Dear Dr. Minhan Dai,

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 and the supplement with changes highlighted at the end of this pdf.)

Interactive comment on "Predicting the* denitrification capacity of sandy aquifers from in situ measurements using push-pull 15N tracer *tests" by W. Eschenbach and R. Well

Anonymous Referee #1

Received and published: 2 January 2015

General Comments. Groundwater denitrification research is moving beyond merely measuring rates to determining what electron donors contribute to denitrification and how long can the electron donor supplies last. This paper considers both, but emphasizes the latter. Determining the denitrification capacities of aquifer materials is an important and pragmatic goal. Herein, Eschenbach, Well, and Walther make a novel contribution by trying to predict the denitrification capacities of sandy aquifers using push-pull tracer tests with 15N, a scientific issue within the scope of this journal.

Specific Comments. The paper is ordered properly and the text is clear; the scientific methods are clearly outlined and the use of mass spectrometry in the field application was documented.

1. Page 16532, line 10 and following: Perhaps push-pull studies may be used to estimate SRC; however, collecting aquifer material for analysis should always be considered as a way *to "ground-truth" these estimates.*
Regarding this valuable Note on data validation, we inserted the following sentence to the text:
"Nevertheless, individual aquifer samples should always be analysed to verify these estimates repeatedly.*"*

2. Page 16545, Section 3.2, and Page 16548 Section 4.1: These sections include regional comparisons and also comparisons with other push-pull tests; however they should also include information from other in situ denitrification tests so that this work appeals to a larger audience.
Tesoriero and Puckett (2011) reviewed the published rates of denitrification and concluded that denitrification by sulphide oxidation was faster than with carbon oxidation. They considered studies that used monitoring-well transects along hypothesized groundwater flow paths.
Green et al. (2010) showed that non-Gaussian dispersion influenced apparent denitrification reaction rates and isotopic fractionation along flow paths. Such mixing would influence the results discussed herein about pull-push tests; a discussion of these influences would be appropriate.
Korom et al. (2012) argued that such mixing would apparently not influence rates measured by their in situ mesocosms (Korom et al., 2005) because advection and mechanical dispersion are minimal. Issues influencing apparent denitrification rates and fractionation are currently important in the groundwater denitrification literature and the authors need to better explain their results in this context.
We added the following discussion to section 4.1.1:

"Denitrification rates can also be derived from the analysis of groundwater samples from monitoring-well transects along hypothesized groundwater flow paths. Therefore, Tesoriero and Puckett (2011) selected 12 study sites with monitoring- well transects within the U.S. The study areas represented a wide range of sedimentary environments and climatic conditions.

Tesoriero and Puckett (2011) generalized the determined denitrification rates broadly into three categories: low rates (< 0,02 µg N kg^{-1} d^{-1}), medium rates (0,02-0,06 µg N kg^{-1} d^{-1}) and high rates (> 0,6 µg N kg^{-1} d^{-1}). Low to were found in areas with elevated O_2 concentrations in the groundwater, medium rates in the presence of low O_2 concentrations and high denitrification rates when changes in the lithology resulted in a sharp increase in the supply of electron (Tesoriero and Puckett (2011), p. 13). Overall, the denitrification rates obtained from theses monitoring-well transects are below the mean D_r(in situ) of the various data subsets in this study (Table 4). For example the mean D_r(in situ) of non-sulphidic aquifer material was 1 µg N kg^{-1} d^{-1} (Table 4) and thus even higher than the high denitrification rates reported by Tesoriero and Puckett (2011). The O_2 concentrations in the ambient groundwater at these push-pull locations were mostly clearly above 1 mg L^{-1}, which is near the reported apparent threshold for the onset of denitrification in aquifers (Green et al., 2008, 2010; McMahon et al., 2004; Tesoriero and Puckett, 2011) (see Sect. 4.3). Mean D_r(in situ) of data subsets of push-pull test at locations with low O_2 concentrations (transition-zone and NO_3^--free zone) (Table 2) were 9 and 8 µg N kg^{-1} d^{-1}, respectively, and thus by the factor of 10 higher as the high rates reported by Tesoriero and Puckett (2011).

Green et al. (2010) showed that groundwater mixing due to advection and mechanical dispersion can strongly influence the derived apparent denitrification rates along flow paths in a way that these transport processes tend "to create the appearance of lower reaction rates and fractionation parameters when measured at larger scales and longer flow paths" (Green et al. 2010, p 12). Green et al. (2010) showed that mixing effects increase with the mean travel distances of groundwater and they conclude "that effects of transport and scale should be considered when comparing reaction rates in different aquifer systems, or when comparing reaction rates in different parts of the same system".

In contrast, Korom et al. (2005) reported clearly higher zero-order denitrification rate of 35.6 µg N kg^{-1} d^{-1} measured by an aquifer mesocosm, this rate is comparable with the highest D_r(in situ) measured in this study (Table 2). Korom et al. (2012) argued that, contrary to monitoring-well transects, such transport depending mixing processes would not influence denitrification rates measured by aquifer mesocosms, since advection and mechanical dispersion are negligible. The influence of advection and mechanical dispersion on the measured apparent denitrification rates by push-pull test should be higher compared to in situ mesocosms. On the contrary during push-pull tests, mixing processes by advection and mechanical dispersion should be significantly lower in comparison to monitoring-well transects, since the flow path of the injected tracer solution in the aquifer is in a decimeter or

maximum meter range during a push-pull test, which is very short compared to flow-paths of hundreds of meters or several kilometres in case of monitoring-well transects. (Additionally the mixing of the injected tracer solution with ambient groundwater was taken into account by the addition of Br^- as conservative tracer to the tracer solution (see Sect. 2.6) to minimise the influence of mixing effects.)

The observed differences in denitrification rates measured in this study with denitrification rates derived from monitoring-well transects (Tesoriero and Puckett 2011) might thus be attributed to effects of transport along long flow paths. We think that these effects should also be considered when denitrification rates are compared that have been derived with different methods."

References. Green, C.T., Böhlke, J.K., Bekins, B.A., Phillips, S.P., 2010. Mixing effects on apparent reaction rates and isotope fractionation during denitrification in a heterogeneous aquifer. Water Resources Research 46, W08525, doi: 10.1029/2009WR008903, 1-19.
Korom, S.F., A.J. Schlag, W.M. Schuh, and A.K. Schlag (2005), In situ mesocosms: denitrification in the Elk Valley aquifer. Ground Water Monit. R. 25(1), 79–89.
Korom, S.F., W.M. Schuh, T. Tesfay, and E.J. Spencer (2012), Aquifer denitrification and in situ mesocosms: modeling electron donor contributions and mesasuring rates. J. Hydrol. 422-423, 112-126, doi:10.1016/j.jhydrol.2012.02.023.
Tesoriero, A. J., and L. J. Puckett (2011), O2 reduction and denitrification rates in shallow aquifers, Water Resour. Res., 47, W12522, doi:10.1029/2011WR010471.

Technical Corrections.

1. *Page 16540, line 21: Change "were" to "where."*

We didn't changed it, because we think „were" is right.

2. *Page 1651, line 24: Change "begin" to "initiation."*

16531, line 24: We changed „begin" to „initiation" as suggested.

3. Page 1652, line 6: The meaning of the sentence beginning on this line is unclear.

16532, line 6: We changed the sentence from (see also comment 2 referee 2):
„At all the possible **influence** of the location of push-pull tests within aquifers regarding the presence or absence of NO3- on measured in situ denitrification rates in groundwater has not *been addressed so far."*
to:
"So far, the effect of different ambient redox conditions, i.e. the presence or absence of NO_3^- in groundwater, on the outcome of push-pull tests, has been insufficiently considered."
Hopefully this clarifies the meaning of the sentence.

4. *Page 16533, line 13: "North" should be "north."*

Changed as suggested

5. *Page 16536, line 21: Change the sentence beginning on this line to, "The multilevel* wells in the FFA were sampled every 12 h during the night and every 3 to 4 h during *the day to investigate more detailed temporal patterns."*

Changed as suggested

6. Page 165*36, line 24: Change "maximal 72 h" to "a maximum of 72 h."*

Changed as suggested.

7. *Page 16536, line 27: Change "sampling" to "sample" and "form" to "from."*

Changed as suggested

8. *Page 16544, line 11: Change "sub data sets" to "subsets."*

Changed as suggested

Interactive comment on Biogeosciences Discuss., 11, 16527, 2014.

Predicting the denitrification capacity of sandy aquifers from in situ measurements using push-pull ^{15}N tracer tests

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Knowledge about the spatial variability of in situ denitrification rates (D_r(in situ)) and their relation to the denitrification capacity in nitrate-contaminated aquifers is crucial to predict the development of groundwater quality. Therefore, 28 push-pull ^{15}N tracer tests for the measurement of in situ denitrification rates were conducted in two sandy Pleistocene aquifers in Northern Germany.

The ^{15}N analysis of denitrification derived ^{15}N labelled N_2 and N_2O dissolved in water samples collected during the push-pull ^{15}N tracer tests was performed by isotope ratio mass spectrometry (IRMS) in the lab and additionally for some tracer tests online in the field with a quadrupole membrane inlet mass spectrometer (MIMS), in order to test the feasibility of on-site real-time ^{15}N analysis. Aquifer material from the same locations and depths as the push-pull injection points was incubated and the initial and cumulative denitrification after one year of incubation (D_{cum}(365)) as well as the stock of reduced compounds (SRC) was compared with in situ measurements of denitrification. This was done to derive transfer functions suitable to predict D_{cum}(365) and SRC from D_r(in situ).

D_r(in situ) ranged from 0 to 51.5 µg Nk g^{-1} d^{-1}. Denitrification rates derived from onsite isotope analysis using membrane-inlet mass spectrometry satisfactorily coincided with laboratory analysis by conventional isotope ratio mass spectrometry, thus proving the feasibility of in situ analysis. D_r(in situ) was significantly higher in the sulphidic zone of both aquifers compared to the zone of non-sulphidic aquifer material. Overall, regressions between the D_{cum}(365) and SRC of the tested aquifer material with D_r(in situ) exhibited only a modest linear correlation for the full data set. But the predictability of D_{cum}(365) and SRC from D_r(in situ) data clearly increased for aquifer samples from the zone of NO_3^--bearing groundwater.

In the NO_3^--free aquifer zone a lag phase of denitrification after NO_3^--injections was observed, which confounded the relationship between reactive compounds and in situ denitrification activity. This finding was attributed to adaptation processes in the microbial community after NO_3^--injections. ~~Exemplarily, I~~it was also demonstrated that the microbial community in the NO_3^--free zone ~~close~~ just below the NO_3^--bearing zone can be adapted to denitrification by ~~amending wells with~~ NO_3^--injections into wells for an extended period. In situ denitrification rates were 30 to 65% higher after pre-conditioning with NO_3^-. Results from this study suggest that such pre-conditioning is crucial for the measurement of D_r(in situ) in deeper aquifer material from the NO_3^--free groundwater zone and thus for the prediction of D_{cum}(365) and SRC from D_r(in situ).

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). NO_3^- is quantitatively the most abundant reactive nitrogen (Nr^1) species. Diffuse NO_3^- emissions from the agricultural sector are the dominant source of Nr fluxes to aquifers. Denitrification in aquifers, reviewed e.g. by Korom (1992), Hiscock et al. (1991), Burgin and Hamilton, (2007) and Rivett et al. (2008), ranges from 0 to 100% of total NO_3^- input with a high spatial variability (Seitzinger et al., 2006). This leads to the question~~,~~ of how individual aquifers will respond to the anthropogenic NO_3^- pollution ~~of~~ in groundwater. ~~Not only the questions~~This problem not only depends on how rates of denitrification will respond to Nr loading (Seitzinger et al., 2006) but also on ~~and~~ where and how long denitrification in aquifers can remediate NO_3^- pollution (Kölle et al., 1985)~~ are of importance~~. ~~Since~~ ~~e~~Continuous NO_3^- input via seepage water~~s~~ leads to ongoing exhaustion of the reductive capacity of aquifers. This can be a problem for keeping ~~the standard for~~ NO_3^- in drinking water below the limit of ~~(<~~50 mg L^{-1}~~,~~ (Drinking Water Directive 98/83/EC) and also be problematic due to possible eutrophication of surface water~~s bodies~~ (Vitousek et al., 1997). But NO_3^- can also mobilise ~~unforeseen~~ deposits of ~~species such as~~ uranium (U) in aquifers, which can be mobilised if NO_3^- reaches reduced aquifer zones (Senko et al., 2002; Istok et al., 2004). Therefore, knowledge about the denitrification capacity of aquifers is needed to predict the possible development of groundwater quality.

The presented study continues previous research on denitrification rates measured in two sandy Pleistocene aquifers in Northern Germany (Fuhrberger Feld aquifer (FFA) and the aquifer of Großenkneten (GKA)). Frind et al. (1990) reported that due to lithotrophic denitrification, NO_3^- has a half-life of 1 to 2 years in the deeper zone (below 5 to 10 m) of the well investigated Fuhrberger Feld aquifer. Weymann et al. (2010) reported very low denitrification rates with values as low as 4 µg N kg^{-1} d^{-1} in the uppermost ~~surface near~~ groundwater, in the organotrophic denitrification zone of the same aquifer. In a recent study, Eschenbach and Well (2013) measured median denitrification rates of 15.1 and 9.6 mg N kg^{-1} yr^{-1} during one year of anaerobic incubations of FFA and GKA aquifer samples, with significantly higher denitrification rates in the deeper parts of both aquifers. This study showed that the cumulative denitrification after prolonged incubation of aquifer samples is correlated to the stock of reduced compounds (SRC). Similar results had been obtained earlier

~~obtained~~ for ~~an~~other aquifers in Northern Germany (Konrad, 2007). While we found close correlations between initial laboratory denitrification rates and the SRC in aquifer zones where NO_3^- is present in groundwater, samples from NO_3^--free groundwater zone showed a lag time of denitrification of several weeks during incubations (Eschenbach and Well, 2013) possibly due to the initial absence of denitrifying enzymes. These findings demonstrate, that the SRC can be estimated from denitrification rates in case the microbial community is adapted to denitrification (Eschenbach and Well, 2013).

In situ denitrification rates can be measured using single well push-pull tests where a test solution containing solutes of interest is rapidly injected into a well (push-phase) and process information is obtained from analysing groundwater collected during the subsequent pull-phase. These tests, perhaps first used for in situ measurement of denitrification rates by Trudell et al. (1986), have proven to be a relatively low-cost ~~instrument~~ technique to obtain quantitative information about several aquifer properties. This method was applied in a variety of studies to derive in situ denitrification rates indirectly by the measurement of NO_3^- depletion during push-pull tests (Trudell et al., 1986; Istok et al., 1997, 2004; Schroth et al., 2001; McGuire et al., 2002; Harris et al., 2006). In comparison only a limited number of studies directly measured denitrification rates from the gaseous denitrification products (Sanchez-Perez et al., 2003; Kneeshaw et al., 2007; Well and Myrold, 2002, 1999; Addy et al., 2002; Well et al., 2003; Addy et al., 2005; Kellogg et al., 2005; Konrad, 2007). ~~Beside~~ Aside from ~~the study of~~ Konrad (2007), these push-pull tests were only conducted in ~~surface near~~uppermost groundwater.

Well et al. (2005) showed that in situ denitrification rates measured with the push-pull ^{15}N tracer method in the saturated zone of hydromorphic soils agreed relatively well with denitrification rates measured in parallel soil samples. Konrad (2007) reported a close correlation between in situ denitrification rates and the cumulative denitrification after at least one year of incubation based on a small number of only 5 comparisons, so only a~~the~~ relatively small data set was ~~relatively small~~used to derive ~~robust~~ transfer functions.

Since denitrification is a microbially mediated ~~redox~~ reaction, the composition, activity and amount of microbes in aquifers should directly influence the measured denitrification rates during single well push-pull tests. It is known that steep gradients in the composition of microbial communities occur in aquifers resulting from the distribution and availability of electron donors and acceptors in aquifers (Kölbelboelke et al., 1988; Griebler and Lueders, 2009; Santoro et al., 2006). Law et al. (2010) reported substantial changes in the microbial community composition after the ~~begin~~ initiation of denitrification and the transition from

denitrification to Fe(III)-reduction within incubated aquifer material. Higher microbial activities after bio—stimulation of indigenous microorganisms by the injection of electron donors into aquifers was reported by Istok et al. (2004), Kim et al. (2005) and Kim et al. (2004). Compared with preceding push-pull tests at the same groundwater monitoring wells, the multiple injection of electron donors increased the reduction rates of NO_3^-, pertechnetate (Tc(VII)) and U(VI) measured during subsequent push-pull tests in a shallow unconfined silty-clayey aquifer (Istok et al., 2004). Trudell et al. (1986) found increasing denitrification rates during a 12 day push-pull test in NO_3^--free groundwater suggesting that the microbial community needed a certain time to adapt to the electron acceptor NO_3^- before denitrification could proceed at a rate equivalent to the availability of reduced compounds. So far, the effect of different ambient redox conditions, i.e. the presence or absence of NO_3^- in groundwater, on the outcome of push-pull tests, has been insufficiently considered. ~~At all the possible influence of the location of push-pull tests within aquifers regarding the presence or absence of NO_3^- on measured in situ denitrification rates in groundwater has not been addressed so far.~~

Overall, the performance of previous push-pull studies suggests that this approach may be suitable to deliver in situ denitrification data that reflect the reduction capacity of the aquifer, i.e. it might be used to estimate SRC without the need for collecting aquifer material. Nevertheless, individual aquifer samples should always be analysed to verify these estimates repeatedly.

