Environmental Systems Microbiology of Contaminated Environments

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5.1.6

While sharing many common approaches and analytical experimental tools, environmental systems microbiology differentiates itself from microbial ecology and molecular microbiology in the applied outcomes of its fundamental research agenda. These outcomes are anthropocentric in nature, ultimately directed toward understanding and ameliorating undesired environmental impacts, such as pollution stress events or predicting the consequences, quantifying risk and developing control strategies for chemical or microbial releases and discharges to the environment. Consequently, basic research questions are directed to environmental perturbation effects and the effect of the microbial community in modulating perturbations within a system that provides ecosystem services and protection for receptor populations, including humans.

In addition to anthropogenic underpinnings, the system framework is an important attribute of environmental microbiology, which draws from both systems biology and ecological system science. The rise of modern molecular tools and approaches has greatly enhanced the understanding of the composition, complexity, and function of environmental microbial communities. However, the suggestion that understanding the meta-community and its interactome at the molecular level will provide reliable predictive capability of the dynamic community response to anthropogenic perturbations remains a hazardous assumption akin to predicting the stock market. Consequently, a systems ecology perspective of experimental design and hypothesis testing is needed to provide an analytical reference for dynamic, complex, and perhaps emergent features in environmental microbiology.

There is little to be accomplished in environmental microbiology without an intimate experimental and field relationship with the environmental system. This is true regardless of whether the target is acid mine drainage, a hazardous waste site, melting permafrost, wastewater treatment, cultural eutrophication, petroleum oil spills, high UV exposure, drinking water biofilms, a groundwater plume, agricultural crop contamination, or a host of other relevant environmental scenarios. No amount of lab experimentation or microcosm simulation is meaningful unless the design permits back-referencing to the field with meaningful validation in terms of replication and seasonal validation. The goal is to delineate not only the process but also its dynamics and variation. This becomes one of the most challenging aspects of environmental systems microbiology—extending testable hypotheses evaluated under highly controlled and replicable lab experiments to field-scale relevant experimental designs. Accomplishing this is a cost, logistics, materials, and design challenge that must be met to obtain meaningful data on complex system function for robust hypothesis testing. In this chapter we cover environmental systems microbiology using biodegradation/biotransformation as the primary focus with examples, including hypotheses and aims, sampling, measurements, analysis, and modeling.

SYSTEMS FRAMEWORK

What is the systems framework for environmental microbiology? The framework is an integration of systems biology thinking with ecological and engineering modeling from a complex systems perspective. Systems biology focuses on an understanding of all the parts of a system with the rationale that if the genetic and molecular components and interactions of the system can be completely described, this knowledge allows science to scale up from the genome or transcriptome to the whole organisms, populations of organisms, communities, and eventually functional ecosystems. This bottom-up approach (Fig. 1) is balanced with a top-down approach from systems ecology that embraces the dynamics and variations within the system and the emergence of new system properties based on temporal or spatial differences in communities and physical chemical influence from an unknown state. From an ecological perspective, these systems are uncontrolled and would be expected to exhibit complex behavior with emergent properties of unknown origin. From an engineering perspective anthropogenic influences and/or control (chemical additions, hydrological, temperature, etc.) would determine the state of the system, lending itself amenable to state-space relationship analysis.

Consequently while the systems biology perspective provides insight into the phylogenetic and taxonomic structure of the community and diversity, abundance, mobility, and expression of functional genes (i.e., biogeochemical cycling, toxin production, antibiotic resistance, quorum sensing), the environmental systems microbiology (ESM) perspective contributes to understanding the resilience of the systems, recruitment and maintenance of introduced species and genes, and dynamic variability of system performance and change. These later areas may well require both new experimental and analytic approaches and hypotheses to accurately

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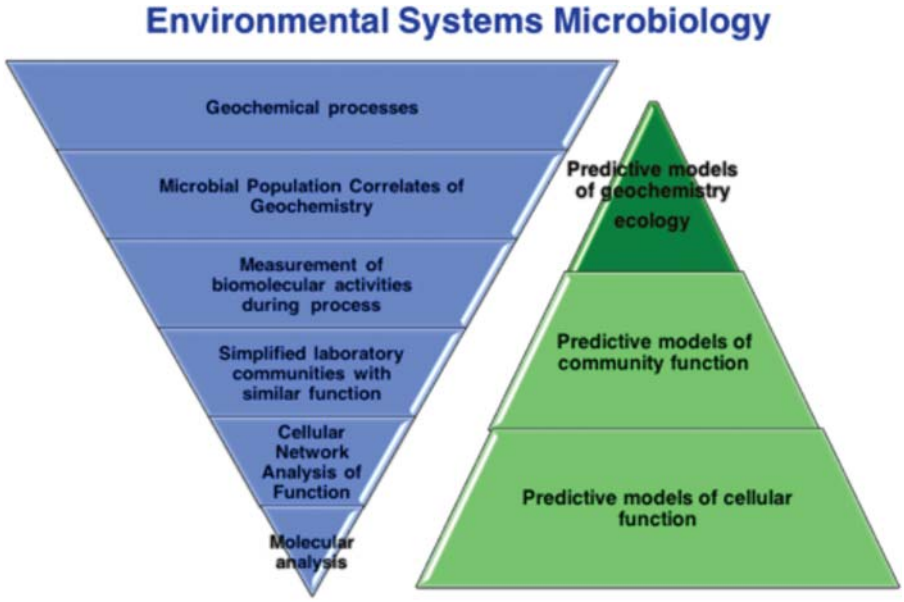


FIGURE 1 Bottom-up approach balanced with a top-down approach from systems ecology that aims to capture the dynamics and variations within a system. doi:10.1128/9781555818821.ch5.1.6.f1

predict environmental scenarios, identify control points, and optimize system function. Because of the complexity of ESM, in terms of timing and resources (equipment, personnel, funding, permits, etc.), it requires very careful planning. We have found that a rigorous field test plan, which is a dynamic document, is the best vehicle for ESM studies.

ESM FIELD TEST PLAN

ESM field test plans must incorporate the following components: (1) hypotheses to be tested and why, (2) boundaries of environment system, (3) measurements to be made, (4) sampling (protocols, holding times, storage, etc.), (5) resources needed (costs, equipment, people, time, etc.), (6) the field test plan summarizing the best possible scenario of items 2–5 to accomplish item 1 (including permits, safety, protocols, responsibilities, budgets, priorities, expected outcomes, reports and publications, data management plan, and science of opportunity), (7) feedback to the field test plan, which involves mobilization, implementation, demobilization, and final analyses and models for reports and publications (Fig. 2).

