

# Life in the Lava Tubes: Bacterial, Archaeal and Eukaryotic Communities in a Mars Analog Environment

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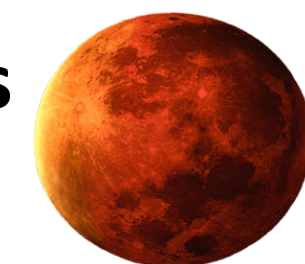


## Abstract

Lava caves are prime targets of interest in the search for extant or past life on rocky planets. Here on Earth, they harbor diverse and abundant microbial communities, which are often associated with secondary minerals formed on host basalt rock. Here, we will present results from an ongoing NASA planetary analog project, Biological and Resource Analog Investigation in Low Light Environments (BRILLE), underway at Lava Beds National Monument (Northern CA, USA). Using 16S and 18S rRNA gene sequencing, we characterized the distribution, diversity, and putative metabolic capabilities of microbial and eukaryotic communities within several caves with a variety of physical parameters such as length, depth, number of entrances, and human visitation. We sampled distinct morphologies in the caves, ranging from soft sticky “ooze”, to microbial films, secondary mineral features (“speleothems”), and what appeared to be bare basalt. The soft-material samples, ooze and microbial biofilms, had lower relative abundances of Actinobacteria and higher relative abundances of Proteobacteria than mineral features. The bacterial community structure did not differ significantly between features, but the community in soft features was higher than in mineral features. The three unclassified archaeal genera, from the families *Nitrosotaleaceae*, *Nitrosopumilaceae*, and *Woesarchaeales*, tend to co-occur and correlate inversely with the genus *Candidatus Nitrosocomicus*. These taxa and abundant bacterial genera, such as *Nitrospira*, are likely involved in nitrogen cycling, and the presence of nitrogen metabolism is further supported with metagenomic data. Our study provides insight into an essential nutrient cycle in a relevant Mars analog environment and may inform future sample collection in the search for life on other planets.

