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Methane and Dissolved Organic Carbon Sustain an Ecosystem within a Density Stratified Coastal Aquifer of the Yucatan Peninsula, Mexico. Evidence for a Subterranean Microbial Loop?

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In coastal karst terrains, anchialine caves that meander in density stratified aquifers provide an exceptional opportunity for scientists to study *in situ* biogeochemical processes within the groundwater. The Caribbean coast of Mexico's Yucatan Peninsula contains over 1000 km of mapped cave passages, the densest known accumulation of anchialine caves in the world. A decades-old study based on the simple observation of ^{13}C-depleted biomass in the cave-adapted fauna suggested biogeochemical processes related to methane-linked carbon cycling and/or other chemoautotrophic pathways as a source of energy and carbon.

In this study, we utilized cave diving and a novel sampling device (the Octopipi) to obtain cm-scale water column profiles of methane, DOC and DIC concentrations and stable carbon isotope ratios to identify the energy sources and microbial processes that sustain life in these subterranean estuaries. High concentrations (up to 9522 nM) low-δ^{13}C (as low as -67.5 permil) methane near the ceiling of the cave (in the fresh water section of the stratified water column) and evidence for methane oxidation in the brackish water portion of the water column suggest methane availability and consumption. Profiles obtained by the Octopipi demonstrate that virtually all of the methane ($\sim$99%) is oxidized at the interface of anoxic freshwater and hypoxic brackish water masses. The high-methane water mass near the ceiling also contained elevated concentrations of DOC (851 μM) that displayed comparatively high δ^{13}C (-27.8 to -28.2 permil), suggesting terrestrial organic matter input from the overlying soils. Low-methane brackish and saline water was characterized by lower DOC concentration (15 to 97 μM), yet with similar δ^{13}C (-25.9 to -27.2 permil), suggesting significant terrestrial organic matter consumption or removal with increasing depth, from fresh to saline water, within the water column. The presence of ^{13}C-depleted fatty acids (e.g., C16:1ω7c with δ^{13}C-values as low as -54.1 permil) and deuterium-depleted δD values (e.g., as low as δD = -225 permil) from tissues of cave-adapted shrimps suggest that methanotrophic bacteria contributed a substantial fraction of their diet. Molecular microbial community analyses are underway to identify the taxonomic associations and syntrophy effects within a subterranean microbial loop that provides carbon and energy to the anchialine food web. These findings provide novel insight into the carbon cycle and methane dynamics for a largely unknown, yet widespread coastal habitat beneath the Earth's surface.