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Investigating the role of microbes in secondary mineral deposits in lava caves as analogs for subsurface Mars

PROJECT REPORT

Lava caves and tubes found in Craters of the Moon National Monument and Preserve (COTM) are important analogs to martian basalts, and can be used to understand microbial community structure and function, as well as biosignature production and preservation. To investigate whether chemolithotrophic microbes play a role in secondary mineral deposition in COTM caves, samples for molecular sequence, organic and mineral composition analyses were collected. The **research goals** were to (i) determine microbial community structure and seasonal dynamics and (ii) examine the metabolic and biochemical interactions within microbial communities to predict their impact on geochemical processes. **Research significance**: Understanding the biochemical mechanisms underlying microbial evolution and function in basalt subsurface environments provides insight into biosignature identification and detection for inferring past or present life on Earth and other volcanic terrains like Mars.

To address the research objectives, I planned and undertook two expeditions: the first took place August 9th-13th 2017 and the second January 7th-10th 2018. Samples were collected from (i) two publicly accessible caves: Indian Tunnel and Buffalo Caves, (ii) Arco Tunnel, and (iii) three Wilderness Caves: Hidden Cave, Needles Cave and Last Chance Cave. With the exception of Arco Tunnel, I had previously collected samples from all the caves visited during these two expeditions, allowing for seasonal and annual comparisons.

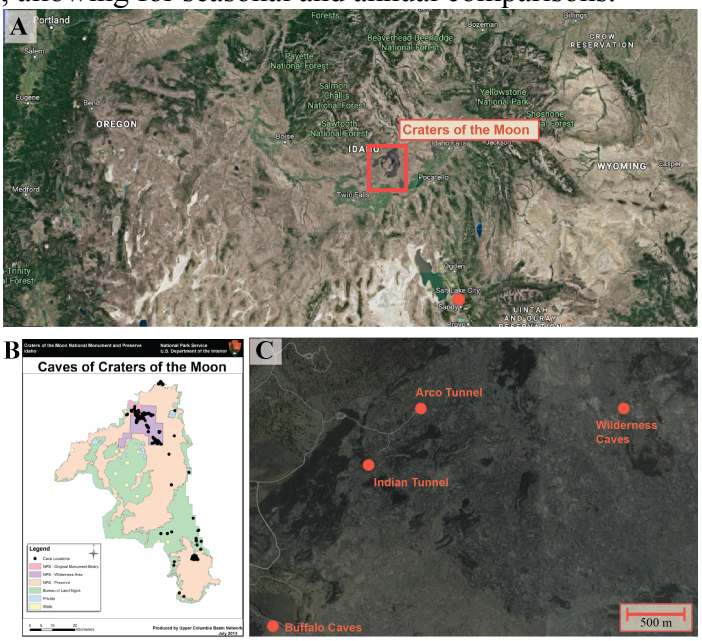

Figure 1. Sampling site location. (**A**) Geographic location of COTM, demarcated by the orange square; the orange circle marks Salt Lake City. (**B**) NPS map showing known cave locations at COTM; colours indicate NPS and BLM management boundaries, with the purple area designated as the Wilderness Area, managed by NPS. (**C**) Location of caves from which samples were collected during the August 2017 and January 2018 expeditions; Wilderness Caves sampled are located in close proximity to each other, and are indicated by a single dot, closest to Needles Cave.

In addition to myself, the expedition teams consisted of Kate Craft and Julie Bevilacqua in the summer, and Steve Squyres and Nicole Wagner in the winter. The summer expedition coincided with NASA FINESSE and BASALT fieldwork, and members of the program joined our team for one, or more, days in the field. National Park Service (NPS) biologist Todd Stefanic

accompanied our team to Arco Tunnel during both expeditions, and guided us to the Wilderness Caves in the winter. We followed safety procedures agreed upon with NPS staff, and had appropriate permits for cave access and sample collection. To mitigate the risk of spreading White Nose Syndrome, we followed recommended decontamination procedures and wore NPS protective coveralls. In the winter, we took care not to disturb hibernating bats and avoided known hibernacula.

Figure 2. Surface conditions on basalt terrain during the summer (right, photo credit: K. Craft), and winter (left, photo credit: E. Zaikova)

Samples for molecular analyses were collected in at least duplicate, with up to 9 replicates where possible, and were placed in a primed cryoshipper for transport to the lab, where they were stored at -80°C. Samples for organic and mineral analysis were collected into DNA-free glass jars and placed in a cooler and subsequently stored in a freezer. External and internal rock samples were collected for chemical characterization of parent rock material. Samples were collected as inconspicuously as possible to minimize the impact of scientific activity. Aseptic technique and sterile utensils were used to collect samples, and each sample was documented. Contextual data, including relative humidity, air and cave wall temperature were measured at each sample site.

