Biogeosciences Discuss., 7, 503–543, 2010
www.biogeosciences-discuss.net/7/503/2010/

Biogeosciences Discussions

This discussion paper is/has been under review for the journal Biogeosciences (BG).
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Kinetics of N_2O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments

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Received: 3 December 2009 – Accepted: 27 December 2009 – Published: 20 January 2010

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

Abstract

Knowledge of the kinetics of N_2O production and reduction in groundwater is essential for the assessment of potential indirect emissions of the greenhouse gas. In this study, we investigated this kinetics using a laboratory approach. The results were compared to field measurements in order to examine their transferability to the in situ conditions. The study site was the unconfined, predominantly sandy Fuhrberger Feld aquifer in Northern Germany. A special characteristic of the aquifer is the occurrence of the vertically separated process zones of heterotrophic denitrification in the surface groundwater and of autotrophic denitrification in the deeper groundwater, respectively. The kinetics of N_2O production and reduction in both process zones was studied during long-term anaerobic laboratory incubations of aquifer slurries using the ^{15}N tracer technique. We measured N_2O, N_2 and NO_3^- concentrations as well as parameters of the aquifer material that were related to the relevant electron donors, i.e. organic carbon and sulfur. The anaerobic incubations showed a low denitrification activity of heterotrophic denitrification with initial rates between 0.0002 and 0.0133 mg N kg^{-1} day^{-1}. The process was carbon limited due to the poor availability of its electron donor. In the autotrophic denitrification zone, initial denitrification rates were considerably higher, ranging between 0.0303 and 0.1480 mg N kg^{-1} d^{-1} and NO_3^- as well as N_2O were completely removed within 60 to 198 days. N_2O accumulated during heterotrophic and autotrophic denitrification, but maximum concentrations were substantially higher during the autotrophic process. The results revealed a satisfactory transferability of the laboratory incubations to the field scale for autotrophic denitrification, whereas the heterotrophic process less reflected the field conditions due to considerably lower N_2O accumulation during laboratory incubation. Finally, we applied a conventional model using first-order-kinetics to determine the reaction rates of the NO_3^--to-N_2O step and the N_2O-to-N_2 step, and evaluated the reaction rate constants for both steps. The model yielded fits to the experimental data that were of limited goodness, indicating that a more sophisticated approach is essential to describe the investigated reaction

kinetics satisfactorily.

1 Introduction

The atmospheric concentration of nitrous oxide (N_2O), a trace gas contributing to global warming and to the depletion of stratospheric ozone, has increased substantially since preindustrial times and continues to do so (IPCC, 2006). Agricultural ecosystems are considered to be a significant source of N_2O emissions due to the prevalent application of mineral and organic fertilisers (Mosier et al., 1998). In aquifers of these ecosystems, elevated N_2O concentrations of up to more than three orders of magnitude above the concentration in water equilibrated air were found in the surface groundwater (Spalding and Parrott, 1994; Well et al., 2005a; von der Heide et al., 2008). Thus, N_2O in groundwater was assumed to be a potential source contributing to atmospheric N_2O emissions (Rice and Rogers, 1993; Mosier et al., 1998; Hefting et al., 2003). Despite numerous recent studies on N_2O emissions originating from groundwater and agricultural drainage water (Groffman et al., 1998; Heincke and Kaupenjohann, 1999; Hiscock et al., 2003; Reay et al., 2003; Weymann et al., 2008), the significance of these indirect emissions is still uncertain. By and large, this could be attributed to two crucial subjects: firstly, N_2O accumulation in groundwater is complexly controlled. N_2O is an intermediate product of denitrification, the major process yielding to the occurrence of N_2O in oxygen depleted groundwater. Thus, N_2O emissions are a net result of the balance between simultaneously running N_2O production and reduction to N_2. This balance is permanently influenced by different enzyme kinetics of various denitrifying communities according to a number of regulating factors. The complex reaction kinetics may lead to a high variability of N_2O concentrations in groundwater (von der Heide et al., 2008) and to wide ranges of groundwater N_2O emission factors (Hack and Kaupenjohann, 2002; Weymann et al., 2008). Secondly, it is a challenge to combine research on the reaction kinetics of N_2O with transport parameters. Clough et al. (2005) stated that the movement and the ultimate fate of N_2O in subsurface environments are still poorly

understood. For example, knowledge of the consumption of N_2O in groundwater is scarce (Clough et al., 2007). Moreover, the fate of groundwater-derived N_2O passing the unsaturated zone has not been succesfully investigated (Weymann et al., 2009).

Denitrification has been frequently investigated during laboratory incubation studies using the ^{15}N tracer or the acetylene blockage technique, mainly to determine the denitrification capacity of soils and aquifer sediments (Smith and Duff, 1988; Ambus and Lowrance, 1991; Paramasivam et al., 1999; Well et al., 2005b). However, laboratory experiments to study the occurrence of N_2O and its reaction kinetics in groundwater are comparatively rare. Obenhuber and Lowrance (1991) observed NO_3^- removal and an accumulation of N_2O in flow-through microcosms within a period of 302 days, especially in the treatments with glucose amendment. Jacinthe et al. (1998) designed a similar experiment with two types of aquifer material over 132 days. The authors reported that heterogeneously distributed "patches" of organic matter induced denitrification in the poorly drained aquifer material, whereas the second type of aquifer material – without these patches – showed no denitrification activity. Furthermore, added dissolved organic carbon (DOC) was obviously not an electron donor for the reduction of NO_3^-. N_2O production rates of the poorly drained aquifer material were highest between days 20 and 30 in the NO_3^- amended treatments and substantially higher than the production rates of N_2. Blicher-Mathiesen and Hoffmann (1999) conducted an experiment with continuously permeated columns as well as static incubations. In both cases, they observed considerable NO_3^- removal and net N_2O production, but they also questioned the transferability of these results to parallel investigated field conditions which did not exhibit N_2O accumulation due to an efficient reduction of N_2O to N_2. Differences in net N_2O production between field and laboratory studies were also observed and discussed by Well et al. (2003). By comparing the N_2O fractions of total denitrification, the laboratory incubation yielded substantially higher values than the field study. Thus, this result confirms the observation of Blicher-Mathiesen and Hoffmann (1999). In contrast, other studies reported a good agreement of laboratory experiments and field methods related to the occurrence of N_2O (Obenhuber and Lowrance, 1991; Hénault et al.,

2001). As becomes clear at this point, it is uncertain whether laboratory investigations of the kinetics of N_2O production and reduction are applicable to field conditions.

In this study, we investigated the kinetics of N_2O production and reduction in an unconsolidated sandy aquifer in Northern Germany. This aquifer consists of vertically separated denitrification zones according to the availibility of electron donors, i.e. organic carbon and reduced sulfur (von der Heide et al., 2008). This provides the opportunity to investigate not only the kinetics of N_2O production and reduction during heterotrophic denitrification as was done in previous studies, but also during the autotrophic pathway.

The specific objectives of this study are (i) to determine the time courses of NO_3^-, N_2O and N_2 during long-term laboratory incubation of aquifer material samples, (ii) to evaluate kinetic rate constants of N_2O production and reduction during heterotrophic and autotrophic denitrification using a conventional k_1-k_2-model that follows first-order-kinetics and (iii) to assess the validity of the laboratory experiments for the relevant in situ processes.

2 Materials and methods

2.1 Study site

The Fuhrberger Feld aquifer (FFA) in Northern Germany is located about 30 km northeast of the city of Hannover. The unconfined aquifer consists of pleistocene, highly permeable carbonate-free sands and gravels with a thickness of 20–40 m underlain by impermeable cretaceous clays. More information about the soils, the hydrology and the land use of the research site is given by Frind et al. (1990), Deurer et al. (2008) and von der Heide et al. (2008). The FFA has been a subject of extensive research activities since the 1980s (reviewed in Korom, 1992), since the catchment is in an area of conflict between its key function for drinking water supply on the one hand and agricultural activities causing considerable inputs of pollutants via seepage, especially of nitrate, on

the other (Kölle et al., 1985; Frind et al., 1990). In the FFA, substantial microbially mediated processes and reactions like denitrification and desulfurication occur, strongly influencing groundwater geochemistry. Autotrophic denitrification with reduced sulfur compounds as an electron donor was identified as the dominant microbial reaction for NO_3^- elimination in the deeper aquifer (Kölle et al., 1985) in depths beyond 2–3 m below the groundwater table (Böttcher et al., 1992). The process was stoichiometricly described by Kölle et al. (1985) and Böttcher et al. (1990) as a reaction mediated by the bacteria *Thiobacillus denitrificans*:

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \quad (1)$$

Kölle et al. (1985) conducted an incubation experiment in order to evaluate the sulfate formation capacity of nitrate amended aquifer slurries from different depths. They found an ongoing sulfate formation during a 284-days-period and calculated schematically the potential of autotrophic denitrification on the basis of pyrite oxidation.

In the case of the surface groundwater, von der Heide et al. (2008) confirmed former assumptions that heterotrophic denitrification with organic carbon as an electron donor replaced autotrophic denitrification due to an exhaustion of the reduced sulfur compounds (Kölle et al., 1983; Böttcher et al., 1991):

$$5C + 4NO_3^- + 2H_2O \rightarrow 2N_2 + 4HCO_3^- + CO_2 \quad (2)$$

Autotrophic denitrification in the deeper aquifer is much more efficient for NO_3^- reduction than heterotrophic denitrification in the surface groundwater. With respect to denitrification efficiency, Weymann et al. (2008) revealed the considerable difference between heterotrophic and autotrophic denitrification by determination of "excess nitrogen" in groundwater samples. Hence, high NO_3^- concentrations are limited to the top few metres of the aquifer, but the deeper groundwater is almost NO_3^--free (Frind et al., 1990; von der Heide et al., 2009).

Recently, research activities in the FFA focused on the occurrence of N_2O in the groundwater. Deurer et al. (2008) investigated the accumulation and dynamics of N_2O

near the groundwater table and its transfer into the unsaturated zone from an exchange zone extending 0.55±0.22 m below the groundwater table. They reported that this zone may also act as a sink for N_2O. An extremely high spatial variability of N_2O concentrations in the surface groundwater of the FFA was postulated by von der Heide et al. (2008). The authors identified the land use and the distance of the groundwater level to the soil surface as factors governing the magnitude of N_2O concentrations in the surface groundwater. Weymann et al. (2008) determined groundwater N_2O emission factors with respect to initial NO_3^- concentrations and assessed these factors related to N_2O accumulation during different stages of the denitrification progress. All recent studies were conducted within a groundwater flowpath strip equipped with multilevel sampling wells (Deurer et al., 2008). In this study, we investigated the groundwater and the aquifer material of the multilevel sampling wells B1 and I1 (von der Heide et al., 2009). The main characteristics of the aquifer material are shown in Table 1.

