

COMBINING GEOCHEMISTRY AND MICROBIAL ECOLOGY TO UNDERSTAND PROCESSES IN THE DEEP TERRESTRIAL BIOSPHERE

Lily Momper¹, Caitlin P. Casar¹, Brittany Kruger², Theodore M. Flynn³ and Magdalena R. Osburn¹,

¹Department of Earth and Planetary Sciences, Northwestern University, Evanston, IL

²Desert Research Institute, Las Vegas, NV

³California Department of Water Resources, West Sacramento, CA

lily.momper@northwestern.edu

Introduction: If there is or ever was life on an extraterrestrial planetary body, evidence of that life would likely be found below the surface, protected from harsh exposed conditions. The deep terrestrial subsurface is a key analog for subsurface settings on other rocky planets. In Earth's deep subsurface biosphere (DSB), water, organic carbon and chemical energy are often scarce. However, it has been shown that archaea and bacteria are able to persist in the DSB to at least 3.5 km below surface [1]. Understanding how organisms persist, and what markers they may leave behind, is critical in predicting which extraterrestrial bodies may have harbored, or harbor, similar life.

In the current study we investigated life down to 1.5 km below surface. Our portal to the DSB is a legacy gold mine in Lead, South Dakota—now home to the Deep Mine Microbial Observatory (DeMMO, consisting of six sample sites, 800-4850 feet below surface) hosted within a sulfide-rich, Paleoproterozoic iron-formation [2]. Previous work by our group used thermodynamic modeling from measured geochemistry to predict energy-yielding metabolic reactions and showed evidence for abundant exergonic chemolithoautotrophic metabolisms [3]. Here, we build upon that work by combining metagenomic sequences with geochemical and 16S rRNA gene sequences, culminating in 5 years of physical and biological data. Synthesizing these datasets of different types and from different time points provides a more holistic understanding of how life persists in Earth's DSB, thereby enabling better prediction of what sorts of life may be in the subsurface of extraterrestrial bodies.

Samples for this study were collected between September, 2013 and September, 2018 from boreholes located between 0-4850 feet (~1.5 km) below surface. On each expedition, geochemical data were collected concomitantly with DNA for 16S and/or metagenomic sequencing.

Results: Preliminary 16S rRNA analyses revealed a change in microbial community composition as a function of depth, including a progression from *Proteobacteria*-dominated and candidate phyla-dominated communities near the surface to *Firmicutes*-dominated communities at depth. Nearly 20 candidate phyla were identified in DeMMO fluids, which are divisions for which we have no cultured representative. Members of the OP3 (*Ca. Omnitrophica*) and Hadesarchaeota were particularly abundant in fluids collected at 800 and 4850 feet deep, respectively.

Further investigation using whole genomic DNA amplification- shotgun metagenomics- enabled complete and near-complete genome reconstruction of surface fluids flowing above DeMMO and fluids circulating within the subsurface. We reconstructed nearly 500 genomes from 8 fluid samples. In some cases, the metagenomic data recapitulated the 16S datasets, enabling us to reconstruct full genomes for abundant members, such as candidate phylum OP3. In other fluids we were able to construct complete genomes for members of the rare biosphere. For example, we reconstructed the most complete genome for candidate phylum NKB19 currently available [4]. Gene prediction and metabolic mapping revealed that putative anaerobic metabolisms, potentially using H₂ as an electron donor, were very common. The transformation of oxidized and reduced sulfur and/or nitrogen species were also very common potential energy sources among genomes. Nearly half of the reconstructed genomes had all genes necessary for carbon fixation via the relatively energy inexpensive, and obligately anaerobic reductive acetyl-CoA pathway. In general, the phylogeny and metabolisms of genomes collected from the surface water above DeMMO were different from those found in fluids collected 800-4850 feet below surface and consisted mostly of aerobic and phototrophic metabolisms. Conversely, the majority of microbes in subsurface borehole fluids retain metabolic capability for chemolithotrophic and autotrophic metabolisms. These results, along with geochemical data and hydrologic modeling, lead us to conclude that the deeply circulating fluids at DeMMO have little if any continuity with the overlying surface fluids, supporting the possibility of subsurface life independent from the surface on other planetary bodies.

Broader implications: Our study of microbial communities in a terrestrial subsurface setting informs the prediction about the location and possible biosignatures of communities on extraterrestrial bodies. Analyzing geochemical metadata with phylogenetic diversity and metagenomic metabolic mapping may enable us to predict what metabolic pathways would be likely in the subsurface of other potentially habitable planets such as Mars.

References: [1] Baker J. B. et al. (2003) *Environ. Microbiol.*, 5, 267–277. [2] Osburn M. R. et al. *Front. in Earth Sci* (in review) [3] Osburn M. R. et al. (2014) *Front. in Microbiol.*, 5. [4] Momper L. et al. *Front. in Microbiol.*, (2018)