ENIGMA: Dissecting Microbial Nitrogen Cycling In The Subsurface Using Tailored Reactor Schemes

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

The unresolved interplay of biotic and abiotic factors drives material and energy transformations in the subsurface environment. Understanding has been limited by the lack of appropriate model systems to simulate the complex matrix of attached and unattached components of the subsurface. Therefore, the ENIGMA consortium has invested in three types of reactor systems that are designed to simulate different features of the subsurface. Simulations of these features, including fluid dynamics as well as temporal and spatial heterogeneity of processes and community composition, are being used to enrich previously uncharacterized activities and quantify the interplay of governing principles.

Abstract:

Microbial activity in the subsurface is driven by a complex interplay between abiotic and biotic processes and is limited by various nutrients over differing spatial and temporal scales. The dynamic nature of the subsurface environment has made deciphering mass and energy transformations by these activities challenging and thus they remain poorly understood (For more information on nitrogen cycling work see “Metabolomics and Transcriptomics for Environmental Systems Biology: Molecular Mechanisms of Reduced Sulfur Caused Growth Inhibition of Field-Isolated Nitrate-Reducing Bacterium” by Majumder et. al.). Specifically, many governing factors of the nitrogen cycle remain to be identified or characterized, including chemodenitrification and intermediate cycling. The Field Research Center (FRC), near Oak Ridge National Lab in Tennessee, provides a contextually relevant site for examining the effects of nitrogen cycle perturbation in field. Historical activities at the FRC led to the deposition of large quantities of heavy metal laden nitric acid that has resulted in a spatially and temporally dynamic environment of varying pH (4-7) and nitrate concentrations (µg/l to g/l). By comparing microbial activity and community structure across the environments within the FRC and

laboratory simulations performed across ENIGMA, we aim to identify and quantify microbial processes that govern nitrogen cycling in the subsurface.

Physical, chemical, and biotic dynamics of the subsurface create a diverse collection of environments that may select for microbial activities. Environmental simulations using a single reactor type miss key components required to reproduce field phenomena. However, many of the dynamics of the subsurface can be separated using three primary reactor designs: a planktonic-based chemostat, a fluidized bed reactor (FBR), and a packed bed reactor (PBR). Planktonic-based chemostats simulate the pore water of the subsurface, support steady state kinetic analyses, and when implemented in the field capture the impact of dynamic events and perturbations of the incoming community structure. However, planktonic reactors are subject to washout when a population is unable to grow at the operating dilution rate. Packed bed reactors most directly simulate the complex subsurface environment (flow through porous media) and are capable of replicating *in situ* flow rates and providing surfaces for attached populations that may grow slower than that required for planktonic populations. However, these packed bed systems also may be complicated by channeling resulting from cell growth and synthesis of microbial products. Fluidized bed reactors simulate a more homogeneous subsurface environment where particle-attached growth is exposed to a uniform fluidized local microenvironment. These general concepts are being used across ENIGMA to recapitulate environmental phenomena in a variety of reactor constructs, including packed bed reactors, fluidized bed reactors, lysimeters, and CDC-type reactors containing sediment coupons (For more details see "Using in-field bioreactors to monitor microbial community dynamic shifts with geochemical perturbations" by Wilpiszeski et. al.). Major environmental parameters, including shear and impact of changing nutrient availability or nutrient transients can be isolated by comparing these reactor systems to better understand the driving forces of community assembly and mass and energy transformations in the subsurface.

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