

## **ENIGMA: Metabolomics and Transcriptomics for Environmental Systems Biology: Molecular Mechanisms of Reduced Sulfur Inhibition of Field-Isolated Nitrate-Reducing Bacterium**

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**Project Goals:** ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

This work explores the field observation of exclusion of nitrate and sulfate reducing bacteria on the molecular level in the field isolate *Intrasporangium calvum* while demonstrating the incorporation of multi-omic monitoring in environmental systems.

### **Abstract:**

The mutual exclusion of sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) from discrete depths was observed in a sediment bore hole experiment based on the taxa and the respiration activity measured at the Oak Ridge Field Research Center (FRC) (Pilot 2017 experiment). Targeted isolations were carried out from various regions of the FRC, and a nitrate-reducing bacterium that aligned with *Intrasporangium calvum* was isolated. Characterization of growth of the isolate on nitrate-reducing conditions noted growth inhibition when reduced-sulfur containing molecules were added to the medium. Both cysteine and sulfide inhibited growth, but with distinct phenotypes. The mechanisms of this growth inhibition were investigated using various physiological assays coupled with transcriptomics and metabolomics. Data analysis is still in progress, but we currently have one proposed mechanism. From global pathway mapping of overlaid metabolomics and transcriptomics data, we saw that branched chain amino acid biosynthesis was downregulated in the cysteine inhibited samples. To confirm that cysteine inhibition of branched chain amino acid biosynthesis was responsible for the cysteine samples growth phenotype, we performed a feed-in experiment with branched-chain amino acids and found the addition rescued the growth inhibition phenotype. We are continuing experiments in order to test other observed dysregulated pathways (genes and metabolites) based on transcriptomics and metabolomics data. The data are revealing on a molecular level how the end-products of SRB respiration inhibit and therefore exclude NRB in a field-relevant bacterium (non-model organism system). In other ENIGMA posters presented here, we are using reactor studies for larger scale view of these processes (see Hunt et al. 'Dissecting microbial nitrogen cycling in the subsurface using tailored reactor schemes' and 'Using in-field bioreactors to monitor microbial community dynamic shifts with geochemical perturbations' by Wilpiseski et al.).