

104. Temporal Variation in Groundwater Geochemistry and Microbial Community Structure at the Oak Ridge Field Site

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Project Goals: The goal of the ENIGMA field microbiology component is to identify key microbial populations and determine the community events and mechanisms of these populations that impact and control environmental activities of interest, ultimately predicting how perturbations of the environment may affect community structure and function. From these results, we can develop models that can be applied to microbial populations overlaid with geochemical parameters and engineering controls. An example of such a model is described in the 100-well survey where we demonstrated the ability to utilize natural bacterial communities as in situ environmental sensors that capture environmental perturbations and elucidate key systems biology features (Smith et al., submitted). Here, we expand upon the survey to capture the microbial community response to temporal changes in the groundwater geochemistry to (1) provide for temporal tuning of predictive models, and (2) to determine if and to what extent geochemical variation in groundwater affects microbial community, activity, and genetic diversity along different well depths.

Across aquatic and terrestrial environments, numerous studies have sought to characterize key microbial communities and to identify factors that drive changes in microbial community structure and activity. While these studies enable us to further understand and potentially uncover key correlations between the composition of microbial communities and their environment, information regarding temporal community dynamics is often limited or in many cases lacking. One such example is the Oak Ridge Field Research Center (ORFRC) where there has been a large focus to characterize the spatial distribution of groundwater and soil microbial communities across different geochemical transects (e.g. Uranium-Nitrate-pH). In this time-series study, we aim to bridge this gap by capturing the spatio- and temporal variation of geochemistry on the overall structure, function, and genetic diversity of the groundwater microbial communities in the groundwater wells at the ORFRC. Here, we present our findings from two pilot studies conducted during November 2013 and from November 2014 - January 2015, respectively, at the ORFRC background field site.

One of our main objectives of the temporal study is to determine how resilient (or volatile) microbial communities are to daily and weekly changes in groundwater geochemistry. To capture changes in microbial community structure and geochemical constituents, we initially sampled two deep and two shallow groundwater wells over the course of three weeks during November 2013. For each well and time-point, groundwater samples were collected for geochemical and microbial communities analyses. Nucleic acids were collected by filtering water through a 10.0µm pre-filter and 0.2µm membrane filter and then extracted using a Modified Miller method. Results from the study showed that geochemical measurements across all the wells remained fairly stable over the course of the study. However, a decrease in pH and increase in conductivity measurements was observed in the shallow wells during

small rain events. Unlike the geochemistry, the 16S rRNA sequencing of the microbial community structure within each well varied on a daily basis in both the 0.2 μm and 10.0 μm size fraction. Statistical analysis of the 16S data using Adonis indicated that there were statistically significant differences (p-value = 0.0001) in the community structure between wells throughout the study. Nonmetric multidimensional scaling analysis of the community structure did not show distinct differences between communities present in shallow versus deep well depth. However, analysis indicated that communities present in wells FW-300 (shallow) and GW-460 (deep) were much more variable throughout the time course.

Due to the stability of the geochemistry in the 2013 pilot study, we hypothesize that the variation in microbial communities is a result of both a sampling effect and from the inclusion of communities associated with biofouling in the well casing and sloughing off during sampling. To determine if the daily variation in groundwater microbial community profiles in the first study were naturally occurring within the aquifer or if (and to what extent) the variation in community structure is a result of factors associated with biofouling or sampling artifacts, we sampled six wells from the background site from November 2014 – January 2015. Of the six wells, we physically and chemically cleaned four wells to remove biofilm and attached particulates from the well casing. The remaining two wells that weren't cleaned, served as controls. Prior to cleaning, all wells were sampled to establish a baseline microbial community profile. Post- cleaning, each well was sampled a total of twelve times. For each well and time-point, groundwater samples were collected for geochemical and microbial community analyses. Currently, the microbial and geochemical data are still being analyzed although the preliminary results indicate geochemical variation in response to rain events during the course of the study.

Overall, results from both pilot studies suggest evidence of geochemical and microbial response within select wells in response to rain events. Findings from these will enable ENIGMA campaigns for more specialized questions on microbial community structure, provide for temporal tuning on environmental models, and further our understanding of the natural temporal variations versus external factors, such as biofouling or sampling-related effects on microbial communities.

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