

Resolving Biotic and Abiotic Controls of Nitrous Oxide Flux in a Subsurface Site Contaminated with High Nitrate Concentrations.

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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.

Abstract:

Linking field observations with laboratory studies, and vice versa, is essential for advancing predictive understanding of environmental systems and for stewardship of those systems. We are developing tools and identifying systems that capture phenomena observed in the field in a reproducible, minimally disruptive, and dissectible manner (see poster by Smith *et. al.*). In turn, those observations serve to direct more refined laboratory studies designed to more completely resolve the roles of microbial ecology and abiotic processes in observable systems-level processes (see poster by Valenzuela *et. al.*).

Microbial activity in the field can be a challenge to quantify due to spatial and temporal heterogeneity and the cost/methodological constraints of real time observations without perturbation. Stable isotopic analysis of biogeochemically active substrates and products can circumvent some of these limitations. The field research center (FRC) at Oak Ridge, TN contains a site that has been contaminated with low pH (3-7), heavy metal laden nitrate (~10 g/l) for decades from historical activities. To understand the microbial processes of this site, we analyzed stable isotopes of ground water as well as dissolved nitrate and nitrous oxide collected from a total of 27 different wells over 3 different areas. These isotopic analyses are well suited for this investigation given the strong process signals (denitrification vs nitrification vs dilution) imprinted on $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of different nitrogen species. Previous measurements of nitrous oxide flux indicated major subsurface production of nitrous oxide, which are supported here by $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and site preference of nitrous oxide samples analyzed from groundwater. Analyses revealed a variable distribution of activity across the site (horizontally and with depth) and implicated both denitrification and chemodenitrification in nitrous oxide production. These data are being contextualized by complementary molecular, biological, and chemical characterization of groundwater and sediment traps recovered from the 27-well survey, together contributing to a framework for developing a more predictive understanding of biotic and abiotic controls of local and systems-level processes.

High concentrations and fluxes of nitrous oxide measured in the subsurface without associated surface emissions indicated significant microbial activity driven by both the production and consumption of this high energy electron acceptor. Two populations of nitrous oxide reducers (clade I and II) were observed to be stratified with depth, a distribution suggested to be controlled by either nitrous oxide concentration

or response to inhibitory factors, including competition for oxygen. To understand these processes and populations, nitrous oxide reducers are being isolated and will provide field relevant kinetic and physiological parameters to evaluate the roles of the activities and variable distribution of clade I and II nitrous oxide reducers in controlling nitrous oxide emissions from the subsurface (see poster by Gushgari-Doyle *et. al.*).

This material by ENIGMA- Ecosystems and Networks Integrated with Genes and Molecular Assemblies a Science Focus Area Program at Lawrence Berkeley National Laboratory is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological & Environmental Research under contract number DE-AC02-05CH11231