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## The Identification of Sulfide Oxidation as a Potential Metabolism Driving Primary Production on Late Noachian Mars.

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**Introduction:** The surface of Mars cannot sustain liquid water today, but there is evidence for the extended presence of liquid water during the Noachian era [1-2]. The transition of the martian climate from the wet Noachian to the dry, late Hesperian would have resulted in saline and sulfur-rich surface waters [1-4]. Terrestrial analogue environments that possess a similar chemistry to these proposed waters can be used to develop an understanding of the diversity of organisms that could have persisted under such conditions. Combining this with laboratory simulation experiments, which enable a greater level of accuracy regarding the chemical environment, allows for concepts regarding diversity and function to be developed.

Here we present the chemistry and microbial community of the highly reducing sediment of the springs of Colour Peak, a sulfidic and saline spring system located within the Canadian High Arctic [2]. We also present details of the viability of this microbial community when grown in defined, simulated martian fluid chemistries based on the chemistry of Rocknest at Gale crater in combination with basaltic and iron enriched martian simulants.

**Methodology:** In this study, the elemental composition of the fluids and sediment porewater of Colour Peak was determined by ICP-OES. This data was compared with a range of fluid chemistries, including those from other analogue environments and martian brines, the composition of which were determined based on the chemistry of the “Rocknest” sand sample at Yellowknife Bay, Gale crater (Mars) by thermochemical modelling [5]. The fluid chemistry derived from the thermochemical modelling was used to calculate Gibbs energy values to identify metabolic pathways that could be energetically feasible. Molecular techniques were also used to investigate the microbial community of the sediment of the Colour Peak Springs. Both DNA and RNA were extracted from the microbes in the sediment using a novel extraction technique that was developed to overcome issues associated with low biomass and the high concentrations of inhibitory substances (e.g. salt and sulfide). The microbial community was characterised by the amplification and sequencing of 16S rRNA gene amplicons produced from the extracted nucleic acids. The ability of the microbial community to grow under defined martian chemical conditions was tested using the modelled fluid chemistry in combination with simulants repre-

sentative of either a general martian chemistry (OUCM-1, also based on the chemistry of Rocknest) or an iron enriched chemistry (OUHR-1, based on the chemistry of Haematite Slope) [6]. Enrichments were incubated at 10 °C for 28 days with a headspace of H<sub>2</sub>/CO<sub>2</sub> (80:20) at one bar pressure. Growth was monitored using microscopy with Live/Dead staining and the enriched communities were characterised by sequencing of 16S rRNA gene amplicons produced from the extracted DNA.

**Results and Discussion:** Analysis of the chemistries of the Colour Peak fluids confirmed a chemical composition that was similar to the thermochemically modelled fluid derived from *in-situ* measurements of the chemistry of Gale crater sediments (Figure 1). This similarity in elemental composition confirms the classification of Colour Peak as an appropriate analogue environment to investigate the habitability of former martian aqueous environments.

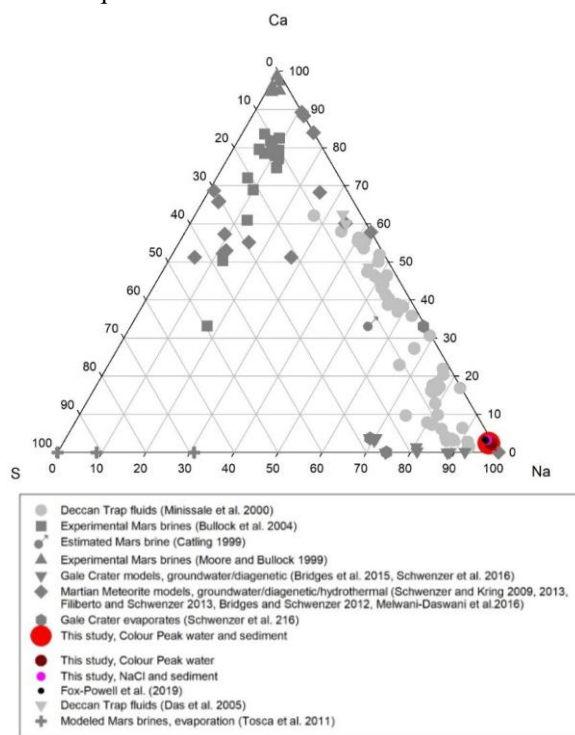


Fig. 1 Ternary plot showing the concentrations of Na, Ca and S in waters from the Colour Peak spring and sediment compared to fluids from the Deccan traps and modelled Mars brine chemistries.

