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## Investigating comammox *Nitrospira* in rapid sand filters via metagenomics and single-cell genomics

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### Introduction

Throughout the last century, nitrification was assumed to be a two-step process executed by two different functional groups, ammonia oxidizing prokaryotes (AOP) and nitrite oxidizing bacteria (NOB). Recently, several articles have shown the capability of a single microorganism (belonging to the *Nitrospira* genus) to carry out the complete oxidation of ammonia to nitrate (comammox) (Daims *et al.*, 2015; van Kessel *et al.*, 2015; Pinto *et al.*, 2015, Palomo *et al.*, 2016). Despite the detection and enrichment of comammox *Nitrospira* from different nitrifying environments, the ecological relevance of comammox remains unknown. Nitrification is often the dominant microbial process in rapid gravity sand filters (RSF), which are engineered microbial systems used for the production of drinking water. High abundance of *Nitrospira* and unusual *Nitrospira*/AOP ratios have been observed in these systems (Albers *et al.*, 2015; LaPara *et al.*, 2015, Gülay *et al.*, 2016). In this study, we applied a combination of metagenomics and single-cell genomics to microbial communities from various locations within an aerated groundwater-fed RSF, where *Nitrospira* are present at high relative abundance (Gülay *et al.*, 2016).

### Material and Methods

Genomic DNA was extracted from six different locations within a groundwater-fed rapid sand filter. DNA was sequenced using Illumina HiSeq 2000 giving 100 bp paired-end reads. High quality reads were assembled using IDBA-UD. Contigs larger than 1,000 nucleotides were clustered into putative taxonomic groups based on pentanucleotide signatures using VizBin. *Nitrospira* single cell sorting was achieved using an in-house developed cell extraction strategy that enabled the disruption of *Nitrospira* cell clusters attached to the mineral coating of the sand. Extracted cells were identified via fluorescence *in situ* hybridization (FISH) with *Nitrospira*-specific 16S rRNA probes and were sorted via fluorescence-activated cell sorting (FACS). Genomic DNA of sorted cells was amplified with multiple displacement amplification (MDA), and screened using *Nitrospira*-specific 16S rRNA gene primers. Selected *Nitrospira* genomes were subject to whole-genome sequencing.

### Results and Conclusions

From the metagenomic data set, fourteen draft genomes were reconstructed and functionally characterized. The organisms represented by draft genomes had the capability to oxidize ammonium, nitrite, hydrogen sulfide, methane, potentially iron and manganese as well as organic compounds. Interestingly, a highly abundant microdiverse composite genome of the *Nitrospira* genus was recovered harboring the genetic potential for complete ammonia oxidation. To evaluate the potential relevance of comammox *Nitrospira* in the removal of ammonium within the studied RSF, we investigated the phylogenetic diversity and abundance of *amoA* as a marker gene of ammonium oxidation. The RSF metagenome *amoA* sequences separated into 4 different clusters. The majority of the *amoA* genes were associated with comammox *Nitrospira* (81% and 52% in top and bulk). Seven and 1% (top and bulk filter, respectively) of *amoA* genes were related to a cluster of canonical beta-proteobacterial AOB.

AOA-associated *amoA* were rarely detected, making up less than 1% in top and bulk samples. *amoA* associated with heterotrophic ammonia oxidizers made up 12% and 46% of all *amoA* (top and bulk filter, respectively). (Figure 1). Cell extraction from sand samples followed by FACS and single-cell sequencing enabled assembly of *Nitrospira* genomes from the RSF (Figure 2). Single cell genomes confirmed the presence of a complete ammonia oxidation pathway in *Nitrospira* and revealed clear taxonomic differences between comammox *Nitrospira* in the investigated RSF and other recently described comammox *Nitrospira* genomes.

The high abundance of comammox *Nitrospira* spp. and the dominance of *Nitrospira*-associated *amoA* sequences in the metagenome suggest an essential role in ammonia oxidation of this novel comammox *Nitrospira* in the investigated RSF.

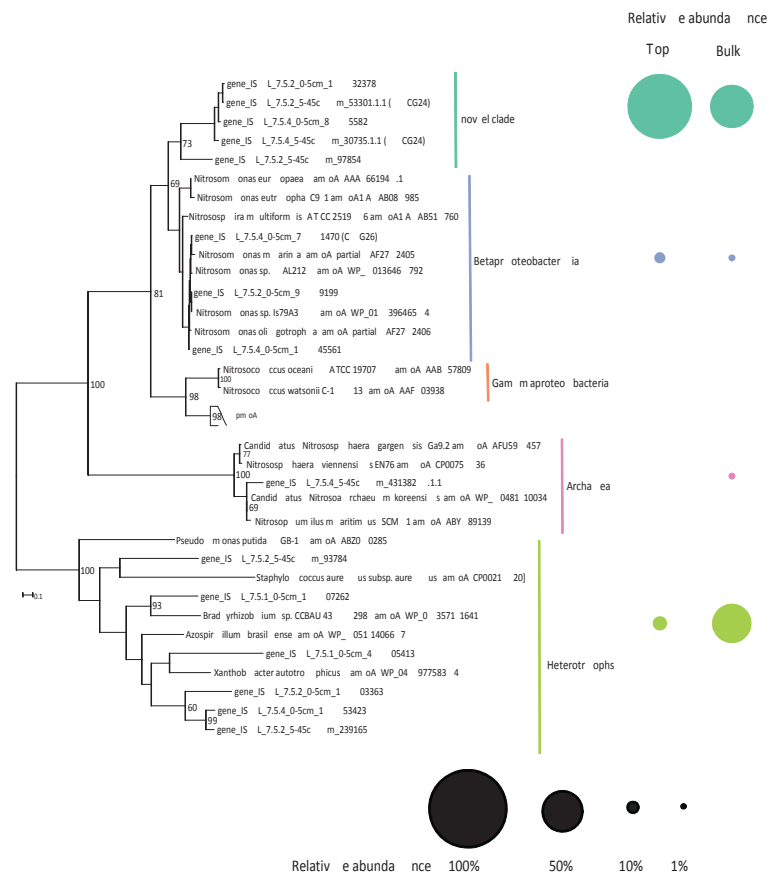


Figure 1. Phylogenetic reconstruction and abundance of *amoA* sequences recovered from the metagenomes (bold).

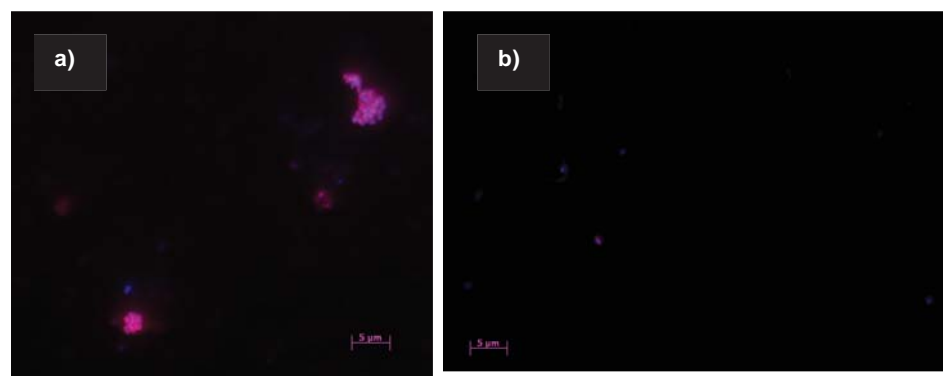


Figure 2. FIHS images showing *Nitrospira* clusters (a) and single cells (b) (in pink).

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