To test if ^{15}N push-pull tests could be evaluated during the course of experiments directly in the field, a membrane inlet mass spectrometer (MIMS) was used during 5 push-pull tests at two monitoring wells for direct field measurements of ^{15}N labelled denitrification products (see Supplement). The main advantages of MIMS with respect to the conventional IRMS approach is that MIMS is low-priced compared to IRMS and results can be obtained ~~in the course of~~during experiments directly in the field. Sampling intervals can thus be adapted to get more precise rates. Moreover, the length of the ~~pull-~~pull-phase can be limited to the duration of clearly increasing $(N_2+N_2O)_{den}$ concentrations to save ~~working time~~hours of labour. Finally, the relatively low cost and simple handling of the MIMS system are favourable to enable extensive application of the ^{15}N push-pull approach to explore denitrification capacities of aquifers.

This study is the second part of a combined approach (a) to quantify exhaustibility of the denitrification capacity in aquifers, (b) to investigate controlling factors and derive predictive models during incubation experiments, and (c) to check if the cumulative denitrification measured after one year of incubation ($D_{cum}(365)$) (Eschenbach and Well, 2013) can be

derived from in situ denitrification rates measured with push-pull tracer tests. Here a study on objective (c) is presented. The specific objectives of this study are (i) to measure in situ denitrification rates with push-pull ^{15}N tracer tests at groundwater monitoring wells, (ii) to develop regression models to predict $D_{cum}(365)$ as well as the stock of reduced compounds from in situ denitrification rates, and (iii) to test an approach to adapt the microbial community in NO_3^--free aquifer zones to NO_3^- as a newly available electron donor during experiments as a means of conditioning prior to subsequent push-pull ^{15}N tracer tests. Additionally (iv) the suitability of MIMS for online field analysis during ^{15}N tracer tests was tested (Supplement).

2 Materials and methods

2.1 Study sites

In situ measurements of denitrification were conducted in the Fuhrberger Feld aquifer (FFA) and the Großenkneten aquifer (GKA). Both aquifers are located in drinking water catchment areas in the ~~North~~ north of Germany. The FFA is situated about 30 km NE of the city of Hanover and the GKA about 30 km SW of the city of Bremen. Both aquifers consist of carbonate free, ~~Quaternary~~ quaternary sands and the deeper parts of the GKA additionally of carbonate free marine sands (Pliocene). The thickness of the FFA and GKA is 20 to 40 and 60 to 100 m, respectively. Both aquifers are unconfined and contain unevenly distributed amounts of microbially available sulphides and organic carbon. Intensive agricultural land use leads to considerable NO_3^- inputs to the groundwater of both aquifers (Böttcher et al., 1989; van Berk et al., 2005; Schuchert, 2007). 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 of an intense ongoing denitrification within the FFA is given by NO_3^- 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). The geological structure of the GKA is described in Howar (2005) and Wirth (1990). Extended zones with oxidizing and

reducing conditions in the groundwater are evident in the GKA (van Berk et al., 2005) but their distribution within the aquifer is more complex ~~as~~ than in the FFA and denitrification is known to occur in the zone of reduced groundwater (van Berk et al., 2005). Own excess-N_2 measurements (Well et al., 2012) at monitoring wells prove intense denitrification within the GKA. But there are no studies on the type of denitrification in this aquifer.

2.2 Single well push-pull ^{15}N tracer tests

2.2.1 Well types and sampling procedure

To quantify in situ denitrification rates (D_r(in situ)), a total of 28 single well push-pull ^{15}N tracer tests, afterwards referred to as push-pull tests, were performed in the FFA and GKA (Table 1) by injecting ^{15}N labelled NO_3^- tracer solution into groundwater monitoring wells. In the FFA, push-pull tests were conducted at multilevel wells consisting of PE tubings (4 mm ID) (Böttcher et al., 1985). Each of these ~~tubings~~ tubes were connected to a filter element at the respective depth. In the GKA, two types were used, (i) conventional groundwater monitoring wells (101 mm ID) with 1 to 4 m long filter screens and (ii) multilevel wells (CMT multilevel system, Soilinst, Georgetown, Canada) consisting of PE pipes with 3 individual channels (13 mm ID) with 25 cm long filter screens at the end. Each channel ended in a different depth. To allow a direct comparison with a previous laboratory incubation study (Eschenbach and Well, 2013), wells from the same locations and with filter screens at the same depth where the aquifer samples had been collected were selected in the FFA and GKA. In situ experiments were conducted principally as described in previous studies (Addy et al., 2002; Trudell et al., 1986; Well et al., 2003).

For sampling multilevel wells, groundwater and tracer solution were extracted with a peristaltic pump (Masterflex COLE-PARMER, Vernon Hills, USA). A submersible pump (GRUNDFOS MP1, Bjerringbro, Denmark) was used for common groundwater monitoring wells. During sampling, an outflow ~~tubing~~ tube with the extracted groundwater or tracer solution was placed at the bottom of 26 or 120 ml serum bottles (multilevel wells and common groundwater monitoring wells, respectively). After an overflow of at least three times the volume of these bottles, the tubing was removed and the bottles were immediately sealed air tight with grey butyl rubber septa (ALTMANN, Holzkirchen, Germany) and aluminium crimp caps. ~~4~~ Four replications were collected per sampling. Groundwater was sampled from the injection depth prior to each push-pull test.

2.2.2 Push-pull tests

A single well push-pull test consists of the injection of a tracer solution into a monitoring well (push-phase) and the extraction of the mixture of test solution and groundwater from the same well (pull-phase).

Push-phase

To prepare the tracer solution, 50 L of groundwater were extracted from multilevel wells (FFA and GKA) or 220 L at common groundwater monitoring wells (GKA) for each push-pull test (Fig. 1). The groundwater was pumped to a stainless steel storage container (Type BO 220 L, SPEIDEL, Ofterdingen, Germany), which was equipped with a floating lid to avoid gas exchange with the atmosphere and thus maintain the dissolved gas composition of the extracted groundwater. After extraction, a stock solution of deionised water (100 ml) with dissolved ^{15}N labelled potassium nitrate (KNO_3 with 60 atom % ^{15}N) and potassium bromide (KBr) was added to attain a concentration of 10 mg ^{15}N labelled NO_3^- -N L^{-1} and 10 mg Br^- L^{-1}, respectively. The mixture of the stock solution and the extracted groundwater is hereinafter referred to as tracer solution. The tracer solution was mixed for 1 h with a submersible pump (Gigant, Eijkelkamp, Giesbeek, ~~the~~ Netherlands) within the stainless steel storage container. The extracted groundwater from the NO_3^- bearing groundwater zone (NO_3^--bearing zone) contained varying concentrations of NO_3^- (Table 2). Consequently, the NO_3^- in the tracer solution of these push-pull tests was a mixture of natural and ^{15}N enriched NO_3^- and NO_3^- concentrations in these tracer solutions were > 10 mg NO_3^--N L^{-1} (see discussion about influence of NO_3^- concentrations on denitrification rates in Sect. 4.2 and in Eschenbach and Well, 2013).

During injection, the outflow of the stainless steel storage container was connected with Tygon® tubings to the selected depths of the multilevel wells. For common groundwater monitoring wells the submersible pump was connected with a pump riser pipe and an inflatable packer (Packer set, UIT Umwelt- und Ingenieurtechnik GmbH, Dresden, Germany). The packer was installed within the groundwater monitoring well to prevent mixing of the injected tracer solution with the water column in the groundwater monitoring well (Fig. 1). The packer was inflated with air to a pressure of 1 bar above the pressure of the overlying water column. The inflated packer and the pump riser pipe remained during the entire tracer

test within the groundwater monitoring well. The pump riser pipe was connected with a PVC hose (13 mm ID) to the stainless steel container. For both types of monitoring wells, the tracer solution was injected gravimetrically. For common wells injections took 30-45 min, for the CMT multilevel system 45-80 min and for the multilevel wells in the FFA 150-240 min.

Pull-phase

The common groundwater monitoring wells in the GKA were constantly sampled at 12 h intervals. The multilevel wells in the FFA were sampled every 12 h during night and every 3 to 4 h during day to investigate more detailed temporal patterns ~~more detailed~~. The multilevel wells were more suitable for this, due to their smaller dead volumes and lower extraction rates. The pull phases of the conducted tracer tests lasted a maximum of~~maximal~~ 72 h. The first sampling was performed immediately after injection. Prior to each sampling, an amount of tracer solution sufficient to replace the dead volume of the groundwater monitoring well was extracted. In total, 4 and 30 to 60 L were extracted per sampl~~e~~ing f~~o~~rom multilevel and groundwater monitoring wells, respectively. For common groundwater monitoring wells the sampling volume differed because of different lengths of filter screens and resulting different dead volumes. During extraction, groundwater temperature, dissolved oxygen, pH and electrical conductivity were measured with sensors (pH/Oxi 340i and pH/Cond 340i, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) installed in a flow-through chamber.

2.3 Incubation of aquifer material

Laboratory experiments were performed to compare denitrification rates measured during laboratory anaerobic incubation ($D_r(365)$) with in situ denitrification rates. The incubated aquifer material originated from the same location and depths as the filter screens of the push-pull test injection points. The aquifer material was sampled and incubated as described in detail in Eschenbach and Well (2013).

Briefly, aquifer material from both aquifers ~~were~~ was collected between 2 to 68 m below ~~soil~~ ground surface. The aquifer samples were incubated in transfusion bottles, in 3 to 4 replications. ^{15}N labelled KNO_3 solution was added and the transfusion bottles were sealed airtight. To ensure anaerobic conditions during incubation, the headspaces of the transfusion bottles were evacuated and flushed with pure N_2. Afterwards, the samples were incubated for one year in the dark at 10 °C, which is approximately the groundwater temperature in both

aquifers. The transfusion bottles were shaken manually two times a week to mix sediment and batch solution. The headspace and the supernatant batch solution in the transfusion bottles were sampled at days 1, 2, 7, 84, 168 and 365 of incubation.

2.4 Pre-conditioning of wells in the NO_3^--free zone of the FFA

To stimulate denitrification in the NO_3^- -free zone with suspected lack of active denitrifiers (Eschenbach and Well, 2013) groundwater monitoring wells were amended by repeated injections of groundwater with added NO_3^- of natural ^{15}N abundance. Injections were designed to maintain elevated NO_3^- levels in the vicinity of the filter screens during a period of several weeks. This was done to test if in situ denitrification rates measured in these wells after pre-conditioning would reflect the average denitrification rates measured during one year of incubation of corresponding aquifer samples (Eschenbach and Well, 2013).

Pre-conditioning was performed at 4 depths in the NO_3^--free groundwater zone at multilevel well B4 in the FFA, ~~that~~ which had been previously tested without pre-conditioning. Therefore 800 L of NO_3^--free reduced groundwater were extracted from a groundwater monitoring well, with a filter screen at 7 to 8m depth below ~~soil~~ ground surface, which is located 30 m west of multilevel well B4, into a 800 L tank ~~container~~ (IBC Tank Wassertank Container 800 L, Barrel Trading GmbH & Co. KG, Gaildorf, Germany) using a drill pump (Wolfcraft Bohrmaschinenpumpe 8 mm Schaft, Wolfcraft GmbH, Kempenich, Germany). The drill pump was connected with a PVC hose (13 mm ID) to the groundwater monitoring well and to the 800 L tank ~~container~~. The extracted groundwater was supplemented with KNO_3 of natural ^{15}N abundance to a concentration of 10 mg NO_3^--N L^{-1}. Approximately 40 L of this mixture were injected weekly into each of the depths 7, 8, 9 and 10 m below ~~soil~~ ground surface, respectively, at multilevel well B4. The injection rate was approx. 1 L min^{-1}. For 7 and 8m depth the peristaltic pump and for 9 and 10 m depth the drill pump were used for injection and both pumps were connected with Tygon® tubings to the selected depths of the multilevel well. The first injection took place on 22 February 2011 and the last on 22 March 2011. In total, 5 pre-conditioning injections were conducted at the 4 depths. Subsequently, 4 push-pull tests were performed in the previously pre-conditioned injection depths as described above between 29 March and 1 April 2011.

2.5 Analytical techniques

2.5.1 Isotope analysis of dissolved N_2 and N_2O

Water samples sampled during push-pull tests were adjusted to 25 °C and a headspace was generated within the serum bottles by the injection of 15 or 40 ml of ambient air into the 26 and 115 ml serum bottles, respectively, replacing the same volume of sample solution. The replaced solution was directly transferred into 20 ml PE vials and frozen for later NO_3^- and SO_2^{4-} analysis. After headspace generation the serum bottles were agitated for 3 h on a horizontal shaker at a constant temperature of 25 °C to equilibrate the dissolved gases with the headspace gas. Finally, 13 ml of the headspace gas of each serum bottle were extracted with a plastic syringe and then transferred to an evacuated 12 ml sampling vial (Exetainer® Labco, High Wycombe, UK), giving a slight positive pressure within the sampling vial. The sampled nitrogen gases in the 12 ml vials were then a mixture of N_2 and N_2O gained from atmosphere and from denitrification, respectively.

The ^{15}N analysis of gas samples was performed by isotope ratio mass spectrometry (IRMS) at the Centre for Stable Isotope Research and Analysis in Göttingen, Germany using a Delta V advantage IRMS (Thermo Scientific, Bremen, Germany) following the method described in Well et al. (2003). Analysis included reduction of N_2O to N_2 prior to IRMS entrance. The sum of N_2 and N_2O isotopologues was thus detected as N_2 in the mass spectrometer. In the following, the sum of denitrification derived N_2 and N_2O is referred to as $(N_2+N_2O)_{den}$. The ^{15}N abundance of (N_2+N_2O) was derived from the measured 29/28 molecular ion mass ratio. We analysed replicate samples, one was equilibrated by electrodeless discharge and the other untreated (Well et al., 1998). This allowed calculating $(N_2+N_2O)_{den}$ as well as the ^{15}N abundance in NO_3^- undergoing denitrification. N_2O was measured using a gas chromatograph (Fisons GC 8000, Milan Italy) equipped with a split-injector and an electron capture detector and a HP-Plot Q column (50 m length x 0.32mm ID; Agilent Technologies, Santa Clara, USA) kept at 38 °C. Gas analysis was completed within two weeks after the respective push-pull tests. The concentrations of denitrification derived ^{15}N labelled N_2 and N_2O in the gas samples were calculated as described by Well and Myrold (1999) and Well et al. (2003), respectively. The concentration of N_2O in the added atmospheric air was taken into account when calculating denitrification derived N_2O in the sample. The measured molar concentrations of N_2 and N_2O in the headspace samples were converted into dissolved gas concentrations using gas solubilities given by Weiss (1970) and Weiss and Price (1980) and

taking into account the temperature, headspace pressure and the liquid-to-headspace volume ratio during equilibration of dissolved gases with the headspace gases in the serum bottles.

Additionally to the standard IRMS analysis of $(N_2+N_2O)_{den}$ ^{15}N labelled denitrification products were measured with a membrane inlet mass spectrometer (MIMS) during 5 push-pull tracer tests directly in the field (see Supplement).

2.5.2 Analysis of NO_3^-, SO_4^{2-} and Br^-

NO_3^- concentrations in the water samples were determined photometrically with a continuous flow analyser (Skalar, Erkelenz, Germany). SO_4^{2-} concentrations were analysed by potentiometric back-titration of excess ~~Ba^{2}~~ Ba^{2+} ions remaining in the solution after addition of a defined amount of $BaCl_2$ in excess to SO_4^{2+}. SO_4^{2-} precipitated as $BaSO_4$~~^{2}~~. The original SO_4^{2-} concentration was then analysed by potentiometric back-titration of the excess ~~Ba^{2}~~ 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. Bromide (Br^-) was analysed with an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES, Spectro Analytical Instruments, Kleve, Germany) after stabilizing the aliquot of the analysed water samples with 10% HNO_3.

2.6 Calculations of denitrification rates

Measured concentrations of $(N_2+N_2O)_{den}$ were converted from the unit (µg N L^{-1}) to (µg N kg^{-1}) under the following assumptions: (i) the average density of the solid aquifer material is 2.65 g cm^{-3} and (ii) the effective porosity of the aquifer material was estimated to be 0.3 from literature values for sediments of similar grain size distribution (Kollmann, 1986), with a range of uncertainty of 0.2 to 0.4, respectively.