2.2 Sampling procedures

Groundwater was collected from the multilevel sampling wells (Böttcher et al., 1985) in order to measure the denitrification related parameters N_2O, NO_3^- and SO_4^{2-}. The groundwater samples were collected in September 2005, December 2005 and March 2006 from the multilevel sampling well B1 using a peristaltic pump (Masterflex, COLE-PARMER, Vernon Hills, USA) as described in detail by Weymann et al. (2008). At the multilevel sampling well I1, a single sampling event was conducted in March 2006. Here, we collected the groundwater for N_2O, NO_3^- and SO_4^{2-} analysis with a plastic syringe, applying the method introduced by Deurer et al. (2008). At both wells, the depth resolution was 0.2 m in the surface groundwater (0.1 m–2.1 m below the groundwater table) and 1.0 m in the deeper groundwater down to a depth of 10 m below the soil surface.

Aquifer material was collected at the well B1 and at the plot appendant to well I1 for laboratory incubations to derive the parameters of the N_2O reaction kinetics. This was done using a hand-operated bailer boring auger set (EIJKELKAMP, Giesbeek,

The Netherlands) consisting of a stainless steel bailer, casing tubes (OD of 10 cm) and a tube clamp. At the multilevel sampling well B1, we collected aquifer material in October 2005 from three depth intervals in the zone of heterotrophic denitrification: 2.0–2.6 m, 2.6–3.0 m and 3.4–4.0 m below the soil surface. At the plot of the multilevel sampling well I1, the aquifer material was sampled at three spots that were spatially arranged as described by von der Heide et al. (2008). Sampling took place in October 2005 from the depth intervals 1.5 m–2.0 m, 2.0 m–2.5 m and 2.5 m–3.0 m below the soil surface (heterotrophic denitrification zone). To sample the autotrophic zone, a PVC pipe (OD of 100 mm) was installed at 6.5 m depth at one spot very close to the well using a drilling rig (WELLCO-DRILL, WD 500, Beedenbostel, Germany) with a hollow-stem auger (OD of 205 mm, ID of 106 mm). Samples were collected using the bailer. During sampling, the bottom part of the PVC pipe was continuously refilled with surrounding aquifer material. Samples thus originated from an undefined area in the vicinity of the pipe bottom. Hence, we were able to collect samples differing in texture and chemical composition from a single spot. The sampling of the autotrophic zone was conducted in December 2005.

The collected aquifer material was transferred from the bailer to 16 L plastic buckets. We filled the buckets until the supernatant groundwater overflowed. Subsequently, the buckets were closed airtight with a lid. From the heterotrophic denitrification zone, we filled one bucket per depth interval. From the autotrophic denitrification zone, 11 buckets were collected from the same depth interval. The aquifer material was stored at groundwater temperature (10 °C) and batched for laboratory incubations within four weeks.

2.3 Laboratory incubations

We performed a laboratory method using the ^{15}N tracer technique that reaches back to the seminal study of Nõmmik (1956) who quantified the gaseous denitrification products from soils receiving $K^{15}NO_3$ by mass spectrometry. The approach of anaerobic incubation of NO_3^- amended slurries has been extensively used for measuring denitri-

fication and N_2O production (Tiedje, 1994; Hénault et al., 2001; Well et al., 2003; Well et al., 2005a). In detail, 500 g of each aquifer material were transferred as slurries in 4 replications to 1125-mL transfusion bottles and amended with 400 mL of a $K^{15}NO_3$ test solution (10 mg N L^{-1}; 60 atom% ^{15}N). The transfusion bottles were sealed with rubber septa and aluminium screw caps. The gravimetric water content of the slurries was 0.19 g g^{-1}, resulting in a dry weight of 405 g. The volume of the solid matter was 153 mL, assuming a particle density of 2.65 g cm^{-3}. Taking the water content of the slurries into account, we determined the liquid volume in the bottles as 495 mL. Consequently, the headspace volume was 477 mL. We established anaerobic conditions by three cycles of evacuation and refilling with N_2, respectively. Subsequently, the samples were incubated at 10 °C, which is the approximate groundwater temperature as estimated from the mean annual air temperature. Gas and water samples were collected following a flexible sampling schedule according to the progress of denitrification. Prior to each sampling, the liquid and the gas phase were equilibrated by vigorous shaking for 3 h. 24 mL of the headspace gas were sampled using a double syringe system consisting of two 30-mL plastic syringes equipped with 3-way Luer-lock stop cocks (BRAUN, Melsungen, Germany) which were connected to each other. After mixing the gas sample within the syringe system, 12 mL from each of the separate syringes were transferred into fully evacuated Exetainers™ (LABCO, High Wycombe, UK). One Exetainer™ was stored for the measurement of N_2O by gas chromatography, the other for the $^{15}(N_2O+N_2)$ analysis by mass spectrometry and both were analysed within 3 weeks. To retain normal pressure in the serum bottles, we re-injected an equivalent volume of pure N_2 after sampling. The resulting dilution of the headspace gas was taken into account in the calculation of the $^{15}(N_2O+N_2)$ concentrations. Water samples were collected with a syringe. Routinely, we withdrew an 15-mL aliquot for NO_3^- analysis. Subsequently, an equivalent amout of the oxygen-free $K^{15}NO_3$ test solution was re-injected. The NO_3^- concentration of the test solution was adjusted according the actual NO_3^- concentration.

2.4 Analytical techniques

The particle size distribution was determined gravimetrically after separating the fractions by sieving and sedimentation following the Atterberg-method (Schlichting et al., 1995). Total organic carbon (C_{org}) and total N of the pulverised and carbonate-free aquifer material was measured using the elemental analyser vario MAX CN (ELEMENTAR ANALYSENSYSTEME GmbH, Hanau, Germany) equipped with a thermal conductivity detector. The precision of the analysis was 0.5%. Sulfur in the identical samples was analysed with a vario EL III elemental analyser (ELEMENTAR ANALYSENSYSTEME GmbH, Hanau, Germany) equipped with a thermal conductivity detector and an UV-absorption photometer. The precision of the analysis was 0.1%. DOC in cold-water extracts and hot-water soluble organic carbon (C_{hws}) were analysed as described by Well et al. (2005b). NO_3^- and SO_4^{2-} in the groundwater samples collected from the multilevel sampling wells were determined by ion chromatography (ICS-90, DIONEX, Idstein, Germany) with a precision of 5%. NO_3^- of the water samples from the laboratory incubations was analysed photometricly using a continuous flow analyser (Skalar, Erkelenz, Germany). The measurement precision was 5%.

N_2O was measured using a gas chromatographer equipped with an electron capture detector and an auto sampler that was described earlier (Well et al., 2003). The ^{15}N analysis of (N_2O+N_2) in the headspace gas was conducted following the method specified in Well et al. (1998, 2003). The gas concentrations of the sample solutions (dissolved N_2O and N_2) were calculated according to Henry's laws from the headspace concentrations using the Bunsen absorption coefficients of N_2O and N_2, respectively (Weiss, 1970; Weiss and Price, 1980). The calculation was described in detail by Well and Myrold (1999) and Well et al. (2003).

2.5 Reaction kinetics

First-order kinetics is frequently used to model processes in the field of groundwater biogeochemistry. For example, Böttcher et al. (1989) applied this kinetics to estimate

field denitrification rates in the FFA. In case of our laboratory approach, we consider a two-step reaction chain for N_2O production and N_2O reduction in order to characterise the heterotrophic and autotrophic denitrification process:

$$NO_3^- \xrightarrow{k_1} 1/2N_2O \xrightarrow{k_2} 1/2N_2 \quad (3)$$

Hoehener et al. (2003) presented an analytical solution following first-order kinetics. This k_1-k_2-standard model is described by the following differential equations for NO_3^- and N_2O, respectively:

$$\frac{dC_{NO_3}}{dt} = -k_1 \cdot C_{NO_3}, \quad (4)$$

$$\frac{dC_{N_2O}}{dt} = F \cdot k_1 \cdot C_{NO_3} - k_2 \cdot C_{N_2O} \quad (5)$$

The analytical solutions are:

$$C_{NO_3}(t) = C_0 \cdot \exp(-k_1 \cdot t), \quad (6)$$

$$(k_1 \neq k_2): \quad C_{N_2O}(t) = F \cdot C_0 \cdot \frac{k_1}{(k_2 - k_1)} \cdot [\exp(-k_1 \cdot t) - \exp(-k_2 \cdot t)], \quad (7)$$

$$(k_1 = k_2): \quad C_{N_2O}(t) = F \cdot C_0 \cdot k_1 \cdot t \cdot \exp(-k_1 \cdot t), \quad (8)$$

where F is the stoichiometric factor and C_0 is the initial nitrate concentration. We note that the sum of N_2 and N_2O is only a function of k_1 and the analytical solution follows by mass balance considerations:

$$C_{sum}(t) = C_{N_2}(t) + C_{N_2O}(t) = F \cdot (C_0 - C_{NO_3}(t)). \quad (9)$$

A Marquardt-Levenberg fit was conducted to all heterotrophic and autotrophic data sets, where the analytical solutions are used as fitting function. All calculations were carried out with the mathematical software Mathematica 6.0. For each data set three

different fits were conducted: (i) a 1-step 3-parameter fit, (ii) a sequential (or 2-step) 3-parameter fit, and (iii) a sequential 2-parameter fit. These fits are indicated in Figs. 1–3. The fitting parameters for the 3-parameter fits were C_0, k_1 and k_2, respectively.

To further evaluate the control of NO_3^- reduction by denitrification we also used a simpler approach which did not include the distinction between N_2O production and reduction and was based on zero-order-kinetics. Reaction rates (D) were derived from the slope of (N_2O+N_2) over time in order to correlate denitrification with the independent parameters of the aquifer material. Initial values of D (D_i) were obtained from the first 7 days of incubation. Maximum values of D (D_{max}) were calculated from the maximum slopes of the (N_2O+N_2)-curve. Finally, we used the maximum N_2O concentration during incubation (cN_2O_{max}) and the ratio between N_2O and (N_2O+N_2) at maximum N_2O concentration (cN_2O_{max}-to-[N_2O+N_2]) as qualitative indicators for the balance between production and reduction of N_2O.