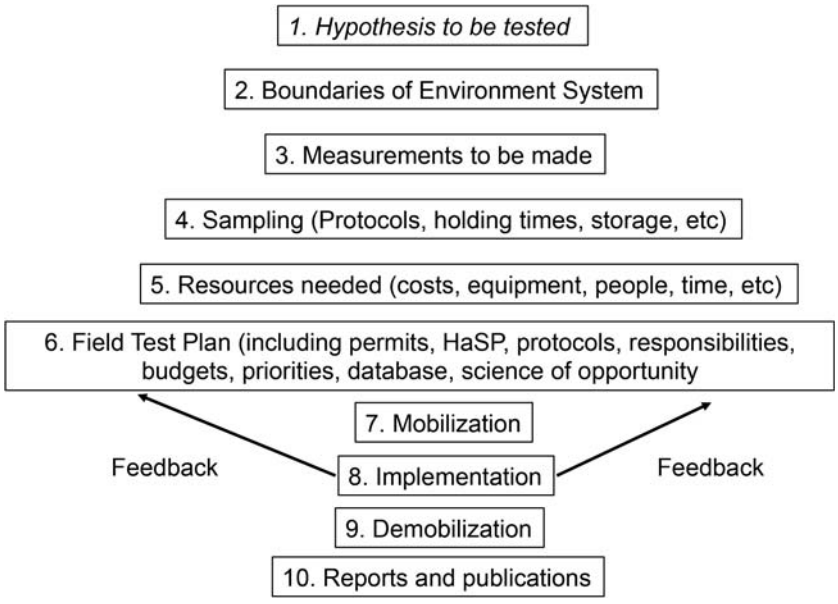


FIGURE 2 Environmental systems microbiology framework. doi:10.1128/9781555818821.ch5.1.6.f2

Hypotheses

Recently there has been a justified wave of discovery science using formally developed molecular tools and analytical methods of the postgenomic period to survey soils, aquatic habitats, plants, gut communities, and wastewater treatment systems, among others. This has led to definitions of community structure and complexity far more diverse than previously described in part due to the ability to assess what some call "microbial dark matter": community members and/or genes that resist routine laboratory isolation, cultivation, and characterization (1, 2). In some instances, there have been attempts to define core metagenomes (3) with untested arguments that the unculturable organisms represent the true metabolic function of the community. While this testable hypothesis is welcome, there have been calls for far greater levels of hypothesis development and testing within the context of ecological theory to move beyond the discovery science and descriptive analysis of environmental microbiology (4).

Boundaries of Environmental System Required for Rigorous Hypotheses

Due to the spatial and temporal scales of environmental microbiology, sampling, and analysis, it is generally acknowledged (if not spoken) that many studies are inherently not reproducible. For most habitats it is virtually impossible to ensure that conditions for a sampling period at a given time and location are identical to a previous sampling interval, not withstanding the fact that all measurable environmental parameters appear the same. This places an extra burden on researchers to amass sufficient time series and/or replicative data at the time of sampling to account for inherent variation. A further requirement is maintaining constancy of analytical methods over long periods of time, during which personnel turnover occurs, instruments change, electrical power fluctuates (e.g., ship power). As a consequence, hypothesis testing can be rather granular with in-depth mechanistic analysis requiring more experimentally controlled and replicated laboratory or microcosm experimentation. This is another characteristic of environmental microbiology the need to cycle back and forth between field analysis, laboratory experimentation, and field verification in hypothesis testing (as below). This requirement is shared in common with microbial ecology and has been well summarized (5).

In the current "omics" era, it is not at all clear that hypotheses are necessarily formulated with these realities in mind. Additionally, there appear far too many hypotheses developed in which the microbial community will be different or will change in response to some state change in X, Y, or Z and then process inferences are drawn from order- or family-level composition or abundance changes with no experimental validation. Such approaches appear hazardous given the mathematical and statistical biases imposed by primer designs, trimming, binning, and clustering protocols often indiscriminately used. Omics is not the only answer to ESM. Too often we have seen "shotgun" approaches to ESM that involve only 16S RNA sequencing or terminal restriction fragment length polymorphism or more recently metagenomes from single samples, at single points in time with no environmental metadata. We know from various studies that each omics platform has large number of steps that can introduce a variety of biases at each step, thus questioning whether our conclusions are really correct (6) (Fig. 3).

Prosser (7, 8) has argued that in many cases more depth of sequencing may not provide the essential temporal or spatial information for resolving phenotypic diversity needed for appropriate hypothesis testing in complex microbial systems. Ultimately it is time to move more aggressively to incorporating descriptive analysis in complex and dynamic perceptions of environmental microbial communities, taking greater

