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"Who Gives a Frack? Life in the Deep Shale Biosphere"

Abstract: The terrestrial deep biosphere is one of the least explored and understood ecosystems on Earth. Although deep shale formations have been previously shown to harbor microbial life, there is a paucity of information on how microorganisms adapt and make a living in such environments. Here, we have leveraged natural gas extraction activities to gain access to samples from these environments (~2000 m depth), where microbial activities can aid in methane recovery, contribute to well souring, and catalyze infrastructure corrosion. Hydraulic fracturing, colloquially known as "fracking", is employed for effective gas and oil recovery in deep black shales, and exposes microorganisms to a range of extreme environmental conditions including high pressures, elevated temperatures, chemical additives and biocides, and brine-level salinities. In this talk, I will discuss our use of assembly-based metagenomics to create a metabolic blueprint of the microbial community from energyproducing Marcellus shale wells over a 328-day period. Using this approach we ask the question: "What abiotic and biotic factors drive microbial metabolism and thus biogeochemical cycling during energy extraction?" We found that after 49 days, increased salinity in produced waters corresponded to a shift in the microbial community. Only organisms that encode adaptations to high salinities are detected after this point. We posit the increased salinity (and the thermodynamic constraints it causes) ensure biogenic methane is derived from C1 methyl compounds and not from hydrogen or acetate. Beyond abiotic constraints, our genomic investigations revealed that viruses

target key members of the microbial community, potentially impacting the biogeochemistry of the system. This predation is likely significant, such that the persisting and dominant microbes all encode a CRISPR-mediated viral defense system. Collectively our genomic results indicate that adaptation to high salinity, metabolism in the absence of oxidized electron acceptors, and viral immunity are controlling factors mediating microbial metabolism during hydraulic fracturing of the deep subsurface.