3 Results

3.1 Multilevel well measurements

At the investigated wells, each of the vertical concentration gradients of NO_3^- and N_2O showed a similar pattern. In the surface groundwater, NO_3^- concentrations initially increased downwards in both profiles to a mean value of 34 mg N L^{-1} in a depth of 3.8 m below the soil surface at B1 and to 30 mg N L^{-1} in a depth of 3.2 m below the soil surface at I1, respectively (Fig. 1). Below 4 m, where the autotrophic denitrification mainly governs NO_3^- reduction, NO_3^- concentrations decreased continuously and reached zero in a depth of 7 m at both wells. In the case of N_2O, we identified two layers where the concentrations were highest: first, there is a zone of N_2O accumulation in the uppermost groundwater coinciding with an "exchange zone" that was recently reported by Deurer et al. (2008). We observed N_2O concentrations up to 1.84 mg N L^{-1} in a depth of 2.0 m below the soil surface at B1 and 1.63 mg N L^{-1} in a depth of 1.6 m below the

soil surface (0.54 m below the groundwater table) at I1. Second, Fig. 1 shows a sharp-cut concentration peak in both profiles, consisting of an outstanding value in 5 m and 6 m depth, respectively. Between these layers, N_2O concentrations in the groundwater were substantially lower at both wells, but still up to three orders of magnitude higher than the N_2O concentration in water equilibrated air. In the deeper groundwater, N_2O concentrations declined rapidly after the sharp-cut peak and were undetectable in 6 m at B1 and 7 m at I1, respectively. In contrast to the vertical concentration gradients of NO_3^- and N_2O, the SO_4^{2-} concentration pattern was different at the investigated wells. At I1, we observed an abrupt increase from 67 mg L^{-1} in a depth of 5 m to 113 mg L^{-1} in a depth of 6 m coinciding with the concentration peak of N_2O. Furthermore, the SO_4^{2-} concentrations remained elevated in the deeper groundwater compared to the surface groundwater. At B1, these phenomena did not occur during all sampling events (further details will be given in the discussion section).

3.2 Denitrification rates and time courses of the N-species during long-term laboratory incubation

The concentration courses of N_2O, of the total denitrification products (N_2O+N_2) and of NO_3^- are represented in Figs. 2 and 3, respectively. Whereas Fig. 2 refers to heterotrophic denitrification in the surface groundwater, Fig. 3 shows the results for the autotrophic case that is dominant in the deeper groundwater. N_2O and (N_2O+N_2) were detectable in all samples proving the general occurrence of denitrification. However, there were substantial differences in denitrification activity and the kinetics of N_2O production and reduction between heterotrophic and autotrophic denitrification, and also within these two groups. Calculated rates of autotrophic denitrification (D_i, D_{max}, Table 2), symbolised by the slopes of the (N_2O+N_2) curves (Fig. 3), were typically one order of magnitude higher than the rates of heterotrophic denitrification. The coincidence of NO_3^- reduction and (N_2O+N_2) production indicate that the mass balance was satisfactory (Figs. 2 and 3). Consequently, NO_3^- concentrations decreased con-

tinuously until complete elimination of NO_3^- during autotrophic denitrification (Fig. 3), whereas the decrease of NO_3^- concentrations during heterotrophic denitrification was marginal and the residual NO_3^- pool was much greater than the reduced one (Fig. 2).

The balance of N_2O production and reduction yielded a characteristic course of the N_2O concentration curve as it was reported by Holtan-Hartwig et al. (2000) and Well et al. (2005a): the majority of samples showed an increase to a maximum concentration (cN_2O_{max}) followed by a decrease that resulted in complete N_2O reduction in the case of autotrophic denitrification. However, the N_2O concentration courses and cN_2O_{max} values were highly variable and the standard deviations partially indicate corresponding uncertainties.

In the heterotrophic denitrification zone, our sampling method enabled collection and laboratory incubation of slurries from different depth intervals (Table 1). The time courses of N_2O showed an increase of cN_2O_{max} with depth (Fig. 2, Table 2). Consequently, cN_2O_{max} was highest in 2.5–3.0 m at I1-S1, in 2.5–3.0 m at I1-S3 and in 3.4–4.0 m at B1 with 0.08, 0.08 and 0.06 mg N_2O-N kg^{-1}, respectively. In contrast, cN_2O_{max} was lowest in the topmost depth intervals where it ranged between 0.0024 and 0.023 N_2O-N kg^{-1}. The results showed that N_2O concentrations were close to the cN_2O_{max} values for a period >100 days in the majority of cases and decreased slowly towards the end of the incubation period.

Despite the slurries from the autotrophic denitrification zone were collected from the same depth interval, these samples exhibited not only a large variation of N_2O concentrations during anaerobic incubation, but also distinct differences in organic carbon, total sulfur and texture. This demonstrates that the aquifer material obtained by our sampling procedure exhibited heterogeneous properties. For example, sample I1-6, the sample with the highest content of organic carbon and clay in the data-set (Table 1), did not show considerable accumulation of N_2O during the entire experiment and exhibited the lowest cN_2O_{max}-to-(N_2O+N_2) ratio (Fig. 3, Table 2). Furthermore, this sample showed by far the highest D_i. cN_2O_{max} of the sample I1-10 was 0.00773 N_2O-N kg^{-1} and thus comparable with the highest cN_2O_{max} values we observed in the sam-

ples of the heterotrophic denitrification zone. Apart from these two samples, all the other ones were characterised by considerably higher cN_2O_{max} values (Fig. 3, Table 2) between 0.24 mg N_2O-N kg^{-1} (sample I1-5) and 1.70 mg N_2O-N kg^{-1} (sample I1-4).

3.3 Correlations

We conducted Spearman rank tests for the partial data-sets of the heterotrophic and the autotrophic zone in order to evaluate correlations between the parameters that were introduced in Tables 1 and 2, respectively.

The correlation coefficients (R_S) for the relationships between cN_2O_{max}, the cN_2O_{max}-to-(N_2O+N_2) ratio, D_i, D_{max} and the independent soil properties are shown in Table 3. In the case of heterotrophic denitrification, a significant correlation at the 0.05 probability level was found between organic carbon and D_i. The relationship between the water-extractable C-species (DOC, C_{hws}) and the denitrification rates (D_i, D_{max}) did not reveal a significant correlation. However, the correlation coefficient for the relationship between D_i and C_{hws} was comparatively high (R_S=0.55). In contrast, DOC was negatively correlated with cN_2O_{max} at the 0.01 probability level. In the case of autotrophic denitrification, the denitrification rates (D_i, D_{max}) were found to be significantly correlated with the potential reductant sulfur, but also with organic carbon. Organic carbon was highly correlated with the clay content, but not with sulfur. DOC and C_{hws} did not correlate with D_i and D_{max}, respectively. Furthermore, we found no significant relations between cN_2O_{max} and the other parameters of the "autotrophic" data set.

3.4 Kinetic rate constants of N_2O production and reduction

All data sets with the calculated rate constants and the corresponding fitting parameters are listed in Table 4 (1-step 3-parameter fit) and Table 5 (sequential 3-parameter fit). As expected from the time courses of the NO_3^- and the (N_2O+N_2) concentrations, the obtained rate constants were higher for autotrophic denitrification. The means for k_1

and k_2 showed a difference of about one order of magnitude, when the heterotrophic and the autotrophic process are compared (Table 4). The rate constants of autotrophic denitrification exhibited a larger variability (Tables 4 and 5). For example I1-6, the sample with the highest D_i and practically no N_2O accumulation, yielded an outstanding high value for k_2, indicating intensive N_2O reduction.

To analyse the fitting results, we chose the data set "I1-S1 2.0–2.5" as a representative example that is shown in Fig. 4. Since the time courses of N_2O concentration result from the competition between production (k_1) and reduction of N_2O (k_2), i.e. between nitrate reduction and di-nitrogen production, one would expect that the rate constants describe consistently both the time course of nitrate (Eq. 6) and the time course of (N_2O+N_2) (Eq. 9). As shown in Fig. 4b, the 1-step 3-parameter fit underestimated the total concentration of the gaseous species. This clearly demonstrates the necessity of an independent measurement in order to prove the rate constants obtained by the kinetics describing the N_2O-curve. We emphasize that fitting for best agreement of measured and modeled N_2O curves (compare 1-step 3-parameter fit in Fig. 4 with experimental data) did not yield a satisfactory agreement for the NO_3^- and (N_2O+N_2) curves, respectively.

In order to ensure that the cumulative curve of (N_2O+N_2) is reproduced reasonable well, we used a second fitting procedure, namely the sequential fit, i.e. in a first fitting step we determined k_1 by the cumulative curve of N_2O+N_2 and in a second step we determined k_2 by the N_2O-curve. As can be seen in Fig. 4b, we then obtained an excellent fit to the time course of (N_2O+N_2). However, the goodness of fit of the N_2O-data has been deteriorated (Fig. 4a), i.e. the early-time behaviour exhibits an increase that is too steep. Nevertheless, the profile in its entirety is still reasonably satisfactory.

As shown in Fig. 4c, both fitting approaches yielded a very low initial nitrate concentration which deviated considerably from the experimental data. The ratio of the theoretical and experimental initial concentration for the the sample I1-S1 2.0–2.5 is 0.02 and 0.03, respectively (Tables 4 and 5). In principle, one would use only the rate constants as fitting parameters, and vary the initial nitrate concentration. This was

used as the starting point. For the sequential 2-parameter fit, the best fits are shown as dashed curves in Fig. 4. The agreement both to the N_2O- and to the (N_2O+N_2) curves was insufficient, indicating that the constant C_0 (Eqs. 7–9) is not given by the initial nitrate concentration. This is also indicated by the magnitude of the deviation between the experimental data and the theoretical curve (Fig. 4c).

4 Discussion

4.1 Field measurements reveal the zones of denitrification and N_2O accumulation

The special characteristic of the studied aquifer is the occurrence of the vertically separated process zones of heterotrophic and autotrophic denitrification. The results of the laboratory incubations showed that these processes generate a different nitrate removal efficiency and thus reaction kinetics. This was also confirmed in a recent study of Weymann et al. (2008) in the FFA by determining excess N_2 dependent upon the depth. Whereas excess N_2 from denitrification was found to be low in the shallow groundwater, i.e. in the heterotrophic zone, the authors reported highest values for excess N_2 (predominantly between 10 and 15 mg N L^{-1}) in depths beyond 5 m below the soil surface, i.e. in the autotrophic zone. Against this background, the question arises to what extent the different nitrate removal efficiencies influence the accumulation of N_2O under field conditions. As the multilevel well measurements indicate, the different reaction kinetics of heterotrophic and autotrophic denitrification yielded a large range and a huge variability of N_2O concentrations in the investigated in-situ profiles at the wells B1 and I1 (Fig. 1). More precisely, we identified a zone of considerable N_2O accumulation close to the groundwater surface which has been already reported by Deurer et al. (2008). Elevated N_2O concentrations were also found up to 2 to 3 m below the water table. A previous study has shown that this layer probably equates with the zone of heterotrophic denitrification (von der Heide et al., 2008). As the laboratory incu-

bations indicate and Weymann et al. (2008) confirmed, this zone is characterised by low nitrate removal efficiency. The occurrence of N_2O accumulation or N_2O emission combined with low nitrate removal efficiency has also been described in other studies. Hefting et al. (2006) found significant N_2O emissions along a flowpath with low nitrate removal efficiency in a riparian buffer zone. Van Cleemput (1998) stated that conditions causing an inhibition of denitrification, i.e. causing a low nitrate removal efficiency, are favourable for N_2O accumulation. In this context, a key factor for heterotrophic denitrification is the availability of organic carbon. The sandy aquifer material of the FFA contains low amounts of organic carbon (Table 1) and the microbial bioavailability can be strongly assumed to be poor (Böttcher et al., 1991). Beside this, it is known that low pH and high NO_3^- levels favour N_2O accumulation due to inhibited N_2O reduction to N_2 (Blackmer and Bremner, 1978; van Cleemput, 1998; Šimek and Cooper, 2002). In case of the FFA, it has been previously assumed that the pH of <5.5 (Deurer et al., 2008) and the high NO_3^- concentrations (von der Heide et al., 2008) are further factors supporting the accumulation of N_2O in the surface groundwater of the FFA in addition to the low availability of organic organic carbon. Confirming the results of Hefting et al. (2006) and van Cleemput (1998), we can thus conclude that the combination of (i) the limited carbon (bio)availability, (ii) the low pH and (iii) high NO_3^- concentrations explains the low nitrate removal efficiency in the surface groundwater as well as the considerable N_2O accumulation.

