Biodiversity, biogeography, and microbial carbon cycling of hot spring communities in the high Andean plateau with implications for early Mars

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Introduction and Motivation

Whether exploring Mars for extant life or for biomarkers indicative of extinct life, one of the fundamental questions driving astrobiological studies of Mars is how putative Martian microorganisms could derive energy. The early collapse of the Martian magnetic dynamo and significant loss of its atmosphere likely transformed Mars into a hyper-arid desert before a metabolism resembling oxygenic photosynthesis was able to emerge. However, Mars may have still supported habitable surface environments from the late Noachian to early Hesperian in locations where impacts and/or volcanic activity allowed for the formation of geothermal springs (1). Such environments could have provided a source of energy for microbes through emission of reduced trace gases (e.g., CH_4, H_2, and CO) which could support chemolithoautotrophic life. Recent measurements by SAM onboard Curiosity show seasonality of multiple gases (e.g., CH_4, O_2, CO) influenced by unknown processes (2), suggesting biological uptake could be a sink. Estimates of surface gas fluxes indicate that these reduced species have the potential to support active microbial communities in the Martian shallow subsurface (3, 4).

Figure 1. (A) Surveying and taking pictures of Polloquere Hot Spring. (B) Dr. Nicole Trefault and Zachary Garvin collecting soil samples at the edge of the hot spring.

Field Work and Methodology

To study the potential for trace gas metabolisms to support Martian life, soils surrounding the gas-emitting Polloquere Hot Springs in the Andean Altiplano of northern Chile were sampled. The site serves as a compelling analog environment for the surface of early Mars due to the low temperature and high UV flux, salinity, and sulfur content. Funds from the NASA Astrobiology Early Career Collaboration Award allowed for me to travel to Santiago, Chile, to meet with collaborators from the Center for Genomics, Ecology & Environment (GEMA) at Universidad Mayor who have expertise in the biogeochemistry and

microbial ecology of the environmental gradients in northern Chile. Our team traveled to the Salar de Surire and Polloquere Hot Springs where we surveyed the field site and acquired soil samples for downstream analyses (Fig. 1).

A subset of soil samples were prepared for 16S rRNA sequencing at Universidad Mayor to assess the diversity within and among samples collected at varying distances and locations around the hot spring. The remaining samples were shipped back to Princeton University to conduct additional analyses. Specifically, microcosm experiments were performed to characterize the microbial gas consumption and production. Ten grams of each sample were placed in 160 mL serum vials and exposed to typical Earth atmospheric mixing ratios of trace gases (2 ppmv CH_4. 500 ppbv H_2, and 100 ppbv CO; Fig 2). Changes in headspace gas composition were measured after 1 week via gas chromatography with FID (CH_4) and RCP (H_2, CO) detectors. Additional short-term microcosms were performed with equal headspace concentrations of CO and H_2 to calculate biological oxidation rates. Soil samples were also extracted for lipids via microwave-assisted extraction.

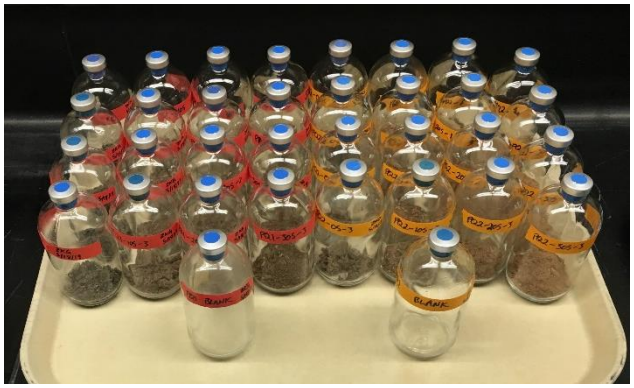

Figure 2. Set of soil microcosms, including triplicate biological samples, killed controls (autoclaved), and blanks (empty vials without soil).

Results and Future Plans

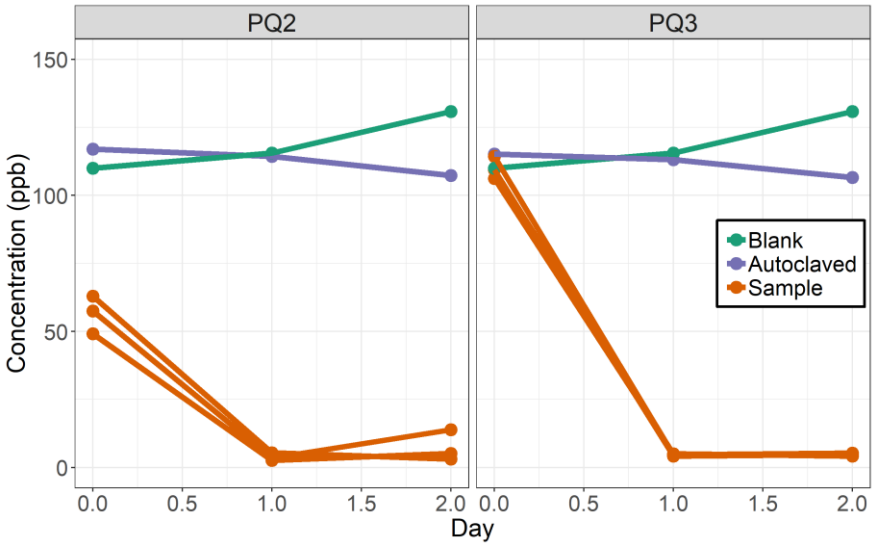

Figure 3. Short-term microcosm results for H_2. Soil samples were collected 30 m from the edge of the hot spring. PQ2 and PQ3 represent samples acquired perpendicular to and downwind of the prevailing wind direction, respectively.

After 1 week, most soils from the microcosm experiments exhibited nearly complete depletion of H_2 and CO within the detection limits of the GCs. The short-term microcosms revealed particularly rapid uptake of H_2, achieving total consumption of trace levels within 24 hours (Fig. 3). Hourly monitoring of microcosms is ongoing, allowing for the calculation of oxidation rates, rate constants, and substrate affinities for the soil gas uptake. Early results suggest trends in both gas oxidation rates and lipid distributions with increasing distance from the hot spring. Sequencing data appear to confirm the presence of known trace gas-metabolizing organisms, particularly

showing high abundance within the *Proteobacteria* phylum. The observed consumption of H_2 and CO supports the plausibility of geothermal springs as sources for trace gas metabolisms in Mars-like environments.

Beyond the additional microcosms, DNA will be extracted from the remaining frozen samples to perform metagenomic sequencing. This will allow for more specific identification of microbes in the soils and the metabolic pathways they utilize for energy and carbon acquisition. Further characterization of lipids, particularly fatty acids, is also planned to assess biosignature potential and distributional trends along environmental gradients around the spring. Long-term goals include returning to the field site to conduct in situ experiments and analyze more springs in the region.

Results from this project have been presented at the NASA-supported *Mars Extant Life: What's Next?* meeting in Carlsbad, NM, as an oral presentation in November 2019. Project updates will also be presented at the *AGU Fall Meeting* in December 2019 as part of the "Getting the Most Out of Astrobiological Data: Overcoming the Too Little, Too Rare, and Too Different" poster session. Funding support from the NASA Astrobiology Early Career Collaboration Award enabled this project to be initiated and the early samples/data to be collected. The award also provided the opportunity to establish new international collaboration between our lab and the GEMA group at Universidad Mayor, which has already proven to be fruitful and a key component of this work. I would like to thank the NASA Astrobiology Institute for the support that has allowed me to pursue this research and connect with the astrobiology community.

References

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