OIL SPILL SCIENCE

SEA GRANT PROGRAMS OF THE GULF OF MEXICO

NEW DISCOVERIES IN MICROBIOLOGY, GENOMICS, AND OIL SPILL IMPACTS

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After the Deepwater Horizon oil spill, impacts to some of the Gulf of Mexico's tiniest residents took center stage. New advances in technology and scientific understanding about microbe communities emerged as millions of gallons of oil spilled into the Gulf. Scientists studying Deepwater Horizon applied these technologies, leading to exciting findings, including new species of oil-degrading bacteria. The combination of an unprecedented environmental disaster and technological breakthroughs led to discoveries that will help scientists and responders better prepare for and respond to future environmental disasters.

SYNTHESIS SERIES

The purpose of this publication is to exclusively reflect findings from synthesis activities supported by the Gulf of Mexico Research Initiative (GoMRI). GoMRI synthesis documents are the primary references for this publication. The summary may also include peer-reviewed publications and other reports cited in the GoMRI synthesis activities that help to provide foundation for the topic.

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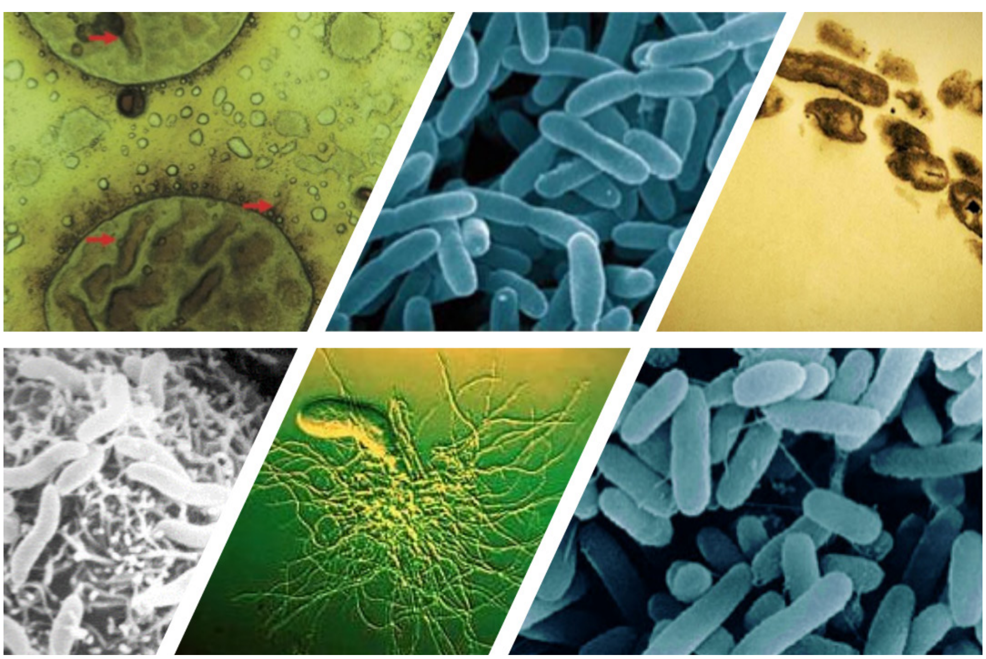
MICROBES AT THE BASE OF EVERY ECOSYSTEM

Microscopic organisms sit at the base of all ecosystem food webs and provide the foundation for more complex life. Called microbes, they are a diverse group that includes bacteria, fungi, viruses, and another group called **archaea**. Microbes are the great decomposers in ecosystems around the world, responsible for providing essential services like breaking down dead and dying organisms and recycling the released nutrients. These nutrients are used by all life forms and include carbon and nitrogen.[a]



Microbes have evolved over billions of years, are highly adaptable, and are found in every environment on Earth. Many microbes live in cooperative populations called microbial communities that can share the work of carbon breakdown, nutrient cycling, and other essential roles.