The concentrations of $(N_2+N_2O)_{den}$ measured during the push-pull tests were corrected for dilution caused by dispersion, diffusion and the tortuosity of the pores. To do this the dilution factor (F_{dil}(ti)) (Eq. 1) was derived from the concentration changes of the conservative tracer Br^- during the push-pull tests as proposed by Sanches-Perez et al. (2003):

$$F_{dil}(ti) = \frac{[Br^-]_{t0}}{[Br^-]_{ti}} \quad (1)$$

where Br^-_{t0} and Br^-_{ti} are the Br^- concentrations of the injected tracer solution and the sampled tracer solution at sampling time ti, respectively. The encountered dilution factors ranged from

1 to 20 and were below 5 in 18 push-pull tests. Only during 4 push-pull tests the dilution factors were between 5-10 and during 2 in the range of 10 to 20. The conventional wells (GKA) showed on average higher dilution factors compared with the CMT multilevel system and the multilevel wells in the FFA. Dilution factors were near 1 for most of the push-pull tests in the FFA, i.e. the injected tracer solution interfered little with the surrounding ambient groundwater.

The corrected concentrations of $(N_2+N_2O)_{den}$ are then obtained by multiplying the uncorrected concentrations of $(N_2+N_2O)_{den}$ at time ti with $F_{dil}(ti)$. Denitrification rates were calculated from the tangent of dilution corrected time courses of $(N_2+N_2O)_{den}$ concentrations at time intervals with the steepest increase during the respective push-pull test (Sanchez-Perez et al., 2003; Istok et al., 2004). This method was used because we suppose that the section of the steepest increase of measured denitrification products during a push-pull test is the best approximation of the maximal denitrification rate possible in the aquifer at the very location of the respective push-pull test. The rationale behind this is: We suppose that the maximal possible denitrification rate measurable at a certain point in an aquifer is dependent on the amount of reduced compounds in the aquifer material capable of supporting denitrification. Also the measured denitrification rate during a push-pull test is dependent on the state of the microbial community in the aquifer material at the location of the conducted push-pull test. For example, if in situ denitrification rates are measured in the zone of NO_3^- free groundwater, microbes might need time to express the appropriate enzymes to start to denitrify after injection of the NO_3^- containing tracer solution. Since it is unknown how long this adaption time might be, the highest measurable denitrification rate during a push-pull test should give an approximation for the maximal possible denitrification rate at the very point of the push-pull test (see also Sect.4.1.2 and 4.2).

2.7 Detection limit and precision of $(N_2+N_2O)_{den}$ measurements

The detection limit of ^{15}N analysis was calculated as the minimum amount of ^{15}N labelled $(N_2+N_2O)_{den}$ 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:

$$r_{sa} - r_{st} \geq 3 \times sdr_{st} \quad (2)$$

where r_{sa} and r_{st} are the $^{29}N_2/^{28}N_2$ ratios in sample and standard, respectively and sdr_{st} is the SD standard deviation (SD) 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 $(N_2+N_2O)_{den}$ was 5 and 1 µg N L^{-1} for samples in 26 and 115 ml serum bottles, respectively, depending on the different ratio of liquid sample to headspace in the respective serum bottles.

The mean coefficient of variation (CV) of concentration measurements of $(N_2+N_2O)_{den}$ (µg N L^{-1}) in 3 replicates per sampling event during all push-pull tests was 0.18 %. The conversion of concentration data from the unit (µg N L^{-1}) to (µg N kg^{-1}) increased the mean CV significantly to 0.49 %. (The mean CV after conversion to (µg N kg^{-1}) was calculated from the 3 concentrations resulting from the range of effective porosity values (see Supplement).)

2.8 Statistical analysis and modelling

Statistical analysis and regression modelling was conducted with WinSTAT for MS Excel Version 2000.1 (R. Fitch Software, Bad Krozingen, Germany). Experimental data (x) was converted into Box–Cox transformed data (f B-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 performed with the statistic software STATISTICA 8 (StatSoft, Tulsa, USA). Simple linear regression analysis was conducted to evaluate quantitative relations between in situ denitrification rates (D_r(in situ)) and various sediment parameters of corresponding aquifer material measured in the laboratory (Eschenbach and Well, 2013). Normal distribution of the measured parameters within the different data sets and the residuals of linear regressions were 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 was checked with scatter plots of residuals vs. independent variables of the respective regression analysis. This was done to ensure homoscedasticity during regression analysis, i.e. to ensure

that the least-squares method yielded best linear estimators for the modelled parameter. To use the regression functions given in the result section with own data, the experimental values have to be transformed according to Eq. (3) with the lambda coefficients given in Table S2 in the Supplement.

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).

2.9 Model sediment properties using regression functions with D_r(in situ)

In situ denitrification rates (D_r(in situ)) measured during push-pull tests were used to model parameters of the investigated aquifer samples measured in the laboratory. These parameters were: (i) the cumulative denitrification after one year of incubation ($D_{cum}(365)$), (ii) the stock of reduced compounds (SRC) and (iii) several sediment parameters like water soluble organic carbon (C_{hws}), the fraction of $KMnO_4$ labile organic carbon (C_l), total sulphur (total-S) and total organic carbon (C_{org}). $D_{cum}(365)$ is the cumulative amount of denitrification products per kg dry weight of incubated aquifer material at the end of one year of anaerobic incubation (mg N kg^{-1}). The SRC is the amount of sulphides and C_{org} converted into N equivalents (mg N kg^{-1}) according to their potential ability to reduce NO_3^- to N_2 (Eschenbach and Well, 2013). These sediment parameters and denitrification rates were analysed during a laboratory incubation study with aquifer samples from the FFA and GKA (Eschenbach and Well, 2013). The aquifer samples were collected from drilled material obtained during well construction of groundwater monitoring and multilevel wells in the FFA and GKA. The analysed aquifer samples originated from depth intervals of approximately 1 m above to 1 m below filter screens or filter elements of respective groundwater monitoring or multilevel wells, used for push-pull tests (Table 1).

3 Results

3.1 Grouping of push-pull test measuring points

Push-pull tests ~~Wells~~ were grouped into data subsets according to the redox state of groundwater ~~–~~and chemical properties of the aquifer material from the vicinity of the filter

screens of groundwater monitoring wells used for the respective push-pull tests ~~properties into the sub data sets~~(aquifer material was collected during well construction) (see also Eschenbach and Well (2013) Sect. 3.1). These data subsets consist of data from ~~are of push-pull tests in~~ wells with filter screens in ~~situ denitrification rates (D_r(in situ)) measured in~~ the NO_3^-—~~-~~bearing and NO_3^--free groundwater zone (NO_3^--bearing and NO_3^--free zone, respectively) and ~~into push pull tests D_r(in situ) measured~~ wells in the zone of non-sulphidic, sulphidic, and transition zone aquifer material (Tables 1 and 2).

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} in the various monitoring wells (Table 2). Therefore, 0.4 mg NO_3^--N L^{-1} was the lowest NO_3^- concentration of groundwater to be considered nitrate bearing in this study. Sulphidic and non-sulphidic aquifer material was distinguished using the sulphate formation capacity (SFC (mg S kg^{-1} yr^{-1})) of incubated aquifer material from the vicinity of the respective filter screen of the used monitoring wells (Eschenbach and Well, 2013). Aquifer ~~S~~samples with a SFC > 1 mg SO_4^{2-}-S kg^{-1} yr^{-1} during incubation were assigned sulphidic and push-pull tests conducted at wells with filter screens in this zone were accordingly assigned to the sulphidic zone. The transition zone was defined as zone within the aquifer where aquifer material still contains~~from the region where~~ sulphides ~~were present~~, but groundwater still contained NO_3^-. It follows, that the NO_3^--bearing groundwater zone comprises the zone of sulphidic aquifer material and the transition zone. ~~For a detailed description of the classification of aquifer material see Eschenbach and Well (2013).~~

3.2 In situ denitrification rates and time courses of denitrification products

D_r(in situ) ranged from 0.0 to 51.5 µg N kg^{-1} d^{-1}. Mean D_r(in situ) in the FFA (9.1 µg N kg^{-1} d^{-1}) were almost 4 to 5 times higher than in the GKA, but differences between aquifers were not significant (Figs. 2 and 3, Tables 3 and 4).

The non-sulphidic zone of both aquifers exhibited the lowest mean D_r(in situ) (1.04 µg N kg^{-1} d^{-1}) of all partial data sets (Table 4) and statistical significant differences (kw: $P < 0.05$) occurred with the full and all partial data sets except D_r(in situ) measured in the GKA and in the NO_3^--bearing zone of both aquifers. The other partial data sets exhibited no significant differences between one another. Mean D_r(in situ) of the transition zone (9.32 µg N kg^{-1} d^{-1}) was slightly higher than in the sulphidic zone of both aquifers.

Except for the multilevel well B6 in 6 m depth, all push-pull injection points with O_2 concentrations above 1 mg O_2 L^{-1} in the groundwater exhibited D_r(in situ) below 0.75 µg N

kg^{-1} d^{-1} (Tables 2 and 3) and aquifer material from this locations were assigned to non-sulphidic aquifer material during laboratory incubations (Eschenbach and Well, 2013).

D_r(in situ) measured after pre-conditioning of push-pull injection points at multiple well B4 (FFA) (67.83 to 152.70 µg N kg^{-1} d^{-1}) were 30 to 65 times higher than D_r(in situ) measured one year before without pre-conditioning (2.76 and 2.28 µg N kg^{-1} d^{-1}) (Table 3).

Among the total of 28 push-pull tests, 24 were conducted without pre-conditioning from which twelve were located in the NO_3^--bearing and twelve in the NO_3^--free zone of both aquifers, respectively. Among the 12 push-pull tests in the NO_3^--free zone all of the 5 FFA locations showed an exponential increase of $(N_2+N_2O)_{den}$ during push-pull tests, whereas in the GKA this was only the case in two to three of the 7 GKA locations. In contrast to this, only 2 out of 12 push-pull tests in the NO_3^--bearing zone of both aquifers exhibited exponential increases and these push-pull tests were located in the transition zone of multilevel well B2. The two push-pull tests at multilevel well B4 (NO_3^--free zone of the FFA) showed an exponential increase of $(N_2+N_2O)_{den}$. All other push-pull tests in the NO_3^--bearing zone exhibited almost linear trends. After preconditioning at the same depths of multilevel well B4 in the NO_3^--free zone, the time course of denitrification products was drastically different compared to the initial tests with a much steeper and initially almost linear trend (Fig. 4).

3.3 Relationship between D_r(in situ), D_{cum}(365) and aquifer parameters

3.3.1 Comparison of D_r(in situ) and D_{cum}(365)

D_r(in situ) was compared with mean denitrification rates during 365 days of laboratory incubation (D_r(365)) (Eschenbach and Well, 2013) with aquifer material collected from the locations of the monitoring wells (see Sect. 2.3). D_r(365) was obtained by dividing cumulative $(N_2+N_2O)_{den}$ production (D_{cum}(365)) by incubation time (365 d). D_r(in situ) was generally lower than D_r(365) (Fig. 3 and Table S1 Supplement). The means of the D_r(in situ)-to-D_r(365) ratio were calculated for the different partial data sets giving a range of 0.05 to 0.47, with the lowest and highest ratios for the data sets of GKA and transition zone push-pull tests, respectively (Table 4). In the transition zone, D_r(in situ)-to-D_r(365) ratios were significantly higher compared to the other data sets (kw: $P < 0.05$). D_r(in situ) of FFA aquifer material was statistical significant closer related to D_r(365) than D_r(in situ) measured in the GKA. The

mean D_r(in situ)-to-D_r(365) ratio from the NO_3^--bearing zone of both aquifers (0.23) was significantly larger than in the NO_3^--free zone of both aquifers (0.1) (Table 4).

D^r(in situ) after pre-conditioning (well B4, FFA) was comparable or higher than D_r(365) with D_r(in situ)-to-D_r(365) ratios of 0.73 to 2.76 (Fig. 3 and Table 4). D_r(in situ) was 30 to 65 times higher compared to values obtained without pre-conditioning at the same wells (Fig. 5 and Table 3).

3.3.2 Regression models to predict D_{cum}(365), SRC and denitrification relevant aquifer parameters from D_r(in situ)

Simple linear regression analysis was applied to obtain regression models for the prediction of D_{cum}(365) from D_r(in situ) for the full and partial data sets. ~~The goodness of fit of the regression models given by the correlation coefficient (R) and the average ratio of calculated D_{cum}(365) to measured D_{cum}(365) for the full and partial data sets.~~The correlation coefficient (R) and the average ratio the average ratio of calculated D_{cum}(365) to measured D_{cum}(365) are used to evaluate the goodness of fit of the regression models.

The goodness of fit of regression models to predict D_{cum}(365) by D_r(in situ) varied for the various data subsets~~sub data sets~~ from no fit in the sulphidic zone ~~and~~ to a good approximation of D_{cum}(365) by D_r(in situ) in the NO_3^--bearing zone (R = 0.04 and R = 0.84, respectively, Table 5). For the full data set, the ~~goodness~~ quality of the fit was modest (R = 0.62) resulting in a wide range of deviations between calculated and measured D_{cum}(365) from -49.1 to 18.1 mg N kg^{-1} in the different data subsets~~sub data sets~~. Linear relationships between D_r(in situ) ~~and~~ and D_{cum}(365) were better for GKA in comparison to FFA aquifer material. Aquifer material which was not ~~jet~~ yet in contact with NO_3^--bearing groundwater (NO_3^--free zone and most of sulphidic zone material) exhibited D_r(in situ) values which were clearly less correlated with D_{cum}(365) than aquifer material which was already in contact with NO_3^--bearing groundwater (non-sulphidic zone, transition zone and NO_3^--bearing zone) (Table 5).

The goodness of the fit of regression models to calculate the SRC from D_r(in situ) was on average slightly worse than the one of regression models to predict D_{cum}(365) from D_r(in situ). As for the prediction of D_{cum}(365) the best goodness of fit of regression models was obtained ~~for the sub data sets of~~from the GKA data sets, the transition zone and the NO_3^--bearing zone with coefficients of determination of R = 0.75, 0.77 and 0.50 (Table 5). Like D_{cum}(365) also for SRC the prediction was best for zones of both aquifers where the aquifer

material had already beenwas already in contact with NO_3^--bearing groundwater in situ prior to the push-pull tests. Contrary to other partial data sets, the data subsetsub set of D_r(in situ) measured in sulphidic aquifer material exhibited a clearly better goodness of fit between D_r(in situ) and SRC than between $D_{cum}(365)$ and D_r(in situ), R = 0.41 and R = 0.04, respectively.

As already mentioned above pre-conditioning of multilevel well B4 strongly increased the measured D_r(in situ). Here, regressions between D_r(in situ) and $D_{cum}(365)$ and between D_r(in situ) and SRC exhibited a modest goodness of fit (R = 0.54 and R =0.53, respectively) (Table 5).

Regression analysis between several denitrification relevant parameters of aquifer material (Eschenbach and Well, 2013) and D_r(in situ) revealed that for some partial data sets, the linear regressions between some of these parameters and D_r(in situ) were even better than the regression between D_r(in situ) and $D_{cum}(365)$ (Table S3 Supplement in comparison to Table 5). For GKA aquifer material, $D_{cum}(365)$ was in closest linear correlation with D_r(in situ). Contrary to this, for FFA aquifer material D_r(in situ) was closer related to $SO_4^{2-}{}_{extr}$ and C_{hws} than to $D_{cum}(365)$ or SRC. For data subsetssub data sets grouped according to the sulphate formation capacity of the incubated aquifer material, several parameters were inhad better or at least the sameequal linear correlation to D_r(in situ) than $D_{cum}(365)$. These parameters were C_{org} and total-S in the non-sulphidic zone, $SO_4^{2-}{}_{extr}$ and total-S in the sulphidic zone, C_{org} and total-S in the transition zone, C_{org} and $SO_4^{2-}{}_{extr}$ in the NO_3^--bearing zone, and $SO_4^{2-}{}_{extr}$ and C_l in the NO_3^--free zone.

