

## Communal Metabolism of Methane and the Rare Earth Element Switch

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**Project Goals: This project addresses the structure and function of microbial communities active in methane consumption, using lake sediment as a model. Through manipulations of synthetic communities and systems biology approaches, we are striving to understand the molecular mechanisms that form a basis for specific interspecies interactions in microbial oxidation of methane. In this phase of the project our goals are 1) Evaluate behavior of multispecies model synthetic communities comprised of major functional guilds defined in prior research, and assess performance of these communities; 2) Determine the metabolic networks governing microbial consortia, through identification of specific enzymes/ pathways/ factors involved in interspecies interactions; 3) Apply machine learning for predictive modeling of community function.**

Metabolism of methane is an important part of biogeochemical cycling of carbon. Methane is also a major contributor to climate change. A specialized group of microbes (the methanotrophs) that consume methane, gaining both energy and carbon from this chemically inert compound, represent a natural filter preventing an even faster accumulation of methane in the atmosphere. While methanotrophy has been studied for the past hundred years as a metabolic feature of individual pure cultures, a concept of communal function in methanotrophy has been gaining momentum. However, the mechanistic details are still missing of how and why the methanotrophs share their hard-earned carbon with other species, and whether and what they gain in return. This current project, initiated in August 2016, builds upon results from prior funding by the DOE (DE-SC-0010556).

We are using Lake Washington sediment community as a model. We manipulated complex natural communities using methane as the sole source of carbon, to determine species persisting in methane-consuming communities (the top-down approach). We also built synthetic communities of pure cultures of methanotrophs and non-methanotrophs and tested their behavior under a variety of environmental conditions (the bottom-up approach). We sequenced multiple (meta)genomes and (meta)transcriptomes to gain insights into the genomic potentials and gene expression patterns in relevant microbes.

Through microcosm manipulation, using methane as the sole source of carbon, followed by metagenomic analysis, we established key species active in methane consumption as the bacteria of the family *Methylococcaceae*. We further established the primary and most abundant satellite types, the non-methanotrophic methylotrophic bacteria of the family *Methylophilaceae*. Two other persistent but less abundant types were identified as members of Burkholderiales and Flavobacteriales. Through manipulation of synthetic communities, followed by transcriptomic analysis, we identified at least one metabolic node at which community cross-talk takes place, the methanol oxidation step that involves alternative methanol dehydrogenase enzymes, one requiring calcium as a cofactor, another requiring rare earth elements (REE), one of the first demonstrations of a biological function

for this group of metals. Enzyme choice, in turn, appears to be determined by a number of environmental factors, such as oxygen and methane partial pressures, as well as sources of nitrogen.

We conclude that methanol must be the major carbon compound that the methanotrophs share with other community members, and that carbon flow is regulated by the REE switch, presenting an unexpected and unprecedented example for the important role of REEs in complex biological systems. Overall, our data shed new light on social lives of microbes involved in metabolism of methane in natural habitats and highlight some of the metabolic links among the community partners.

### **Publications**

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