

## **Dynamics of nitrate reduction through process partitioning in a synthetic community**

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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. Denitrification is presumed to be a major anaerobic microbial process in the low-oxygen zones of the contaminated subsurface of the Oak Ridge Field Research Center (FRC) due to the high concentrations of nitrate. To simulate in the laboratory the exchange of nitrogen intermediates presumed to be occurring in this and other subsurface environments, we established a denitrifying co-culture composed of isolates from this field site. This co-culture provides a controlled system to help inform the ecological, energetic, and functional significance of denitrification pathway complementation. In this and future work, this model system is being utilized to address fundamental questions regarding the importance of nitrogen intermediate exchange in denitrifying populations. Key questions include the relevance of this type of metabolic specialization to population dynamics, community assembly, gene expression, resource partitioning, and system resiliency.**

### **Abstract:**

Perturbations, whether from natural phenomena or anthropogenic pollutants, can have severe effects on community function and ecosystem health. In polluted environments, such as the Fields Research Center (FRC), changes in community state can disrupt the cycling of nutrients like nitrogen and lead to the production of the greenhouse gases such as nitrous oxide. Here we highlight efforts to characterize the physiology of a nitrate reducing synthetic community and the environmental implications of pathway partitioning in the context of community function and resilience. We show that partitioning of the denitrification pathway into smaller functional units appears to be a common strategy among organisms isolated from diverse environments. We also show how two particular isolates obtained from the FRC, *Rhodanobacter R12* and *Acidovorax 3H11*, function independently and as a community in nitrate reducing conditions. Independently,

neither organism can completely reduce nitrate to nitrogen gas but together they complement one another to perform complete denitrification. Using kinetic models, transcriptomics, and estimates of community relative abundance we show how *Rhodanobacter R12* and *Acidovorax 3H11* are able to cooperate to enhance community growth and perform complete denitrification. We highlight how each organism responds to variations in nitrate concentration and the role of nitrite in modulating species growth. We also highlight efforts to develop and refine metabolic models for each organism and the insights these models provide. This model community for bidirectional metabolite exchange is now being used as a basis for evaluating biotic and abiotic controls of denitrification in the contaminated subsurface of the Oak Ridge FRC.

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