Dear Dr. Neftel,

thank you for your decision and comments and for allowing us to submit a revised version of our manuscript. 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.

With kind regards

Wolfram Eschenbach

(We attached a version of the manuscript with changes highlighted at the end of this pdf.)

Responses to reviewer 1

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 paper addresses relevant scientific questions within the scope of Biogeoscience. It includes a rather large data set and analysis that should be helpful to the scientific community on aquifer denitrification. However, I have several comments and concerns:

1. **1.1** In the first three pages I noted two apparent typographical errors. On line 11 of the abstract in indicates that the "long-term" denitrification capacities ranged from 0.18... However, in Table S2 it appears that this lower range value should be 0.19. Furthermore on page 8810, line 10 "amphiboles" is misspelled as "amphibols." I encourage the authors to review the manuscript again for errors.

   We have reviewed the manuscript thoroughly and hope to have eliminated all remaining errors.

   At the end of the introduction we now provide a small paragraph, which introduces the limitations of this research. In this paragraph we also refer to the sections 4.4 and 4.5 where the mentioned limitations are discussed in more detail.

2. **2.1** The stated goals of the research included (page 8811, line 28) "to quantify exhaustibility of long-term denitrification capacity in aquifers." What is "long-term" in the authors' view? As mentioned above, long-term seems to be until the denitrification capacities of the sediment are exhausted. This idea is repeated in the paragraph beginning on page 8811, line 16. However, "long-term" from the methodology seems to mean 1 year incubation experiments [page 8812, line 4; page 8814, line 14, D_{cap} is the "cumulative amount of denitrification... at the end of one year of incubation (page 8817, line 26 and following)]. Assuming that using data from incubating sediment samples for one year will result in reliable estimates for minimum lifetimes of denitrification (page 8818, line 21 and following) of up to 66.5 years (Table S2) is a big assumption. In my view, "long-term" from the perspective of aquifer denitrification needs to be > 10 years. Again, I think the data provided are helpful, but the assumptions made and the related limitations of this research need to be more clearly stated.

   We agree, long-term denitrification capacity is the capacity until the denitrification capacity of the sediment is exhausted. Therefore we changed the phrase denitrification capacity (D_{cap}) to cumulative denitrification after one year of incubation ($D_{cum}(365)$) throughout the whole manuscript.

   We rewrote section 4.4 and included following sections into the manuscript in order to make the underlying assumptions and limitations of this study more clearly. (see also our response to question 2.2)

We added the following to section 4.4:

"*Two key assumptions were made for the assessment of the lifetime of denitrification in both aquifers from our incubation experiments. There are relations between (i) the measured $D_{cum}(365)$ and the stock of reduced compounds (SRC) and (ii) between the SRC and the denitrification capacity.*

(i) The measured $D_{cum}(365)$ was a good predictor for the SRC for the whole data set and GKA samples. The SRC was also predictable for sulphidic and NO_3^--free samples. Contrary, $D_{cum}(365)$ was a poor indicator of the SRC for aquifer material from already oxidized parts of both aquifers with relatively low amounts of SRC (Table 6). Since the conducted incubations were not able to exhaust the denitrification capacity of the aquifer samples, the real fractions of the SRC available for denitrification (aF_{SRC}) in the incubated samples and even more so the in situ aF_{SRC} remained unknown."

(see also our response to comment 1 of reviewer 2)

2.2 How do we know that all of the organic C and sulphur present in the sediments is able to be oxidized?

We don't know and we didn't assumed this. We assumed that only 5 % of the stock of reduced compounds (SRC) in the samples was able to be oxidized during microbial denitrification. This value was estimated from the intensive incubations. We added the following sentences to the beginning of section 4.4 of the manuscript: "*Since the conducted incubations were not able to exhaust the denitrification capacity of the aquifer samples the real fractions of the SRC available for denitrification (aF_{SRC}) in the incubated samples remained unknown…*"

In sediments I am familiar with, we have organic C in the unsaturated zone (below the soil zone), but little to no pyrite. Knowing that both organic C and pyrite exists below the water table suggests that the organic C above the water table is resistant to oxidation. Could it be that organic C below the water table is also resistant to oxidation?

Surely, there are parts of organic carbon below the water table that are resistant to oxidation. To make this point clearer we added the following to section 4.4:

"*(ii) The low total-S values in the upper parts of both aquifers (Table S1) suggest that most of the sulphides present in both aquifers (see section 4.3.1) are not resistant to*

oxidation. Moreover, sulphides are supposed to be the dominant reduced compound supporting denitrification in the FFA (Kölle et al. 1983). Both aquifers (FFA and GKA) still contain reduced compounds in form of organic matter in their oxidized upper parts. So obviously, certain fractions of the whole SRC are resistant to oxidation. But it is unknown how the ratio of oxidizable to none-oxidazable C_{org} may change with depth in both aquifers. During this study we found that the C_l/C_{org} ratio was higher for deeper (sulphidic) aquifer samples compared with non-sulphidic samples from the upper region in both aquifers. This suggests that the proportion of organic C which is recalcitrant is higher in the already oxidized zone (see section 4.3.1). A reason for this might be that the proportion of mineral associated organic carbon to total organic carbon is higher in this zone.

(Mineral association of organic matter is assumed to increase the recalcitrance fraction of total organic matter (Eusterhues et al., 2005). Eusterhues et al. (2005) reported for a dystric cambisol and a haplic podzol from northern Bavaria that 80 – 95 % of the total organic carbon content of the particle size fraction (< 6.3 µm) in the C horizon is mineral associated organic matter and Fe oxides were identified as the most relevant mineral phases for the formation of organo-mineral associations.) Fe oxides can form during autotrophic denitrification with pyrite and they are known to exist frequently in oxidized aquifers.)"

3. With the comments of #2 above, I recommend that the title be changed to "Predicting long-term denitrification capacity of sandy aquifers from shorter-term incubation experiments and sediment properties.

   We followed this suggestion and changed the title accordingly.

4. **4.1** Sulphur was measured as total S (page 8815, line 20) and assumed to be pyrite (page 8818, line 11). Is this a good assumption?

   We believe this is as a sufficiently good assumption for both aquifers, especially for the reduced parts. In these deeper parts the occurrence of sulphate minerals are not reported. The total S values of aquifer samples from the reduced parts of both aquifers are at least 10 times higher than the ones measured in the upper oxidized region of the FFA and GKA. See also our replies below.

   4.2 Why not measure inorganic S instead of total S?

   On this issue, we replied the following to one of the reviewer (during the first short review process). Hopefully this answers the question sufficiently:

We used total-S as an inexpensive estimate for sulphide content. This is reasonable because previous investigations in comparable aquifers and the Fuhrberger Feld aquifer showed that total-S values were to a large extent identical with sulphides.

In Line 776 to 779 of the submitted manuscript (open discussion paper) we referred to this:

"Bergmann (1999) and Konrad (2007) investigated the distribution of S species in aquifer material from sandy aquifers in North Rhine-Westphalia and Lower Saxony, Germany, respectively, and found that 80 to over 95% of the total-S value is represented by sulphide-S."

Kölle et al. (1982) reported from 23 aquifer samples from different locations in the Fuhrberger Feld Aquifer a mean lignin content of 0.26 % by weight and a pyrite content of lignin of 5.6 % (chemical and x-ray analysis) giving 77.7 mg FeS_2-S kg^{-1}. The median total-S values of 72 mg S kg^{-1} of our Fuhrberg samples (Table S1, supplementary material) are comparable to the values given by Kölle et al. (1983).

We assume that in the deeper parts of both aquifers aluminium hydroxide and aluminium hydroxysulfates minerals are negligible. Gypsum mineral are for different reasons unlikely in the investigated sediments. The SO_4^{2-} and Ca concentrations in the groundwater of both aquifers are far below equilibrium concentration with gypsum of approximately 2 g L^{-1}. Precipitation of gypsum minerals in the groundwater is therefore unlikely. Gypsum rock fragments are not reported for both aquifers and microcrystalline gypsum minerals if initially present should have already dissolved since deposition of the unconsolidated rock aquifers. Because of this we are relatively sure that the gypsum content is negligible.

4.3 On line 6 on this same page it mentions that the possible sulphate produced by dissolution of sulphate minerals was accounted for, but were the amounts significant?

We corrected for pore water SO_4^{2-} and possible dissolution of sulphate minerals. The amounts were significant. We added the following sentences at the relevant point at the manuscript (section 2.5):

"*For the aquifer samples from the NO_3^- free zone of both aquifers and for non-sulphidic samples these initial SO_4^{2-}-S concentrations accounted for 25,4 % and 90 % of the final SO_4^{2-}-S concentrations in the batch solutions. These initial SO_4^{2-}-S concentrations originated supposedly mainly from pore water SO4. The SO_4^{2-}*

concentrations of the groundwater at the origin of the samples reached 5 to 60 mg S l^{-1} in both aquifers (data not shown).”

5. In section 3.6.1 (page 8824), the authors noted that D_{cap} was not predictable by the seven-day denitrification rate (except for non-sulphidic samples) (see also page 8832, line 11 and following); however, D_{cap} was predicted well with the eighty-four-day denitrification rate. If goal c (page 8812, line 1 and following) is to use push-pull tests to check “long-term” denitrification this presents a problem because push-pull tests generally cannot be used for 84 days?

   That is true and a result of this as well as a second study to follow, were we conducted push-pull test at the origin of the sampled aquifer material. During this second study we also tested push pull test with pre conditioning of the aquifer material. These tests resulted in a better agreement between measured laboratory and in situ denitrification rates.

   In the conclusions we already referred to this problem: *“In the deeper zones that had not yet been in contact with NO_3^-, $D_{cum}(365)$ was poorly related to initial denitrification rates. Only after prolonged incubation of several weeks denitrification rates could predict $D_{cum}(365)$ of these samples.*”

6. On page 8828 (line 8 and following) the authors write, “The ultimate goal of our research is to predict long-term denitrification capacity (D_{cap}) from initial denitrification rates.” But this assumes that a one-year long D_{cap} effectively predicts “long-term” denitrification capacity (as in quantifying its exhaustability).

   To emphazise our assumptions and the limitations of this research more clearly, we changed the beginning of section 4.2 to:

   “An important goal of denitrification research is to predict long-term denitrification capacity of aquifers from initial denitrification rates.
   The conducted incubations showed that there are significant quantitative relations between $D_{cum}(365)$ and the SRC of the incubated aquifer samples (Table 6) and it can be assumed that the SRC represents a maximum estimate of the long-term denitrification capacity of aquifer material. Taking this into account it was tested if initial denitrification rates can predict $D_{cum}(365)$.”

7. The question discussed in section 4.5 (page 8840, line 4 and following) are very good. However, I don't find compelling the authors' responses. The only way I know to adequately answer these questions is to have in situ studies. And push-pull tests Apparently won't help achieve the authors' goal (see my comment 5 above). Apparently, the only long-term in situ tests that would work appear to be like those described by Korom et al. (2005). They could be used to test in situ some estimated minimal lifetime of denitrification values given on Table S2 (2-5+ years). They also may help determine what electron donors take part in the denitrification and for how long.

   To emphasize the limitations in drawing conclusions from laboratory incubations to the in situ process, we rewrote the section 4.5.3 to clarify the limitations of our approach and added: *"Linear regressions showed that there are quantitative relations at least between $D_{cum}(365)$ and the SRC of the incubated aquifer samples from the reduced zone in both aquifers (Table 6) and it can be assumed that the SRC in a certain degree determines the long-term denitrification capacity of aquifer material. From this, one- year incubations may give minimum estimates of the denitrification capacity of aquifer sample. Furthermore one year of incubation seems long enough to overcome microbial adaptation processes encountered at the beginning of the conducted incubations (see section 4.2)."*

   But we think the questions as well as the associated conclusions drawn from this study, are nonetheless helpful for future studies.

Bergmann, A.: Hydrogeochemische Untersuchungen anoxischer Redoxprozesse in tiefen Porengrundwasserleitern der Niederrheinisccchen Bucht - Im Umfeld des Tagebaus Garzweiler I, Bochumer geol. geotechn. Arb. 51, 59. Abb., 27. Tab.; Bochum, Germany, 167, 1999.
Eusterhues, K., Rumpel, C., and Kogel-Knabner, I.: Organo-mineral associations in sandy acid forest soils: importance of specific surface area, iron oxides and micropores, Eur. J. Soil Sci., 56, 753-763, 10.1111/j.1365-2389.2005.00710.x, 2005.
Kölle, W., Werner, P., Strebel, O., and Bottcher, J.: DENITRIFICATION BY PYRITE IN A REDUCING AQUIFER, Vom Wasser, 61, 125-147, 1983.
Konrad, C.: Methoden zur Bestimmung des Umsatzes von Stickstoff für drei pleistozäne Grundwasserleiter Norddeutschlands, 161, 2007.

Responses to reviewer 2

According to the reviewer 1 comment 2.1, we changed the phrase denitrification capacity (D_{cap}) to cumulative amount of denitrification after one year of incubation ($D_{cum}(365)$) throughout the whole manuscript.

Referee 2

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 ($D_{cum}(365)$) 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) ^{15}N labelling of NO_3^- was used because denitrification was assumed to be the dominant process of NO_3^- 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 NH_4^+ 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 $^{15}NO_3^-$ labelling approach

^{15}N labelling of NO_3^- with subsequent analysis of produced ^{15}N labelled N_2 and N_2O did not exclude the possible contribution of dissimilatory nitrate reduction to ammonium (DNRA) since ^{15}N of NH_4 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 ^{15}N labelled N_2 from the labelled NO_3^- during anaerobic incubations. Hence, despite the fact that previous investigations reported denitrification as the dominant process of NO_3^- 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 NO_3^- 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 NO_3^- 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 NH_4^+ 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 l^{-1} and 0,5 mg Mn l^{-1}. Only some transition zone samples showed Fe concentrations 4 and 7 mg Fe l^{-1} 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 l^{-1} and < 0.1 mg Mn l^{-1} in the oxidized zone of both aquifers. Only in the reduced NO_3^- free zone of both aquifers the concentrations of Fe(II) and Mn(II) are higher (1 to 7 mg Fe l^{-1} and <0,1 mg Mn l^{-1} in the GKA and 4 to 16 mg Fe l^{-1} and 0.1 to 1 mg Mn l^{-1} in the FFA). Therefore, only solids like e.g. pyrite ore are possible sources for the electron donors for NO_3^- 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 NH_4^+ 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 NH_4^+ is not a significant electron donor during NO_3^- 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):
"C_{org} 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

"C_{org} 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 S^-) and (6) (electron donor Fe^{2+}) 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 $^{15}NO_3^-$ labelling approach

For the quantification of denitrification ^{15}N labelled NO_3^- was used during the conducted anaerobic incubations. ^{15}N 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 ^{15}N labelled N_2 from the labelled NO_3^- during anaerobic incubations.

Under strict anaerobic conditions, DNRA is an alternative pathway for the reduction of NO_3^-. But DNRA is seldom reported to be the dominant process of NO_3^- 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 NO_3^- concentrations and high C:NO_3^- ratios. But denitrification was presumably not NO_3^- limited since NO_3^- concentrations were always above 1 mg N l^{-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 l^{-1}, samples from B2 in depth 8-10 m ≈ 1 mg N l^{-1}) of NH_4^+. Also NH_4^+ accumulation was generally not observed during the conducted experiments. Since the incubations were anaerobic NH_4^+

accumulation should be expected if DNRA was a significant contributing process, except anammox consumed the possibly produced NH_4^+ immediately. If significant N_2 production via anammox occurred, this would have been difficult to observe since NH_4^+ and NO_2^-, the educts of this process, came from the same ^{15}N labelled NO_3^- pool in the batch solution. (At the beginning of incubation NO_2^- concentrations were below detection and NH_4^+ concentrations < 0,5 mg N l^{-1}, respectively.) If anammox contributed significantly to N_2 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 NO_3^- consumption by denitrification from coupled DNRA-anammox during anaerobic incubation experiments ^{15}N labelled NO_2^- might be used.

The groundwater in both aquifers NH_4^+ sometimes contains low concentrations of NH_4^+. In the GKA NH_4^+ 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 l^{-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 D_{cap} (= 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, D_{cap} 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, $D_{cum}(365)$ 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 NO_3^- l^{-1} (Well et al., 2012) (see also section 4.5.1)."

16. Page 8814. Line 8. Does that mean 60% ^{15}N-NO_3^- and 40% ^{14}N-NO_3^-? And where was the ^{15}N material from?

That is correct 60% ^{15}N-NO_3^- and 40% ^{14}N-NO_3^-. This ^{15}N labelled KNO_3 was obtained from

Chemotrade Chemiehandelsgesellschaft mbH
Marschallstr. 19
D-40477 Düsseldorf

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

We changed the respective sentence to: *"^{15}N labelled KNO_3 with 60 atom% ^{15}N (Chemotrade Chemiehandelsgesellschaft mbH, Düsseldorf, Germany) was dissolved in deionized water (200 mg ^{15}N labelled NO_3^- 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 N_2 in

the sampled 12 ml sample vials but found it in the range of blank signals (N_2 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 conducted 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 SO_4^{2-}

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 (N_2+N_2O) production was determined, is given in the supplement."

We added the following to the supplement:
"Quantification of total N_2+N_2O production

The molecular ion masses 28 and 29 ($^{28}N_2$, $^{29}N_2$) were recorded for IRMS analysis of denitrification derived ^{15}N labelled N_2 and N_2O. The N_2O in the headspace samples was reduced to N_2 in a reduction column prior to the mass spectrometer entrance. The headspace samples were a mixture of unlabeled N_2 und denitrification denitrified ^{15}N labelled N_2 and N_2O. On condition that (i) the ^{15}N abundance of the denitrified NO_3^- is known, (ii) denitrification is the sole gaseous nitrogen forming process, and (iii) the amount of N_2 evolved from the ^{15}N labelled NO_3^- pool is small compared with the unlabelled N_2 in the sample, the fraction of denitrified N_2 in a given mixture can be determined by measuring only $^{29}N_2/^{28}N_2$ ratios using the equations provided by (Mulvaney, 1984) (see also discussion in: (Mulvaney, 1984) and (Eschenbach and

Well, 2011)). For the measurement of the ^{15}N abundance of the denitrified NO_3^- 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 N_2 isotopologue distributions according to the ^{15}N abundances of the unlabelled N_2 and the ^{15}N labelled denitrification derived (N_2+N_2O), respectively. A high frequency discharge unit was then used for online equilibration of N_2 molecules prior to isotope analyses. After equilibration the measured samples consisted of one binomial distribution of N_2 isotopologues according to the total ^{15}N abundance of the mixture. The ^{15}N abundance of denitrified NO_3^- can then be calculated from the measurement of the $^{29}N_2/^{28}N_2$ 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 NO_3^--N l^{-1} was the lowest measured NO_3^- concentration above the limit of detection of 0,2 mg NO_3^--N l^{-1}. Therefore, 0,4 mg NO_3^--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 O_2 threshold for denitrification in a heterogeneous aquifer and found that an apparent O_2 threshold obtained from groundwater sample analysis of < 40 O_2 µmol l^{-1} is consistent with an intrinsic O_2 threshold of < 10 µmol l^{-1}. This apparent threshold of 40 µmol O_2 l^{-1} corresponds well with the threshold of minimal and maximal dissolved O_2 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 O_2 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 C_{hws} and $D_{cum}(365)$ of non-sulphidic material (Table 3) and NO_3^--bearing samples (r_s: 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 C_{hws} is higher in upper part. The non- correlation between C_{hws} and D_{cap} in the sulfidic aquifer might simply be because denitrification and thus D_{cap} is sulphide dependent in this region.

We change the respective section to:

"The close correlation between C_{hws} and $D_{cum}(365)$ in the non-sulphidic aquifer material and not for deeper sulphidic aquifer material is distinctive and but difficult to interpret since C_{hws} represents not an uniform pool of organic matter. The missing correlation between C_{hws} and $D_{cum}(365)$ 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.

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 aF_{SRC} 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.

45. Also show e.g., nitrate concentration decrease during your incubations. Does the amount of nitrate consumed fit with N_2 production?