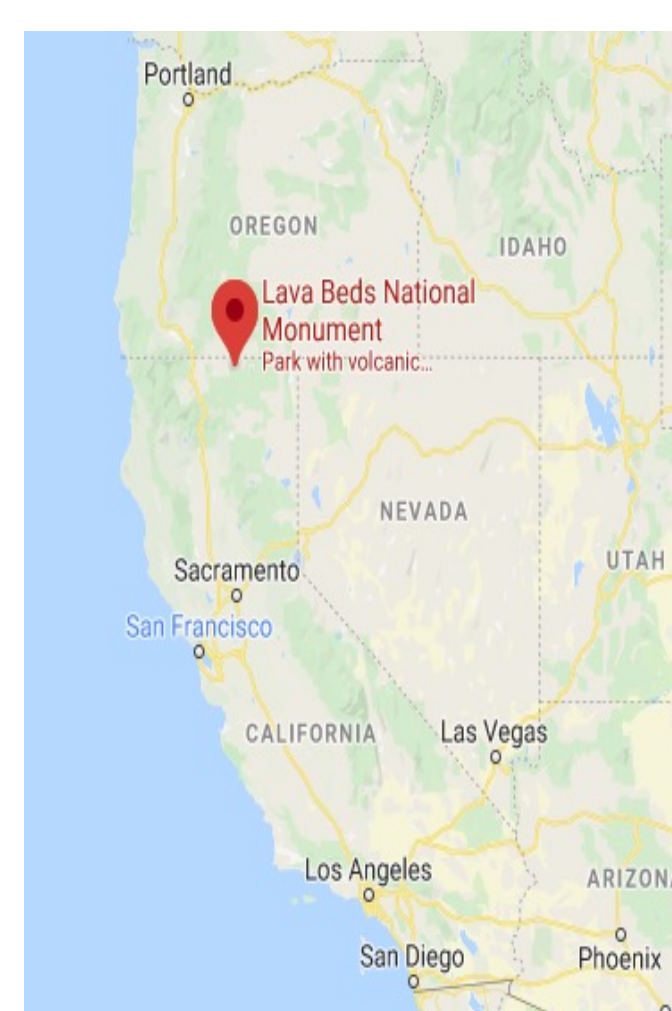
## Introduction

### Why are lava caves a good Mars Analog environment?



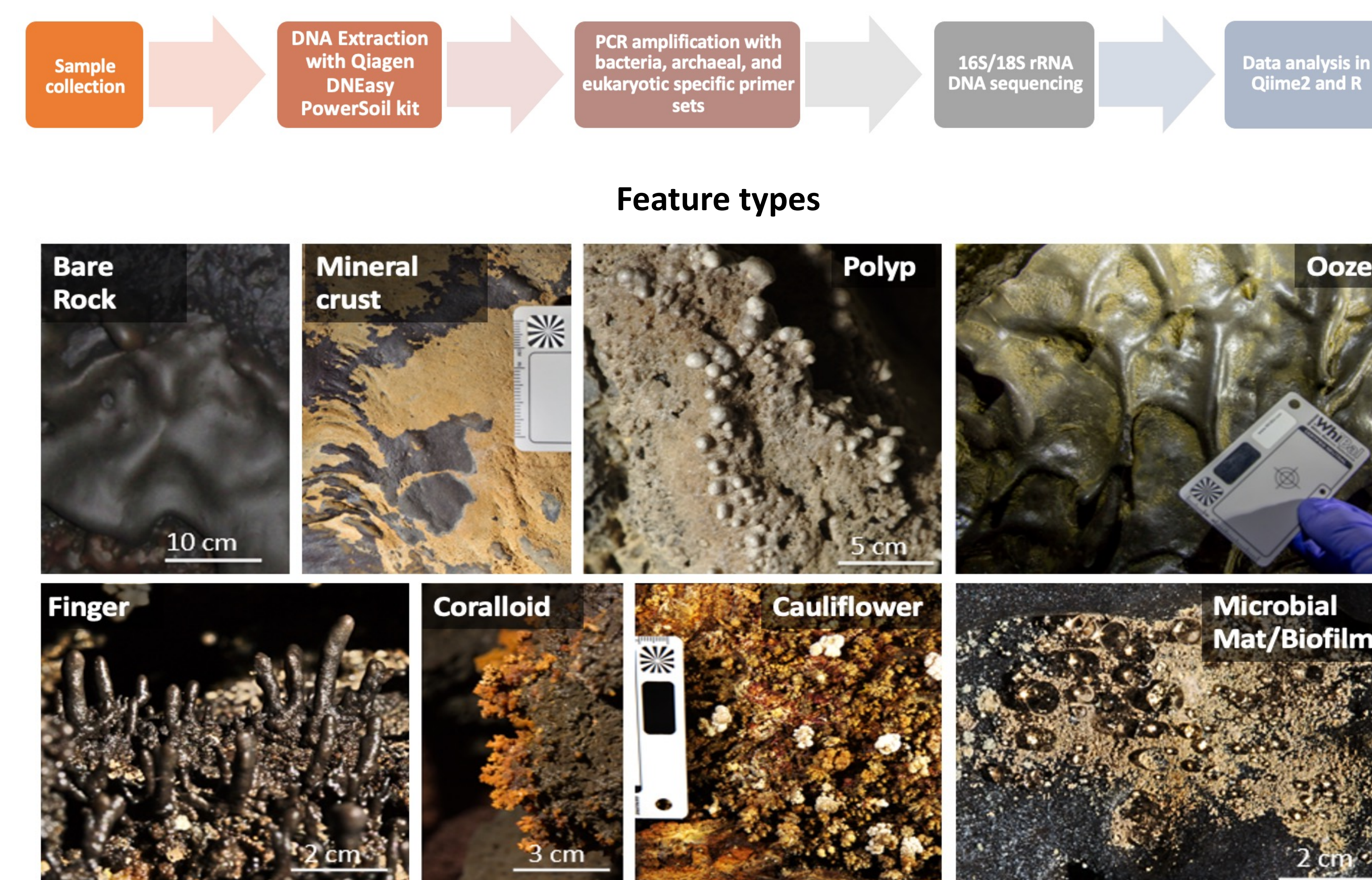
- Harsh surface conditions of Mars: ionizing radiation, oxidation, and variable temperature
- Caves are insulation from surface conditions and could potentially preserve biosignatures.
- Liquid water on Mars exists in the subsurface
- Lava tubes exist on Mars, and could be potentially explored by an autonomous rover in future NASA mission

