The Development, Progress, and Cross-Campaign Investigation of the Abiotic Influences on Denitrification Processes Partitioned Between Synthetic Communities

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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 Modelling Campaign to simulate, model, predict, and characterize mechanistic underpinnings of N_2O emissions in varying ecological contexts of shifts in pH, oxygen, metal availability and community organization.* Insights from these studies will inform the efforts of additional campaigns when performing field studies to test mechanistic hypotheses that may explain observed field phenomenon.

Abstract:

Legacy activities at the Field Research Center (FRC) at Oak Ridge, TN has led to some of the highest recorded subsurface nitrate concentrations [>10g/L] on record. This concerningly large pool of subsurface nitrate is often reduced to the greenhouse gas nitrous oxide (N_2O) via denitrification or from dissimilatory nitrate reduction to ammonia (DNRA), which eventually can be converted to N_2O from a variety of microbial processes. It is important to understand the environmental conditions that favor complete denitrification (N_2 emission) or incomplete denitrification (e.g., N_2O emission) in the subsurface. 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 a rainfall events revealed a sudden decline in pH up to 1.5 units over a matter of hours. This phenomenon has been observed in other soil environments, and during such perturbations, denitrification processes may be stimulated, leading to the increased production of N_2O and N_2. We therefore, hypothesized that if the process of complete denitrification is partitioned among incomplete denitrifiers, it is subject to specific abiotic influence that may lead to increased N_2O off-gassing. To test this hypothesis, we have organized a cross-campaign effort to elucidate different mechanisms of abiotic control, in particular pH shifts, C/N ratios, microaerobic environments, and metal availability. Here we have established a synthetic community (SynCom) of two field isolates --*Rhodanobacter sp*. R12 and *Acidovorax sp*. 3H11-- which together can perform complete denitrification. 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. By

analyzing transcriptional profiles, we aim to model, predict, and characterize mechanistic underpinnings of N_2O emissions in varying ecological contexts. Ongoing experiments are focused on additional environmental controls such as, shifts in pH at differing C/N ratios, oxygen, and metal availability (e.g., Ni). Insights from these studies will inform field studies that may validate model-driven hypotheses.

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