We added a figure showing the cumulative nitrate decrease to the supplement. We added the following to the supplement:
"The NO_3^- decrease during incubations showed the same pattern as the measured production of (N_2+N_2O) by GC-IRMS. The measurement of (N_2+N_2O) production by GC-IRMS was more precise and had a lower detection limit compared to the measurement of NO_3^- consumption (compare Fig. 1a and Fig. S3a).
The N balance between the NO_3^- content at the start of incubations and the sum of NO_3^- consumption and in the (N_2+N_2O) during incubation was for most of the incubated samples < 1 mg N / batch assay. The samples with the highest measured production of (N_2+N_2O) showed also the highest deviation between the amount of NO_3^- consumed and the measured production of (N_2+N_2O) (compare Fig. 1c and Fig. S3c)."

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Predicting the denitrification capacity of sandy aquifers from shorter-term incubation experiments and sediment properties

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Abstract

Knowledge about the spatial variability of denitrification rates and the lifetime of denitrification in nitrate-contaminated aquifers is crucial to predict the development of groundwater quality. Therefore, regression models were derived to estimate the measured cumulative denitrification of aquifer sediments after one year of incubation from initial denitrification rates and several sediment parameters, namely total sulphur, total organic carbon, extractable sulphate, extractable dissolved organic carbon, hot water soluble organic carbon and potassium permanganate labile organic carbon.

For this purpose, we incubated aquifer material from two sandy Pleistocene aquifers in Northern Germany under anaerobic conditions in the laboratory using the ^{15}N tracer technique. The measured amount of denitrification ranged from 0.19 to 56.2 mg N kg^{-1} yr^{-1}. The laboratory incubations exhibited high differences between non-sulphidic and sulphidic aquifer material in both aquifers with respect to all investigated sediment parameters. Denitrification rates and the estimated lifetime of denitrification were higher in the sulphidic samples. For these samples, $D_{cum}(365)$ exhibited distinct linear regressions with the stock of reduced compounds in the investigated aquifer samples. The cumulative denitrification measured during one year of incubation ($D_{cum}(365)$) was predictable from sediment variables within a range of uncertainty of 0.5 to 2 (calculated $D_{cum}(365)$/measured $D_{cum}(365)$) for aquifer material with a $D_{cum}(365)$ > 20 mg N kg^{-1} yr^{-1}. Predictions were poor for samples with lower $D_{cum(365)}$ like samples from the NO_3^- bearing groundwater zone, which includes the non-sulphidic samples, from the upper part of both aquifers where denitrification is not sufficient to protect groundwater from anthropogenic NO_3^- input. Calculation of $D_{cum}(365)$ from initial denitrification rates was only successful for samples from the NO_3^--bearing zone, whereas a lag-phase of denitrification in samples from deeper zones of NO_3^- free groundwater caused imprecise predictions. $D_{cum}(365)$ exhibited distinct

In our study, $D_{cum}(365)$ of two sandy Pleistocene aquifers was predictable using a combination of short-term incubations and analysis of sediment parameters. Moreover, the protective lifetime of denitrification sufficient to remove NO_3^- from groundwater in the investigated aquifers is limited which demonstrates the need to minimize anthropogenic NO_3^- input.

1 Introduction

Denitrification, the microbial mediated reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to the nitrogen gasses nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) is important to water quality and chemistry at landscape, regional and global scales (Groffman et al., 2006). Since 1860 the inputs of reactive nitrogen (Nr)[1] to terrestrial ecosystems have increased from 262 to 389 Tg N yr^{-1} (Galloway et al., 2004). The production of reactive nitrogen via the Haber-Bosch process contributed approximately with 100 Tg N yr^{-1} to this tremendous increase. In the European Union diffuse emissions of Nr range from 3 to >30 kg N ha^{-1} yr^{-1} from which 51 to 85% are derived from agriculture (Bouraoui et al., 2009). Diffuse Nr emissions from the agricultural sector are therefore the dominant source of NO_3^- fluxes to aquatic systems which leads to the questions, how rates of denitrification will respond to Nr loading (Seitzinger et al., 2006) and where and how long denitrification in aquifers can remediate the anthropogenic NO_3^- pollution of groundwater (Kölle et al., 1985).

NO_3^- pollution of groundwater has become a significant problem due to eutrophication of water bodies (Vitousek et al., 1997) and potential health risks from NO_3^- in drinking water. The latter causes increasing costs for keeping the standard for NO_3^- in drinking water (< 50 mg l^{-1}, Drinking Water Directive 98/83/EC) (Dalton and Brand-Hardy, 2003; Defra, 2006). Therefore, knowledge about the denitrification capacity of aquifers is highly needed. The term denitrification capacity of aquifers or aquifer material used in this study refers to the amount of NO_3^- that can be denitrified per m^3 aquifer or per kg of aquifer material until significant denitrification activity stops because of exhaustion of electron donors.

Denitrification in groundwater is mainly depending on the amount and microbial availability of reduced compounds in the aquifers, capable to support denitrification and is of a high spatial variability, ranging from 0 to 100% of the NO_3^- input (Seitzinger et al., 2006). The main constituents of reduced compounds acting as electron donor during denitrification are organic carbon (organotrophic denitrification pathway), reduced iron and reduced sulphur compounds (lithotrophic denitrification pathway). Iron sulphides are known to be an important electron donor for autotrophic denitrification (Kölle et al., 1985), 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.

[1] The term reactive nitrogen is used in this work in accordance to Galloway et al. (2004) and includes all biologically or chemically active N compounds like reduced forms (e.g., NH_3, NH_4^+), oxidized forms (e.g., NO_x, HNO_3, N_2O, NO_3^-) and organic compounds (e.g., urea, amines, proteins . . .).

Denitrification in groundwater can be a very slow to fast process. Frind et al. (1990) reported that litotrophic denitrification has a half-life of 1 to 2 yr in the deeper zone (5 to 10 m below soil surface) of the well investigated Fuhrberger Feld aquifer (FFA). Contrary to the high denitrification rates in deeper reduced parts of this aquifer (litotrophic denitrification zone) Weymann et al. (2010) reported very low denitrification rates with values as low as 4 μg N kg^{-1} d^{-1} in the surface near groundwater (organotrophic denitrification zone) of the same aquifer. Denitrification rates in the organotrophic zone were one to two orders of magnitude lower than in its deeper parts and altogether too low to remove NO_3^- from groundwater.

While there are numerous laboratory incubation studies evaluating denitrification rates of aquifer sediments, there are only few studies reporting the amount of denitrification measured over several months of incubation and/or the stock of reactive compounds capable to support denitrification in the investigated aquifer sediments (Kölle et al., 1985; Houben, 2000; Mehranfar, 2003; Weymann et al., 2010; Well et al., 2005). Even less investigations tried to develop stochastic models to estimate the measured denitrification from independent sediment variables (Konrad, 2007; Well et al., 2005). Mehranfar (2003) and Konrad (2007) estimated the availability of a given stock of reduced compounds within sediments during incubation experiments that lasted at least one year, showing that approximately 5 to 50% of sulphides were available for denitrification during incubation. However, in both studies incubation time was insufficient for complete exhaustion of reductants within the experiments.

Since laboratory investigations of denitrification rates in aquifer material are time consuming and expensive, in situ measurements are helpful to increase knowledge about the spatial distribution of denitrification in aquifers. In situ denitrification rates can be derived from concentration gradients (Tesoriero and Puckett, 2011), in situ mesocosms (Korom et al., 2012) and from push-pull type ^{15}N tracer tests (Addy et al., 2002; Well and Myrold, 1999). Well et al. (2003) compared in situ and laboratory measurements of denitrification rates in water saturated hydromorphic soils and showed that both methods were over all in good agreement. Konrad (2007) proposed to estimate long-term denitrification capacity of aquifers from in situ push-pull tests as an alternative to costly drilling of aquifer samples with subsequent incubations. A good correlation between in situ denitrification rates and the cumulative amount of denitrification during incubation based on a small number of comparisons was reported (Konrad, 2007), but the data set was too small to derive robust transfer functions.

Since the oxidation of reduced compounds in aquifers is an irreversible process, the question arises, how fast ongoing NO_3^- input will exhaust denitrification capacity of aquifers and to which extent this may lead to increasing NO_3^- concentrations. Two studies attempted to answer this. Kölle et al. (1985) calculated a maximum lifetime of autotrophic denitrification in the FFA of about 1000 yr by a mass balance approach. Houben (2000) modelled the depth shift of the denitrification front in a sandy aquifer in Western Germany giving a progress rate of approximately 0.03 m yr^{-1}.

Overall, there is very limited information on long-term denitrification capacity of aquifer sediments because there are virtually no direct measurements. Because of this predictions based on stochastic models are hampered by the lack of suitable data sets. Therefore, knowledge about the spatial distribution of denitrification rates is highly demanded (Rivett et al., 2008).

To progress knowledge in this field, we combine established methods with the testing of new concepts. Our goals are (a) to get estimates of the exhaustibility of denitrification capacity in aquifers from incubation experiments, (b) to investigate controlling factors and derive predictive models and (c) to check if laboratory ex situ denitrification rates can be derived from actual in situ rate measurements using push-pull tests at groundwater monitoring wells. Here we present an approach to tackle (a) and (b). In a second study we will present results to (c). The specific objectives are (i) to measure denitrification during one year anaerobic incubation of sediment material from two aquifers, (ii) to estimate the total stock of reactive compounds in these samples and their availability for denitrification as well as influencing sediment parameters, (iii) to develop regression models to estimate the measured cumulative denitrification from initial denitrification rates and from sediment properties and (iv) to estimate the minimal lifetime of denitrification in the investigated aquifer material.

2 Materials and methods

2.1 Study sites

Aquifer material was collected in the Fuhrberger Feld aquifer (FFA) and the Großenkneten aquifer (GKA), two drinking water catchment areas in Northern Germany (Fig. S1 in the supplent). The FFA is situated about 30 km NE of the city of Hannover and the GKA about 30 km SW of the city of Bremen. Both aquifers consist of carbonate free, Quaternary sands and the GKA additionally of carbonate free marine sands (Pliocene). The thickness of the FFA and GKA is 20 to 40 m and 60 to 100 m, respectively. Both aquifers are unconfined and contain unevenly distributed amounts of microbial available sulphides and organic carbon. Intense agricultural land use leads to considerable nitrate inputs to the groundwater of both aquifers (Böttcher et al., 1990; van Berk et al., 2005). Groundwater recharge is 250 mm yr^{-1} in the FFA (Wessolek et al., 1985) and 200 to 300 mm yr^{-1} in the GKA (Schuchert, 2007).

Evidence for intense ongoing denitrification within the FFA is given by nitrate and redox gradients (Böttcher et al., 1992) as well as excess-N_2 measurements (Weymann et al., 2008). The FFA can be divided into two hydro-geochemical zones, the zone of organotrophic denitrification near the groundwater surface with organic carbon (C_{org}) as electron donor and a deeper zone of predominantly lithotrophic denitrification with pyrite as electron donor (Böttcher et al., 1991, 1992). Detailed information about the FFA is given by Strebel et al. (1992), Frind et al. (1990) and von der Heide et al. (2008). Extended zones with oxidizing and reducing conditions in the groundwater are also evident in the GKA (van Berk et al., 2005) but their distribution within this aquifer is more complex as in the FFA. The geological structure of the GKA is described in Howar (2005) and Wirth (1990). Intense denitrification is known to occur in the zone of reduced groundwater (van Berk et al., 2005). This was proven by excess-N_2 measurements at monitoring wells within the GKA (Well et al., 2012). But there are no studies on the type of denitrification in this aquifer.

2.2 Sampling procedures

The aquifer material used in this study originated from depths between 3–18 m and 8–68 m below soil surface of the FFA and GKA, respectively.

The aquifer material from the FFA was drilled with a hollow stem auger (OD of 205 mm, ID of 106 mm, WELLCO-DRILL, WD 500, Beedenbostel, Germany) and the core samples were

immediately transferred into 2 l glass bottles. The remaining headspace within these bottles was filled with deionised water until it overflowed. Then the bottles were sealed airtight with rubber covered steel lids. Aquifer material from the GKA was drilled by percussion core drilling. The aquifer samples were collected with a double core barrel with an inner PVC liner (OD 95.8 mm, ID 63.4 mm, HWL (HQ) Wireline core barrel, COMPDRILL Bohrausrüstungen GmbH, Untereisesheim, Germany). After sampling, the liner was removed from the core barrel and sealed airtight at both ends with PVC lids. In the laboratory, the aquifer material from the PVC liner was transferred into glass bottles as described above. The aquifer samples were stored at 10 °C (approximately the mean groundwater temperature in both aquifers) in the dark. After sampling of aquifer material, groundwater monitoring wells and multilevel wells were installed in the borings. 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.

2.3 Laboratory incubations

2.3.1 Standard treatment

Anaerobic incubations were conducted to measure the cumulative denitrification and the denitrification rates of the investigated aquifer material as described by Weymann et al. (2010). In total, 41 samples from both aquifers collected between 2 to 68 m below soil surface were incubated. From each sample, 3 to 4 replicates of 300 g fresh aquifer material were filled in 1125 ml transfusion bottles. ^{15}N labelled KNO_3 with 60 atom% ^{15}N (Chemotrade Chemiehandelsgesellschaft mbH, Düsseldorf, Germany) was dissolved in deionized water (200 mg ^{15}N labelled $NO_3^- l^{-1}$). (The natural nitrate concentrations in both aquifers are in the range of 0 to 250 mg $NO_3^- l^{-1}$ (Well et al. 2012) (see also section 4.5.2).) 300 ml of this solution was added to each transfusion bottle and then the bottles were sealed airtight with 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. The mixture of the labelled KNO_3 solution and pore water of the aquifer samples is referred to as batch solution below. The headspace of each transfusion bottle was evacuated for 5 min

and then flushed with pure N_2. This procedure was repeated 5 times to ensure anaerobic conditions within the bottles. Samples were incubated for one year in the dark at 10 °C.

The water content of the investigated aquifer material was determined gravimetrically using parallels of the incubated material. The dry weight, the volume of the incubated sediment (assuming a particle density of 2.65 g cm^{-3}), the liquid volume and the headspace volume were calculated for each replicate independently. Samples of the headspace gas and the supernatant batch solution were taken at days 1, 2, 7, 84, 168 and 365 of incubation. The transfusion bottles were shaken on a horizontal shaker at 10 °C for 3 h prior to sampling to equilibrate headspace gasses with the dissolved gasses in the batch solutions. 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. Subsequently, 20 ml of the supernatant solution were sampled with a syringe and transferred into a PE bottle and frozen until analysis. To maintain atmospheric pressure within the transfusion bottles, 13 ml pure N_2 und 20 ml of O_2 free ^{15}N labelled KNO_3 solution were re-injected into every transfusion bottle after sampling. The ^{15}N-labelled KNO_3 solution was stored in a glass bottle, which was sealed air tight with a rubber stopper. Prior to re-injection of the KNO_3 solution into the transfusion bottles, the solution was purged with pure N_2 through a steel capillary for 1 h to remove dissolved O_2. The headspace in the glass bottle was sampled to check O_2 contamination and was always found to be in the range of O_2 signals of blank samples (N_2 injected into evacuated 12 ml sample vials).

2.3.2 Intensive treatment

A modified incubation treatment was conducted for aquifer samples with high content of C_{org} and sulphides, to increase the proportion of reduced compounds that are oxidized during incubation. 30 g aquifer material and 270 g quartz sand were filled in transfusion bottles and prepared for anaerobic incubations as described above for the “standard” treatment. 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. The incubation temperature was 20 °C and samples were permanently homogenized on a rotary shaker in the dark. 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. In total, 9 aquifer samples were selected

from the FFA and GKA and incubated in 4 replications. Additionally, 4 transfusion bottles were filled only with the pure quartz sand to check for possible denitrification activity of this material, which was found to be negligible.

2.4 Analytical techniques

The particle sizes distribution in the aquifer sediments was determined by wet sieving. The silt and clay fractions were determined by sedimentation following the Atterberg method (Schlichting et al., 1995). Contents of total sulphur (total-S), total nitrogen (total-N) and total organic carbon (C_{org}) of the carbonate free aquifer sediments were analysed with an elemental analyser (vario EL III, ELEMENTAR ANALYSESYSTEME, Hanau, Germany).

For hot water soluble organic carbon (C_{hws}) 10 g aquifer material and 50 ml deionized water were boiled for 1 h and then filtrated (Behm, 1988). Cold water extracts were used for the determination of extractable dissolved organic carbon (DOC_{extr}) and extractable sulphate ($SO_4^{2-}{}_{extr}$). C_{hws} and DOC_{extr} in the extracts were measured with a total carbon analyser (TOC 5050, Shimadsu, Kyoto, Japan). To measure the fraction of $KMnO_4$ labile organic carbon (C_l) 15 g aquifer material and 25 ml 0.06M $KMnO_4$ solution were shaken on a rotating shaker for 24 h and then centrifuged by 865RCF (Konrad, 2007). 1 ml of the supernatant was sampled and diluted in 100 ml dionized water. C_l was then determined as the decolourization of the $KMnO_4$ solution by means of a photometer (SPECORD 40, Analytic Jena, Jena, Germany). NO_3^-, NO_2^- and NH_4^+ concentrations were determined photometrically in a continuous flow analyser (Skalar, Erkelenz, Germany). For the determination of SO_4^{2-} concentrations in the batch solutions and SO_4^{2-} extracts, a defined amount of $BaCl_2$ solution was added in excess to the samples and SO_4^{2-} precipitated as $BaSO_4$. The original SO_4^{2-} concentration was then analysed by potentiometric back-titration of the excess Ba^{2+}-ions remaining in the solution using EDTA as titrant. Possible interfering metal cations were removed from the samples prior to this analysis by cation exchange.

The major cations in the batch solution (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{4+} , Fe^{3+} and Al^{3+}) were measured by means of Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Spectro Analytical Instruments, Kleve, Germany) after stabilizing an aliquot of the batch solution samples with 10% HNO_3.

N_2O was measured using a gas chromatograph (Fisons GC8000, Milan, Italy equipped with an electronic capture detector as described previously by Weymann et al. (2009). O_2 was

analysed with a gas chromatograph equipped with a thermal conductivity detector (Fractovap 400, CARLO ERBA, Milan, Italy) described in Weymann et al. (2010).

The ^{15}N analysis of denitrified (N_2+N_2O) was carried out by a gas chromatograph (GC) coupled to an isotope ratio mass spectrometer (IRMS) at the Centre for Stable Isotope Research and Analysis in Göttingen, Germany within two weeks after sampling, following the method described in Well et al. (2003). The concentrations of ^{15}N labelled denitrified N_2 and N_2O in the gas samples were calculated in the same way as described in detail by Well and Myrold (1999) and Well et al. (2003). A brief explanation, how total (N_2+N_2O) production was determined, is given in the supplement.

From the obtained molar concentrations of denitrification derived N_2 and N_2O in the gas samples, which are equal to the molar concentrations in the headspace of the transfusion bottles, the dissolved N_2 and N_2O concentrations in the batch solutions were calculated. This was done according to Henry's law using the solubilities for N_2 and N_2O at 10 °C given by Weiss (1970) and Weiss and Price (1980). The detection limit of ^{15}N analysis was calculated as the minimum amount of ^{15}N labelled denitrification derived (N_2+N_2O) mixed with the given background of headspace N_2 of natural ^{15}N abundance necessary to increase the measured $^{29}N_2/^{28}N_2$ ratio to fulfil the following equation:

$$r_{sa} - r_{st}st \geq 3 \times s.d.r_{st} \quad (1)$$

where r_{sa} and r_{st} are the $^{29}N_2/^{28}N_2$ ratios in sample and standard, respectively and s.d.r_{st} is the standard deviation of repeated r_{st} measurements. The r_{st} values were analysed with IRMS by measuring repeated air samples. Under the experimental conditions, the detection limit for the amount of denitrification derived ^{15}N labelled (N_2+N_2O) was 15 to 25 µg N kg^{-1}.

Dissolved oxygen, pH and electrical conductivity (pH/Oxi 340i and pH/Cond 340i, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) were measured in the groundwater from the installed groundwater monitoring wells.