### Study Site: Lava Beds National Monument



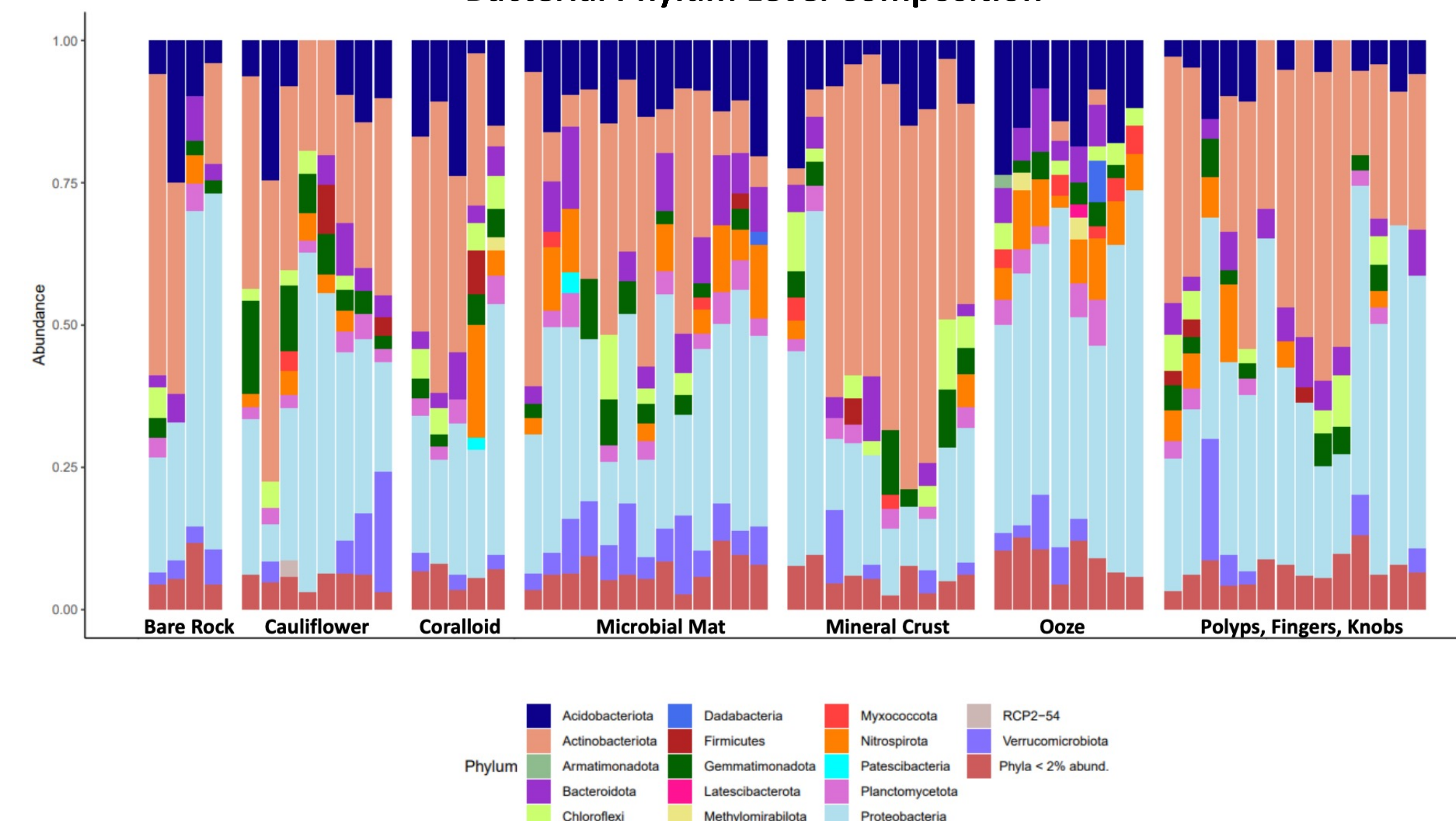
- Contains over 800 caves, making this site the most concentrated region of lava caves in North America
- Caves formed 10,500 to 65,000 years ago by volcanic eruption
- Variety of suspected biomineral features (shown in figure 1)
- Previous microbiological studies indicate surface and cave microbes are distinct (Lavoie et al. 2017)

