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\(^1\), C. Henny\(^3\), F. J. Stewart\(^1\), T. J. DiChristina\(^1\), J. A. Brandes\(^5\), D. A. Fowlie\(^6\), S. A. Crowe\(^1\), 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. \(^7\)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-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.](image)


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