predict an outcome or timeline. Simple models might be integrated to provide a more complex and comprehensive modeling tool, if key metabolic reactions, players, and processes are identified.

The development and application of biogeochemical models are currently impeded by a limited understanding of regulatory factors and their relationships. Also, such models must be rigorously tested and verified before their predicted results are applied, which can be a daunting challenge.

Models of metabolic pathways for specific organisms have been developed. However, microbial community and ecosystem function models are far more complex and are still beyond our reach. Furthermore, significant knowledge gaps preclude translation of genomics data into an integrative biogeochemical model for hydrocarbon degradation and will require the following data:

Physical-Chemical-

- Physical properties and their distribution in the water column and sediments, e.g., salinity, temperature, and density
- Geochemical properties and their distribution in the water column and sediments, e.g., nutrients, oxygen, and temperature
- MOS particle formation and settling rates
- Oil (liquid and gas) composition and behavior in water, including phase partitioning with time, mixing, and degradation

Microbial-

- Microbial growth rates
- Quantitative gene and transcript data
- Toxicities and rates of degradation for key hydrocarbon compounds related to microbial species, genes, and pathways
- Linkage of metabolic genes and pathways of hydrocarbon degradation
- Microbial community dynamics and time-series data for functional succession
- Contribution of anaerobic cycling coupled with hydrocarbon degradation in sediment and particles
- Function of microbial species integrated into community and ecosystem dynamics on regional and global scales

Most importantly, researchers need to be able to translate genomics data into a measurable assessment of ecosystem function. Coupling laboratory and field studies with machine learning tools is a promising approach, where powerful computational algorithms can be developed to identify key microbial and environmental drivers of ecosystems.

Models accurately reflecting environmental impact and microbial influences will require the collaboration of oceanographers, microbiologists, and computational scientists. Such collaborations will be essential to interpret and integrate diverse physical and biological processes involved in biogeochemical cycling.

3. Translating Omics into Function for Biogeochemical Models

Genomics data indicate a significant role of microbial communities in ecosystem services but do not directly reflect microbial activities. Omics data require further validation with quantitative data from laboratory and field studies to confirm predictions of biogeochemical functions.

Gene and Enzyme Characterization

The majority of omics data collected to date is in the form of metagenomes, which provide information on metabolic potential but not activity. Transcriptomics and proteomics reveal functions that are currently active. Integrating multi-omics data will allow more accurate prediction and development of model ecosystem functions under specific conditions.

Omics analysis can identify novel genes and proteins, but too often, their functions are unknown. Function can be inferred from sequence and structural similarities to known metabolic enzymes of reference species. However, in many cases, the enzymes may have unique or unknown specificity, such as a:

- Broader range of hydrocarbon substrate
- Specific subset of hydrocarbon compound affinities
- Novel function

To truly predict function, the novel genes and enzymes must be characterized, and this is accomplished usually with traditional cultivation, biochemical assays, or gene knockout experiments. These methods are currently indispensable yet underutilized because they are labor-

intensive and time-consuming. In fact, ~30 to 50% of genes in marine microbiomes are uncharacterized. New high-throughput strategies are needed to annotate these genes and enzymes and link omics data with functional rates of activity at the enzymatic and cellular levels.

Oil Degradation Rates

Knowing how quickly metabolic enzymes and pathways break down specific hydrocarbon compounds is important to predict when the compounds will be fully degraded. To inform models, the metabolic rates should be examined on many levels, from the purified enzyme, single cells, and individual microbial species, to the entire community.

Although laboratory studies can reveal metabolic potential under controlled conditions, they may not reflect in situ conditions, as environmental factors like pressure, temperature, nutrients, salinity, oil concentration, etc., strongly influence degradation rates. Thus, laboratory studies provide only a rough approximation, and field studies of microbial communities in proper environmental context are necessary to measure actual and relevant metabolic rates. These rates are likely to be site specific, differing widely from surface seawater to deep-sea sediments, and they should be measured in diverse ecosystems with time course studies to inform biogeochemical models.

These same principles also apply to other processes involved in ecosystem function, such as rates of nitrogen fixation.

