

LINKING IRON REDUCTION TO ANAEROBIC METHANE OXIDATION IN AN ANCIENT OCEAN ANALOG. M. S. Bray<sup>1</sup>, B. C. Reed<sup>1</sup>, J. Wu<sup>1</sup>, C. B. Kretz<sup>2</sup>, F. J. Stewart<sup>1</sup>, T. J. DiChristina<sup>1</sup>, J. A. Brandes<sup>3</sup>, D. A. Fowle<sup>4</sup>, S. A. Crowe<sup>5</sup>, J. B. Glass<sup>1,2\*</sup>

<sup>1</sup>School of Biology, Georgia Institute of Technology, Atlanta, GA, USA; <sup>2</sup>School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA; <sup>3</sup>Skidaway Institute of Oceanography, Savannah, GA, USA; <sup>4</sup>Department of Geology, University of Kansas, Lawrence, KS, USA; <sup>5</sup>Department of Earth, Ocean, & Atmospheric Sciences; Department of Microbiology & Immunology, University of British Columbia, Vancouver, BC, Canada; \*jennifer.glass@eas.gatech.edu

The Archean ocean was likely O<sub>2</sub>-poor but rich in CH<sub>4</sub> and dissolved Fe(II). Methane and Fe(III), generated from photoferrotrophy or aerobic Fe(II) oxidation, could have been used by ancient microorganisms as energy sources. Modern species of bacteria and archaea that couple anaerobic oxidation of CH<sub>4</sub> (AOM) to NO<sub>x</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup> reduction, respectively, have been characterized. AOM coupled to Fe(III) reduction (Fe-AOM) is a thermodynamically favorable reaction that might support microbial growth. This reaction may have operated in the Archean oceans, and geochemical evidence suggests it plays a role in CH<sub>4</sub> oxidation in modern ecosystems. However, characterization of the microbes that perform this putative metabolism remains elusive. Isolation of extant microorganisms mediating Fe-AOM would demonstrate the reaction and its microbial mediation, and would provide insight into the possible mechanisms of ancient CH<sub>4</sub> cycling.

In this study, we attempted to enrich for microorganisms mediating Fe-AOM in sediments from Lake Matano, Indonesia, a stratified tropical lake with anoxic CH<sub>4</sub>-rich deep waters. Lake Matano sediments receive abundant Fe(III) due to erosion of lateritic soils and scarce (<100 nM) SO<sub>4</sub><sup>2-</sup> and NO<sub>x</sub><sup>-</sup>, making this environment an excellent Archean ocean analog. Fe(III)-amended Lake Matano sediments were incubated in the presence and absence of CH<sub>4</sub>. CH<sub>4</sub> stimulated Fe(III) reduction in initial incubations by 8-fold over N<sub>2</sub> controls. The dominant 16S rRNA gene sequences in CH<sub>4</sub>-amended enrichments (79, 40 and 27% in sediment layers from 0-5, 5-10 and 10-15 cm depth, respectively) were Betaproteobacteria in the Rhodocyclaceae family. Cultured species of Rhodocyclaceae perform diverse metabolisms, including respiration of O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, ClO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, SeO<sub>4</sub><sup>2-</sup> or Fe(III), and oxidation of a wide range of organic electron donors. Some aerobic Rhodocyclaceae species oxidize C1 compounds including methanol, methylamines, formate and formaldehyde, but none are known to oxidize CH<sub>4</sub>. The enrichment with highest AOM activity measured by <sup>13</sup>CH<sub>4</sub> incorporation into dissolved inorganic carbon was dominated by 16S rRNA gene sequences with highest similarity to the ClO<sub>4</sub><sup>-</sup>-reducing bacterium *Azospira suillum* strain PS. Ongoing work is focused on culture isolation and characterization by coupling metagenomics, metatranscriptomics and microscopy.