

Probing the Deepest Limits of the Earth's Biosphere

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Several important questions regarding the deep continental biosphere remain largely unanswered, including: 1) What factors control the lowest depth limit of life? 2) What are the sources of carbon for deep life? and 3) What processes regulate the energy flux for deep life? Opportunities to address these important questions are limited by the cost of drilling to great depth. As an alternative to drilling from the surface, we've been sampling extremely deep fracture water (1-4 km) in the Kaapvaal Craton of South Africa via boreholes that are already in place in gold, diamond, and platinum mines due to mining activities. The craton's geothermal gradient of 9 to 25°C/km, enables mining at these depths. We have found that 1) cell turnover times are ~1-2 years at 3 km and 55°C, 2) deep saline fracture waters, ~2.0 Ga, are rich in radiolytically generated H₂ and abiogenic hydrocarbons, 3) H₂ flux is stimulated by seismic activity, 4) CH₄ and CO₂ are the primary carbon sources for microbial ecosystems, and 5) more complex organic C appears to be generated in situ by microbes growing on these energy sources and carbon substrates; plant- and soil-derived dissolved organic carbon appears to be absent. Culture-independent analyses (16S rDNA pyro-tag sequencing, metagenomic sequencing, transcriptomic sequencing, single cell genome sequencing, and proteomics) have revealed indigenous, novel, anaerobic microbial communities. Primary producers are chemoautotrophs, including hydrogenoclastic methanogens and sulfate reducing bacteria (SRB). ANME sequences suggest that some methanogens carry out anaerobic methane oxidation. SRB include *Candidatus Desulforudis audaxviator*, which appears to be a cosmopolitan subsurface dweller, having also been detected in other deep fracture waters, e.g., in the Great Basin Desert, Finland, and off axial marine ridge basalt. Sulfur-oxidizing denitrifiers metabolize sulfide generated by SRB. Collectively, these findings support the existence of an actively metabolizing H₂-driven subsurface lithoautotrophic microbial ecosystem (SLiME) functioning entirely independently from life at Earth's surface, i.e., from photosynthetic products.