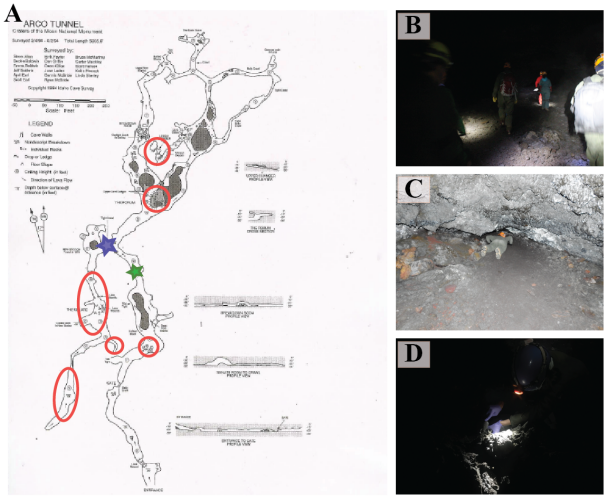

Figure 3. Sample collection in Arco Tunnel. (**A**) Detailed map of Arco Tunnel, provided to us by NPS at COTM. Orange circles indicate areas where samples were collected. The green star corresponds to location shown in image (**B**) and the blue star indicates the tight crawl entrance to the zones 6 and 7 of the cave including to the "Senate" room shown in panel (**C**). (**D**) Sample collection in Arco Tunnel using DNA-free utensils, gloves and aseptic sampling technique. Photo credit K. Craft (C and D) and E. Zaikova (B).

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The daily activities were as follows:

- August 9th, 13th and January 7th and 10th: travel days.
- August 10th: sampling in Arco Tunnel, time in field – 6:30 am to 5 pm. This is an extensive cave, ~1.8 km in length, with no light penetration and many different environments (air temperature ranged between 10 and 18 °C, humidity also varied between 79-87% RH). Deposits sampled (11) ranged in texture and colour,.
- August 11th, time in field – 9 am to 6 pm: sample collection of altered and unaltered basalt from lava flows at Big Craters and Highway flow for molecular and GC analyses to complement BASALT biosignatures characterization and better interpretation of cave samples.
- August 12th, time in field – 9 am to 6:00 pm: sampling in Indian Tunnel and Buffalo Caves. These two caves are publicly accessible and have some degree of light penetration in all parts of the cave and noticeable external airflow. Unexpected medical delays in the field prevented sampling Wilderness Caves.
- January 8th: sampling in Needles, Last Chance and Hidden Caves, time in field – 8 am to 4:30 pm. Samples were collected from the darkest portions of each cave. Several mounds of white sulfate deposits described in scientific literature were observed and sampled in Needles and Hidden Cave.
- January 9th: sampling in Arco Tunnel, time in field – 8 am to 3 pm. Dripping water was observed throughout the cave. Three hibernating bats were observed in one cave zone that we did not sample in. I planned to collect samples from Indian Tunnel on this day as well, but as weather conditions worsened throughout the day, I prioritized team safety and forwent this portion of the day.

The time in field listed is the approximate total time at COTM, including hike to the cave and equipment check at NPS office. No obvious differences between the types and amounts of deposits were observed. Although both wall and air temperatures inside the caves were lower in the winter than the summer, their variation was much smaller than external conditions. Relative humidity was slightly lower in the winter, however dripping water or ice were observed in the winter in location that were dry in the summer.

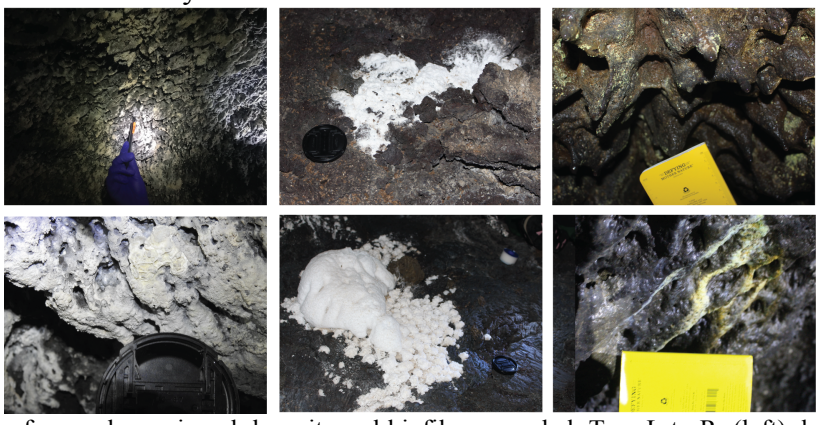

Figure 4. A subset of secondary mineral deposits and biofilms sampled. Top, L to R: (left) deposits on ceiling and walls of Buffalo Caves, pencil is shown for scale, photo credit K. Craft; (middle) white powder-like deposit on the floor of Needles Cave, lens cap for scale, photo credit E. Zaikova; (right) green deposits/biofilm in Arco Tunnel, field notebook for scale, photo credit: E. Zaikova. Bottom, L to R: (left) wall and ceiling deposit in Indian Tunnel, lens cap is shown for scale, photo credit K. Craft; (middle) white deposit on floor of Hidden Cave, one of several

mounds of similar size, lens cap is shown for scale, photo credit E. Zaikova; (right) pigmented biofilm in Arco Tunnel, field notebook shown for scale, photo credit E. Zaikova.

DNA was extracted and sequenced from samples collected in summer 2017, and comparisons with 2016 data are being performed. DNA extraction from the 2018 samples is underway. Protein and RNA sequencing, microscopy as well as mineralogy analyses will be carried out shortly.