

Investigating Microbial Ecophysiology of Aerobic and Anaerobic Microbes Through the ENIGMA Environmental Atlas

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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.

Microbes have diverse and innovative metabolisms that allow for the transformation of carbon and other geochemically relevant elements in the environment. Understanding how these metabolisms intimately link microbes to their habitats is critical for understanding geochemical processes in the environment. As part of ENIGMA, we take an integrated approach to investigate microbes catalyzing key ecophysiological processes at the Oak Ridge Reservation Field Research Center (ORR-FRC) including diverse cultivation strategies to obtain new isolates and supplement our increasing strain repository, genomic characterization of newly obtained isolates to inform metabolic poise, and physiological characterization to confirm metabolic functions *in vivo*.

Here, we highlight two ENIGMA Environmental Atlas projects that exemplify our research goals and interdisciplinary approaches. First, we present an analysis of seven distinct strains of a single genus, *Arthrobacter*, isolated from varying depths of a single ORR FRC sampling location and adjacent groundwater well, linking niche ecotype to genotype and phenotype. *Arthrobacter* are a highly prevalent aerobic subsurface bacterial genus known for complex carbon metabolisms, and are abundantly present at ORR-FRC. Through analysis of circularized high-quality genomes and laboratory phenotyping, we were able to group these isolates into several ecotypes that demonstrate functional and genomic capacities related to carbon degradation that are specific to their biogeochemical conditions of origin, and that genes found in plasmids and predicted genomic islands support several of these functions. We then performed a pangenome analysis to compare the genomic diversity of our strains to other environmental *Arthrobacter* isolates from diverse environments. These data suggest that volatile accessory genomes of *Arthrobacter* sp. likely contribute to the genus' high potential for adaptability in the environment.

Second, we report on our efforts towards understanding the influence that minerals have on anaerobic microbial communities that impact carbon transformations in the subsurface. Methanogenic archaea in the

subsurface tend to be outcompeted for electron donors by other anaerobes such as sulfate-reducing bacteria. However, geochemical analysis of sediment cores collected from ORR FRC suggest the coexistence of methanogenesis and sulfate reduction within similar depth horizons, where semiconductive iron minerals were also abundant. We hypothesized that iron minerals may serve as an electron conduit that supports methanogenesis through extracellular electron transport despite the presence of competing sulfate-reducing bacteria. Amending ORR FRC sediments with iron minerals ferrihydrite and hematite using butyrate as the primary electron donor, we observed that the presence of hematite stimulated both methanogenesis and sulfate reduction whereas ferrihydrite only stimulated sulfate reduction. We posit that sulfide-derived electrons are being transferred to methanogens through hematite mineral conduits. Future work examining both the composition of the microbial communities *via* 16S analysis as well as the redox and coordination state of the iron minerals using synchrotron-based methods will help us better understand how conductive minerals aid in sustaining complex and competing microbial metabolisms in nature. These projects demonstrate two examples of the research performed with aerobic and anaerobic bacteria from ORR-FRC under the aims of the ENIGMA Environmental Atlas.

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