## Methods



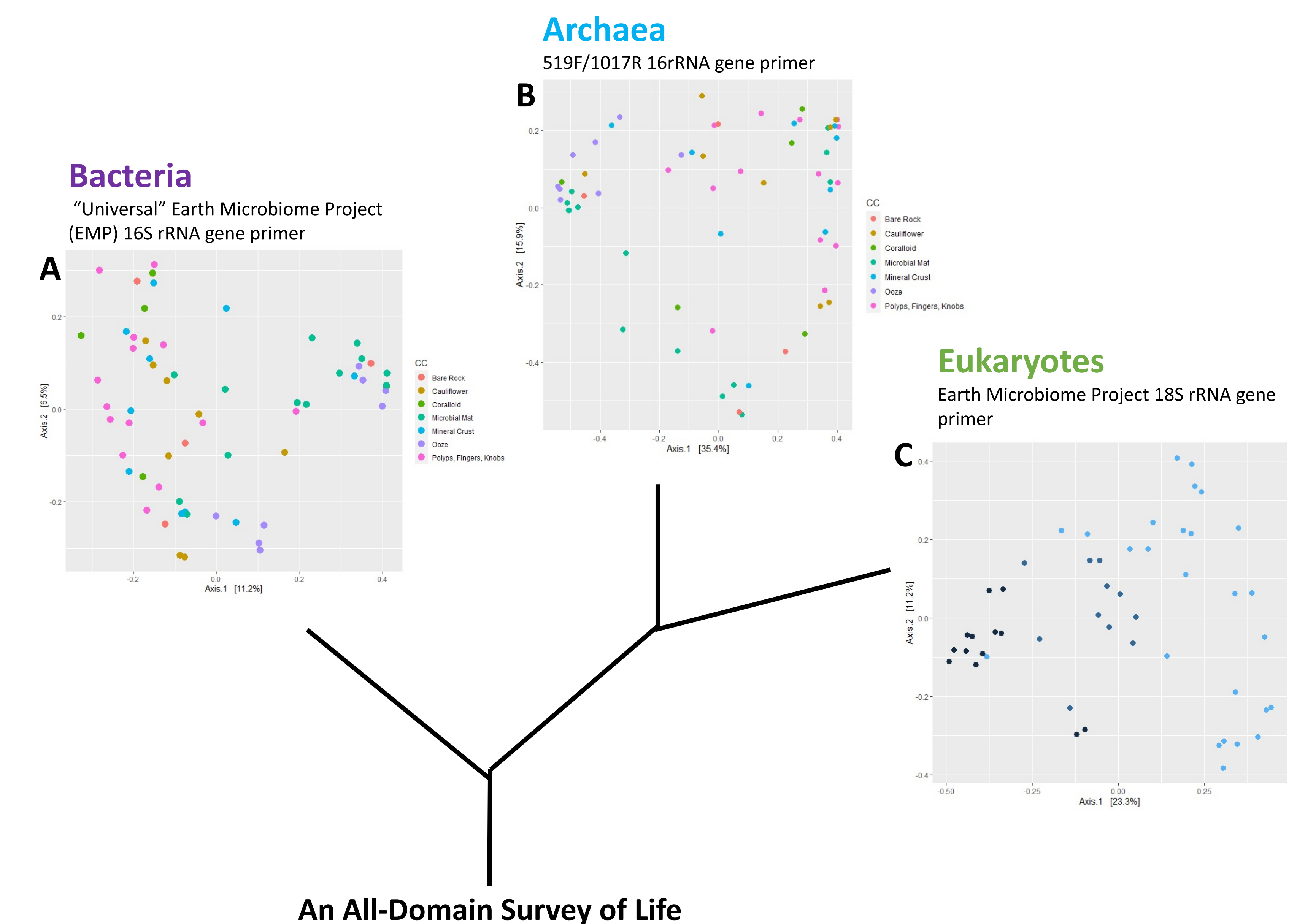
**Figure 1.** Representative photos of the eight feature types found at Lava Beds National Monument, CA. These included secondary mineral features such as mineral crusts, which vary in composition and lack vertical extensiveness; polyps, small, round, smooth shapes; fingers, long, finger-shaped slender deposits; coralroids, coral-like deposits with branching morphology; cauliflower, a floret that resembles a head of cauliflower; bare rock, which shows no macroscopic evidence of microbial life or minerals deposits, as well as features with clear microbial component, including ooze, a soft, sometimes slimy, wall deposits; microbial mats/biofilms, clusters of microbial colonies that can be thin or thick deposits on the walls and are often hydrophobic.

