Shifting microbial communities sustain multi-year iron reduction and methanogenesis in ferruginous sediment incubations.

M. S. Bray[1], J. Wu[1], B. C. Reed[1], C. B. Kretz[2], R. L. Simister[3], C. Henny[4], F. J. Stewart[1,2], T. J. DiChristina[1,2], J. A. Brandes[5], D. A. Fowle[6], S. A. Crowe[3], J. B. Glass[1,2#]. [1]School of Biology; [2]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, 30332, USA.[3]Departments of Microbiology & Immunology and Earth, Ocean, & Atmospheric Sciences, University of British Columbia, Vancouver, BC, Canada. [4]Research Center for Limnology, Indonesian Institute of Sciences, Cibinong, Indonesia. [5]Skidaway Institute of Oceanography, Savannah, GA, 31411, USA. [6]Department of Geology, University of Kansas, Lawrence, KS, 66045, USA. [#]Correspondence: Jennifer.Glass@eas.gatech.edu

Introduction: Decreased solar luminosity during the Archean is thought to have been remedied by abundant greenhouse gases in the atmosphere, in particular methane (CH_4). In addition, ferruginous conditions in the Archean ocean likely led to significant deposition of solid Fe(III) to sediments. Reactive Fe(III) minerals can influence CH_4 emissions by inhibiting microbial methanogenesis or by stimulating anaerobic CH_4 oxidation. The balance between Fe(III) reduction, methanogenesis, and methane oxidation in ferruginous Archean oceans would have controlled CH_4 fluxes to the atmosphere, thereby regulating the capacity for CH_4 to warm the early Earth under the Faint Young Sun. In this study, we examined CH_4 and Fe cycling in anoxic incubations of ferruginous sediment from the Archean ocean analogue Lake Matano, Indonesia.

Study Site: Lake Matano, Indonesia is one of the only modern analogues for ferruginous Archean oceans. Despite the presence of Fe(III) oxides, CH_4 accumulates to 1.4 mM in anoxic deep waters of the lake [1]. Methanotrophy is a key carbon fixation process in Lake Matano's oxic-anoxic transition zone, and the dearth of other oxidants (<100 nM nitrate and sulfate) suggests that Fe(III) might be the primary electron acceptor in methanotrophy [2,3].

Methods: Anoxic incubations of Lake Matano sediments were sampled over three successive transfers (500 days total). Incubations contained CH_4 headspace, and 10 mM ferrihydrite or goethite. Iron reduction and methanogenesis were measured throughout the incubation by ferrozine and gas chromatography, respectively. In addition, incorporation of $^{13}C\text{-}CH_4$ into the DIC pool was measured as a proxy for CH_4 oxidation. Amplicon sequencing of the 16S rRNA gene was used to track microbial community changes over time.

Results: After three dilutions, Fe^{3+} reduction persisted only in bottles with ferrihydrite. Methanogenesis continued in the presence of goethite, suggesting that reactive Fe(III)-oxides (ferrihydrite) inhibit methanogenesis (Fig. 1). Methane oxidation was observed throughout the incubations, but the stoichiometry of CH_4 oxidized to Fe^{3+} reduced suggested that oxidation was linked to additional electron acceptors besides Fe^{3+}. Microbial diversity decreased markedly over the course of incubation with ultimate dominance of unclassified members of the order Desulfuromonadales in all treatments, and Rhodocyclaceae in treatments amended with CH_4.

Implications: These results suggest that Fe(III)-oxide mineralogy and availability of electron donors could have led to spatial separation of Fe(III)-reducing and methanogenic microbial communities in ancient ferruginous marine sediments. Methanogenic microbial communities could have persisted in sediments dominated by goethite or other crystalline Fe(III)-oxides while ferrihydrite would have allowed Fe(III)-reducing communities to outcompete methanogenic ones for a limited electron donor supply.

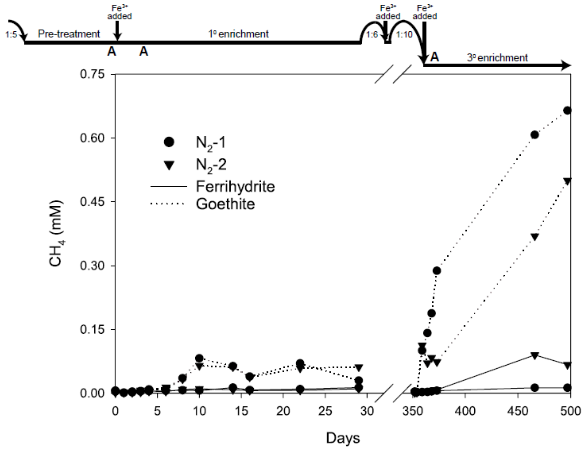

Figure 1. Accumulation of CH_4 in the headspace of sediment enrichments. Timeline at top shows transfer dates and dilution ratios. All treatments were run in duplicate.

References: [1] Crowe, S.A., et al. (2008) *Limnology and Oceanography*, 53, 319-331. [2] Crowe, S., et al., (2011) *Geobiology*, 9, 61-78. [3] Sturm, A, et al. (2016) *Biogeosciences Dicussions, 1-34*

This research was funded by NASA Exobiology grant NNX14AJ87G and supported by the NASA Astrobiology Institute (NNA15BB03A). SAC was supported through NSERC CRC, CFI, and Discovery grants.