

Habitability of the Terrestrial Deep Subsurface: Sanford Underground Research Facility, South Dakota, USA. M. R. Osburn¹, J. P. Amend^{2,3}, D. E. LaRowe², L. M. Momper³, G. P. Wanger^{4,5}, B. K. Reese⁶, M. Y. El-Naggar⁷, R. Bhartia⁴, V. J. Orphan⁵, Y. Jangir⁷, and D. Moser⁸, ¹Department of Earth and Planetary Sciences, Northwestern University, Evanston, IL 60208, USA (maggie@northwestern.edu), ²Department of Earth Sciences, University of Southern California, ³Department of Biological Sciences, University of Southern California, ⁴Jet Propulsion Lab, ⁵Division of Geological and Planetary Sciences, California Institute of Technology, ⁶Department of Life Sciences, Texas A&M University, Corpus Christi, ⁷Department of Physics, University of Southern California, ⁸Desert Research Institute.

Introduction: Subsurface environments on rocky planetary bodies are among the most promising candidates for harboring extraterrestrial life. However, the extent and diversity of the terrestrial subsurface environments on Earth remain poorly characterized. Our NASA Astrobiology Institute team—*Life Underground*—uses a multilateral approach to study the subsurface biosphere at the Sanford Underground Research Facility (SURF) in South Dakota, USA. At this site, 500 km of tunnels cut through metamorphosed paleoproterozoic iron formation host rock to a depth of 8100 ft (~2470 m) [1]. Mine drifts and boreholes can intersect waterbearing fractures, allowing for direct sampling of otherwise inaccessible hard rock environments.

Geochemistry: Geochemical analyses of borehole fluids and pools from the 300 to 4850 ft levels at SURF have revealed enormous geochemical diversity. Fluids range from fresh to brackish, correlating with a transition from oxidizing to reducing conditions (ORP from 330 to -275 mV). The concentrations of redox sensitive species also vary considerably (e.g., Fe²⁺ from bdl to 6.2 mg/L and ΣS²⁻ from 7 to 305 μg/L). The composition of these fluids reflects mixing between an isolated paleometeoric end-member and fresher relatively modern meteoric fluids [2].

Energetics: Variable geochemical composition produces wide-ranging energetic yields for chemolithotrophic microbial metabolisms. Consistent with the canonical electron tower, reactions with O₂, NO₃⁻, and MnO₂ as the electron acceptors are the most energy-yielding when evaluated per mole of electrons. If instead we take into account limiting reactants in the systems and report energy density (J/kg fluid), then S⁰, HS⁻ and Fe²⁺ oxidation reactions are the most exergonic [3].

Cores: The majority of our research at SURF has thus far focused on preexisting (i.e., legacy) boreholes. In 2014, however, we took part in a core drilling operation, and then deployed a UV fluorescence mapping instrument (MOSAIC, a precursor to the Mars 2020 mission flight instrument SHERLOC). MOSAIC was set up underground at the drill site to map the distribution of organics in the rocks as soon as the cores were

extracted from the ground. The instrument detected and mapped organics, especially ringed compounds, but true biological signatures were difficult to identify, possibly due to low (or no?) biomass in these cores. Fresh rock samples were preserved for downstream analyses, including DNA extractions and lab cultivations.

Microbiology: Based on 16S and metagenomic sequencing, the microbial community composition changes from Proteobacteria-dominated near the surface to Firmicutes-dominated at depth; Thaumarchaeota and so-called ‘dark matter’ lineages (especially OP3) were also found at several depths. Analysis of lipid biomarkers, specifically intact polar membrane lipids that include phospholipids, aminolipids, glycolipids, and GDGTs, in porewater and biofilm samples further characterize the microbial communities at SURF. Water and rock samples are being used as inocula to cultivate subsurface organisms with an array of batch and continuous culture techniques. Of particular note is the *in situ* deployment of a bioelectrochemical cultivation platform that relies on physical electrodes that function as electron donors or electron acceptors in microbial respiration. With this technique, we aim to enrich possible chemolithotrophs present at SURF as predicted by our energetic studies.

Synthesis: The *Life Underground* team is combining geochemical, electrochemical, microbiological, and energetic analyses of fluids and rocks at SURF to characterize the habitability of terrestrial subsurface environments. Although Earth’s terrestrial subsurface is vast and diverse, this approach, when applied to more locations, can help to better quantify the habitability of Earth’s deep subsurface. With a more expansive view of ‘intraterrestrial’ life on Earth, we can then better anticipate the types of extraterrestrial locations that could support life.

References: [1] Caddey S. W. (1991) *USGS Bull*, 1857-J, 1-67. [2] Murdoch L. C. et al. (2011) *Hydrogeol J*, 20, 27-43. [3] Osburn M. R. et al. (2014) *Frontiers Microbiol*, 5, doi:10.3389/fmicb.2014.00610.