As mentioned in Sect. 3.1, we observed a sharp-cut N_2O concentration peak in both profiles in the deeper groundwater (Fig. 1). Here, in depths of 5 m and 6 m, respectively, the autotrophic process governs the production and reduction of N_2O. In contrast to heterotrophic denitrification, the nitrate removal in the autotrophic process zone is much more intensive. This has been revealed by the results of the laboratory incubations and was shown previously (Frind et al., 1990; Weymann et al., 2008). Due to the low nitrate removal efficiency in the heterotrophic denitrification zone, the NO_3^- load of the groundwater was still high (concentrations between 11 and 23 mg N L^{-1}) when it came in contact with the reduced sulfur compounds of the deeper aquifer. Ac-

cordingly, N_2O was produced within an intensive nitrate removal caused by autotrophic denitrification. But, in contrast to the N_2O accumulation in the surface groundwater, we conclude that the sharp-cut N_2O concentration peak in both profiles is an indicator for rapid N_2O reduction which hampered an accumulation of N_2O in the sense of the heterotrophic denitrification zone. Finally, the high nitrate removal efficiency in the autotrophic denitrification zone resulted in a complete reduction of NO_3^- and N_2O in the deeper groundwater in depths below 5 m and 6 m, respectively. Thus, the deeper aquifer clearly functioned as a sink for N_2O. This is comparable with the findings of Blicher-Mathiesen and Hoffmann (1999) who reported an effective nitrate removal in a riparian fen without N_2O accumulation.

In summary, the vertical courses of NO_3^- and N_2O concentrations at the investigated wells plausibly reflect the occurrence of the separated denitrification zones in the aquifer. Taking the SO_4^{2-} concentrations into account (Fig. 1), this conception is only confirmed by the gradient of I1. At this well, the increase of the SO_4^{2-} concentrations reflect the considerable sulfate formation capacity of the autotrophic zone (Kölle et al., 1985). This was not observed at well B1. Low potassium concentrations (data not shown) indicate that the deeper groundwater at this well is charged with groundwater that originated from forest or pasture. This groundwater is characterised by significantly lower concentrations of SO_4^{2-}, N_2O and NO_3^- than groundwater under arable land (von der Heide et al., 2008). Hence, we assume that dilution attenuated the concentrations of the investigated parameters in the deeper groundwater at well B1.

4.2 Kinetics of N_2O production and reduction during long-term laboratory incubation

The results showed convincingly the substantial difference between the N_2O kinetics of heterotrophic and autotrophic denitrification. Among the factors governing denitrification, the initial NO_3^- concentration, O_2, and pH had been kept constant by our set-up of anaerobic incubation. Variation in process dynamics was thus mainly caused by

the differences in the electron donors, i.e. organic carbon and reduced sulfur and their microbial availability.

We attribute the low activity of heterotrophic denitrification to the limited supply of organic carbon due to its low content and poor microbial availability, respectively. This is supported by NO_3^- concentrations that remained close to initial concentrations during the incubation period, indicating that the electron acceptor was not a limiting factor for the process. Carbon limitation in sand and gravel aquifers was also demonstrated by Smith and Duff (1988), Obenhuber and Lowrance (1991) and Paramasivam et al. (1999). In fact, the organic carbon content in the samples of the heterotrophic zone was very low (Table 1) compared to the results of other incubation studies (Paramasivam et al., 1999; Well et al., 2005a). Besides the NO_3^- analyses, we regularly measured DOC concentrations in the "heterotrophic" water samples (data not shown). Initial concentrations were found to be between 6 and 25 mg C L^{-1} and were predominantly higher than the critical lower threshold of about 2–7 mg C L^{-1} that was reported to be necessary to promote denitrification (Spalding et al., 1978; Groffman et al., 1996). We did not observe significant DOC consumption within the whole incubation period in any sample of the heterotrophic zone. Furthermore, the correlation analysis yielded no significant relationships between extractable DOC and the denitrification rates (Table 3). Both findings indicate a poor bioavailability of DOC for denitrification in the heterotrophic zone, supporting the results of a previous field study in the FFA (Deurer et al., 2008) as well as the results of Jacinthe et al. (1998) and Siemens et al. (2003). However, von der Heide et al. (2010) reported significant negative correlations between DOC and N_2O concentrations in the surface groundwater of the FFA, a relationship that was also observed in this study (Table 3). The authors attributed this relationship to a promotion of N_2O accumulation by decreasing bioavailability of DOC. This would require that DOC functions as an electron donor for the NO_3^--to-N_2O step of denitrification, but to lesser extent for the N_2O-to-N_2 step. As our data supply no evidence to confirm or contradict this, further research into the effect of DOC on N_2O accumulation in groundwater is needed.

In contrast to heterotrophic denitrification, the autotrophic process was not limited by its electron donor reduced sulfur, but by its electron acceptor NO_3^-. The availability of reduced sulfur was sufficient to eliminate NO_3^- and N_2O completely in all samples. Hence, we stress that the laboratory incubations also confirm the role of autotrophic denitrification to function as a sink for NO_3^- and N_2O.

The aquifer material of the autotrophic zone was more variable in texture and organic carbon than the homogeneous sands of the heterotrophic zone (Table 1). The samples with the highest contents of organic carbon and clay, i.e. I1-5, I1-6 and I1-9, respectively, showed the highest denitrification activity (Fig. 3). These observations and the positive correlation between organic carbon and the "autotrophic" denitrification rates (Table 3) indicate that the kinetics of denitrification was apparently governed by these parameters. Against this background, the question arises whether heterotrophic denitrification also occurs in the deeper aquifer. On the one hand, lignitic pebbles which are nonuniformly distributed throughout the deeper aquifer (Frind et al., 1990), could function as "patchy" hot spots (Parkin, 1987; Jacinthe et al., 1998; Gold et al., 1998) providing organic carbon serving as the electron donor and probably causing the small scale spatial variability of denitrification activity and N_2O accumulation (von der Heide et al., 2010). This organic carbon is also used as an electron donor to reduce sulfate in the deeper groundwater of the FFA (Böttcher et al., 1989; Frind et al., 1990). Korom (1991) showed thermodynamically, that organic carbon used as an electron donor in the sulfate-reducing zone of the FFA would preferentially be used by bacteria for heterotrophic denitrification. On the other hand, Böttcher et al. (1991) stated that as long as reduced sulfur compounds are available in the FFA, simultaneous heterotrophic denitrification is unlikely for several reasons. For example, the authors emphasized that the microbial availability of the organic lignitic pebbles is probably poor and might superimpose the thermodynamic "advantage" of heterotrophic denitrification. However, this has not been proven until now. The reactivity of the lignitic pebbles and the question, to what extent a possible heterotrophic process in the deeper aquifer potentially contributes to total denitrification, remain subjects of uncertainty. Further investigations

into the deeper groundwater will be necessary to overcome this lack of knowledge.

Kinetic constants k_1 and k_2 of the first-order approach roughly reflected the different reaction rates of heterotrophic and autotrophic denitrification, as a comparison of their mean values revealed (Tables 4 and 5). The outstanding high k_2-value in the case of sample I1-6 reflects the fact that the balance between N_2O production and reduction was clearly at the reduction side, yielding negligible N_2O accumulation. Here, the rate constants also described the experimental data plausibly. However, in most cases the goodness of fit as given by R^2 of k_2 was not satisfactory. This can also be seen from the strong deviation between fitted and measured initial NO_3^- concentration (Tables 4 and 5). The initial nitrate concentration fitted by the 3-parameter fits (C_0, Tables 4 and 5) was much too low and not in agreement with the experimental data for the samples of heterotrophic denitrification (example in Fig. 4c). We assume that the exhaustion of available organic carbon is the reason for this deviation, because organic carbon was not taken into account by the model as a factor that limited the reaction. Instead, the first-order model assumed that process rates were controlled by the decreasing availability of NO_3^- which did not occur during our experiments due to the poor nitrate removing efficiency of heterotrophic denitrification. Furthermore, the predominantly linear time courses of NO_3^- and (N_2O+N_2) during autotrophic denitrification (Fig. 3) indicate that the reaction kinetics is rather described by a zero-order than by a first-order model. Pätsch (2006) and Konrad (2007) reported in agreement that both kinetics can occur in one aquifer. Therefore, using only one modeling approach may include uncertainties (Pätsch, 2006) and an improved model should be flexible enough to include both reaction types.

These considerations reveal that for an improved modeling approach (i) the electron donors have to be taken into account and (ii) zero-order and Michaelis-Menten kinetics should also be applied in order to describe production and reduction of N_2O more precisely.

4.3 Transferability of laboratory incubations to field conditions

Did the laboratory experiments and the respective kinetic constants reflect the process kinetics that are present in groundwater of the FFA? Generally, this question is subject to an ongoing controversy in groundwater literature, about whether or not batch experiments effectively describe field scale reactions (Dykaar and Kitanides, 1996; Ginn et al., 2002; McQuarrie and Sudicky, 2001). Kelly et al. (1996) showed by a comparison between derived kinetic parameters from batch experiments and column experiments that the derived kinetic constants deviated significantly. For example, column experiments yielded V_{max}-values for Benzene between 0.037 and 0.219 1/h, but a batch-experiment revealed a V_{max}-value that was 0.049 1/h. On the other hand, Schirmer et al. (2000) had shown that kinetics derived from batch experiments can describe an in-situ tracer test. As already mentioned in the introduction section, the question of transferability led also to conflicting statements related to denitrification and to the occurrence of N_2O (Hénault et al., 2001; Obenhuber and Lowrance, 1991; Blicher-Mathiesen and Hoffmann, 1999; Well et al., 2003). Thus, to find an unambiguous and general answer seems to be impossible. Rather, we should assess the question as the case arises. Taking the present results of this study into account, it becomes obvious that we have to distinguish between heterotrophic and autotrophic denitrification if the transferability of the laboratory incubations should be assessed.

