Nitrification moves: stratification patterns in rapid sand filters for drinking water production

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Anaerobic groundwater is treated with aeration followed by rapid sand filtration (RSF) for drinking water production. Theoretically, the introduction of oxygen onsets the simultaneous oxidation of the most common groundwater contaminants – ammonium and iron (II). However, decades of full-scale operational data show nitrification to be strongly hindered in the presence of iron, and to occur only upon its complete removal. In this work, we aim to understand the as-of-yet unknown mechanism hindering ammonium oxidation. To this end, we first quantified the *in-situ* and grain-specific maximum removals along the depth of full-scale RSFs, and applied metagenomic and metaproteomic analysis to characterize the taxonomic and functional profile of the corresponding microbial communities. Next, we employed parallel lab-scale columns to elucidate the direct impact on nitrification of the individual species involved in iron oxidation.

We observed a sharp stratification of both the *in-situ* ammonium and iron (II) concentration profiles, and the estimated maximum nitrification rates along the full-scale RSF. This stratification contrasts starkly with a relatively homogeneous genome-based distribution of nitrifying guilds. By quantifying the proteins expressed by the microbial community ("metaproteome") along the filter height, we resolved this seemingly contradictory observation. The proteomic-based distribution of core functional guilds, iron (II) and ammonia oxidizers, greatly varied along the full-scale filter height, in accordance to the measured concentration and activity profiles (Fig. 1). Iron oxidizers were the most abundant organisms at the top of the RSF, and their presence decreased along the filter depth. Conversely, in line with the measured maximum nitrification rates, nitrifying biomass abundances were the highest in the lower filter layers, where iron (II) oxidation was complete. The homogenous genome-based microbial distribution is likely the result of frequent media-filter mixing during backwash, while the stratified proteomic and activity profiles reflect a much faster metabolic response of the underlying versatile microbiome.

At reaction level, the iron (III) flocs - the final product of iron (II) oxidation - are the primary cause of hindered nitrification in RSFs. Iron (II) or the common by-products of its oxidation, namely reactive oxygen species, had limited to no impact on ammonium oxidation. Importantly, the impact of iron (III) flocs, both biologically produced *in-situ* or added externally, was significant at concentrations as low as 6 mg/L, well below the values typically found in groundwaters. Also, the degree of inhibition increased with the flocs concentration.

In conclusion, our findings provide the first proteomic-based functional-profiling of a full-scale RSF, and identify iron (III) flocs as the major cause of nitrification suppression. Altogether, these results set the groundwork to individually control the removal of major groundwater contaminants (*e.g.* switching to iron (II) oxidation reactions that do not produce flocs) towards more efficient and high-rate RSFs.

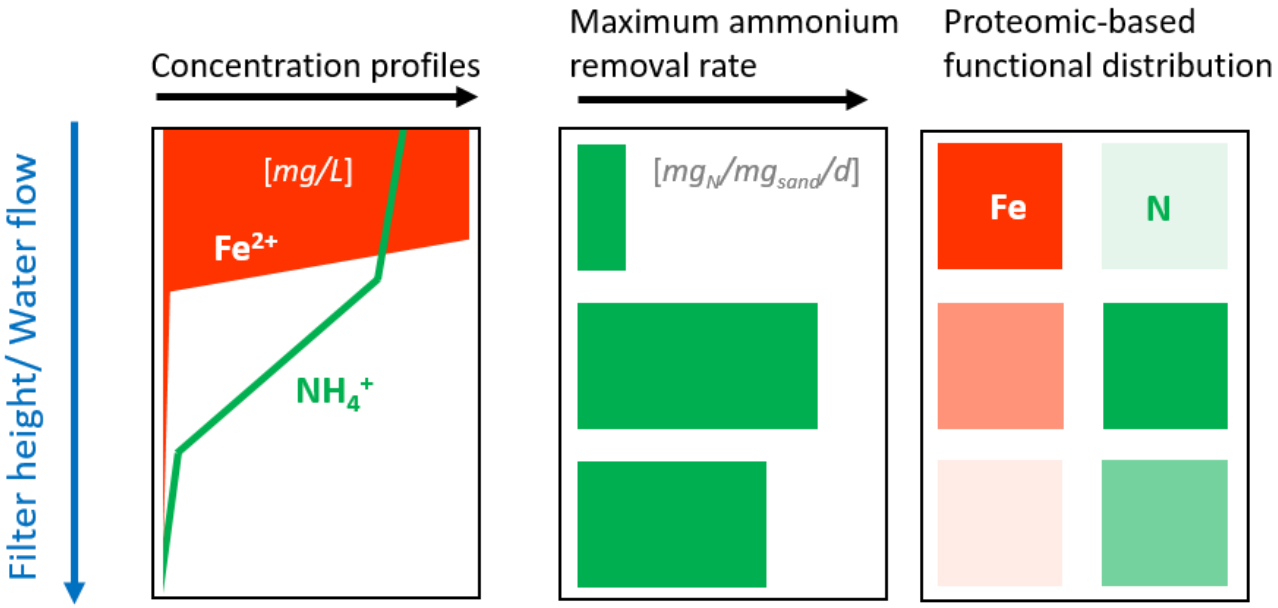

Figure 1. Schematic representation of (left) the water concentration profiles of iron(II) and ammonia, (center) maximum grain-specific ammonia removal rates and (right) proteomic-based functional distribution of the microbial community in a groundwater-fed rapid sand filter. Color intensity is proportional to the represented parameter.

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