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Biogeosciences Discussions

Interactive comment on "Predicting long-term denitrification capacity of sandy aquifers from incubation experiments and sediment properties" *by* W. Eschenbach and R. Well

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Dear Anonymous Referee,

thank you for your decision and comments on our manuscript bg-2012-136. We greatly appreciate the detailed and constructive comments of the two reviewers which helped us to improve the manuscript.

Overall, we addressed all comments of the reviewers and hope that we adequately solved the requests.

(The changed and added Figures are also given in the supplement to this response.)

With kind regards

Wolfram Eschenbach

Responses to reviewer 3

According to the reviewer 1 comment 2.1, we changed the phrase denitrification capacity (Dcap) to cumulative amount of denitrification after one year of incubation (Dcum(365)) throughout the whole manuscript.

Referee 3

Referee(s)' Comments to Author:

"Predicting long-term denitrification capacity of sandy aquifers from incubation experiments and sediment properties", by W. Eschenbach and R. Well

This manuscript presents results from ex situ incubations to determine the long-term denitrification capacity of two sandy aquifers. The relatively large dataset and conclusions have important implications for local water resource management and pollution control. Furthermore the manuscript provides a framework for further attempts to predict long-term denitrification capacity with relatively small effort (short-term incubations and sediment parameter analysis). I recommend its publication in Biogeosciences. However, I have a few questions and concerns.

General concerns

1. Generally, the authors should make clear from the beginning what the limitations in their method are, e.g., ex situ incubations for predicting in situ rates; one year incubations for predicting several decades etc.. Maybe already in the title the misleading "long-term" should be replaced.

We changed the title to: "Predicting the denitrification capacity of sandy aquifers from shorter-term incubation experiments and sediment properties" (see also reviewer 1 comment 3) Now we provide a small paragraph, which introduces the limitations of this

in the material and method section. In this paragraph we also refer to the sections 4.4 and 4.5 where the mentioned limitations are discussed in more detail. (see also our response to reviewer 1 comment 2.1 and response to reviewer 3 comment 3)

We added: “2.7 Basic assumption and methodical limitations of the presented approach

The underlying assumptions of the presented study are that there are quantitative relations between the measured cumulative denitrification during one year of incubation (Dcum(365)) and the stock of reduced compounds (SRC) of aquifer material and between the SRC and the denitrification capacity. The basic limitations of the presented approach are: (i) in situ processes are estimated from ex situ incubations, (ii) one year incubations are used for predicting the lifetime of denitrification in the investigated aquifers over several decades and (iii) 15N labelling of NO3ËĽ was used because denitrification was assumed to be the dominant process of NO3− reduction, in the two aquifers. The limitations of the presented investigation are further discussed in section 4.4 and 4.5. This work focuses on organotrophic and sulphide depended denitrification in both aquifers, this seems appropriate taking into account previous investigations (Kölle et al. 1983, Kölle et al. 1985, Hansen 2005) and the evaluation Fe, Mn and NH4+ in the batch solutions during incubation and in situ in both aquifers (see the supplement: other possible electron donors).”

We added also a small paragraph to section 4.5

“4.5.1 Limitations of the 15NO3− labelling approach

15N labelling of NO3− with subsequent analysis of produced 15N labelled N2 and N2O did not exclude the possible contribution of dissimilatory nitrate reduction to ammonium (DNRA) since 15N of NH4 was not checked. Moreover, our approach was not suitable to identify a possible coupling of DNRA with anaerobic ammonium oxidation (anammox) with subsequent formation of 15N labelled N2 from the labelled NO3ËĽ during anaerobic incubations. Hence, despite the fact that previous investigations re-

ported denitrification as the dominant process of NO3ËĽ attenuation in the FFA (Kölle et al. 1983, Kölle et al. 1985), a certain contribution by DNRA-annamox can not be excluded. DNRA is seldom reported to be the dominant process of NO3ËĽ reduction in groundwater systems (Rivett et al. 2008). To our knowledge there are no studies about anaerobic ammonium oxidation (anammox) in fresh water aquifers. The possible contribution of DNRA-anammox to NO3ËĽ consumption during incubation is discussed in more detail in the methodical part of the supplement.”

2. 2.1 Another major concern is that the authors focus on organotrophic and sulphide-dependent denitrification only. However, there are other electron donors such as Fe(II), Mn(II) or ammonium.