4 Discussion

4.1 Quantifying D_r(in situ) with push-pull tests

4.1.1 Ranges of D_r(in situ) and comparison with previous studies

To compare previous D_r(in situ) data with our measurements, all data denitrification rates were converted to the dimension µg N kg^{-1} d^{-1} assuming an effective pore space of 0.3 and an average density of dry aquifer solids of 2.65 g cm^{-3}. D_r(in situ) values measured in the FFA and GKA (Table 3) are comparable with D_r(in situ) (2.3–27.1 µg N kg^{-1} d^{-1}) measured by Konrad (2007) in two Pleistocene sandy aquifers in Northern Germany (aquifers of Thülsfelde and Sulingen, about 40 km west and 30 km south of the city of Bremen,

respectively). Also D_r(in situ) reported by ~~(~~Addy et al., (2002) and ~~(~~Addy et al., (2005) show a similar range of denitrification rates with 2.1–121.2 and 0.5–87.9 µg N kg^{-1} d^{-1}, respectively. Those values were measured in two riparian sites and a site with marsh sediments ~~on~~ in Rhode Island USA. Somewhat larger spans of D_r(in situ) were reported by Well et al. (2003) for water-saturated mineral sub-soils from various locations in Northern Germany and by Konrad (2007) for the sandy to silty aquifer of Wehnsen (about 30 km southeast of the FFA) with D_r(in situ) from 0–300 and 45–339 µg N kg^{-1} d^{-1}, respectively. These larger spans also cover ~~also~~ the full range~~magnitude~~ of D_r(in situ) values measured at multilevel well B4 in the FFA after pre-conditioning (Table 3). Sanches-Perez (2003) measured D_r(in situ) from 22.1 to 7646.4 µg N kg^{-1} d^{-1} with the acetylene inhibition method in 2 shallow sandy aquifers in France and Spain. Overall, there is a wide range of reported D_r(in situ) in aquifers.

Denitrification rates can also be derived from the analysis of groundwater samples from monitoring-well transects along hypothesized groundwater flow paths. Therefore, Tesoriero and Puckett (2011) selected 12 study sites with monitoring- well transects within the U.S. The study areas represented a wide range of sedimentary environments and climatic conditions. Tesoriero and Puckett (2011) generalized the determined denitrification rates broadly into three categories: low rates (< 0,02 µg N kg^{-1} d^{-1}), medium rates (0,02-0,06 µg N kg^{-1} d^{-1}) and high rates (> 0,6 µg N kg^{-1} d^{-1}). Low to were found in areas with elevated O_2 concentrations in the groundwater, medium rates in the presence of low O_2 concentrations and high denitrification rates when changes in the lithology resulted in a sharp increase in the supply of electron (Tesoriero and Puckett (2011), p. 13). Overall, the denitrification rates obtained from theses monitoring-well transects are below the mean D_r(in situ) of the various data subsets in this study (Table 4). For example the mean D_r(in situ) of non-sulphidic aquifer material was 1 µg N kg^{-1} d^{-1} (Table 4) and thus even higher than the high denitrification rates reported by Tesoriero and Puckett (2011). The O_2 concentrations in the ambient groundwater at these push-pull locations were mostly clearly above 1 mg L^{-1}, which is near the reported apparent threshold for the onset of denitrification in aquifers (Green et al., 2008, 2010; McMahon et al., 2004; Tesoriero and Puckett, 2011) (see Sect. 4.3). Mean D_r(in situ) of data subsets of push-pull test at locations with low O_2 concentrations (transition-zone and NO_3^--free zone) (Table 2) were 9 and 8 µg N kg^{-1} d^{-1}, respectively, and thus by the factor of 10 higher as the high rates reported by Tesoriero and Puckett (2011).

Green et al. (2010) showed that groundwater mixing due to advection and mechanical dispersion can strongly influence the derived apparent denitrification rates along flow paths in a way that these transport processes tend "to create the appearance of lower reaction rates and

fractionation parameters when measured at larger scales and longer flow paths" (Green et al. 2010, p 12). Green et al. (2010) showed that mixing effects increase with the mean travel distances of groundwater and they conclude "that effects of transport and scale should be considered when comparing reaction rates in different aquifer systems, or when comparing reaction rates in different parts of the same system".

In contrast, Korom et al. (2005) reported clearly higher zero-order denitrification rate of 35.6 µg N kg^{-1} d^{-1} measured by an aquifer mesocosm, this rate is comparable with the highest D_r(in situ) measured in this study (Table 2). Korom et al. (2012) argued that, contrary to monitoring-well transects, such transport depending mixing processes would not influence denitrification rates measured by aquifer mesocosms, since advection and mechanical dispersion are negligible. The influence of advection and mechanical dispersion on the measured apparent denitrification rates by push-pull test should be higher compared to in situ mesocosms. On the contrary during push-pull tests, mixing processes by advection and mechanical dispersion should be significantly lower in comparison to monitoring-well transects, since the flow path of the injected tracer solution in the aquifer is in a decimeter or maximum meter range during a push-pull test, which is very short compared to flow-paths of hundreds of meters or several kilometres in case of monitoring-well transects. (Additionally the mixing of the injected tracer solution with ambient groundwater was taken into account by the addition of Br^- as conservative tracer to the tracer solution (see Sect. 2.6) to minimise the influence of mixing effects.)

The observed differences in denitrification rates measured in this study with denitrification rates derived from monitoring-well transects (Tesoriero and Puckett 2011) might thus be attributed to effects of transport along long flow paths. We think that these effects should also be considered when denitrification rates are compared that have been derived with different methods.

~~But there is not only a strong local variability in D_r(in situ) of aquifers also D_r(in situ) can change substantially during push-pull tests itself. During a push-pull test conducted by Trudell et al. (1986) in situ denitrification rates increased strongly. During the 12 day lasting pull-phase of this tracer test in the O_2- and NO_3^--free groundwater zone of a shallow sandy aquifer in south western Ontario Canada D_r(in situ) increased from 30.3 to 504.6 µg N kg^{-1} d^{-1} (Trudell et al., 1986).~~

4.1.2 Temporal and spatial variability of in situ denitrification rates ~~Time course of $(N_2+N_2O)_{den}$ and pre-conditioning~~

~~But T~~there is~~seem to be~~ not only a strong local In addition to possible ~~,~~systematic differences between different methods with respect to the derived denitrification rates, it has to take into account that ~~also variability in D_f(in situ) of aquifers also~~ D_f(in situ) can show a considerable temporal variability ~~change substantially~~ during push-pull tests itself. ~~During a push-pull test conducted by Trudell et al. (1986) in situ denitrification rates increased strongly.~~ This was evident d~~D~~uring the 12 day ~~lasting~~long pull-phase of a push-pull test conducted by Trudell et al. (1986)~~this tracer test~~ in the O_2 and NO_3^--free groundwater zone of a shallow sandy aquifer in south western Ontario Canada, where D_f(in situ) increased from 30.3 to 504.6 µg N kg^{-1} d^{-1} (Trudell et al., 1986).

Comparable to the results of Trudell et al. (1986), in this study most of the push-pull tests ~~I~~in the NO_3^--free zone showed~~,~~ an exponential increase of $(N_2+N_2O)_{den}$ with time, i.e. increasing denitrification rates~~was observed during most of the push-pull tests~~. ~~Sections~~ Periods of an exponential ~~time courses~~increase of dilution corrected denitrification products ~~observed~~ during tracer tests were also previously reported (Eschenbach and Well, 2011; Konrad, 2007). In the study of Konrad (2007), 5 out of 13 push-pull tests showed an exponential increase of dilution corrected denitrification products. 4 of these 5 push-pull tests were located in the NO_3^--free groundwater zone. Contrarily, push-pull tests in the NO_3^--free zone (consisting of the data subsets of non-sulphidic aquifer material and the transition zone) showed approximately constant denitrification rates during the push-pull tests. The ~~N~~non-sulphidic aquifer material exhibited very low denitrification rates during the push-pull tests presumably ~~also~~ because the aquifer material was depleted in the reduced compounds capable of supporting (Table S1 in the Supplement and Eschenbach and Well (2013) Sect. 4.2) and dissolved O_2 in groundwater inhibited NO_3^- reduction. Dissolved O_2 concentrations in the ambient groundwater and therefore also in the injected test solutions were > 1 mg O_2 ~~l~~L^{-1} at 6 out of 8 injection points in the non-sulphidic zone of both aquifers (Table 2) which is near or above the apparent threshold for the onset of denitrification in aquifers (see Sect. 4.3 below) whereas in the transition zone O_2 concentrations were far below this threshold.~~,~~ In relation to the amount of reduced compounds of transition zone aquifer material (Table S1 in the Supplement and Fig.2 in Eschenbach and Well (2013)), which was almost as low the one of non-sulphidic aquifer material, the measured in situ denitrification rates were comparatively

high (Table 3). Despite the clearly lower SRC content in situ denitrification rates in the transition zone were on average higher than in the NO_3^--free zone (Table 3). ~~As a result, i~~We suspect that the clearly different activity of denitrification in relation to the SRC in both data subsets is becaus the microbial community in the NO_3^--free zone is not ready to denitrify since it needs time to adapt to NO_3^- as possible electron acceptor.

Therefore, it is concluded, that the exponential increase of denitrification products observed during push-pull tests in our study and previous studies can probably be attributed to growth and stimulation of denitrifiers by the injection of NO_3^- into aquifer zones that have previously not been in contact with NO_3^-. Trudell et al. (1986) found an increase of denitrifying bacteria species during the 12 day ~~lasting~~ long tracer test which was accompanied by a 17-fold increase of measured denitrification rates. Several other investigations showed increasing microbial activity after bio stimulation of aquifer sediments by the injection of electron donors to monitoring wells (Istok et al., 2004; Kim et al., 2004, 2005). Istok et al. (2004) reported that the viable biomass on solid samplers installed in monitoring wells more than doubled compared with samplers installed in monitoring wells without electron donor addition.

To establish an active denitrifying microbial community in the strict anaerobic zone of an aquifer we injected NO_3^- as newly available electron acceptor in the NO_3^- -free zone at Multilevel well B4 in the FFA. To our knowledge, pre-conditioning of aquifer material prior to a push-pull ^{15}N tracer test by the injection of only NO_3^- ~~NO_3^- as new available electron acceptor~~ was firstly used in ~~the presented~~this study. ~~This was done to establish a denitrifying microbial community in the strict anaerobic zone of an aquifer.~~ Pre-conditioning at multilevel well B4 (see Sect. 2.4) ~~in the FFA~~ resulted in a 30- to 65-fold increase in measured in situ denitrification rates compared with push-pull tests without pre-conditioning at the same depths of multilevel well B4 (Table 3 and Fig. 5). It can be concluded that pre-conditioning in the NO_3^--free zone of the FFA led to growth of the community of active denitrifiers in the aquifer material in the vicinity of the respective injection points. The increase of D_r(in situ) due to pre-conditioning might be a combined effect from the increase of active denitrifiers and a higher denitrification rate per microbial cell due to synthesis of enzymes for denitrification. Pre-conditioning ~~resulted~~ lead not only ~~in~~ to higher denitrification rates but also the time course of $(N_2+N_2O)_{den}$ did not show a ~~section~~ period of a distinct exponential increase compared with prior measurements without pre-conditioning (Fig. 4). This might show that denitrifiers in the tested aquifer material after pre-conditioning were ready to denitrify and that there was a stable denitrifying community, see also Sect. 4.2.

Pre-conditioning also improved the comparability of in situ and laboratory denitrification rates. All in all the measured ~~Higher average~~ denitrification rates during one year of incubation (D_r(365)) were on average higher in comparison to denitrification rates derived with normal push pull tests (D_r(in situ)). This may have resulted from several factors including the stimulation of denitrification in the lab due to disturbance of aquifer material, establishment of strictly anaerobic conditions and the adaptation of the microbial community over time. The ratio between D_r(in situ) and D_r(365) was highly variable within the data set. Interestingly, it was lowest in the non-sulphidic and NO_3^--free zones of both aquifers (Table 4). ~~Non-sulphidic aquifer material exhibited low denitrification rates during the push-pull tests also because dissolved O_2 inhibited NO_3^- reduction. Dissolved O_2 concentrations in the ambient groundwater and therefore also in the injected test solutions were > 1 mg O_2 l^{-1} at 6 out of 8 injection points in the non-sulphidic zone of both aquifers (Table 2).~~ In case of non-sulphidic aquifer material dissolved O_2 (Table 2) might have inhibited NO_3^- reduction and in the zone of NO_3^- free groundwater. D_r(365) of non-sulphidic aquifer material measured during anaerobic incubation in the laboratory (Eschenbach and Well, 2013) can therefore be seen as a potential activity which is only partly effective under in situ conditions due to a low ~~consumption~~ reduction rate of dissolved O_2 in groundwater. This is also reflected by the low D_r(in situ) to D_r(365) ratio in the non-sulphidic wells (Table 4).

The mean D_r(in situ)-to-D_r(365) ratio in the NO_3^--bearing zone were twice as high compared to the NO_3^--free zone (Table 4 and Fig. 3). This probably reflects the need for microbial adaptation to NO_3^- discussed in the previous section. Mean D_r(in situ) and the ratio of D_r(in situ)-to-D_r(365) of 0.47 were highest in the transition zone, showing that in the transition zone D_r(in situ) and D_r(365) were in closer agreement compared with other zones. During the push-pull tests in the transition zone the ambient concentration of dissolved O_2 ~~were~~ was always below 0.13 mg L^{-1} and NO_3^- was always detectable in the ambient groundwater at the 5 injection points in the transition zone (Table 2). Denitrification was therefore presumably not inhibited by dissolved O_2 and the microbial population had already adapted to NO_3^- as an available electron acceptor. Hence, denitrifying conditions during push-pull tests and during laboratory incubation were similar, resulting in closer agreement in denitrification rates.

4.2 Interpretation of observed time courses of produced $(N_2+N_2O)_{den}$

Figure 6 sums up our interpretation of the results from push-pull tests in the NO_3^--free zone. Immediately after the injection of the ^{15}N tracer in the NO_3^--free zone of both aquifers there

seems to follow a time interval with little to no ~~or negligible~~ production of ^{15}N labelled $(N_2+N_2O)_{den}$ (=lag-phase) (compare with Figs. 2 and 4). During this time, denitrifiers might still have to synthesise enzymes for denitrification and are not yet ready to denitrify.

After the lag-phase follows a phase of exponential increase of $(N_2+N_2O)_{den}$ during which the amount of active denitrifiers and or their activity might adapt to the newly available electron acceptor NO_3^-. The growth of denitrifiers might depend on the microbially available stock of reduced compounds (SRC), i.e. on the surface area of ~~reactive~~ reduced compounds (saRC) present in the aquifer material. If the denitrifying community is adapted to NO_3^- and had colonized the saRC, denitrification rates should be relatively constant. Hence a zero order reaction model should fit the measured data during the relatively short duration of a push-pull test (Fig. 6, linear response phase). It is suspected that these conditions apply to the NO_3^--bearing zone but not to the NO_3^--free zone. After preconditioning at multilevel well B4, $(N_2+N_2O)_{den}$ was initially high and there was no subsequent exponential increase, while the opposite was the case during previous tests at the same well without pre-conditioning (Fig. 4). This probably reflects ~~a~~ the more constant activity of denitrifiers during the push-pull tests after pre-conditioning (Fig. 6, linear response phase). Similar adaptation effects have been reported previously, where bio-stimulation by injecting electron donors like ethanol, glucose, propane or fumarate resulted in constant activity, thus allowing the use of zero-order reaction models to derive reduction rates during push-pull tests (Istok et al., 2004; Kim et al., 2004, 2005). This supports our interpretation that pre-conditioning leads to a kind of equilibrium between the denitrifying community, the injected NO_3^- and the saRC present in the aquifer material, ultimately resulting in relatively constant reaction rates while NO_3^- is not limiting (Fig. 6, linear response phase). In our experiments, the latter condition was fulfilled, because NO_3^- concentrations during the pull-phase were always clearly above 1.0 mg NO_3^--N L^{-1}, which is assumed to be the threshold of NO_3^- concentrations limiting denitrification rates reported by Wall et al. (2005).

From the dynamics of microbial adaptation outlined above it follows, that preconditioning prior to push-pull tests in the zone of NO_3^- free groundwater is needed to allow estimating the stock of reduced compounds from in situ denitrification rates.

4.3 Predicting $D_{cum}(365)$ and SRC of aquifer sediments from D_r(in situ)

The main objective of this study is to predict the cumulative denitrification measured during one year of laboratory incubation of aquifer samples ($D_{cum}(365)$) and the stock of ~~reactive~~

reduced compounds (SRC) from in situ denitrification rates (D_r(in situ)). In comparison to costly drilling of aquifer material and laboratory measurement of D_{cum}(365) and SRC, D_r(in situ) can be measured with relatively low cost push-pull tests at existing groundwater monitoring wells, which would thus allow spatial mapping of denitrification activity within aquifers.

There are only scarce data comparing the stock of reduced compounds (SRC) or longer-term denitrification rates (e.g. D_r(365)) with D_r(in situ)). Well et al. (2003) showed for denitrification in the saturated zone of hydromorphic soils that laboratory derived denitrification rates after 24 h of anaerobic incubation were in good agreement with in situ denitrification rates, but the study was limited to near-surface groundwater. Konrad (2007) tested this approach in deeper aquifer zones with a small data set of pairs of D_r(in situ) vs. D_{cum}(4 push-pull ^{15}N tracer tests and incubations of corresponding aquifer material) and found that both quantities were related (spearman rank correlation coefficients of $R \geq 0.8$).

