

## **Novel genomes reveal carbon cycling potential of microbes living in an Archean iron formation**

CODY S. SHEIK<sup>1</sup>, JONANTHAN P. BADALAMENTI<sup>2</sup>, DANIEL R. BOND<sup>2,3</sup>, JEFFERY A. GRALNICK<sup>2,3</sup>, AND BRANDY M. TONER<sup>2,4</sup>

<sup>1</sup>Department of Biology and the Large Lakes Observatory, University of Minnesota Duluth, Duluth, MN, 55812 [cssheik@d.umn.edu]

<sup>2</sup>BioTechnology Institute, University of Minnesota, St. Paul, MN, 55108 [jbadalam@umn.edu]

<sup>3</sup>Plant and Microbial Biology, University of Minnesota, St. Paul, MN, 55108 [dbond@umn.edu, gralnack@umn.edu]

<sup>4</sup>Department of Soil, Water and Climate, University of Minnesota, St. Paul, MN, 55108 [toner@umn.edu]

The deep terrestrial biosphere is estimated to contain up to 135 Pg of carbon (C) in living microbial biomass. This subsurface microbial C pool is associated with crustal aquifers with mean residence times that span millions to billions of years and is decoupled from the rapid exchange processes occurring between the Earth surface and atmosphere. Microbes inhabiting the deep biosphere face unique challenges due to their isolation from the Earth's surface. Carbon sources, terminal electron acceptors, nutrients, and metal cofactors that are necessary to drive metabolism must be solely derived from water-rock-microbe interactions.

At the Soudan iron mine in northern Minnesota, legacy boreholes drilled circa 1960 to explore the iron formation and subsequently abandoned provide unique access to the microbial life living in the cold, highly reduced, fracture brine fluids. Total dissolved organic carbon (DOC) of these fluids is low yet several boreholes emit CH<sub>4</sub> and CO<sub>2</sub>. Previous 16S rRNA sequencing show these borehole communities are low in diversity and structure correlates with fluid chemistry. Pilot metagenomes were sequenced from three boreholes with varying redox values. After assembly, we recovered approximately thirty partial to near full genomes from each borehole. Interestingly, the primary methanogen recovered were associated with the methylotrophic methanogen genus *Methanolobus*. This suggests methane generation is driven through methylated compounds produced as metabolic byproducts other microorganisms or biomass turnover. As evident by the low DOC in Soudan brines, tight regulation of carbon cycling to methane is likely and suggests microbe-microbe and/or syntrophic interactions may be vital to efficient carbon turnover in these subsurface communities