We added the following to the end of the introduction:

“This work focuses on organotrophic and sulphide depended denitrification in both aquifers, this seems appropriate taking into account previous investigations (Kölle et al. 1983, Kölle et al. 1985, Hansen 2005) and the evaluation Fe, Mn and NH4+ in the batch solutions during incubation and in situ in both aquifers (see the supplement: other possible electron donors).”

We added the following to the supplement:

“Other possible electron donors

During incubations Fe and Mn concentrations in the batch solution were always mostly far) below 1 mg Fe lËĽ1 and 0,5 mg Mn lËĽ1. Only some transition zone samples showed Fe concentrations 4 and 7 mg Fe lËĽ1 during incubation. The measured concentrations of Fe(II) and Mn(II) in the groundwater at the origin of the samples are below <0.5 mg Fe lËĽ1 and < 0.1 mg Mn lËĽ1 in the oxidized zone of both aquifers. Only in the reduced NO3ËĽ free zone of both aquifers the concentrations of Fe(II) and Mn(II) are higher (1 to 7 mg Fe lËĽ1 and <0,1 mg Mn lËĽ1 in the GKA and 4 to 16 mg Fe lËĽ1 and 0.1 to 1 mg Mn lËĽ1 in the FFA). Therefore, only solids like e.g. pyrite ore

are possible sources for the electron donors for NO3ËL' reduction in both aquifers and it is assumed that pyrite is the major source for Fe(II). Recently Korom et al. (2012) indicated that non-pyritic ferrous iron might play a more important role for denitrification than considered up to now. They assume that ferrous iron from amphiboles contributed to denitrification with 2–43% in a glaciofluvial shallow aquifer in North Dakota. The NH4+ concentrations in the groundwater at sample origin are below detection limit in the GKA and below 0.5 at multilevel well N10 in the FFA, it is assumed that NH4+ is not a significant electron donor during NO3ËL' reduction in both aquifers (see also section 4.5.1 of the manuscript and below)."

The contribution of Fe(II) coming from pyrite is included in our calculations. (see section 2.5. To make this clearer, we change the sentence (section 2.5): "Corg was converted according to Eq. (4) given in Korom (1991) and total-S values (in form of pyrite) according to Eqs. (5) and (6) given in Kölle et al. (1983)." to "Corg was converted according to Eq. (4) (electron donor organic C) given in Korom (1991) and total-S values (in form of pyrite) according to Eqs. (5) (electron donor SËL') and (6) (electron donor Fe2+) given in Kölle et al. (1983)."

2.2 How would for example anammox (the anaerobic oxidation of ammonium) influence the results? What is the potential for this process in the two examined aquifers? How can the authors predict how much ammonium will be available in the sediments in the future? E.g., coming from organic matter remineralisation?

We respond to 2.2 below (response to comment 3 below).

3. Finally, the authors did not address the possibility that nitrate could be reduced to ammonium (DNRA) by e.g. sulphide oxidation. This pathway would result in partial N recycling, and in a significant donor loss.

To address this possible turn over processes we added the following to the Supplement and refer to this at the end of the introduction (see comment 1 above):

"Limitations of the 15NO3ËL' labelling approach

For the quantification of denitrification 15N labelled NO3ËL' was used during the conducted anaerobic incubations. 15N labelling of nitrate can not completely exclude the possible contribution of dissimilatory nitrate reduction to ammonium (DNRA) followed by anaerobic ammonium oxidation (anammox) to the formation of 15N labelled N2 from the labelled NO3ËL' during anaerobic incubations. Under strict anaerobic conditions, DNRA is an alternative pathway for the reduction of NO3ËL'. But DNRA is seldom reported to be the dominant process of NO3ËL' reduction in groundwater systems (Rivett et al., 2008) and chemical modelling by van de Leemput et al. (2011) suggested that DNRA is rather of importance under low NO3ËL' concentrations and high C:NO3ËL' ratios. But denitrification was presumably not NO3ËL' limited since NO3ËL' concentrations were always above 1 mg N lËL'1 (Korom et al., 2005;Morris et al., 1988;Wall et al., 2005) during the incubations. DNRA is presumably not an important process during this investigation because the batch solutions contained only small amounts (< 0,5 mg N lËL'1, samples from B2 in depth 8-10 m $\approx$ 1 mg N lËL'1) of NH4+. Also NH4+ accumulation was generally not observed during the conducted experiments. Since the incubations were anaerobic NH4+ accumulation should be expected if DNRA was a significant contributing process, except anammox consumed the possibly produced NH4+ immediately. If significant N2 production via anammox occurred, this would have been difficult to observe since NH4+ and NO2ËL', the educts of this process, came from the same 15N labelled NO3ËL' pool in the batch solution. (At the beginning of incubation NO2ËL' concentrations were below detection and NH4+ concentrations < 0,5 mg N lËL'1, respectively.) If anammox contributed significantly to N2 production than also DNRA must have been a significant process with half the turnover rate of anammox. Contrary to marine environments, where high rates of anammox are reported (Canfield et al., 2010), in freshwater systems there is not much evidence for anammox (van de Leemput et al., 2011;Burgin and Hamilton, 2007). To our knowledge, there are no studies about anammox in fresh water aquifers, whereas it is reported to exist in wastewater treatment systems, marine sediments and lakes (Jetten et al., 1998;Schubert et