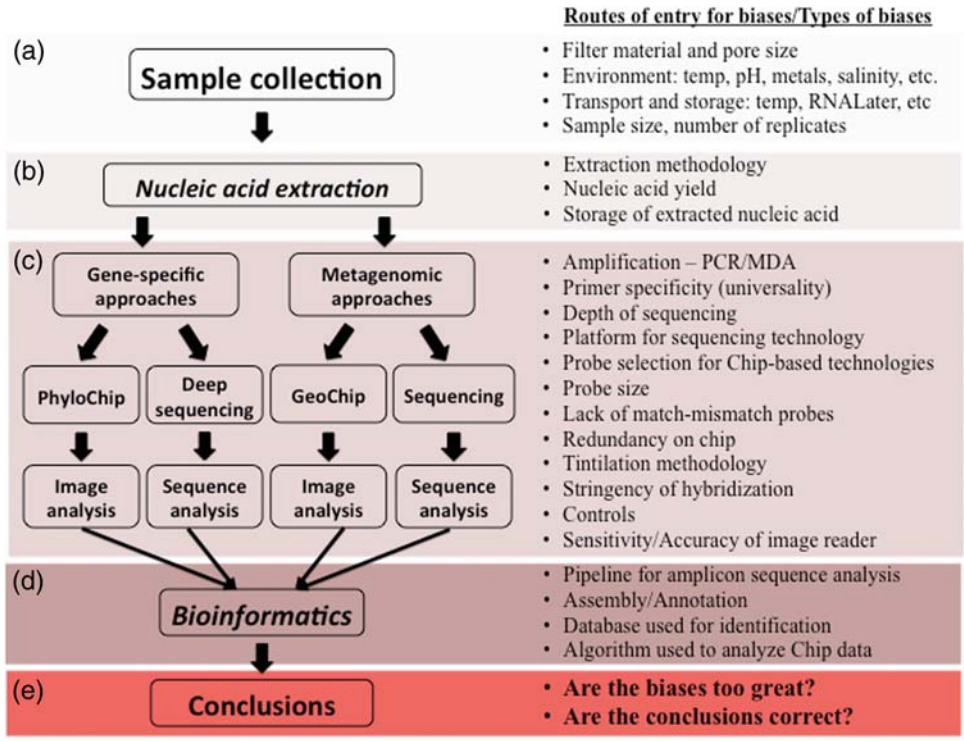


FIGURE 3 Biases associated with nucleic acid analyses (reused from (6), with permission). doi:10.1128/9781555818821.ch5.1.6.f3

advantage of our powerful molecular tools, "top-down conceptual framework," and bounding the environmental system where we are testing our hypotheses.

This framework puts more emphasis on the dynamic community as a contributing factor to the apparent diversity of the community. Even the most stable microbial community experiences input dynamics exhibiting periodicity, which is then interactively transposed to a dynamic system output by a multitude of molecular, biochemical, network, and population-level effects. Such an approach can lead to a more causal or mechanistic understanding with less correlative or associative trend analysis. Environmental complexity becomes a driving force than is amenable to lab-scale simulation and testing (9, 10). Myriad stress responses for microbes have illustrated this effect.

Measurements, Sampling, and Resources

An important consideration is that the output periodicity of a community can dramatically change if a tipping point is crossed, and these are difficult to predict from purely correlative analysis (11) at which time research focusing on causal networks within complex systems (12) should take on greater prominence. Of course from an environmental microbiology perspective, this requires more and greater mutual collaboration with modelers and numerical analysts. However, analytical tools exist for state, space, nonparametric, time series, empirical dynamic simulation analysis, and so on are being applied in areas such as dormancy in complex communities (13), disturbance effects in community colonization (14), and time series analysis of community complexity (15). It is clear that ESM requires multiple lines of evidence at multiple scales, see Fig. 4 for a few examples at different scales. An example of how this approach has been utilized in an ESM study is Fig. 5, which shows the actual measurements decided on for the Deepwater Horizon oil spill (17).

Analysis of the types of measurements that need to be made to experimentally test any given hypotheses requires careful evaluation of the parameters that can be measured and the accuracy, precision and cost (time and money), with available resources. Availability of resources and budget for the overall project is also critical and may require detailed analysis and iteration depending on various limits and hypotheses. Thus it is essential to develop protocols, test sampling, and measurements prior to initiation of a field campaign. Prioritization should be given to the measurements and sampling techniques used in terms of repeatability, cost, and ability to test the hypotheses chosen. Table 1 provides an example of various measures, the measurement type, data format, and highest priority data for a typical ESM. Data management is also a critical concern for ESM projects since large amounts of data will be collected that must have "cradle-to-grave" quality assurance and quality control.

For one ESM at a DOE field site contaminated with metal and radionuclides and mixed waste, Fig. 6 gives an example of how a flow diagram was developed for accomplishing the project in a given period of time. This project ultimately provided evidence that bacterial community structure could significantly predict a large number of geochemical parameters over a broad range of contaminant concentrations (18) (Fig. 7).

Field Test Plan

Once the hypotheses, environmental boundaries, experimental designs, measurements, sampling, cost, time, personnel, and equipment resources are agreed on by the project team, development of a field test plan can begin in earnest. The field test plan is a dynamic document and may require several iterations or fundamental changes before being implemented. The plan needs to define the hypotheses to be tested, environmental boundaries, measurements to be made and

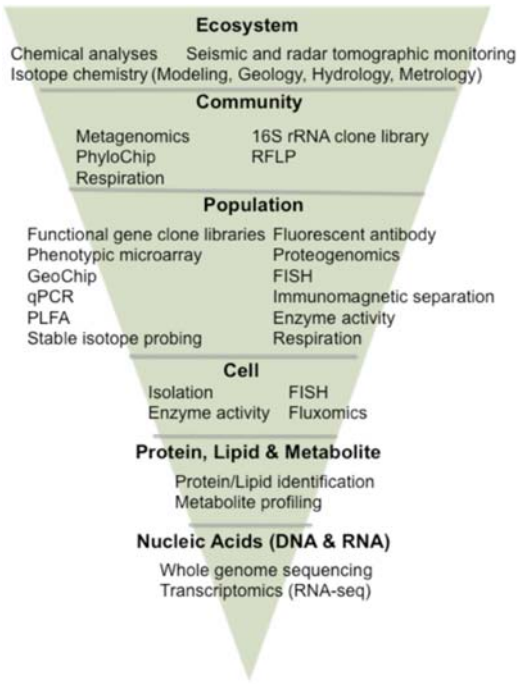


FIGURE 4 Different levels of analyses that can be performed and examples of analytical methods that can be used (reused from (16), with permission). doi:10.1128/9781555818821.ch5.1.6.f4

