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Topic: Applying -omics approaches to understand microbial functioning

Metagenomics and single-cell genomics reveal high abundance of comammox *Nitrospira* in a rapid gravity sand filter treating groundwater.

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The recent discovery of complete ammonia oxidizing (comammox) Nitrospira has revealed that the metabolic division of labor in nitrification is not obligate as was assumed during the last century. Despite the detection and enrichment of commamox Nitrospira from different nitrifying environments, the ecological relevance of comammox remains unknown. In this study, we analyzed the microbial communities from various locations within a groundwater-fed rapid sand filter (RSF), where Nitrospira were at very high relative abundances. Through metagenomics, a highly abundant composite multi-genome of Nitrospira genus was recovered harboring metabolic capacity for complete ammonia oxidation. We developed a cell extraction strategy that enables the disruption of *Nitrospira* cell clusters attached to the mineral coating of the sand. Individual cells were identified via fluorescent in situ hybridization (FISH) with Nitrospira-specific 16S rRNA probes and sorted via fluorescence-activated cell sorting (FACS). Sorted cells were screened and selected Nitrospira spp. were subject to whole-genome sequencing. The single cell genomes confirmed the genomic presence of a complete ammonia oxidation pathway and revealed clear taxonomic differences with the recently described comammox *Nitrospira* genomes. The high abundance of comammox *Nitrospira* spp. together with the low abundance of canonical ammonia oxidizing prokaryotes in the investigated RSF system suggests the essential role of this novel comammox Nitrospira in the RSFs and potentially other nitrifying environments.