Title: Metagenomic Insights into Key Nitrogen-Cycling Microbial Taxa within the Terrestrial Subsurface at Riverton, WY

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Project Abstract: Naturally-reduced zones (NRZs), formed within contaminated DOE legacy site floodplains in the upper Colorado River Basin, contain large inventories of nitrogen (N) and uranium (U). Microbial N-cycling processes, like nitrification and denitrification, have the capability to "unlock" the biogeochemical nutrient supply stored within NRZs, drive carbon cycling, and liberate U to the aquifers. However, despite their biogeochemical importance, remarkably little is known regarding N-cycling microbial communities within terrestrial subsurface sediments, let alone NRZs. To address this knowledge gap, we have performed detailed metagenomic analysis of microbial communities within sediment samples collected from the Riverton, WY subsurface. In particular, we have employed cutting-edge 'binning' approaches to generate metagenome-assembled genomes (MAGs) from 12 subsurface depths within a ~2.4 m sediment depth profile. This resulted in the generation of 100s of draft- to high-quality MAGs, many of which correspond to N-cycling taxa. Since our recent work highlighted the importance of ammonia-oxidizing archaea (AOA) in the Riverton subsurface (based on N-cycling functional genes and 16S rRNA amplicon sequencing), a major goal of this project was to definitively link key AOA functional genes (e.g., *amoA*, *nirK*, etc.) to their corresponding 16S rRNA sequences through the analysis of thaumarchaeal MAGs. Our binning efforts resulted in over 20 distinct thaumarchaeal MAGs spanning 11 different depths. Phylogenetic analysis of these genomes, based on ribosomal proteins and multiple functional genes, revealed truly remarkable AOA diversity. Interestingly, comparison of these MAGs to reference genome sequences revealed that, while some genomes were most closely related to known terrestrial AOA (e.g., *Nitrososphaera*, *Nitrosocosmicus*), others either represent completely novel lineages or are most closely to AOA from aquatic and even marine environments. We also observed islands of AOA gene clusters unique to Riverton MAGs. Interestingly, pangenome analysis revealed a clear distinction between AOA genomes derived from 'above' versus 'below' the water table, highlighting the underappreciated importance of hydrology in controlling AOA distribution and diversity in this environment. Finally, several nitrite-oxidizing bacterial genomes (e.g., *Nitrospirota*) were also obtained (containing *nxr* genes), which likely oxidize the nitrite produced by AOA to nitrate in these sediments. Overall, this project is yielding unprecedented genomic and ecophysiological insights into the microbial communities responsible for N-cycling, and especially nitrification, in a terrestrial subsurface environment that is directly influenced by hydrological fluctuations.