Objectives

The overall goal of this project was to generate high-resolution data for understanding patterns in microaerophilic biological methane cycling across the Archaean/Proterozoic boundary, linked to the rise of atmospheric O_2 and climate ~2.9-1.8 billion years ago (Ga). Specific aims were to reconstruct microaerophilic microbial methane oxidation kinetics and ultimately to derive empirical models for the activity of these microorganisms with the evolution of ocean chemistry. Copper geochemistry and traditional microbiological culture work was to be used to answer five key questions: 1) Does methanotrophic bacterial growth on various Cu-doped ferrihydrite minerals result in significant Cu stable isotope fractionation between biomass and mineral phases? 2) Did dissolved oceanic Cu concentrations change, following the development of oxygenated surface waters during the early stages of the Great Oxidation Event? 3) Are copper isotopes fractionated between ferrihydrite minerals and organic matter deposited in a range of Precambrian banded iron formations? 4) Do copper isotopic signatures in various iron formations correlate with methanotrophic bacterial activity? 5) Can Cu isotopic fractionation signatures be used as biomarkers for tracing the link between Precambrian atmospheric CH_4 concentrations and the rise of atmospheric O_2?

i) **Microbial culture analysis.** Various Cu-doped ferrihydrite minerals were synthesized and analyzed by powdered X-ray diffraction (XRD) for mineralogical composition. Cu/Fe molar ratios were determined using inductively coupled plasma optical emission spectrometry (ICP-OES). Two types of methanotrophs were successfully grown on these various minerals, which acted as a sole source of copper (Fig. 1a-b). Two key aspects pertinent to the project became clear. 1) The Cu/Fe molar ratios conditionally affect growth and methane oxidation rates, controlling the type of enzyme used for methane oxidation (Fig. 1c-d). Sufficient mineral-Cu favoured the expression of the di-Cu enzyme. In low Cu the di-Fe enzyme was employed for methane oxidation (Fig. 1e).

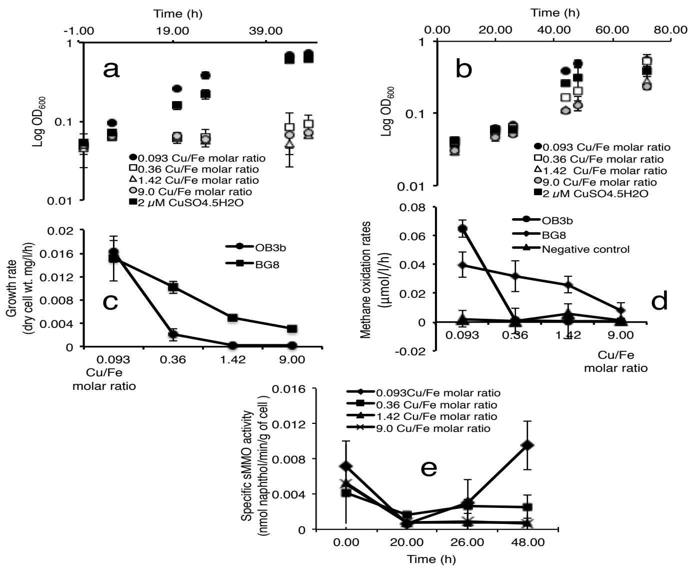

Fig. 1. The activity of two distinct methanotrophic bacteria grown on synthetic ferrihydrite minerals doped with varying Cu/Fe molar ratios. ***a**, Growth curve for the type II di-Fe-enzyme containing* Methylosinus trichosporium *OB3b.* ***b**, Growth curve for the type I di-Cu-dependent* Methylomicrobium album *BG8.* ***c-d**, Growth and CH_4 oxidation rates for* M. album *BG8 and* M. trichosporium *OB3b.* ***e**, sMMO (di-Fe enzyme) activity during type II growth.*

2) Cells fractionate copper during methane oxidation. The lighter copper isotopes are associated with biomass and heavy copper enriched in the residual ferrihydrite mineral. These fractionation patterns were proportional to growth and methane oxidation rates.

ii) **Rock analysis:** Copper distribution in shale samples deposited between 2.6 and 1.6 Ga, indicated that oceanic copper concentrations might have increased dramatically after the great oxidation event (GOE), at ~2.45 Ga. This was followed by a dramatic decline at ~2.1 Ga, towards pre-GOE levels (Fig. 2a). The Cu trends were suggested to reflect patterns in organic matter deposition through the same time interval (Fig. 2b-c). Together with records in banded iron formations, deposited 3.7-0.6 billion years ago (inset, Fig. 2), a three-stage change in the evolution of Cu in the Archaean/Proterozoic oceans could be inferred.

Potential impact of results: Potential autotrophic pathways that would likely dominate in a methane-rich atmosphere, as was the case in the early atmosphere, especially before the rise of atmospheric oxygen, was studied. We found that pathways favouring Cu-dependent methane oxidation likely were important for organic carbon deposition (Fig. 3a-b). Combining this information with the results gained on the growth of the various archetypical methanotrophs on different Cu/Fe molar ratios and the Cu/Fe molar ratios measured for the geological materials, including those reported in the public literature for banded ironstones deposited 3.0-1.6 billion years ago, three key scenarios arose.