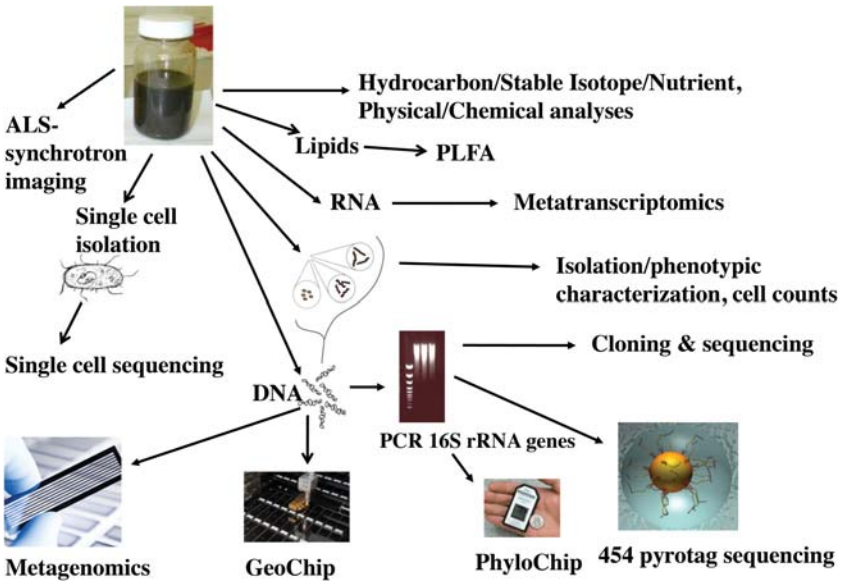
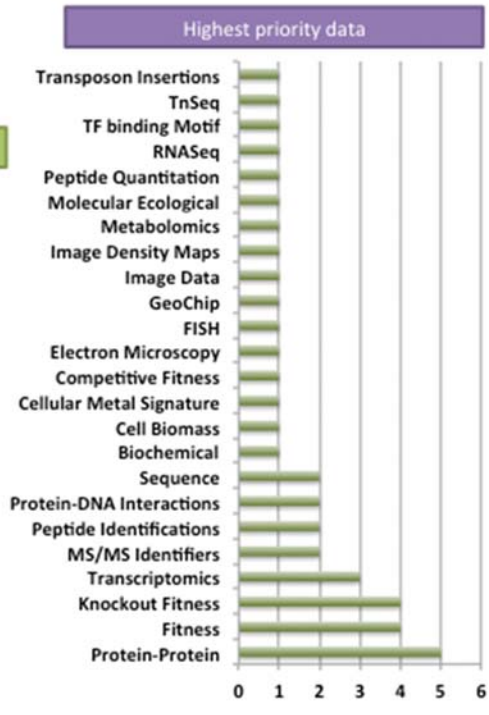


FIGURE 5 Examples of critical measurements made during the Deepwater Horizon oil spill (17). An environmental systems microbiology approach was used by performing analyses at different scales, measurements, and methods. doi:10.1128/9781555818821.ch5.1.6.f5

their priority (including appendances for all measurement protocols to be followed), health and safety plans, all permits required (including sampling, handling, and transport), personnel responsibilities, funding, contingencies for unexpected cost and time variances, communication plan for field and lab operations (including regular meetings with personnel on progress, problems, and highlights), and communication plans for the sponsor, scientific community, and public. Depending on the size and the scope of the project, this may also require mobilization, implementation, and

TABLE 1 Prioritization of measurements for a typical ESM, data types, measurement types, data formats, and highest priority data (arranged by columns). Data priority established by importance to hypothesis testing.

Data Types	Measurement Type	Data Format	Highest priority data
FISH	Abundance of enriched DNA in IP complexes	QIIME and Muther.	Transposon Insertions
16sRNA sequences	Cell Growth	csv	TnSeq
Cell density	Cell Number	DM3 format	TF binding Motif
Cellular Metal Signature	DNA Abundance	excel	RNASeq
Competitive fitness	DNA sequence	fasta	Peptide Quantitation
Fitness	Fluorescence Intensity	bam	Molecular Ecological
Fluorescence microscopy	Functional associations	matrix	Metabolomics
Functional gene sequences	Gene expression	mysql	Image Density Maps
Genome sequencing	Image data 2D and 3D	openxml	Image Data
Growth rate	Micrographs	mzxml	GeoChip
Imaging	Motif detection	tab-delimited text	FISH
Metabolomics	mRNA abundance	Genome Browser Tracks	Electron Microscopy
Metagenomic sequences	MS/fragmentation patterns	Cytoscape	Competitive Fitness
Metal Elution Profile	MS/intensity	text	Cellular Metal Signature
Metalloprotein Predictions	MS/m/z	TIFF	Cell Biomass
Mutant Fitness	MS/MS Spectra	Velvet	Biochemical
Network Model	MS/MS Spectra of metabolites	Newbler	Sequence
Phenotype data	MS/Retention time	graph	Protein-DNA Interactions
Primer sequences	Read counts		Peptide Identifications
Protein Abundance	Metalloprotein Predictions		MS/MS Identifiers
Protein-DNA interaction	Sequencing Reads		Transcriptomics
Protein-Protein Interaction			Knockout Fitness
Proteomic localization			Fitness
Proteomics			Protein-Protein
Short genomic sequences			
Transcriptomics			