The different denitrification capacities of the heterotrophic and autotrophic zones in the FFA were reflected by the incubation experiments. The anaerobic incubations showed only marginal nitrate removal efficiency in the heterotrophic zone. In contrast, the rapid nitrate removal related to autotrophic denitrification yielded a capacity that is about one order of magnitude higher. Both observations are in agreement with the field data (Fig. 1) and with a previous field study (Weymann et al., 2008). Hence, this finding confirms the results of Well et al. (2003) who also reported a satisfactory agreement of laboratory and in situ measurements of denitrification.

If we regard the occurrence of N_2O, the subject of transferability has to be con-

sidered more differentially. For the autotrophic zone that was investigated at well I1, the field measurements yielded a maximum N_2O concentration of 1.05 mg N L^{-1} at a depth of 6 m (Fig. 1) which equates to 0.26 mg N kg^{-1} assuming a pore volume of 40%. The median of the cN_2O_{max} values measured during laboratory incubation (Table 2) was 0.52 mg N kg^{-1}. This comparison shows that laboratory and field data were in one order of magnitude and thus in satisfactory agreement. Furthermore, laboratory and field investigations showed correspondingly that the autotrophic zone functions as a sink for N_2O if the NO_3^- pool is exhausted, since N_2O was completely consumed during the last stage of denitrification progress (Figs. 1 and 3). On the other hand, cN_2O_{max} measured during laboratory incubation of the heterotrophic aquifer material were considerably lower than the maximum N_2O concentrations we evaluated in the field. Whereas the median of the cN_2O_{max} values measured during laboratory incubation (Table 2) was 0.02 mg N kg^{-1}, the averaged maximum N_2O concentrations at B1 and I1 were 1.74 mg N L^{-1} (Fig. 1), which equates to 0.43 mg N kg^{-1}. This observation is in contrast to the findings of Well et al. (2003) who reported greater N_2O-fractions as a result of laboratory incubation in most of the investigated soils. Blicher-Mathiesen and Hoffmann (1999) also observed higher N_2O concentrations during their laboratory experiments due to a differing reduction pattern that supported N_2O accumulation. Another disagreement between laboratory and field data is exhibited by the increasing cN_2O_{max} values of N_2O with depth during laboratory incubation (Fig. 2), whereas the field data indicate the highest N_2O accumulation in the uppermost groundwater (Fig. 1; Deurer et al., 2008).

What are the reasons causing the poorer transferability of the "heterotrophic" incubations to the field scale related to the kinetics of N_2O production and recuction? One explanation could be that the aquifer slurries are subject to a certain disturbance for a short time according to the laboratory method, i.e. physical disruption and aerobic conditions during collection. This may alter the composition of the microbial communities. In fact, the influence of these processes seemed to be negligible in the case of autotrophic denitrification, because the laboratory incubations reflected the field data.

This might be explained by the high abundance of reduced sulfur that seems to be easily accessible to the autotrophic denitrifier *Thiobacillus denitrificans* (Böttcher et al., 1991) and by the fact that the electron donor was not sustainably altered by temporal contamination with atmospheric oxygen and physical disturbances during sampling. To the contrary, we assume that the small pool of available organic carbon in the heterotrophic zone might be sensitive to disturbances, e.g. by oxidation with atmospheric oxygen. This could lead to some loss of denitrification capacity and might explain the observed deviations in N_2O accumulation. Another reason for the discrepancy between laboratory and field-based studies was reported by Smith et al. (1996). The authors identified the differences between spatial and temporal scales as a reason for this discrepancy. Furthermore, sampling of a small amount of aquifer slurry for laboratory incubations may miss patches and hotspots of available organic carbon and heterotrophic denitrification activity (Jacinthe et al., 1998). Finally, in-situ N_2O accumulation in the heterotrophic zone is affected by the fluctuating groundwater level and day-scale infiltration events. These dynamics are not provided by static incubation experiments. To sum up, we note that the kinetics of N_2O production and reduction in the heterotrophic denitrification zone tends to be susceptible to effects connected with sampling and the static laboratory approach conducted at the microscale. In contrast, the autotrophic denitrification seems to be a more robust process. The availability of its uniformly distributed eletron donor induces high denitrification activity which hampers changes of in situ processes and reaction kinetics during laboratory investigations yielding a good agreement of field and laboratory results.

5 Conclusions

N_2O is produced in the surface groundwater of the FFA as an intermediate of heterotrophic denitrification as well as in the deeper groundwater due to autotrophic denitrification. The heterotrophic process is limited by the availability of the electron donor organic carbon yielding a low denitrification capacity. Field measurements indicated

considerable N_2O accumulation especially in the uppermost groundwater. In contrast, laboratory incubations of aquifer material showed substantially lower N_2O concentrations than measured in the field. Thus, the laboratory results are hardly transferable to the field scale. We conclude that the discrepancy is due to the susceptibility of the sensitive heterotrophic process to sampling activities and differences in spatial scales between field and laboratory conditions. The autotrophic process is characterised by a high denitrification capacity and not limited by its electron donor, reduced sulfur. Laboratory and field data were found to be in good agreement showing that the autotrophic zone functions as a sink for N_2O. The application of a conventional k_1-k_2-model following first-order-kinetics revealed rate constants that roughly confirmed the experimental data, i.e. for example the difference between the reaction rates of heterotrophic and autotrophic denitrification. However, the fitting results to the experimental time courses of the N-species were partly unsatisfactory. In conclusion, we note that a more sophisticated approach will be necessary to describe the kinetics of N_2O production and reduction succesfully.

Acknowledgement. Financial support from the German Research Foundation (DFG) is gratefully acknowledged. We thank I. Ostermeyer and K. Schmidt for their assistance in the laboratory and L. Szwec and R. Langel (Centre for Stable Isotope Research and Analysis, University of Göttingen) for the stable isotope analyses. Sampling would not have been possible without the help of G. Klump and the drilling crew from the Federal Institute for Geosciences and Natural Resources in Hannover. Finally, we thank J. Böttcher and W. Duijnisveld for fruitful discussions.

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This Open Access Publication is funded by the University of Göttingen.

References

Ambus, P. and Lowrance, R.: Comparison of denitrification in two riparian soils, Soil Sci. Soc. Am. J., 55, 994–997, 1991.

Blackmer, A. M. and Bremner, J. M.: Inhibitory effect of nitrate on reduction of nitrous oxide to molecular nitrogen by soil microorganisms, Soil Biol. Biochem., 10, 187–191, 1978.

Blicher-Mathiesen, G. and Hoffmann, C. C.: Denitrification as a sink for dissolved nitrous oxide in a freshwater riparian fen, J. Environ. Qual., 28, 257–262, 1999.

Böttcher, J., Strebel, O., and Duijnisveld, W. H. M.: Kinetik und Modellierung gekoppelter Stoffumsetzungen im Grundwasser eines Lockergesteins-Aquifers, Geol. Jahrb. Reihe C, 51, 3–40, 1989.

Böttcher, J., Strebel, O., Duijnisveld, W. H. M., and Frind, O.: Reply (to a comment of Scott F. Korom), Water Resour. Res., 27, 3275–3278, 1991.

Böttcher, J., Strebel, O., and Kölle, W.: Redox conditions and microbial sulfur reactions in the Fuhrberger Feld sandy aquifer, in: Progress in Hydrogeochemistry, edited by: Matthess, G., Frimmel, F. H., Hirsch, P., Schulz, H. D., and Usdowski, E., Springer, Berlin and Heidelberg, 219–226, 1992.

Böttcher, J., Strebel, O., Vörkelius, S., and Schmidt, H.: Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer, J. Hydrol., 114, 413–424, 1990.

Clough, T. J., Addy, K., Kellog, D. Q., Nowicki, B. L., Gold, A. J., and Groffman, P. M.: Dynamics of nitrous oxide in groundwater at the aquatic-terrestrial interface, Glob. Change Biol., 13, 1528–1537, 2007.

Clough, T. J., Sherlock, R. R., and Rolston, D. E.: A review of the movement and fate of N_2O in the subsoil, Nutr. Cycl. Agroecosys., 72, 3–11, 2005.

Deurer, M., von der Heide, C., Böttcher, J., Duijnisveld, W. H. M., Weymann, D., and Well, R.: The dynamics of N_2O in the surface groundwater and its transfer into the unsaturated zone: A case study from a sandy aquifer in Germany, Catena, 72, 362–373, 2008.

Dykaar, B. and Kitanides, P.: Macrotransport of biologically reacting solute through porous media, Water Resour. Res., 32, 302–320, 1996.

Frind, E., Duijnisveld, W. H. M., Strebel, O., and Böttcher, J.: Modeling of multicomponent transport with microbial transformation in groundwater: The Fuhrberg case, Water Resour. Res., 26, 1707–1719, 1990.

Geistlinger, H., Jia, R., Eisermann, D., and Stange, C. F.: Spatial and temporal variability of dissolved nitrous oxide in near-surface groundwater and its correlation to dissolved oxygen and groundwater fluctuations, J. Plant Nutr. Soil Sci., accepted, 2009.
Ginn, T. R., Wood, B. D., Nelson, K. E., Scheibe, T. D., Murphy, E. M., and Clement, T. P.: Processes in microbial transport in the natural subsurface, Adv. Water Resour., 25, 1017–1042, 2002.
Gold, A. J., Jacinthe, P. A., Groffman, P. M., Wright, W. R., Puffer, R. H.: Patchiness in groundwater nitrate removal in a riparian forest, J. Environ. Qual., 27, 146–155, 1998.
Grant, R. F. and Pattey, E.: Modelling variability in N_2O emissions from fertilized agricultural fields, Soil Biol. Biochem., 35, 225–243, 2003.
Groffman, P. M., Gold, A. J., and Jacinthe, P. A.: Nitrous oxide production in riparian zones and groundwater, Nutr. Cycl. Agroecosys., 52, 179–186, 1998.
Groffman, P. M., Howard, P., Gold, A. J., and Nelson, W. M.: Microbial nitrate processing in shallow groundwater in a riparian forest, J. Environ. Qual., 25, 1309–1316, 1996.
Hack, J. and Kaupenjohann, M.: N_2O discharge with drain water from agricultural soils of the upper Neckar region in Southern Germany, in: Non-CO_2 Greenhouse Gases: Scientific understanding, control options and policity aspects, edited by: van Ham, J., Baede, A. P. M., Guicherit, R., and Williams-Jacobse, J. G. F. M., Millpress, Rotterdam, 185–190, 2002.
Hefting, M. M., Bobbink, R., and de Caluwe, H.: Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones, J. Environ. Qual., 32, 1194–1203, 2003.
Hefting, M. M., Bobbink, R., and Janssens, M. P.: Spatial variation in denitrification and N_2O emission in relation to nitrate removal efficiency in a N-stressed riparian buffer zone, Ecosystems, 9, 550–563, doi:10.1007/s10021-006-0160-8, 2006.
Heincke, M. and Kaupenjohann, M.: Effects of soil solution on the dynamics of N_2O emissions: a review, Nutr. Cycl. Agroecosys., 55, 133–157, 1999.
Hénault, C., Chéneby, D., Heurlier, K., Garrido, F., Perez, S., and Germon, J. C.: Laboratory kinetics of soil denitrification are useful to discriminate soils with potentially high levels of N_2O emission on the field scale, Agronomie, 21, 713–723, 2001.
Hiscock, K. M., Bateman, A. S., Mühlherr, I. H., Fukada, T., and Dennis, P. F.: Indirect emissions of nitrous oxide from regional aquifers in the United Kingdom, Environ. Sci. Technol., 37, 3507–3512, 2003.
Hoehener, P., Duwig, C., Pasteris, G., Kaufmann, K., Dakhel, N., and Hauke, H.: Biodegradation of petroleum hydrocarbon vapors: laboratory studies on rates and kinetics in unsaturated