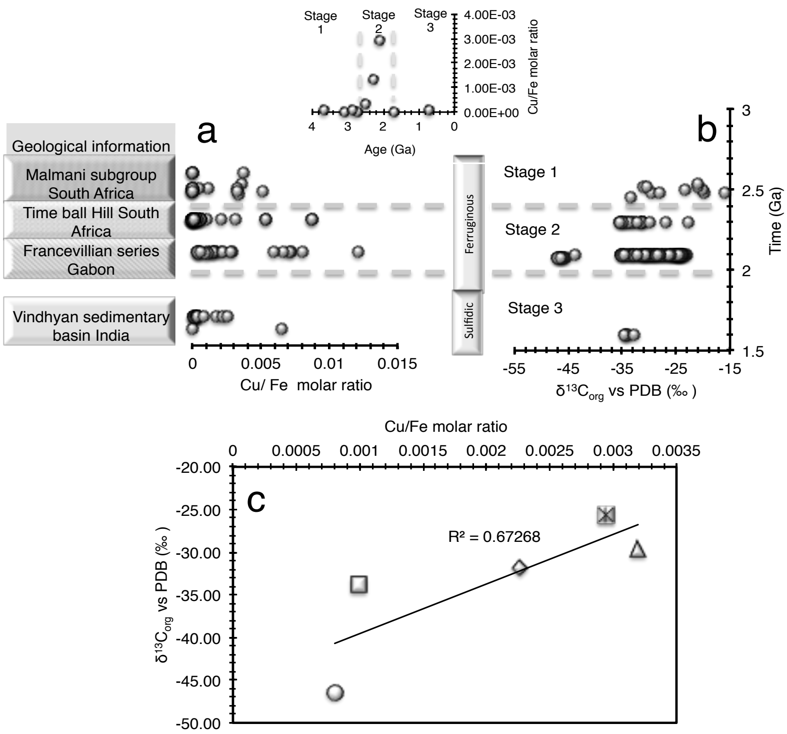

*Fig. 2. Established rrelationship between Cu/Fe molar ratios, time (a), and $\delta^{13}C_{org}$ stable-isotopic records (b), in black shales deposited ~2.6-1.65 billion years ago. Inset represents averages of Cu/Fe molar ratios inferred from 238 data points reported in the public literature for marine banded iron formations and ironstones deposited between 3.7-0.6 Ga **c**, averaged Cu/Fe molar ratios vs. averaged $\delta^{13}C_{org}$. Ringed, ~2,084. Squared, ~1.65 Ga. Diamond, ~2.3 Ga. Starred, square ~2.5 Ga. Triangle, ~2.1 Ga. The Cu/Fe molar ratio is considered because changes in Fe levels over time, a predominant metal in the Archaean ferruginous oceans and through much of the Proterozoic, would have influenced Cu and life as a whole*

1) Methanotrophic bacteria activity likely increased substantially at the onset of the GOE, coupled to increased copper bioavailability. 2) This activity likely peaked at ~2.1 Ga (Fig. 3c). 3) They were active from the start to the end of the Palaeoproterozoic glaciations, suggesting a role in the regulation of both atmospheric methane concentrations, facilitation of the rise of atmospheric oxygen and dramatic fluctuations in the Palaeoclimate

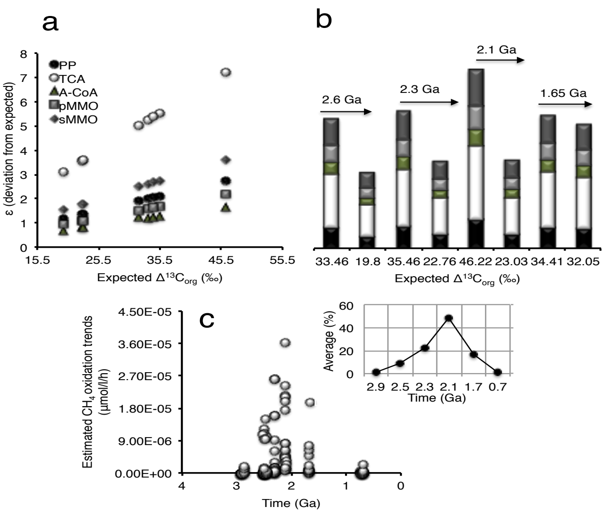

*Fig. 3. Predicted Precambrian autotrophic carbon fixation and MMOX trends reconstructed from $\delta^{13}C_{org}$ shale records and published $\delta^{13}C_{org}$ fractionation patterns for modern reference microbial processes. **a**, Predicted deviation trends for key autotrophic carbon fixation pathways calculated for $\delta^{13}C_{org}$, 2.6-1.65 Ga. **b**, Proportions for the highest and lowest $\Delta^{13}C$-values measured at each time point. It is assumed that if the $\delta^{13}C$ isotopic composition at anytime was influenced by a certain carbon fixation pathway, there will be a smaller deviation from the reference (modern value). If not, a wider deviation will be observed. **c**, potential trends for a Cu-dependent methanotroph living in a Precambrian ocean, calculated from a standard curve of CH_4 oxidation rates in growth experiments vs. different Cu/Fe molar ratios. The slope of the curve (R^2=0.9) was used to extrapolate CH_4 oxidation trends from Cu/Fe molar ratios reported in the public literature for banded ironstones. Insert is averaged CH_4 oxidation trends (%) through time. PP: pentose phosphate. TCA: reductive tricarboxylic acid cycle. A-CoA: Reductive acetyl CoA. sMMO &*