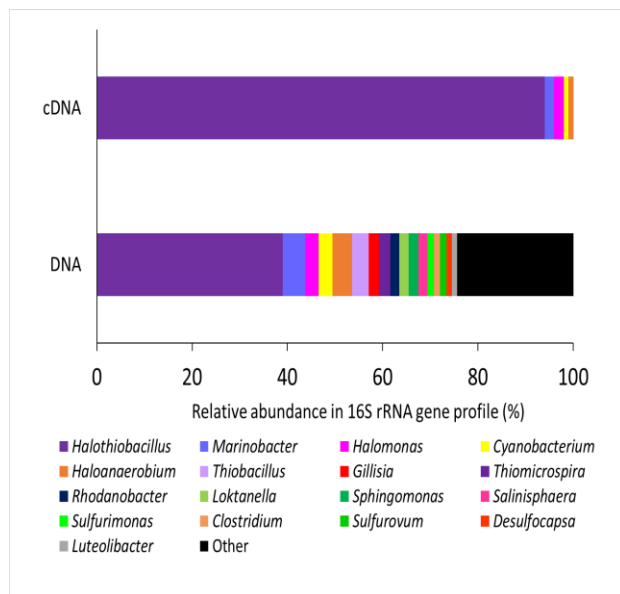


Fig. 2 16S rRNA gene and 16S rRNA community profiles of Colour Peak (CP) sediment. Sequences were revealed by amplicon sequencing of 16S rRNA gene amplicons retrieved by PCR from DNA and RNA extracted from sediment samples collected from CP. All genera pictured are present at >1% relative abundance. DNA refers to an averaged community profile of three replicate 16S rRNA gene profiles.

16S rRNA gene profiling of the Colour Peak microbial community (Figure 2) revealed it was dominated by bacteria associated with the oxidation of reduced sulfur species and the fixation of carbon dioxide (autotrophy). Gibbs energy values demonstrated that the oxidation of reduced sulfur species was a viable metabolism in this chemical environment under both oxic (using modern day concentrations of oxygen in the martian atmosphere [7]) and anoxic (denitrification-enabled [8]) conditions. The enrichments performed under simulated martian chemical conditions confirmed that the Colour Peak Spring sediment contained microbes that were viable under reconstructed martian chemical conditions. However, whilst sulfide oxidising bacteria were viable in the enrichments, sulfate reducing bacteria dominated the enriched communities.

In the unenriched community, non-autotrophic, fermentative bacteria were also detected as being active. Given the low concentration of carbon in the sediment and the persistence of bacteria that are dependent on an exogenous supply of organic carbon, the community profile suggests that the sulfur oxidising bacteria may be driving primary production in this environment. The simulation experiments support the possible role of primary producers supporting the persistence of additional diversity, with the enrichments comprising clades of bacteria associated with autotro-

phy and additional heterotrophic bacteria. The potential for the autotrophic sulfur-cycling bacteria to enable the survival of heterotrophic bacteria within the sediment has implications for the viability of metabolisms on Mars, since syntrophy may facilitate a greater diversity of metabolisms.

**Conclusion:** This study highlights the potential role of oxidation of reduced sulfur species as a metabolism on Mars using either oxygen or nitrate as an electron acceptor. This needs further characterisation with regards to its viability in a martian context. It also shows the importance of community dynamics and the role of syntrophy when considering the viability of metabolisms under terrestrial and martian chemical conditions.

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