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Hotspots of anammox and nitrification in oligotrophic limestone aquifers

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Despite the high relevance of karstic aquifers as drinking water reservoirs, sources and sinks of nitrate in these aquifers and the interconnecting biogeochemical processes are still not fully understood. We assessed nitrogen transformation processes and key microbial players across an oligotrophic limestone aquifer system in the Hainich Critical Zone Exploratory (CZE; Germany) sampled at 5 to 88 m depth. The setting of the Hainich CZE allows to follow groundwater flow along a 6 km hillslope transect, with preferential groundwater recharge areas located under different land use types, including forest, pasture, and cropland. Metagenomics- and metatranscriptomics-based analyses along with functional gene targeted quantitative approaches revealed that the groundwater microbiomes are mainly driven by nitrogen cycling, along with a high spatial heterogeneity of nitrogen transformation reactions across the two aquifer assemblages. Under suboxic conditions with low availability of organic carbon and long groundwater travelling times, anaerobic ammonium oxidation (anammox) contributed an estimated 84% to total nitrogen loss. Under oxic conditions, high abundances of amoA genes including those related to Nitrospira sp. capable of complete oxidation of ammonia (comammox) suggested a contribution of both canonical nitrification and comammox to an overall nitrification activity of 14.4 nmol NO_x per liter and day. Potential vertical transfer of soil-borne N cycling microbiota was assessed by comparison of nitrifier communities between groundwater and surface soils and seepage water from the respective recharge areas, revealing strong shifts towards groups with high substrate affinities in the groundwater. The observed high spatial heterogeneity in nitrogen transformation processes and their microbial drivers across the different aquifer assemblages is likely driven by the different types of land use at groundwater recharge areas as well as differences in groundwater residence times and oxygen supply.