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Identifying novel nitrifying bacteria in rapid gravity sand filters using stable isotope probing

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Introduction

Nitrification is often the dominant microbial process in rapid gravity sand filters (RSF), which are used as the sole treatment of aerated groundwater to produce drinking water in Denmark. Near complete removal of ammonium and nitrite is required due to strict regulatory limits that enable high water stability in the distribution system. RSFs are a unique environment harboring diverse microbial communities including a range of nitrifying bacteria; Betaproteobacterial ammonia oxidizers (*Nitrosomonas*, *Nitrospira*; AOB), ammonia oxidizing archaea (AOA), diverse heterotrophs potentially capable of ammonia and/or nitrite oxidation and a large fraction of *Nitrospira* spp., recently shown to comprise both nitrite oxidizers (NOB) as well as comammox *Nitrospira* spp. (Gülay et al., 2016, Palomo et al., 2016). We sought to develop a better understanding of the autotrophic microbes involved in nitrification in RSFs using DNA and RNA stable isotope probing (SIP) based on ¹³C-labeled bicarbonate incorporation.

Material and Methods

A lab-scale column system that mimics full-scale RSF characteristics (Tatari et al., 2013) was subject to defined loadings of ammonium or nitrite as sole energy sources. Loadings were informed by full-scale conditions. Eight sand-packed columns were fed with either ammonium or nitrite and labelled or unlabeled bicarbonate spiked in effluent water from the parent full-scale RSF. To enable the separate identification of ammonia and nitrite oxidizers, allylthiourea (ATU) and chlorate were applied in ammonium fed columns to attempt inhibition of ammonium oxidation and nitrite oxidation, respectively. Columns were run for 15 days, then sampled and subject to DNA and RNA extraction followed by ultracentrifugation and fractionation to separate labelled and unlabeled DNA extracted from each column. 16S rRNA and 16S rRNA genes from gradient fractions were amplified using universal bacterial primers and subject to Illumina tag-based sequencing. Sequencing data underwent quality control and OTU clustering, and the microbial communities were analyzed for changes in OTU distribution across fractions between ¹³C-incubated and control samples to identify organisms assimilating HCO₃⁻ during ammonium or nitrite oxidation.

Results and Conclusions

Our analysis revealed the assimilation of HCO₃⁻ during nitrification by phylogenetically diverse organisms. Autotrophic carbon uptake during ammonium oxidation within the filters was associated with both *Nitrosomonas* spp. and Thaumarchaeota (AOA), as well as *Nitrospira*, *Herbaspirillum*, *Comamonas*, *Xanthomonadaceae*, *Acidovorax*, *Janthinobacterium*, *Halomonas*, *Pseudomonas*, and *Methylobacterium*. Nitrite oxidation was

associated with *Nitrospira*, clade OM27, *Woodsholea*, *Methylobacter*, *Aquabacterium*, *Xanthomonadales*, and *Hyphomicrobium*. A number of the organisms that were found to take up the ^{13}C label during nitrification, such as *Herbaspirillum*, *Xanthomonadaceae*, *Pedobacter* and *Janthinobacterium* have not previously been associated with nitrification and are typically characterized as heterotrophs. It is not clear whether these species are capable of autotrophy or nitrification, or if they are cross-feeding on labelled carbon products produced by true nitrifiers. Importantly, our results support the previous observation from metagenomic sequencing that certain *Nitrospira* spp. present in the RSFs are capable of complete ammonia oxidation. The detection of strong labelling of this clade during both ammonium and nitrite oxidation further support this observation.

Our results reveal that nitrification may be carried out by more diverse organisms than previously realized, in particular comammox *Nitrospira*, which are abundant in this system, but also by poorly characterized AOs that are typically viewed as heterotrophs. Ongoing work aims to elucidate the ecophysiology of different groups of ammonia oxidizers in RSFs to provide a more complete understanding of nitrification.

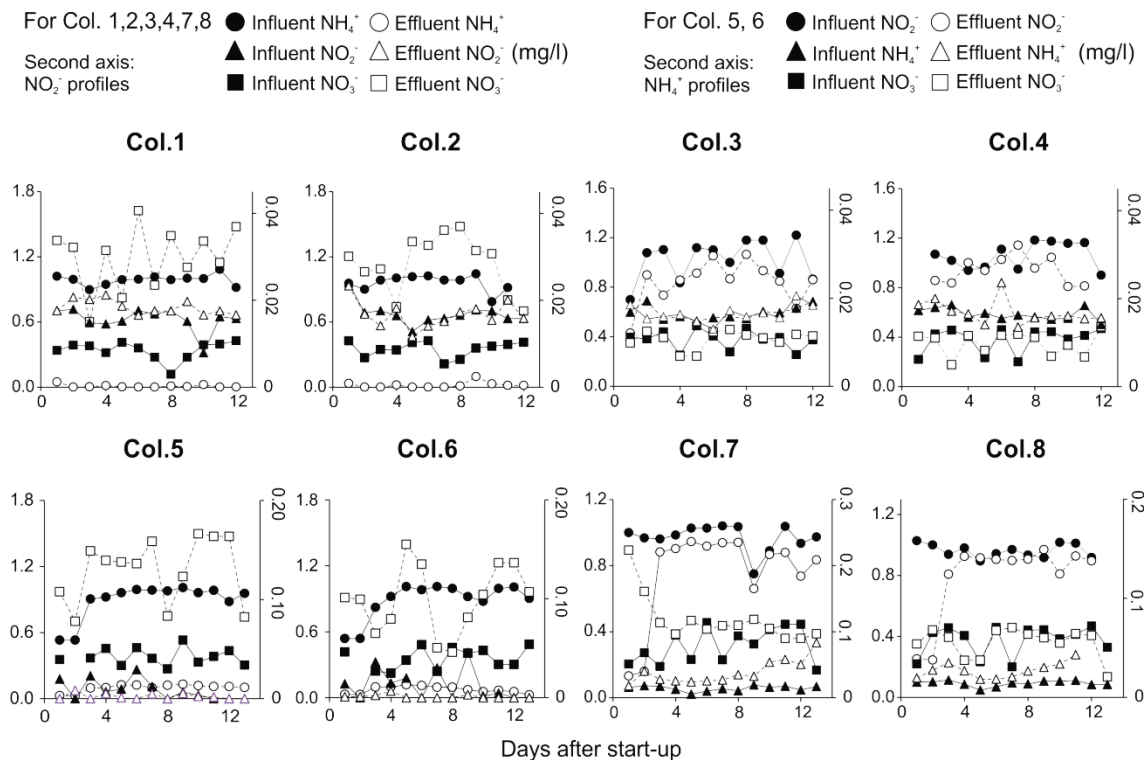


Figure 1. Concentrations of influent and effluent NH_4^+ , NO_2^- , and NO_3^- in control and $\text{H}^{13}\text{CO}_3^-$ fed columns. Columns 1-4, 7-8 were fed with ammonium and labelled (1, 3, 8) or unlabelled (2, 4, 7) HCO_3^- . Columns 3 & 4 include ATU, Columns 7 & 8 include chlorate. Columns 5 & 6 were fed with nitrite and labelled (5) or unlabelled (6) HCO_3^- .

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