Microbial Population Growth and Decline

Microbes metabolize and reproduce as they consume hydrocarbons, generating an even larger population of cells to consume this metabolic fuel. Such population growth has an impact on the rate of hydrocarbon and nutrient depletion in the environment. Therefore, the growth rates of key organisms and consortia are important for predictive biogeochemical models. Unfortunately, there is only a very limited quantitative understanding of such growth and mortality rates over time.

Rates of growth and mortality can be measured in the laboratory to derive parameters for models, but field studies more accurately reflect conditions in different natural environmental contexts. Environmental variables like temperature, oxygen or trace metal concentration, and biological factors, such as the presence of microbial partners or competitors, will influence microbial population growth and decline. None of these factors can be adequately recapitulated in laboratory experiments. Field studies are essential to derive applicable quantitative measures of microbial growth rates for predictive biogeochemical modeling.

Tools to Link Genotype to Ecosystem Function

Research tools to bridge the gap between omics and quantitative measures of ecosystem function are currently under development. These approaches provide some insight but rely on labor-intensive and time-consuming laboratory methods that limit their utility. New high-throughput strategies are needed. GoMRI research established, advanced, and integrated many approaches, resulting in more powerful and versatile bioinformatics tools. Strategies and directions recommended for further improvement include the following:

Genome Models- Genomic profiling of individual species is used to link taxonomy to function. Taxonomy infers a species' ecosystem functions, but closely related strains can differ dramatically in genetic content and hence metabolic capability, adaptation to different niches, and other ecological implications. A variety of tools are available to generate genome-scale models:

- Genome assembly integrating multiple sets of omics data into complete genomes
- Long-read sequencing (Nanopore, PacBio) to improve reconstruction of genomes from metagenomic data
- Cultivation, purification, and genome sequencing of microbial species in the laboratory to directly link genotype to functions for genome-scale metabolic models

Stable Isotope Probing- Biogeochemical processes can be tracked and metabolic steps and/or pathways assigned to specific microbes in complex communities using isotope-labeled substrates, e.g., ^{13}C (or heavy isotopes of N, O, or H). Stable isotope probing (SIP) enables researchers to simplify complex communities by separating and studying the metabolically

active microbes. Active organisms consume isotopically labeled substrate, which can be followed to determine the by-products produced, while also assessing cross-feeding in laboratory or field samples. This enables the following:

- Identification of novel metabolic pathways and individual metabolic steps. This can be done without prior knowledge of the microbes, genes, or pathways involved.
- Discovery of new microbial species and/or strains that play key metabolic roles and, potentially, their isolation for additional genomic and functional analyses
- Discovery of novel metabolic capabilities of previously known microbes, i.e., some *Methylophaga* strains can oxidize alkanes
- Confirmation of genomic predictions to update gene and enzyme functional annotations
- Measurement of rates of biotransformation for quantitative modeling

Bioorthogonal Noncanonical Amino Acid Tagging- SIP is an extremely tedious and time-consuming method that is limited to labeling and tracking small molecules such as methane or other hydrocarbons. Bioorthogonal noncanonical amino acid tagging (BONCAT) is a similar approach, with fewer drawbacks, in that it can be used to track metabolic activity through assessing protein synthesis of individual microbes, and consortia, in their natural environment (Figure 10). BONCAT can also be used to determine metabolic activity and ecosystem function contributed by specific members of the community under controlled conditions. This relatively new tool was unavailable 10 years ago, that is to GoMRI researchers, but holds great promise for advancing this field in the future.

