

Temporal Variability and Microbial Activity in Groundwater Ecosystems

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Project Goals: A fundamental goal in the field of microbial ecology is to link the activity and structure of microbial populations and communities to processes occurring within an ecosystem. This project aims to identify the drivers of community structure and succession by identifying the metabolically active fraction of microbial communities from both pristine and contaminated groundwater habitats at the Field Research Center (FRC) at Oak Ridge National Laboratory (ORNL). It is hypothesized that community function is independent of phylogeny and that functionality will be altered as a result of environmental perturbations. The use of geochemically distinct wells in combination with the enumeration and sequencing of translationally-active microorganisms, activity assays, carbon utilization profiles, and geochemical measurements will allow for the elucidation of the mechanisms shaping community structure and function in terms of turnover of natural organic matter and major contaminants (*e.g.*, NO₃⁻). Multiple assay comparisons will be used to achieve an accurate characterization of the active fraction of groundwater microbial communities and will ultimately be applied to continuous sediment cores from both contaminated and pristine locations.

Abstract: Saturated subsurface environments are estimated to contain approximately 40% of the prokaryotic biomass on Earth, and due to the complexity of these habitats they support highly diverse microbial communities. In addition, it is estimated that over 98% of the Earth's consumable and available freshwater is in the subsurface as groundwater. However, the factors that determine microbial community assembly, structure, and function in groundwater systems and the impact on water quality and contaminant transport remain poorly understood. Three non-contaminated background wells were sampled for groundwater geochemistry and microbial diversity approximately 3 times a week over a period of three months. Community analysis via ss-rRNA paired-end sequencing and distribution-based clustering revealed temporal differences in richness, diversity, and variability in the groundwater communities. Microbial community composition of a given well was on average >50% dissimilar to any other well at a given time point. Similarities in community structure across wells were observed with respect to the presence of 20 cosmopolitan populations in all samples in all wells; however, wells differed in the relative abundances of these taxa. Similarity percentage (SIMPER) analysis revealed that temporal variability was explained by lowly abundant and transient populations or more highly abundant and frequently present taxa in a sample-dependent manner.

Based on the observations of temporally and spatially variable groundwater communities, we aim to use a combination of methodological approaches to determine how functional groups of microorganisms relate to habitat as well as structure/composition heterogeneity. Specifically, we identified the active fraction of microbial communities and quantified rates of activity from both contaminated and pristine groundwater habitats. Total cell abundances, quantification of translationally-active microorganisms, ^3H -leucine incorporation, carbon utilization profiles, and community sequencing of active microorganisms (SSU rRNA and sorted translationally-active cells) were used to investigate four groundwater wells representing geochemical extremes. Two previously studied background wells and two contaminated wells were sampled November 2016 – January 2017. Contaminants in non-background wells include: radionuclides (U, Sr, and Tc), metals (Sr, Cd, Ba, B, Hg, Cr), volatile organic contaminants (VOCs), and nitrate. Of particular interest for this study is the U-nitrate-pH gradient present within contaminated groundwater wells.

Bioorthogonal non-canonical amino acid tagging (BONCAT) was used to identify translationally-active bacterial and archaeal cells for microscopic evaluation and sequencing. Incubations with additions of amino acid L-azidohomoalanine (AHA) followed with fluorescent tagging of AHA-containing cellular proteins can identify newly synthesized proteins. For contaminated wells, large proportions of the community were identified as translationally-active; however, specific rates of activity were low based on labeled amino acid incorporation. Total cell abundances ranged from $1.11\text{--}2.07 \times 10^5$ cells/mL with 73-84% of the community being translationally active. Rates of ^3H -leucine incorporation for the two contaminated groundwater samples were 14.5 ± 2.4 ng C/d and 45.1 ± 7.4 ng C/d, respectively.

The objectives of this study are part of a larger program that aims to understand the geospatial relationships between hydrogeology, geochemistry, and microbiology. Ultimately, we aim to apply these methodologies to attached biofilm communities from continuous sediment cores spanning depths from 0-50 m. It is known that free living microbial assemblages are substantially different than attached sediment populations. Due to sampling constraints, groundwater has been more routinely studied as it is difficult to obtain representative sediment samples. Studies that have evaluated species diversity in sediment boreholes have observed significant variation; however, it is not known how species variation relates to variation in function or activity. Additionally, *in situ* measurements from this study will aid in the development a predictive framework for understanding large scale biogeochemical cycling from groundwater environments.

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