Cryptic Chemosynthetic Pathways Support an Ecosystem within Subterranean Karst Estuaries

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Subterranean karst estuaries extend inland into density-stratified coastal aquifers that contain distinct mixtures of meteoric freshwater and saline groundwater. The underground water masses are separated by chemoclines creating sharp interfaces between oxic and anoxic sub-habitats. Access through flooded cave passages provided the basis for discovering a surprising diversity of endemic animals (the anchialine fauna) in this highly oligotrophic subsurface environment. The Caribbean coast of Mexico's Yucatan Peninsula contains over 1,000 km of mapped cave passages, the densest accumulation of coastal caves and the largest known continuous anchialine habitat in the world. Our initial investigation of this subsurface ecotone reveals high concentrations of methane (~ 6,500 nM) and dissolved organic carbon (DOC; ~ 700 µM), including methanol (< 100 µM) and ethanol (< 40 µM). Moreover, stable isotope-based evidence from cave-adapted shrimp and water shows that a microbial loop supports the food web with methane and DOC as the primary sources of energy and carbon. 16S rRNA gene amplicon sequencing and respiratory quinone analysis reveals the presence of a diverse microbial community capable of mediating heterotrophy, methanotrophy, methylotrophy and chemoautotrophy. Microbes that mediate chemoautotrophic carbon fixation include methanogens, sulfur-oxidizers, sulfate-reducers and ammonia-oxidizers represented by taxa of both marine and terrestrial origins. Our observations suggest that subterranean karst estuary habitats hold promising opportunities for studying diverse biogeochemical processes, including cryptic chemoautotrophic pathways, and their importance in supporting the globally distributed anchialine fauna.