25. Natural and Synthetic Ecology in ENIGMA: Determining the links between Microbial Community Structure and Function

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Project Goals: The overarching goal of the Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to understand environmentally-relevant microbial community structure and function through a series of integrated field-to- laboratory campaigns. The Natural and Synthetic Ecology campaign is designed as an interdisciplinary platform to elucidate the fundamental ties between structure and function, as well as determine the environmental influences on these ties. Our initial focus has been the development of in-field bioreactors as a method of capturing temporal fluctuations in the *in-situ* community due to either natural or induced influences. Going forward we will determine the influence of naturally-occurring carbon sources, invasive species and selected pressures on microbial activities such as nitrate- and metal- reduction.

One of the most difficult aspects of studying microbial ecology is determining and understanding the fundamental ties between microbial community structure (the organism biodiversity and their relative abundances that comprise a given microbial community) and the observed functions (the detectable biochemical activities that support survival of the observed species). Although microorganisms are important in controlling the fate of contaminants in the subsurface, information on the basis of how why microbial communities respond to contaminants is lacking. Hence, it is important to characterize microbial communities, establish linkages between biodiversity and function, and study interactions between different species. As a part of the overall ENIGMA goal to link genotypes to phenotypes, the overall objective of this campaign is to obtain deep understanding of the composition, structure, function, activity and interaction of subsurface microbial communities at DOE contaminated sites (i.e. Oak Ridge Integrated Field Research Center). We have developed a bioreactor system for manipulating and temporally monitoring the *in-situ* microbial community in the field so as to maintain the *in-situ* community structure. Community structure was measured through sequencing, PCR and qPCR for selected genes, cell counts and total protein before and after the cells entered the bioreactor system. Temporal community function was qualified by alterations in the concentration of 53 metals, 12 organic acids, 14 anions and 4 sugars, pre- and post- bioreactor exposure. Near future experimental plans include determining an adequate naturally-occurring carbon source that will allow for an increase in total biomass with minimal alteration to the relative abundances of the major phylogenetic groups within the community. Finally, in order to establish the environmental relevance of new field isolates from this site, a pilot study using the bioreactor system will be conducted in collaboration with the Metals Metabolism campaign of ENIGMA. The bioreactors will use synthetic groundwater mimicking the geochemistry of the ORNL wells and will be inoculated with groundwater supplemented with various Mo concentrations since a current hypothesis is that a lack of Mo may inhibit nitrate-reduction. The experimental duration is expected to be 30 days with temporal

measurements of metals (53 elements), metabolites, 16S rRNA to determine changes in community structure, and by qPCR of key denitrification genes. End-point samples will be used for the isolation and characterization of new denitrifying strains. This work is highly collaborative, involving several ENIGMA campaigns including the 100 Well Survey, Microparticle Mesogenomics, Microbial Isolations and Characterizations and, Metals with down the line benefits to the Predictive Biology and Printable Worlds campaigns.

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