Many new groups of hydrocarbon-degrading microbes, like those pictured here, were discovered using new technology that began to emerge around the time of the Deepwater Horizon oil spill. Clockwise from the upper left, *Candidatus* Macondimonas diazotrophica, *Cycloclasticus* spp., *Alcanivorax* spp., *Colwellia* spp., *Oceanospirillaceae* spp., and *Marinobacter hydrocarbonoclasticus.* (Joye & Kostka, 2020)

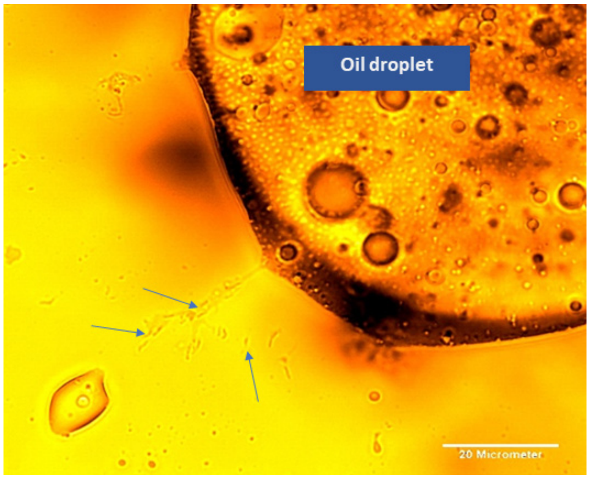


FIGURE 1. Microbes (arrows) swim toward an oil droplet using chemotaxis, a process where organisms are drawn toward (or away from) increased concentrations of a chemical. (Berkeley Synchrotron Infrared Structural BioImaging Program Archives, Lawrence Berkeley National Laboratory)

They also share the **metabolites**, the leftovers created as a byproduct of decomposing and other activities. By sharing in the effort, individuals or groups of similar individuals can specialize in one type of task, and others can do different tasks.

Every living organism, including microbes, possesses genes that contain specially encoded instructions for making proteins. Proteins generally do the work that cells need to function, grow, and survive. Many of an organism's genes are unique to the types of tasks it handles and help the microbes to not waste energy on genes that are rarely used. A good analogy might be a neighborhood potluck; no single family is responsible for everything, but everyone brings a dish to help make a complete meal. That way everyone gets to share in the bounty.

MICROBES AS FIRST RESPONDERS

Oil is a fossil fuel, meaning it is made up of decayed plant and animal material. Oil is a complex mixture of many compounds, most of which are hydrocarbons, which as the name implies are made up of carbon and hydrogen. Carbon and hydrogen molecules can be arranged in multiple ways, creating many unique hydrocarbons with different chemical characteristics. Learn more about oil and its components in ***Frequently asked questions: Oil edition.***

Microbes, like all organisms, need carbon to live and grow. Though carbon is found in many sources, including plants and animals, most carbon is not in a useable form for organisms until it is decomposed by microbes. Some microbes, called hydrocarbon degraders, have evolved over millions of years to break down oil as a source of energy and carbon. Though hydrocarbon degraders are found all over the world, they are typically rare and found in very small numbers when oil is not present. However, after an oil spill, hydrocarbon degraders sense the presence of oil using **chemotaxis** (**Figure 1**) and react quickly by moving toward this new source of carbon. Once they encounter oil, the hydrocarbon degraders can grow to great numbers.

In this way, microbes serve as first responders, working as emergency cleanup crews and facilitating the growth of other microbes that feed on the leftover metabolites. Scientists have found that the response of a microbial community to an oil spill will vary depending on several factors, like the environmental conditions, the microbial community that is present, the composition

NUTRIENTS, ENERGY, AND METABOLISM

Metabolism is a term that is used to describe all chemical reactions needed to maintain living cells. It includes the process of breaking down molecules for energy and the making of other compounds used by cells to function. How organisms **metabolize** compounds is closely linked to the availability of different nutrients, the environment they are living in, and the genes that they contain. To put it another way, humans gain energy and grow by eating food. But what humans can use as food is different than other organisms; we cannot eat only grass and expect to survive for very long, but cows do. The differences in our abilities to breakdown and use different food sources is controlled by many factors, including our genes.

