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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 comammox *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.