In this study, transfer functions were developed to predict D_{cum}(365) from D_r(in situ) measurements with a larger data set in different redox zones typically present in aquifers. Moreover, pre-conditioning was evaluated by addition of NO_3^- to aquifer material and the subsequent measurement of in situ denitrification rates.

Only a modest goodness of fit ($R = 0.62$) was found using linear regression between D_r(in situ) and D_{cum}(365) for the full data set (Table 5). Without Box–Cox transformations of input data the correlation coefficient was even lower ($R = 0.1$). This shows that it was necessary to transform the input data to approach normal distribution and homoscedasticity for regression analysis. Otherwise the ordinary least squares method did not find the best or efficient linear estimators for regression coefficients.

Like in the previous laboratory study (Eschenbach and Well, 2013) grouping of D_r(in situ) measuring points by locality or according to hydro-geochemical zones increased the predictive power of D_r(in situ) with respect to the measured D_{cum}(365) and SRC of aquifer material for some partial data sets. Altogether, D_r(in situ) was the best predictor for D_{cum}(365) and SRC of the partial data set of GKA aquifer material with correlation coefficients of 0.82 and 0.75, respectively. For the FFA the predictive power of D_r(in situ) for D_{cum}(365) and SRC was significantly lower compared to the GKA (Table 5). This finding mirrors results of laboratory incubations with FFA and GKA material reported by Eschenbach and Well (2013) (Table 4 of the cited study), in which initial denitrification rates (D_r(7)) of GKA material were a better predictor of D_{cum}(365) than in case of FFA material. Contrary to the GKA aquifer samples, the SRC of the FFA samples was not predictable by D_r(in situ). One reason might be

a different microbial availability of organic carbon (C_{org}), which is one major constituent of SRC in both aquifers (Eschenbach and Well, 2013). The ratio of KMnO4 labile organic carbon (C_l) to C_{org} was almost twice as high in the GKA material compared to the FFA material (Eschenbach and Well, 2013), suggesting that the proportion of C_{org} available for microbes is higher in the GKA aquifer material and on the other hand that a significant proportion of C_{org} is unavailable for denitrification in the FFA.

Grouping of aquifer material according to hydro-geochemical zones or sediment parameters resulted in better regressions between D_r(in situ) and D_{cum}(365) and SRC for partial data sets where NO_3^- is still present in the groundwater, i.e. in the transition and NO_3^--bearing zone (Table 5). Konrad (2007) reported similar relationship between D_r(in situ) and D_{cum}(365) under comparable conditions. Relatively weak fits were obtained for data sets with push-pull measuring points located completely or mostly in the zone of NO_3^- free groundwater (NO_3^--free zone and sulphidic aquifer material, respectively) and in the non-sulphidic zone (Table 5). For the NO_3^--free zone this is attributed to a missing adaptation of the microbial community to NO_3^- as electron acceptor as discussed above. In the study of Trudell et al. (1986) it took at least 8 days until measured denitrification rates stopped to increase during the push-pull test. In our study, such long pull-periods were not possible because of comparatively higher groundwater velocities in both aquifers. At some injection points in the FFA, the tracer plume had already moved away with groundwater ~~already~~ within 35 h ~~after~~ of the injection.

The goodness of fit in the modelling of ~~D_r~~D_{cum}(~~in situ~~365) and SRC using linear regression functions was highly variable among partial data-sets. The mean ratios of calculated D_{cum}(365) to measured D_{cum}(365) and calculated SRC and measured SRC were best for the transition zone with ratios near 1. ~~and worst for the sulphidic and NO_3^--free zone (Table 5)~~. We suppose the reasons for this might be (i) that residual reduced compounds, that could support denitrification were still present in the aquifer material, (ii) the O_2 concentrations in the ambient groundwater (Table 2) were far below the reported apparent threshold of < 40 - 60 µmol L^{-1} (≈ 1.5 – 2.3 mg O_2 L^{-1}) for the onset of denitrification in aquifers (Green et al., 2008, 2010; McMahon et al., 2004; Tesoriero and Puckett, 2011) (see also Sect. 4.1 in Eschenbach and Well (2013)) and (iii) NO_3^- was present in the ambient groundwater of the transition zone. Therefore we expect that the microbial community was already adapted to NO_3^-, i.e. ready to denitrify, and denitrification was not inhibited by dissolved O_2. Conversely, in the non-sulphdic zone higher O_2 concentrations might have inhibited denitrification and this might have been limiting for D_r(in situ) than the limited

content of reduced compounds. This might explain the poor fit between calculated and measured values (Table 5) in the non-sulphidic zone. In the NO_3^--free zone the groundwater was almost O_2 free and, in comparison to the other zones, the aquifer material had a larger stock of reduced compounds (Table S1 in the Supplement). But nonetheless the correlation coefficients between D_r(in situ) and D_{cum}(365) and D_r(in situ) and the SRC were very low and the developed regression functions ~~D_r(in situ)~~ underestimated especially D_{cum}(365) and SRC of deeper aquifer samples with high values of D_{cum}(365) and SRC to a large extent (Table 5). We suppose the reason for this is ~~apparently because of~~ the lack of adaptation of the microbial community to NO_3^-, as already discussed above.

Pre-conditioning at multilevel well B4 led to a clearly better fit of D_r(in situ) and D_r(365) (Table 4). This indicates that pre-conditioning should increase the predictability of D_{cum}(365) and probably also of SRC from Dr(in situ) measurements in the NO_3^--free zone.

5 Conclusions

The possibility to predict the capacity of aquifer zones to remove NO_3^- inputs over extended time periods based on in situ measurement of denitrification rates ~~(D_r(in situ))~~ was evaluated in two Pleistocene aquifers in Northern Germany. This was done by comparison of D_r(in situ) with denitrification parameters determined in aquifer material samples, i.e. the stock of reduced compounds (SRC) and the cumulative denitrification measured during one year of incubation in the laboratory (D_{cum}(365)).~~Direct comparison of in situ push-pull tests and laboratory incubations to determine the cumulative denitrification measured during one year of incubation (D_{cum}(365)) and the stock of reduced compounds (SRC) of aquifer material proved to be a suitable approach to calibrate linear regression models.~~

Prediction of D_{cum}(365) and SRC from D_r(in situ) for data sets containing data from both aquifers was only satisfactory in the aquifer zones where NO_3^- was present. This type of in situ tests might thus be suitable for mapping D_{cum}(365) and SRC in NO_3^- bearing zones of Pleistocene sandy aquifers using existing monitoring wells. It is thus a promising and low-cost method to estimate D_{cum}(365) of aquifer material from aquifer zones where NO_3^- is still present in the groundwater. Our results also indicate that the push-pull technique (without pre-conditioning) is not suited to derive the SRC or D_{cum}(365) of aquifer samples from in situ denitrification rates under conditions where the groundwater is nitrate-free. Moreover, future routine applications of this approach could be facilitated by online field analysis using

membrane inlet mass spectrometry, which we demonstrated to be feasible and precise ~~(see Supplement)~~. Still, the correction for dilution of the injected tracer solution with ambient groundwater is ~~requested~~ necessary when using membrane inlet mass spectrometry in the field (see Sect. 2.6 and the Supplement).

In the NO_3^--free aquifer zone increasing denitrification rates were observed during the conducted push-pull tests, which ~~was~~ were interpreted as the result of adaptation processes of the denitrifying communities following NO_3^- injections. Also D_r(in situ) without pre-conditioning ~~were~~ was generally lower than average denitrification rates after one year of incubation (D_r(365)) in the laboratory. This was especially the case for D_r(in situ) measurements in the NO_3^- free groundwater zone. In this study it was demonstrated exemplarily that the microbial community in the NO_3^--free zone ~~close~~ just below the NO_3^--bearing zone can be adapted to denitrification by amending wells with NO_3^- injections for an extended period. In situ denitrification rates measured after this pre-conditioning reflected the D_{cum}(365) and SRC more satisfactorily. From this findings it is assumed that microbial adaptation after NO_3^- injection confounded the relationship between reactive compounds present in the tested aquifer material and D_r(in situ) measured during push-pull tests, which resulted in poor prediction of D_{cum}(365) and SRC from D_r(in situ). Therefore we assume that pre-conditioning is a prerequisite for the measurement of in situ denitrification rates using push-pull tracer tests in the NO_3^- free groundwater zone. Further research is needed to check if this microbial adaptation would also work in deeper layers far below the NO_3^--bearing zone.

Acknowledgements

This research was made possible by financial support from the Deutsche Bundesstiftung Umwelt (DBU). The authors would like to thank I. Ostermeyer and K. Schmidt for their assistance in the laboratory, L. Szwec and R. Langel (Centre for Stable Isotope Research, University of Göttingen) for stable isotope analyses, R. Hoppe and W. Raue (enercity Hannover) for support during pre-conditioning and W. Duijnisveld (Federal Institute for Geosciences and Natural Resources (Hannover), M. Dietze and R. Liedl (Institut für Grundwasserwirtschaft (Dresden)) for technical support the push-pull tests. We would also like to thank Jeremy Smith for suggestions to improve the language. Two anonymous reviewers and the associate editor of this journal are gratefully acknowledged for their valuable comments on a previous version of this manuscript.

The Supplement related to this article is available online at doi:10.5194/bgd-11-16527-2014-supplement.

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Tables:

Table 1. Overview of the conducted push-pull ^{15}N tracer tests, the used wells and the depth range of the respective filter screens in both aquifers. Push-pull test with and without pre-conditioning were conducted at multilevel well B4 ~~in 7, 8, 9 and 10 m below soil surface~~.

	Fuhrberg (multilevel wells)					Großenkneten (conventional monitoring and multilevel wells)					
Monitoring well	B1	B2	B4	B6	N10	Gro 326	Gro 327	S1	S2	CMT 1	CMT2
	filter screen m below ~~soil~~ ground surface										
non-sulphidic zone		2.95-3.05		3		8.0-10.0					8.15-8.40
(NO_3^--bearing zone)		4.15-4.25		6							22.65-22.90
Transition zone		7.95-8.05			5						
(NO_3^--bearing zone)		8.95-9.05									
		9.95-10.05			8						
Sulphidic zone	6.95-7.05	13.95-14-05	6.95-7.05*				35.0-39.0	66.0-67.0	26.0-27.0		26.65-26.90
(NO_3^--free zone)	7.95-8.05		8.75-8.85*							29.15-29.40	
			9.85-9.95*							31.15-31.40	
			9.95-10.05*							33.35-33.60	

1175 * Push-pull tests with pre-conditioning.

Table 2. Background conditions of the groundwater ~~at~~ from the injection depths of the ~~locations of~~ push-pull ^{15}N tracer tests.

Location	inj.	aquifer zone	O_2	N_2O	SO_4^{2-}	pH	redox	con[b]

	depth[a]		NO_3^-						
	m		mg L^{-1}	mg N L^{-1}	µg N L^{-1}	mg S L^{-1}		mV	µS cm^{-1}
FFA B1	6.95-7.05	sulphidic	0.67	< 0.25	n.d.	27.64	6.00	-171	473
FFA B1	7.95-8.05	sulphidic	0.76	< 0.25	n.d.	24.73	6.04	-175	440
FFA B2	2.95-3.05	non-sulphidic	3.66	41.47	1.59	15.07	4.66	273	563
FFA B2	4.15-4.25	non-sulphidic	0.96	27.59	68.31	36.94	4.83	209	564
FFA B2	7.95-8.05	transition zone	0.16	12.58	0.03	32.52	4.48	341	553
FFA B2	8.95-9.05	transition zone	0.13	7.09	0.05	38.41	4.65	367	488
FFA B2	9.9510.05	transition zone	0.06	1.0	n.d.	43.30	4.75	374	458
FFA B2	13.95-14.05	sulphidic	0.40	0.63	n.d.	42.51	6.75	117	453
FFA B4	7.95-8.05	sulphidic	0.22	< 0.25	1.14	42.30	5.28	-38	432
FFA B4	8.95-9.05	sulphidic	0.12	< 0.25	0.70	51.19	5.43	-	-
FFA B6	2.95-3.05	non-sulphidic	9.51	6.10	0.02	13.95	5.70	365	255
FFA B6	5.95-6.05	non-sulphidic	1.28	19.55	10.66	22.45	5.18	349	441
FFA N10	4.95-5.05	transition zone	0.12	13.12	184.8	59.87	4.61	341	660
FFA N10	7.95-8.05	transition zone	0.16	0.4	1.03	52.03	5.60	3	463
GKA 326	8.0-10.0	non-sulphidic	6.30	3.06	0.12	4.67	4.10	374	105
GKA CMT2	8.15-8.40	non-sulphidic	6.10	3.14	0.12	5.06	4.40	387	100
GKA CMT2	22.65-22.90	non-sulphidic	5.70	3.98	0.56	12.09	5.10	276	163
GKA CMT2	26.65-26.90	sulphidic	0.10	< 0.25	0.01	18.57	5.40	30	221
GKA S2	26.0-27.0	sulphidic	0.30	< 0.25	n.d.	17.85	5.30	161	217
GKA CMT1	29.15-29.40	sulphidic	0.20	< 0.25	n.d.	18.16	5.50	-24	240
GKA CMT1	31.15-31.40	sulphidic	0.14	< 0.25	n.d.	17.91	5.20	134	195
GKA CMT1	33.35-33.60	sulphidic	0.20	< 0.25	n.d.	18.60	5.10	122	272
GKA 327	35.0-39.0	sulphidic	0.10	< 0.25	0.13	10.85	5.30	26	275
GKA S1	66.0-67.0	non-sulphidic	0.13	< 0.25	0.02	5.10	5.72	-54	103

FFA Fuhrberger Feld aquifer;

GKA Großenkneten aquifer;

[a] injection depth (the absolute depth can vary by a few cm);

[b] conductivity.

Table 3. In situ denitrification rates (D_r(in situ)) and minimum and maximum values of D_r(in situ) in dependence of the range of estimated effective porosities (0.2 to 0.4). D_r(in situ) were calculated from a regression line through the $(N_2+N_2O)_{den}$ concentrations at time intervals with the steepest increase of $(N_2+N_2O)_{den}$ during the respective push-pull test. Tracer tests after pre-conditioning are marked with *.

Location	Injection depth[a]	Aquifer zone	D_r(in situ)	D_r(in situ) Max	D_r(in situ) min	R[a] R[b]
	M m		µg N kg^{-1} d^{-1}			
FFA B1	6.95-7.05	sulphidic[c,d]	17.59	27.361	10.261	0.94
FFA B1	7.95-8.05	sulphidic[c,d]	1.512	2.352	0.882	0.92
FFA B2	2.95-3.05	non-sulphidic[b,c]	0.120	0.186	0.070	0.14
FFA B2	4.15-4.25	non-sulphidic[b,c]	0.065	0.102	0.038	0.01
FFA B2	7.95-8.05	transition zone[b,c]	0.429	0.667	0.250	0.95
FFA B2	8.95-9.05	transition zone[b,c]	1.415	2.201	0.825	0.90
FFA B2	9.95-10.05	transition zone[b,c]	8.650	13.456	5.046	0.99
FFA B2	13.95-14.05	sulphidic[c,d]	51.47	80.078	30.029	0.82
FFA B4	7.95-8.05	sulphidic[c,d]	2.755	4.286	1.607	0.98
FFA B4	8.95-9.05	sulphidic[c,d]	2.278	3.544	1.329	0.86
FFA B6	2.95-3.05	non-sulphidic[b,c]	0.057	0.089	0.033	0.02
FFA B6	5.95-6.05	non-sulphidic[b,c]	4.998	7.774	2.915	0.96
FFA N10	4.95-5.05	transition zone[b,c]	12.89	20.052	7.520	0.95
FFA N10	7.95-8.05	transition zone[b,c]	23.19	36.074	13.528	0.99
FFA B4*	6.95-7.05	sulphidic[c,d]	152.6	237.527	89.073	0.94
FFA B4*	7.95-8.05	sulphidic[c,d]	67.83	105.514	39.568	0.99
FFA B4*	8.95-9.05	sulphidic[c,d]	145.5	226.481	84.930	0.98
FFA B4*	9.95-10.05	sulphidic[c,d]	150.7	234.530	87.949	1.00
GKA 326	8.0-10.0	non-sulphidic[b]	0.747	1.162	0.436	0.96
GKA CMT2	8.15-8.40	non-sulphidic[b]	0.051	0.079	0.030	0.02
GKA CMT2	22.65-22.90	non-sulphidic[b]	0.009	0.013	0.005	0.00
GKA CMT2	26.65-26.90	sulphidic[c,d]	1.233	1.918	0.719	0.70
GKA S2	26.0-27.0	sulphidic[c,d]	0.860	1.338	0.502	0.99
GKA CMT1	29.15-29.40	sulphidic[c,d]	4.427	6.886	2.582	0.78
GKA CMT1	31.15-31.40	sulphidic[c,d]	0.504	0.784	0.294	0.63
GKA CMT1	33.35-33.60	sulphidic[c,d]	2.002	3.114	1.168	0.77
GKA 327	35.0-39.0	sulphidic[c,d]	6.192	9.632	3.612	0.99
GKA S1	66.0-67.0	non-sulphidic[c,d]	2.271	3.533	1.325	1.00

FFA Fuhrberger Feld aquifer; GKA Großenkneten aquifer; [a](the absolute depth can vary by a few cm), [a] [b] correlation coefficient of the regression line;

[b] [c] NO_3^--bearing zone; [c] [d] NO_3^--free zone.

