Iron(II)- and sulfur-driven autotrophic denitrification in a pyrite-rich limestone aquifer

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The removal of nitrate by microbial denitrification in freshwater aquifers is often limited by the lack of suitable electron donors, i.e. low concentrations of organic carbon. In these oligotrophic ecosystems chemolithoautotrophic nitrate-reducing bacteria that can use inorganic compunds such as Fe(II) (NRFeOx) or reduced sulfur (NRSOx) as electron donors could play a major role in nitrate degradation. However, autotrophic NRFeOx bacteria have not been isolated from this type of environment and as such there are no model cultures to study the rate-limiting factors of this potentially environmentally relevant process. Geochemical analysis of groundwater originating from a pyrite-rich limestone aquifer (≥6.3 mg FeS_2/g) from Southern Germany showed catchment-scale variations in nitrate concentrations from >50 mg/L (a guideline value for drinking water) to <0.5 mg/L, suggesting the occurrence of denitrification. To study the potential for microbial nitrate reduction coupled to pyrite oxidation two anaerobic autotrophic NRFeOx and NRSOx enrichment cultures were isolated from one groundwater well in the contaminated aquifer. 16S rRNA gene-based sequencing of the *in situ* groundwater microbial community and the NRFeOx enrichment culture originating from the same low nitrate well showed that in both cases the community is dominated by bacteria belonging to the *Gallionellaceae* family highlighting the potential contribution of autotrophic Fe(II)-oxidizing bacteria to nitrate turnover in groundwaters. Here, we present a novel enrichment culture which can be use as model organisms to study NRFeOx in aquifers and determine rate-limiting factors of nitrate turnover.