

Population filtering in sediment biofilms from dynamic, source planktonic communities

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Project Goals: The goal of Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to push the frontier of knowledge on the impact of microbial communities on ecosystems and to elucidate microbial assembly rules. As part of this, we must link genetic capacities to ecological function. In these sites, the microbial communities are often predominantly attached to sediment particles as a biofilm. We have characterized temporal dynamics of bacterial groundwater communities and compared to the establishment of particle-associated bacterial communities on native sediments incubated down-well. In pursuing this goal, inter-laboratory collaboration facilitated by ENIGMA has led to the identification of aquifer population distributions between the liquid/solid boundaries *in situ*.

Understanding the factors that determine microbial assembly, composition, and function in subsurface environments are critical to assessing contributions to biogeochemical processes such as carbon cycling and bioremediation. However, these factors are still not fully understood. In this study, surrogate sediment samples were incubated for 3 months in 3 wells (FW-301, FW-303, FW-305) within the background site of the Oak Ridge Field Research Center in Oak Ridge, TN. Local sediment biofilm communities were compared to those of the groundwater (source diversity). Groundwater samples from each well were collected approximately 3 times a week in order to identify members of planktonic communities. Field well geochemistry was also measured. Multiple sediment samples (n=12) were used per well to determine inter- and intra-well variation. Geochemical measurements reveal that organic acids and anions remained relatively stable in all wells over the studied time period. Wells had similar median values for oxidation-reduction potential but not for conductivity, pH, and dissolved oxygen. Additionally, the temporal stability of conductivity, pH, and dissolved oxygen was different for each well, with FW-303 being the most stable and FW-301 being the most dynamic. Community analysis of local and source diversity via ss-rRNA paired-end sequencing and distribution-based clustering revealed higher richness, diversity, and variability in source groundwater communities compared to sediment-associated communities. Groundwater sequences displayed transitory predominance unique to each

well over time, and were characterized by periodic blooms of different populations (e.g. *Curvibacter*, *Dechloromonas* in FW-301, *Anoxybacillus*, *Methylophilus* in FW-305). In sediment samples, 20-40% of the communities consisted of populations that were abundant at less than 5% of the total sampled diversity. Inter-well sediment biofilms (across wells) were also distinct from each other. Intra-well sediment biofilms showed much less variability, although sediment from FW-305 was the most variable. For all wells, some populations observed at low abundances in planktonic communities became predominant in the biofilm (e.g. *Aquabacterium*, *Pseudomonas*). However, these populations were represented by unique sets of OTUs in each well. Ordination analysis revealed sediment biofilm communities were distinct from corresponding groundwater communities, with the exception of FW-305 (the youngest well), which showed greater similarity between within-well planktonic and sediment communities. Intra-well population networks (SPARCC) with strong associations (correlation cutoff $\geq \pm 0.8$) revealed that FW301 had the greatest number of positive associations between sediment populations, as well as the most negative associations between groundwater and sediment populations. Many more correlations were observed in biofilms than corresponding groundwater populations, and may have been a consequence of temporal changes. Conversely, FW-305 had fewer associations overall, and none that were negative. These results indicate a shift in local community structure that is influenced by the available source community as well as hydrology.

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