## Distinct Depth-discrete Profiles of Microbial Communities and Geochemical Insights in the Subsurface Critical Zone

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Microbial assembly and metabolic potential in the Subsurface Critical Zone (SCZ) is substantially impacted by sediment geochemical gradients and subsurface hydrogeology, selecting for microbes distinct from those in surface soils. Hydraulic conditions are one of the key drivers influencing microbial communities in the SCZ, causing changes in bioavailability of substrates, and resulting in distinct microbial diversity, and activity here as compared to the vadose zone above.

In this study, we coupled metagenomics and geochemical analyses to elucidate how microbial community composition and metabolic potential are shaped and impacted by geochemistry in the SCZ of background site at Oak Ridge Field Research Center in Oak Ridge, Tennessee. Six sediment core segments were taken at different depth, including the shallow subsurface (0.1-0.9 m below ground surface (bgs)), vadose zone (0.9-1.5 m, 1.5-2.4 m, 2.4-3.0 m bgs), capillary fringe (3.0-4.0 m bgs), and saturated zone (4.0-4.6 m bgs). Our results demonstrate that the subsurface microbes are highly localized and communities are rarely inter-connected. The community composition varies even over very short vertical distances. and are strongly correlated, or "co-vary" with the differences in a subset of environmental variables including pH, CEC, TOC, DOC, TN, nitrate, P, and five metals. FT-ICR MS results indicate that the SCZ is generally a low-carbon environment with a recalcitrant DOC pool. The relative abundance of recalcitrant carbon such as condensed aromatic-like compounds increases from 3.4% in the shallow subsurface to 12.3% in the saturated zone, selecting microbes from copiotrophs to oligotrophs. Archaea (Euryarchaeota, Thaumarchaeota, Crenarchaeota) and members of the phyla Acidobacteria, Chloroflexi, GAL15 and Rokubacteria predominate, likely equipped of utilizing recalcitrant C. This strongly suggests that sediment geochemistry is vital in selecting distinct microbial communities in the SCZ- a dynamic and critical zone that plays an important role in ecological carbon and nitrogen cycles.