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Table 4. Means, standard deviation and ranges of D_r(in situ) of the data sets. Statistical significant differences (kw: $P < 0.05$) between D_r(in situ) values measured in the various data subsetssub data sets occurred only between D_r(in situ) measured in the non-sulphidic zone and some other partial data sets.

Data set	D_r(in situ)[a] ($\mu g\ kg^{-1}\ N\ d^{-1}$) N[c]	means	range	non-sulphidic[d]	D_r(in situ) / D_r(365)[b] N[e]	means	Range
Whole data set	24	6.07±11.36	0.00 – 51.48	s[1]	34	0.15±0.20	0.00 – 0.60
FFA	14	9.10±14.20	0.06 – 51.48	s[1]	16	0.26±0.24	0.01 – 0.60
GKA	10	1.83±2.02	0.00 – 6.19	Ns	18	0.06±0.06	0.00 – 0.20
non-sulphidic zone	8	1.04± 1.78	0.00 – 5.00	-	11	0.05±0.08	0.00 – 0.23
sulphidic zone	14	8.59±13.67	0.43 – 51.48	s[2]	23	0.20±0.22	0.01 – 0.60
transition zone	5	9.32±9.32	0.43 – 23.19	s[1]	8	0.47±0.14	0.25 – 0.60
NO_3^--bearing zone	12	4.38±7.24	0.00 – 23.19	Ns	17	0.23±0.24	0.00 – 0.60
NO_3^--free zone	16	7.76±14.53	0.50 – 51.48	s[1]	17	0.10±0.10	0.01 – 0.37
B4 pre-conditioned	4	128.1±43.4	67.8 – 152.7	-	4	1.87±0.84	0.72 – 2.76
B4 un-conditioned	2	2.52±0.34	2.28 – 2.76	-	2	0.04±0.02	0.02 – 0.05

[a] all D_r(in situ) measurements, [b] only D_r(in situ) measurements with corresponding incubated aquifer samples [c] number of D_r(in situ) measurements; [d] statistical differences between non-sulphidic and other data sets (s significant differences; ns not significant differences; [1] differences significant at the 0.05 probability level; [2] differences significant at the probability level; [3] differences significant at the 0.001 probability level); [e] number of comparisons between D_r(in situ) and corresponding incubated aquifer samples.

Table 5. Simple regressions between D_r(in situ) and $D_{cum}(365)$ and SRC from anaerobic incubations with corresponding aquifer material. $f^{B\text{-}C}(X) = A + B \times f^{B\text{-}C}(D_r(\text{in situ}))$.

	calculated/measured	Deviation

						(mg N kg^{-1} yr^{-1})			
Data set	X[a]	N[b]	A	B	R[c]	mean	range	mean	range
Whole data set	D_{cum}(365)	34	2.878	0.603	0.62	2.29±4.19	0.16 – 22.96	-3.07±14.67	-47.2 – 12.8
Whole data set	SRC	34	6.123	0.152	0.40	1.51±1.31	0.12 – 5.19	-671.2±2091	-7734 – 1379
FFA	D_{cum}(365)	16	2.640	0.578	0.52	2.83±4.90	0.13 – 19.18	-3.08±14.71	-49.1 – 7.0
FFA	SRC	16	3.772	0.006	0.07	1.22±0.82	0.11 – 2.92	-377.8±1375	-5317 –413.7
GKA	D_{cum}(365)	18	3.046	0.818	0.82	1.34±0.92	0.26 – 3.85	-2.25±12.28	-30.8 – 15.5
GKA	SRC	18	8.024	0.613	0.75	1.43±1.23	0.178 – 4.47	-617.0±2179	-5780 – 2390
non-sulphidic	D_{cum}(365)	11	1.050	0.156	0.40	2.25±3.20	0.26 – 10.65	-0.10±2.41	-5.2 – 1.8
non-sulphidic	SRC	11	8407	752.8	0.43	1.50±0.84	0.46 – 3.19	31.54±240.7	-553 – 272.6
sulphidic	D_{cum}(365)	23	4.185	-0.033	0.04	1.33±0.90	0.30 – 4.19	-3.32±15.13	-39.4 – 13.1
sulphidic	SRC	23	21.40	-1.372	0.41	0.30±0.18	0.03 – 0.61	-1823±2313	-8564 – -144
transition zone	D_{cum}(365)	8	1.109	0.581	0.53	1.03±0.26	0.74 – 1.43	-0.36±2.84	-4.5 – 3.3
transition zone	SRC	8	5.349	-0.602	0.77	1.05±0.41	0.58 – 1.92	-50.11±340.6	-518.7 – 561
NO_3^--bearing	D_{cum}(365)	17	2.132	0.454	0.84	2.21±3.76	0.13 – 15.17	-0.67±2.52	-6.3 – 2.7
NO_3^--bearing	SRC	17	193.3	16.32	0.55	1.36±0.75	0.41 – 2.76	-19.35±365.2	-929 – 462.6
NO_3^--free	D_{cum}(365)	17	7.774	2.036	0.36	1.47±0.88	0.31 – 3.00	-1.69±16.23	-38.7 – 18.1
NO_3^--free	SRC	17	77.61	8.421	0.21	1.78±1.46	0.27 – 4.47	-485.4±2494	-6077 – 2095
pre-conditioned[1]	D_{cum}(365)	4	14.402	0.099	0.54	1.06±0.35	0.62 – 1.47	0.12±9.49.79	-12.95 – 9.41
pre-conditioned[1]	SRC	4	319.5	4.895	0.53	1.12±0.52	0.51 – 1.77	5.5±462	-638.0 – 464

[1] experimental data of pre-conditioned push-pull tracer tests was not Box-Cox transformed before regression analysis, because of the small number of data pairs. For these data pairs the following equation applies: $X = A + B \times D_r$(in situ).

[a] Independent sediment parameter

[b] number of samples

[c] correlation coefficient.

Figure captions:

Fig. 1. Schematic of push-pull ^{15}N tracer tests at groundwater monitoring and multilevel wells.

Fig. 2. Time courses of denitrification derived $(N_2+N_2O)_{den}$ and dissolved O_2 during ^{15}N push-pull tests in the FFA (**A** and **C**) and GKA (**B** and **D**). FFA = Fuhrberger Feld aquifer; GKA = Großenkneten aquifer; ns non-sulphidic; s sulphidic and tZ transition zone aquifer material.

Fig. 3. Relation between in situ denitrification rates determined by ^{15}N push-pull tracer tests and average denitrification rates during one year of incubation (Eschenbach and Well, 2013). FFA Fuhrberger Feld aquifer; GKA Großenkneten aquifer; ns non-sulphidic; s sulphidic and tZ transition zone aquifer material.

Fig. 4. Time courses of $(N_2+N_2O)_{den}$ during push-pull tests without pre-conditioning (**A**) (grey diamonds) and with pre-conditioning **B** (black diamonds) at multilevel well B4 in the FFA. The push-pull tests without pre-conditioning at B4 was conducted in April 2010. One year later in April 2011 the aquifer material of the respective depths was conditioned over 5 weeks with NO_3^- amended groundwater of natural ^{15}N abundance prior to the ^{15}N push-pull tests.

Fig. 5. D_r(in situ) after 5 weeks of pre-conditioning of aquifer material (black diamonds) in comparison to D_r(in situ) without pre-conditioning. The small diagram shows the difference between D_r(in situ) after pre-conditioning and unconditioned D_r(in situ) at multilevel well B4 in the FFA.

1205 **Fig. 6.** Schematic time courses of denitrification during push-pull tests in the NO_3^--free groundwater zone. (D_r = measured in situ denitrification rates, saRC = surface area of reduced compounds present in the ~~testet~~ investigated aquifer.)

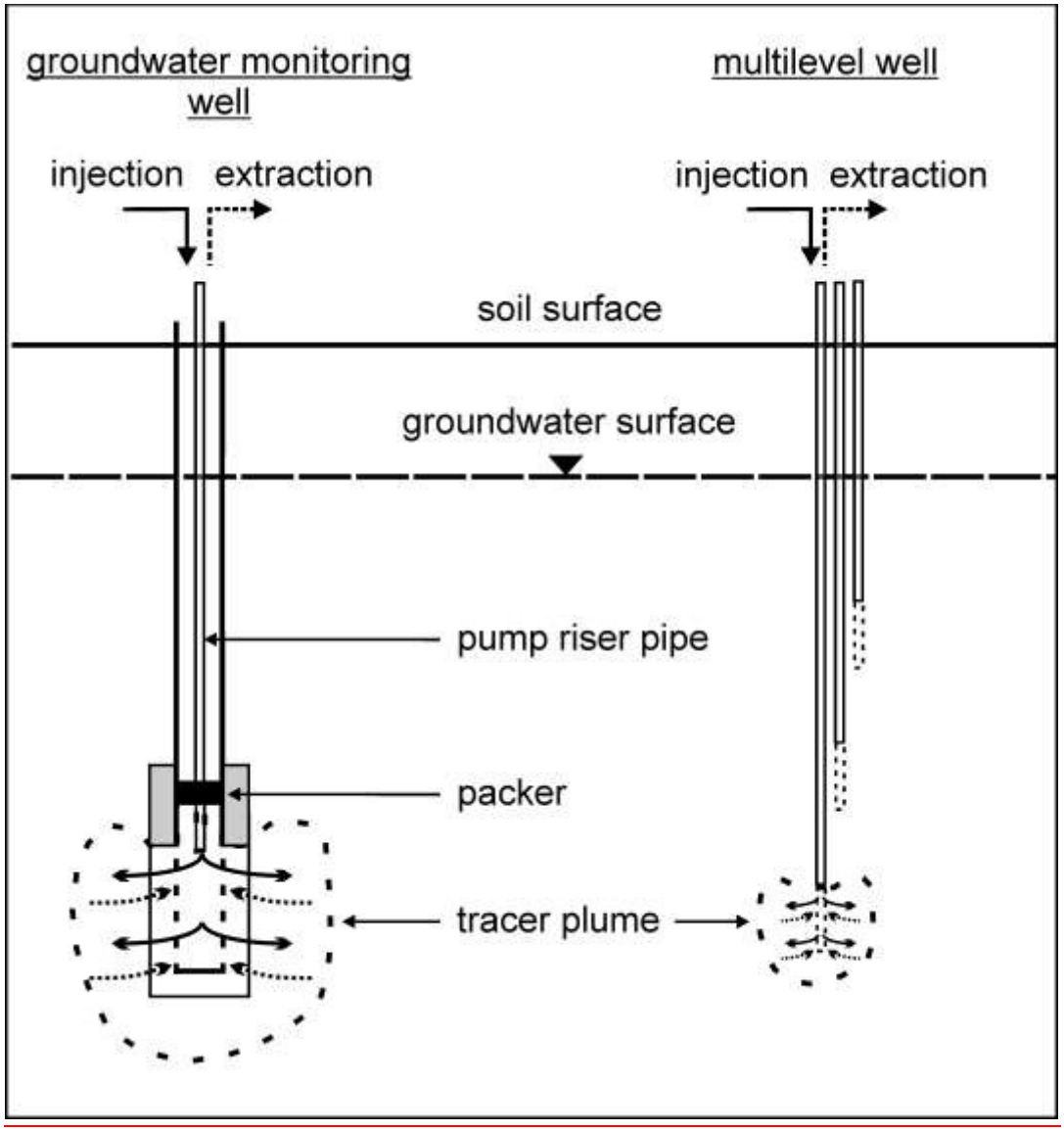
groundwater monitoring well
multilevel well
injection
extraction
injection
extraction
soil surface
groundwater surface
pump riser pipe
packer
tracer plume

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Fig. 1.

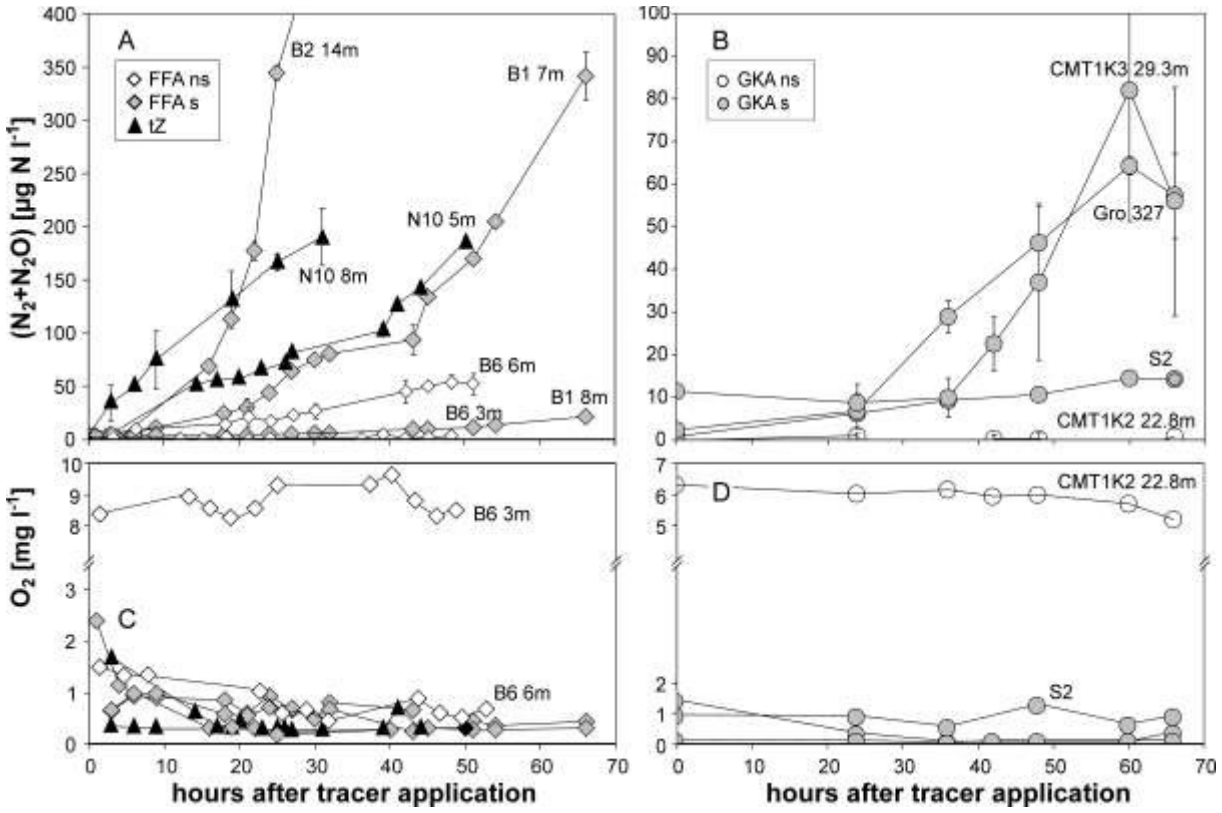
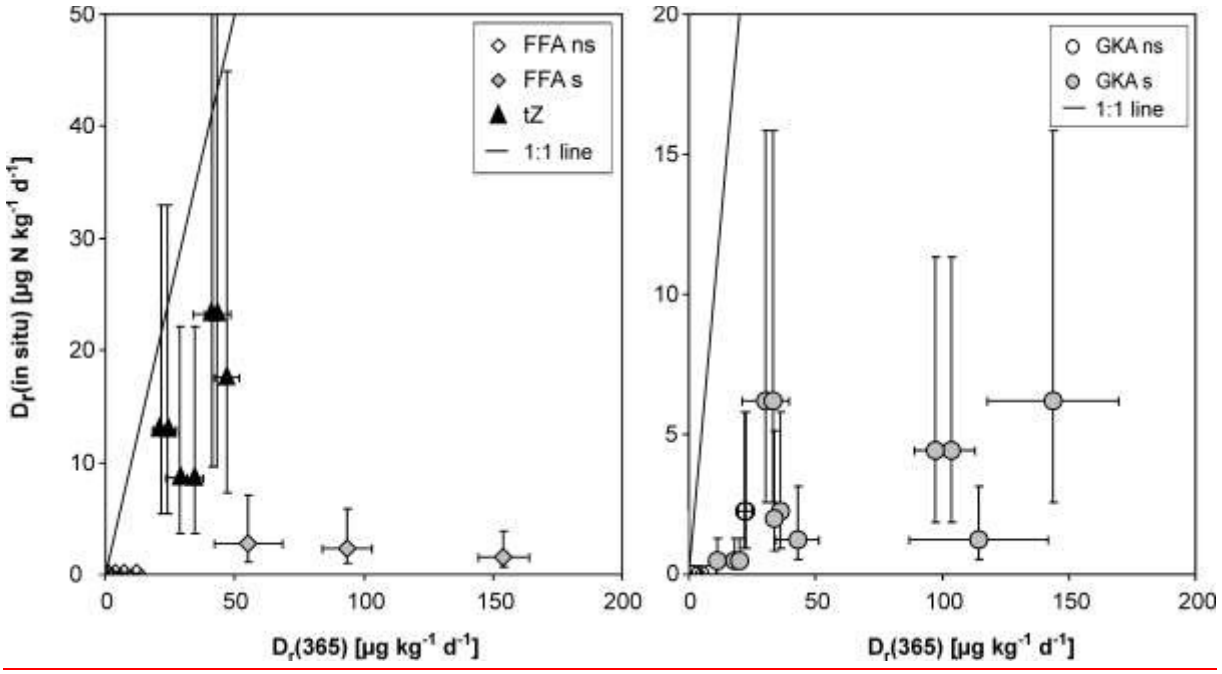


Fig. 2.



