A Multi-Laboratory Effort to Use Synthetic Communities to Discover, Characterize, and Dissect Key Microbial Processes Relevant to Field Observations

J.J. Valenzuela[1]*(jvalenzu@isbscience.org), A.V. Carr[1], J.A. Wilson[1], K. Hunt[2], H.J. Smith[3], F.L. Poole[4], X. Ge[4], C.M. Gionfriddo[5], R.L. Wilpiszeski[5], V. Li[6], A. M. Deutschbauer[6], T. R. Northen[6], M. W.W. Adams[4], R. Chakraborty[6], D. A. Elias[5], D. A. Stahl[2], M. W. Fields[3], G. E. Siuzdak[7], **N. S. Baliga[1,2], A. P. Arkin[6,8]** and **P. D. Adams[6,8]**

[1]**Institute for Systems Biology, Seattle, WA**; [2]University of Washington, Seattle, WA; [3]Montana State University, Bozeman, MT; [4]University of Georgia, Athens, GA; [5]Oak Ridge National Lab, Oak Ridge, TN; [6]Lawrence Berkeley National Lab, Berkeley CA; [7]Scripps Research Institute, La Jolla, CA; [8]University of California at Berkeley, CA

http://enigma.lbl.gov

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 the core efforts of the Environmental Simulation and Modeling campaign (EnvSim) to establish synthetic communities to discover, characterize, and dissect key microbial processes relevant to field observations, in particular as they relate to denitrification and the emission of the greenhouse gas nitrous oxide (N_2O).*

The Environmental Simulation and Modeling campaign (EnvSim) has strategically aligned efforts across ENIGMA to address two major sets of hypotheses relevant to ecologically important phenomena observed at the Oak Ridge Field Research Center (FRC). The first hypothesis, which originated from field observations, was that sulfate respiration and nitrate respiration processes operate in mutual exclusivity down the transect of a sediment core. Using the field isolate (*Intrasporangium calvum* C5) this phenomenon was simulated and the mechanistic underpinnings delineated through laboratory investigations. Insights from this study are currently being tested back at the FRC using groundwater chemostats to demonstrate the "field-to-lab-to-field" iteration model employed across ENIGMA for investigating a phenomenon that emerged through the investigations of complex interactions within a microbial community. The second set of hypotheses being tested by the EnvSim campaign involve the interplay of biotic and abiotic factors that lead to N_2O emissions at the FRC. It has been shown that in FRC wells which have a lower pH (~6.5 - 3) tend to have higher concentrations of N_2O. The EnvSim campaign has deduced four potential mechanisms that may account for the N_2O emissions at the FRC and are currently being investigated by labs across ENIGMA. Denitrification at the FRC[1] may be driven by complete denitrifiers, however, their NosZ enzymes, which catalyze the final step in denitrification by converting N_2O to N_2, may be sensitive at lower pHs. Additionally, the denitrification process could be partitioned among organisms and some may have pH-sensitive NosZ genes. Another factor contributing to variable N_2O emissions relates to the metal co-factors involved in the denitrification pathway[2,3]. For instance, excess Cu, Al, Mn, U, Ni, Co, Cu, and/or Cd may have inhibitory effects on multiple enzymatic steps during denitrification, while the enzymatic production of nitrite, the precursor of N_2O, can be limited by the essential metal Mo. Lastly, abiotic production of N_2O may occur as a result of chemodenitrification, in which metals like Fe, Mn, and some organic compounds can drive redox reactions that convert nitrogen cycle intermediates to N_2O under the right conditions[4].

Here, we describe an important subset of ongoing collaborative projects that are dissecting the above hypotheses. We highlight the complementary nature of each project and how the use of different reactor systems answer distinct questions, which provides important information and data that can be funneled into our predictive models. For instance, we are using column reactors and highly controlled, constantly stirred, planktonic reactors to study how microbial community structure and function changes in response to pH shifts, oxygen concentrations, and sulfurous compounds. Batch culture experiments are being used to study the abiotic and biotic impacts of metals on denitrification processes. In addition, transcriptomic analysis of these experiments are aiding the construction of a metabolic and gene regulatory network model. Importantly, the work being showcased illustrates the field-to-lab and lab-to-field framework of the ENIGMA campaign strategy.

References

1. Alvarez, L., Bricio, C., Blesa, A., Hidalgo, A. and Berenguer, J., 2014. Transferable denitrification capability of Thermus thermophilus. Appl. Environ. Microbiol., 80(1), pp.19-28.
2. Glass, J. and Orphan, V.J., 2012. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. Frontiers in microbiology, 3, p.61.
3. Tavares, P., Pereira, A.S., Moura, J.J.G. and Moura, I., 2006. Metalloenzymes of the denitrification pathway. Journal of inorganic biochemistry, 100(12), pp.2087-2100.
4. Zhu-Barker, X., Cavazos, A.R., Ostrom, N.E., Horwath, W.R. and Glass, J.B., 2015. The importance of abiotic reactions for nitrous oxide production. Biogeochemistry, 126(3), pp.251-267.

This material by ENIGMA- Ecosystems and Networks Integrated with Genes and Molecular Assemblies a Scientific 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