al., 2006;Dalsgaard et al., 2005). To distinguish NO3ËL' consumption by denitrification from coupled DNRA-anammox during anaerobic incubation experiments 15N labelled NO2ËL' might be used. The groundwater in both aquifers NH4+ sometimes contains low concentrations of NH4+. In the GKA NH4+ concentrations are mostly below detection limit and in the reduced zone at multilevel well N10 in the FFA between 0,3 and 0,5 mg lËL'1 (own measurements), since that, the possible occurrence of DNRA or anammox can not strictly be excluded in both aquifers.

Specific questions and comments

4. Page 8808. Line 25. Are the authors that confident that Dcap (= Denitrification capacity during 1 year) can always be predicted by short-term incubations and sediment analyses? At least, the result presented in this study do NOT prove that the long-term denitrification capacity can be predicted. The sentence should be rephrased to e.g., "We use our results from short-term incubations and analysis of sediment parameters to predict the long-term denitrification capacity of sandy Pleistocene aquifer." Or: "In our study, Dcap of two sandy Pleistocene aquifers was predictable using a combination of short-term incubations and analysis of sediment parameters."

We agree with this and changed the respective sentence as suggested to: "In our study, Dcum(365) of two sandy Pleistocene aquifers was predictable using a combination of short-term incubations and analysis of sediment parameters."

5. Page 8810. Lines 5, 17, 18. "organotrophic" instead of "heterotrophic".

We have changed as proposed, and accordingly also in the whole manuscript.

6. Page 8810. Lines 6, 7, 13, 15. "lithotrophic" instead of "autotrophic". (The correct scheme is: hetero- vs. auto- in terms of carbon substrate used for growth; and organo- vs. litho- in terms of electron donor.)

We have changed as proposed, and accordingly also in the whole manuscript.

7. Page 8811. Line 19. "...calculated a maximum..." instead of "the".

Corrected

8. Page 8812. Line 2. Write "...from actual in situ rate measurements using..."

Changed as proposed

9. Page 8812. Line 3. I don't understand. (c) was goal (as stated above) but is not addressed in this study?

We will present the results to goal (c) in a second study. Since both studies are close related to each other we refer already here to this second study. To make this clearer we inserted the following sentence: "In a second study we will present results to (c)."

10. Page 8812. Line 21. "is" instead of "has been estimated".

Changed as proposed

11. Page 8812. Line 23. "Evidence for intense ongoing denitrification...".

Corrected to: for....

12. Page 8812. Line 26. "organotrophic" instead of "heterotrophic" if you speak about electron donor.

Corrected to "organotrophic"

13. Page 8813. Line 1. "lithotrophic" instead of "autotrophic".

Corrected to "lithotrophic"

14. Page 8813. How much time passed between sampling and the start of incubation experiments? Also state in what year and month the cores were drilled.

We added the requested information into section 2.2 of the manuscript: "FFA aquifer samples from depths between 2 to 5 m below soil surface were sampled in April and Mai 2008 and deeper samples in the FFA in June 2007. GKA samples were drilled in December 2008. GKA samples and samples from depths up to 5 m in the FFA were

incubated within 4 week after sampling. Deeper FFA samples were incubated 3 to 6 months after sampling."

15. Page 8814. Line 8. What is the natural range for nitrate concentrations in the 2 aquifers?

We added the following at the respective point of the manuscript: "The natural nitrate concentrations in both aquifers are in the range of 0 to 250 mg NO3− lËL'1 (Well et al., 2012) (see also section 4.5.1)."