1215

Fig. 3.

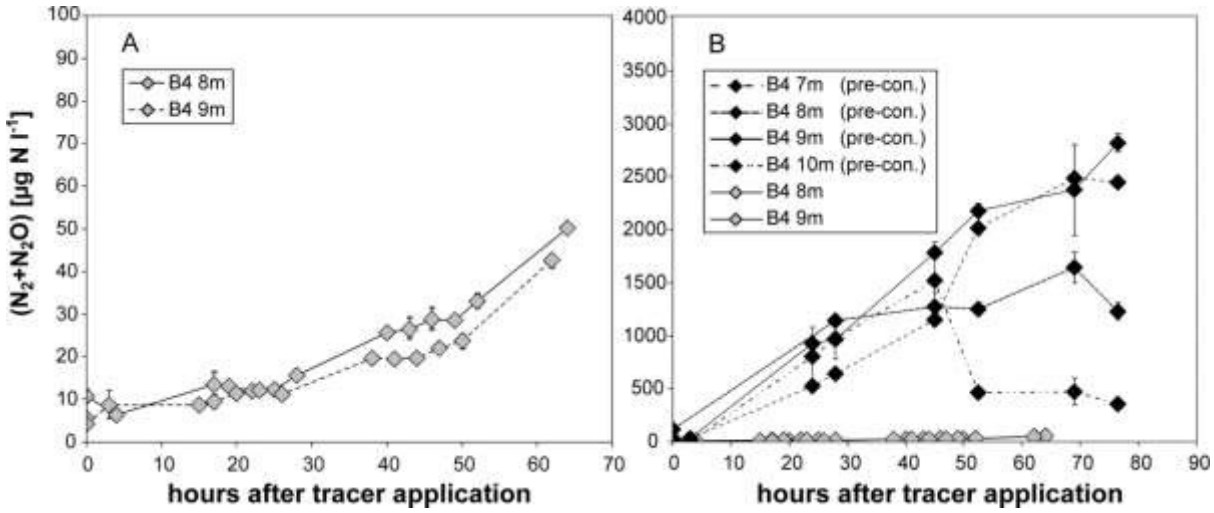

A
B4 8m
B4 9m
(N2+N2O) [µg N l-1]
hours after tracer application
B
B4 7m (pre-con.)
B4 8m (pre-con.)
B4 9m (pre-con.)
B4 10m (pre-con.)
B4 8m
B4 9m
hours after tracer application

Fig. 4.

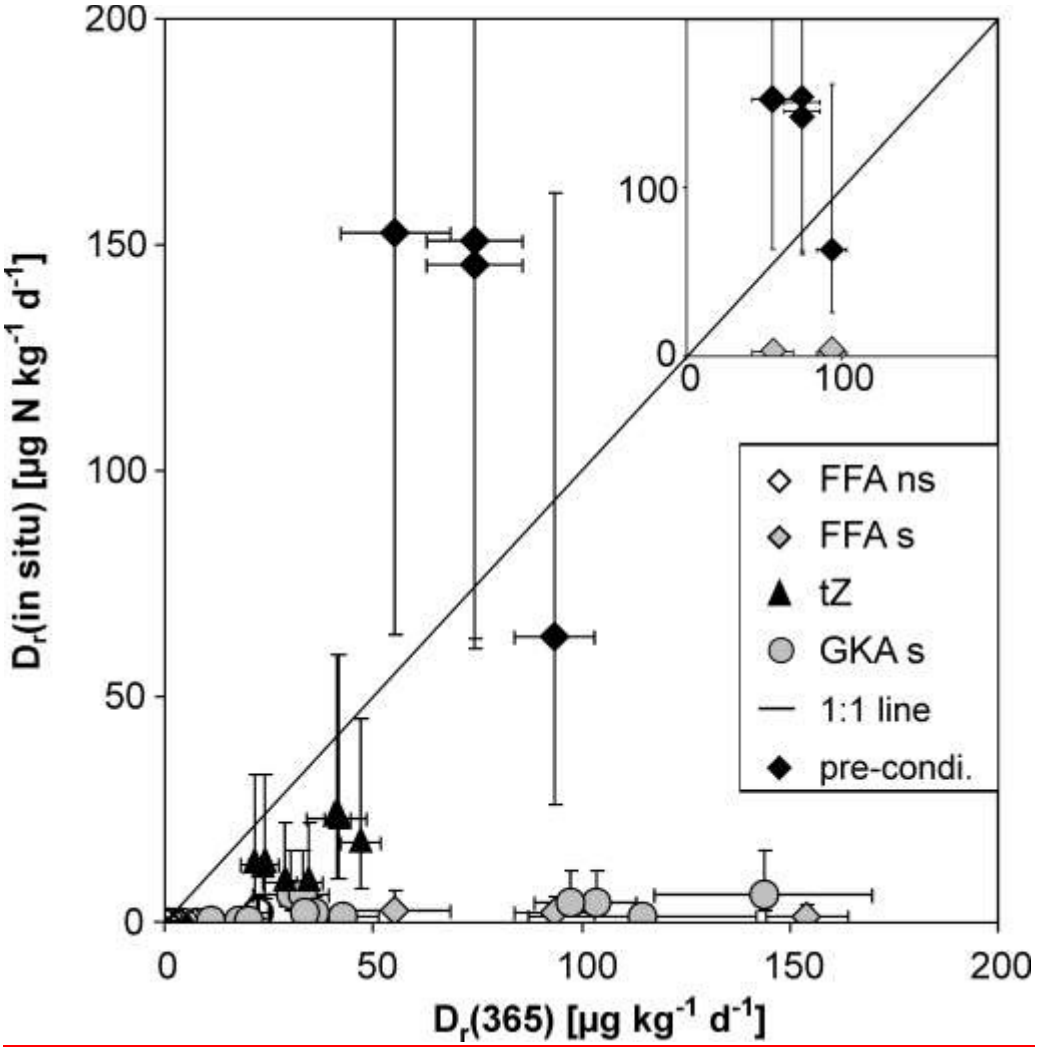
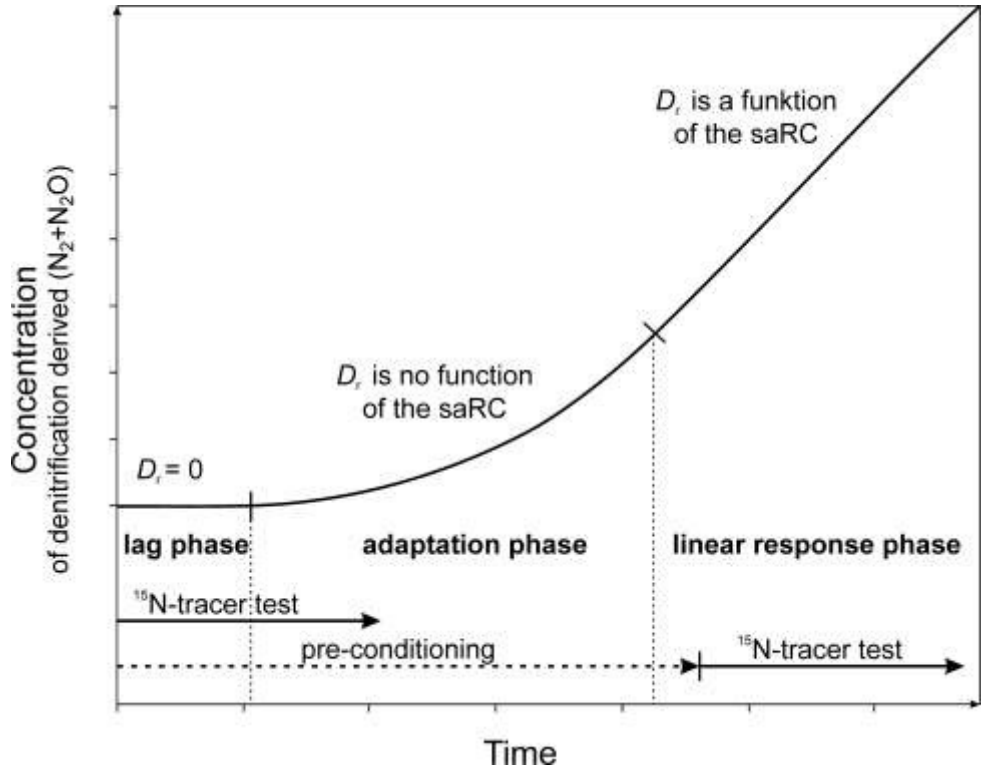

Dr(in situ) [µg N kg-1 d-1]
Dr(365) [µg kg-1 d-1]
FFA ns
FFA s
tZ
GKA s
1:1 line
pre-condi.

Fig. 5.



1220

Fig. 6.

Supplementing information for:

Predicting the denitrification capacity of sandy aquifers from in situ measurements using push-pull ^{15}N tracer tests

S1 In situ isotope analysis using MIMS

At two multilevel wells in the Fuhrberger Feld aquifer (FFA) (multilevel well B1 and B2) the concentration of ^{15}N labelled denitrification products in the sampled tracer solution was measured during the conducted push-pull tests directly in the field, using a membrane inlet mass spectrometer (MIMS) as described in Eschenbach and Well (2011). These measurements were done to compare online field MIMS measurements with the offline laboratory analysis by isotope ratio mass spectrometry (IRMS) as described in section 2.5.1.

The instrumental set up was similar to the laboratory setup described in Eschenbach and Well (2011) and installed inside a van. Briefly, it consisted of the quadrupole mass spectrometer a cryotrap, a membrane inlet, a cryostatic water bath, 2 peristaltic pumps, a reduction furnace and a T-connection.

After injection of tracer solution into the respective depths of the monitoring wells, samples of the tracer solution were extracted using a peristaltic pump (masterflex COLE-Parmer, Vernon Hills, USA) (see section 2.2). A subsample of the sampled tracer solution was then pumped through a T-connection using a second peristaltic pump (ISMATEC, BVP-Standard, Wertheim-Mondfeld, Germany). The T-connection was directly connected via stainless steel tubing with the membrane inlet of the mass spectrometer (described in detail in Eschenbach and Well (2011)). The dissolved gasses in the sampled tracer solution diffused in the membrane inlet through the gas permeable membrane into the high vacuum of the mass spectrometer. A copper reduction furnace and a cryotrap were placed in the vacuum line between membrane inlet and the ion source of the mass spectrometer. N_2O was reduced to N_2 within the reduction furnace. Therefore ^{15}N labelled denitrification derived N_2 and N_2O was analyzed as (N_2+N_2O) on the molecular ion masses 28, 29 and 30 as $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$. The cryotrap was filled with liquid N_2 in order to remove water vapour and CO_2 (see also Fig. 1 Analyser side, in Eschenbach and Well (2011)). The membrane inlet and a flask containing air-equilibrated standard water were placed within a cryostatic water bath (Thermo Haake, HAAKE AG, Karlsruhe, Germany) to ensure constant membrane inlet, sample and air-equilibrated standard water temperatures. The air-equilibrated standard water was manufactured as described in Kana et al. (1994) and used to calibrate the MIMS.

5 push-pull tests (at multilevel well B1 and at B2, respectively) with parallel online MIMS measurements were conducted, in the depths of 7, 8 (B1) and 8, 9 and 10 m (B2) below soil surface (Table 1). Overall, there were 58 pairs of IRMS and MIMS measurements. Both online field MIMS and offline laboratory IRMS measurement were in close agreement

(Fig. S1). The averaged concentrations of the sum of ^{15}N labelled denitrification derived N_2 and N_2O ($(N_2+N_2O)_{den}$) measured with both methods ranged from 0.9 to 99 and 0.3 to 16 µg N l^{-1}, in samples from B1 and B2 respectively. Maximal differences between MIMS and IRMS measurements of $(N_2+N_2O)_{den}$ were 6.6 and 2.5 µg N l^{-1} for samples from B1 and B2 respectively (Fig. S2).

The Bland-Altman-method for method comparison was used to evaluate the agreement of both methods (Bland and Altman, 1986) because correlation and regression analysis can result in the context of method comparison to significant misinterpretations (Altman and Bland, 1983; Bland and Altman, 2003, 1995, 1986). Denoting the results of the IRMS and MIMS measurement of $(N_2+N_2O)_{den}$, as $(N_2+N_2O)_{IRMS}$ and $(N_2+N_2O)_{MIMS}$, respectively. The differences between measurements of individual samples with both methods $[(N_2+N_2O)_{IRMS} - (N_2+N_2O)_{MIMS}]$ were plotted against the average of both measurements $[(N_2+N_2O)_{IRMS} + (N_2+N_2O)_{MIMS}]/2$ (Fig. S2). Furthermore the average of differences ($\bar{d}$), the 95 %-limits of method agreement and 95 %-confidence intervals were calculated as described in Bland and Altman (1986).

The distribution of the magnitude of differences in Figure S2 suggests that there is no substantial increase in variance between both methods with increasing magnitude of measurement, which is a prerequisite for the calculation of method bias and 95 %-limits of method agreement without the need of transforming the data. The average of differences ($\bar{d}$) of all parallel measurements (= estimated method bias) was rather small ($\bar{d}$= 0.6 µg N l^{-1}; Fig. S2). The 95 %-limits of method agreement calculated as described in Bland and Altman (1986) were $\bar{d}$±4 µg N l^{-1}. This means that 95 % of observed differences are expected to fall within these limits. The confidence bands for ($\bar{d}$) and the 95 %-limits of method agreement are narrow (Fig. S2) with values of $\bar{d}$±0.46 and 95 %-limits±0.8 µg N l^{-1}, respectively, showing that sample size was sufficient for the calculation of relative precise values for the estimated method bias and estimated limits of method agreement.

The comparison of online field measurements using MIMS with laboratory offline measurements (IRMS) thus showed a good agreement between both methods (Figs. S1 and S2) with only minor bias under the experimental conditions such as those encountered during this study, i.e. were $(N_2+N_2O)_{den}$ was in the range of 0.9 to 99 µg N l^{-1} and ^{15}N abundances of denitrified NO_3^- were between 45 and 60 atom % ^{15}N. This close agreement is in line with our previous study where offline IRMS and online MIMS measurement were compared under laboratory conditions (Eschenbach and Well, 2011). This shows that in situ application does not alter the precision of the MIMS system.

In summary, the MIMS system was suitable for isotope analysis precise enough for the full range of measured concentrations, showing that this analytical system is suitable for in situ analysis during ^{15}N push pull tests. But still the correction for dilution of the injected tracer solution with ambient groundwater is necessary (see Sect. 2.6). Possibly this can be achieved with an additional inert gas like helium (He), which might be added to the tracer solution by stripping it with He before injection. Helium can then be measured online with the mass spectrometer. Or dilution correction might be achieved by the use of a tracer solution with a different salinity compared to the ambient groundwater.

The main advantages with respect to the conventional IRMS approach is that results can be obtained in the course of experiments directly in the field. Sampling intervals can thus be adapted to get more precise rates. Moreover, the length of the pull phase can be limited to the duration of clearly increasing $(N_2+N_2O)_{den}$ concentrations to save working time. Finally, the relatively low cost and simple handling of the MIMS system are favourable to enable extensive application of the ^{15}N push-pull approach to explore denitrification capacities of aquifers.