The same is true for microbes. Different groups of microbes have their own energy and nutrient requirements. In this way an ecosystem can support a diversity of microbes, each capable of using what they need. However, during an oil spill, nutrients and energy sources shift to favor microbes with genes that allow them to use oil as an energy source,[b] meaning they build molecules they need using the energy stored in the oil. Some of these oil-degrading microbes have metabolisms that can break down different chemicals in oil faster than others.

of the oil, and the circumstances of the oil spill itself.

Despite their importance, scientists knew relatively little about hydrocarbon-degrading microbes living in the Gulf of Mexico prior to the Deepwater Horizon oil spill (DWH) nor about the effects of hydrocarbons on the microbe communities in general.[a] It was known that microbes could play an important role in oil spill response, as observed during the Exxon Valdez spill in Alaska.[1]

Why does nitrogen need to be "fixed"?

Nitrogen is the most abundant element in Earth's atmosphere, making up around 78% of the air we breathe. Nitrogen is a key component of DNA, meaning that all life on Earth requires nitrogen to grow and reproduce.[2] However, not all nitrogen is created equally. Most nitrogen is not useable for many organisms, including microbes, in its basic form. Similar to how the raw ingredients we use to make meals might not be suitable for eating until they have been cooked, some sources of nitrogen need to be transformed through a process called fixation.[2] Nitrogen fixation is a chemical process that transforms nitrogen from the gas form (N_2) found abundantly in the atmosphere into forms that are useable by organisms (**Figure 2**). This process is performed artificially in factories to produce nitrogen fertilizers, a form of fixed nitrogen, that can be used on farm fields to grow crops. In contrast to artificial fertilizers, microbes are unique in that they can fix nitrogen naturally through biochemical reactions. For example, legumes such as alfalfa or clover have bacteria in their roots that do the job of fixing nitrogen for the plant. Many types of bacteria in soil and water can fix nitrogen, giving them an advantage in places where nitrogen is not readily available.[b]

In the world's oceans, usable nitrogen is often present in limited quantities relative to its need by organisms. This means that the quantity of useable nitrogen limits the growth of organisms that cannot fix their own. Petroleum contains only small amounts of major nutrients like nitrogen and phosphorus. Prior to DWH, scientists thought that oil-degrading microbes were often unable to degrade oil because of nitrogen limitation. For example, during the Exxon Valdez oil spill, responders applied nitrogen fertilizers to the water to help speed up microbe growth and increase the breakdown of oil.[b] Not until DWH did scientists begin to see evidence that the same microbes that were degrading oil could also fix nitrogen, meaning they did not always need the addition of fertilizers as previously believed.[b,c]

ADVANCES IN TECHNOLOGY LEAD TO NEW DISCOVERIES

Many microbial species are difficult to isolate from their environment and nearly impossible to grow in a laboratory and so have been underreported and understudied in the environment.[c] The DWH oil spill and the subsequent formation of the Gulf of Mexico Research Initiative (GoMRI) coincided with the arrival of new tools for studying microbial communities. These tools reduced, or even removed, the need to grow microbial species in a laboratory by focusing on microbial genes. Scientists extract genes from a water or soil sample, replicate them multiple times in a machine, and then