2.5 Calculated parameters

The following parameters describing the denitrification dynamics during anaerobic incubation were calculated from the measurements described above. Denitrification rates D_r(X) were calculated as the cumulative amount of denitrification products formed until the day of sampling divided by the duration of incubation until sampling (mg N kg^{-1} d^{-1}), with X as the day of sampling. We calculated denitrification rates for day 7, 84, 168 and 365 of incubation,

$D_r(7)$, $D_r(84)$, $D_r(168)$ and $D_r(365)$, respectively. $D_r(7)$ is also referred to as the initial denitrification rate. $D_{cum}(365)$ is the cumulative amount of denitrification products per kg dry weight of incubated aquifer material at the end of one year of incubation (mg N kg^{-1} yr^{-1}). $D_r(365)$ multiplied by 365 d equals $D_{cum}(365)$, so we refer only to $D_{cum}(365)$ below. The sulphate formation capacity (SFC) (Kölle et al., 1985) was derived from the measured increase of SO_4^{2-} concentrations in the batch solution between the first sampling (day 1) and the end of incubation (day 365). To correct the SFC value for dissolution of possible SO_4^{2-}-minerals and/or SO_4^{2-} from the pore water of the incubated aquifer material we subtracted the SO_4^{2-} concentrations in the batch solution after two days of incubation from the finally SO_4^{2-} concentration after one year. For the aquifer samples from the NO_3^- free zone of both aquifers and for non-sulphidic samples these initial SO_4^{2-}-S concentrations accounted for 25,4% and 90% of the final SO_4^{2-}-S concentrations in the batch solutions. These initial SO_4^{2-}-S concentrations originated supposedly mainly from pore water SO_4^{2-}. The SO_4^{2-} concentrations of the groundwater at the origin of the samples reached 5 to 60 mg S l^{-1} in both aquifers (data not shown).

The stock of reactive compounds (SRC) was estimated from total-S and C_{org} data. For simplicity it was assumed that C_{org} corresponds to an organic substance with the formula CH_2O (Korom, 1991; Trudell et al., 1986) and that all sulphur was in the form of pyrite (FeS_2) (see section 4.3.1). C_{org} and total-S values were converted into N equivalents (mg N kg^{-1}) according their potential ability to reduce NO_3^- to N_2. C_{org} 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 S^-) and (6) (electron donor Fe^{2+}) given in Kölle et al. (1983). The fraction of SRC which is available for denitrification during incubation (aF_{SRC}) (%) was calculated as the ratio of the measured $D_{cum}(365)$ to the SRC of the incubated aquifer material. The share of total-S values contributing to the aF_{SRC} was calculated from the measured SFC during incubation. The remaining portion of the aF_{SRC} as assigned to microbial available C_{org} compounds in the aquifer samples.

The estimated minimum lifetime of denitrification (emLoD) was calculated as follows:

$$emLod = \frac{A_{dw} \times (SRC \times aF_{SRC} \times 0.01)}{nitrate\ input}\ [yr\ m^{-1}] \quad (2)$$

where the dry weight of 1 m^3 aquifer material (A_{dw}) (kg m^{-3}) is multiplied with the fraction of its SRC (mg N kg^{-1}) content available for denitrification during one year of incubation. This value is then divided by the nitrate input (mg NO_3^--N m^{-2} yr^{-1}) giving the estimated minimal lifetime of denitrification for one m^3 of aquifer material. To calculate A_{dw} a porosity of 35% and an average density of the solid phase of 2.65 g cm^{-3} of the aquifer material was assumed, giving an A_{dw} of 1722.5 kg m^{-3}. Furthermore, an average aF_{SRC} of 5% was used to calculated emLoD (see Sect. 4.4). The NO_3^- input to the aquifer coming with the groundwater recharge was assumed from literature data on N leaching. Köhler et al. (2006) measured mean NO_3^- concentrations in the groundwater recharge under arable sandy soils between 40 and 200 mg NO_3^- l^{-1}. For a conservative estimate of emLoD we use the maximum value 200 mg NO_3^- l^{-1}. This value gives a nitrate input of 11.3 g NO_3^--N m^{-2} yr^{-1} (= 6.6 mg NO_3^--N kg^{-1} yr^{-1}) to the aquifer under condition of a groundwater recharge rate of about 250 mm yr^{-1} as reported for the GKA and FFA by Schuchert (2007) and Renger et al. (1986), respectively.

2.6 Statistical analysis and modeling

Statistical analysis and modelling was performed with WinSTAT for MS Excel Version 2000.1 (R. Fitch Software, Bad Krozingen, Germany). Differences between partial data sets were considered significant at the $P < 0.05$ level (Kruskal-Wallis test (kw), with the null hypothesis that both partial data sets belong to the same population). Spearman rank correlations (r_s) were used to determine significant correlations between sediment parameters and $D_{cum}(365)$. Simple and multiple linear regression analysis were performed to evaluate quantitative relations between $D_{cum}(365)$ and the sediment parameters and to predict $D_{cum}(365)$ from these parameters. Simple linear regressions and multiple linear regressions are in the following referred to as simple regression and multiple regressions. Normal distribution of the measured parameters within the different data sets was tested with the Kolmogorov-Smirnov-Test, normal distribution was assumed at the $P > 0.05$ level, with the null hypothesis that the tested parameter was normal distributed. The uniform distribution of residuals of regressions were checked with scatter plots of residuals vs. independent variables of the respective regression analysis. This was done to ensure homoscedasticity during regression analysis, to ensure that the least-squares method yielded best linear estimators for the modelled parameter.

Experimental data (x) was converted into Box-Cox transformed data ($f^{B\text{-}C}(x)$) according to Eq. (3) using different lambda coefficients (λ) to achieve a normal like distribution of experimental data within the different data sets.

$$f^{B-C}(x) = \frac{x^{\lambda} - 1}{\lambda} \quad (3)$$

Box-Cox transformations were conducted with the statistic software STATISTICA 8 (StatSoft, Tulsa, USA). To use the regression functions to model $D_{cum}(365)$, input data have to be transformed according to Eq. (3) with the lambda coefficients given in Table S5 (see the Supplement).

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 ($D_{cum}(365)$) 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) ^{15}N labelling of NO_3^- was used because denitrification was assumed to be the dominant process of NO_3^- 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 NH_4^+ in the batch solutions during incubation and in situ in both aquifers (see the supplement: other possible electron donors).

3 Results

3.1 Incubations and independent variables: grouping of aquifer material

For data analysis, the aquifer material was grouped by locality (FFA and GKA aquifer material). Moreover, chemical sediment properties (non-sulphidic and sulphidic samples) and groundwater redox state at the sample origin (samples from NO_3^- free and NO_3^- bearing groundwater zone of both aquifers were assigned to NO_3^--free and NO_3^--bearing sub-groups, respectively) were taken into account for further differentiation. (0.4 mg NO_3^--N l^{-1} was the lowest measured NO_3^- concentration above the limit of detection of 0,2 mg NO_3^--N l^{-1}. Therefore, 0,4 mg NO_3^--N l^{-1} was the lowest concentration to be considered nitrate bearing in this study.) Finally, a transition zone sub-group was defined for samples from the region where sulphides were present, but groundwater still contained NO_3^-. Sulphidic and non-sulphidic samples are distinguished using the sulphate formation capacity (SFC (mg S kg^{-1} yr^{-1})) of the incubated aquifer material. Samples with SFC > 1 mg SO_4^{2-}-S kg^{-1} yr^{-1} were assigned sulphidic. The groundwater at the origin of sulphidic samples had always dissolved O_2 concentrations below 1.5 mg O_2 l^{-1} (see section 4.1). The groundwater at the origin of NO_3^--free samples was completely anoxic in both investigated aquifers. In our data set, subgroups of non-sulphidic and NO_3^--bearing as well as sulphidic and NO_3^--free samples were almost identical (Tables S1 and S2 in the Supplement). Moreover, statistically significant differences were only found in $D_{cum}(365)$ with higher values for NO_3^--bearing in comparison to non-sulphidic samples. NO_3^--free and sulphidic samples differed only in their total-S values significantly, with higher total-S contents in NO_3^--free samples. Therefore, we discussed the partial data sets of NO_3^--free and NO_3^--bearing samples only when significant differences to subgroups according to sediment properties occurred.

3.2 Time course of denitrification products, denitrification rates and cumulative denitrification at the end of incubations

The denitrification rates of non-sulphidic and NO_3^--bearing samples where significantly lower than those of sulphidic and NO_3^--free samples (kw: P <0.01) (Table 2 and Fig. 1). Almost all of the transition zone samples exhibited a clear flattening of the slopes of denitrification derived (N_2+N_2O) concentration curves, i.e. showed decreasing denitrification rates over time (Fig. 1b). Non-sulphidic samples showed a relative constant production of

(N_2+N_2O) (Fig. 1a), but denitrification rates where highly significant (kw: $P < 0.001$) lower compared to sulphidic samples (Table 2, Fig. 1).

Both FFA and GKA aquifer material had nearly the same median initial denitrification rates ($D_r(7)$) with values of 33.8 and 31.2 µg N kg^{-1} d^{-1}, respectively, whereas the maximal $D_r(7)$ of GKA material was over 50% higher compared to the FFA material (Table 2). At the end of incubation, samples from the FFA and GKA had a comparable range of $D_{cum}(365)$ (up to 56 mg N kg^{-1} yr^{-1}). Sulphidic samples had significantly higher median $D_r(7)$ and $D_{cum}(365)$ (35.6 µg N kg^{-1} d^{-1} and 15.6 mg N kg^{-1} yr^{-1}, respectively) than non-sulphidic samples (11.5 µg N kg^{-1} d^{-1} and 1.6mg N kg^{-1} yr^{-1}, respectively) (kw: $P < 0.001$) (Table 2). Non-sulphidic samples exhibited higher initial denitrification rates ($D_r(7)$) than average denitrification rates ($D_r(365)$), whereas this was vice versa for sulphidic samples. Transition zone samples were similar in $D_r(7)$ compared to sulphidic material, but $D_{cum}(365)$ was about 25% lower.

After the intensive treatment incubated aquifer samples were 1 to 17 times higher in $D_r(7)$ (data not shown) and between 3.6 to 17 times higher in $D_{cum}(365)$ compared to the standard treatment (Table S2 in the Supplement, multiplying the aF_{SRC} from intensive treatment by the SRC and 0.01 gives $D_{cum}(365)$ of intensive treatment). But the intensive treatment did not lead to a complete exhaustion of the stock of reactive compounds during incubations, i.e. samples still exhibited denitrification rates at the end (Fig. 1d).

3.3 Sediment parameters

C_{org} exhibited large ranges of similar magnitude in both aquifers (203–5955 and 76–8972 mg C kg^{-1} in the FFA and GKA aquifer samples, respectively) (Table 1). The same applied for total-S, (29–603 and 36–989 mg S kg^{-1}) and $SO_4^{2-}{}_{extr}$ (0 to 25 and from 0.3 to 20 mg S kg^{-1}). GKA samples contained significantly lower median DOC_{extr} values than FFA material (9.2 and 6.1 mg C kg^{-1}, respectively). $SO_4^{2-}{}_{extr}$ and DOC_{extr} decreased with depth in the FFA (r_s: R = −0.83 and R = −0.86, respectively, $P < 0.001$) and in the GKA (r_s: R = −0.54 and R = −0.59, respectively, $P < 0.05$). The ranges of C_{hws} were comparable for FFA and GKA material (Table 1). C_l values of FFA and GKA samples were statistical not different from each other, but maximum values in GKA samples were almost 3 times higher than in FFA material (Table 1). In median, 17% and 26% of the C_{org} in the GKA and FFA aquifer material, respectively, belonged to the fraction of C_l. Statistical significant differences (kw: $P < 0.05$) occurred between the groups of non-sulphidic and sulphidic aquifer material with a

ratio of C_l to C_{org} by 0.17 and 0.24, respectively. Similar differences and ratios applied for the groups of NO_3^--bearing and NO_3^--free samples (Table 1). Except for values of total-S and DOC_{extr}, the investigated sediment parameters exhibited no significant differences between FFA and GKA aquifer material (Fig. S2 in the Supplement). All sediment variables showed significant differences (kw: $P < 0.05$) between the 3 groups of non-sulphidic, sulphidic and transition zone samples (Fig. S2 in the Supplement). On average, transition zone samples had lower ranges in all sediment parameters than sulphidic material except in C_{hws} and DOC_{extr}. Non-sulphidic samples exhibited lower average concentrations in the independent sediment variables compared to transition zone samples, except for $SO_4^{2-}{}_{extr}$ and DOC_{extr} for which the opposite was the case (Table 1, Fig. S2 in the Supplement).

3.4 The stock of reactive compounds and its availability for denitrification during incubation

3.4.1 Standard treatment

The stock of reduced compounds (SRC) of FFA and GKA aquifer material differed not significantly from each other (0.22–6.0 and 0.97–8.9 g N kg^{-1}, respectively) (Table 2 and Fig. 2a). In contrast, the median SRC of sulphidic aquifer material (1.3 g N kg^{-1}) was 2 and 5 times higher compared to the non-sulphidic (0.24 g N kg^{-1}) and transition zone material (0.67 g N kg^{-1}). The fraction of SRC available for denitrification during incubation (aF_{SRC}) in the FFA material ranged from 0.08 to 5.44% and was significantly higher than the range of aF_{SRC} of GKA material (0.36 to 1.74% aF_{SRC}) (Fig. 2b). Transition zone samples exhibited the highest median aF_{SRC} values (1.65%), followed by sulphidic (1.16%) and non-sulphidic aquifer material with the lowest aF_{SRC} values (0.47%). Statistical significant differences were only found between non-sulphidic samples and the previous two groups (Fig. 2b).

3.4.2 Intensive treatment

Since we used parallel samples for the intensive and standard treatments, the SRC was identical for both treatments. Also the intensive treatment was not able to exhaust the denitrification capacity of the incubated aquifer material during incubation (Fig. 1). The aF_{SRC} derived from intensive incubations was 3.6 to 17 times higher compared to the standard

treatment (Table S2 in the Supplement, aF_{SRC} values of the intensive treatment are given in parentheses).

3.5 Relationship between the cumulative denitrification and sediment parameters

Correlations between $D_{cum}(365)$ and sediment parameters showed substantial differences among the various partial data sets (Table 3). For the whole data set C_{org} exhibited the closest correlation (r_s: R = 0.72, $P < 0.001$) with $D_{cum}(365)$. In the FFA aquifer material, DOC_{extr} and $SO_4^{2-}{}_{extr}$ showed highly significant negative relations to $D_{cum}(365)$ (Table 3). The relation between these parameters and $D_{cum}(365)$ was only poor or not significant for the rest of sub data sets. C_{hws} exhibited the highest positive correlations with $D_{cum}(365)$ in the partial data sets with samples containing relatively low concentrations of sulphides (Table 1), i.e. the data sets of non-sulphidic and transition zone samples (r_s: R = 0.85 and R = 0.60, respectively, $P < 0.001$). C_l was in closest relation with $D_{cum}(365)$ in GKA and non-sulphidic samples (r_s: R = 0.87 and R = 0.73, respectively, $P < 0.01$). C_{hws} and C_l were more closely related to $D_{cum}(365)$ compared to C_{org} within sub-groups of aquifer material with no or only low contents of total-S. In contrast to GKA, the FFA aquifer material exhibited good correlations between C_{hws} and $D_{cum}(365)$ (r_s: R = 0.58, $P < 0.01$) (Table 3). In all data sets, the silt content was significantly positively correlated with $D_{cum}(365)$, except for transition zone aquifer material where this relation was not significant. For the whole data set and FFA and GKA data sets, total contents of C_{org} and sulphur were in closest positive correlation with $D_{cum}(365)$. In the partial data sets which were differentiated according to chemical parameters, these relations were less pronounced or not significant.

3.6 Regression models to predict $D_{cum}(365)$

3.6.1 Predicting $D_{cum}(365)$ from intial denitrification rates

Initial denitrification rates derived after 7 days of incubation ($D_r(7)$) exhibited only good linear relations with $D_{cum}(365)$ for non-sulphidic samples (with sub-sets of FFA and GKA non-sulphidic samples) and for the group NO_3^--bearing samples with correlation coefficients > 0.86 (Table 4). For the other data sets, $D_{cum}(365)$ was not predictable by $D_r(7)$ (Table 4 and Fig. 3). Moreover, especially sulphidic and NO_3^--free samples, exhibited a considerable lag-

phase at the beginning of incubation, which resulted in poor predictions of $D_{cum}(365)$ from $D_r(7)$. In contrast to $D_r(7)$, the average denitrification rate after 84 days of incubation, i.e. at the next sampling time $D_r(84)$, showed good to excellent regressions ($R > 0.78$) with $D_{cum}(365)$ for the whole and most of the partial data sets. An exception were the transition zones samples which showed declining denitrification rates during incubation (Fig. 1).

3.6.2 Predicting $D_{cum}(365)$ from sediment parameters

Simple regression and multiple regression analysis was performed to predict $D_{cum}(365)$ from independent sediment variables, i.e. the silt content, C_{org}, total-S, $SO_4^{2-}{}_{extr}$, DOC_{extr}, C_{hws} and C_l. The goodness of fit between modelled and measured $D_{cum}(365)$ is given by correlation coefficients, the ratio of calculated to measured $D_{cum}(365)$ ($R_{c/m}$) and the average deviation of $R_{c/m}$ from the mean in the various sub data sets.

Simple regression models yielded a significant lower goodness of fit than multiple regressions (Table 5, Tables S3 and S4 in the Supplement). Simple regressions with individual sediment parameters demonstrated that C_{org} and C_l yielded best predictions of $D_{cum}(365)$ when the whole data set was analysed (Table S3 in the Supplement). Regression analysis of partial data sets grouped according to chemical properties, i.e. groups including samples from both aquifers, resulted in R values below 0.8 for all tested variables. For the sulphidic samples, C_{org} or C_l values were the best individual sediment parameters to model $D_{cum}(365)$ when considering partial data sets including samples from both aquifers. For the individual aquifers, some single sediment parameters were very good estimators ($R > 0.8$) for $D_{cum}(365)$, e.g. total-S and DOC_{extr} in the FFA data set and C_{org}, total-S and C_l for GKA. C_{org} was clearly less correlated with $D_{cum}(365)$ in those sub-groups of aquifer material with low contents of SRC, i.e. the non-sulphidic aquifer material.

Combinations of total-S and C_{org} did not substantially increase the goodness of fit of the regression models to predict $D_{cum}(365)$ in comparison to simple regressions with these two variables (Table 5, selection I in comparison to Table S3 and S4 in the Supplement), in some cases the goodness of fit even worsened. Only for the partial data sets of non-sulphidic samples a linear combination of these two variables was slightly better than a simple regression with one of the independent variables.

Table 5, selection II lists the combinations including C_{org}, total-S, C_l, and $SO_4^{2-}{}_{extr}$ which revealed the highest correlation coefficient with $D_{cum}(365)$ for the corresponding data sets. Compared to simple regressions these linear combinations improved correlation coefficients

of regressions for most partial data sets. Also the range of deviations of calculated from measured $D_{cum}(365)$ values ($R_{c/m}$) was smaller (Table S4 in the Supplement). For the whole data set and the sulphidic samples for example, the correlation coefficient R increased from 0.80 to 0.86 and from 0.66 to 0.79, respectively, if instead of regressions between C_{org} and $D_{cum}(365)$ the combination of C_{org}-C_l was used to model $D_{cum}(365)$. This combination was also better than regressions with C_l alone (Table 5 in comparison to Table S4 in the Supplement). The combination of total-S and $SO_4^{2-}{}_{extr}$ improved the correlation coefficient with $D_{cum}(365)$ in comparison to simple regression with total-S clearly for all sub data sets containing sulphidic aquifer material. For FFA samples this combination raised R of the simple regressions from 0.83 to 0.89.

For all data sets, except the sub data set of sulphidic material, multiple regressions between $D_{cum}(365)$ and all 7 independent sediment parameters (direct multiple regression) yielded correlation coefficients R > 0.92 (data not shown), i.e. over 84% of the variance of the measured $D_{cum}(365)$ values could be explained with this regression. For sulphidic aquifer material, R was 0.83. A stepwise multiple regression, which gradually adds the sediment parameters to the regression model according to their significance yielded results which were almost identical to the results of direct multiple regression (Table 5, selection III). The stepwise multiple regression model reduced the number of needed regression coefficients (i.e. the number of needed sediment variables) to model $D_{cum}(365)$ from 7 to 3 or 5. The goodness of fit as indicated by mean $R_{c/m}$ values close to 1 and small ranges of $R_{c/m}$ values was usually the best with multiple regression analysis, especially for samples with $D_{cum}(365)$ values below 20 mg N kg^{-1} yr^{-1} (Table S4 in the Supplement).

