Investigating the abiotic control of denitrification processes using synthetic communities and laboratory simulations

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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 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. Thus, ENIGMA has been organized into several campaigns involving multiple institutes with varying expertise. *Here we describe an overarching goal of the Environmental Simulations and Modeling Campaign to simulate, model, predict, and characterize mechanistic underpinnings of* N_2O *emissions in varying ecological contexts (pH, metal availability, oxygen, etc.) using field isolates assembled into synthetic communities.*

Abstract: (Please limit to 2 pages.)

The Field Research Center (FRC) at Oak Ridge, TN has some of the highest subsurface nitrate concentrations [>10g/L] ever recorded. This concerningly large pool of subsurface nitrate, which is a remnant of legacy activities, can end up as the greenhouse gas nitrous oxide (N_2O) via incomplete denitrification or as nitrogen gas (N_2) when completely denitrified. It is critical to understand the environmental drivers that favor either complete denitrification (N_2 emission) or incomplete denitrification (e.g., N_2O emission) in the subsurface so that models can predict the fate of excess nitrate. For instance, at the FRC we have observed that wells with a pH below neutral and high nitrate levels emit large amounts of N_2O. In addition, monitoring wells after rainfall events revealed a sudden decline in pH up to 1.5 units over a matter of hours. We therefore hypothesized that if the process of complete denitrification is partitioned among multiple organisms (i.e., incomplete denitrifiers), this coupled reaction is subject to disruption by abiotic factors that may lead to increased N_2O off-gassing. To test this hypothesis, we have established a synthetic community (SynCom) of two field isolates --*Rhodanobacter sp*. R12 and *Acidovorax sp*. 3H11-- which together can perform complete denitrification but cannot independently. Therefore, we have generated a cross-campaign initiative to elucidate different mechanisms of abiotic control including pH shifts, microaerobic environments, and metal availability. Using time course experiments we determined that a shift in pH from neutral pH 7 to pH 6 is enough to decouple the complete denitrification process of the SynCom resulting in

significant increases in N_2O emissions. Transcriptome analysis of the SynCom at differing pH conditions, suggest dynamic changes in community composition and physiological states. Transmission electron microscope images suggest very different morphologies between the two field isolates that may play an important role in carbon, nitrogen, and phosphorus fluxes between the organisms. Current experiments are focused on shifts in pH at differing C/N ratios, oxygen, and metal availability (e.g., Ni) that can shift complete denitrification to incomplete. Insights from these studies can be utilized to define reaction terms for predictive modeling at the field site.

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