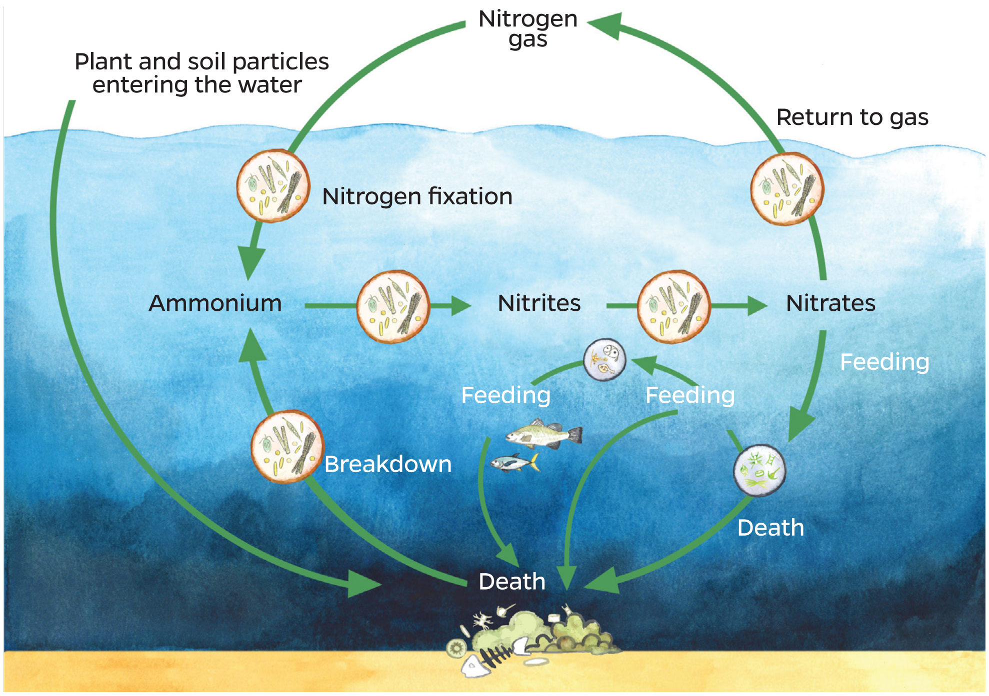


FIGURE 2. Nitrogen gas in the atmosphere is "fixed" by marine microbes for use during growth and reproduction. After fixation, several chemical steps are needed to convert the nitrogen into different forms; microbes (circled in orange) are involved in each step. These new forms of nitrogen are then available to larger microscopic marine life to live and grow. When marine life dies, microbes help to break that material back down and the cycle begins again. (Anna Hinkeldey)

MANY (TINY) HANDS MAKE LIGHT WORK

During DWH, emergency responders applied 1.8 million gallons of dispersants on the surface waters of the Gulf of Mexico and injected them directly into the wellhead. Dispersants break up oil into smaller droplets to reduce or even prevent surface slicks. As small oil droplets mix with the surrounding water, hydrocarbon-degrading bacteria can latch onto the droplets, further speeding up oil degradation. To learn more about the use of dispersants during the response to DWH, read ***Frequently asked questions: Dispersants edition***.

However, the unprecedented use of dispersants during DWH and the subsequent public concern led scientists to investigate the development of alternative, natural dispersants.[b] It turns out that some microbes can produce their own **biosurfactants** to breakup and blend oil with water, just like dispersants. Using technologies developed over the past decades, scientists can help responders in their decisions to use chemical dispersants by 1) identifying the microbes that are present near a spill, 2) determining if microbes present possess the genes to create biosurfactants, and 3) providing information on how to boost their oil-degrading potential if they are present. Scientists even think they could use their newfound knowledge to create new types of oil eating microbes by adding genes from some microbes that produce biosurfactants to others that do not.[b] This process already happens naturally between microbes through a process called **horizontal gene transfer**, allowing some groups to pick up new genes from others.[b] While this technology is still being developed, scientists believe it could be an effective alternative to the use of traditional chemical dispersants in the future.

put them back together in a process called gene sequencing. Think of this like cutting up the title of a book into individual words, copying those words over and over, then putting the copies of the title back together again. The words are the genes, the order is the sequence.

Scientists can sequence an entire microbial **genome** using rapid and relatively inexpensive **genomic** tools.[c] They can go even further and sequence all the genes for all the organisms in a sample through a process called **metagenomics**. They can also determine if and when the genes turn on and begin producing different proteins through **proteomics**. These types of tools are referred to generically as omics tools. Omics tools allow researchers to search for genetic information about the types of microbes in a sample, the types of genes they possess, how those genes are being activated, and the metabolic work the microbes are performing.[b,c] This is to say, scientists can find out who the microbes are, what abilities they have, and what they have been eating.