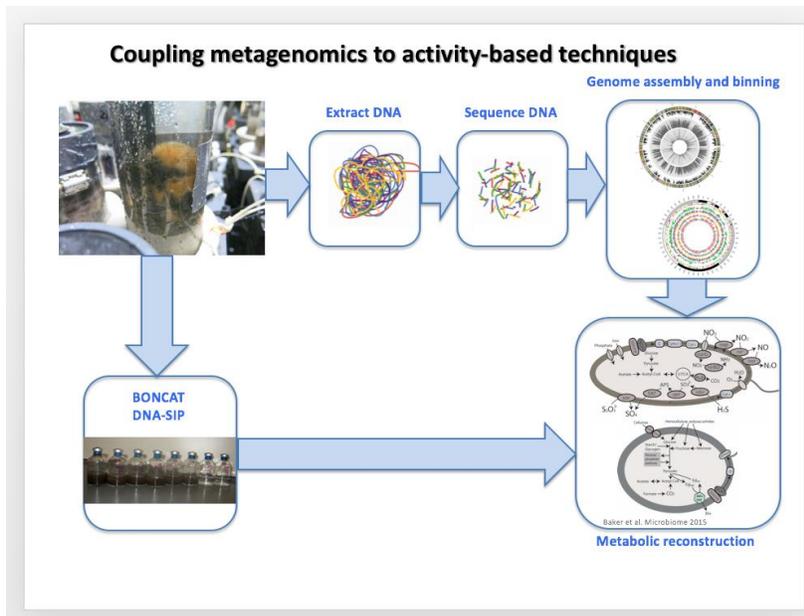


Figure 10. Bioorthogonal noncanonical amino acid tagging (BONCAT) represents an exciting new approach that can be coupled with omics methods to track metabolic activity through assessing protein synthesis of individual microbes, and consortia, in their natural environment (Hatzenpichler et al, 2016).

Microcosms, Mesocosms, and Other Experimental Systems- Microcosm and mesocosm systems that mimic natural conditions are lacking and difficult to develop but essential to conduct research that links omics information to functional measures. These experimental systems allow researchers to tease apart and quantify the many factors that contribute to biogeochemical processes, thereby verifying and calibrating conclusions from field observations. Developing such systems is a major undertaking that requires detailed recapitulation of environmental conditions and dynamics to reflect real-world ecosystems accurately, including their microbial components. They can range in scale from a microcosm, e.g., a tube or bottle on the benchtop (hundreds of milliliters to ~5 liters) to >5-liter mesocosms, depending on the system and hypothesis. To ensure that these systems provide results transferable to real-world situations, they must be extensively validated by comparing lab-based measurements to field data.

For example, the porous sands that often dominate on beaches and in shallow subtidal ecosystems are difficult to reproduce in the lab. Thus, researchers placed considerable effort into

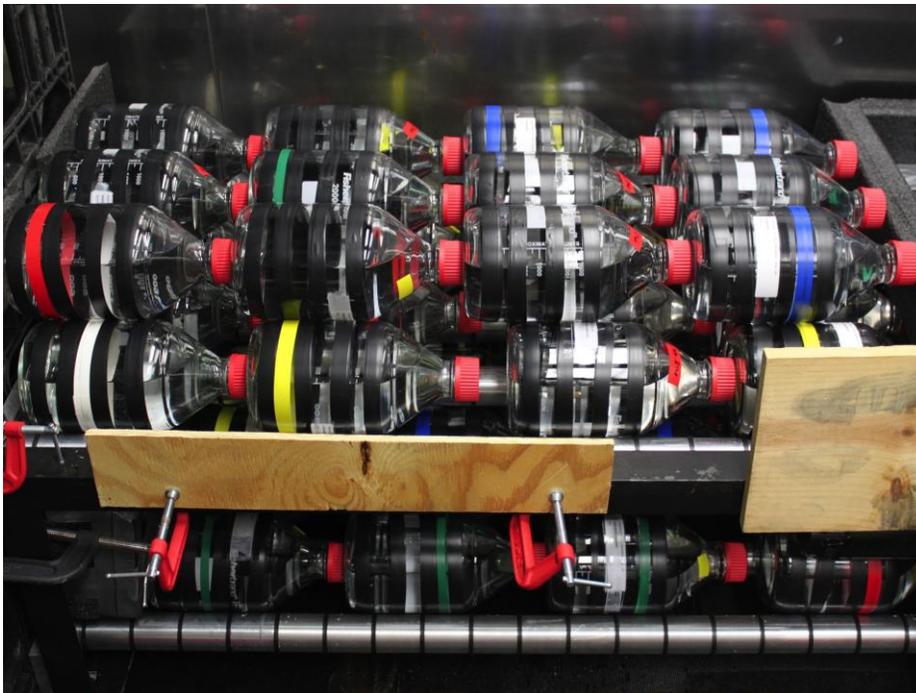
developing a system of flowthrough chambers that mimic *in situ* wave and current conditions (Figure 11). These sand mesocosms were employed to effectively verify field observations of hydrocarbon biodegradation in beach sands of the Gulf of Mexico. For planktonic environments, SINTEF (Norwegian: Stiftelsen for industriell og teknisk forskning) developed a mesocosm carousel system that mimics subsurface water and mixing (Figure 11). Many different systems will be needed to study biogeochemistry in different environments, locations, and disturbances, from crude oil spills in deep waters in the Gulf of Mexico to climate change in the Arctic.