3.7 Predicting the stock of reduced compounds (SRC) from $D_{cum}(365)$ and estimation of the minimal lifetime of denitrification (emLoD)

The mean $D_{cum}(365)$ values of the 3 to 4 replications per aquifer sample were used to predict the SRC of the aquifer with samples simple regressions (Table 6). For the whole data set the measured $D_{cum}(365)$ values exhibited good linear relations with the SRC of the incubated aquifer samples (R = 0.82). $D_{cum}(365)$ of GKA samples showed good to excellent and clearly better regressions with the SRC than the $D_{cum}(365)$ FFA samples. The prediction of SRC from $D_{cum}(365)$ was also clearly better for sulphidic and NO_3^--free samples compared to samples from already oxidized parts of both aquifers (Table 6).

The minimal lifetime of denitrification (emLoD) of the incubated aquifer material was estimated for a nitrate input of 11.3 g NO_3^--N m^{-2} yr^{-1} as described in Sect. 2.5. With this nitrate input and an assumed fraction of the SRC available for denitrification during incubation (aF_{SRC}) of 5% the calculated emLoD of one m^3 of aquifer material ranged between 0.7–8 and 2.4–67 yr m^{-1} for non-sulphidic and sulphidic aquifer material, respectively (Tables 2 and S2 in the Supplement). The estimated median emLoD of sulphidic material was 5 times higher then the one of non-sulphidic samples. FFA and GKA samples differed statistical not significantly in their emLoD values (kw: $P < 0.05$) (median emLoD values of NO_3^--free aquifer samples from the FFA and GKA are 19.8±15 yr and 10.5±20 yr, respectively; see also Table S2 in the Supplement).

4 Discussion

4.1 Groundwater redox state and sample origin

The non-sulphidic aquifer material in this study, which exhibited low denitrification rates, originated generally from aquifer regions with dissolved O_2 concentrations > 1.5 mg l^{-1} (=42 µmol O_2 l^{-1}) and is already largely oxidized. This aquifer parts could be referred to as aerobic (1–2 mg O_2 l^{-1}, Rivett et al., 2008). In laboratory experiments with homogeneous material the intrinsic O_2 threshold for the onset of denitrification is between 0 and 10 µmol O_2 l^{-1} (Seitzinger et al., 2006). Reported apparent O_2 thresholds for denitrification in aquifers are between 40 to 60 µmol l^{-1} (Green et al., 2008, 2010; McMahon et al., 2004; Tesoriero and Puckett, 2011). Green et al. (2010) modelled the apparent O_2 threshold for denitrification in a heterogeneous aquifer and found that an apparent O_2 threshold obtained from groundwater sample analysis of < 40 O_2 µmol l^{-1} is consistent with an intrinsic O_2 threshold of < 10 µmol l^{-1}. This apparent threshold of 40 µmol O_2 l^{-1} corresponds well with the threshold of minimal and maximal dissolved O_2 concentrations at the origins of non-sulphidic and sulphidic aquifer material, respectively, in both aquifers. The sulphides that occur in zones where O_2 is still measurable in the groundwater might represent residual sulphides from poorly perfused micro areas within the aquifer material.

4.2 Predicting $D_{cum}(365)$ from initial denitrification rates and time course of denitrification

An important goal of denitrification research is to predict long-term denitrification capacity of aquifers from initial denitrification rates.

The conducted incubations showed that there are significant quantitative relations between $D_{cum}(365)$ and the SRC of the incubated aquifer samples (Table 6) and it can be assumed that the SRC represents a maximum estimate of the long-term denitrification capacity of aquifer material. Taking this into account it was tested if initial denitrification rates can predict $D_{cum}(365)$. This was done to facilitate determination of $D_{cum}(365)$ since laboratory measurements of initial denitrification rates ($D_r(7)$) are more rapid and less laborious and expensive compared to one-year incubations to measure $D_{cum}(365)$. Moreover, initial denitrification rates can also be measured in situ at groundwater monitoring wells (Konrad, 2007; Well et al., 2003) and can thus be determined without expensive drilling for aquifer material. Konrad (2007) tested this approach with a small data set (13 in situ measurements) and 26 pairs for $D_r(7)$ vs D_r(in situ) and only 5 pairs for D_r(in situ) vs. $D_{cum}(365)$. One objective of this study is to develop transfer functions to predict $D_{cum}(365)$ from $D_r(7)$. The next step would be to compare in situ denitrification rates (D_r(in situ)) from push-pull experiments at the location of the incubated aquifer samples with their $D_{cum}(365)$ measured in this study and to check how precise $D_{cum}(365)$ can be derived from D_r(in situ).

By and large, the measured range of $D_{cum}(365)$ values agreed well with previous incubations studies, which investigated the denitrification activity of aquifer material from comparable Pleistocene sandy aquifers. Well et al. (2005) and Konrad (2007) report total ranges for D_{cum} of 9.5 to 133.6 mg N kg^{-1} yr^{-1} and 0.99 to 288.1 mg N kg^{-1} yr^{-1}, respectively. Weymann et al. (2010) conducted incubations with aquifer material from one location within the FFA, reporting ranges of $D_{cum}(365)$ of heterotrophic (≈ non-sulphidic) and autotrophic (≈ sulphidic) aquifer material between 1–12.8 and 14.5–103.5 mg N kg^{-1} yr^{-1}, respectively (calculated from reported denitrification rates). All of these denitrification capacities are comparable to our findings (Table 2), indicating that the selection of our sites and sampling location represent the typical range of denitrification properties of this kind of Pleistocene sandy aquifers.

Two aspects have to be considered when using $D_r(7)$ as an indicator for $D_{cum}(365)$: (aspect i) the availability of reactive compounds may change during incubation and (aspect ii) different microbial communities resulting from the availability of different electron donors and acceptors may be evident in samples from different aquifer redox zones (Griebler and Lueders, 2009; Kölbelboelke et al., 1988; Santoro et al., 2006) and possible shifts within the microbial community during incubation have thus to be taken into account (Law et al., 2010).

With respect to (aspect i), it is straightforward that the availability of reduced compounds for denitrification in aquifer material directly influences the measured denitrification rates since denitrification is a microbially mediated process and the significant majority of microbes in aquifers are attached to surfaces and thin biofilms (Griebler and Lueders, 2009; Kölbelboelke et al., 1988). Therefore, the area of reactive surfaces of reduced compounds within the sediment might control the amount of active denitrifiers in an incubated sample and thus the measured denitrification rates and vice versa. Therefore, denitrification rates are an indirect measure of the availability of reduced compounds for denitrification and the availability of reduced compounds may reduce due to oxidation during incubation. On the contrary, growth of the microbial community may change the apparent availability of reduced compounds due to the increase of the area of "colonised" reduced compounds within the incubated aquifer material and thus leading to increasing denitrification rates during incubation.

The almost linear time-course of denitrification in non-sulphidic and sulphidic samples (Fig. 1a, c) indicate minor changes of the availability of reduced compounds during incubation. The linear-time-courses also suggest a pseudo-zero-order kinetic of denitrification where denitrification rates are independent from changes of NO_3^- or reduced compounds during the incubations. NO_3^- concentrations in the batch solution of incubated samples were always above 3.0 mg NO_3^--N l^{-1} during the whole incubation period and thus above the reported threshold of 1.0 mg NO_3^--N l^{-1}, below which denitrification is reported to becomeNO_3^- limited (Korom et al., 2005; Morris et al., 1988; Wall et al., 2005).

The small denitrification rates measured in the non-sulphidic samples may then be the result of only small amounts of organic carbon oxidized during denitrification. The consumed fraction of available organic carbon might release fresh surfaces which can further be oxidized during denitrification. The relative stable denitrification rates of non-sulphidic samples may then reflect that the area of microbial available surface of reduced compounds exhibits negligible change during incubation. This is plausible for the case that the surface of

the organic matter is relatively small in comparison to its volume, which applies to the lignitic pebbles in the FFA (Frind et al., 1990).

Most of the sulphidic aquifer samples from the zone of NO_3^--free groundwater in both aquifers showed also relative constant linear increase of denitrification products during incubation (Fig. 1c). This aquifer material was not yet in contact with dissolved O_2 and NO_3^- from the groundwater. Hence, the reduced compounds, if initially present in the solid phase, are supposed to be not yet substantially depleted. The relative constant linear increase of denitrification products of these samples suggests that the denitrifying community had a relative constant activity during incubation, implying a constant amount of denitrifying microbes and thus constant areas of reactive surfaces. In contrast, almost all transition zone samples exhibited clearly declining denitrification rates during incubation (Fig. 1b). This group represents aquifer material already depleted in reduced compounds (Table 1 and Fig. 2a) but still containing residual contents of reactive sulphides and therefore showing a SFC > 1 mg SO_4^{2-}-S kg^{-1} yr^{-1}. These residual sulphides might be relatively fast exhausted during incubation leading to a loss of reactive surfaces and in the following to a flattening of the slope of measured denitrification products (N_2+N_2O).

The intensive incubation experiment gave up to 17 times higher denitrification rates than the standard incubations (Table S2 in the Supplement) and differed from the standard incubations only in three points: (i) dilution of aquifer material with pure quartz sand, (ii) higher incubation temperatures (20 °C instead of 10 °C) and (iii) continuous shaking of the incubated sediments on a rotary shaker. The denitrification activity of the added pure quartz was found to be negligible. Well et al. (2003) evaluated the temperature effect on denitrification rates measured during laboratory incubations. An increase of incubation temperature from 9 to 25 °C resulted in 1.4 to 3.8 times higher denitrification rates. In contrast to this the intensive incubation experiment presented in this study gave up to 17 times higher denitrification rates than the standard incubations. This suggests that not only higher temperatures but also the continuous shaking of the incubated aquifer material may have led to higher denitrification rates by the enlargement of the surfaces of reduced compounds within the aquifer material due to physical disruption of pyrite and/or organic carbon particles. The latter was visible as black colouring of the batch solution which was not noticeable at the beginning of intensive incubations and also not during the standard incubations. But in contrast to our expectations, the intensive treatment did not lead to a faster decline of denitrification rates during incubation (Fig. 1d). The reasons for this might be that the loss of reactive surfaces of reduced compounds due to consumption during denitrification was small compared to their amount.

Also the shaking might have contributed to the creation of reactive surfaces and thus may have supported denitrification. A possible temperature effect on the suit of active denitrifiers during incubations and from this on the resulting denitrification rates was not investigated during this study, but should be considered in further studies.

With respect to the importance of changes in the availability of electron acceptors for the communities of active microbes present in aquifer material (aspect ii), we assume that in the sulphidic samples from the zone of NO_3^--free groundwater, the population of denitrifiers had to adapt to the addition of NO_3^- as a new available electron donor, e.g. by growth of denitrifying population and changes in the composition of the microbial community (Law et al., 2010). This adaptation processes require time and might be a reason for the missing correlation between $D_r(7)$ and $D_{cum}(365)$ during incubation of sulphidic samples in both aquifers, whereas $D_r(84)$ was a good predictor for $D_{cum}(365)$ (Fig. 3 and Table 4). This explanation is in line with the fact that spatial heterogeneity of microbial diversity and activity is strongly influenced by several chemical and physical factors including the availability of electron donors and acceptors (Griebler and Lueders, 2009; Kölbelboelke et al., 1988; Santoro et al., 2006). Santoro et al. (2006) investigated the denitrifier community composition along a nitrate and salinity gradient in a coastal aquifer. They conclude that for the bacterial assemblage at a certain location, “steep gradients in environmental parameters can result in steep gradients (i.e. shifts) in community composition”.

The observed adaptation phase is in accordance to results given by Konrad (2007) who found also only after 84 days of incubation good relations between mean denitrification rates and $D_{cum}(365)$, whereas the sampling after day 21 of incubation gave poor correlations. We conclude that 7 days of incubation were not sufficient to get reliable estimates of $D_{cum}(365)$ from $D_r(7)$ for aquifer samples from deeper reduced aquifer regions in both investigated aquifers, whereas there are good transfer functions to predict $D_{cum}(365)$ from $D_r(84)$ for all partial data sets.

We conclude that prediction of denitrification from initial denitrification rates ($D_r(7)$) during incubation experiments is possible for non-sulphidic samples, which were already in contact with groundwater NO_3^-. The denitrification capacity of these samples must have been exhausted to some extent during previous denitrification or oxidation and the laboratory incubations reflect the residual stock of reductants. Contrary, the denitrification capacity of sulphidic samples was not predictable from $D_r(7)$. These samples were not yet depleted in reduced compounds and therefore these samples exhibited significantly higher denitrification rates during incubation. With respect to in situ measurements of denitrification rates with

push-pull tests in the reduced zones of aquifers the required adaptation time of the microbial community to tracer NO_3^- might lead to an underestimation of possible denitrification rates.

4.3 Predicting $D_{cum}(365)$ of aquifer sediments, correlation analysis and regression models

4.3.1 Sediment parameters and their relation to $D_{cum}(365)$

Correlation analysis

C_{org}, $SO_4^{2-}{}_{extr}$, C_{hws} and C_l exhibited no significant differences between both aquifers, whereas the amount of total-S was significantly higher and DOC_{extr} values significantly lower for GKA compared to FFA samples. But in contrast, the opposite groups of non-sulphidic to sulphidic aquifer material differed significantly in all of the analysed independent sediment variables (kw: $P < 0.05$) (Table 1 and Fig. S2 in the Supplement). The same applies also for the opposite groups of NO_3^--free and NO_3^--bearing aquifer material (data not shown).

The measured range of DOC_{extr} (4.7 to 11.6 mg C kg^{-1}) for FFA and GKA aquifer samples are in the range of recently reported values (Weymann et al., 2010) for aquifer samples from the same site at comparable depths. The DOC_{extr} values clearly decreased with depth in both aquifers (Table S1 in the Supplement) and exhibited partly significant negative correlations with the $D_{cum}(365)$ of the incubated aquifer material (Table 3) (r_s: $P < 0.05$). Similarly, von der Heide et al. (2010) reported significant negative correlation between DOC and the concentrations of N_2O as an intermediate during reduction of NO_3^- to N_2 in the upper part of the FFA. From these findings we suppose that the reactive fraction of DOC is increasingly decomposed or immobilized with depth in both aquifers. Moreover, the negative correlation between the DOC_{extr} and the measured $D_{cum}(365)$ suggests that the contribution of DOC_{extr} to denitrification capacity of the aquifers is relatively small, which is consistent with findings of Tesoriero and Puckett (2011) and Green et al. (2008).

The highest concentrations of $SO_4^{2-}{}_{extr}$ were measured in samples from the upper parts of both aquifers (Table 1). The measured range of $SO_4^{2-}{}_{extr}$ (Table 1) exhibited significant negative correlations between $D_{cum}(365)$ of FFA and GKA aquifer material (r_s: R = −0.82 and R = −0.49, respectively, $P < 0.05$) (Table 3). $SO_4^{2-}{}_{extr}$ values decreased with depths in both aquifers (Table S1 in the Supplement) and thus exhibited an inverse concentration gradient compared with total-S values. The range of $SO_4^{2-}{}_{extr}$ of FFA and GKA material is comparable

to $SO_4^{2-}{}_{extr}$ values (20.5± 16.7mg SO_4^{2-}-S kg^{-1}) of aquifer samples from North Bavaria, from a deeply weathered granite with a sandy to loamy texture (Manderscheid et al., 2000). All measured $SO_4^{2-}{}_{extr}$ values above 10 mg S kg^{-1} from FFA and GKA samples (except for the samples from 25.9–26.9 m and 27–28.3 m below surface in the GKA) originated from zones within these two aquifers with pH values of the groundwater between 4.39 and 5.6 (von der Heide unpublished data and own measurements). According to the pH values, the groundwater from these locations is in the buffer zone of aluminium hydroxide and aluminium hydroxysulphates (Hansen, 2005). It is known that hydroxysulphate minerals can store SO_4^{2-} together with aluminium (Al) in acidic soils (Khanna et al., 1987; Nordstrom, 1982; Ulrich, 1986) and aquifers (Hansen, 2005). Therefore, dissolution of aluminium hydroxysulphate minerals may have lead to the higher values of $SO_4^{2-}{}_{extr}$ in samples from the upper already oxidized parts of both aquifers.

$KMnO_4$ labile organic carbon (C_l) measured in the aquifer material was closely related to C_{org} (r_s: R = 0.84, $P < 0.001$). GKA samples showed a much wider range of C_l values (0.9 to 2504.7 mg C kg^{-1}) than FFA aquifer material (2.7 to 887 mg C kg^{-1}) (Table 1). The total average of C_l/C_{org} ratios of 0.24 for the whole data set is comparable to the mean ratio of 0.3 reported by Konrad (2007) for 3 comparable sandy aquifers, showing that typically less than half of C_{org} in Pleistocene aquifers is $KMnO_4$ labile. The higher C_l/C_{org} ratio in the sulphidic samples might indicate that the C_l fraction of C_{org} in the upper non-sulphidic parts of both aquifers is already oxidized to a larger extent (Table 1). Konrad (2007) assumes that C_l represents the proportion of C_{org} which might be available for microbial denitrification. A stoichiometric $CH_2O_{(Corg)}/NO_3^-$-N ratio of 1.25 (Korom, 1991) leads to the conclusion that the amount of C_l was always higher than the measured amount of denitrification after one year of incubation ($D_{cum}(365)$) of the several aquifer samples. This shows that a significant fraction of C_l did not support a fast denitrification. It can thus be assumed that C_l represents rather an upper limit for the bioavailable organic carbon in the incubated sediments. However, among the sediment parameters C_l was the best predictor of $D_{cum}(365)$ for GKA samples and non-sulphidic aquifer material and also a comparatively good predictor with respect to the whole data set (Table 3).

The values of hot water extracts (C_{hws}) from FFA and GKA aquifer material with the ranges of 0.01–42.6 and 14.9–58.5 mg C kg^{-1}, respectively, are comparable to the range of C_{hws} of 6.2 to 141 mg C kg^{-1} given by Konrad (2007). C_{hws} represents on average a proportion of 6.5% of the entire C_{org} pool in the aquifer material from FFA and GKA. This value is similar to the proportion of 5% C_{hws} of the entire C_{org} reported by Konrad (2007), with significantly

(kw: $P < 0.05$) higher percentages in the non-sulphidic (12.5%) compared to the sulphidic samples (3.7%). We found strong and highly significant correlations between C_{hws} and $D_{cum}(365)$ of non-sulphidic material (Table 3) and NO_3^--bearing samples (r_s: R = 0.85 and R = 0.74, respectively, $P < 0.001$). Studies on C_{hws} stability in soil organic matter revealed that C_{hws} is not completely bioavailable (Chodak et al., 2003; Sparling et al., 1998). Moreover, these authors conclude that C_{hws} is not a better measure of the available soil organic carbon than total C_{org} values. Balesdent (1996) concluded from natural ^{13}C labelling technique (long-term field experiments with maize) that coldwater extracts contain amounts of slowly mineralizable "old" C_{org} pools and this can also be expected for hot water extracts. The close correlation between C_{hws} and $D_{cum}(365)$ in the non-sulphidic aquifer material and not for deeper sulphidic aquifer material is distinctive and but difficult to interpret since C_{hws} represents not an uniform pool of organic matter. The missing correlation between C_{hws} and $D_{cum}(365)$ might indicate that denitrification in this zone is sulphide dependent.

The measured C_{org} values of FFA and GKA aquifer material (Table 1) are comparable to ranges reported by Konrad (2007), Strebel et al. (1992) and Hartog et al. (2004) (Pleistocene fluvial and fluvio-glacial sandy aquifers in Northern Germany and the eastern part of The Netherlands). The total sulphur contents of FFA and GKA aquifer samples are also comparable to the ranges reported by these authors, except Hartog et al. (2004) who reported 4 to 5 times higher total-S contents. Bergmann (1999) and Konrad (2007) investigated the distribution of S species in aquifer material from sandy aquifers in North Rhine-Westphalia and Lower Saxony, Germany, respectively, and found that 80 to over 95% of the total-S value is represented by sulphide-S.