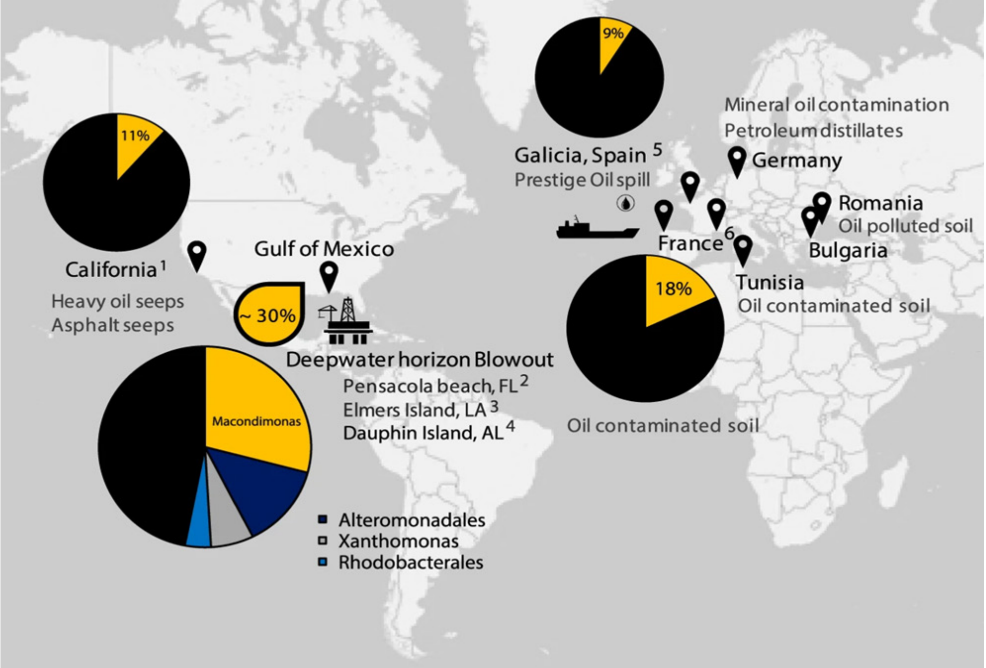


FIGURE 3. A new organism isolated through GoMRI-supported research, tentatively named *Ca. Macondimonas diazotrophica* (yellow wedges), can be found in oil-contaminated sediments around the world. (Adapted from Karthikeyan et al., 2019)

The DWH oil spill was the first major environmental disaster where omics tools were used.[c] Scientists widely applied these techniques to track the response of the ecosystem to oil contamination in unprecedented detail.[c] Because these techniques are generally less expensive than traditional laboratory procedures, scientists could take many more samples over a variety of distances and time spans, allowing them to see differences in microbial communities between days or even hours.[c]

New species, new genes, new understandings

Over the past decade scientists have discovered many new groups of microbes that grow well in the presence of oil.[b] In fact, researchers even discovered that many well-known types of microbes had the hidden ability to break down oil, like the bacterial group *Bacteroidetes* most often associated with the human gut. During an oil spill, these typically low-abundance microbes and some opportunistic others sense the presence of hydrocarbons and use chemotaxis to move toward the source, where they flourish and reproduce rapidly.[a] The sudden growth of the microbial community is often referred to as a bloom. Blooms of microbes feed on hydrocarbons, sometimes transforming the hydrocarbons into other products that are harder to break down. After depleting the easier-to-consume hydrocarbons, the bloom species frequently die off and other groups that can break down these new products take over. This changeover, or **succession**, of species continues until microbes digest all the hydrocarbons and the hydrocarbon degraders become rare again.[b]

GoMRI researchers discovered that a newly identified microbe — tentatively named *Candidatus Macondimonas diazotrophica* — contains genes to fix nitrogen, degrade hydrocarbon, and produce biosurfactants.[3] By studying samples from across the world, researchers also found that this same microbe, abbreviated *Ca. M. diazotrophica*, is a member of a newly discovered genus of Gammaproteobacteria, a group that includes the human gut bacterium *Escherichia coli (E. coli)* and the occasional pathogen *Salmonella*.[c] Scientists also found evidence of *Ca. M. diazotrphica* in hydrocarbon-contaminated sediments from coastal ecosystems across the globe (**Figure 3**). In fact, microbes containing this sequence often made up about 30% of their total communities but were almost absent in non-oiled sediments or seawater. Microbes in this family play a key ecological role in the natural response to oil spills in coastal environments around the world and could prove useful for studying impacts of future oil spills.[c]