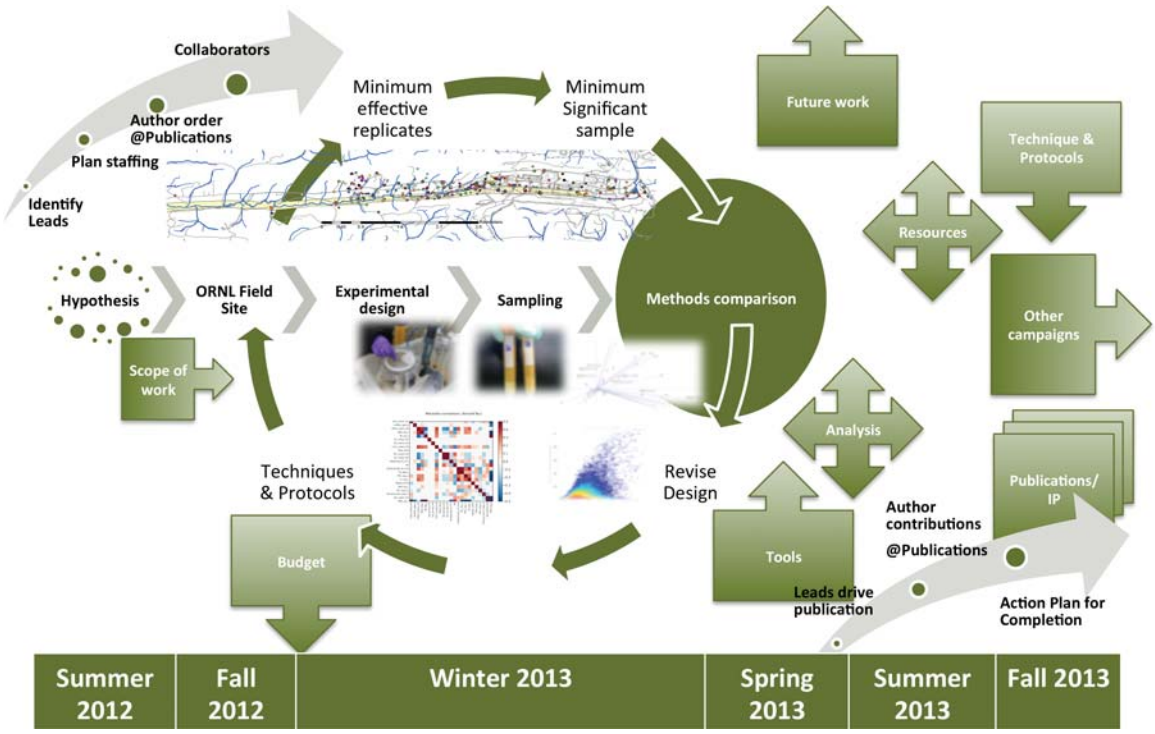


FIGURE 6 Flow chart for ESM study at Oak Ridge National Laboratory Field Site (18). doi:10.1128/9781555818821.ch5.1.6.f6

demobilization plans. Since this is a dynamic document, regular feedback to modify the field test plan is critical to get the best results for the ESM project.

MODELS AND ESM CASE STUDIES

Conceptual and numerical models allow ESM projects to take maximum advantage of the large data sets collected simultaneously from the ecosystem level to the molecular level. The following are case studies of ESM projects that have resulted in new understandings in ESM.

The Single Organism Ecosystem

The hypothesis was that bacteria could survive in the deep subsurface for millennia and this might suggest the possibility

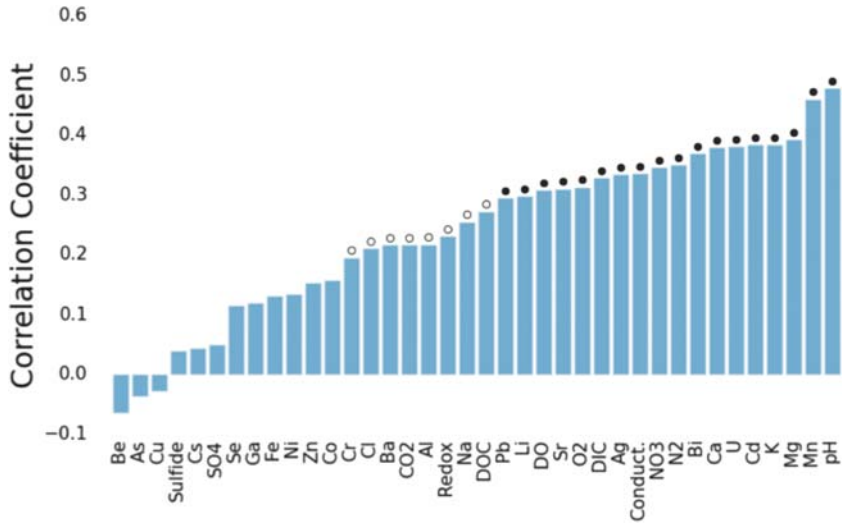


FIGURE 7 Bacterial DNA can be used to quantitatively predict many geochemical features. Besides classification, we can use 16S sequence data to predict quantitative values for a variety of geochemical measurements at each well sampled. Correlation coefficient (Kendall's tau) between true and predicted values. Eighteen of these correlations are highly significant ($p < 0.0001$, indicated by •), 8 are significant ($p < 0.01$, indicated by o), and 12 of these correlations are not significant (reused from (18), with permission).
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of life on other planets in the deep subsurface. Samples were taken from pore waters at 8,000 ft. in South African gold mines. Measurements included hydrology, geology, geochemistry, several isotopes, culture work, direct cell counts, and large volumes of water filtered and frozen for later extraction and sequencing (19–21). Though bacteria could be observed, they were nonculturable. Hydrology, geology, isotopic analysis, and geochemistry indicated that these bacteria had been isolated from any surface intrusions for at least 12 million years. Multiple lines of evidence showed that they were alive and not just dormant or spores (19). Multiple isotopic analysis of compounds in the cells collected indicated that these compounds had turnover times from 40 to 400 years, that is, suggesting a very slow metabolism (20). Sequence analysis showed this was not a previously described thermophilic sulfate-reducing bacterium with the metabolism that would support life under these extreme conditions (19). The hydrology, geology, isotopic analysis, and geochemistry enabled a conceptual model to be developed for how this bacterium lived. Natural uraninite decayed, causing radiolysis of water and bicarbonate, this in turn provided H as an energy source, carbonate as a carbon source, and oxidation and dissolution of pyrite by the H_2O_2 produced, which in turn produced sulfate and Fe^{3+}. This allowed the precipitation of iron oxide and release of phosphate. Nitrogen was supplied by the dissolution of calcite and release of ammonium from smectite minerals. Thus this bacterium had also the basic requirements for life but supplied on a very slow basis. This bacterium was obligately anaerobic with no stress repair mechanisms, hence its difficulty to culture. It also had incorporated a number of Archaea genes and was in fact a single clone (19), see Fig. 8.

Deep-Sea Oil Plume During the Deepwater Horizon Oil Spill

The hypothesis was that even at 1,100 m where a cloud of oil was moving to the southwest, oil degraders would be found and were degrading the oil. Because of all the media attention the spill was causing, it was imperative that this ESM be extremely well planned and take every measurement possible (Fig. 5). Oceanospiralles was found by clone library, 16S rRNA, and metagenomics to dominate the oil-contaminated