16. Page 8814. Line 8. Does that mean 60% 15N-NO3− and 40% 14N-NO3−? And where was the 15N material from?

That is correct 60% 15N-NO3− and 40% 14N-NO3−. This 15N labelled KNO3 was obtained from

Chemotrade Chemiehandelsgesellschaft mbH Marschallstr. 19 D-40477 Düsseldorf

But to our knowledge they didn't trade 15N labelled nitrate anymore. Maybe since 2 years.

We changed the respective sentence to: "15N labelled KNO3 with 60 atom% 15N (Chemotrade Chemiehandelsgesellschaft mbH, Düsseldorf, Germany) was dissolved in deionized water (200 mg 15N labelled NO3− l−1). 300 ml of this solution was...."

17. Page 8814. Line 9. How do you know it was airtight? What kind of rubber septa were used? Were they made anoxic before use (as e.g., described in Canfield et al. 2010)? Most stoppers are not completely oxygen-tight, which might be significant if incubations take as long as 1 year. Did you check for oxygen contaminations in your incubations?

We used natural rubber septa because of their good resealability properties after multiple injections. These septa had a thickness of 2 cm. We added to the manuscript:" ... natural rubber septa of 2 cm thickness and aluminium screw caps. These septa were

used because they kept good sealing after multiple needle penetrations from repeated sampling.". Small amounts of oxygen entering the transfusions bottles are difficult to detect, because they will be reduced during incubation. Occasionally, we measured the N2 in the sampled 12 ml sample vials but found it in the range of blank signals (N2 injected into evacuated 12 ml sample vials).

We added to the supplement:

"Recommendations for future anaerobic incubations

Control of air contamination during incubation experiments Canfield et al. (2010) recommended to de-aerate rubber septa by boiling them for 24 hour in water and store them in a He atmosphere before use. An elegant way to check for possible air contamination is the measurement of Ar in the headspace of the transfusion bottles during incubation. Increasing Ar concentrations are indicator of air contaminations during incubation. Unfortunately we were not able to measure Ar during the incubations, due to instrumental restrictions."

18. Page 8814. Line 14. ". . .for up to one year. . ."

The duration of all incubations was one year. That is why we did not change the respective sentence (=Samples were incubated for one year in the dark at 10 °C.).

19. Page 8814. Line 22. 13 ml gas was transferred into 12 ml exetainers?

To make this point clearer, we changed the respective sentence to: "For the gas sampling, 13 ml headspace gas were extracted with a syringe and transferred to evacuated 12 ml sample vials (Exetainer® Labco, High Wycombe, UK). By doing so, the gas sample was slightly pressurized within the vial."

20. Page 8815. Line 15. ". . . to check for possible denitrification. . ."

Changed as suggested

21. Page 8815. I understand that the "intensive treatment" experiments were con-

ducted to speed up electron donor usage. Can you add a reference why and how much this is faster at 20C? And please explain in a sentence why adding quarts sand.

I have no reference how much faster it is at 20°C, only 9 compared to 25°C (Well et al., 2003). They report that, during anaerobic incubations the 25 °C treatment yielded denitrification rates which were between 1.4 and 3.8 times the rates at 9 °C We added the following sentence at the respective point of the manuscript: "Well et al. (2003) reported that during anaerobic incubations a raise of incubation temperatures from 9 to 25°C resulted in 1.4 to 3.8 higher denitrification rates."

We added the following two sentences at the respective point to the manuscript: "The quartz sand was added to increase the permeability of fine grained parts of the incubated aquifer material. This was done to increase the reactive surface area, i.e. the contact area between tracer solution and reduced compounds."

22. Page 8815. Line 26. "were" instead of "where"

Changed as suggested

23. Page 8816. Line 11. Delete "to SO42Âŕ

Changed as suggested

24. Page 8816. Line 24. What masses were measured on the IRMS? Although you cite Well et al., please give a brief explanation of how you determined total N2 production in your incubations.

We added the following at the respective point of the manuscript: "A brief explanation, how total (N2+N2O) production was determined, is given in the supplement."

We added the following to the supplement: "Quantification of total N2+N2O production

The molecular ion masses 28 and 29 (28N2, 29N2) were recorded for IRMS analysis of denitrification derived 15N labelled N2 and N2O. The N2O in the headspace samples was reduced to N2 in a reduction column prior to the mass spectrometer entrance.

