Determination and quantification of the abiotic vs. biotic extent of nitrate/nitrite reduction by Fe(II) under anoxic conditions based on $\delta^{15}N$ isotope analysis

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Nitrate is considered to be a major pollutant in environments which have been influenced by agricultural processes. The denitrification pathways within anoxic systems in these areas, e.g. aquifers, are currently not fully understood. Particularly an extensive application of liquid manure or other synthetic fertilizers lead to higher concentrations of nitrate in the groundwater due to the fact that NO_3^- that is not used by plants is washed out from the soil and therefore reaches the groundwater. Furthermore, factors which control the formation and degradation of the intermediate nitrite, which develops during the first reductive step of denitrification and which is not only toxic to bacteria but also poses considerable threat to human health, are still unclear. Recent studies showed that chemodenitrification is a driving force in these systems and that nitrite concentrations can be kept low due to Fe(II) oxidation *(1).* Moreover, high levels of nitrate might stimulate nitrate-reducing Fe(II)-oxidizing microorganisms, which are also shown to accumulate nitrite under organic-rich and Fe(II) deficient conditions. It has also been demonstrated that the nitrite which is produced is able to further react with Fe(II) leading to Fe(III) and N_2O/N_2 formation *(2, 3).*

In this study we are quantifying the extent of biotic vs. abiotic nitrate reduction coupled to Fe(II) oxidation by cultivating several nitrate-reducing Fe(II)-oxidizers (e.g. *Acidovorax* sp. strain BoFeN1 *(2)*) on Fe(II)-containing growth medium. The nitrate-reducing Fe(II)-oxidizing enrichment culture KS, which is currently considered to be probably the only true autotrophic culture with this metabolism, is used to compare heterotrophically grown and autotrophically grown nitrate-reducing Fe(II)-oxidizing bacteria. In addition to that, we are also testing abiotically set up batches without bacteria to quantify and determine the abiotic reaction pathway. The main focus in these experiments is on the measurement of $\delta^{15}N$ isotopes (IRMS) of each N-containing product in the multi-step reaction in order to determine the differences between abiotically and biotically driven nitrate reduction. These measurements are supplemented with the quantification of Fe(II)/Fe(III) and NO_2^-/NO_3^- which will be carried out by using the ferrozine assay and flow injection analysis respectively, in order to quantify the extent of Fe(II) oxidation and NO_3^- reduction Ultimately this study will allow us to understand the extent of different mechanisms of nitrate reduction in natural anoxic systems and also to distinguish between abiotic and biotic driven reactions.

References:

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