Establishment of the Deep Mine Microbial Observatory (DeMMO). M. R. Osburn[1*], C. P. Casar[1], B. Kruger[2], T. M. Flynn[3] and J. P. Amend[4], [1]Northwestern University (2145 Sheridan Rd, Evanston, IL [*]maggie@earth.northwestern.edu), [2]Desert Research Institute (Las Vegas, NV), [3]Argonne National Laboratory (Argonne, IL), [4]University of Southern California (Los Angeles, CA).

Motivation: Earth's deep subsurface hosts a diverse microbiome rich in potential for the study of conditions analogous to exoplanetary interiors and corresponding microbial intraterrestrials. Access to the subsurface is often transient, however, and involves major disruptions to the very environments we seek to study. To overcome these challenges we have established a network of six packered boreholes that serve as stable sampling ports at the Sanford Underground Research Facility (SURF), South Dakota, USA. Our network extends from 250-1500 m below the surface, allowing access to deep subsurface fluids across a large range of ages and compositions. By tapping these directly, we are poised to install long-term experimental apparatus and conduct targeted experiments to further characterize the ecology and physiology of Earth's deepest inhabitants.

Methods: The deep subsurface of SURF has been previously shown to contain diverse microbial life and geochemical potential [1, 2]. We selected the DeMMO sites from a subset of previously-studied boreholes while including two new sites, at 610 m and 1250 m, to provide additional coverage across the depth gradient. Each site exhibits consistent water outflow rates, limited evidence for subsurface interaction with mine workings, and is regularly accessible. We capped each borehole using custom designed and machined expandable packers composed of high-density plastic (Delrin) and polyurethane rather than the standard stainless steel manifolds because these latter corrode rapidly in the subsurface, affecting both their lifespan and the geochemical integrity of the associated waters, thereby also affecting native microbial community structure. Prior to packering, boreholes were drilled out and reamed to remove old concrete plugs and create a stable surface against which the packers could seal. Drilling and packer installation was carried out at 3 of 6 DeMMO holes (DeMMO 3, 4, and 5) from May to July 2016. Geochemical and microbiological monitoring of fluids from each site began prior to hole modification and has continued since on an approximately bimonthy basis. Analyses include major cations and anions, redox sensitive solutes (DO, Fe^{2+}, NO_3^-, NH_4^+, S^{2-}), DIC, DOC, dissolved gas composition, water isotopes, and sequencing of 16S rRNA genes of cells filtered from borehole fluids.

Results: Continued monitoring of DeMMO boreholes from December 2015 to December 2016 has identified each site as a unique and stable biogeochemical island.

Geochemistry. Geochemical data reveal consistent fluid compositions at individual stations. Similar compositions of nonreactive ions between sets of boreholes suggest that each pair of sites is hydrologically connected. This observation is consistent with the spatial orientation of the boreholes and expected hydrologic flow paths. There is no apparent seasonal trend in either flow rate or major element chemistry, consistent with the long residence time of these fluids in the subsurface. A gradient of decreasing DOC with depth is consistent with the gradual consumption of surface-derived organic matter by native microbes.

Microbiology. DNA sequencing of borehole fluids revealed significant microbial diversity within the SURF site as well as substantial differences between boreholes. Drilling was found to impart a significant but transient perturbation to the microbial community in a particular borehole. Holes that were not subjected to drilling display a remarkably consistent community composition over the course of months. Significant populations of candidate phyla (e.g. OP3) and deeply divergent strains of known phyla are present at most sites. Of the sequences from characterized phyla, those most closely related to microbes involved in sulfur and iron cycling are well-represented as are relatives of known thermophiles. Continued monitoring of all sites will allow us to evaluate the longer-term impact of drilling on the deep subsurface microbiome as well as further illuminate the connections between geochemistry and microbiology in this unique environment.

Experiments aimed at culturing and characterizing microbial inhabitants are underway at a number of DeMMO sites. In particular, we are interested in replicating the intra-fracture biofilm community by cultivating microorganisms on solid substrates using flow-through columns as well as more traditional approaches using synthetic growth media.

Future directions: We aim to establish collaborations at other deep subsurface research sites, most notably in the South African and Canadian Shields, to establish nodes of DeMMO worldwide.

References: [1] Osburn M. R. (2014) *Frontiers in Microbiology, 5*, 1-14 [2] Momper, et al. (submitted) *Environmental Microbiology Reports*.