Evaluating Bulk δ[13]C and δ[15]N Values of Acidic Hydrothermal Sediments– Implications for Mars Astrobiology.

Chance Sturrup (csturru1@vols.utk.edu), Anna Szynkiewicz (aszynkie@utk.edu), University of Tennessee, Knoxville

Introduction: The search for signs of past life on Mars is one of the most pressing pursuits in planetary sciences. Numerous observations and experiments have been conducted to assess habitable environments on Mars using orbiter, lander, rover as well as Earth analog data [e.g., 1, 2]. Generally, it is widely accepted that if life as we know it ever existed on Mars, it would have been mainly controlled by microbial processes [3]. Hydrothermal systems are believed to be places where microbial life first arose on Earth. Strong evidence for past hydrothermal processes has been also found on Mars by the Mars Exploration Rovers in Gusev Crater and Meridiani Planum, though orbital data suggests other locales may have experienced past hydrothermal activity and alterations [e.g., 4, 5]. Therefore, many modern hydrothermal settings (e.g., hot springs, mud pots, fumaroles) on Earth have been used as analogs to study geochemical processes related to microbial activity and preservation of biosignatures [2].

δ^{13}C and δ^{15}N are useful biosignatures because biologic and abiotic processes lead to distinctive isotope fractionations on Earth [6]. The NASA Curiosity rover is equipped with SAM instrument to measure δ^{13}C and δ^{15}N, though it is limited to bulk compositions [1]. While terrestrial hydrothermal settings are valuable analogs for assessing past Martian habitability and biosignatures, the influence of Earth's modern biosphere is oftentimes overlooked. For example, it is unclear whether isotope biosignatures associated with thermophile activity are sufficiently preserved in the bulk sediments of surface hydrothermal settings. Because plant matter and products of its decomposition are abundant on modern Earth and can be physically transported to the hydrothermal settings by water and/or wind, the initial isotope biosignature originating from microbial processes can be altered by inputs of allochthonous material. To address these knowledge gaps, this research aims to assess if distinctive bulk δ^{13}C and δ^{15}N related to microbial/thermophile processes are preserved in the relevant Mars analog environments.

Methods: Four distinctive geographic and volcanic locations of Iceland and United States (Lassen, Yellowstone, Valles Caldera) were chosen for bulk δ^{13}C and δ^{15}N analysis in the sediments of hydrothermal features. They all share similar elevated volcanic S-rich emissions and low pH comparable to various past settings on Mars but differ in the abundance and type of vegetative cover [7]. The reported measurements of bulk δ^{13}C and δ^{15}N fall within the capabilities of the Curiosity's SAM instrument, therefore making direct comparisons to the Martian in situ analysis possible.

In total 77 samples were collected. Of these 55 samples were hydrothermal sediments of hot springs and mudpots with low pH <3, 17 were forest- and 5 water-endmember samples. Hydrothermal sediments were collected by submerging sterile, plastic 55 mL centrifuge tubes into the hot spring/mud pot sediment. In some cases, a plastic spatula was also used to scoop the sediment into the tube. Afterward, the collected sediments were placed on ice for transport and frozen at -22°C by the end of the day to prevent biodegradation during storage. Main biologic endmembers comprised of organic-rich soils and plants were also collected by hand from the surrounding forests adjacent to the study sites. Additionally, representative water samples were collected to analyze δ^{13}C of dissolved inorganic (DIC) and organic (DOC) carbon. Samples were filtered into 60mL sterilized polyethylene bottles using 60mL Luer-Lock syringes and 0.45 µm nylon filters.

All solid samples have been freeze-dried and ground with an agate mortar and then placed into tin capsules for measurements of bulk C and N concentrations, δ^{13}C and δ^{15}N on the Delta Plus XL isotope ratio mass spectrometer (IRMS) in the Stable Isotope Laboratory, University of Tennessee. The Costech elemental analyzer coupled with the IRMS combusted the samples at ~900°C to CO_2 and N_2O gases. Additionally, the δ^{13}C values of DIC and DOC in the water samples from Valles Caldera and Yellowstone were measured using IRMS coupled to the Thermo-Scientific Gas Bench II instrument.

Results: Bulk C concentrations of acidic hydrothermal sediments were generally higher (0.1 to 4.8 wt.% C) in the forested sites of United States compared to lower concentrations (0.1 to 0.5 wt.%) in sporadically vegetated (mainly mosses and grass) Iceland. Conversely, bulk N concentrations varied in similar range of 0.01 to 0.2 wt.% in all sites.

Bulk δ^{13}C of sediments was lower (-35 to -20 ‰) in United States compared to higher values (-23 to -15 ‰) in Icelandic sites (Fig. 1A). The δ^{13}C of plant endmembers ranged from -30 to -25 ‰, as expected of the Calvin-Cycle metabolism found in the C3 plant species of the studied regions. The δ^{13}C of DOC in the Valles Caldera and Yellowstone hot spring and mud pot water varied from -23 to -19 ‰ compared to more negative values of -32‰ in non-hydrothermal waters.

Bulk δ^{15}N values were less distinctive in Iceland, Valles Caldera, and Yellowstone and varied in a wide range from –18 to +5 ‰. Because elevated NH_4^+ concentrations were measured in the hot spring and mud pot water of these sites, <1 to 478 mg/L [7], the measured bulk $\delta^{15}N$ of sediments was likely from volcanic NH_4^+ that precipitated out of pore water during drying of the sediment samples for isotope analysis. For example, post cation analysis of sediment samples using a Dionex ion chromatograph (IC) detected elevated NH_4^+ that re-dissolved in the leaching solution. Additionally, positive correlations between NH_4^+ concentrations and bulk wt.% N were apparent in multiple locations.

