High nitrous oxide emissions from a nitrate contaminated subsurface indicate significant metabolic activity.

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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.

Linking field observations with laboratory studies and vice versa is essential for advancing predictive understanding of environmental systems and for stewardship of those systems. However, some environments that provide critical ecosystem services, such as the subsurface and groundwater systems, are extremely difficult to sample and monitor in real-time, and doing so is both expensive and invasive. Thus, nondestructive approaches to process analyses are essential tools for connecting lab and environment. To this end, we are developing protocols that use the flux and isotopic composition of the greenhouse gas nitrous oxide to resolve alternative biotic (e.g., nitrification and denitrification) and abiotic processes, such as iron catalyzed reduction of nitrite, that contribute to its emission from the subsurface. ENIGMA has developed a program to connect measurements of nitrous oxide to biologically mediated processes through field observations (For more information on ENIGMA field observations see "Spatiotemporal Dynamics of Groundwater and Sediment: Geochemistry, Microbial Communities and Activities in a Contaminated Aquifer" by Walker et. al.) and laboratory simulations (For more information on ENIGMA lab to field plan see "A Multi-Laboratory Effort to Use Synthetic Communities to Discover, Characterize, and Dissect Key Microbial Processes Relevant to Field Observations" by Valenzuela et. al.), thereby establishing a noninvasive metric for quantifying activity without destructive sampling.

Contamination by nitrogen species is a concern in many terrestrial and aquatic environments impacted by past and current human activities, including release associated with intensive agriculture and industrial activity, and from wastewater treatment. This contamination has been shown to lead to altered plant, animal, and microbial communities and to increased production of the greenhouse gas nitrous oxide, primarily through either nitrification or denitrification. The subsurface of the Field Research Center (FRC), near Oak Ridge National Lab in Tennessee, has been contaminated with low pH (3-7), heavy metal laden nitrate (~10 g/l) for decades. To understand how this contamination has influenced subsurface processes we are investigating environmental variables influencing nitrous oxide emissions, considering both biotic and abiotic contributions to this important greenhouse gas. By using flux analysis and isotopic

characterization of nitrous oxide, combined with complementary molecular and chemical characterization of multiple observation wells, we anticipate developing a more predictive understanding of the controlling variables. Current analyses are focused on wells positioned at different depths and spanning a range of pH, nitrate contamination, and metals contamination to resolve biotic and abiotic sources of production and to identify controlling environmental variables. Initial data sets have revealed that subsurface fluxes are orders of magnitude higher than those observed in other systems, including agricultural soils and the marine oxygen minimal zone. In contrast, the observed surface fluxes are in the range observed for other sites, indicating additional consumptive processes within the vadose zone that mitigate surface emissions.

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