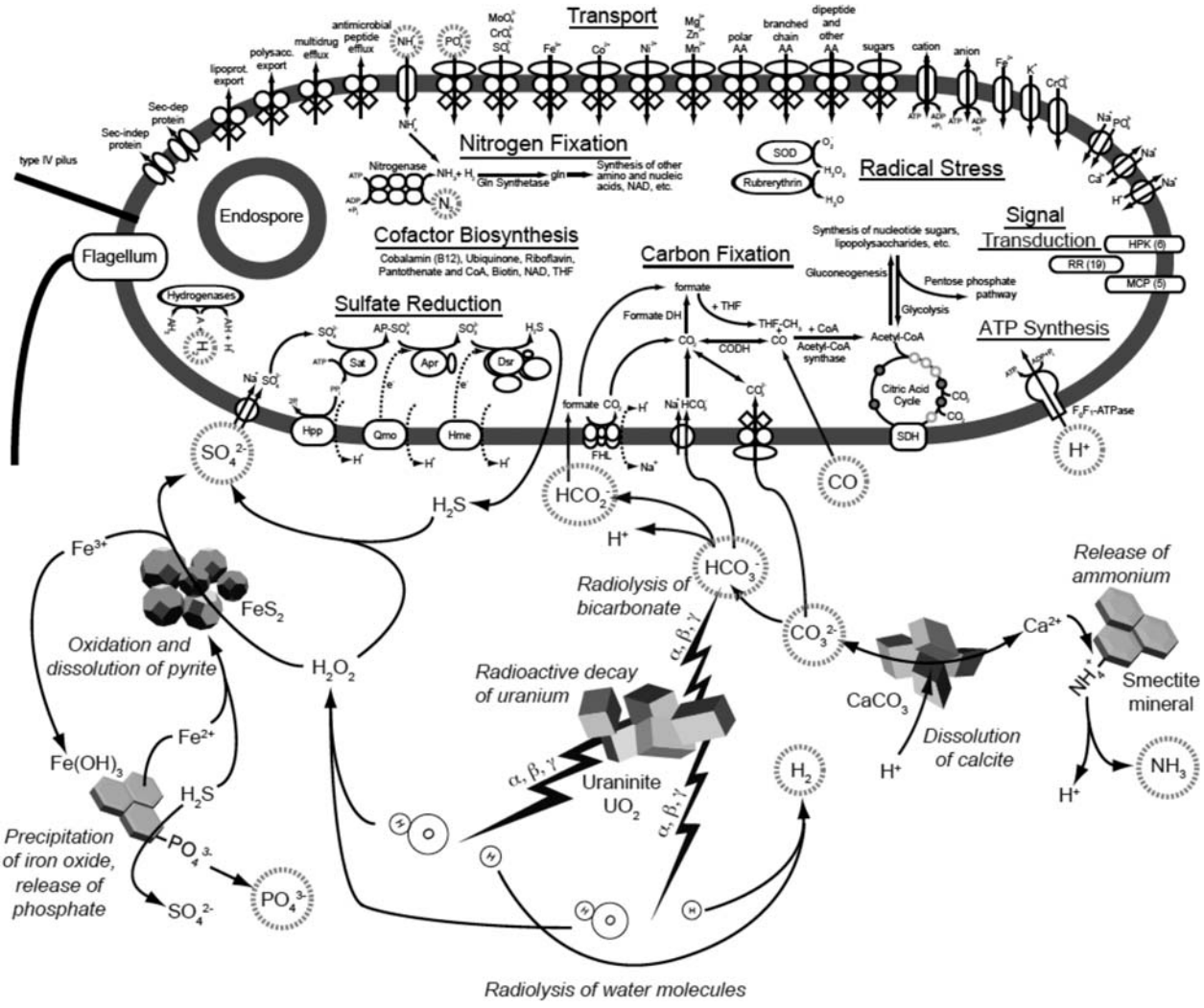

FIGURE 8 Single organism living is an isolation mechanism for survival. Metabolic networks determined from metagenomic sequencing. Metabolic models and stable isotopic analyses of geochemistry determined sources nutrients and energy (19, 20).
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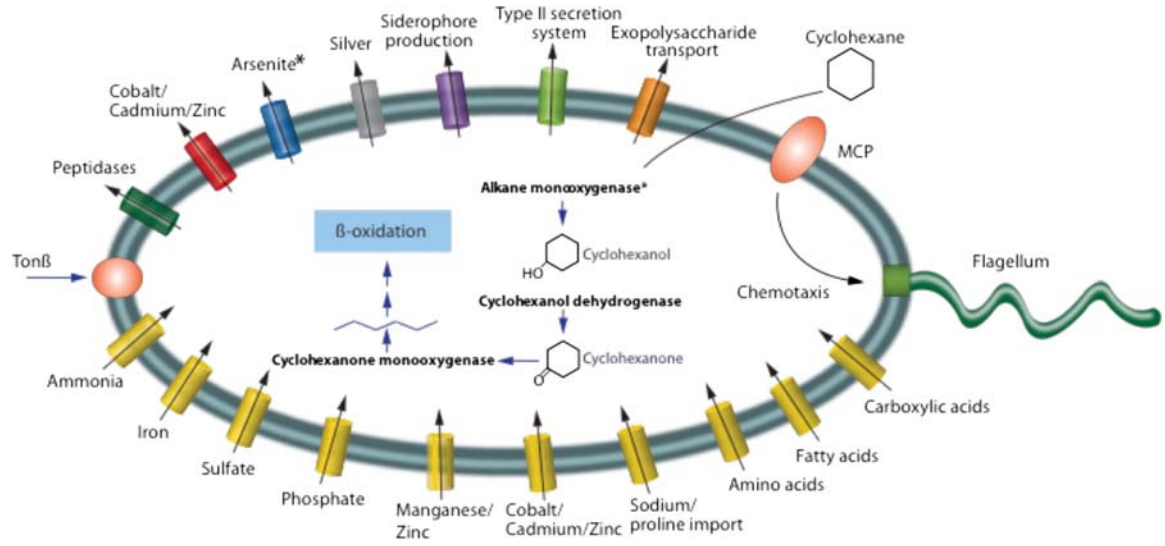