Figure 11. Laboratory microcosm and mesocosm approaches were effectively used to validate field observations made after the Deepwater Horizon oil spill. (Image Credit: Kostas Konstantinidis, Georgia Institute of Technology).



Studying beach oiling after the Deep Water Horizon (Image Credit: Markus Huettel, Florida State University)



Lab microcosms to study the fate of oil in deep water plumes (Image Credit: Samantha Joye, University of Georgia)

Defined Microbial Consortia- Models based on single microbial species have limited utility because microbes in natural ecosystems do not act in isolation but form interactive, often cooperative and synergistic, communities. Accounting for these complex microbial interactions is a major challenge in modeling. Researchers might start by paring back the community into a smaller subset of key players to study ecosystem functions in a fully tractable manner in the laboratory. These consortium models can serve as tools to study a subset of underlying microbial interactions that dictate partnerships within the larger community.

First, researchers will need to define the key community members that together dictate a given ecosystem function. These consortia will likely differ based on the function, environment, and location of interest. For example, who are the key players that degrade methane or alkanes, in deep water versus beach sands, in the Gulf of Mexico versus the Arctic? Once defined, these consortia can then inform more complex, and accurate, functional models, such as:

- Genome-scale models for key players that can be integrated through bioinformatics to describe metabolic pathways and other functions accomplished through cooperative microbial interactions
- Multiple species grown together in the laboratory to define cooperative metabolic rates and link meta-omics parameters with quantitative functional data.

Time-Series Field Studies- Models derived solely from laboratory studies may be misleading, as microbes can act very differently in the lab compared to the environments. Therefore, field studies are critical to confirm laboratory-based hypotheses and generate biogeochemical models that reflect real-world situations.

To describe actual rates of community evolution, hydrocarbon degradation and other ecosystem functions will require extensive time course field studies across diverse environments. Such studies involve repeated sampling from the same site over the course of many months to years and coordinated analysis of microbial population data and metabolic substrates. Such field studies will provide invaluable quantitative and functional information, which can be integrated with laboratory-based data, to inform both empirical and conceptual models.

4. Facilitating Progress through Open-Access Resources

In the last decade, GoMRI researchers have generated vast data sets composed of omics and environmental information from both field studies and laboratory experiments. These data sets represent a wide variety of locations, timelines, and environmental conditions pertaining to the DWH spill and are of great value to the research community. In the event of another spill or disaster, these GoMRI data sets can be mined for information to predict consequences and guide better response plans. In addition, these data sets contain undiscovered clues and lessons about biogeochemical processes, with the potential to advance both basic and applied ocean science research, as well as other disciplines.

Prior to the GoMRI program, data generated by independent research groups were often underutilized due to a lack of open-access resources. In some cases, valuable data sets and the protocols and techniques used to collect them were confined within research groups and not available to the community. Robust data and metadata sharing procedures are necessary to accelerate progress in interdisciplinary research, preventing scientists from wasting time, money, and effort to reinvent the wheel at every step.

Some scientists have resorted to constructing their own in-house open-access databases, which is extremely time-consuming and must be made available (e.g., GitHub) for public scrutiny and feedback. Many individual researchers have established such databases focused on specific metabolic pathways such as sulfur metabolism or hydrocarbon degradation. However, most databases created to date are boutique style, far from comprehensive, and often not fully accessible to the greater scientific community. Further, one investigator's pipeline may be incompatible with independent bioinformatics tools, altogether limiting their utility. Additional challenges exist in sharing genomic, proteomic, and metabolomic data, which need to be curated and annotated for hydrocarbon-degrading microbes, pathways, genes, and enzymes in consistent ways across research teams.