The headspace samples were a mixture of unlabeled N2 und denitrification denitrified 15N labelled N2 and N2O. On condition that (i) the 15N abundance of the denitrified NO3− is known, (ii) denitrification is the sole gaseous nitrogen forming process, and (iii) the amount of N2 evolved from the 15N labelled NO3− pool is small compared with the unlabelled N2 in the sample, the fraction of denitrified N2 in a given mixture can be determined by measuring only 29N2/28N2 ratios using the equations provided by (Mulvaney, 1984) (see also discussion in: (Mulvaney, 1984) and (Eschenbach and Well, 2011)). For the measurement of the 15N abundance of the denitrified NO3− and to check for the conditions mentioned above, replicate samples were measured as described in detail in (Well et al., 1998). The headspace samples represented a mixture of two binomial N2 isotopologue distributions according to the 15N abundances of the unlabelled N2 and the 15N labelled denitrification derived (N2+N2O), respectively. A high frequency discharge unit was then used for online equilibration of N2 molecules prior to isotope analyses. After equilibration the measured samples consisted of one binomial distribution of N2 isotopologues according to the total 15N abundance of the mixture. The 15N abundance of denitrified NO3− can then be calculated from the measurement of the 29N2/28N2 ratios of unequilibrated and equilibrated replicate samples (Well et al., 1998)."

25. Page 8820. Line 15. What was the minimum nitrate concentration to be considered "nitrate-bearing"?

We added the following to the manuscript in section 3.1: "(0.4 mg NO3−-N l−1 was the lowest measured NO3− concentration above the limit of detection of 0,2 mg NO3−-N l−1. Therefore, 0,4 mg NO3−-N l−1 was the lowest concentration to be considered nitrate bearing in this study.)"

26. Page 8820. Line 22. 1.5 mg O2 L is quite high for being called "sulfidic"...

We discussed this in section 4.1.: "Green et al. (2010) modelled the apparent O2 threshold for denitrification in a heterogeneous aquifer and found that an apparent O2

threshold obtained from groundwater sample analysis of < 40 O2 μmol l$-$1 is consistent with an intrinsic O2 threshold of < 10 μmol l$-$1. This apparent threshold of 40 μmol O2 l$-$1 corresponds well with the threshold of minimal and maximal dissolved O2 concentrations at the origins of non-sulphidic and sulphidic aquifer material, respectively, in both aquifers."

We added the following sentence in section 4.1 and refer now at the named point in the manuscript to section 4.1.: "The sulphides that occur in zones where O2 is still measurable in the groundwater might represent residual sulphides from poorly perfused micro areas within the aquifer material."

27. Page 8820. Line 17. Spell "denitrification".

Corrected

28. Page 8828. Line 20. Rephrase this sentence.

We rephrased this sentence to: "By and large, the measured range of Dcum(365) values agreed well with previous incubations studies, which investigated the denitrification activity of aquifer material from comparable Pleistocene sandy aquifers."

29. Page 8832. Line 11. "were" instead of "where".

Changed as suggested

30. Page 8833. Line 23. Remove brackets around citations.

Improved as suggested

31. Page 8835. Line 12. Delete "high to very high and". Or do you mean by "high to very high and highly significant"? The correlations are just highly significant (no matter whether p<0.001 or p<0.01).

We changed the respective sentence into: "We found strong and highly significant correlations between Chws and Dcum(365) of non-sulphidic material (Table 3) and

NO3$-$-bearing samples (rs: R = 0.85 and R = 0.74, respectively, P < 0.001)."

32. Page 8835. Line 20 to 23. I do not agree with the conclusion, that the bioavailable fraction of Chws is higher in upper part. The non- correlation between Chws and Dcap in the sulfidic aquifer might simply be because denitrification and thus Dcap is sulphide dependent in this region.

We change the respective section to:

"The close correlation between Chws and Dcum(365) in the non-sulphidic aquifer material and not for deeper sulphidic aquifer material is distinctive and but difficult to interpret since Chws represents not an uniform pool of organic matter. The missing correlation between Chws and Dcum(365) might indicate that denitrification in this zone is sulphide dependent."

33. Page 8836. Line 23. "were" instead of "where".

Changed as suggested

34. Page 8838. Line 20. "too short" instead of "to short".

Changed as suggested

35. Page 8840. Line 4. Change this title to e.g., "Are laboratory incubation studies suitable for predicting in situ processes?"

