METAGENOMIC INVESTIGATION OF THE UNIQUE ROPE-LIKE MICROBIAL COMMUNITIES OF THE FRASASSI CAVE SYSTEM.
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Abstract: The subsurface environment of Mars and Europa is a possible refuge for microbial life and should be targeted for future exploration. On Earth, microbial life can thrive hundreds to thousands of meters below the surface, and this deep biosphere likely hosts novel microbial taxa and unique metabolic adaptations. Caves represent windows to Earth's subsurface that are relatively accessible to human exploration, and could also represent a target for initial subsurface exploration on extraterrestrial bodies.

Expeditions into the Frasassi cave system, Italy, have revealed rope-like microbial communities in the anoxic waters of stratified cave lakes. These unusual microbial formations occur in multiple seemingly-disconnected locations throughout the cave system. The biofilms contain diverse microbial communities dominated by *Deltaproteobacteria*, *Chloroflexi*, and *Planctomycetes*, and have similar community composition despite different morphologies and locations. Metagenomics, bulk isotope fractionation, and geochemistry of one of the rope-like biofilms suggests that carbon fixation via the Wood-Ljungdahl pathway is a possible mechanism for primary production in the anoxic ecosystem. Metagenomic evidence suggests members of the *Deltaproteobacteria* and *Chloroflexi* have the capacity for carbon fixation, and that sulfate reduction coupled to hydrogen or organic carbon oxidation seems to be a dominant energy metabolism in the community. Attempts at fluorescence *in situ* hybridization (FISH) indicated low microbial activity in the communities, and ongoing research using metatranscriptomics will further explore active microbial populations and processes in the biofilms. A deeper understanding of microbial life strategies in the energy-limited, anoxic Frasassi ecosystem provides important insight into analogous ecosystems that would have been more prevalent during certain periods of Earth's early history.