Temporal Monitoring of Microbial Community Dynamics Under Iron- and Sulfate- Reducing Conditions via "Now-Generation" DNA Sequencing-Enabled Molecular Environmental Microbiology

ANL SFA (Laboratory Research Manager: Carol S. Giometti)

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Understanding the biotic component of any environmental system is challenging, owing to the dynamic spatial and temporal scales that evolving microbial communities exhibit. Over the past 20 years, studies of microbial community composition have been predicated on obtaining inventories of the 16S ribosomal RNA (rRNA)-encoding genes present in a sample as proxies for the organisms present and their relative abundances. Presently, the application of so-called "next-generation" DNA sequencing technologies (effectively state of the art, hence "now-generation") has enabled a new level of relative low-cost, high-throughput sampling that is commensurate with the genomic capacity of the system being studied. Fully exploiting this technological potential is dependent upon realizing scale-effective sampling of the system. One additional benefit of the advent of "now-generation" DNA sequencing is the routine interrogation of all genes present in a sample (effectively the "metagenome" of the microbial community). The underlying computation for annotating the sequencing reads (i.e. comparing them with a database of known genes) at this scale is non-trivial, but strategies developed by members of the Mathematics and Computer Science Division (MCS) and the Institute for Genomics and Systems Biology (IGSB) at Argonne have made this possible. In-house DNA sequencing capabilities (including the Illumina HiSeq2000, GAIIx, and MiSeq platforms, and 454 GS-FLX) and data analysis (via the MG-RAST metagenomics analysis server; http://metagenomics.anl.gov) are unique capabilities at Argonne that can facilitate integration of microbial community dynamics with the synchrotron- and lab-based biogeochemical investigations within the Argonne SBR SFA.

In two recent studies, we have investigated the impact of electron donors (acetate, lactate, and glucose; see companion poster by O'Loughlin et al.) and an electron shuttling compound (2,6-anthraquinone disulfonate [AQDS]) on the evolution of microbial communities over time in iron-rich materials seeded with field sediments from the US DOE SBR Old Rifle IFRC site. For both experiments, mixed batch systems were characterized by microbial community analysis (using 454-based tag sequencing of the V3-V4 region of the 16S rRNA-encoding gene) and geochemical measurements (wet chemical and synchrotron-based analyses) to elucidate natural biogeochemical processes, particularly those related to C, Fe, and S dynamics. Geochemical characterization of an acetate-amended system supplemented with or without AQDS identified an increase in the concentration of reduced iron and a decrease in the concentration of sulfate with time. An increase in the relative abundance of the Deltaproteobacteria was observed early in the experiment (post Day 2) for both systems. For the AQDS-amended system the majority of the community was dominated early by *Geobacter* whereas a greater increase in the relative abundance of Bacteroidetes was observed for the batch not containing AQDS. In both systems a transition occurred (post Day 23) when the Firmicutes became dominant (specifically members of the genus *Desulfosporosinus*). This work is the first detailed laboratory analysis of microbial community development over time in the presence of an exogenous electron shuttle. Many dynamics observed in our experimental batch systems containing acetate as the available electron donor with (or without) the electron shuttle AQDS overlapped with previous observations in the field.

Deep sequencing inventories of the organisms present in a community allows for a more appropriate scale for capturing the complexity of the community structure present in a sample. Standardization of sampling strategies and concomitant automation of sample processing allows the interrogation of a suite of samples at a low cost that spatial and temporal dynamics can now be addressed with the appropriate experimental design. Combining these two key capabilities means that the ebb and flow of individual microbial population abundances within a community can be monitored owing to changes in environmental conditions over time, without *a priori* knowledge of what microorganisms are present. More pointedly, the sensitivity of this overall approach implies that in conjunction with chemical and physical characterizations of the samples, specific triggering events can be identified that dictate assembly of the community.

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