Changed as suggested

36. Page 8840. Line 15. "within the range" instead of "between".

Changed as suggested

37. Page 8841. Line 8. "Decreasing concentrations" instead of "A decreasing concentrations".

Changed as suggested

38. Page 8841. Line 17. Spell “investigated”.

Corrected

39. Page 8842. Line 25. “were” instead of “where”.

Corrected

40. Table 3. Is it necessary to distinguish between p<0.001 and p<0.01

We followed Weymann et al. 2010. They also distinguish between p<0.001 and p<0.01 in their correlation analysis between different parameters obtained during similar incubations.

41. Figure 1. Please add a legend (open symbols, closed symbols, crosses) to the figure. Also consider using black as the fill color. As the figure is now it is hard to distinguish between open and closed symbols.

We changed this as suggested. (We attached this Figure)

42. Figure 1 caption. “denitrified” instead of “denitrivied”.

Corrected

43. Figure 2. What does A, B, a, and b stand for?

In the figure caption of Figure 2 we rewrote the sentence: “Different uppercase letters above the box-plots indicate significant differences between FFA and GKA material, different small letters show significant differences between nS, S and tZ (Kruskal-Wallis-Test, P < 0.05).

To: “Different uppercase letters above the box-plots indicate significant differences between SRC and aFSRC values of FFA and GKA material and small letters show significant differences of this two parameters between nS, S and tZ samples (Kruskal-Wallis-Test, P < 0.05).” Hopefully this explains what A, B and a... stand for.

44. Supplemental material: A map indicating the sampling locations would be helpful.

We added a map to the supplemental material, indicating the sampling locations within both Fuhrberger Feld and Großenkneten catchments. (We attached this Figure)

45. Also show e.g., nitrate concentration decrease during your incubations. Does the amount of nitrate consumed fit with N2 production?

We added a figure showing the cumulative nitrate decrease to the supplement. We added the following to the supplement: “The NO3− decrease during incubations showed the same pattern as the measured production of (N2+N2O) by GC-IRMS. The measurement of (N2+N2O) production by GC-IRMS was more precise and had a lower detection limit compared to the measurement of NO3− consumption (compare Fig. 1a and Fig. S3a). The N balance between the NO3− content at the start of incubations and the sum of NO3− consumption and in the (N2+N2O) during incubation was for most of the incubated samples < 1 mg N / batch assay. The samples with the highest measured production of (N2+N2O) showed also the highest deviation between the amount of NO3− consumed and the measured production of (N2+N2O) (compare Fig. 1c and Fig. S3c).” (We attached this Figure)

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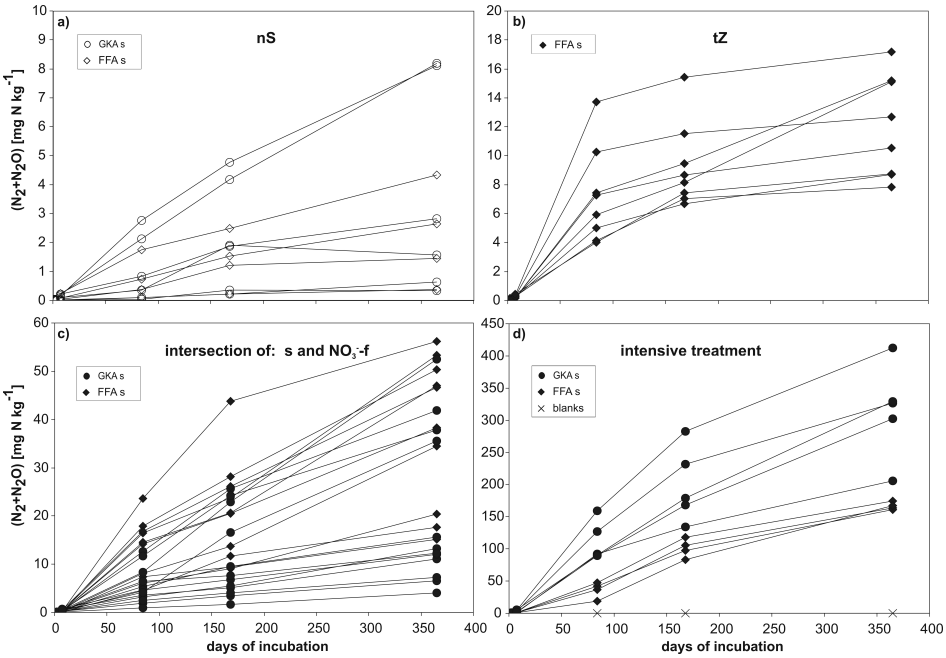

