Nitrate sources and sinks in oligotrophic groundwater

Martina Herrmann\textsuperscript{1,2}, Markus Krüger\textsuperscript{1}, Bo Thamdrup\textsuperscript{3}, and Kirsten Küsel\textsuperscript{1,2}

\textsuperscript{1}Friedrich Schiller University Jena, Institute of Biodiversity, Aquatic Geomicrobiology, Jena, Germany (martina.herrmann@uni-jena.de)
\textsuperscript{2}German Center for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
\textsuperscript{3}Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark

Despite the high relevance of karstic aquifers as drinking water reservoirs, nitrate pollution of groundwater is posing an increasing threat on a global scale. Under anoxic conditions, nitrate can be converted to \( \text{N}_2 \) by denitrification or anaerobic ammonia oxidation (anammox) and thus be removed from the system. However, in the presence of oxygen, nitrification may continue in the groundwater, supported by the activity of ammonia oxidizing bacteria (AOB), archaea (AOA), and the recently discovered complete ammonia oxidizers (comammox bacteria). We aimed to disentangle different sources and sinks of nitrate and key microbial players involved in nitrogen transformation processes in oligotrophic limestone aquifers of the Hainich Critical Zone Exploratory (CZE; Germany). Assessment of process rates using \(^{15}\text{N}\)-labeling techniques revealed a variance of nitrification rates by two orders of magnitude across six oxic groundwater wells. Surprisingly, wells with nitrate concentrations higher than 300 \( \mu \text{mol} \text{ L}^{-1} \) showed only very low nitrification activity of less than 2 nmol \( \text{NO}_3^- \text{ L}^{-1} \text{ d}^{-1} \), pointing to surface inputs rather than in situ production. In turn, maximum nitrification activity of 127 nmol \( \text{NO}_3^- \text{ L}^{-1} \text{ d}^{-1} \) coincided with a consistently large fraction of comammox bacteria of more than 70\% in the groundwater nitrifier community. Estimated per cell activities of ammonia oxidation suggested that a contribution from comammox was needed to sufficiently explain the observed nitrification rates. Anaerobic ammonia oxidation (anammox) and denitrification as potential nitrate or nitrite sinks varied within a smaller range of 1 to 5 nmol \( \text{N}_2 \text{ L}^{-1} \text{ d}^{-1} \) across anoxic wells and were dominated by anammox, most likely linked to a low availability of organic carbon and suitable inorganic electron donors for chemolithoautotrophic denitrification. Differences in activities agreed well with 100 times higher transcriptional activity of \( hzsA \) genes involved in anammox compared to \( nirS \) genes involved in denitrification. Our findings provide strong evidence for nitrification supported by comammox bacteria in oligotrophic groundwater and for anammox as the dominating N removing process.