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Assessing the effect of nitrate on microbial communities in water filled karst caves.

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Extensive surface water-groundwater interactions in karst aquifers result in dynamic water-rock interactions, form extensive cave networks and impact biogeochemical cycling. Nitrate (NO_3^-) contamination is an expanding concern in the Upper Floridan Aquifer, one of the most productive freshwater ecosystems in the world where water discharges from over 1,000 springs. Over the last 40 years, there has been a ~50-fold increase in NO_3^- due to agricultural runoff which accounts for 60-70% of nitrate contamination. Elevated NO_3^- levels can alter groundwater chemistry, including dissolved oxygen (DO) availability, potentially affecting microbial community structure and function. In this study, we tested the hypothesis that the concentration of groundwater NO_3^- affects microbial communities in both groundwater and associated with sediments and minerals. Here, we study caves in one of the most impacted watersheds in the UFA, the Suwannee River Basin (SRB), which has springs with groundwaters that range from 0.66 to 4.36 mg/L NO_3^-. Our analysis identified 69 bacterial phyla, 13 archaeal phyla and an estimated 22,903 individual taxa across the 7 cave and groundwater communities analyzed. Additionally, non-metric multidimensional scaling (NMDS) showed that community composition varied significantly across sites, with distinct assemblages associated with groundwater, clay and coarse-grained sediments, goethite and other Fe-oxide minerals, and carbonate rocks. DO and NO_3^- are strongly negatively correlated (Spearman correlation, $r = -0.61$, $p < 0.0001$), weaky correlated to diversity and richness ($r < 0.3$) and explained the largest amount of variability in the microbial communities (PERMANOVA, 20.8% and 15.1%, respectively). The distinct microbial community structures we have documented in UFA cave environments emphasizes the need for physiological observations that can determine the impact of NO_3^- on biogeochemical cycling of nitrogen and carbon in karst aquifers.