To address these issues, the GoMRI program generated a highly collaborative and open-access model. GoMRI supported the development of a data clearinghouse, a great resource for data and metadata, although without analysis and synthesis capabilities

(<https://data.gulfresearchinitiative.org/>). Another example of a highly successful publicly available platform that facilitates all kinds of omics-based analysis is Anvi'o, an open-source, community-driven platform for analysis and visualization of omics data (<https://github.com/merenlab/anvio/releases>). In the case of Anvi'o, support and training are available through online tutorials and workshops that occur frequently in the United States and abroad. The scientific community will be better served with more open-access and standardized tools to work across platforms.

Open-access resources allow scientists to examine existing data in new ways that can yield important new discoveries. With these resources, scientists can:

- Apply novel bioinformatics tools and skillsets
- Examine different biological and physical phenomena and test different hypotheses
- Bring unique perspectives and lessons from diverse fields of study
- Compile multiple data sets for comprehensive analysis with greater statistical power
- Perform cross-study comparisons to reveal commonalities or differences among locations, environments, time points, etc.
- Test and validate novel data analysis tools on diverse data sets

In medical research, the implementation of open-access databases, bioinformatics tools, and other resources over the past decade has facilitated rapid advances in basic biological sciences and clinical medicine. These resources allow scientists from different backgrounds to mine existing data sets for novel discoveries, maximizing the impact of data that have already been collected. The establishment of similar resources for microbial omics within ocean sciences and biogeochemistry would similarly propel the field forward.

Scientists, funding agencies, and research institutions need to invest in open-access infrastructure to facilitate research advances and impact across biogeochemical and ocean science fields. This includes devoting resources not only to create these resources, but also to adequately maintain them for decades to come. These services are invaluable to the scientific community, yet entirely undervalued by academic institutions, which focus instead on publication records. These

institutions need to adjust antiquated views to incentivize and compensate scientists for their contributions to these essential community resources.

The Oil Spill Microbiome Project

An initiative is suggested to establish a comprehensive, searchable database of microbial genomes, metagenomes, metagenome assembled genomes (MAGs), and genes (*alkB*, PAH dioxygenase ROcker profiles) associated with oiled samples across the world. This will involve the following tasks:

- Curating all publicly available (meta)genome data associated with the DWH spill from various sources
- Assembling and generating individual microbial genomes and community metagenomes
- Characterizing genomes taxonomically and functionally to determine specific patterns in ecosystems
- Comparing data with uncontaminated locations across the globe to assess the potential for oil biodegradation

Toward this end, MiGA is a publicly available Web server (<http://microbial-genomes.org/>) that allows researchers to classify unknown genomes against all classified genomes at the genome level. Efforts like MiGA could be expanded, with integration of the appropriate metadata, to allow researchers not only to identify their sequences taxonomically, but also to assess where these organisms were previously documented and under which conditions, etc. (Figure 12).



MAG ID	Mason_Bins_BP350_AllReads_006
Closest hit	Colwellia psychrerythraea (NC_003910), 69.3 (AAI)
Location	28.74, -88.37
Date	10/17/10
Sample type	deep sea sediment
Depth	1500m below sea level
Macondo oil present	Y
TPH (mg/kg)	31.8
PAH	3.14
Total Aromatics	17.19
Total Inorg. N (mg/l)	3.584
Distance from MC252 wellhead	0.6km
Abundance ?	
MAG also found at:	Locations detected
Any other metadata recovered can be added here	

Figure 12. Example of Potential Oil Spill Microbiome Project Entry. MAG = metagenome assembled genome; TPH = total petroleum hydrocarbons; PAH = polycyclic aromatic hydrocarbons; MC252 refers to the leasing site from which the oil from the Deepwater Horizon accident was discharged. (Figure Credit: Rodriguez-R and Konstantinidis, Georgia Institute of Technology)

Challenges to Implementation

Without standardized protocols for data collection and curation, integration of data sets into a single comprehensive open-access database will be difficult. Data set content and resolution can be poorly matched, with major informational gaps between them, such that comparing data sets across groups is like comparing apples to oranges, *per se*.

