Core Values: Spatial Variation in Microbial Function, Activity, and Community Assembly in Groundwater and Sediment from a Contaminated Subsurface Aquifer

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Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses 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.

Primary motivations for studying the subsurface are to expand what is known about Earth's microbial diversity and the subsurface microorganisms under low nutrient conditions that significantly impact C, S, N, P and mineral cycles. However, on an ecosystem scale, there is limited information regarding the exact relationship between microbial diversity, environmental parameters, and biogeochemical processes between subsurface groundwater and porous media (*i.e.*, sediment). The Department of Energy ENIGMA Scientific Focus Area seeks to map the causal interactions that constrain microbial community assembly, functionality, and dispersal in chemically and physically complex environments. We recently initiated an in depth study of microbial distribution and activity throughout sediment and water compartments in the shallow subsurface at the Oak Ridge Field Research Center, a site of nuclear weapon development during the Manhattan Project.

We hypothesize that strong gradients of pH, heavy metals, nitrate, and other contaminants at the site influence the distribution, structure, and activity of microbial communities. We performed large-scale analysis of two sediment cores and associated groundwater for which we produced depth-index data sets of physical, chemical, bulk biological and sequencing measurements. One core (466 cm) was from a region outside the area of heavy chemical contamination and the other core (815 cm) from within the contaminated area. We divided the cores into ~23 cm segments for processing, resulting in 56 segments which allowed a finer-grained analysis of the vertical transect as compared to other subsurface studies.

We observed differences in diversity and distribution of dominant metabolisms between the heavily contaminated and less contaminated cores. The heavily contaminated core was less diverse as measured by 16S amplicon sequencing. Approximately 250 exact sequence variants (ESVs) accounted for half the observed reads in the heavily contaminated core as compared to 660 from the uncontaminated core, suggesting strong selective pressure from contamination. In general, there is little overlap in ESVs between the two cores (~300-350 meters apart). Analysis of ESV distribution and inferred functional potential using FAPROTAX suggests that the less contaminated core has strongly ordered communities with well-defined functional zones for nitrification, denitrification, methanogenesis, and sulfate reduction, and that these functions are carried out by specific diverse subcommunities differentially distributed with depth. There was a clear interplay among communities mediating denitrification, methanogenesis, and sulfate reduction within the variably saturated zone. These metabolisms are also correlated with the presence of key chemical constraints, such as uranium, nitrate, and pH, not just location in the core. Conversely, the heavily contaminated core has a far more heterogenous population structure with little evident intersegmental transfer (*i.e.*, little mixing between adjacent layers). Within the heavily contaminated core we observed two distinct cosmopolitan communities: (1) a large and diverse community enriched in denitrifying organisms and (2) a less diverse highly abundant community not clearly enriched in any metabolism. We found that the ENIGMA isolate collection is not representative of all of the organisms in the cores, but in some cases we have greater than 80% coverage of the strains present in a subcommunity. Based on the isolates we have and the strong chemical and physical correlates to specific community compositions, we are currently in the process of generating enrichments and synthetic communities representative of each of the identified subcommunities. From activity assays, we identified a competitive exclusion of sulfate reduction and denitrification; species representative of these metabolisms are found and enriched in different locations in the core. This exclusion was further supported by shotgun metagenomics. From 22 groundwater and 91 sediment shotgun metagenomes, we have successfully circularized 8 genomes and have over 50 genomes with >98% completeness and <2% contamination. We are tracking these genomes with depth in the core and analyzing the potential functional roles of these organisms in denitrification and sulfate reduction.

To distinguish intact and potentially viable cells from "relic" DNA, we used complementary culture-independent methods to determine respiratory rates and identify the active fractions of microbial assemblages from groundwater and sediment. Groundwater from the less contaminated area had higher diversity and three- to four-fold higher activity than heavily contaminated samples. Additionally, in the less contaminated area, the activity on a per cell basis was two to three-fold greater for planktonic cells compared to particle associated organisms, with small cells (<0.1μm) contributing up to 19% of total activity. Conversely, in heavily contaminated aquifers, activity was greater for sediment-associated cells. To understand the distribution of the active ESVs across the ORNL landscape we are analyzing the top active ESVs and checking their abundance in historic 16S rRNA sequence data from nearly 100 wells from ORFRC.

This study integrates over 12 measures of microbial community composition, activity, and environmental controls to provide new insights into how contamination impacts the distribution of activity between attached and planktonic populations in subsurface microbial communities. The application and refinement of *in situ* measurements spanning different scales will aid in the development predictive frameworks for understanding large scale biogeochemical cycling from groundwater environments.

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