Microbially driven N turnover processes contribute to carbon autotrophy in limestone aquifers

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Despite the increasing problem of groundwater pollution with nitrate, only little is known about microbially driven nitrogen turnover processes and the corresponding microbial groups in aquifers. In this study we investigated spatial and temporal distribution patterns of microbial communities involved in nitrification, autotrophic and heterotrophic denitrification, and anaerobic ammonia oxidation (anammox) in two superimposed pristine limestone aquifers along a groundwater observation transect of eight wells (12 to 88 m depth) located in the Hainich region (Thuringia, Germany). Since these processes are partially or exclusively carried out by autotrophic organisms, we also wanted to evaluate their potential to support carbon autotrophy in these pristine systems. Concentrations of oxygen (up to 300 μmol L^{-1}) and nitrate (up to 520 μmol L^{-1}) were higher in the lower aquifer compared to the mostly anoxic upper aquifer. Quantitative PCR targeting bacterial and archaeal *amoA* genes encoding ammonia mono-oxygenase revealed a higher genetic potential for nitrification in the lower, oxic aquifer at nitrification rates of 0.6 nmol NO_x L^{-1} h^{-1}. Incubation experiments revealed a potential for nitrate reduction with both inorganic and organic electron donors, pointing to autotrophic and heterotrophic denitrification in both aquifers. Abundances of *nirS* genes encoding nitrite reductase and anammox-related 16S rRNA genes showed maximum abundances of 3.6 x 10^7 and 1 x 10^7 L^{-1}, respectively, in the anoxic upper aquifer where nitrate and ammonium co-occurred at concentrations of 30 μmol L^{-1}. A potential contribution of anammox to nitrate reduction was further supported by maximum concentrations of [3] -ladderane [FAME] (2.6 ng L^{-1}) indicative of active anammox bacteria at the same sites. Our results clearly show that pristine limestone aquifers harbor the potential for microbially driven oxic and anoxic nitrogen transformation reactions and that autotrophic denitrification and anammox might play an unexpectedly important role.