4.3.2 Predicting $D_{cum}(365)$ from sediment variables

Single sediment parameters like C_{org}, C_l or total-S are partly good to very good estimators for the measured $D_{cum}(365)$ in our data set (Table S3 in the Supplement). Grouping of aquifer material according to hydro-geochemical zones strongly increases the predictive power of single independent sediment parameters with respect to the measured denitrification during incubation (S3 in the Supplement). For example, C_{org} and C_l values are very good parameters to predict $D_{cum}(365)$ for GKA aquifer material, which almost linearly increased with measured C_{org} and C_l values. The predictability of $D_{cum}(365)$ with simple regressions, linear combinations of two sediment parameters and multiple regressions was best when these models were applied to partial data sets of one aquifer, whereas predictions were always

worse when samples from both aquifers were included (Tables 5 and S3 in the Supplement). For example, total-S values exhibited good simple regressions (R > 0.8) with partial data sets that contain only aquifer material from one aquifer. Conversely, the linear correlation coefficients between total-S and $D_{cum}(365)$ of sulphidic aquifer material and NO_3^--free samples (both groups contain FFA and GKA aquifer material) were relatively low with R of 0.4 and 0.32, respectively. The proportion of total-S in SRC of the GKA samples was 3 times higher than in samples from the FFA, whereas the share of sulphides contributing to the measured denitrification capacity was almost the same in FFA and GKA material during incubation (Fig. 2b). This shows that samples from both sites were distinct in the reactivity of sulphides which may be related to the geological properties of the material including the mineralogy of the sulphides and the origin of the organic matter.

C_{org} and total-S can be seen as integral parameters with no primary information about the fraction of reactive and non-reactive compounds (with regard to denitrification) represented by these parameters. As already discussed above, C_l might be an upper limit for the fraction of microbial degradable organic carbon as part of total organic carbon (C_{org}) in a sample of aquifer material. In our data set, C_l exhibited better regressions with $D_{cum}(365)$ than C_{org} for aquifer material with relatively low $D_{cum}(365)$, i.e. non-sulphidic aquifer material and transition zone samples (Table S3 in the Supplement). In these two partial data sets it can be assumed that the reduced compounds available for denitrification are already depleted by oxidation with NO_3^- and dissolved O_2. The median C_{org} contents of non-sulphidic and transition zone samples were only about 20% and 60% of the one of NO_3^--free samples (Table 1). Hence, C_{org} in non-sulphidic and transition zone samples might represent less reactive residual C_{org} compared to aquifer material which was not yet in contact with groundwater NO_3^- or dissolved O_2. This might be the reason for the comparatively low correlation of C_{org} and $D_{cum}(365)$ in the depleted aquifer material of non-sulphidic and transition zone samples. Similar to this finding, Well et al. (2005) reported poor correlations between C_{org} and the measured amount of denitrification for hydromorphic soil material with low measured denitrification activity during incubation.

Multiple regression analysis clearly enabled the best prediction of $D_{cum}(365)$. Except for sulphidic samples, correlation coefficients > 0.91 were achieved for all other partial data sets (Table 5). But multiple regression models are of limited practical use because the measurement of several sediment parameters is time consuming and expensive.

The goodness of fit of the regression models was highly variable. Simple regressions, linear combinations of two sediment variables and multiple regression analysis could predict the

order of magnitude of $D_{cum}(365)$. The uncertainty of calculated $D_{cum}(365)$ as given by the ratio of calculated $D_{cum}(365)$ vs. measured $D_{cum}(365)$ ($R_{c/m}$) was within a range of 0.2 to 2 for aquifer material with a measured $D_{cum}(365)$ > 20 mg N kg^{-1} yr^{-1} when simple regressions models and multiple regressions were applied (Table S4 in the Supplement). In case of less reactive aquifer material ($D_{cum}(365)$ < 20 mg N kg^{-1} yr^{-1}), only multiple regressions were able to predict $D_{cum}(365)$ close to this range of uncertainty, whereas simple regressions models yielded poor fits. Well et al. (2005) performed long-time anaerobic incubations with soil material of the saturated zone of hydromorphic soils from Northern Germany in order to measure and calculate denitrification during incubations. They used multiple regressions models to model cumulative denitrification from independent sediment variables. Similar to our finding, they report that prediction of denitrification with regression models was unsatisfactory for samples with low measured denitrification rates (< 36.5 mg N kg^{-1} yr^{-1}, this threshold fits also to our data) and they presumed that a considerable variability in the fraction of reactive organic carbon in the measured C_{org} is the reason for this observation.

4.4 From $D_{cum}(365)$ and SRC to the assessment of the lifetime of denitrification within the investigated aquifers

As already defined above the denitrification capacity can be defined as the part of the SRC capable to support denitrification. The lifetime of denitrification in aquifer material depends on the combination of the denitrification capacity, i.e. the stock of available reduced compounds, the NO_3^- input and the kinetics of denitrification .

Two key assumptions were made for the assessment of the lifetime of denitrification in both aquifers from our incubation experiments. There are relations between (i) the measured $D_{cum}(365)$ and the stock of reduced compounds (SRC) and (ii) between the SRC and the denitrification capacity.

(i) The measured $D_{cum}(365)$ was a good predictor for the SRC for the whole data set and GKA samples. The SRC was also predictable for sulphidic and NO_3^--free samples. Contrary, $D_{cum}(365)$ was a poor indicator of the SRC for aquifer material from already oxidized parts of both aquifers with relatively low amounts of SRC (Table 6). Since the conducted incubations were not able to exhaust the denitrification capacity of the aquifer samples, the real fractions of the SRC available for denitrification (aF_{SRC}) in the incubated samples and even more so the in situ aF_{SRC} remained unknown.

(ii) The low total-S values in the upper parts of both aquifers (Table S1) suggest that most of the sulphides present in both aquifers (see section 4.3.1) are not resistant to oxidation. Moreover, sulphides are supposed to be the dominant reduced compound supporting denitrification in the FFA (Kölle et al. 1983). Both aquifers (FFA and GKA) still contain reduced compounds in form of organic matter in their oxidized upper parts. So obviously, certain fractions of the whole SRC are resistant to oxidation. But it is unknown how the ratio of oxidizable to none-oxidazable C_{org} may change with depth in both aquifers. During this study we found that the C_l/C_{org} ratio was higher for deeper (sulphidic) aquifer samples compared with non-sulphidic samples from the upper region in both aquifers. This suggests that the proportion of organic C which is recalcitrant is higher in the already oxidized zone (see section 4.3.1). A reason for this might be that the proportion of mineral associated organic carbon to total organic carbon is higher in this zone.

(Mineral association of organic matter is assumed to increase the recalcitrance fraction of total organic matter (Eusterhues et al. 2005). Eusterhues et al. (2005) reported for a dystric cambisol and a haplic podzol from northern Bavaria that 80 – 95% of the total organic carbon content of the particle size fraction (< 6.3 µm) in the C horizon is mineral associated organic matter and Fe oxides were identified as the most relevant mineral phases for the formation of organo-mineral associations. Fe oxides can form during autotrophic denitrification with pyrite and they are known to exist frequently in oxidized aquifers.)

With regard to assumption (ii) a further assumption for the assessment of the lifetime of denitrification is: The ratio of SRC to $D_{cum}(365)$ during incubations is a rough measure to estimate the aF_{SRC} capable to support denitrification in situ.

Since the real value of aF_{SRC} remained unknown, the estimated minimal lifetime of denitrification (emLoD) was to calculated with an average aF_{SRC} of 5% was assumed. This value was assumed from intensive incubation with median aF_{SRC} of 6.4% and the fact that denitrification did not stop during all incubations (Fig. 1) and thus the real aF_{SRC} of the incubated aquifer samples were higher than the measured ones (Table S2 in the Supplement).

The data set provides spatial distribution of $D_{cum}(365)$ and SRC values in both aquifers. From this data the lifetime of denitrification (Eq. 2) as well as the the depth shift of the denitrification front in both aquifers were estimated. The simplified approach of calculating emLoD with Eq. 2 implicitly assumes that the residence time of groundwater in 1 m^3 aquifer material is sufficient to denitrify the nitrate input coming with groundwater recharge, if the amount of microbial available SRC is big enough to denitrify the nitrate input.

If the residence time is too short, NO_3^- would reach the subsequent m^3 of aquifer material with groundwater flow, even if the first m^3 still posses an SRC available for denitrification. This means the denitrification front would have a thickness of more than 1 m and the real lifetime of denitrification within one m^3 would be longer then predicted by Eq. 2. This was the case at multilevel wells B2 and N10 in the FFA in the depths between 8–10 and 4.5–8.6 m, respectively. At this depths the groundwater still contains NO_3^-, although the measured $D_{cum}(365)$ of the aquifer material during incubation was higher than the nitrate input (6.6 mg N kg^{-1} yr^{-1}). Two reasons might explain this, either the nitrate input is considerably higher than $D_{cum}(365)$ of these aquifer material or there are flow paths through the aquifer, where reduced compounds are already exhausted.

All non-sulphidic samples were in the NO_3^--bearing zone of both aquifers, i.e. their $D_{cum}(365)$ values were too low to remove the nitrate input during groundwater passage. Therefore, the protective lifetime of denitrification in the investigated aquifers was estimated from the thickness of the NO_3^--free zone in both aquifers and the amount of microbial available SRC (Table S1 in the Supplement). The median emLoD of NO_3^--free aquifer samples from the FFA and GKA are 19.8±15 and 10.5±20 yr m^{-1}, respectively. The high standard deviation of the calculated emLoD values reflects the high heterogeneity of the SRC distribution in both aquifers. These median values of emLoD are equal to a depth shift of the denitrification front of 5 to 9.5 cm yr^{-1}, respectively, into the sulphidic zone, if groundwater flow would only have a vertical component. Since real groundwater flow has a vertical and horizontal component at a given location, the real depth shift of the oxidation front should be lower, depending on the relation of vertical to horizontal groundwater flow velocity.

With respect to the thickness of the NO_3^--free zone at multilevel well N10 in the FFA and at the investigated groundwater wells in the GKA, of 16 and 42 m, respectively, this gives a protective lifetime of denitrification of approximately 315 yr and 440 yr, respectively. These values are conservative estimates, on condition that only 5% of the SRC are available for denitrification and the nitrate input is 11.3 g N m^{-2} yr^{-1}. According to Eq. 2, emLoD is inverse to nitrate input and thus would increase with decreasing nitrate input. From SFC measurements and assuming a nitrate input of 4.5 g N m^{-2} yr^{-1} Kölle et al. (1985) estimated a protective lifetime of denitrification of about 1000 yr summed up over the depth of the FFA aquifer at one location, giving 50 yr lifetime of denitrification per depth meter. Using the same nitrate input as in our estimation (11.3 g NO_3^--N m^{-2} yr^{-1}) the data given by Kölle et al. (1985) would give a lifetime of denitrification of about 20 yr per depth meter. With respect to

the high spatial heterogeneity of SRC values this value fits well to our data for sulphidic aquifer material (Table S2 in the Supplement).

Taking this into account the above stated limitations of the assessment of the emLoD within the investigated aquifers from shorter-term incubations, the calculated emLoD should be validated by long-term in situ test as described by Korom et al. (2005).

4.5 Are laboratory incubation studies suitable for predicting in situ processes?

In the following a few conclusions from the presented study are given, trying to contribute to this question. Therefore, a couple of sub-problems arising from this question are discussed in the following.

4.5.1 Limitations of the $^{15}NO_3^-$ labelling approach

^{15}N labelling of NO_3^- with subsequent analysis of produced ^{15}N labelled N_2 and N_2O did not exclude the possible contribution of dissimilatory nitrate reduction to ammonium (DNRA) since ^{15}N of NH_4 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 ^{15}N labelled N_2 from the labelled NO_3^- during anaerobic incubations. Hence, despite the fact that previous investigations reported denitrification as the dominant process of NO_3^- 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 NO_3^- 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 NO_3^- consumption during incubation is discussed in more detail in the methodical part of the supplement.

4.5.2 Are the NO_3^- concentrations during incubation comparable to those in situ and what is their influence on the measured denitrification rates?

The NO_3^- concentrations in the FFA range from 0–43 (median 8.5) mg N l^{-1} and in the GKA from 0–57.6 (median 7.2) mg N l^{-1} (Well et al., 2012). The nitrate concentrations at the beginning of the batch experiments were in the range of 35 to 43 mg N l^{-1}, depending on the amount of pore water in the incubated sediments diluting the added tracer solution. During the

incubation experiments the measured NO_3^- concentrations were always within the ranges of NO_3^- concentrations found in both aquifers.

The almost linear time course of denitrification products (see Sect. 4.2) accompanied by a parallel decrease of NO_3^- concentrations in the batch solutions suggests that the NO_3^- concentrations were of no or only minor importance for the measured denitrification rates during the conducted incubation experiments, i.e. the kinetics of denitrification were zero-oder. The presented experimental results are in accordance to several workers who reported that the kinetics of denitrifcation at NO_3^- concentrations above 1 mg N l^{-1} are zero-order, i.e. independent of the nitrate concentration, which suggest that the supply of electron donors controls the denitrification rates (Rivett et al., 2008). In a recent publication Korom et al. (2012) stated that denitrification in aquifers appears to be most often reported as zero-order. This statement was based on Green et al. (2008) and Korom (1992) and citations therein. Similarly, Tesoriero and Puckett (2011) found that in most suboxic zones of 12 shallow aquifers across the USA in situ denitrification rates could be described with zero-order rates.

In accordance to the cited studies, the experimental results indicate that the supply of electron donors controlled the measured denitrification rates during the conducted incubation experiments, rather than NO_3^- concentrations. Presumably this can also be expected in situ in both aquifers, if the observation period of rate measurements is short enough, so that the consumption of electron donors does not change the supply of denitrifiers with electron donors significantly. Decreasing concentrations of reduced compounds supporting denitrification would lead to decreasing denitrification rates, i.e. to first-order rates. From this findings it might be concluded that the comparability of laboratory and in situ denitrification rates is less affected by the concentration of NO_3^- als long as denitrification becomes not NO_3^- limited, i.e. at NO_3^- concentrations > 1 mg N l^{-1}.

4.5.3 Is one year incubation suitable to predict the denitrification capacity over many decades in an aquifer?

Our experiments are an approach to narrow down the real denitrification capacity of the investigated aquifer. Longer incubation periods would have been better, but there are always practical limits and incubation experiments could not be conducted over several decades.

Linear regressions showed that there are quantitative relations at least between $D_{cum}(365)$ and the SRC of the incubated aquifer samples from the reduced zone in both aquifers (Table 6) and it can be assumed that the SRC in a certain degree determines the long-term

denitrification capacity of aquifer material. From this, one- year incubations may give minimum estimates of the denitrification capacity of aquifer sample. Furthermore one year of incubation seems long enough to overcome microbial adaptation processes encountered at the beginning of the conducted incubations (see section 4.2). During the intensive incubation experiment 4.6 to 26.4% of the stock of reduced compounds (SRC) of the incubated aquifer material was available for denitrification with median values of 6.4% (Table S2 in the Supplement). From the results of standard and intensive incubations it was assumed that 5% of the SRC is available for denitrification in the investigated sediments. The SRC of aquifer material from the zone of NO_3^--bearing groundwater was only 40% compared to the SRC present in aquifer material from the zone of NO_3^--free groundwater in both aquifers (Table 2), suggesting that an availability of 5% of the SRC did not over estimated the denitrification capacity of the investigated aquifers. Nonetheless, quantitative relations between $D_{cum}(365)$, SRC and the long-term denitrification capacity of aquifers can only be verified by long-term in situ experiments, for example like those described by Korom et al. (2005).

4.5.4 Did laboratory incubation studies really indicate what happens in situ?

They can not exactly retrace all processes contributing to the reduction of NO_3^- to N_2 and N_2O and their interaction under in situ conditions. But laboratory incubations might allow to get estimates of the amount of reduced compounds present in the incubated aquifer material that are able to support denitrification. And laboratory incubations should be compared with short-term and long-term in situ measurements to check the meaningfulness of laboratory incubations for the in situ process as well as the predictability of long-term in situ processes from short-term measurements. In a second study to follow we will compare laboratory incubations and in situ measurements at the origin of the incubated aquifer material.

5 Conclusions

We investigated the relationship between the cumulative denitrification after one year of anaerobic incubation ($D_{cum}(365)$) and, initial laboratory denitrification rates, different sediment parameters and the stock of reduced compounds (SRC) of incubated aquifer samples from two Pleistocene unconsolidated rock aquifers. This was done to characterize denitrification capacity of sediment samples from the two aquifers and to further develop approaches to predict exhaustion of denitrification capacity and $D_{cum}(365)$.

Measured denitrification rates and ranges of the investigated sediment parameters coincided with previous studies in comparable aquifers suggesting that these results derived in this study are transferable to other aquifers.

$D_{cum}(365)$ appeared to be a good indicator for the long-term denitrification capacity of aquifer material from the reduced zone of both aquifers since it was closely related to the SRC.

$D_{cum}(365)$ could be estimated from actual denitrification rates in samples that originated from regions within both aquifers that were already in contact with NO_3^- bearing groundwater, i.e. where the microbial community is adapted to NO_3^- as an available electron acceptor for respiratory denitrification. These regions are thus favourable for the determination of $D_{cum}(365)$ from short-term laboratory experiments. Based on these findings, we expect that in situ measurement of actual denitrification rates will be suitable to estimate $D_{cum}(365)$ in the zone of NO_3^- bearing groundwater, if denitrification is not limited by dissolved O_2. In the deeper zones that had not yet been in contact with NO_3^-, $D_{cum}(365)$ was poorly related to initial denitrification rates. Only after prolonged incubation of several weeks denitrification rates could predict $D_{cum}(365)$ of these samples.

$D_{cum}(365)$ could also be estimated using transfer functions based on sediment parameters. Total organic carbon (C_{org}) and $KMnO_4$-labile organic C (C_l) yielded best transfer functions for data sets containing aquifer material from both sites, suggesting that transfer functions with these sediment parameters are more transferable to other aquifers when compared to regressions based on total-S values. $D_{cum}(365)$ could be predicted relatively well from sediment parameters for aquifer material with high contents of reductants. Conversely, samples depleted in reductants exhibited poor predictions of $D_{cum}(365)$, probably due to higher microbial recalcitrance of the residual reductants.

We conclude that best predictions of $D_{cum}(365)$ of sandy Pleistocene aquifers results from a combination of short-term incubation for the non-sulphidic, NO_3^--bearing zones and analysing the stock of reduced compounds in sulphidic zones which are to date not yet depleted by denitrification processes.

During incubations only samples from the transition zone between the non-sulphidic and NO_3^--free zones showed clearly declining denitrification rates and therefore it was difficult to predict $D_{cum}(365)$ of these samples. The declining denitrification rates of theses aquifer samples resulted possibly from the small contents of residual reduced compounds that might get available due to physical disruption during sampling and incubation. For non-sulphidic aquifer material and all sulphidic aquifer samples from the zone of NO_3^--free groundwater denitrification rates could be described with zero-order kinetics, suggesting that

denitrification was independent from the NO_3^- concentration during incubation of these samples. For the progressing exhaustion of reductants in denitrifying aquifers we suspect that the temporal dynamics is governed by the loss of reactive surfaces leading to reduced microbial habitats in the incubated sediment and to reduced denitrification rates, but this needs to be confirmed.

The protective lifetime of denitrification is limited in the investigated locations of the two aquifers but is expected to last for several generations where the NO_3^--free anoxic groundwater zone extends over several meters of depth. But where this zone is thin or contains only small amounts of microbial available reduced compounds it is needed to minimize anthropogenic NO_3^- input.

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Table 1. Sediment parameters of the incubated aquifer material (medians with ranges in brackets).