Understanding of the role of microbes in the fate of oil spills has been limited by the lack of genomic data, which includes gene sequence data and information on organization and expression of those genes. GoMRI-funded researchers have produced a lasting legacy for future oil spill research by creating a huge database called the Genome Repository of Oil Systems.[3,4] This public database contains the genome sequences of microbial species found in natural ecosystems and from oil spills all over the world. This list of oil-degrading bacteria helps to link the microbes to environmental data and provides the locations where they can be found. Despite the tremendous progress of GoMRI-funded researchers, this new database reveals that more than 95% of the oil-degrading bacteria discovered with omics methods are new to science. This means that science has only scratched the surface of the potential roles of these microbes following oil spills.

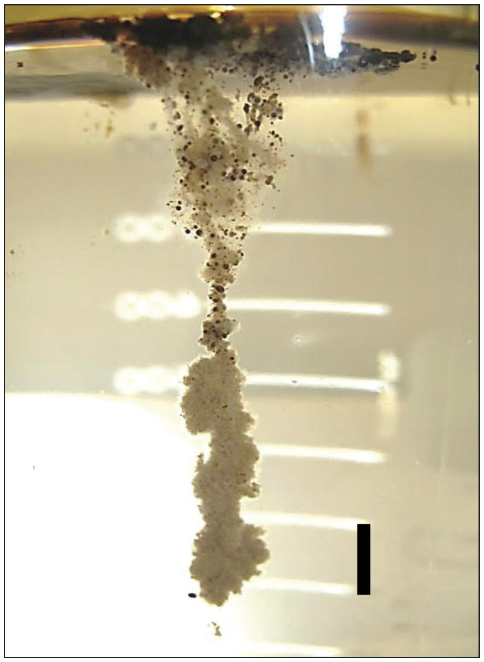


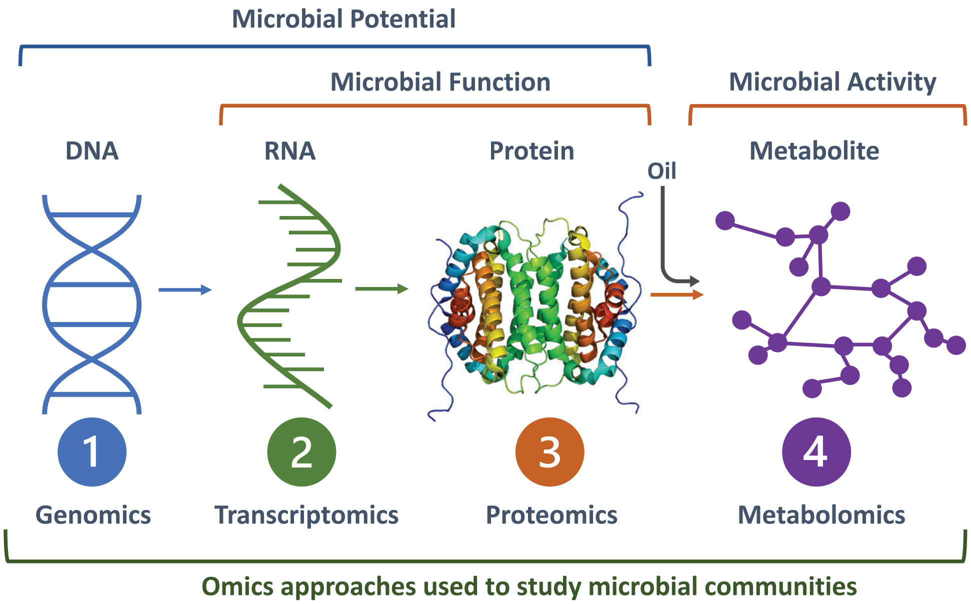
FIGURE 4. Oil droplets (dark brown) are trapped inside a web of sticky strings created by microbes inside a laboratory. Sticky clumps like this were also created in the ocean after Deepwater Horizon spill, trapping soil and plant particles along with oil droplets, and then sinking to the sea floor. (Ziervogel et al., 2012)

