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Comammox *Nitrospira* are key nitrifiers in diverse groundwater-fed drinking water filters

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Nitrification is a dominant process in groundwater-fed rapid sand filters (RSFs) used for drinking water purification. Near complete removal of ammonium and nitrite is required in the EU and Denmark 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 ammonia oxidizers (AOs); Betaproteobacterial ammonia oxidizers (*Nitrosomonas*, *Nitrosospira*), ammonia oxidizing archaea, diverse heterotrophs and a large fraction of *Nitrospira* spp., which in one studied filter have been shown to comprise both nitrite oxidizers as well as complete nitrifying (comammox) *Nitrospira* spp. (Palomo et al. 2016). We developed a new qPCR assay for the quantification of the comammox *Nitrospira amoA* gene which amplifies both clades A and B and applied this assay to the study of 12 drinking water treatment plants across Denmark. We further sequenced amplicons of the 16S rRNA gene of total Bacteria and *amoA* gene of *Nitrospira* to examine the microbial biodiversity present in the filters. Our results show that comammox *Nitrospira* are present in high abundance making up an average of 19% of the microbial communities in the examined filters. While members of both clades A (41 sequence variants) and B (47 sequence variants) were both present in high abundance, the majority of comammox diversity (70-90% in each filter) was made up by clade B. Ordination analysis with variance partitioning was performed on the total microbial communities and the comammox *Nitrospira* communities to identify physicochemical parameters of the influent water, filter material, or operational parameters which influenced the community structures in an effort to understand the success of comammox *Nitrospira* in these filters. Temperature as well as the sulfate and calcium content of the influent water made significant contributions towards explaining both the total and comammox community structures, while the iron content of the filter material made a significant contribution to explaining only the structure of the comammox *Nitrospira* communities. Further examination of groundwater-fed RSFs with higher variability in microbial communities and physicochemical parameters may provide further information on the ecology of comammox *Nitrospira* and explain their success in the groundwater-fed filters examined in this study. Together this work provides a new assay for the simultaneous detection of clade A and B comammox *Nitrospira* and expands our current knowledge of the diversity of comammox *Nitrospira*, while attempting to explain the success of comammox *Nitrospira* in these groundwater-fed filters.