Data set	$SO_4^{2-}{}_{extr}$ [a]	DOC_{extr} [b]	C_{hws} [c]	C_l [d]	C_{org} [e]	Total-S [f]	C_l/C_{org}
	mg S kg^{-1}	mg C kg^{-1}				mg S kg^{-1}	
FFA	5.36 (0-25.2)	9.21 (5.7-11.6)	29.4 (0.1-42.6)	172.5 (2.7-887)	715.8 (203-5955)	72.3 (28.8-603)	0.165 (0.011-0.42)
GKA	10.52 (0.3-20.2)	6.11 (4.7-9.9)	29.1 (14.9-59)	239.8 (0.9-2505)	802.7 (75.9-8972)	509.6 (36.2-989)	0.264 (0.012-0.60)
non-sulphidic	14.46 (0.3-25.3)	8.96 (5.2-11.6)	21.6 (14.9-59)	91.2 (0.9-260)	236.7 (75.9-1047)	46.1 (28.8-196)	0.165 (0.011-0.42)
sulphidic	4.9 (0-20.2)	6.11 (4.7-10.8)	30.3 (0-42.6)	294.4 (38-2505)	1114.0 (232-8972)	463.7 (44.8-988.8)	0.239 (0.058-0.60)
transition zone	3.55 (0-12.8)	8.21 (6.2-10.8)	32.0 (22-42.5)	138.8 (82.2-463)	664.7 (311-1625)	53.2 (47.1-175.7)	0.226 (0.058-0.36)
NO_3^--bearing	11.05 (0-25.3)	9.21 (6.2-11.6)	27.6 (14.9-44)	116.9 (0.9-463)	538.3 (75.9-1625)	49.3 (28.8-175.7)	0.191 (0.011-0.42)
NO_3^--free	4.91 (0.3-20.2)	5.69 (4.7-9.9)	31.1 (0-59)	377.4 (37-2505)	1161.5 (232-8972)	510.4 (44.8-988.8)	0.267 (0.092-0.60)

[a] Extractable sulphate-S;
[b] extractable dissolved organic carbon;
[c] hot-water soluble organic carbon;
[d] KMnO4 labile organic carbon;
[e] total organic carbon;
[f] total sulphur.

Table 2. Initial denitrification rates, long-term denitrification capacity, stock of reduced compounds, sulphate formation capacity and estimated minimal lifetime of enitrification (medians with ranges in brackets).

Data set	$D_r(7)$[a]	$D_{cum}(365)$[b]	SRC[c]	SRC_C[d]	SRC_S[e]	aF_{SRC}[f]	SFC[g]	emLoD[h]
	µg N kg^{-1} d^{-1}	mg N kg^{-1} yr^{-1}	g N kg^{-1}			% yr^{-1}	mg S kg^{-1} yr^{-1}	yr m^{-1}
FFA	33.8 (1.3-69.9)	15.1 (0.19-56.2)	0.70 (0.2-6.0)	0.67 (0.2-5.6)	50.50 (0.0-0.4)	1.5 (0.1-5.4)	5.3 (0-39.4)	5.3 (1.6-45)
GKA	31.16 (0.7-109)	9.6 (0.34-52.5)	1.10 (0.1-8.9)	0.75 (0.1-8.4)	0.36 (0.0-0.7)	0.8 (0.4-1.7)	4.2 (0-30.0)	8.3 (0.7-67)
non-sulphidic	11.5 (0.7-35.3)	1.6 (0.19-8.2)	0.24 (0.1-1.0)	0.22 (0.1-1.0)	0.03 (0.0-0.1)	0.47 (0.1-1.7)	0.3 (0-1.3)	1.8 (0.7-8)
sulphidic	35.6 (12.3-109)	15.6 (4.09-56.2)	1.3 (0.3-8.9)	1.04 (0.2-8.4)	0.32 (0.0-0.7)	1.16 (0.4-5.4)	8.1 (1.2-39)	9.7 (2.4-67)
transitions Zone	36.48 (20.3-61)	11.6 (7.8-17.2)	0.67 (0.3-1.6)	0.62 (0.3-1.5)	0.04 (0.0-0.1)	1.65 (0.6-4.6)	2.9 (1.5-7)	5.05 (2.5-12)
NO_3^--bearing	21.05 (0.7-61)	4.3 (0.19-17.2)	0.54 (0.1-1.6)	0.50 (0.1-1.5)	0.035 (0.0-0.1)	0.80 (0.1-4.6)	1.0 (0-6.9)	4.1 (0.7-12)
NO_3^--free	33.89 (12.3-109)	20.2 (4.1-56.2)	1.44 (0.3-8.9)	1.08 (0.2-8.4)	0.36 (0.0-0.7)	0.94 (0.4-5.4)	9.4 (0.7-39)	10.80 (2.4-67)

[a] Initial denitrification rate after day 7;
[b] long-term denitrification capacity;
[c] stock of reactive compounds;
[d] concentration of reduced compounds derived from measured C_{org};
[e] concentration of reduced compounds derived from total-S values;
[f] fraction of SRC available for denitrification during one year of incubation;
[g] sulphate formation capacity;
[h] estimated minimal lifetime of denitrification.

Table 3. Spearman rank correlation coefficients between $D_{cum}(365)$ and sediment parameters for the whole data set and partial data sets.

	$SO_4^{2-}{}_{extr}$	DOC_{extr}	C_{hws}	C_l	total-N	C_{org}	Total-S	Sand	Silt
Whole data set	-0.63[c]	-0.59[c]	0.36[a]	0.68[c]	0.55[c]	0.72[c]	0.64[c]	-0.38[b]	0.63[c]
FFA	-0.82[c]	-0.87[c]	0.58[b]	0.38n.s.	0.34n.s.	0.64[c]	0.82[c]	-0.44[a]	0.64[c]
GKA	-0.49[a]	-0.40n.s.	0.13n.s.	0.87[c]	0.78[c]	0.88[c]	0.88[c]	-0.40[a]	0.73[c]
non-sulphidic	-0.38n.s.	-0.53[a]	0.85[c]	0.73[b]	0.32n.s.	0.43n.s.	0.65[a]	-0.81[b]	0.72[b]
sulphidic	-0.45[a]	-0.18n.s.	0.24n.s.	0.46[a]	0.59[c]	0.61[c]	0.33[a]	-0.28n.s.	0.42[a]
transition zone	-0.52[b]	-0.59[b]	0.60[c]	-0.74[c]	-0.59[c]	-0.61[c]	0.13n.s.	-0.01n.s.	0.52n.s.

[a] Correlation significant at the 0.05 probability level;
[b] correlation significant at the 0.01 probability level;
[c] correlation significant at the 0.001 probability level;
n.s. not significant.

Table 4. Simple linear regressions between $D_{cum}(365)$ and $D_r(t)$, $f^{B\text{-}C}(D_{cum}(365)) = A + B \times f^{B\text{-}C}(D_r(t))$.

		$D_r(7)$			$D_r(84)$			$D_r(168)$		
Data set	N[a]	R[b]	A	B	R[b]	A	B	R[b]	A	B
Whole data set	151	0.59	1.075	1.969	0.95	-0.361	0.962	0.96	0.065	1.085
FFA	86	0.57	2.005	2.705	0.94	-0.345	0.984	0.96	-0.015	1.123
GKA	65	0.68	1.613	2.565	0.94	0.452	1.503	0.94	-0.050	1.102
non-sulphidic	44	0.88	-0.391	1.264	0.95	-0.867	0.792	0.85	-0.216	1.160
transition zone	28	0.01	-3.866	-0.025	0.78	-1.556	1.156	0.69	-0.020	1.963
sulphidic	107	0.10	-2.521	0.304	0.82	0.047	1.697	0.91	1.326	2.514
NO_3^--bearing	64	0.86	0.815	1.818	0.98	-1.446	0.427	0.94	-0.771	0.748
NO_3^--free	87	0.15	-1.757	0.217	0.91	-0.613	0.750	0.94	0.183	1.394
FFA non-sulphidic	20	0.94	-2.125	0.239	0.97	-2.015	0.205	0.82	-1.527	0.441
FFA sulphidic	66	0.08	-1.928	0.880	0.82	-0.351	1.373	0.90	-0.462	0.785
GKA non-sulphidic	24	0.86	1.608	2.583	0.98	-0.546	0.926	0.87	1.007	1.877
GKA sulphidic	41	0.30	-1.684	1.028	0.86	2.147	2.863	0.91	2.353	3.343
FFA NO_3^--free	38	0.58	-0.340	0.613	0.95	-0.754	0.675	0.89	0.027	1.279
GKA NO_3^--free	49	0.31	-1.423	0.454	0.85	-0.462	0.808	0.93	0.125	1.374

[a] Sample number;
[b] correlation coefficient.

Table 5. Results of multiple linear regression analysis between $D_{cum}(365)$ and various selections of sediment parameters. To achieve normal distribution, all variables in the different data sets were Box-Cox transformed. Regression coefficients are given for the equation $f^{B\text{-}C}(D_{cum}(365)) = C_1 + C_2 \times f^{B\text{-}C}(\%\ silt) + C_3 \times f^{B\text{-}C}(C_{org}\ mg\ kg^{-1}) + C_4 \times f^{B\text{-}C}(total\text{-}S\ mg\ kg^{-1}) + C_5 \times f^{B\text{-}C}(SO_4^{2-}{}_{extr}\ mg\ S\ kg^{-1}) + C_6 \times f^{B\text{-}C}(DOC_{extr}\ mg\ C\ kg^{-1}) + C_7 \times f^{B\text{-}C}(C_{hws}\ mg\ C\ kg^{-1}) + C_8 \times f^{B\text{-}C}(C_l\ mg\ C\ kg^{-1})$.

Data set	N[a]	R[b]	F[c]	Regression coefficients							
				C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
Selection I: C_{org} and total-S											
Whole data set	151	0.82	153.1	-9.739	*	2.008	0.302	*	*	*	*
FFA	86	0.83	96.1	-17.950	*	1.366	5.565	*	*	*	*
GKA	65	0.86	85.6	-0.431	*	0.015	0.027	*	*	*	*
non-sulphidic	44	0.80	37.4	-204.2	*	0.586	247.877	*	*	*	*
sulphidic	107	0.66	40.5	-3.229	*	1.328	-5.0E-5	*	*	*	*
NO_3^--bearing	64	0.71	30.3	-205.28	*	0.302	236.599	*	*	*	*
NO_3^--free	87	0.80	76.9	-7.192	*	2.018	-0.003	*	*	*	*
transition zone	28	0.72	15.5	-446.52	*	-5.474	712.716	*	*	*	*
Selection II: Two sediment parameters giving the highest correlation coefficient											
Whole data set	111	0.86	154.1	-8.529	*	1.849	*	*	*	*	0.164
FFA	46	0.89	84.6	-18.935	*	*	7.553	-0.044	*	*	*
GKA	65	0.93	204.7	-5.326	*	1.274	*	*	*	*	0.204
non-sulphidic	44	0.80	37.4	-204.2	*	0.586	247.877	*	*	*	*
sulphidic	67	0.79	53.9	-6.399	*	2.254	*	*	*	*	-0.363
NO_3^--bearing	56	0.80	51.2	-184.96	*	*	216.915	-0.191	*	*	*
NO_3^--free	55	0.89	102.2	-9.437	*	2.963	*	*	*	*	-0.927
transition zone	20	0.74	12.8	193.30	*	-2.692	*	*	*	*	-181.402
Selection III: stepwise multiple regression with all sediment parameters											
Whole data set	111	0.93	172.9	-0.090	*	1.415	*	-0.154	-3.169	*	0.146
FFA	46	0.95	105.9	0.466	-0.350	*	*	*	-0.309	0.299	0.166
GKA	65	0.97	188.4	-4.953	-0.545	*	0.014	-0.191	4.926	*	0.306
non sulphidic	44	0.96	122.7	-85.481	*	-0.525	*	*	-0.479	127.635	0.032
sulphidic	67	0.84	31.5	-6.166	-0.211	2.333	0.001	-0.091	*	*	-0.522
NO_3^- zone	56	0.93	112.0	2.589	*	*	*	-0.167	-0.142	*	0.240
NO_3^--free	55	0.91	68.2	-8.581	*	2.581	0.003	-0.325	*	*	-0.754
transition zone	20	0.91	23.1	72.50	0.756	-18.033	*	-0.299	*	-0.186	*

*: Variable not included in the regression model;
[a] number of included samples;
[b] correlation coefficient;

[c] f-coefficient.

Table 6. Simple regression between $D_{cum}(365)$ and SRC, $f^{B\text{-}C}(SRC) = A + B \times f^{B\text{-}C}(D_{cum}(365))$. $D_{cum}(365)$ is the mean of 3 to 4 replications per aquifer sample.

Data set	N[a]	R[b]	A	B
Whole data set	40	0.82	5.186	0.302
FFA	22	0.76	3.560	0.064
GKA	18	0.95	5.635	0.785
non-sulphidic	11	0.36	4940.4	1618.2
sulphidic	29	0.73	9.006	2.292
NO_3^--bearing	17	0.49	134.13	26.763
NO_3^--free	23	0.79	28.971	5.068
transition zone	8	0.58	5.034	-0.415

[a] Sample number
[b] correlation coefficient

Figure captions:

Fig. 1. Time courses of denitrification products (N_2+N_2O) (average of 3 to 4 replicas per depth) from different groups of aquifer material during standard (**a** to **c**) and intensive treatment **(d)**. Open and closed symbols denote non-sulphidic and sulphidic aquifer material, respectively. Circuits and diamonds represent GKA and FFA material, respectively. Crosses indicate blanks of intensive treatment. nS, S, tZ and NO_3^--*f* indicate non-sulphidic and sulphidic samples, transition zone material and NO_3^--free samples, respectively. Error bars were omitted for clarity, but were small in comparison to measured concentrations of denitrivied (N_2+N_2O).

Fig. 2. FFA, GKA, nS, S and tZ indicate Fuhrberger Feld-, Großenkneten-, non sulphidic-, sulphidic- and transition zone aquifer material, respectively. White circular segments represent fractions derived from C_{org} and black segments fractions derived from total-S values. Different uppercase letters above the box-plots indicate significant differences of SRC and sF_{SRC} values between FFA and GKA material, different small letters show significant differences between nS, S and tZ (Kruskal-Wallis-Test, $P < 0.05$). **(a)** The stock of reduced compounds (SRC) and its composition in the various groups of aquifer material. The composition of SRC was calculated from C_{org} and total-S values (Sect. 2.5). **(b)** Fraction of SRC available for denitrification during incubation (aF_{SRC}). The aF_{SRC} and its composition was calculated as described in Sect. 2.5.

Fig. 3. Relation between denitrification rates determined during 7 ($D_r(7)$), 84 ($D_r(84)$) or 365 ($D_r(365)$) days of incubation. **(a)** $D_r(7)$ vs. $D_r(365)$ of FFA samples. **(b)** $D_r(84)$ vs. $D_r(365)$ of FFA samples. **(c)** $D_r(7)$ vs. $D_r(365)$ of GKA samples. **(d)** $D_r(84)$ vs. $D_r(365)$ of GKA samples.

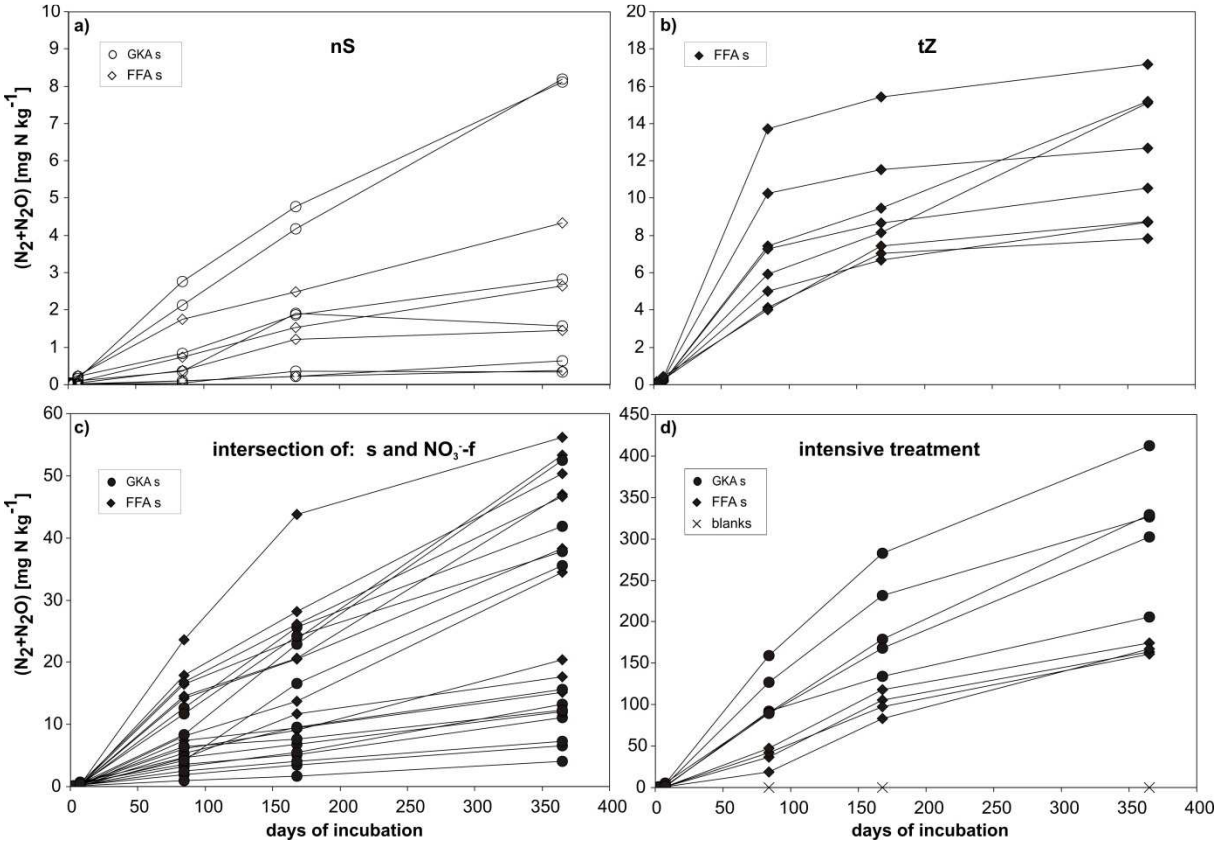
a)
nS
GKA s
FFA s
b)
tZ
FFA s
c)
intersection of: s and NO3-f
GKA s
FFA s
d)
intensive treatment
GKA s
FFA s
blanks
(N2+N2O) [mg N kg-1]
days of incubation

Fig. 1

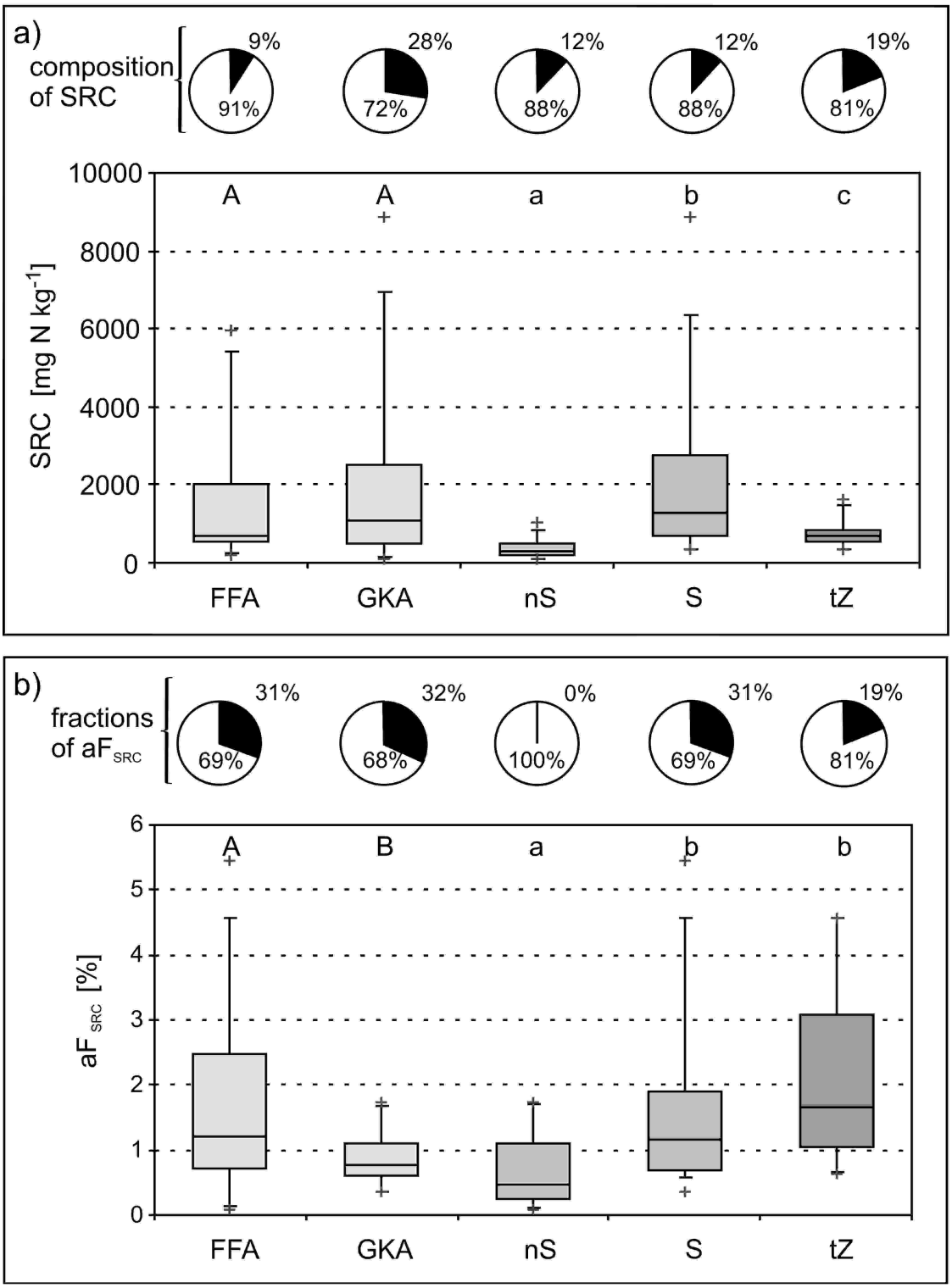
a)
composition of SRC
9%
91%
28%
72%
12%
88%
12%
88%
19%
81%
SRC [mg N kg-1]
10000
8000
6000
4000
2000
0
A
A
a
b
c
FFA
GKA
nS
S
tZ
b)
fractions of aFSRC
31%
69%
32%
68%
0%
100%
31%
69%
19%
81%
aFSRC [%]
6
5
4
3
2
1
0
A
B
a
b
b
FFA
GKA
nS
S
tZ

