FISHING IN THE DESERT: ACCESSING THE DEEP BIOSPHERE THROUGH CONTINENTAL WELLS.

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Introduction: Study of the subsurface biosphere allows for a better understanding of microbial community adaptations to environmental stressors analogous to those found in extraterrestrial environments (temperature, radiation, redox extremes, etc.) and for the beta application of novel life detection and investigation mechanisms. A major challenge in this field lies in obtaining access to subsurface environments without imparting significant anthropogenic disturbance to the system. In an effort to mitigate those anthropogenic influences, the Life Underground node of the NASA Astrobiology program (NAI-LU) has developed a long-term field site at a deep well (~755 m below land surface (mbls)) in the Mojave Desert, BLM-1. Drilled in 2007 and left undisturbed since a high-volume pumping test in 2011, this well taps a vast regional aquifer in fractured Paleozoic carbonates, which is hydrologically distinct from shallower perched aquifers (as demonstrated by unique geochemical and microbial community signatures for each). Our experimental and monitoring activities at BLM-1 started in 2014.

Approach: The research effort at BLM-1 includes aqueous geochemical characterization, investigation of microbial colonization on solid surfaces of varying redox reactivity, assessment of microbial community structure change after carbon source addition and characterization of down-hole community shifts over time (next generation DNA sequencing), characterization of colonized organisms via multiple microscopic and spectroscopic techniques (deep-UV fluorescence microscopy, Raman spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX)), metagenomic characterization of the planktonic community, and development and deployment of a custom designed in-situ pressure sampling device.

Long Term Monitoring Results: Geochemical characterization of BLM-1 fluids reveals an environment that experiences downhole (water surface to max depth) gradients in temperature (28ºC to 57ºC) oxidation-reduction potential (+50 mV to -250 mV), pH (9.2 to 6.7), and conductivity (1000 to 2300 µS). Discrete analysis of select dissolved metals, major ions, and carbon species at two depths (579 mbls and 755 mbls) reveal unique zones of inorganic and organic carbon availability, redox conditions, and concentrations of conservative ions. In comparison, concentrations of major conservative ions are much lower at a shallower well nearby (BLM-1a, ~200 mbls max depth) which accesses an overlying alluvial aquifer. In addition, various redox-sensitive dissolved gases, including methane, carbon dioxide, and hydrogen, were identified at BLM-1 fluids. Note that the role of hydrogen in this system may be enhanced by its production resulting from corrosion of the low-carbon steel casing that lines the well to a depth of ~750 mbls.

Using next generation DNA sequencing (Illumina, v4 region, 16S rRNA gene), we are conducting long term monitoring of downhole microbial communities that colonize synthetic sponge material. Numerous incubations of sponges (3-6 months at a time) along a line extending from the surface to the bottom of the hole support remarkably consistent microbial communities at the discrete depths through time. Carbon addition, in the form of sterile collagen-rich sponges, at 580 and 750 mbls resulted in a spike in relative abundance of Candidate Division OP8 and Thermotogae; their abundance, however, diminished over time. One OTU with >99% sequence similarity to the 16S rRNA gene of *Candidatus* Desulforudis audaxviator was observed at relative abundances approaching 30% in planktonic samples from 750 mbls. While, in general, major composition of sponge-colonized communities were very similar to that in discretely sampled planktonic communities over the course of this monitoring, this composition differs substantially from historically analyzed planktonic populations obtained during 2007 and 2011 pumping tests. The two spatially distinct aquifers accessed by BLM-1 and BLM-1a revealed distinctly different microbial community compositions on incubated sponges.