

## Metagenomic characterization of subsurface Thaumarchaeota populations in hydrologically-variable floodplain sediments

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The terrestrial subsurface microbiome contains vastly underexplored phylogenetic diversity and metabolic novelty, with critical implications for global biogeochemical cycling. Among key microbial inhabitants of subsurface soils and sediments are Thaumarchaeota, an archaeal phylum encompassing the ammonia-oxidizing archaea (AOA). Although AOA have been studied extensively in topsoils, our understanding of the diversity, ecophysiology, and activity of these critically important nitrogen-cycling organisms in deeper soils/sediments is limited. Our recent analysis of subsurface microbial communities (based on both 16S rRNA and *amoA* ammonia monooxygenase genes) within hydrologically-variable floodplain sediments in the Wind River Basin near Riverton, WY revealed that AOA were the predominant ammonia-oxidizers and that their community structure shifted dramatically with depth. In order to understand the ecophysiological adaptations of these subsurface AOA, we used genome-resolved metagenomics to examine Thaumarchaeota populations spanning 11 distinct depths along a 234 cm depth profile at Riverton site KB1. Phylogenomic analysis of metagenome-assembled genomes (MAGs) indicated a pronounced shift in AOA populations: in the well-drained top ~100 cm of the profile, the ‘terrestrial’ Nitrososphaerales lineage was dominant; whereas in deeper, moister, oligotrophic sediment layers, members of the typically ‘marine’ Nitrosopumilales lineage were most pervasive. This vertical zonation in thaumarchaeal population structure was similarly evident in the relative abundances of lineages, estimated based on read mapping against MAGs. Our results suggest that hydrological variables, particularly proximity to the water table, impart a strong control on the ecophysiology of Thaumarchaeota in alluvial sediments. To complement our detailed spatial (vertical) characterization of AOA communities at site KB1, we recently investigated subsurface microbial community composition over both time and space (depth) at a nearby Riverton site (Pit2) through a full seasonal hydrologic cycle of water table rise, flooding, and summer drought. In particular, we obtained 16S rRNA gene amplicon data from samples collected monthly (April to September 2017) from 7 distinct subsurface layers [topsoil, evaporite, sand, evaporite/clay, transiently-reduced zone (TRZ), clay, and aquifer], allowing spatiotemporal AOA dynamics to be examined within the context of the overall microbial communities and a suite of hydrogeochemical measurements. We also selected 40 of these Pit2 samples for metagenomic sequencing, which has resulted in the generation of >3000 high-quality MAGs, many of which correspond to diverse AOA as well as nitrite-oxidizing bacteria. Overall, this study is yielding unprecedented genomic and ecophysiological insights into the microbial communities responsible for nitrification in subsurface floodplain sediments directly influenced by hydrological fluctuations.