ENIGMA Environmental Overview: Field to Lab to Field

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Project Goals: ENIGMA working hypotheses**:** 1) Key transects in the environment provide constraints on community composition and activity that are discernable at multiple scales. The uranium/nitrate/pH gradient may provide one such transect and we will examine the communities and activities in different *in situ* (and laboratory simulated) uranium/chromium contaminated environments. 2) These environmental constraints change in time due in part to the structure and function of these communities and causal relationships may be discovered and quantified. 3) Community structure is established and maintained by varying factors that include transient populations, niche diversification, optimized interactions, and resource competition. We will be attempting to dissect these by field perturbation and time series studies and laboratory simulations of the environment. 4) There are important stable communities that achieve the above, which we can dissect at the level of molecular interactions. We will drive toward isolation of key organisms, discovery of key activities and interactions, and dissect cellular networks that control when and how these activities are expressed.

The implications of the above hypotheses suggest: 1) There is a core set of metabolic factors that are the direct effectors of metal-reduction in soil and, while exact microbial species may vary, the molecular network will be relatively constant. This effort includes the identification of the biomolecular determinants of metal reduction in key populations that directly and/or indirectly interact with other populations that have programmed responses to important environmental parameters. 2) There are particular variants of these keystone microbes that adapt them for different metals and different concentrations and these are deployed in planktonic and attached communities over time during the reductive and reoxidative processes following stimulation by substrate amendment. 3) At a particular site there are core, relatively stable sub-communities of microbes whose interspecific interactions are responsible for the stable reduction (and ultimate reoxidation) of metals. There is also a core community structure of necessary functional classes of microbes that form a stable "food web" to exploit the available energy in the environment nearly optimally. For example at the Hanford 100-H study site, microbes from soil samples differed from the corresponding groundwater organisms (even at the phyla level) and were more diverse ($p=0.001$). While many of the populations were observed in both groundwater and surrogate sediments, the respective matrices appeared to enrich for particular OTUs. Results do not indicate a large shift in dominant organisms in soil from pre- to post- injection, and this may be due to the microbes remaining dominant from the

first stimulation. However, a prevalence of core genera and rare genera were observed across 34 samples while urban and rural genera were less abundant.

Background and Significance. ENIGMA is planning on shifting the principle field focus from Hanford to Oak Ridge. For the last 12 years ORNL has been characterizing, monitoring, and conducting field experiments at DOE's BER ORNL Field Research site. The focus has been on elucidating the mechanisms and efficacy of bioreduction and bioimmobilization of U, one of the DOE's most common waste site contaminants. This has involved a number of field studies including pump tests, hydrological modeling, characterization of sediment and groundwater and amendments of ethanol, bromide, and nitrate, etc. More than 300 wells have been established and characterized and are available for analysis in a searchable database.

Research and Design

- Overarching Driver: Determine microbial community structure and function in both *in situ* environments and constructed consortia - environment to the laboratory.
- Elucidate structure to function during key biogeochemical transformations - *immobilization of metals*
- Determine key succession events and mechanisms - *stability in the context of geochemical and thermodynamic constraints*
- Parameterize critical microbes at the phylogenetic and functional level in conjunction with key biogeochemical variables that together, impact and control environmental activities of interest (*e.g.*, metal-reduction; N flow; C flow)
- Identify key populations, directly and/or indirectly related to activities of interest.
    - *Iin situ* and laboratory consortia will be used to explicate levels of biological organization from populations to proteins, and
    - models will be developed with various bioinformatic tools (*e.g.*, AdaptML, random matrix theory, multivariate statistics) commonly used for genes and proteins but applied to populations overlaid with geochemical parameters and engineering controls.

The current tasks for the Environmental Core are as follows:

Task 1. Optimize omics protocols for environmental samples.

Task 2. Use existing data from the ORNL FRC database to design efficient field sampling studies that maximize the geochemical diversity of study sites. This strategy is expected to enhance the resolution of associations between microbial communities and key geochemical features. Toward this end, we have developed a Monte Carlo search algorithm to optimize site selection for geochemical diversity. We are currently looking for 100 wells that we can do a metagenome analysis in cooperation with the existing, planned SBR IFRC program at ORNL.

Overarching principle for Field Studies and Field Linked Resources: With the cooperation of the ORNL IFRC and as a team, establish the hypotheses, experiments, sampling, analyses, and schedule as a test plan, including all protocols, amounts, and responsibilities. Prioritize everything so that time, money, shipping, sample limitations, and unforeseen contingencies will not limit delivery of milestones. This includes 50%, 75%, and 95% evaluations of test plans by the team prior to execution, and fatal-flaw analyses at each step.

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