GoMRI researchers recommend developing common methods and terminology for data collection and requirements for documenting core information to facilitate the creation of comprehensive open-access resources, including:

- Geo-referenced sampling location
- Habitat where samples were collected, such as deep sea, beach sand, marshland, etc.
- Date and time of day the sample was collected, along with information on local conditions (weather, tides, etc.)

- Hypotheses considered in the study design
- Instruments and protocols used to analyze samples and data

These standards will enable appropriate comparisons and integration of multi-omics data across studies.

To maximize the utility and impact of such resources, it is also recommended that:

- Study design information and results be annotated and easily searchable so that anyone can identify studies and data sets relevant to their interest or need
- Studies provide raw data for analysis with novel bioinformatics tools
- Journals require data to be made public to foster open-access science
- Resources be integrated into established open-access databases and websites, like the National Center for Biotechnology Information (NCBI) site, so data and the associated metadata are easily located and accessed by anyone, especially those from diverse fields

5. Facilitating Advances through Multidisciplinary Collaboration

Omics research in the context of broader oceanographic and biogeochemical studies requires extensive knowledge and technical skills, including biochemistry, microbiology, genetics, ecology, bioinformatics, physics, and other aspects of biological, chemical, physical, and computational sciences. No single person or even single research group can bring all of these different expertise to the table. Therefore, multidisciplinary collaborations are essential to advance the field.

Unfortunately, a number of challenges have thus far limited such collaborations and/or their impact. Communication is a major obstacle. Each scientific field has its own language, tools, and culture; these field-specific words, syntax, and plot lines make communication easier and more efficient for scientists within a particular field but act as barriers to outsiders. In particular, bioinformatics is an extremely complex, but essential, language that can be difficult to integrate with the more traditional sciences. To facilitate multidisciplinary collaborations, GoMRI researchers suggest a number of strategies to overcome these communication barriers, as follows.

Bioinformatics Literacy- With the advent of next-generation sequencing and big data, bioinformatics tools are becoming increasingly complex, powerful, and indispensable to all disciplines. Therefore, all researchers across disciplines should have some degree of bioinformatics and computer programming literacy, as it has become the common language of modern research. This foundation will help all researchers analyze their own data to some extent but, more importantly, to effectively communicate with bioinformatics experts who wield more advanced analytical tools that propel the field forward.

Interdisciplinary Training- Although the need for interdisciplinary scientists is increasing, training programs are siloed, denying the next generation of researchers the skills they will need to become future scientific leaders. This is a major flaw that sets the entire field up to stagnate for decades to come. Investing in interdisciplinary training will better serve our students and postdocs, the scientific community, and society as a whole, particularly as we face challenges posed by climate change and other future environmental disasters, including oil spills.

GoMRI researchers suggest restructuring curricula to instill the mentality and skills required to navigate multidisciplinary research, as follows:

- Experiential learning, as opposed to memorization-based learning, to teach critical thinking and problem-solving skills
- Group projects to teach students how to work together and communicate effectively across disciplines
- Teaching students that failure is a part of science. If students are afraid to fail, they will not take the risks required to be innovative.
- Upcoming students must be taught to be multilingual from the beginning, when they are first learning the language of science. Multidisciplinary strategies should be introduced much earlier in the educational pipeline, as soon as high school or middle school, to ingrain these skills. Retraining students later in careers is difficult and much less effective.

Leading research organizations can support interdisciplinary training programs, as follows:

- Education grants from the American Society for Microbiology or National Science Foundation to fund the development, implementation, and distribution of programs for the practice of science
- Institutional incentives for faculty to update curricula
- Student scholars programs, such as that led by GoMRI at the Gulf of Mexico Oil Spill & Ecosystem Science (GoMOSES) conference or other fellowship-based training experiences

Fostering Cross-Pollination- Even if scientists are trained and capable of cross-disciplinary collaboration, they must be given the opportunity and incentive to interact. Fostering cross talk and cross-pollination between and among disciplines in biomedical fields has yielded scientific and clinical advances.