Fig. 2

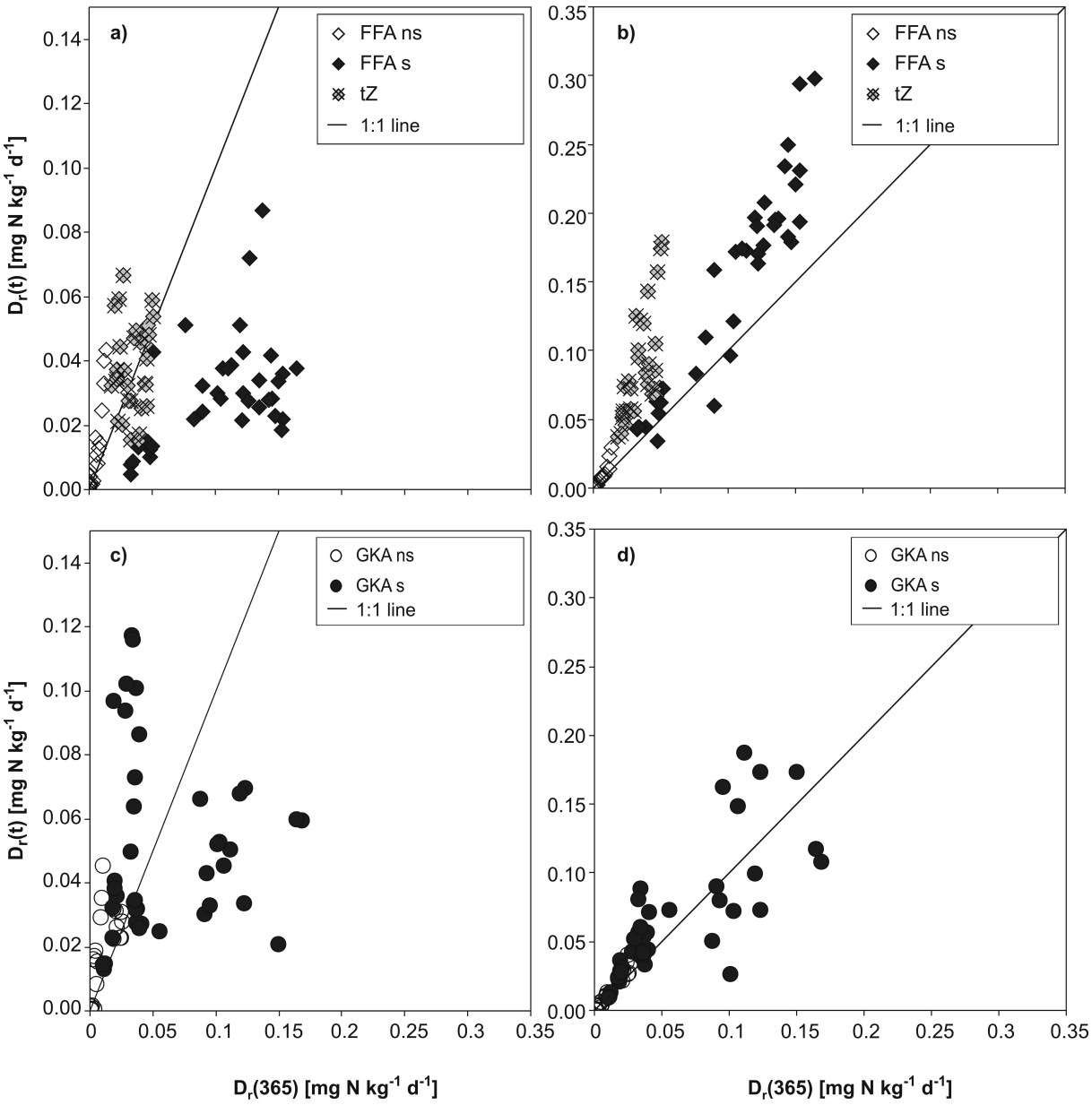
a)
FFA ns
FFA s
tZ
1:1 line
b)
FFA ns
FFA s
tZ
1:1 line
c)
GKA ns
GKA s
1:1 line
d)
GKA ns
GKA s
1:1 line
D_r(t) [mg N kg^-1 d^-1]
D_r(365) [mg N kg^-1 d^-1]

Fig. 3

Supporting information for:

Predicting the denitrification capacity of sandy aquifers from shorter-term incubation experiments and sediment properties

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Other possible electron donors

During incubations Fe and Mn concentrations in the batch solution were always mostly far) below 1 mg Fe l^{-1} and 0,5 mg Mn l^{-1}. Only some transition zone samples showed Fe concentrations 4 and 7 mg Fe l^{-1} 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 l^{-1} and < 0.1 mg Mn l^{-1} in the oxidized zone of both aquifers. Only in the reduced NO_3^- free zone of both aquifers the concentrations of Fe(II) and Mn(II) are higher (1 to 7 mg Fe l^{-1} and < 0,1 mg Mn l^{-1} in the GKA and 4 to 16 mg Fe l^{-1} and 0.1 to 1 mg Mn l^{-1} in the FFA). Therefore, only solids like e.g. pyrite ore are possible sources for the electron donors for NO_3^- 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 NH_4^+ 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 NH_4^+ is not a significant electron donor during NO_3^- reduction in both aquifers (see also section 4.5.1 of the manuscript and below).

Limitations of the $^{15}NO_3^-$ labelling approach

For the quantification of denitrification ^{15}N labelled NO_3^- was used during the conducted anaerobic incubations. ^{15}N 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 ^{15}N labelled N_2 from the labelled NO_3^- during anaerobic incubations.

Under strict anaerobic conditions, DNRA is an alternative pathway for the reduction of NO_3^-. But DNRA is seldom reported to be the dominant process of NO_3^- 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 NO_3^- concentrations and high C:NO_3^- ratios. But denitrification was presumably not NO_3^- limited since NO_3^- concentrations were always above 1 mg N l^{-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 l^{-1},

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samples from B2 in depth 8-10 m ≈ 1 mg N l^{-1}) of NH_4^+. Also NH_4^+ accumulation was generally not observed during the conducted experiments. Since the incubations were anaerobic NH_4^+ accumulation should be expected if DNRA was a significant contributing process, except anammox consumed the possibly produced NH_4^+ immediately. If significant N_2 production via anammox occurred, this would have been difficult to observe since NH_4^+ and NO_2^-, the educts of this process, came from the same ^{15}N labelled NO_3^- pool in the batch solution. (At the beginning of incubation NO_2^- concentrations were below detection and NH_4^+ concentrations < 0,5 mg N l^{-1}, respectively.) If anammox contributed significantly to N_2 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 anammox from denitrification during anaerobic incubation experiments ^{15}N labelled NO_2^- might be used.

NH_4^+ concentrations in the groundwater are mostly below detection limit in the GKA and in the reduced zone at multilevel well N10 in the FFA between 0,3 and 0,5 mg NH_4^+ l^{-1} (own measurements). Therefore, the possible occurrence of DNRA or DNRA-anammox can not strictly be excluded in both aquifers.

Quantification of total N_2+N_2O production

The molecular ion masses 28 and 29 ($^{28}N_2$, $^{29}N_2$) were recorded for IRMS analysis of denitrification derived ^{15}N labelled N_2 and N_2O. The N_2O in the headspace samples was reduced to N_2 in a reduction column prior to the mass spectrometer entrance. The headspace samples were a mixture of unlabeled N_2 und denitrification denitrified ^{15}N labelled N_2 and N_2O. On condition that (i) the ^{15}N abundance of the denitrified NO_3^- is known, (ii) denitrification is the sole gaseous nitrogen forming process, and (iii) the amount of N_2 evolved from the ^{15}N labelled NO_3^- pool is small compared with the unlabelled N_2 in the sample, the fraction of denitrified N_2 in a given mixture can be determined by measuring only $^{29}N_2/^{28}N_2$ ratios using the equations provided by (Mulvaney, 1984) (see also discussion in: (Mulvaney, 1984) and (Eschenbach and Well, 2011)). For the measurement of the ^{15}N

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abundance of the denitrified NO_3^- 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 N_2 isotopologue distributions according to the ^{15}N abundances of the unlabelled N_2 and the ^{15}N labelled denitrification derived (N_2+N_2O), respectively. A high frequency discharge unit was then used for online equilibration of N_2 molecules prior to isotope analyses. After equilibration the measured samples consisted of one binomial distribution of N_2 isotopologues according to the total ^{15}N abundance of the mixture. The ^{15}N abundance of denitrified NO_3^- can then be calculated from the measurement of the $^{29}N_2/^{28}N_2$ ratios of unequilibrated and equilibrated replicate samples (Well et al., 1998).

Fit between NO_3^- consumption and (N_2+N_2O) production

The NO_3^- decrease during incubations showed the same pattern as the measured production of (N_2+N_2O) by GC-IRMS. The measurement of (N_2+N_2O) production by GC-IRMS was more precise and had a lower detection limit compared to the measurement of NO_3^- consumption (compare Fig. 1a and Fig. S3a).

The N balance between the NO_3^- content at the start of incubations and the sum of NO_3^- consumption and in the (N_2+N_2O) during incubation was for most of the incubated samples < 1 mg N / batch assay. The samples with the highest measured production of (N_2+N_2O) showed also the highest deviation between the amount of NO_3^- consumed and the measured production of (N_2+N_2O) (compare Fig. 1c and Fig. S3c).

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.

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Table S1. Sediment parameters and basic properties of all incubated samples

Sample location	Depth interval	SG[a]	SO_4^{2-}[b]	DOC[c]	C_{hws}[d]	C_l[e]	C_{org}	total-S	total-N	Sand	Silt
	[m]		mg S kg^{-1}	mg C kg				mg S kg^{-1}	mg N kg^{-1}	[%]	
FFA B1	6.0-7.0	s^n	3.3	7.2	30.3	82.2	643	86	33	95.0	5.0
FFA B1	7.0-8.0	s	3.3	5.7	32.3	887.0	5955	603	94	94.8	5.2
FFA B2	2.0-3.0	n s^n	10.2	11.5	20.0	2.7	237	29	26	98.9	0.2
FFA B2	3.0-4.0	n s^n	25.3	10.2	17.2	2.7	203	38	23	98.9	0.2
FFA B2	4.0-5.0	n s^n	19.5	8.9	21.6	228.6	545	46	54	96.4	1.3
FFA B2	8.0-9.0	s^n	0.0	6.9	33.8	93.9	1625	176	31	40.4	59.6
FFA B2	9.0-10.0	s^n	0.9	6.2	40.0	116.9	538	156	28	94.7	5.3
FFA B4	7.0-8.0	s	n.d.[1]	n.d.[1]	n.d.[1]	n.d.[1]	483	220	21	97.3	2.7
FFA B4	8.0-9.0	s	n.d.[1]	n.d.[1]	n.d.[1]	n.d.[1]	1114	359	39	95.4	4.7
FFA B6	2.0-3.0	n s^n	17.7	11.6	22.1	259.6	695	56	41	97.8	0.6
FFA B6	3.0-4.0	n s^n	23.3	10.3	21.6	172.5	1047	59	46	97.8	0.4
FFA N10	4.5-5.0	s^n	5.4	9.2	22.2	462.7	1291	50	87	94.9	1.0
FFA N10	5.0-5.5	s^n	3.8	9.6	27.6	206.9	737	49	55	98.0	0.3
FFA N10	5.5-6.0	s^n	12.8	10.8	28.4	160.6	687	49	36	97.4	0.4
FFA N10	7.7-8.3	s^n	n.d.[1]	n.d.[1]	41.2	n.d.[1]	311	57	10	96.3	3.8
FFA N10	8.3-8.6	s^n	n.d.[1]	n.d.[1]	42.5	n.d.[1]	320	47	11	97.9	2.2
FFA N10	10.0-10.4	s	n.d.[1]	n.d.[1]	n.d.[1]	n.d.[1]	310	45	18	96.3	3.7
FFA N10	10.4-10.7	s	n.d.[1]	n.d.[1]	n.d.[1]	n.d.[1]	5627	464	113	96.4	3.6
FFA N10	12.0-13.0	s	n.d.[1]	n.d.[1]	0.0	n.d.[1]	2554	558	64	96.7	3.3
FFA N10	13.0-14.0	s	n.d.[1]	n.d.[1]	39.7	n.d.[1]	1848	588	53	95.1	4.9
FFA N10	16.0-17.0	s	1.1	5.7	42.6	241.0	2608	448	51	97.2	2.8
FFA N10	17.0-18.0	s	n.d.[1]	n.d.[1]	41.1	n.d.[1]	2504	441	48	96.9	3.1
GKA	8.0-9.0	n s^n	14.5	8.1	18.3	1.8	102	54	9	96.8	1.4
GKA	9.0-10.0	n s^n	14.5	9.0	14.9	0.9	76	38	6	97.3	0.9
GKA	22.0-23.0	n s^n	11.1	8.6	43.8	221.3	176	42	15	95.4	1.2
GKA	23.0-24.0	n s^n	10.8	9.4	33.7	50.3	192	36	23	96.0	0.9
GKA	25.9-27.0	s	8.2	6.1	31.1	1021.2	2553	682	69	87.6	12.4
GKA	27.0-28.3	s	4.8	5.8	39.0	1531.1	6373	989	127	79.6	20.4
GKA	28.3-29.3	s	10.3	8.1	27.4	2504.9	4159	883	114	76.8	21.3
GKA	29.3-30.3	s	12.7	6.6	26.2	2205.8	4543	760	96	83.9	14.2
GKA	30.3-31.2	s	13.6	5.2	28.9	347.7	784	509	14	97.6	2.2
GKA	31.3-32.0	s	18.1	9.9	42.6	192.0	834	494	27	96.5	3.2
GKA	32.9-33.7	s	20.2	5.1	20.8	377.4	821	630	23	96.9	2.8
GKA	33.7-34.7	s	15.6	5.3	29.2	150.5	752	510	17	98.5	1.4
GKA	35.7-36.7	s	2.2	5.4	32.0	2391.1	8972	708	120	96.9	3.1
GKA	36.7-37.7	s	5.1	5.5	22.4	37.7	232	677	3	98.8	1.2
GKA	37.7-38.7	s	0.5	4.7	23.2	447.4	1162	379	30	97.8	2.3
GKA	65.1-65.4	s	1.8	6.2	23.7	239.8	1009	716	39	89.4	10.7
GKA	67.1-67.5	n s	0.3	6.9	56.5	132.1	358	196	21	92.1	7.9
GKA	67.5-68.0	n s	3.5	5.2	58.5	n.d.[1]	377	194	44	94.7	5.3

[a] sediment group; [b] extractable sulfate-S; [c] extractable dissolved organic carbon; [d] extractable hot-water soluble carbon; [e] $KMnO_4$ labile organic carbon; [1] n.d.: not determined; n s non-sulphidic; s sulphidic aquifer material, n s and s with the subscript n indicates NO_3^--bearing samples.

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Table S2. Denitrification rates, long-term denitrification capacity, stock of reduced compounds, sulphate formation capacity and estimated minimal lifetime of denitrification of all incubated samples.

Sample location	Depth interval	SG[a]	$D_r(7)$[b]	$D_{cum}(365)$[d]	SRC[e]	SRC_C[f]	SRC_S[g]	aF_{SRC}[h]	SFC[i]	emLoD[j]
	[m]		µg N kg^{-1} d^{-1}	mg N kg^{-1} yr^{-1}	mg N kg^{-1}			% yr^{-1}	mg S kg^{-1} yr^{-1}	yr
FFA B1	6.0-7.0	s^n	51.66	17.18	659.6	599.5	60.1	2.60	6.1	5.0
FFA B1	7.0-8.0	s	33.89	56.24	5974.2	5552.7	421.5	0.94	39.4	44.8
FFA B2	2.0-3.0	n s^n	1.27	0.19	240.8	220.7	20.1	0.08	0.1	1.8
FFA B2	3.0-4.0	n s^n	2.12	0.37	215.4	189.2	26.3	0.17	-0.1	1.6
FFA B2	4.0-5.0	n s^n	35.27	4.34	540.2	508.0	32.2	0.80	1.0	4.1
FFA B2	8.0-9.0	s^n	21.05	10.53	1638.2	1515.5	122.7	$0.64^{(10.0)}$	3.5	12.3
FFA B2	9.0-10.0	s^n	41.41	12.68	610.7	502.0	108.7	$2.08^{(26.4)}$	2.2	4.6
FFA B4	7.0-8.0	s	45.67	20.16	603.6	450.2	153.4	3.34	9.6	4.5
FFA B4	8.0-9.0	s	25.24	34.09	1289.5	1038.9	250.7	2.64	22.0	9.7
FFA B6	2.0-3.0	n s^n	11.53	2.64	687.0	648.9	39.1	0.38	0.3	5.2
FFA B6	3.0-4.0	n s^n	6.93	1.46	1017.4	976.5	40.9	0.14	0.1	7.6
FFA N10	4.5-5.0	s^n	35.97	8.69	1239.0	1204.1	34.8	0.70	1.5	9.3
FFA N10	5.0-5.5	s^n	61.03	8.75	721.6	687.1	34.5	1.21	2.1	5.4
FFA N10	5.5-6.0	s^n	36.99	7.82	674.6	640.3	34.3	1.16	5.2	5.1
FFA N10	7.7-8.3	s^n	33.71	15.04	329.5	290.0	39.5	4.56	1.5	2.5
FFA N10	8.3-8.6	s^n	20.25	15.17	331.5	298.7	32.9	4.58	6.9	2.5
FFA N10	10.0-10.4	s	12.34	17.45	320.6	289.3	31.3	5.44	5.4	2.4
FFA N10	10.4-10.7	s	23.75	50.07	5571.6	5247.7	323.9	0.90	9.4	41.8
FFA N10	12.0-13.0	s	26.47	52.84	2771.3	2381.7	389.6	1.91	37.9	20.8
FFA N10	13.0-14.0	s	35.58	38.04	2134.1	1723.3	410.8	1.78	18.2	16.0
FFA N10	16.0-17.0	s	69.90	46.65	2744.7	2431.5	313.2	$1.70^{(6.3)}$	23.6	20.6
FFA N10	17.0-18.0	s	34.48	46.55	2642.7	2335.0	307.8	$1.76^{(6.3)}$	36.8	19.8
GKA	8.0-9.0	n s^n	0.81	0.63	132.6	95.0	37.6	0.47	0.9	1.0
GKA	9.0-10.0	n s^n	0.71	0.34	97.1	70.7	26.4	0.35	0.4	0.7
GKA	22.0-23.0	n s^n	14.68	1.57	193.3	164.2	29.1	0.81	0.2	1.5
GKA	23.0-24.0	n s^n	31.77	2.83	204.5	179.2	25.3	1.38	0.0	1.5
GKA	25.9-27.0	s	26.36	15.63	2857.4	2381.0	476.4	0.55	1.2	21.4
GKA	27.0-28.3	s	29.43	41.82	6634.0	5943.2	690.8	$0.63^{(4.9)}$	8.3	49.8
GKA	28.3-29.3	s	46.38	37.82	4495.6	3878.5	617.2	$0.84^{(7.3)}$	13.8	33.7
GKA	29.3-30.3	s	57.08	35.49	4766.8	4236.0	530.8	$0.74^{(6.4)}$	8.1	35.8
GKA	30.3-31.2	s	26.07	6.54	1086.9	731.4	355.4	0.60	3.8	8.2
GKA	31.3-32.0	s	14.06	4.09	1122.4	777.7	344.7	0.36	5.0	8.4
GKA	32.9-33.7	s	38.39	7.28	1206.0	765.6	440.4	0.60	10.2	9.1
GKA	33.7-34.7	s	62.14	12.25	1057.4	700.9	356.6	1.16	17.7	7.9
GKA	35.7-36.7	s	64.30	52.46	8861.3	8366.7	494.6	$0.59^{(4.6)}$	30.0	66.5
GKA	36.7-37.7	s	87.51	11.07	689.6	216.7	472.8	1.60	9.2	5.2
GKA	37.7-38.7	s	109.2	12.06	1347.7	1083.1	264.7	$0.89^{(15.3)}$	4.6	10.1
GKA	65.1-65.4	s	33.12	13.22	1441.2	941.3	499.9	0.92	1.3	10.8
GKA	67.1-67.5	n s	30.54	8.18	471.0	333.8	137.2	1.74	1.3	3.5
GKA	67.5-68.0	n s	23.62	8.11	487.1	351.5	135.6	1.67	0.7	3.7

[a] sediment group; [b] initial denitrification rate; [c] average denitrification rate after one year; [d] measurable denitrification capacity after one year; [e] depot of reactive compounds (SRC); [f] concentration of reduced

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compounds derived from measured C_{org}; [g] concentration of reduced compounds derived from total-S values; [h] fraction of SRC available for denitrification during one year of incubation, in parenthesis aF_{SRC} from the intensive treatment; [i] sulphate formation capacity (SFC); [j] estimated minimal lifetime of denitrification; n s non-sulphidic; s sulphidic aquifer material, n s and s with the subscript n indicates NO_3^--bearing samples.