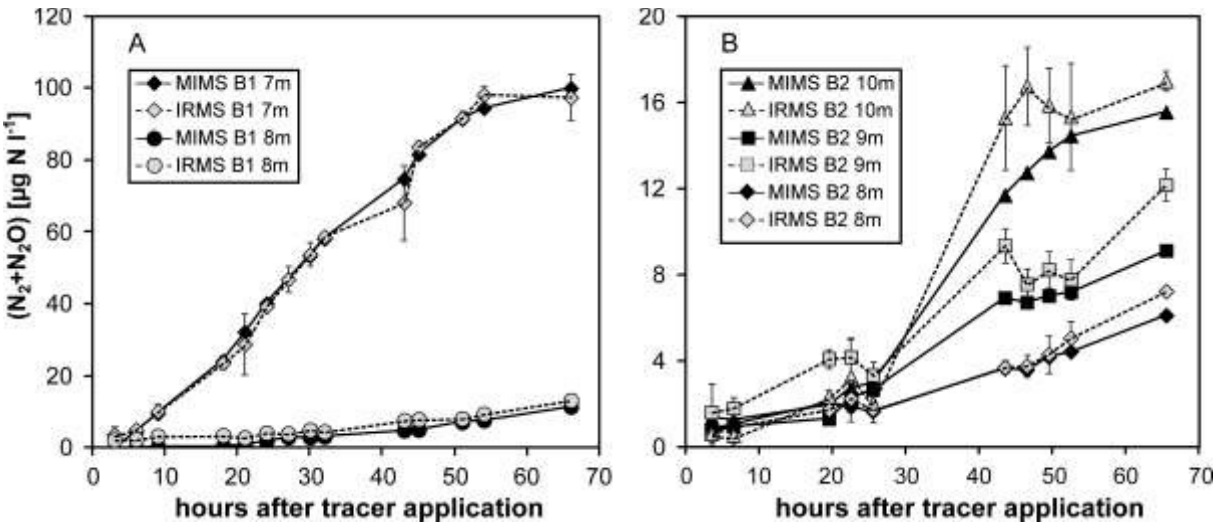


Fig. S1. Comparison of online field measurements of $(N_2+N_2O)_{den}$ from aqueous samples, using a membrane inlet mass spectrometer (MIMS) with standard offline laboratory measurements by means of isotope ratio mass spectrometry (IRMS) at the multilevel wells B1 (A) und B2 (B) for 5 ^{15}N push-pull tracer tests in the Fuhrberger Feld Aquifer.

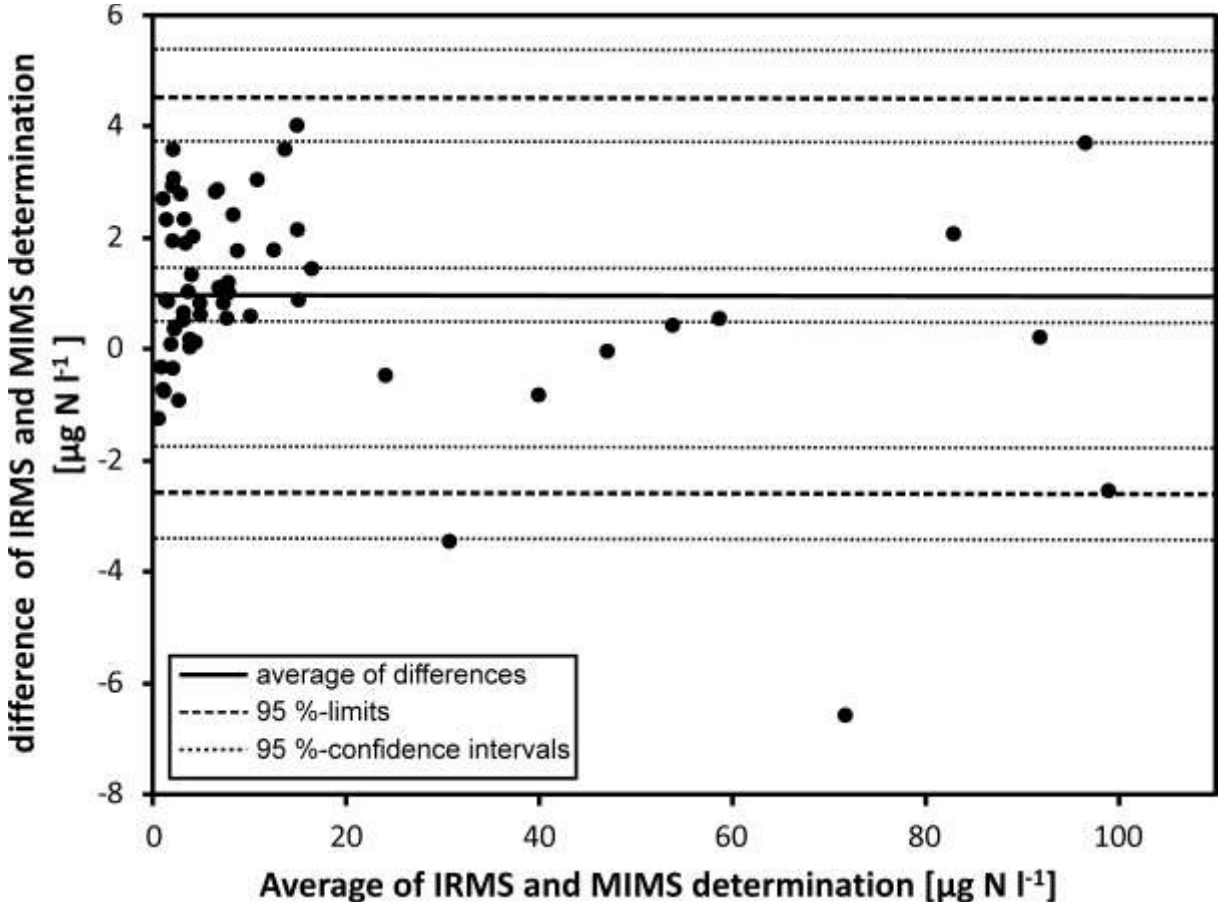


Fig. S2. Bland-Altman-Plot of the differences between online field MIMS analysis and offline laboratory IRMS measurement plotted against the average of both determinations.

S2 Possible confounding factors and uncertainties

Addy et al. (2002) discussed 3 potential confounding factors for the quantification of ^{15}N gas formation during push-pull tests: (i) dilution of denitrification derived gases, (ii) degassing of ^{15}N labelled denitrification derived gasses during the pull-phase of ^{15}N tracer tests (see therefore also discussion in Eschenbach and Well (2011)) and (iii) a lag phase between ^{15}N tracer injection and microbial response. In the following it is briefly referred to (iii).

Microbial adaptation processes after ^{15}N tracer injection might require time especially in the NO_3^--free zone of aquifers (see Sect. 4.2), where aquifer material is brought into contact with NO_3^- for the first time. After pre-conditioning a clear lag phase was not observed during push-pull tests in the NO_3^--free zone at multilevel well B4 in the FFA, therefore it is believed that this is attributed to the stimulation of denitrifiers due to the repeated injections of NO_3^- enriched groundwater at this multilevel well. Therefore, pre-conditioning might be a way to shorten or eliminate the observed lag phases between tracer injections and microbial response.

An additional uncertainty during push-pull tests (iv) is the effective porosity of investigated aquifer sediments. The effective porosity determines the volume of aquifer solids in reaction contact with 1 L test solution. Therefore, this value is needed to relate concentration data of evolved $(N_2+N_2O)_{den}$ from (µg N L^{-1}) to (µg N kg^{-1}). This conversion strongly increases the coefficient of variation (CV) of concentration measurements of $(N_2+N_2O)_{den}$ and thus increases the uncertainty of measured D_r(in situ) because of the uncertainty of the real effective porosity of the tested aquifer material (see Sect. 2.7). The effective porosity at the injection point can be measured with pumping tests prior or after the push-pull ^{15}N tracer test to reduce this source of uncertainty.

S 3. Additional detailed results from laboratory incubations and linear regression models

Table S1. Denitrification rates, cumulative denitrification, stock of reduced compounds, sulphate formation capacity and estimated minimal lifetime of denitrification of incubated samples from both aquifers (Eschenbach and Well, 2013) and corresponding in situ denitrification rates.

Sample location	Depth interval	Aquifer zone[a]	$D_{cum}(365)$[b]	SRC[c]	SRC_C[d]	SRC_S[e]	SFC[f]	D_r (in situ)
	m		mg N kg^{-1} yr^{-1}	mg N kg^{-1}			mg S kg^{-1} yr^{-1}	µg N kg^{-1} d^{-1}
FFA B1	6.0-7.0	transition zone	17.18	659.6	599.5	60.1	6.1	17.59
FFA B1	7.0-8.0	sulphidic	56.24	5974.2	5552.7	421.5	39.4	1.51
FFA B2	2.0-3.0	non-sulphidic	0.19	240.8	220.7	20.1	0.1	0.12
FFA B2	3.0-4.0	non-sulphidic	0.37	215.4	189.2	26.3	-0.1	0.12
FFA B2	4.0-5.0	non-sulphidic	4.34	540.2	508.0	32.2	1.0	0.07
FFA B2	8.0-9.0	transition zone	10.53	1638.2	1515.5	122.7	3.5	8.65
FFA B2	9.0-10.0	transition zone	12.68	610.7	502.0	108.7	2.2	8.65
FFA B4	7.0-8.0	sulphidic	20.16	603.6	450.2	153.4	9.6	2.76
FFA B4	8.0-9.0	sulphidic	34.09	1289.5	1038.9	250.7	22.0	2.28
FFA B6	2.0-3.0	non-sulphidic	2.64	687.0	648.9	39.1	0.3	0.06
FFA B6	3.0-4.0	non-sulphidic	1.46	1017.4	976.5	40.9	0.1	0.06
FFA N10	4.5-5.0	transition zone	8.69	1239.0	1204.1	34.8	1.5	12.89
FFA N10	5.0-5.5	transition zone	8.75	721.6	687.1	34.5	2.1	12.89
FFA N10	5.5-6.0	transition zone	7.82	674.6	640.3	34.3	5.2	12.89
FFA N10	7.7-8.3	transition zone	15.04	329.5	290.0	39.5	1.5	23.19
FFA N10	8.3-8.6	transition zone	15.17	331.5	298.7	32.9	6.9	23.19
FFA N10	10.0-10.4	sulphidic	17.45	320.6	289.3	31.3	5.4	-
FFA N10	10.4-10.7	sulphidic	50.07	5571.6	5247.7	323.9	9.4	-
FFA N10	12.0-13.0	sulphidic	52.84	2771.3	2381.7	389.6	37.9	-
FFA N10	13.0-14.0	sulphidic	38.04	2134.1	1723.3	410.8	18.2	-
FFA N10	16.0-17.0	sulphidic	46.65	2744.7	2431.5	313.2	23.6	-
FFA N10	17.0-18.0	sulphidic	46.55	2642.7	2335.0	307.8	36.8	-
GKA	8.0-9.0	non-sulphidic	0.63	132.6	95.0	37.6	0.9	0.00
GKA	9.0-10.0	non-sulphidic	0.34	97.1	70.7	26.4	0.4	0.00
GKA	22.0-23.0	non-sulphidic	1.57	193.3	164.2	29.1	0.2	0.00
GKA	23.0-24.0	non-sulphidic	2.83	204.5	179.2	25.3	-0.0	0.00
GKA	25.9-27.0	sulphidic	15.63	2857.4	2381.0	476.4	1.2	1.23
GKA	27.0-28.3	sulphidic	41.82	6634.0	5943.2	690.8	8.3	1.23
GKA	28.3-29.3	sulphidic	37.82	4495.6	3878.5	617.2	13.8	4.43
GKA	29.3-30.3	sulphidic	35.49	4766.8	4236.0	530.8	8.1	4.43
GKA	30.3-31.2	sulphidic	6.54	1086.9	731.4	355.4	3.8	0.50
GKA	31.3-32.0	sulphidic	4.09	1122.4	777.7	344.7	5.0	0.50
GKA	32.9-33.7	sulphidic	7.28	1206.0	765.6	440.4	10.2	0.50
GKA	33.7-34.7	sulphidic	12.25	1057.4	700.9	356.6	17.7	2.00
GKA	35.7-36.7	sulphidic	52.46	8861.3	8366.7	494.6	30.0	6.19
GKA	36.7-37.7	sulphidic	11.07	689.6	216.7	472.8	9.2	6.19
GKA	37.7-38.7	sulphidic	12.06	1347.7	1083.1	264.7	4.6	6.19
GKA	65.1-65.4	sulphidic	13.22	1441.2	941.3	499.9	1.3	2.27
GKA	67.1-67.5	non-sulphidic	8.18	471.0	333.8	137.2	1.3	2.27
GKA	67.5-68.0	non-sulphidic	8.11	487.1	351.5	135.6	0.7	2.27

FFA Fuhrberger Feld aquifer; GKA Großenkneten aquifer; [a] sediment characteristic; [b] cumulative denitrification after one year of incubation; [c] stock of reactive compounds (SRC); [d] fraction of organic carbon in the SRC; [e] fraction of total-S in the SRC; [f] sulphate formation capacity (SFC).

Table S2. Lambda values of the Box-Cox transformed D_r(in situ) and variables measured during anaerobic incubation.

Data set	Lamda values		
	D_r(in situ)	D_{cum}(365)	SRC
Whole data set	0.216	0.303	-0.024
FFA	0.214	0.369	-0.185
GKA	0.257	0.236	0.039
non-sulphidic zone	0.041	0.122	1.493
Sulphidic zone	0.190	0.260	0.229
transition zone	-0.150	-0.029	-0.159
NO_3^--bearing	0.099	0.337	0.797
NO_3^--free	0.319	0.670	0.492

Table S3. Simple regressions between D_r(in situ) and individual sediment parameters from aquifer parallels. $f^{B\text{-}C}(X) = A + B \times f^{B\text{-}C}(D_r(\text{in situ}))$. For each sub data set the two sediment parameters with the best correlation coefficient with D_r(in situ) are listed.

Data set	X[a]	N[b]	A	B	R[c]	R^2
Whole data set	SO_4^{2-}	29	3.697	-0.564	0.58	0.33
Whole data set	C_{org}	34	5.516	0.134	0.40	0.16
FFA	C_{hws}	14	19.74	1.754	0.75	0.56
FFA	SO_4^{2-}	11	3.263	-0.472	0.72	0.52
GKA	total-S	18	92.88	17.51	0.75	0.56
GKA	C_{org}	18	5.612	0.324	0.69	0.48
non-sulphidic	total-S	11	5.128	0.150	0.62	0.38
non-sulphidic	C_{org}	11	680.1	51.58	0.42	0.18
sulphidic	total-S	23	543.2	-109.7	0.69	0.48
sulphidic	SO_4^{2-}	18	3.540	-0.614	0.49	0.24
transition zone	total-S	8	0.608	-0.001	0.60	0.36
transition zone	C_{org}	8	5.341	-0.601	0.73	0.53
NO_3^--bearing	C_{org}	17	151.0	12.75	0.55	0.30
NO_3^--bearing	SO_4^{2-}	14	5.612	-0.501	0.53	0.28
NO_3^--free	SO_4^{2-}	15	3.085	-0.844	0.51	0.26
NO_3^--free	C_l	14	34.51	5.418	0.29	0.08

[a] Independent sediment parameter; [b] Sample number; [c] Correlation coefficient; SO_4^{2-} extractable sulphate-S; C_{hws} hot-water soluble organic carbon; C_l $KMnO_4$ labile organic carbon; C_{org} total organic carbon; total-S total sulphur.

Table S4. Lambda values of the Box-Cox transformed sediment parameters.

Data set	Lamda values								
	$D_r(7)$	$D_{cum}(365)$	D_r(in situ)	C_{org}	total-S	$SO_4^{2-}{}_{extr}$	DOC_{extr}	C_{hws}	C_l
Whole data set	0.487	0.303	0.216	-0.050	0.132	0.457	0.946	0.825	0.199
FFA	0.583	0.369	0.214	-0.191	-0.292	0.254	-	0.915	0.513
GKA	0.445	0.236	0.257	-0.052	0.685	0.628	-1.307	-0.203	0.291
non-sulphidic	-0.168	0.122	0.041	1.060	0.062	1.161	-	1.434	0.183
sulphidic	0.375	0.260	0.190	0.162	0.965	0.368	-1.931	1.314	-0.081
transition zone	0.397	-0.029	-0.150	-0.158	-1.649	0.642	-0.012	0.783	-0.834
NO_3^--bearing	0.121	0.337	0.099	0.752	-0.228	0.679	-	2.949	0.492
NO_3^--free	0.364	0.670	0.319	0.378	1.998	0.297	-3.158	0.970	0.452

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Table S5. Lambda values of the Box-Cox transformed variables.

Data set	Lamda values				
	SRC	SRC_C	SRC_S	aF_{SRC}	SFC
Whole data set	-0.024	-0.050	0.132	0.155	0.176
FFA	-0.185	-0.191	-0.291	0.326	0.187
GKA	0.039	-0.052	0.685	-0.139	0.193
non-sulphidic	1.493	1.043	-0.054	0.095	-0.014
sulphidic	0.229	0.159	0.941	-0.313	0.117
transition zone	-0.159	-0.158	-1.650	-0.089	-0.152
NO_3^--bearing	0.797	0.745	-0.307	0.069	0.120
NO_3^--free	0.492	0.375	1.914	-0.266	0.344

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