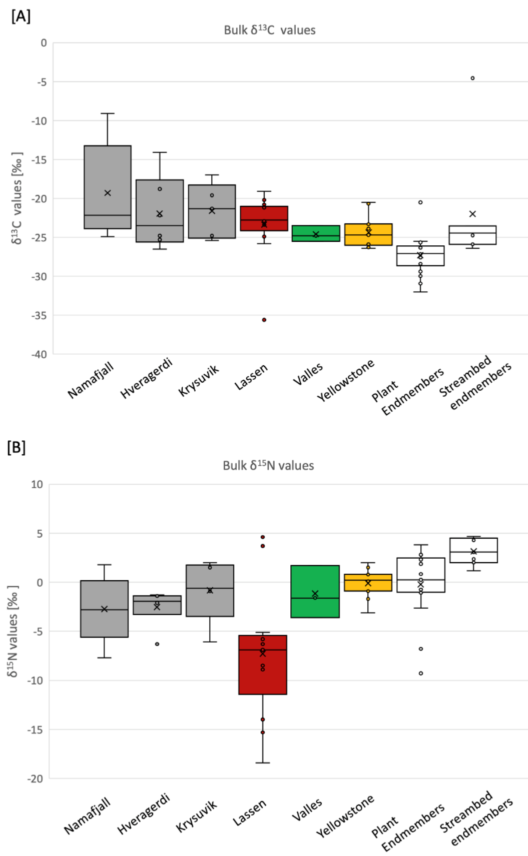

Figure 1: Bulk $\delta^{13}C$ (A) and $\delta^{15}N$ (B) results for the acidic hydrothermal sediments and major organic endmembers from the surrounding forest. Grey boxes are for three different sites sampled in Iceland.

Discussion: Wide variations of the measured bulk $\delta^{13}C$ of acidic hydrothermal sediments can be interpreted in two ways. (1) The $\delta^{13}C$ values may represent mixing of two main organic endmembers consisting of microbial/thermophile matter (and/or DOC) with higher values of -19 ‰ and variable inputs of allochthonous plant matter with lower values of -32 to -25 ‰ from the surrounding forest. For instance, the studied sites in Yellowstone, Valles Caldera and Lassen with denser forest had more negative bulk $\delta^{13}C$ of hydrothermal sediments compared to less vegetated areas of Iceland with higher values (Fig. 1A). Alternatively (2), the measured bulk $\delta^{13}C$ of hydrothermal sediments is representative of refractory carbon from thermal decomposition of existing biomass within the studied hot springs and mud pots. Additional measurements of bulk $\delta^{13}C$ at 550°C and 870°C will be conducted to better understand what type of organic material is preserved in the studied sediment samples.

The measured range of bulk $\delta^{15}N$ values (-18 to +3‰) was consistent with more negative $\delta^{15}N$ of local volcanic gases (-4 to +3 ‰) [8, 9, 10] while plant and streambed endmembers of non-volcanic areas were more positive (-1 to +5 ‰) (Fig. 1B). Because precipitation of dissolved NH_4^+ in pore water occurred during freeze-drying of the studied sediments for isotope analysis, the bulk $\delta^{15}N$ analysis would be capable of detecting isotope signatures of volcanic gases under dry conditions on Mars. Given that the $\delta^{15}N$ expected for thermophilic metabolism and allochthonous material overlap, it is impossible to distinguish between these two N sources using the results of our study.

Conclusion: Our preliminary bulk $\delta^{13}C$ results show the measurable inputs of plant matter from the surrounding forest, thus influencing the C isotope composition of hydrothermal sediments in Valles Caldera, Lassen, and Yellowstone. In contrast, the bulk $\delta^{13}C$ of more barren hydrothermal sites in Iceland were more consistent with hydrothermal microbial metabolism of thermophilic bacteria and archaea. This implies that similar microbial C isotope biosignatures could have been preserved in Martian environments if comparable conditions persisted. There is also potential for $\delta^{15}N$ to help determining if locations on Mars have experienced past hydrothermal activity given that the measured bulk values likely recorded the isotope composition of the NH_4^+ from volcanic emission. Although the inputs of allochthonous matter from the forested areas appear to be considerable increasing the bulk C concentrations in the sediments, the applicability of bulk isotope analysis to detect microbially mediated C in the Martian surface sediments remains strong.

References: [1] Mustard J.F. et al. (2013) *MEPAG report.* [2] Williams A. J. et al. (2021) *Astrobiology, 21,* 60-82. [3] Westall F. et al. (2013) *Astrobiology, 13.* [4] Ruff S. and Farmer J. (2016) *Nat Commun, 7.* [5] Fernández-Remolar D. C. et al. (2008) *Astrobiology, 8,* 1023-1047. [6] Sharp Z. (2017) *Principals of Stable Isotope Geochemistry 2nd Edition.* [7] Ende J. and Szynkiewicz A. (2021) *Icarus 368.* [8] Goff F. and Janik C. J. (2002) *Journal of Volcanology and Research, 116,* 299-323 [9] Janik C. J. and McLaren M. K. (2010) *Journal of Volcanology and Geothermal Research, 189,* 257-277 [10] Sano Y. et al. (1985) *Geochemical Journal, 19,* 135-148