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Biogeosciences Discussions

Interactive comment on "Kinetics of N_2O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments" *by* D. Weymann et al.

Anonymous Referee #2

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General comments

The manuscript of Weymann et al. deals with the question what controls the natural attenuation of nitrate and the reduction product N2O in aquifers. The topic is scientifically and also socially relevant due to the widespread occurrence of nitrate as groundwater contaminant, and the incomplete knowledge what the driving forces for natural attenuation of nitrate in groundwater systems are. Thus, the manuscript is in the scope of Biogeosciences.

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Overall, the manuscript is well structured and clear. However, I found serious weak points which generally prevent the outcome of substantial conclusions:

1. Nitrate reduction to N2 is a rather complicate process consisting of several enzymatic steps which can be carried out by a single organism or by different organism in a microbial community network. The different steps (NO3- to NO2- to NO to N2O to N2) might be differentially regulated by the availability of electron donors, the oxygen concentrations, differences in enzyme activities etc. However, this is not discussed at all. The authors use the relationship between consumed nitrate and produced N2O/N2 to describe the overall nitrate reduction process, which is in my view an oversimplification of processes which actually occur.

2. It is known for a long time that nitrate reduction rates (and also the reduction rates of the more reduced intermediates nitrite and NO) are usually controlled by the amount of oxygen present. The authors show no data for the oxygen concentrations within their microcosms and within the upper aquifer zones they investigated in situ. Therefore, it can not be ruled out that the low nitrate reduction rates the authors observed in their microcosms set up with sediment from the upper aquifer parts and observed in situ in the upper aquifer parts, are related to the oxygen content of the groundwater. The authors widely do not discuss whether oxygen might have influenced the denitrification rates observed in their experiments.

3. The authors assume that in aquifer material taken from the deeper parts of the aquifer, denitrification is mainly coupled to the oxidation of reduced sulphur species (autotrophic denitrification zone) as this process was shown to occur in the investigated aquifer in previous studies. However, at least in the microcosm experiments, this hypothesis was not supported by the experimental data, e.g. production of sulphate coupled to nitrate removal. For me, it remains unclear what have been the electron donors for nitrate reduction in the microcosms.

Therefore, the manuscript needs substantial revision.

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Specific comments

Page 506, lines 1-3 Is it so? I guess in the absence of oxygen, N2O will be rapidly reduced to N2 by most denitrifyers. The crucial role of the presence of oxygen for N2O reduction should be highlighted. By the way, the authors should comment on the fact that substantial amounts of N2O can be produced in aquifers also by partial nitrification of ammonium.

Page 507, lines1-2 As mentioned above, N2O concentrations might be strongly controlled in situ by the oxygen concentrations. This fact should be discussed.

Pages 507-509, chapter 2.1 I suggest moving most parts of this chapter in the introduction or discussion.

Page 510-511, chapter 2.3 I wonder how the authors removed the oxygen during the set up of the experiment. Where the aquifer material has been transferred to the transfusion bottles? I guess it was done in the laboratory under air atmosphere, as no glove-box was mentioned. Did the authors flush the K15NO3 solution with N2 gas to remove the oxygen? I can not believe that three cycles of evacuation and refilling with N2 were sufficient to adjust anoxic conditions in the microcosms. Did the authors analyse the oxygen content of the microcosms? Line 26: 'oxygen-free K15NO3 test solution' – describe in detail how the oxygen was removed from the solution.

Page 513, equation (3) Nitrate reduction to N2O is a rather complicate process consisting of several enzymatic steps. The different steps might be differentially regulated by the availability of electron donors, the oxygen concentrations, differences in enzyme activities etc. I wonder how you can generalize those steps by a single rate constant (k1). Please explain.

Page 515, chapter 3.2 (i) The author's claim that the microorganisms thriving in the aquifer material sampled from deeper zones uses mainly reduced sulphur compounds for nitrate reduction (autotrophic nitrate reduction), since the in situ analyses indicate

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such a process (sulphate was produced in deeper aquifer zones). But why sulphate production was not analysed in the microcosm experiment to verify autotrophic denitrification? (ii) For the correct interpretation of the results, it would have been essential to analyse the oxygen content of the microcosms, at least at the beginning of the microcosm incubation. For me, it seems that nitrate reduction is inhibited in the microcosms made of aquifer material of the upper aquifer zones; and with increasing depth, the inhibition is reduced. Could that be due to the oxygen content of the microcosms?

Page 517, chapter 3.3 As a conclusion, no indications for ongoing autotrophic denitrification in the microcosms by the correlation analyses have been found?

Page 518, line 8 ff. As stated above, nitrate reduction do not result directly in N2O.

Page 519-520, discussion of heterotrophic denitrification (i) Oxygen is a key factor controlling several denitrification steps as well as N2O production/reduction rates. I wonder why the authors did not discuss whether oxygen could influence the in situ denitrification rates in the uppermost layers of the aquifer. Is there anything known about oxygen contents of the heterotrophic denitrification zone? (ii) The authors mention that the pH of the surface groundwater is lower than 5.5, which might inhibit denitrification. What pH is characteristic for deeper parts of the aquifer, e.g. the autotrophic denitrification zone? Is there a difference which might explain the different denitrification rates? (iii) The authors set up their microcosms by mixing aquifer material with K14NO3 solution. What has been the final pH of the microcosms? Is the pH comparable with pH values measured in situ (lower than 5.5)? Page 519-520, discussion of autotrophic denitrification I suggest discussing whether iron-dependent denitrification may contribute to the overall denitrification rate observed in the autotrophic aquifer zone.

Page 521, lines 25-27 The authors do not show any data for pH and oxygen inside their microcosms, but state that both parameters were kept constant during incubation. How do they know?

Page 523, lines 1-5 As stated above, sulphate was not analysed as indicator for

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sulphur-dependent denitrification taking place in the microcosms. How can the authors be sure that the denitrification process was mainly coupled to sulphur oxidation and not coupled to the oxidation of other substances, e.g. carbon compounds or ferrous iron?

Page 524, line 1 As stated above, an analysis of sulphate during the microcosm incubation could have been resulted in experimental evidence for ongoing oxidation of reduced sulphur species linked to denitrification. Why was sulphate not analyzed?

Page 524, lines 24-27 I agree that the availability of electron donors is important for any modelling approach concerning nitrate reduction. But I feel an improved approach might also incorporate kinetics of individual steps during denitrification, e.g. kinetics of nitrate reductase, nitrite reductase and NO reductase, and should consider inhibition effects caused by e.g. pH or oxygen.

Page 526, lines 15-22 The described differences between field and microcosm experiments might be due to different oxygen contents. This should be discussed here.

Page 526, lines 24-28 I do not understand how (short-term?) aerobic conditions and physical disruption could alter the denitrifying community drastically. Most described heterotrophic denitrifyers are facultative aerobes and likely survive in the presence of oxygen.

Interactive comment on Biogeosciences Discuss., 7, 503, 2010.