

## **The Application of Ecosystem Modeling at Subsurface Sites to Study Carbon, Nutrient and Metal Cycling in Microbial Communities**

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**Project Goals: Diverse microbial communities exist in subsurface environments that possess significant metabolic potential to effect global carbon, nitrogen and metal cycles including the transformation of radionuclides. Objectives of this ongoing project are: 1) to apply systems-level biology through application of ‘metaomics’ approaches (collective analyses of whole microbial community DNA, RNA and protein) to the study of microbial environmental processes and their relationship to C, N and metals including the influence of microbial communities on uranium contaminant mobility in subsurface settings undergoing natural attenuation, 2) improve methodologies for data generation using metaomics technologies and analysis and interpretation of that data and 3) use the data generated from these studies towards microbial community-scale metabolic modeling.**

To meet the goals of this ongoing project, two subsurface sites from the Department of Energy (DOE) Rifle Integrated Field Research Challenge Site (RIFRC) are being interrogated using a suite of metaomic approaches. The first site consists of sediments from the Winchester 2007 gallery, ‘JB’ well locations and was chosen due to the occurrence of natural attenuation of uranium (uranium reduction in the absence of biostimulation or other remedial interventions). The second and more recent sites of study within this project have been collected from Colorado River Floodplain (CORFP) sediments representing recent sediment depositions. Overbank deposits in the floodplain have become enriched in C, Fe and S minerals. Aggradation processes have led subsequent burial of these enriched sediments creating ‘hotspots’ of biogeochemical activity which serve as analogs to the buried naturally reduced sediments at the JB sites.

In this current work, we describe the generation of a framework for microbial community modeling that couples collections of genome-scale models of bacterial metabolism from the subsurface ecosystem for the purpose of predicting how a complex microbial community may respond to changes in the subsurface environment. This effort is critical as it provides an important analytical strategy for integrating and interpreting metaomics data as well as the capacity to simulate various interactions between environmental conditions and microbial communities. Guided by results from previous metagenomic and metatranscriptomic analyses from a site identified as undergoing natural attenuation of uranium at the RIFRC (JB-05) and the CORFP site, collections of genome-scale metabolic models representing seven bacterial classes were chosen including (a) Beta-proteobacteria (representative species: *Thiobacillus denitrificans*, *Rhodoferrax ferrireducens* and *Variovorax paradoxus*); (b) Delta-proteobacteria (representative species: *Geobacter metallireducens* and *Desulfovibrio vulgaris*); (c) Bacilli (representative species: *Bacillus subtilis*); (d) Gamma-proteobacteria (representative species: *Pseudomonas stutzeri*); (e) Alpha-proteobacteria (representative species: *Rhodospseudomonas palustris*); (f) Actinobacteria (*Arthrobacter* sp.) and (g) Clostridia (*Clostridium cellulolyticum*), and metabolic models constructed. This framework was then divided into two main components: 1) the

bacterial community and 2) the extracellular subsurface ecosystem. The mixed microbial community model was placed in the subsurface ecosystem (outer environment), and interactions among community members as well as the exchange between the community and the outer environment were captured by a dynamic multi-species metabolic modeling (DMMM) approach.

The ecosystem model indicates that *T. denitrificans* may dominate the community at the JB site due to its ability to use inorganic electron donors for energy and fix CO<sub>2</sub> as its carbon source effectively bypassing any limitations of bioavailable organic carbon. Through electron transport with cytochrome bc<sub>1</sub> complexes and NADH-Q oxidoreductase, a tight coupling between Fe(II) oxidation and nitrate reduction can be established to support CO<sub>2</sub> fixation as the main carbon source. This is in accordance with the bioinformatic analyses of taxonomic and functional profiles from the JB site indicating that *T. denitrificans* possesses a modified complete Calvin cycle for CO<sub>2</sub> fixation, and is the most abundant microorganism available among the seven bacterial classes. In contrast, the ecosystem model shows that in acetate amended sites, the delta-proteobacteria, *G. metallireducens*, would be numerically dominant over other bacterial classes, due to its ability to fix N<sub>2</sub> to complement or overcome limitations of organic nitrogen allowing for rapid growth during biostimulation.

In addition to capture of the community structure in terms of the relative abundance approximating the RIFRC, the ecosystem model is also able to predict how the components of the microbial community may respond to changes in the environment they inhabit by altering conditions in the extracellular subsurface ecosystem component of the model. Results from the model simulations show for example, that the community structure is highly responsive to acetate concentrations in the subsurface. As acetate concentrations increase (from 0 nmol/l to 300 nmol/l) the relative abundance of the gamma-proteobacteria also increases (from ~4%-40%) while that of the beta-proteobacteria decreases (from ~76%-35%). Further, the model suggests that *R. ferrireducens* may become numerically dominant over *T. denitrificans* as acetate concentrations increase, a finding which has been supported by experimental evidence. Similarly, the model simulations show that nitrate concentrations are also important in driving community dynamics. When nitrate concentrations are increased (0 nmol/l to 750 nmol/l) and organic carbon such as acetate is low (180 nmol/l) the model predicts a community structure similar to that measured using metaomic approaches at the JB site in which the beta-proteobacteria class (in particular *T. denitrificans*) are numerically dominant over the other bacterial classes (increasing to ~43%).

Overall, the community model presented here captures, at least in part, the microbial community structure that was observed using metaomic approaches at RIFRC sites and provides an important framework for continued community modeling development. The model as created here is capable of predicting the response of the community structure in changing environments such as anoxic/oxic conditions or limitations by carbon or nutrients which is critical to understanding carbon and energy flows in an ecosystem leading to improved predictions that can be used to design more efficient remediation and management strategies and better understand the implications of environmental perturbations such as climate change.

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