To promote cross-disciplinary collaborations, it is important to facilitate both professional and casual interaction to spark innovative ideas and brainstorming across fields. Established researchers and students are equally important to include in these initiatives. Some strategies for fostering cross-pollination include:

- ***Events-*** seminars, conferences, retreats, hackathons, etc. that include diverse attendees
- ***Spaces-*** research buildings, lounges, coffee, lunch areas, etc. that serve scientists from diverse departments
- ***Cluster hiring-*** new faculty hired to conduct research around a common theme like ocean health rather than a specific discipline like chemistry
- ***Funding-*** grants, fellowships, training programs, etc. that require cross-disciplinary participation

GoMRI is an excellent example of how research funding can bring together diverse groups toward a common goal, yielding advances and real-world impacts that are otherwise unattainable. The GoMRI research model can guide future efforts to assemble and coordinate interdisciplinary research teams that can address big questions in basic and applied sciences. Moving forward, it may be particularly fruitful for biogeochemical researchers to cross-pollinate with human microbiome scientists, who face many of the same scientific questions and

challenges. Backed by medical research funding, this field is at the transformative edge, having already developed and implemented many of the genomic, microbiology, and computational tools to study the complexities of microbial population dynamics. Their tools, hypotheses, and discoveries may be easily adapted to study microbial populations in ocean systems.

Sharing Data and Knowledge- Open-access data resources are critical for cross-disciplinary research, allowing scientists from various disciplines to bring new perspectives and tools that can provide invaluable insights both within and outside their respective fields, as discussed previously.

Standardization- Scientists face many challenges in communicating and/or analyzing data across disciplines due to a lack of standardized terminology, protocols, and database annotations across fields and research groups. Establishing standards for common practices for data collection and database annotation will ensure that all data can be fully utilized by all scientists. Standards for providing summaries of experimental design, driving motivations, and research questions along with data sets will provide the necessary context for others. These efforts will ease collaboration and facilitate progress across fields.

Spill Response- Communication is critical during a disaster to optimize the coordination of response and research efforts. This is essential to maximize resources, including funding, manpower, and availability of research vessels and equipment. This was a problem during the DWH spill, when the National Oceanic and Atmospheric Administration (NOAA and GoMRI funded overlapping research projects instead of communicating to establish complementary approaches. In the future, a disaster response plan should be prepared ahead of time and distributed to researchers, as well as funding agencies and government players, which outlines a course of action and provides appropriate contact information to coordinate disaster responses.

Public Outreach- Researchers need to improve communication with the public, particularly regarding the importance of biogeochemical research and to rally public support, interest, and funding for the field. Highlighting relevant advances and applied solutions will make the field more tangible to those without a scientific background. Despite the importance of public

communication in sustaining the field, communication efforts and skills are often undervalued by the scientific community. Public communication efforts often are not funded or recognized by employers or grants but should be. Students and faculty should be trained in the art of science communication and incentivized to do so.

V. CONCLUSIONS

Microbiology and omics tools played an instrumental role in understanding how the Deepwater Horizon oil spill impacted marine and coastal ecosystems. These new omics-based technologies and strategies enabled an in-depth and comprehensive study of the microbial communities and their biogeochemical function in unprecedented detail.

Using the powerful and constantly evolving tools of genomics, GoMRI researchers discovered novel genes, pathways, organisms, communities, and partnerships involved in oil decomposition. In addition, they documented key shifts in those community structures that dictate essential ecosystem functions and bioremediation services. Altogether, the GoMRI studies revealed new insights and core lessons about how microbial communities respond to environmental disturbances and maintain and restore ecosystem stability during and after an oil spill or other perturbation. The lessons learned inform a scientific understanding of how oil spills impact various ecosystems, from deep water to beach sands to tidal marshes, and allow better prediction and mitigation of damage in the event of future spills around the world.

GoMRI research sets a strong foundation for studying microbial ecosystems beyond the context of oil spills. These efforts established research methodologies, omics tools, bioinformatics strategies, scientific collaborations, and shared resources that scientists now and in the future can build on to study diverse environmental disturbances, including the long-term threat of climate change. Ultimately, these advances will help scientists define omics-based biological indicators for monitoring ecosystem health at the time of natural and man-made disasters. These biological indicators will guide researchers as to when and how biogeochemical processes are disrupted and will advise emergency response plans that protect and restore diverse ecosystems worldwide both effectively and efficiently.

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