PRIMED FOR RESPONSE

Microbial ecosystems in the Gulf of Mexico may have been primed to respond to the presence of oil prior to DWH.[a,b] The Gulf of Mexico is home to hundreds of natural seeps that continually release crude oil and natural gas into the environment.[5] As a result, microbes in the Gulf of Mexico appeared to already contain genes that allowed them to rapidly gear up to degrade hydrocarbons. For example, scientists find genes that help microbes break down hydrocarbons even in oil-free Gulf of Mexico samples more commonly than in other regions without the Gulf's rich oil supplies.[b] Other regions of the world with large oil resources, like the Gulf of California and the Black Sea, may also have this pre-primed microbial community.[b]

What happens to oil and microbes that reach the seafloor?

Small oil droplets may be rapidly colonized by bacteria. These bacterial colonies often produce



Scientists use many different types of genetic tools, collectively called omics, to study the potential, function, and activity of microbes in the environment. 1) Genomics are used to study microbial genes 2) **transcriptomics** reveals how genes are converted into proteins, 3) proteomics aid understanding of which proteins help degrade hydrocarbons, and 4) **metabolomics** help scientists study the metabolites leftover from oil degradation.[b,e,7] (Adapted from Abram, 2015)

sticky compounds that can stream off the colonized oil in long strings. These sticky strings surrounding oil droplets (**Figure 4**) can attach to sinking particles, like soil grains and plant debris, creating heavier clumps that eventually sink to the depths of the seafloor. These clumps are known as marine oil snow (MOS). The formation and eventual sinking of MOS effectively removes oil from the water while increasing the concentration of oil on the seafloor.

Once MOS reaches the seafloor, it can impact organisms that live there. Filter-feeding organisms like bivalves and corals may have their feeding parts covered the sticky, oily particles. Other animals may ingest or even be smothered by oil containing particles. The MOS also moves microbes from the water column to the seafloor where they may compete for resources with the established microbial communities, potentially disturbing the stability of species in the deep sea. To learn more about MOS and how oil and microbes are transported to the seafloor read ***Microbes and oil: What's the connection?***

FUTURE DIRECTIONS

The discoveries by GoMRI scientists and the methodologies used to study microbial communities are useful beyond oil spill research. The establishment of basic principles of microbial ecosystem function and response has laid the groundwork to understand diverse water- and land-based ecosystems in the face of both human-caused disasters such as chemical spills and natural disasters like harmful algal blooms. However, understanding how disturbances impact microbial communities, and by extension the ecosystem as a whole, requires a clearer picture of the starting or pre-disaster conditions.[b] Unfortunately, pre-disaster baseline data on microbial communities is lacking for many regions. Research is usually focused on documenting impacts after a disaster has occurred instead of learning about microbial communities beforehand. In addition, pre-disaster microbial communities are dynamic in terms of composition over seasons and across both large and small distances, requiring extensive sampling to document the extent of the variability.[b] Yet even without detailed baseline information on microbial communities, scientists have devised ways that the knowledge gained from DWH can help us to recognize and respond to future disasters.[a]

Launching microbial research into the future

Microbiology and omics tools played an instrumental role in understanding how the DWH oil spill impacted marine and coastal ecosystems. These new omics tools and strategies aided an in-depth and thorough study of the microbial communities and their function in unprecedented detail. Altogether, the GoMRI-funded studies revealed new insights and lessons about how microbial communities respond to environmental disturbances and maintain and restore ecosystem stability during and after an oil spill or other disturbance. The lessons learned from this research can inform us 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.