alluvial sand, J. Contam. Hydrol., 66, 93–115, 2003.
Holtan-Hartwig, L., Dörsch, P., and Bakken, L. R.: Comparison of denitrifying communities in organic soils: kinetics of NO_3^- and N_2O reduction, Soil Biol. Biochem., 32, 833–843, 2000.
Intergovernmental Panel on Climate Change (IPCC): 2006 IPCC guidelines for national greenhouse gas inventories, Prepared by the National Greenhouse Gas Inventories Programme, edited by: Egglestone, H. S., Buendia, L., Miwa, K., Ngara, T., and Tanabe, K., IGES, Japan, 2006.
Jacinthe, P. A., Groffman, P. M., Gold, A. J., and Mosier, A.: Patchiness in microbial nitrogen transformations in groundwater in a riparian forest, J. Environ. Qual., 27, 156–164, 1998.
Kelly, W. R., Hornberger, G. M., Herman, J. S., and Mills, A. L.: Kinetics of BTX biodegradation and mineralization in batch and column systems, J. Contam. Hydrol., 23, 113–132, 1996.
Kölle, W., Strebel, O., and Böttcher, J.: Formation of sulfate by microbial denitrification in a reducing aquifer, Water Supp., 3, 35–40, 1985.
Kölle, W., Werner, P., Strebel, O., and Böttcher, J.: Denitrifikation in einem reduzierenden Grundwasserleiter, Vom Wasser, 61, 125–147, 1983.
Konrad, C.: Methoden zur Bestimmung des Umsatzes von Stickstoff, dargestellt für drei pleistozäne Grundwasserleiter Norddeutschlands, Ph.D. thesis, Dresden University of Technology, Germany, 157 pp., 2007.
Korom, S. F.: Comment on "Modeling of multicomponent transport with microbial transformation in groundwater: The Fuhrberg case" by E. O. Frind et al., Water Resour. Res., 27, 3271–3274, 1991.
Korom, S. F.: Natural denitrification in the saturated zone: a review, Water Resour. Res., 28, 1657–1668, 1992.
McQuarrie, K. T. B. and Sudicky, E. A.: Multicomponent simulation of wastewater-derived nitrogen and carbon in shallow unconfined aquifers, I. Model formulation and performance, J. Contam. Hydrol., 47, 53–84, 2001.
Mosier, A. R., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S. P., and van Cleemput, O.: Closing the global N_2O budget: nitrous oxide emissions through the agricultural nitrogen cycle, Nutr. Cycl. Agroecosys., 52, 225–248, 1998.
Nõmmik, H.: Investigations on denitrification in soil, Acta Agr. Scand., 6, 195–228, 1956.
Obenhuber, D. C. and Lowrance, R.: Reduction of nitrate in aquifer microcosms by carbon additions, J. Environ. Qual., 20, 255–258, 1991.
Paramasivam, S., Alva, A. K., Prakash, O., and Cui, S. L.: Denitrification in the vadose zone and

in surficial groundwater of a sandy entisol with citrus production, Plant Soil, 208, 307–319, 1999.
Parkin, T. B.: Soil microsites as a source of denitrification variability, Soil Sci. Soc. Am. J., 51, 1194–1199, 1987.
Pätsch, M.: Analyse des Nitratumsatzes und dessen Heterogenität im quartären Grundwasserleiter des Wasserwerkes Thülsfelde – Berücksichtigung bei der Modellierung des Transportes, Ph.D. thesis, Dresden University of Technology, Germany, 223 pp., 2006.
Reay, D. S., Smith, K. A., and Edwards, A. C.: Nitrous oxide emission from agricultural drainage waters, Glob. Change Biol., 9, 195–203, 2003.
Rice, C. W. and Rogers, K. L.: Denitrification in subsurface environments: Potential source for atmospheric nitrous oxide, in: Agricultural ecosystems effects on trace gases and global climate change, ASA special publications, edited by: Harper, L. A. et al., American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, Madison, 121–132, 1993.
Schirmer, M., Molson, J. W., Frind, E. O., and Barker, J. F.: Biodegradation modelling of a dissolved gasoline plume applying independent laboratory and field parameters, J. Contam. Hydrol., 46, 339–374, 2000.
Schlichting, E., Blume, H. P., and Stahr, K.: Bodenkundliches Praktikum, Blackwell Wissenschaft, Berlin, Germany, 1995.
Siemens, J., Haas, M., and Kaupenjohann, M.: Dissolved organic matter induced denitrification in subsoils and aquifers?, Geoderma, 113, 253–271, 2003.
Šimek, M. and Cooper, J. E.: The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years, Eur. J. Soil Sci., 53, 345–354, 2002.
Smith, R. L. and Duff, J. H.: Denitrification in a sand and gravel aquifer, Appl. Environ. Microb., 54, 1071–1078, 1988.
Smith, R. L., Garabedian, S. P., and Brooks, M. H.: Comparison of denitrification activity measurements in groundwater using cores and natural-gradient tracer tests, Environ. Sci. Technol., 30, 3448–3456, 1996.
Spalding, R. F., Gormly, J. R., and Nash, K. G.: Carbon contents and sources in ground waters of the central Platte region in Nebraska, J. Environ. Qual., 7, 428–434, 1978.
Spalding, R. F. and Parrott, J. D.: Shallow groundwater denitrification, Sci. Total Environ., 141, 17–25, 1994.
Tiedje, J. M.: Denitrifiers, in: Methods of Soil Analysis. Part 2, Microbiological and Biochemical

Properties, edited by: Weaver, R. W., Angle, J. S., and Bottomley, P. J., SSSA, Madison, WI, USA, 245–267, 1994.
van Cleemput, O.: Subsoils: chemo- and biological denitrification, N_2O and N_2 emissions, Nutr. Cycl. Agroecosys., 52, 187–194, 1998.
von der Heide, C., Böttcher, J., Deurer, M., Weymann, D., Well, R., and Duijnisveld, W. H. M.: Spatial variability of N_2O concentrations and of denitrification-related factors in the surficial groundwater of a catchment in Northern Germany, J. Hydrol., 360, 230–241, 2008.
von der Heide, C., Böttcher, J., Deurer, M., Duijnisveld, W. H. M., Weymann, D., and Well, R.: Estimation of indirect N_2O emissions from a shallow aquifer: a case study in Northern Germany, J. Environ. Qual., 38, 2161–2171, 2009.
von der Heide, C., Böttcher, J., Deurer, M., Duijnisveld, W. H. M., Weymann, D., and Well, R.: Spatial and temporal variability of N_2O in the surface groundwater: A detailed analysis from a sandy aquifer in Northern Germany, Nutr. Cycl. Agroecosys., doi:10.1007/s10705-009-9310-7, online available at: http://www.springerlink.com/content/587j18011l2pk271/?p=2754fdf3d4964196ad134462fa4dacd4&pi=29, in press, 2010.
Weiss, R. F.: The solubility of nitrogen, oxygen and argon in water and sea water, Deep-Sea Res., 17, 721–735, 1970.
Weiss, R. F. and Price, B. A.: Nitrous oxide solubility in water and sea water, Mar. Chem., 8, 347–359, 1980.
Well, R., Augustin, J., Meyer, K., and Myrold, D. D.: Comparison of field and laboratory measurement of denitrification and N_2O production in the saturated zone of hydromorphic soils, Soil Biol. Biochem., 35, 783–799, 2003.
Well, R., Becker, K. W., Langel, R., Meyer, B., and Reineking, A.: Continuous flow equilibration for mass spectrometric analysis of dinitrogen emissions, Soil Sci. Soc. Am. J., 62, 906–910, 1998.
Well, R., Flessa, H., Jaradat, F., Toyoda, S., and Yoshida, N.: Measurement of isotopomer signatures of N_2O in groundwater, J. Geophys. Res., 110, G02006, doi:10.1029/2005JG000044, 2005a.
Well, R., Höper, H., Mehranfar, O., and Meyer, K.: Denitrification in the saturated zone of hydromorphic soils – laboratory measurement, regulating factors and stochastic modeling, Soil Biol. Biochem., 37, 1822–1836, 2005b.
Well, R. and Myrold, D. D.: Laboratory evaluation of a new method for in situ measurement of denitrification in water-saturated soils, Soil Biol. Biochem., 31, 1109–1119, 1999.

Weymann, D., Well, R., von der Heide, C., Flessa, H., Böttcher, J., and Duijnisveld, W. H. M.: Recovery of groundwater N_2O at the soil surface and its contribution to total N_2O emissions, Nutr. Cycl. Agroecosys., 85, 299–312, 2009.

Weymann, D., Well, R., Flessa, H., von der Heide, C., Deurer, M., Meyer, K., Konrad, C., and Walther, W.: Groundwater N_2O emission factors of nitrate-contaminated aquifers as derived from denitrification progress and N_2O accumulation, Biogeosciences, 5, 1215–1226, 2008, http://www.biogeosciences.net/5/1215/2008/.

Table 1. Location and basic properties of the investigated aquifer materials.

