MICROBIAL DIVERSITY AND FUNCTIONALITY IN EXTREME MARS ANALOG SETTINGS: ACID-SULFATE HYDROTHERMAL SYSTEMS IN COSTA RICA AND ICELAND. Brian M. Hynek1,2, J. Wang1, N. Dragoné3, G. Avard4, G. E. Alvarado5 and L. McHenry6 1Lab. for Atmospheric and Space Physics, University of Colorado Boulder, USA, 2Dept. of Geological Sciences, University of Colorado Boulder, 3Dept. Of Environmental and Evolutionary Biology, University of Colorado Boulder, 4OVSICORI, National University of Costa Rica, 5Centro de Investigaciones Ciencias Geológicas, Universidad de Costa Rica, 6Dept. of Geosciences, Univ. Wisconsin-Milwaukee. hynek@lasp.colorado.edu

**Introduction:** We have been investigating Mars geochemical analog environments in Costa Rica and Iceland where the style of hydrothermal alteration and alteration mineralogy are consistent with Mars' relict hydrothermal systems [e.g. 1-5]. These dynamic sites are all on active volcanoes and exhibit hydrothermal systems operating under a range of temperatures (9-95°C) and low pH (0-4.3) acid-sulfate conditions. In places, we have a time series of samples to assess changing community composition. Martian acid-sulfate hydrothermal systems were likely similarly diverse and equally challenging to life. Further, the martian examples could likely have supported life for long durations, regardless of the global climate conditions. Our results provide an assessment of habitability on early Mars and also explore the limits of life on Earth.

**Field Sites:** After visiting dozens of sites, we focused our work on locales best matching inferred physicochemical conditions, substrates, and mineralogy found at relict martian hydrothermal sites. In Costa Rica, we concentrated on 3 field sites with a time-series of data from 11/2013 and 3/2017: (1) Laguna Caliente, the Poás volcano summit crater lake, (2) Laguna Fumarólica/Las Paílas, and (3) Borinquen source pool. The latter two sites are on the Rincón de la Vieja volcano. In Iceland, 2017 data are shown from an area within the Hellisheid Geothermal Power Station property on the Hengill volcano. In all cases, acid-sulfate alteration of the parent rocks leads to a variety of sulfates, Fe-oxides, and clays akin to those found on Mars in relict hydrothermal settings [1-4,6].

**Methods:** Briefly, fluids from pools/springs/lakes along with entrained and bottom sediment was collected aseptically in duplicate and immediately frozen. DNA was extracted using the MoBio PowerSoil/Max Soil kits following manufacturer’s instructions. DNA from multiple extractions of the duplicate sample was pooled and concentrated with Amicon Ultra-4 spin filters. For sequence-based analyses of 16S rRNA genes, we used the approaches described previously [5,7]. The V4 region of the 16S rRNA gene was PCR-amplified using barcoded primers. Products from duplicate PCR reactions for each sample, as well as “no template” and “DNA extraction” negative controls, were pooled, cleaned, and normalized using the ThermoFisher Scientific SequalPrep Normalization Plate kit, and sequenced on an Illumina MiSeq platform using v2 500-cycle paired-end kits in the Fierer Lab at CU Boulder.

Sequence reads were processed as previously described [5,8]. Briefly, sequences were demultiplexed, forward and reverse reads were merged, and quality-filtered with QIIME and UPARSE. A database of ≥97% similar sequence clusters was constructed in USEARCH (Version 8; [9]). Demultiplexed sequences were mapped against the de novo constructed databases to generate counts of sequences matching clusters (i.e. taxa) for each sample. Taxonomy was assigned to each taxon using the RDP classifier with a threshold of 0.5, trained on the Greengenes database (for prokaryotes; V. 13_8; [10]), and sequences were aligned to known isolates using the BLASTn algorithm [11] and NCBI 16SrRNA database.

**Microbiology Results:** Taxonomy and distribution of the microbial populations are shown in Fig. 1.

Poás Lago Caliente, Costa Rica: This lake is characterized by extremely low pH, widely fluctuating temperatures, and very high concentrations of dissolved ions [5]. Remarkably, in both our 2013 and 2017 samples from the Poás crater lake, ~98% of all of the sequence reads clustered in a single OTU within the genus Acidiphilium (Fig. 1) [5]. Members of the Acidiphilium genus are acidophilic bacteria, with several obligate heterotrophs and at least one facultative chemoautotroph [e.g., 12-13]. Shotgun metagenomics has been completed on this microbe and show it has a multitude of ways to gain carbon and energy (primarily S oxidation), and it can operate under aerobic or anaerobic conditions [14, this conference].

Laguna Fumarólica, Costa Rica: This small lake exhibits low pH, high temperature, and abundant toxic metals (e.g., As). 16s gene analysis of our 2013 samples showed very low diversity, dominated by Thermoprotei Archaea (Fig. 1). However, samples from 2017, when the lake was more acidic but cooler, showed no Archaea present and instead a diverse community of Bacteria (Fig. 1). We infer that the cooler temperature allowed Bacteria to outcompete the Archaea, even given the more acidic conditions.

Borinquen Source Spring, Costa Rica: This near-boiling spring had the most moderate pH (~3.5) of the Costa Rica sites. In both 2013 and 2017, the community
was dominated by 1-2 *Thermoprotei* Archaea that were different from the members at Laguna Fumarolic.

**Hengill, Iceland:** Results are shown from ambient temperature seeps (pH = 3.4) immediately adjacent to a large hydrothermal system (Fig. 1). The biofilms exhibited significantly different color, mineralogy, and microbial communities. The yellow spring’s color is from indigenous S\(^0\) and community structure was dominated by Bacteria and Archaea that metabolize sulfur. H\(_2\)S(g) from below was likely being incorporated into this spring as in the adjacent hot springs. The brown biofilms, 50 cm away, showed an entirely different biological community lacking Archaea and dominated by Bacterial phototrophs.

**Implications for Searching for Life on Mars:** Mars’ accreted sulfur- and iron-rich and identified hydrothermal settings show these enrichments. Thus, understanding Earth’s microbial communities and their functions in Mars analog settings provide a framework for what life might have existed on Mars. Our microbiology studies at acid-sulfate hydrothermal systems have bearing on detecting life in similar relic environments on Mars and how to search for it with rovers like Mars 2020 and Exomars: (1) We showed that chemoliths oxidizing sulfur is a dominant process in these settings. (2) Hydrothermal systems are dynamic settings and in places, the microbial community structure can change dramatically through time due to geochemical changes (Laguna Fumarólica) or remain the same (Poás, Borinquen). (3) These sites can also be spatially diverse over decimeter-scales in terms of their geochemistry, mineralogy, and biological diversity (Hengill). (4) Life can persist in extreme environments (pH ~ 0, or T ~ boiling) (Poás and Borinquen, respectively), although community diversity and biomass dramatically drop off.


**Figure 1.** 16s rRNA genes Bacterial and Archaean diversity in Mars analog hydrothermal settings. Arrows indicate sampling locations.