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Niche partitioning within genus *Nitrospira* is affected by environmental copper concentration

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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. Previous work has revealed that in poorly functioning filters, the addition of trace copper can increase the rate of nitrification, leading to increased removal of ammonium and nitrite to below regulatory limits. 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 and comammox *Nitrospira* spp.. This diversity points towards extensive niche partitioning within the nitrifying guild, and particularly within *Nitrospira* which generally comprises between 10 and 65% of the total filter community. Copper is a co-factor in the ammonia monooxygenase enzyme and is thus an essential and at times limiting nutrient in nitrifying environments. We sought to examine the effects of copper on niche partitioning within the genus *Nitrospira* in full-scale filters. Sand samples from the top of an after-filter that displayed incomplete ammonium oxidation at Nærum waterworks were taken prior to Cu dosing treatment and 4 months following the commencement of low-level Cu dosing ($\sim \mu\text{g Cu L}^{-1}$). Copper treatment had an immediate effect on nitrification, resulting in removal of ammonium and nitrite to below regulatory levels. DNA was extracted from sand samples and was subject to qPCR and amplicon based Illumina sequencing of *Nitrospira nxrB* (nitrite reductase B-subunit) and *amoA* genes using newly designed primers targeting clades A and B comammox. Quantitative PCR revealed that Cu addition resulted in a 4-fold increase of total *Nitrospira*, but a 5-fold decrease in the abundance of comammox *Nitrospira*. However, further examination of the qPCR melt curves and *amoA* sequence data revealed that the reduction in comammox *Nitrospira* resulted from the near complete loss of Clade B comammox, while Clade A comammox were present at similar absolute abundances as in the community prior to copper dosing. The reasons for the loss of Clade B comammox are currently unclear. Higher sensitivity to copper toxicity in Clade B relative to Clade A and nitrite-oxidizing *Nitrospira* seems unlikely due to the extremely low copper concentrations applied. An overall stimulation of the growth of nitrifying bacteria occurred once copper limitation was removed, likely resulting in the out-competition of Clade B *Nitrospira* ammonium oxidizers. These results suggest that copper availability plays a role in determining the diversity and distribution of *Nitrospira* spp. in nitrifying environments.