Sample location	Depth interval [m]	Denitrification zone	Organic C	Nt	C-to-N ratio [m kg^{-1}]	DOC[1]	C_{hws}[2]	Sulfur	Clay %
B1	2.0–2.6	heterotrophic	539.62	17.22	31.34	28.13	n.d.	47.35	0.00
B1	2.6–3.0		587.66	40.94	14.35	16.37	n.d.	45.79	0.00
B1	3.4–4.0		658.61	39.46	16.69	13.27	n.d.	39.65	0.00
I1-S1	1.5–2.0		816.12	53.43	15.27	19.27	167.25	44.61	0.00
I1-S1	2.0–2.5		609.26	40.22	15.15	16.28	111.80	75.78	0.00
I1-S1	2.5–3.0		485.18	67.82	7.15	12.69	109.66	91.55	0.00
I1-S2	1.5–2.0		536.64	23.78	22.57	16.40	91.59	24.76	0.00
I1-S2	2.0–2.5		506.05	32.39	15.62	17.82	101.66	13.57	0.00
I1-S3	1.5–2.0		729.46	42.06	17.34	21.09	113.56	33.72	0.00
I1-S3	2.0–2.5		584.57	36.82	15.88	17.73	103.55	41.67	0.00
I1-S3	2.5–3.0		527.99	41.40	12.75	13.45	94.90	64.33	0.00
I1-1	6.5–7.0	autotrophic	556.00	30.00	18.53	8.77	330.20	302.45	0.70
I1-2	6.5–7.0		437.95	129.84	3.37	7.65	338.39	265.47	0.95
I1-3	6.5–7.0		469.38	52.62	8.92	6.85	351.00	457.96	1.99
I1-4	6.5–7.0		714.68	65.07	10.98	9.88	390.00	430.86	2.22
I1-5	6.5–7.0		1293.73	94.97	13.62	8.46	258.70	379.89	3.44
I1-6	6.5–7.0		1488.87	123.58	12.05	11.87	267.15	396.13	5.09
I1-7	6.5–7.0		685.32	39.72	17.25	10.37	284.05	253.24	1.95
I1-8	6.5–7.0		461.45	45.33	10.18	8.27	247.00	361.88	1.50
I1-9	6.5–7.0		894.72	70.58	12.68	12.27	253.50	376.33	3.55
I1-10	6.5–7.0		545.91	41.64	13.11	7.25	318.50	436.03	2.26
I1-11	6.5–7.0		720.72	55.23	13.05	7.00	278.20	361.84	3.11

[1] extractable dissolved organic carbon
[2] extractable hot-water soluble carbon
n.d.=not determined.

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Table 2. Maximum N_2O concentrations (cN_2O_{max}), cN_2O_{max}-to-(N_2O+N_2) ratio and denitrification rates (D_i, D_{max}) during anaerobic incubation. D_i denotes the initial denitrification rate calculated at day 7. D_{max} is the maximum denitrification rate calculated for the time interval with the steepest increase of the (N_2O+N_2) curve.

Sample location	Depth interval [m]	Denitrification zone	cN_2O_{max} [mg N kg^{-1}]	cN_2O_{max}-to-(N_2O+N_2) ratio	D_i [mg N kg^{-1} d^{-1}]	D_{max} [mg N kg^{-1} d^{-1}]
B1	2.0–2.6	heterotrophic	0.0024	0.0270	0.0005	0.0027
B1	2.6–3.0		0.0128	0.0695	0.0006	0.0025
B1	3.4–4.0		0.0793	0.1096	0.0034	0.0133
I1-S1	1.5–2.0		0.0233	0.0259	0.0086	0.0352
I1-S1	2.0–2.5		0.0368	0.3794	0.0011	0.0040
I1-S1	2.5–3.0		0.0793	0.4148	0.0007	0.0046
I1-S2	1.5–2.0		0.0055	0.0264	0.0016	0.0119
I1-S2	2.0–2.5		0.0194	0.2581	0.0009	0.0047
I1-S3	1.5–2.0		0.0053	0.0041	0.0133	0.0306
I1-S3	2.0–2.5		0.0025	0.0177	0.0004	0.0035
I1-S3	2.5–3.0		0.0585	0.1182	0.0002	0.0065
I1-1	6.5–7.0	autotrophic	1.2579	0.1951	0.0432	0.0770
I1-2	6.5–7.0		0.4827	0.0634	0.0509	0.0612
I1-3	6.5–7.0		0.3305	0.0754	0.0846	0.1776
I1-4	6.5–7.0		1.6980	0.2083	0.0784	0.1665
I1-5	6.5–7.0		0.2391	0.0506	0.0865	0.2577
I1-6	6.5–7.0		0.0111	0.0013	0.1480	0.1566
I1-7	6.5–7.0		0.5202	0.1923	0.0344	0.0613
I1-8	6.5–7.0		0.8362	0.1307	0.0303	0.0397
I1-9	6.5–7.0		0.5256	0.0997	0.0777	0.2836
I1-10	6.5–7.0		0.0773	0.0102	0.0415	0.1004
I1-11	6.5–7.0		0.6470	0.1153	0.0572	0.1685

Table 3. Spearman rank correlation coefficients between the variables within the heterotrophic and the autotrophic data-set. Clay was not detectable in the case of heterotrophic denitrification and was thus excluded from the correlation analysis.

	C_{org}	N_t	C-to-N ratio	DOC	C_{hws}	Sulfur	Clay	cN_2O_{max}	cN_2O_{max}-to-(N_2O+N_2) ratio	D_i
Correlation coefficients between parameters of heterotrophic denitrification										
N_t	0.22 ns									
C-to-N ratio	0.27 ns	−0.69**								
DOC	0.32 ns	−0.34 ns	0.65*							
C_{hws}	0.67*	0.69*	−0.17 ns	0.45 ns						
Sulfur	−0.15 ns	0.44 ns	−0.62*	−0.44 ns	0.33 ns					
cN_2O_{max}	−0.08 ns	0.51 ns	−0.64*	−0.82**	0.02 ns	0.34 ns				
cN_2O_{max}-to-(N_2O+N_2) ratio	−0.56*	0.12 ns	−0.62*	−0.68*	−0.21 ns	0.50 ns		0.69**		
D_i	0.62*	0.24 ns	0.32 ns	0.19 ns	0.55 ns	−0.45 ns		0.15 ns	−0.23 ns	
D_{max}	0.39 ns	0.35 ns	0.16 ns	0.06 ns	0.24 ns	−0.42 ns		0.33 ns	−0.28 ns	0.74**
Correlation coefficients between parameters of autotrophic denitrification										
N_t	0.33 ns									
C-to-N ratio	0.43 ns	0.54*								
DOC	0.57*	0.15 ns	0.29 ns							
C_{hws}	−0.38 ns	−0.11 ns	−0.20 ns	−0.33 ns						
Sulfur	0.15 ns	0.13 ns	−0.28 ns	−0.22 ns	0.19 ns					
Clay	0.82***	0.49 ns	0.07 ns	0.31 ns	−0.43 ns	0.47 ns				
cN_2O_{max}	−0.20 ns	−0.35 ns	0.04 ns	0.11 ns	0.15 ns	−0.35 ns	−0.48 ns			
cN_2O_{max}-to-(N_2O+N_2) ratio	−0.19 ns	−0.52 ns	0.17 ns	0.16 ns	0.25 ns	−0.38 ns	−0.54*	0.94***		
D_i	0.64*	0.66*	0.66*	0.15 ns	0.10 ns	0.53*	0.65*	−0.38 ns	−0.42 ns	
D_{max}	0.65*	0.32 ns	0.32 ns	0.08 ns	−0.08 ns	0.52*	0.72**	−0.17 ns	−0.21 ns	0.73**

* Correlation significant at the 0.05 probability level.
** Correlation significant at the 0.01 probability level.
*** Correlation significant at the 0.001 probability level.

Table 4. Rate constants for heterotrophic and autotrophic denitrification derived from the sequential 3-parameter fit. $R^2(k_1)$ and $R^2(k_2)$ denote the correlation coefficients for the (N_2O+N_2)-data and the N_2O-data, respectively. The initial nitrate concentration C_0 was used as the third fitting parameter. The ratio of the fitting value and the experimental value is given in the last column and SD denotes the standard deviation.

Sample location	Depth interval [m]	k_1 [d^{-1}]	k_2 [d^{-1}]	R^2 (k_1)	R^2 (k_2)	$C_{0\,fit}$ [mg N kg^{-1}]	$C_{0\,fit}/C_{0\,exp}$
B1	2.0–2.6	0.007	0.977	1.000	0.740	0.263	0.018
B1	2.6–3.0	0.008	0.263	1.000	0.270	0.226	0.012
B1	3.4–4.0	0.004	0.162	1.000	0.430	1.698	0.101
I1-S1	1.5–2.0	0.005	0.920	1.000	0.844	3.826	0.213
I1-S1	2.0–2.5	0.004	0.115	1.000	0.840	0.714	0.041
I1-S1	2.5–3.0	0.003	0.031	1.000	0.910	0.945	0.063
I1-S2	1.5–2.0	0.004	1.172	1.000	0.830	1.174	0.098
I1-S2	2.0–2.5	0.006	0.103	1.000	0.910	0.364	0.032
I1-S3	1.5–2.0	0.007	4.881	1.000	0.850	3.768	0.350
I1-S3	2.0–2.5	0.005	1.435	1.000	0.900	0.550	0.044
I1-S3	2.5–3.0	0.005	0.100	1.000	0.270	0.687	0.052
mean		0.005	0.924	1.000	0.709	1.292	0.093
SD		0.001	1.407	0.000	0.255	1.310	0.102
I1-1	6.5–7.0	0.004	0.135	0.980	0.370	15.340	1.550
I1-2	6.5–7.0	0.003	0.230	1.000	0.580	18.880	1.840
I1-3	6.5–7.0	0.020	1.145	0.990	0.390	9.680	1.020
I1-4	6.5–7.0	0.008	0.289	0.990	0.100	10.120	1.080
I1-5	6.5–7.0	0.019	1.824	0.990	0.330	10.490	1.080
I1-6	6.5–7.0	0.021	35.320	1.000	0.400	9.950	1.000
I1-7	6.5–7.0	0.004	0.154	1.000	0.610	12.860	1.390
I1-8	6.5–7.0	0.002	0.239	0.980	0.300	22.140	2.460
I1-9	6.5–7.0	0.021	1.157	0.990	0.270	10.140	1.060
I1-10	6.5–7.0	0.012	3.578	0.990	0.320	9.799	1.060
I1-11	6.5–7.0	0.014	0.621	0.990	0.330	11.250	1.190
mean		0.012	4.063	0.991	0.364	12.786	1.339
SD		0.008	10.418	0.007	0.140	4.237	0.456

Table 5. Rate constants for heterotrophic and autotrophic denitrification derived from the 1-step 3-parameter fit. R^2 denotes the correlation coefficient.The initial nitrate concentration C_0 was used as third fitting parameter. The initial nitrate concentration C_0 was used as the third fitting parameter. The ratio of the fitting value and the experimental value is given in the last column and SD denotes the standard deviation.