FIGURE 9 Single-cell sequence of oceanospiralle dominant strain in the Deep Water Horizon oil spill.
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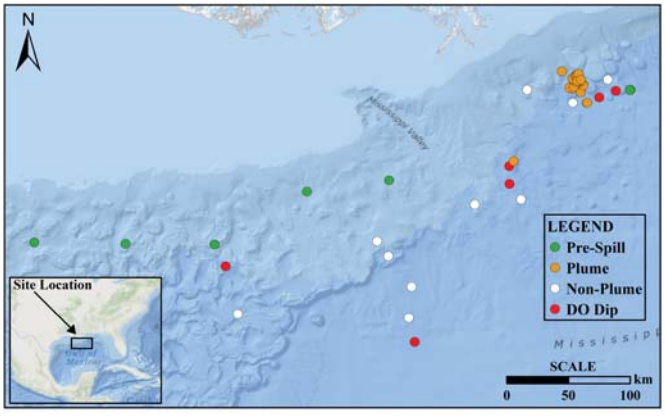
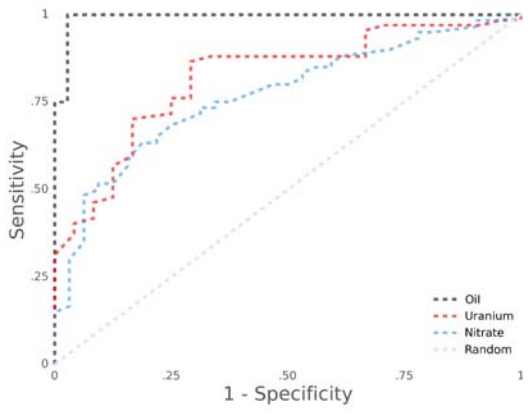
water in the deep plume, and overall the species diversity was greatly reduced in comparison to uncontaminated water at the same depth. It was also found that the half-life of the oil by four different methods was 1.2 to 6.1 days (17). The four methods included two direct water measurements from different laboratories of oil along the length of the plume, taking into account dilution with current measurements, and two lab incubation methods that used water from the same depth as the plume and fresh Macondo oil incubated at 5°C, using first-order rate equation. One of the lab methods involved more classical oil biodegradation where communities were enriched with oil from the site and then incubated with marine minimal media with fresh Macondo oil. The other lab technique was to use fresh uncontaminated water samples from the plume depth incubated with fresh Macondo oil and no other additions. The oil in the deep plume was very dilute and dominated by alkanes, which the oceanospiralles had increased ability, as demonstrated by single-cell sequencing, metagenomics, and transcriptomics, to degrade and was chemotactic to oil (22) (Fig. 9). This ESM project also provided data that allowed a large number of other projects to use to compare and contrast their results on different samples taken during the Deepwater Horizon oil spill and other oil spills (23–33). Additionally it described the succession that occurred in community structure during and after the well was capped (26).

Radionuclide-Contaminated Site and a Model for Bacterial Predictions of Biogeochemistry

In one of our most recent ESM completed, water was collected from 100 of more than 800 possible wells in both



Sampling locations in Gulf of Mexico



Quality of classifiers (oil in black; groundwater chemistry in red, blue)

FIGURE 10 SLiME predictions for groundwater and deep sea contaminants (reused from (18), with permission).
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contaminated and uncontaminated areas at the Oak Ridge National Laboratory Field Site. The wells were geographically isolated and not in direct contact with each other (18) (see Figs 6 and 7). The water collected from each well was filtered at the well head and frozen, and then all samples were extracted at the same time by the same person. The extracted samples were then sequenced for 16S rRNA to obtain the community structure for each sample. Structured learning in microbial ecology (SLiME) was used to correlate the metadata geochemistry with the 16S community structure information. This demonstrated 18 geochemical parameters that were highly significantly correlated, 8 that were significant, and 12 that were not (Fig. 7). Two of the most highly correlated parameters were nitrate and uranium, which are major contaminants at the site. To see if SLiME could show such correlations on completely different contaminants and environments the ESM data from Deepwater Horizon was used (26). It predicted the oil concentrations in the deep water plume even better than it predicted the uranium and nitrate concentrations (Fig. 10). Thus, having ESM data from different sites and environments enabled verification that the SLiME model may have more universal predictive capabilities for many types of environments and contaminants.

