Isotopic evidence for carbon fixation within a lava tube system

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The cycling of C by microbes in the subsurface is a critical component of biogeochemistry on a global scale. Chemolithoautotrophs, microbes that couple inorganic redox metabolisms to carbon fixation, inhabit and are active in the deep subsurface biosphere. However, the contribution of chemolithoautotrophy to the shallow subsurface C cycle is thus far poorly constrained. Lava tubes are accessible conduits through which basalt-hosted biogeochemistry in the shallow subsurface can be directly studied. The walls of these caves are enriched in redox sensitive elements, such as S or Fe, which serve as energy sources for chemolithoautotrophs.

Here, we use compound-specific stable isotope analysis to explore *in situ* C fixation by chemolithoautotrophs in the lava tubes of Lava Beds National Monument, CA. Intact polar lipid (IPL)-derived fatty acids originate from the membranes of living cells. IPL fatty acid profiles provide coarse information about source organisms and their contribution to living biomass. Crucially, comparing their $\delta^{13}C$ values to potential C source reservoirs can elucidate whether C fixation is actively performed by lava tube communities.

IPL fatty acid concentrations and their $\delta^{13}C$ values were determined for a variety of lava tube features, such as biofilms, oozes, cave polyps, and secondary minerals, in addition to surface soil. Extracted IPLs were derivatized into fatty acid methyl esters (FAMEs), which were characterized and quantified by GC-MS. IPL FAME-specific $\delta^{13}C$ values were determined via GC-IRMS and were compared to isotopic measurements of bulk inorganic and organic C phases.

Unlike soils and other cave features, lava tube biofilms are dominated by branched and *trans*-unsaturated IPL FAMEs, indicative of bacterial biomass. These IPL FAMEs from biofilms exhibit the most negative $\delta^{13}C$ values (down to -47.8±0.2‰ vs. VPDB) and are significantly depleted in ^{13}C relative to other cave samples and inferred C source reservoirs. These patterns are reflected in total organic C (TOC) $\delta^{13}C$ values across the studied features. From these data, isotopic mass balance calculations suggest the fixation of C by microbial communities within lava tube biofilms. These results have implications for understanding the role of shallow subsurface chemolithoautotrophy on broader C biogeochemistry.