

Spatiotemporal Dynamics of Groundwater and Sediment: Geochemistry, Microbial Communities and Activities in a Contaminated Aquifer

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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.

Spatiotemporal variability of groundwater levels and sources could greatly impact the geochemistry and the drift, dispersal, and selection of associated microbial communities. As a pilot study, one uncontaminated site and two contaminated sites (Area 2 and Area 3) at the DOE Oak Ridge Reservation Y-12 Complex in Oak Ridge, Tennessee, were selected for bi-weekly sampling. Three groundwater wells at each site were sampled for geochemistry and microbial activity measurements. Groundwater was also filtered in succession through 10 μm , 0.2 μm and 0.1 μm filters for assessment of microbial communities (16S and metagenomic). After completing five sampling time points from March to May 2019, the preliminary results of bacterial communities revealed distinct succession patterns affected by contamination and cell size. For the uncontaminated site and Area 2, total microbial cell counts ranged between 5 to 18 $\times 10^6$ cells/ml and the counts for Area 3 averaged between 2 to 8 $\times 10^6$ cells/ml. Cell counts for most contaminated site (Area 3) were more consistent over time when compared to the other two sites. Total microbial activity was assessed via the uptake of ³H-leucine. Activity measurements changed temporally and were an order of magnitude higher for uncontaminated wells compared to the contaminated wells (1-3 $\times 10^{-5}$ ng C/cell/d versus 0.6-8 $\times 10^{-6}$ ng C/cell/d, respectively). The changes in total microbial activity did not always correlate to changes in microbial cell numbers. These results suggest that not all microbes were active over the same times and places (*i.e.*, mechanisms of dispersal and selection were likely impacting different populations spatiotemporally). The bacterial communities from contaminated wells (Area 2 and 3) were similar in diversity and structure compared to those in uncontaminated wells. The unique ESVs

respective to each well (contaminated or uncontaminated) were typically lower in abundance compared to ESVs detected across areas and wells. The results supported environmental filtering, particularly in contaminated wells, for unique, low-abundance populations. In addition, detected ESVs from the 0.1 μ m filters showed decreased relative abundance of area-specific species over time, but ESVs from larger fractions (0.2 μ m to 10 μ m) did not. While larger-size bacteria from different wells always showed different community structure, small-size bacteria from different areas became convergent after late April. This implicates the influence of migration, corresponding to precipitation changes in April and May that coincided with observed changes in the δO^{18} values of groundwater. While α - and/or β -Proteobacteria generally dominated in larger-size bacteria, the phylum Bacteroidetes significantly increased or even predominated in the small-size fraction after late April, mainly attributed to the genus *Hydrothalea*. Sulfate-reducing bacteria, a relevant functional group at this site, belonged to the orders Syntrophobacterales and Desulfobacterales, which were nearly undetectable in small-size bacteria. In contrast, *Rhodanobacter*, a dominant genus in contaminated wells of this site, was detectable in different size fractions and showed decreased relative abundance after late April. These preliminary data demonstrated the value of more frequent sampling for an in-depth time series analysis.

Starting in July 2019 a comprehensive, high-resolution time series survey of 27 wells was carried out to obtain diurnal and seasonal fluctuations within three levels (mild, moderate, and high) of nitrate and heavy metal contamination. With this data, we aim to model these areas and study changes within the attached and unattached microbial communities in relation to groundwater geochemistry. Measurements were gathered from 27 previously established groundwater wells four days/week over the span of 17 weeks (70 days total, July to December) to build both diurnal and seasonal time series. In-field geochemical measurements were obtained for dissolved oxygen (DO), pH, conductivity, oxidation-reduction potential (ORP), and nitrate concentration. Samples were also taken for metals, anions, organic acids, and total organic and inorganic C/N. Preliminary results show diurnal and seasonal changes in geochemistry with wide variations between each well and levels of contamination. Additionally, one well in each level of contamination (3 wells total) was selected to complete a “deep-dive” analysis by sampling for microbial communities in groundwater (unattached) and sediment (attached). Groundwater was filtered through 8 μ m and 0.2 μ m filters for 16S rRNA and metagenomic analysis for a total of 420 filters. In each of the three “deep-dive” wells, 18 unamended sediment traps were deployed throughout the sampling period in order to complete a time series soil analysis. The attached microbial communities and soil geochemistry will be compared to the unattached communities and groundwater geochemistry. Results for each stage of analysis will be linked to groundwater flow vectors and on-site weather data. With this data, we aim to establish a predictive systems model to understand potential distribution of microbial communities and associated activities in the shallow subsurface.

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