Fig. 1. Fig. 1. Time courses of denitrification products (N2+N2O) (average of 3 to 4 replicas per depth) from different groups of aquifer material during standard (a to c) and intensive treatment (d). Open an

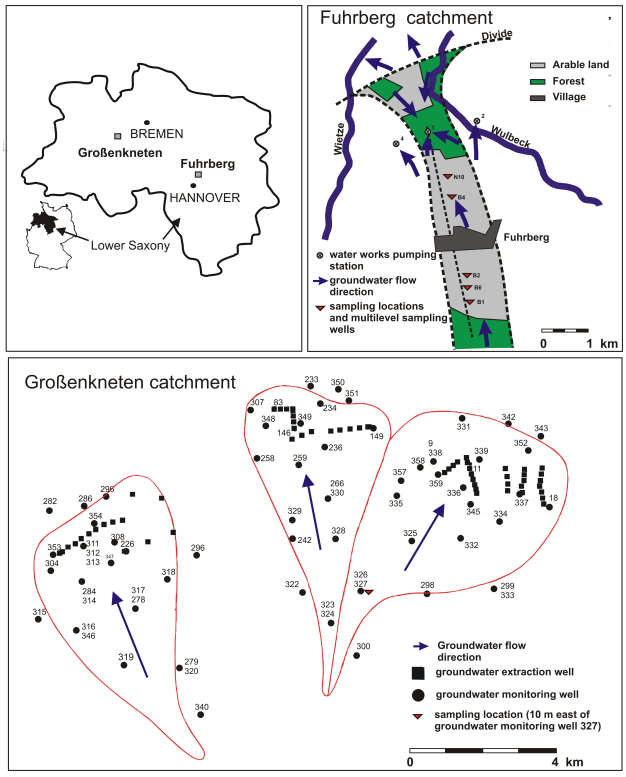

Fig. 2. Fig. S2: Sampling locations within the Fuhrberger Feld and Großenkneten catchment in Lower Saxony (Germany).

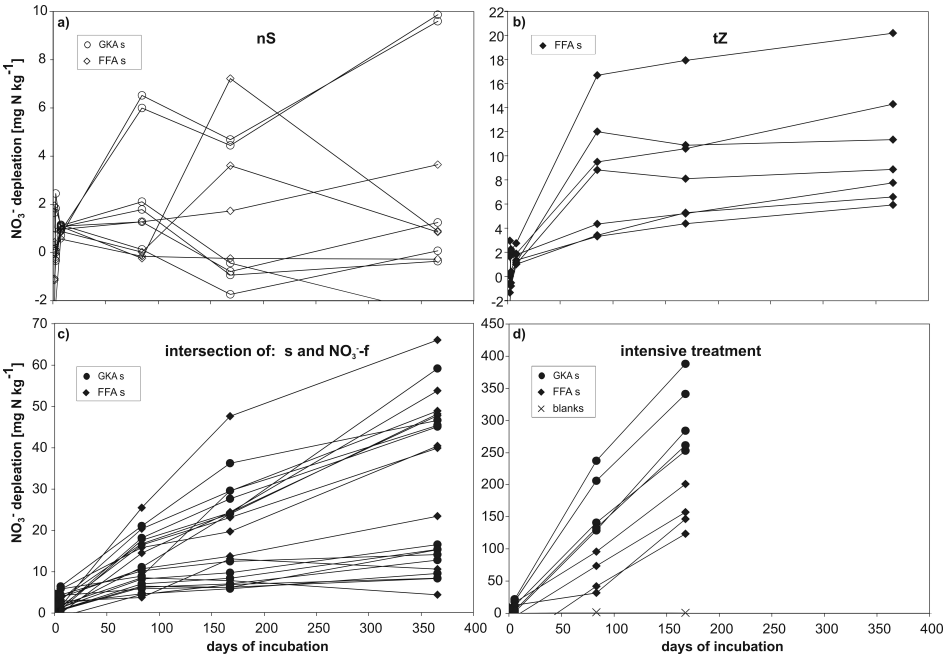

Fig. 3. Fig. S3: Measured NO3− consumption during incubations. (The NO3− concentrations at the last sampling date of intensive incubations were not measured.)