### Bacterial Phylum Level Composition



**Figure 2.** This figures shows the phylum level relative abundance of bacteria in 62 samples, grouped by feature category. These data were generated with the Earth Microbiome Project (EMP) primer set and non-bacterial sequences were filtered out. Actinobacteria (28%±19%) and Proteobacteria (34%±15%) are the most abundant phyla. Note the almost complete lack of Actinobacteria in the ooze, and lower percentage in microbial mats for a total of (16%±18) Actinobacteria for more microbial features. Nitrospirata was also abundant in the ooze.

## Results



**Figure 3.** PCOA ordination of a Bray dissimilarity matrix based on genus level assignments from 16S/18S rRNA sequencing targeting either bacteria (A), archaea (B), or eukaryotes (C). Samples color denotes feature category in A and B, and year in C.

**Bacteria** A total of 20,839 bacterial amplicon sequencing variants (ASVs) were recovered with the 16S rRNA EMP primer set. There is some pattern of more microbial features (ooze and microbial mats) clustering together and the secondary minerals clustering together.

**Archaea** A total of 1,629 archaeal ASVs were recovered with the archaeal specific primer. It show a similar trend to the bacterial dataset, but with a higher percentage of the total community differences explained.

**Eukaryotes** A total of 4915 ASVs 18S rRNA EMP primer set. Instead of grouping by feature, the eukaryotes community change was associated with the three different sampling years.

## Conclusions

- Bacterial and archaeal microbial communities do not clearly cluster by individual feature groups, but do cluster by the broader feature groupings of more microbial (ooze and microbial mat/biofilm) and more mineral (bare rock, cauliflower, coralloid, mineral crust, polyps, fingers, and knobs) features
  - Actinobacteria are more abundant in the more mineral than more microbial features (Figure 2)
- Ooze is the most distinct feature type
  - This feature clusters at the edge of the bacterial and archaeal ordination (Figure 3 A and B)
  - Near absence of Actinobacteria, the second most abundant phylum in the dataset
  - Has higher relative abundance of Nitrospirata (Figure 2), and other nitrogen cycling archaea (data not shown) particularly those associated with ammonia oxidation
- Eukaryotic community structure correlates with year sampled, not feature category.

## Acknowledgements

This work was funded by the NASA PSTAR program, NNN16ZDA001N, and a NASA EPSCoR Space Grant Fellowship 01539. We would like to thank the National Park Service for access to samples.