Table S3. Simple regression between $D_{cum}(365)$ and sediment parameters (X), $f^{B\text{-}C}(D_{cum}(365)) = A + B \times f^{B\text{-}C}(X)$. Regressions with C_{org}, total-S are listed for each partial data set. Regression with a third independent sediment variable are only given, if correlation coefficient were better compared to correlations with C_{org} or total-S.

Data set	X[a]	N[b]	R[c]	A	B
whole data set	C_{org}	151	0.80	-11.022	2.654
whole data set	total-S	151	0.71	-2.397	0.805
whole data set	C_l	111	0.83	-1.028	0.492
FFA	C_{org}	86	0.72	-26.950	8.017
FFA	total-S	86	0.83	-14.879	6.312
FFA	DOC_{extr}	46	0.84	10.503	-0.495
GKA	C_{org}	65	0.93	-9.525	2.457
GKA	total-S	65	0.86	-0.252	0.026
GKA	C_l	65	0.93	-0.730	0.416
non-sulphidic	C_{org}	44	0.52	-5.434	1.205
non-sulphidic	total-S	44	0.77	-231.440	284.854
non-sulphidic	C_{hws}	44	0.77	-164.600	233.898
sulphidic	C_{org}	107	0.66	-3.097	1.293
sulphidic	total-S	107	0.40	2.747	0.001
sulphidic	C_l	67	0.60	-0.119	0.638
NO_3^--bearing	C_{org}	64	0.58	-4.946	0.661
NO_3^--bearing	total-S	64	0.67	-268.670	312.977
NO_3^--bearing	C_l	56	0.73	-0.737	0.267
NO_3^--free	C_{org}	87	0.77	-5.862	1.623
NO_3^--free	total-S	87	0.32	3.741	0.004
transition zone	C_{org}	28	0.58	18.117	-4.020
transition zone	total-S	28	0.20	-178.180	277.350
transition zone	C_l	20	0.73	192.880	-190.340

[a] Independent sediment parameter
[b] Sample number
[c] Correlation coefficient

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Table: S4. Ratios of modelled $D_{cum}(365)$ vs measured $D_{cum}(365)$ (group means with standard deviation, ranges in parentheses) for samples with high (> 20 mg N kg^{-1}) and low $D_{cum}(365)$ (< 20 mg N kg^{-1}).

Data set	Modelled $D_{cum}(365)$/ Measured $D_{cum}(365)$					
	Multiple regressions			Simple regressions		
	Selection I[a]	Selection II[b]	Selection III[c]	C_{org}	Total-S	Best[d]
	$D_{cum}(365) \geq$ 20 mg N kg^{-1} yr^{-1}					
Whole data set	0.88 ±0.33 (0.33 – 1.67)	0.89 ±0.28 (0.39 – 1.26)	0.87 ±0.24 (0.55 – 1.30)	0.86 ±0.32 (0.29 – 1.53)	0.68 ±0.25 (0.42 – 1.54)	0.83 ±0.38 (0.22 – 1.35)
FFA	0.86 ±0.12 (0.71 – 1.26)	0.86 ±0.50 (0.79 – 0.93)	0.84 ±0.07 (0.74 – 0.94)	0.71 ±0.17 (0.30 – 1.08)	0.86 ±0.15 (0.68 – 1.29)	0.57 ±0.06 (0.49 – 0.66)
GKA	0.89 ±0.33 (0.41 – 1.47)	1.14 ±0.18 (0.78 – 1.38)	1.08 ±0.19 (0.79 – 1.34)	1.14 ±0.19 (0.88 – 1.46)	0.84 ±0.30 (0.39 – 1.38)	1.13 ±0.26 (0.67 – 1.51)
sulphidic	0.73 ±0.22 (0.44 – 1.35)	0.78 ±0.16 (0.57 – 1.13)	1.15 ±0.38 (0.81 – 2.05)	0.74 ±0.22 (0.43 – 1.36)	0.33 ±0.09 (0.23 – 0.68)	0.66 ±0.25 (0.28 – 1.19)
	$D_{cum}(365) <$ 20 mg N kg^{-1} yr^{-1}					
Whole data set	2.29 ±3.06 (0.20 – 18.28)	1.90 ±2.27 (0.17 – 11.08)	1.38 ±1.02 (0.34 – 6.23)	2.69 ±4.40 (0.23 – 26.07)	3.03 ±3.85 (0.20 – 18.32)	1.72 ±1.49 (0.23 – 8.79)
FFA	2.52 ±3.03 (0.23 – 12.41)	1.77 ±1.44 (0.34 – 5.69)	1.14 ±0.66 (0.26 – 3.41)	3.56 ±4.90 (0.24 – 20.27)	2.63 ±3.39 (0.25 – 13.64)	2.19 ±2.53 (0.18 – 11.82)
GKA	1.73 ±1.29 (0.31 – 5.51)	1.35 ±0.71 (0.23 – 3.10)	1.19 ±0.43 (0.30 – 2.16)	1.39 ±0.82 (0.23 – 3.99)	1.76 ±1.38 (0.34 – 6.02)	1.35 ±0.68 (0.23 – 3.02)
non-sulphidic	1.36 ±1.04 (0.18 – 5.23)	1.36 ±1.04 (0.18 – 5.23)	1.09 ±0.45 (0.52 – 0.45)	1.94 ±2.39 (0.21 – 10.45)	1.47 ±1.00 (0.18 – 8.25)	1.55 ±0.94 (0.24 – 7.26)
sulphidic	1.49 ±0.84 (0.51 – 4.33)	1.29 ±0.66 (0.33 – 3.13)	1.39 ±0.60 (0.43 – 3.19)	1.48 ±0.84 (0.50 – 4.36)	1.27 ±0.61 (0.69 – 3.69)	1.46 ±0.76 (0.44 – 3.49)
transition zone	1.03 ±0.22 (0.71 – 1.52)	1.03 ±0.22 (0.67 – 1.56)	1.01 ±0.13 (0.84 – 1.27)	1.05 ±0.27 (0.64 – 1.77)	1.07 ±0.32 (0.67 – 1.73)	1.03 ±0.24 (0.72 – 1.58)

[a] C_{org} and total-S;
[b] two sediment parameters giving highest correlation coefficient;
[c] stepwise multiple regression;
[d] simple regression with the sediment parameter giving the best correlations with $D_{cum}(365)$;

Table S5. Lambda values of the Box-Cox transformed sediment parameters

Data set	Lambda values											
	$D_r(7)$	$D_r(84)$	$D_r(168)$	$D_{cum}(365)$	silt	C_{org}	total-S	$SO_4^{2-}{}_{extr}$	DOC_{extr}	C_{hws}	C_l	SRC
whole data set	0.512	0.346	0.341	0.294	0.021	-0.056	0.132	0.700	-0.213	0.040	0.171	-0.024
FFA	0.626	0.441	0.428	0.370	0.007	-0.176	-0.196	0.347	1.426	0.811	0.364	-0.185
GKA	0.503	0.345	0.259	0.208	-0.206	-0.080	0.750	0.670	-0.789	-0.133	0.170	0.039
non-sulphidic	0.220	0.100	0.172	0.106	-0.069	-0.050	-1.217	0.784	0.732	-1.400	0.758	1.492
sulphidic	0.219	0.209	0.305	-0.059	-0.067	-0.111	1.100	0.358	-2.02	0.635	-0.059	0.229
NO_3^--bearing	0.408	0.134	0.221	0.235	-0.210	0.108	-1.145	0.650	1.401	-0.039	0.261	0.797
NO_3^--free	0.160	0.103	0.313	0.144	-0.337	-0.017	0.950	0.214	-2.422	-0.335	0.230	0.492

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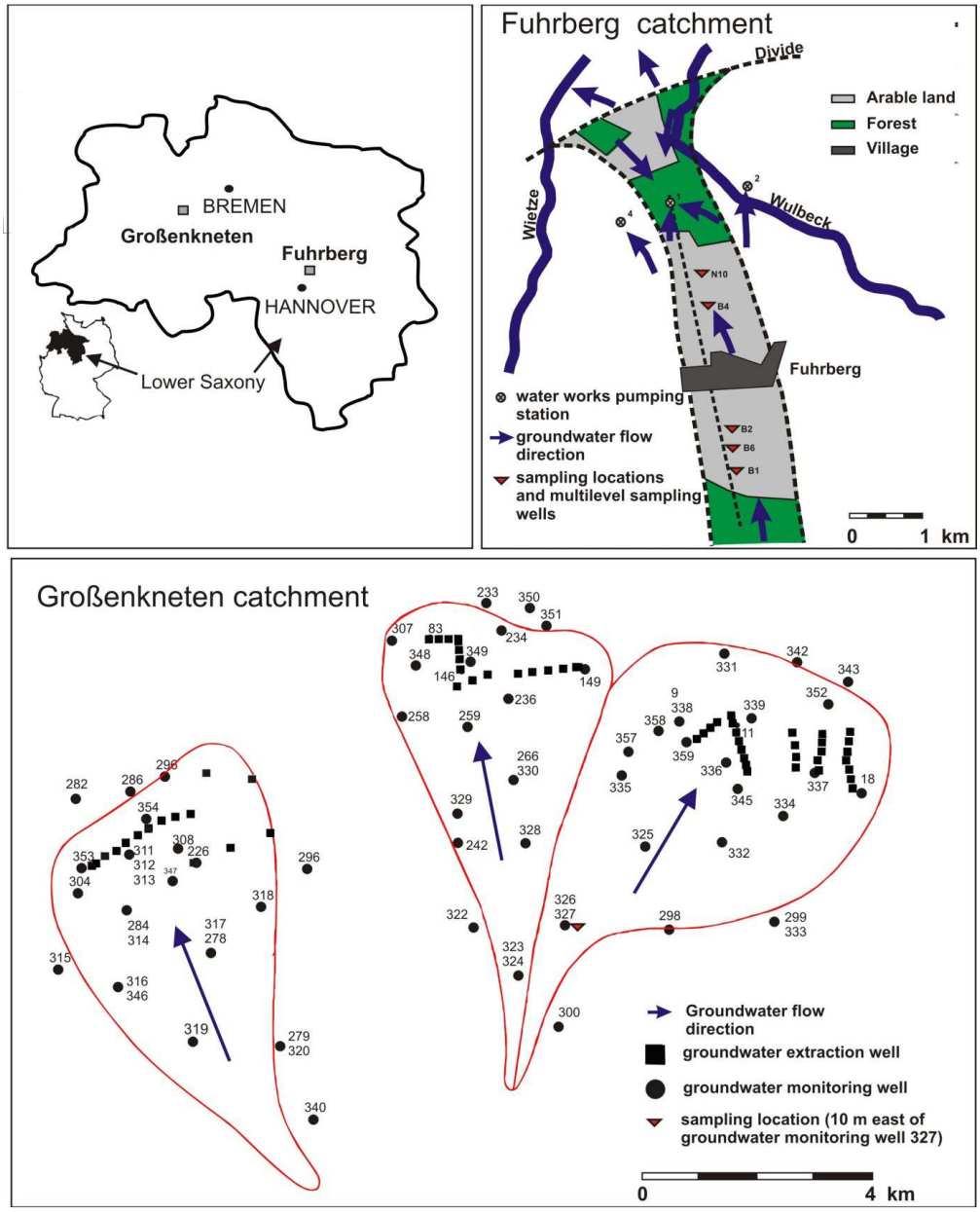

Fig. S2: Sampling locations within the Fuhrberger Feld and Großenkneten catchment in Lower Saxony (Germany).

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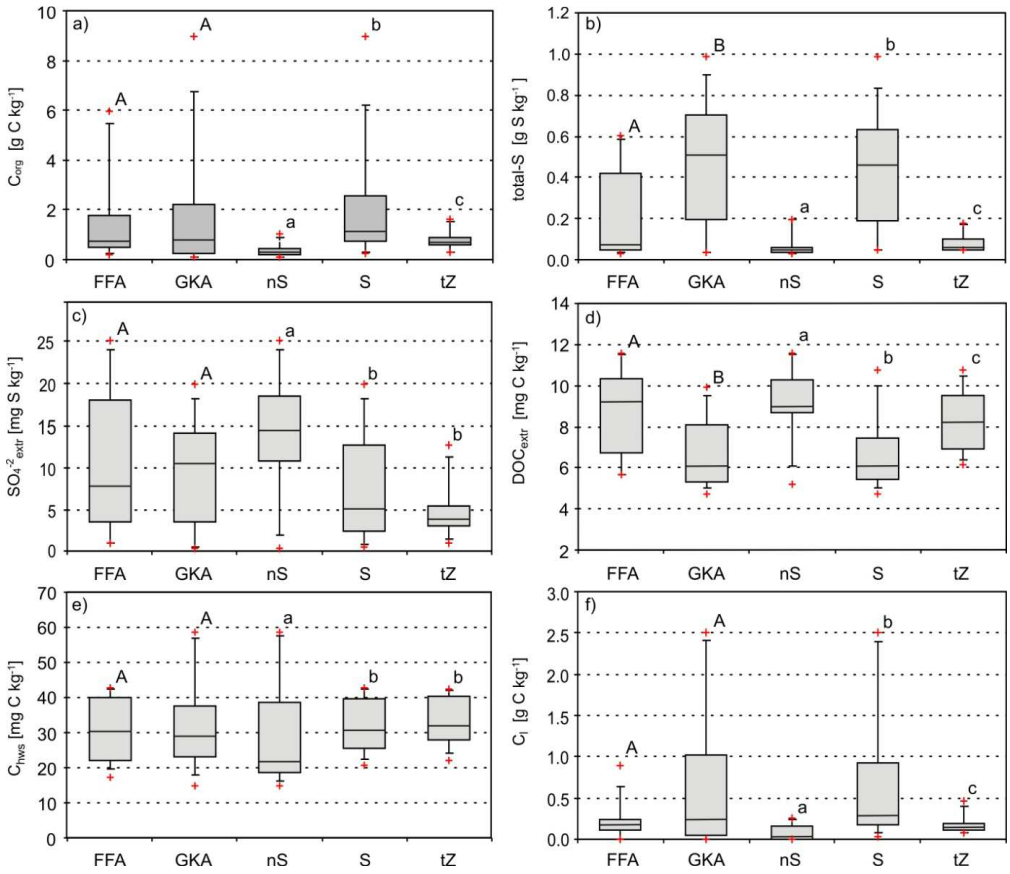

Fig. S2: Distribution of different sediment parameters in the aquifer material from the Fuhrberger Feld aquifer (FFA) and the Großenkneten aquifer (GKA) and in the various established groups of aquifer material: a) organic carbon, b) total sulphur, c) extractable sulphate, d) extractable dissolved organic carbon, e) hot water soluble organic carbon, f) potassium permanganate labile organic carbon. n S, S and tZ indicate non sulphidic -, sulphidic - and transition zone aquifer material, respectively. Different uppercase letters above the box-plots indicate significant differences between FFA and GKA material, different small letters show significant differences between n S, S and tZ (Kruskal-Wallis-Test ($P < 0.05$)).

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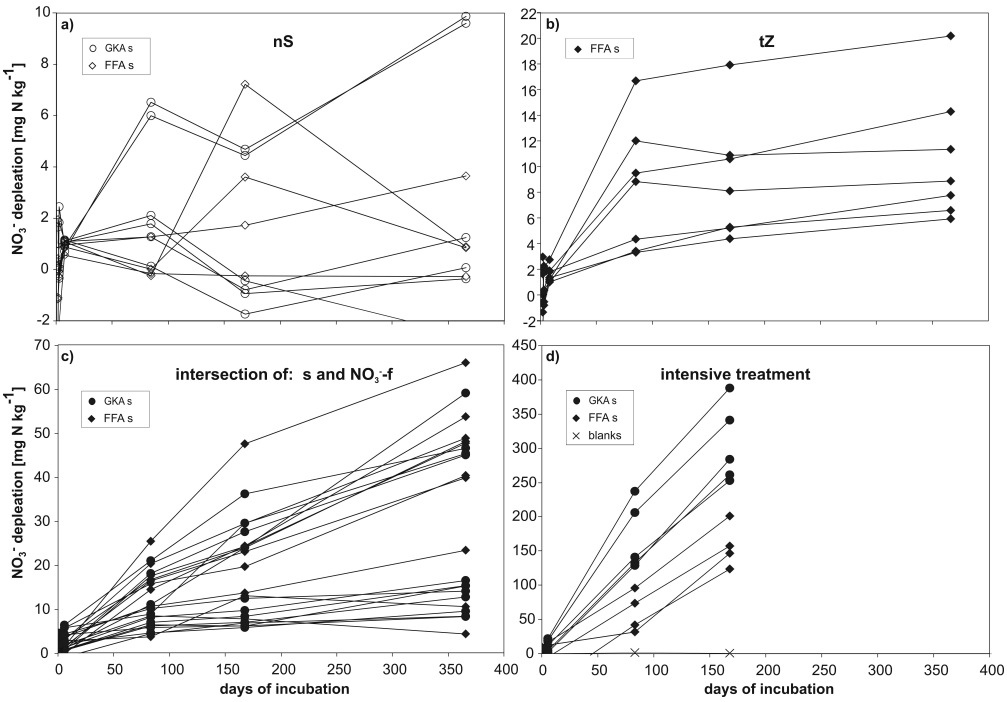

Fig. S3: Measured NO_3^- consumption during incubations. (The NO_3^- concentrations at the last sampling date of intensive incubations were not measured.)

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