

Remarkable diversity of metagenome-assembled genomes from key ammonia- and nitrite-oxidizing organisms recovered from hydrologically-variable floodplain sediments

Christopher Francis^{1*}, Bradley Tolar¹, Linta Reji¹, Emily Cardarelli¹, John Bargar², Kristin Boye², and Nicholas Bouskill³

¹ Department of Earth System Science, Stanford University, Stanford, CA

² SLAC National Accelerator Laboratory, Menlo Park, CA

³ Lawrence Berkeley National Laboratory, Berkeley, CA

Contact: (caf@stanford.edu)

Project Lead Principal Investigator (PI): Christopher Francis

BER Program: ESS

Project: University project DE-SC0019119

Project Abstract: Subsurface microbial communities mediate biogeochemical transformations that drive both local and ecosystem-level cycling of essential elements, including nitrogen (N). Two functional guilds of chemoautotrophic microorganisms are responsible for the first oxidative step of the N cycle, nitrification: ammonia-oxidizing archaea (AOA) and bacteria (AOB) catalyze the oxidation of ammonia to nitrite, while nitrite-oxidizing bacteria (NOB) oxidize nitrite to nitrate. Despite the critical role nitrification plays in N-cycling and removal in terrestrial ecosystems, our understanding of the diversity, ecophysiology, and activity of nitrifying organisms in deeper soils/sediments is quite limited. To address this knowledge gap, our recent work examined the phylogenetic diversity and metabolic potential of subsurface ammonia-oxidizing Thaumarchaeota lineages in hydrologically-variable floodplain sediments in the Wind River Basin near Riverton, WY. Metagenomes obtained from 11 discrete depths along a ~2-m sediment profile at site KB1 yielded diverse Thaumarchaeota MAGs with distinct functional potential. Particularly notable was the shift in phylogenetic identity with depth, which appeared to be linked to soil moisture as well as C:N content. The predominantly ‘terrestrial’ *Nitrososphaerales* were dominant in the top, well-drained (dry) layers with relatively higher total C (and lower C:N), while the typically ‘marine’ *Nitrosopumilales* dominated the deeper, moister layers, including the capillary fringe where total C and N were lowest. All AOA MAG clusters shared the genomic potential for ammonia oxidation (e.g., AMO, NirK) and CO₂ fixation (e.g., 4-hydroxybutyryl-CoA dehydratase); however, surface soils were dominated by relatively more ‘generalist’ AOA capable of utilizing various organic compounds (e.g. urea, cyanate and nitriles), whereas ‘oligotrophic’ AOA lineages became prominent in deeper, moister layers.

We have also examined temporal changes in nitrifying communities within the soil/sediment column at a nearby Riverton site (Pit2), capturing a full seasonal floodplain hydrologic cycle of water table rise, flooding, and summer drought. Genome-resolved metagenomic analysis of these samples yielded 100 MAGs belonging to AOA (Thaumarchaeota) and 22 attributed to major NOB clades (*Nitrospiraceae*, *Nitrospirales*). Although some overlap was observed in AOA MAGs recovered from Riverton sites KB1 and Pit2, a surprisingly large number of AOA MAGs were found exclusively within Pit2 metagenomes; intriguingly, many of these were closely related to marine/estuarine *Nitrosopumilus* and *Nitrosarchaeum* species, as well as MAGs from estuarine sediments, suggesting adaptations to saline conditions may be shared among these AOA. Overall, this study is yielding unprecedented genomic and ecophysiological insights into subsurface nitrifying communities, over both time and space, in floodplain sediments directly influenced by hydrological fluctuations.