CHANGES IN MICROBIAL COMMUNITIES ARE VISIBLE IN BEACH SAND

One way scientists use genetic sequencing is to look at the diversity of microbes in environmental samples, making comparisons between samples that contain oil and those that do not. For example, a sand core collected on June 30, 2010 at Pensacola Beach, Fla., contains an obviously oiled layer (dark brown) (**Figure 5**). More than 50% of the microbes in that layer belonged to genus *Marinobacter*, a known hydrocarbon-degrading group of microbes (in the Alteromonadales order, shown in the large bluish band).[c] The concentration of this microbial group is far more than in sands below and above the oiled layer, as is clear from both the small region of the same color (small bluish band on the far left) and the different colored banding patterns. Having information about the microbial communities above and below the oiled layer allows scientists to not only know which microbial species were impacted by the oil, but also predict what the community should look like once the oil has been degraded.[c]

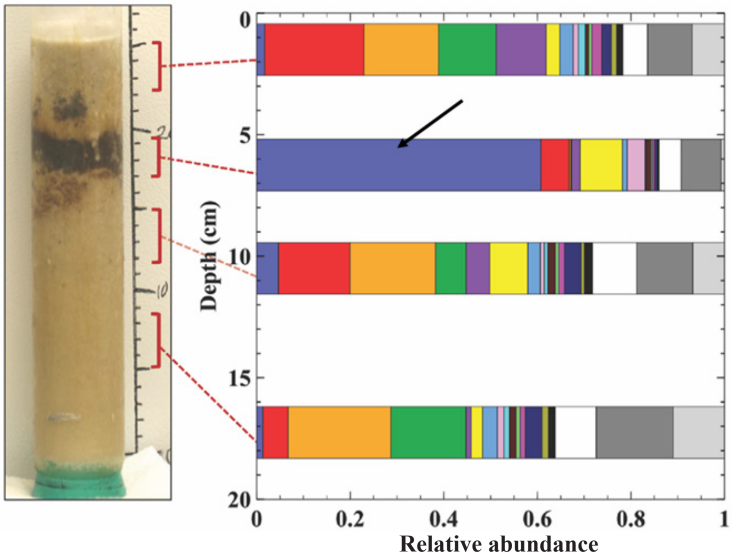


FIGURE 5. A sand core (left) collected from a beach contains a layer of oil (dark brown). Microbial communities were analyzed above, within, and below the oiled layer using genomics tools. The results of these analyses are shown in the center. Different members of the community are visible by the different color bands. The widths of the colored bands indicate the abundance of that group of microbes relative to the rest of the sample. The large bluish band (arrow) from the oiled layer represents a group of known hydrocarbon degraders. (Markus Huettel)

GLOSSARY

Archaea — A group of single-celled organisms once thought to be bacteria but later classified as their own group.

Biosurfactant(s) — Compounds that are produced by microbes that work to break up oil similarly to chemical dispersants.

Chemotaxis — The movement of cells, like bacteria, either toward or away from chemical signatures. Bacteria use chemotaxis to find sources of carbon and nutrients.

Genome(s) — The genetic material found in a cell of an organism.

Genomic(s) — The study of genes and genomes in an organism.

Horizontal gene transfer — The exchange of genetic material from one organism to another without reproduction. This contrasts with vertical transfer, which is the exchange of genes from one generation to the next.

Metabolites — Substances formed during the process of breaking down chemicals by an organism, the byproducts of metabolism.

Metabolize — To process, or break down, a substance through metabolism.

Metabolomics — The study of small molecules called metabolites which are the byproduct of metabolism.

Metagenomics — The sequencing of all the genomes from all organisms in a sample.

Proteomics — The study of proteins produced by genes.

Succession — The progressive changeover of a community of organisms over time.

Transcriptomics — The study of the ribonucleic acid (RNA) molecules in a cell. RNA is responsible for carrying messages coded through DNA to produce proteins.

REFERENCES

Publications resulting from the GoMRI-supported synthesis activities serve as the primary references for this work. Additional supporting literature, either cited in GoMRI synthesis papers or necessary for foundational information about the subject, is also included.

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The GoMRI Research Board established Synthesis & Legacy committees to review 10 years of oil spill science findings. Look for Sea Grant extension publications on these GoMRI synthesis topics:

- Observing and modeling oil plumes and circulation
- Combined ecosystem modeling
- Combined oil spill modeling
- How oil weathers and degrades
- Ecological/ecosystem oil spill impacts
- Human health and socioeconomic oil spill impacts
- Microbiology, genetics, and oil spills
- Dispersant-related impacts from oil spill response

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