Sample location	Depth interval [m]	k_1 [d^{-1}]	k_2 [d^{-1}]	R^2	$C_{0\,fit}$ [mg N kg^{-1}]	$C_{0\,fit}/C_{0\,exp}$
B1	2.0–2.6	0.003	0.113	0.860	0.063	0.004
B1	2.6–3.0	0.006	0.006	0.920	0.023	0.001
B1	3.4–4.0	0.007	0.007	0.830	0.127	0.008
I1-S1	1.5–2.0	0.010	0.070	0.950	0.206	0.011
I1-S1	2.0–2.5	0.008	0.050	0.880	0.230	0.013
I1-S1	2.5–3.0	0.007	0.007	0.970	0.195	0.013
I1-S2	1.5–2.0	0.003	0.091	0.950	0.154	0.013
I1-S2	2.0–2.5	0.005	0.044	0.960	0.175	0.016
I1-S3	1.5–2.0	0.005	0.078	0.960	0.087	0.008
I1-S3	2.0–2.5	0.006	0.061	0.960	0.025	0.002
I1-S3	2.5–3.0	0.003	0.003	0.950	0.130	0.010
mean		0.006	0.048	0.926	0.129	0.009
SD		0.002	0.038	0.048	0.072	0.005
I1-1	6.5–7.0	0.010	0.010	0.560	1.140	0.120
I1-2	6.5–7.0	0.009	0.009	0.810	0.660	0.060
I1-3	6.5–7.0	0.038	0.038	0.690	0.460	0.050
I1-4	6.5–7.0	0.006	0.006	0.370	1.110	0.120
I1-5	6.5–7.0	0.036	0.036	0.610	0.310	0.030
I1-6	6.5–7.0	0.006	87.010	0.500	57.710	5.800
I1-7	6.5–7.0	0.013	0.013	0.830	0.920	0.100
I1-8	6.5–7.0	0.007	0.007	0.470	0.630	0.070
I1-9	6.5–7.0	0.038	0.038	0.530	0.520	0.050
I1-10	6.5–7.0	0.015	0.015	0.650	0.080	0.010
I1-11	6.5–7.0	0.029	0.029	0.580	0.690	0.070
mean		0.019	7.928	0.600	5.839	0.589
SD		0.014	26.228	0.139	17.207	1.729

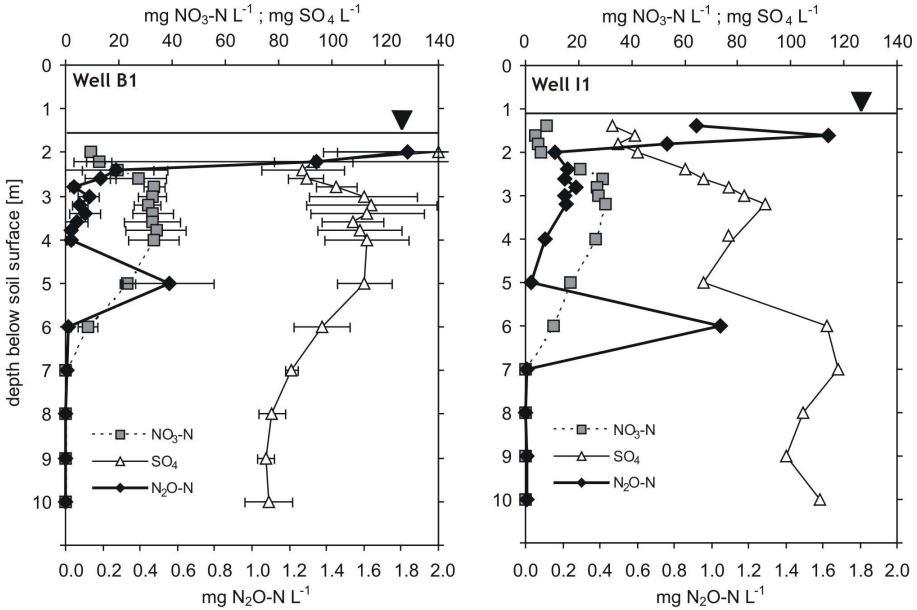

Fig. 1. Vertical concentration gradients of N_2O, NO_3^- and SO_4^{2-} at the wells B1 and I1. The data of well B1 are mean values of three sampling events, the error bars denote the standard deviation.

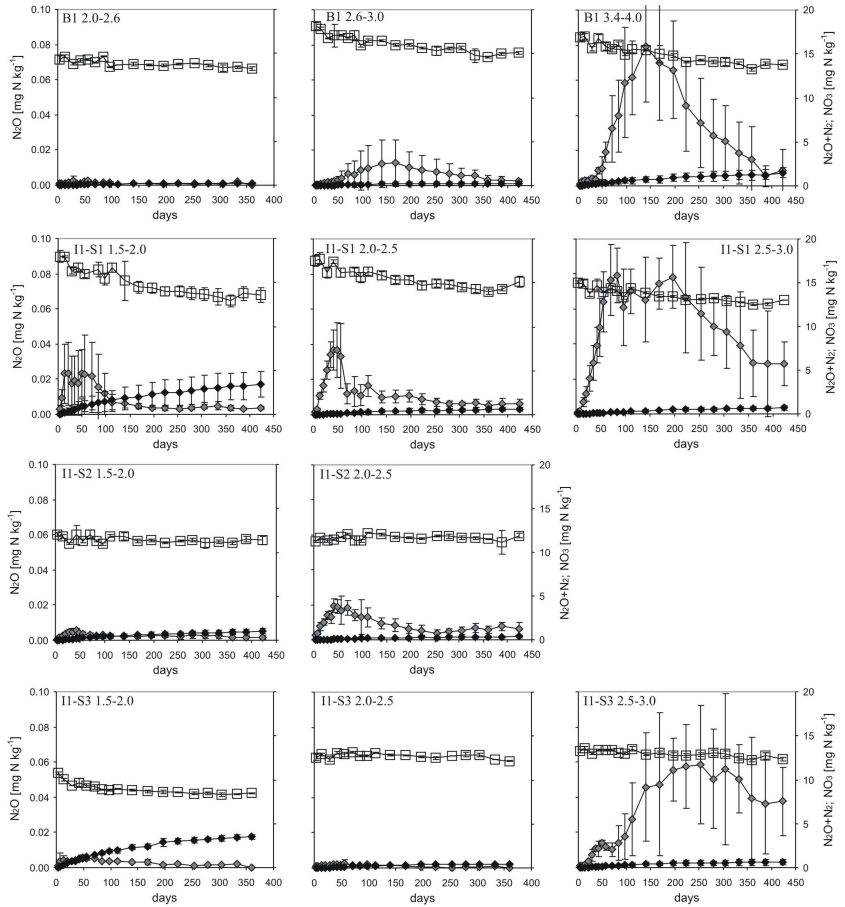

Fig. 2. Concentration courses of —◇— N_2O —◆— N_2O+N_2 —□— NO_3 during long-term anaerobic incubation of aquifer material from the heterotrophic denitrification zone. The symbols denote the means of 4 replications and the error bars represent the standard deviation.

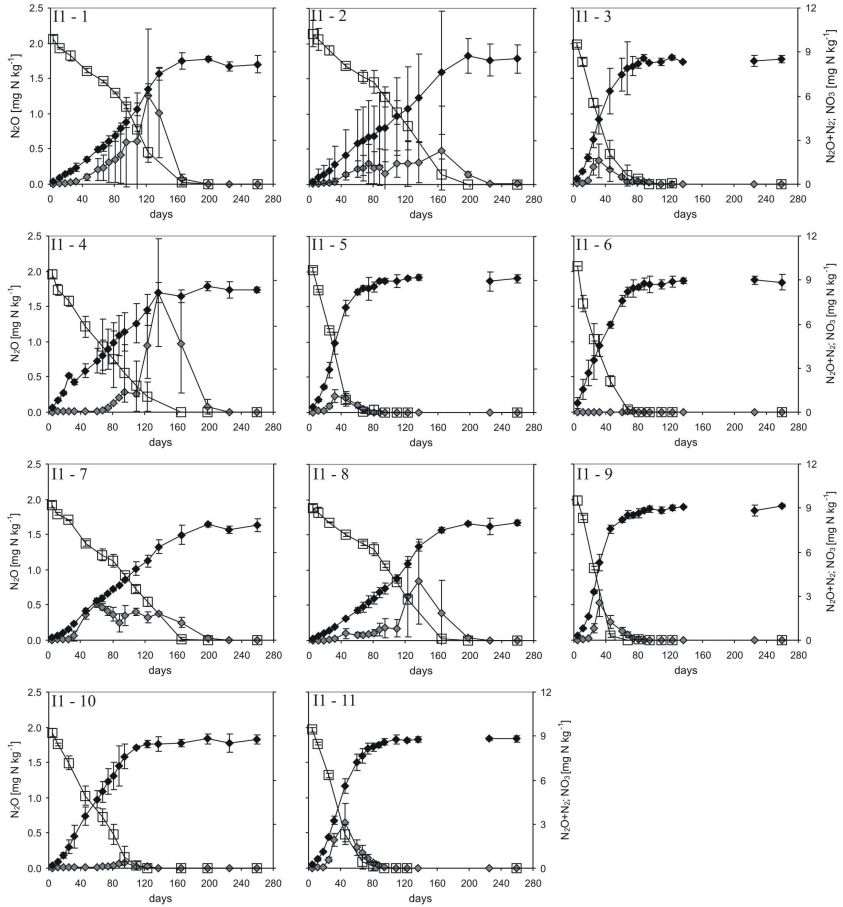

Fig. 3. Concentration courses of —◆— N_2O —◆— N_2O+N_2 —□— NO_3 during long-term anaerobic incubation of aquifer material from the autotrophic denitrification zone. The symbols denote the means of 4 replications and the error bars represent the standard deviation.

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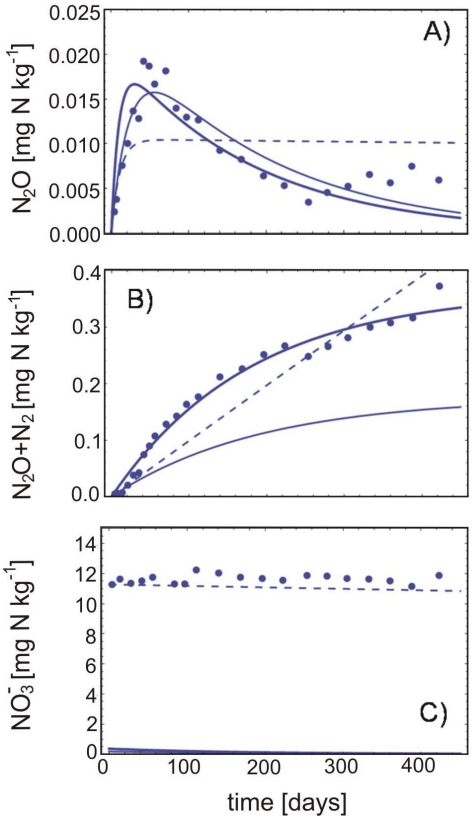

Fig. 4. Comparison between experimental N_2O- **(a)**, (N_2O+N_2)- **(b)**, and NO_3^- **(c)** concentrations (solid circles) and fitting curves (thick solid line: sequential 3-parameter fit; thin solid line: 1-step 3-parameter fit; dashed line: sequential 2-parameter fit) for the data set I1-S1 2.0–2.5 (heterotrophic denitrification). The rate constants k_1 and k_2 as well as the initial nitrate concentration C_0 were used as fitting parameters for the 3-parameter fits.