REFERENCES

1. **Marcy Y, Ouverney C, Bik EM, Losekann T, Ivanova N, Martin HG, Szeto E, Platt D, Hugenholtz P, Relman DA, Quake SR**. 2007. Dissecting biological "dark matter" with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. *Proc Natl Acad Sci USA* **104:**11889–11894.
2. **Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T**. 2013. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* **499:**431–437.
3. **Shade A, Handelsman J**. 2012. Beyond the Venn diagram: the hunt for a core microbiome. *Environ Microbiol* **14:**4–12.
4. **Alivisatos AP, Blaser MJ, Brodie EL, Chun M, Dangl JL, Donohue TJ, Dorrestein PC, Gilbert JA, Green JL, Jansson JK, Knight R, Maxon ME, McFall-Ngai MJ, Miller JF, Pollard KS, Ruby EG, Taha SA**. 2015. A unified initiative to harness Earth's microbiomes. *Science* **350:**507–508.
5. **Jessup CM, Kassen R, Forde SE, Kerr B, Buckling A, Rainey PB, Bohannan BJM**. 2004. Big questions, small worlds: microbial model systems in ecology. *Trends Ecol Evol* **19:**189–197.
6. **Hazen TC, Rocha AM, Techtmann SM**. 2013. Advances in monitoring environmental microbes. *Curr Opin Biotechnol* **24:**526–533.
7. **Prosser JI**. 2012. Ecosystem processes and interactions in a morass of diversity. *FEMS Microbiol Ecol* **81:**507–519.
8. **Prosser JI**. 2013. Think before you sequence. *Nature* **494:**41.
9. **de los Reyes FL, Weaver JE, Wang L**. 2015. A methodological framework for linking bioreactor function to microbial communities and environmental conditions. *Curr Opin Biotechnol* **33:**112–118.
10. **Langenheder S, Bulling MT, Solan M, Prosser JI**. 2010. Bacterial biodiversity-ecosystem functioning relations are modified by environmental complexity. *PLoS One* **5**(5)**:** e10834.
11. **Scheffer M, Bascompte J, Brock WA, Brovkin V, Carpenter SR, Dakos V, Held H, van Nes EH, Rietkerk M, Sugihara G**. 2009. Early-warning signals for critical transitions. *Nature* **461:**53–59.
12. **Sugihara G, May R, Ye H, Hsieh CH, Deyle E, Fogarty M, Munch S**. 2012. Detecting causality in complex ecosystems. *Science* **338:**496–500.
13. **Stolpovsky K, Martinez-Lavanchy P, Heipieper HJ, Van Cappellen P, Thullner M**. 2011. Incorporating dormancy in dynamic microbial community models. *Ecol Model* **222:** 3092–3102.
14. **Vuono DC, Benecke J, Henkel J, Navidi WC, Cath TY, Munakata-Marr J, Spear JR, Drewes JE**. 2015. Disturbance and temporal partitioning of the activated sludge metacommunity. *ISME J* **9:**425–435.
15. **Faust K, Lahti L, Gonze D, de Vos WM, Raes J**. 2015. Metagenomics meets time series analysis: unraveling microbial community dynamics. *Curr Opin Microbiol* **25:** 56–66.
16. **Chakraborty R, Wu CH, Hazen TC**. 2012. Systems biology approach to bioremediation. *Curr Opin Biotechnol* **23:** 483–490.
17. **Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL, Stringfellow WT, Bill M, Conrad ME, Tom LM, Chavarria KL, Alusi TR, Lamendella R, Joyner DC, Spier C, Baelum J, Auer M, Zemla ML, Chakraborty R, Sonnenthal EL, D'Haeseleer P, Holman HYN, Osman S, Lu ZM, Van Nostrand JD, Deng Y, Zhou JZ, Mason OU**. 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* **330:**204–208.
18. **Smith MB, Rocha AM, Smillie CS, Olesen SW, Paradis C, Wu LY, Campbell JH, Fortney JL, Mehlhorn TL, Lowe KA, Earles JE, Phillips J, Techtmann SM, Joyner DC, Elias DA, Bailey KL, Hurt RA, Preheim SP, Sanders MC, Yang J, Mueller MA, Brooks S, Watson DB, Zhang P, He ZL, Dubinsky EA, Adams PD, Arkin AP, Fields MW, Zhou JZ, Alm EJ, Hazen TC**. 2015. Natural bacterial communities serve as quantitative geochemical biosensors. *mBio* **6**(3)**:**e0036-13.
19. **Chivian D, Brodie EL, Alm EJ, Culley DE, Dehal PS, DeSantis TZ, Gihring TM, Lapidus A, Lin L-H, Lowry SR, Moser DP, Richardson PM, Southam G, Wanger G, Pratt LM, Andersen GL, Hazen TC, Brockman FJ, Arkin AP, Onstott TC**. 2008. Environmental genomics reveals a single-species ecosystem deep within earth. *Science* **322:** 275–278.
20. **Lin L-H, Wang P-L, Rumble D, Lippmann-Pipke J, Boice E, Pratt LM, Sherwood Lollar B, Brodie EL, Hazen TC, Andersen GL, DeSantis TZ, Moser DP, Kershaw D, Onstott TC**. 2006. Long-term sustainability of a high-energy, low-diversity crustal biome. *Science* **314:**479–482.
21. **MacLean LCW, Pray TJ, Onstott TC, Brodie EL, Hazen TC, Southam G**. 2007. Mineralogical, chemical and biological characterization of an anaerobic biofilm collected from a borehole in a deep gold mine in South Africa. *Geomicrobiol J* **24:**491–504.
22. **Mason OU, Hazen TC, Borglin S, Chain PSG, Dubinsky EA, Fortney JL, Han J, Holman HYN, Hultman J, Lamendella R, Mackelprang R, Malfatti S, Tom LM, Tringe SG, Woyke T, Zhou JH, Rubin EM, Jansson JK**. 2012. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. *ISME J* **6:**1715–1727.
23. **Atlas RM, Hazen TC**. 2011. Oil biodegradation and bioremediation: a tale of the two worst spills in US history. *Environ Sci Technol* **45:**6709–6715.
24. **Baelum J, Borglin S, Chakraborty R, Fortney JL, Lamendella R, Mason OU, Auer M, Zemla M, Bill M, Conrad ME, Malfatti SA, Tringe SG, Holman HY, Hazen TC, Jansson JK**. 2012. Deep-sea bacteria enriched by oil and dispersant from the Deepwater Horizon spill. *Environ Microbiol* **14:**2405–2416.

25. **Beazley MJ, Martinez RJ, Rajan S, Powell J, Piceno YM, Tom LM, Andersen GL, Hazen TC, Van Nostrand JD, Zhou JZ, Mortazavi B, Sobecky PA**. 2012. Microbial community analysis of a coastal salt marsh affected by the Deepwater Horizon oil spill. *PLoS One* **7**(7)**:**e41305.
26. **Dubinsky EA, Conrad ME, Chakraborty R, Bill M, Borglin SE, Hollibaugh JT, Mason OU, Piceno YM, Reid FC, Stringfellow WT, Tom LM, Hazen TC, Andersen GL**. 2013. Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environ Sci Technol* **47:**10860–10867.
27. **Kimes NE, Callaghan AV, Aktas DF, Smith WL, Sunner J, Golding BT, Drozdowska M, Hazen TC, Suflita JM, Morris PJ**. 2013. Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. *Front Microbiol* **4. 50:**1–17.
28. **King GM, Kostka JE, Hazen TC, Sobecky PA**. 2015. Microbial responses to the Deepwater Horizon oil spill: from coastal wetlands to the deep sea. *Ann Rev Mar Sci* **7:** 377–401.
29. **Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M**. 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Appl Environ Microbiol* **77:**7962–7974.
30. **Lu ZM, Deng Y, Van Nostrand JD, He ZL, Voordeckers J, Zhou AF, Lee YJ, Mason OU, Dubinsky EA, Chavarria KL, Tom LM, Fortney JL, Lamendella R, Jansson JK, D'Haeseleer P, Hazen TC, Zhou JZ**. 2012. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. *ISME Journal* **6:**451–460.
31. **Mason OU, Scott NM, Gonzalez A, Robbins-Pianka A, Baelum J, Kimbrel J, Bouskill NJ, Prestat E, Borglin S, Joyner DC, Fortney JL, Jurelevicius D, Stringfellow WT, Alvarez-Cohen L, Hazen TC, Knight R, Gilbert JA, Jansson JK**. 2014. Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *ISME J* **8:**1464–1475.
32. **Spier C, Stringfellow WT, Hazen TC, Conrad M**. 2013. Distribution of hydrocarbons released during the 2010 MC252 oil spill in deep offshore waters. *Environ Pollut* **173:**224–230.
33. **Valentine DL, Mezic I, Macesic S, Crnjaric-Zic N, Ivic S, Hogan PJ, Fonoberov VA, Loire S.** 2012. Dynamic autoinoculation and the microbial ecology of a deep water hydrocarbon irruption. *Proc Natl Acad Sci USA* **109:** 20286–20291.