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## **Dramatic loss of comammox *Nitrospira* associated with long-term nitrite feeding**

*Marta Kinnunen, Alejandro Palomo, Arnaud Dechesne, Barth F. Smets*

Until recently, nitrification was thought to be a strict two-step process where ammonia was first oxidized to nitrite by ammonia-oxidizing bacteria and/or archaea, and subsequently to nitrate by nitrite oxidizing bacteria (NOB). Recent studies in NOB metabolism, however, have revealed that certain *Nitrospira* are capable of performing both steps, resulting in complete ammonia oxidation (comammox) by single microorganisms. These comammox *Nitrospira* have been detected in drinking water (Pinto *et al.*, 2015; Palomo *et al.*, 2016) and aquaculture systems (van Kessel *et al.*, 2015), as well as deep oil exploration wells (Daims *et al.*, 2015). The discovery of comammox *Nitrospira* has significantly changed our understanding of biogeochemical nitrogen cycle.

The goal of this experiment was to determine the extent of competition between comammox *Nitrospira* and canonical *Nitrospira* in ammonium scarce environment, with nitrite as the main energy source.

Community assembly was monitored on well-established biofilms formed on the grains of rapid sand filter (RSF) for drinking water production. RSF sand was placed in laboratory scale column bioreactors and subjected to continuous feeding of tap water spiked with  $\text{NO}_2^-$  (1 mg-N/L) for 250 days. The biofilms were then characterized by Illumina MiSeq platform, targeting the 16S rRNA gene.

The relative abundance of a putative comammox clade B *Nitrospira* sequence variant (with 100% 16S rRNA gene similarity to comammox CG24\_A assembled genome) identified in the initial RSF sand (Palomo *et al.*, unpublished) at a relative abundance of  $12.4 \pm 1.1\%$ , was not detected in 4 out of 6 replicates after 250 days. Similar trend was observed for other putative comammox clade B *Nitrospira* sequence variants. In contrast, we observed significant increase ( $\text{padj} < 0.001$ ) in canonical *Nitrospira* sequences (100% similarity to uncultured *Nitrospira* sp. clone KC836101 (Pester *et al.*, 2014)).

These observations indicate different behavior of *Nitrospira* in the absence of ammonia and point to a possible competitive advantage of canonical *Nitrospira* in environments where nitrite is the sole nitrogen, as well as energy source. In addition, the results suggest that other comammox *Nitrospira* could also be unable to grow in the only presence of nitrite as it was observed for comammox *Ca. Nitrospira inopinata* (Daims *et al.*, 2015).

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Palomo, A., Jane Fowler, S., Gülay, A., Rasmussen, S., Sicheritz-Ponten, T., and Smets, B.F. (2016) Metagenomic analysis of rapid gravity sand filter microbial communities suggests novel physiology of *Nitrospira* spp. *ISME J.* **10**: 1–13.

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