

Kinetics of N₂O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments

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Abstract. Knowledge of the kinetics of N₂O production and reduction in groundwater is essential for the assessment of potential indirect emissions of the greenhouse gas. In the present 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 near-surface groundwater and of autotrophic denitrification in depths beyond 2–3 m below the groundwater table, respectively. The kinetics of N₂O production and reduction in both process zones was studied during long-term anaerobic laboratory incubations of aquifer slurries using the ¹⁵N tracer technique. We measured N₂O, N₂, NO₃⁻, NO₂⁻, and SO₄²⁻ concentrations as well as parameters of the aquifer material that were related to the relevant electron donors, i.e. organic carbon and pyrite. The laboratory incubations showed a low denitrification activity of heterotrophic denitrification

with initial rates between 0.2 and 13 μg N kg⁻¹ d⁻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 30 and 148 μg N kg⁻¹ d⁻¹, and NO₃⁻ as well as N₂O were completely removed within 60 to 198 days. N₂O 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 N₂O accumulation during laboratory incubation. Finally, we applied a conventional model using first-order-kinetics to determine the reaction rate constants *k*₁ for N₂O production and *k*₂ for N₂O reduction, respectively. The goodness of fit to the experimental data was partly limited, indicating that a more sophisticated approach is essential to describe the investigated reaction kinetics satisfactorily.



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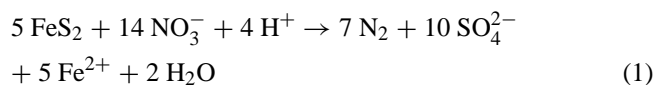
1 Introduction

The atmospheric concentration of nitrous oxide (N₂O), 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 N₂O emissions due to the prevalent application of mineral and organic fertilisers (Mosier et al., 1998). In aquifers of these ecosystems, elevated N₂O concentrations of up to three orders of magnitude above the concentration in water equilibrated air were found in the near-surface groundwater (Spalding and Parrott, 1994; Well et al., 2005a; von der Heide et al., 2008). Thus, N₂O in groundwater was assumed to be a potential source contributing to atmospheric N₂O emissions (Rice and Rogers, 1993; Mosier et al., 1998; Hefting et al., 2003). Despite several recent studies on N₂O 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. This could be attributed to two crucial aspects: firstly, N₂O accumulation in groundwater is complexly controlled. N₂O is an intermediate product of denitrification, the major process yielding to the occurrence of N₂O in oxygen depleted groundwater. Thus, N₂O emissions are a net result of the balance between simultaneously running N₂O production and reduction to N₂. 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 N₂O concentrations in groundwater (von der Heide et al., 2008) and to wide ranges of groundwater N₂O emission factors (Hack and Kaupenjohann, 2002; Weymann et al., 2008). N₂O in the groundwater can also be produced by nitrification as it was shown by Mühlherr and Hiscock (1998) and Hiscock et al. (2003) for British chalk aquifers. Secondly, it is a challenge to combine research on the reaction kinetics of N₂O with transport parameters. Clough et al. (2005) stated that the movement and the ultimate fate of N₂O in subsurface environments are still poorly understood. For example, the consumption of N₂O in groundwater is poorly investigated (Clough et al., 2007), and depends on the coaction of several factors, mainly the electron donor availability, the O₂ concentration, and the pH, respectively. Moreover, the fate of groundwater-derived N₂O passing the unsaturated zone has not been fully understood (Weymann et al., 2009).

Denitrification has been frequently investigated during laboratory incubation studies using the ¹⁵N 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 N₂O and its reaction kinetics in groundwater are comparatively rare. Obenhuber and

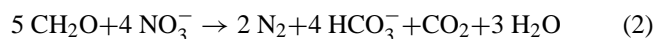
Lowrance (1991) observed NO₃⁻ removal and an accumulation of N₂O in flow-through microcosms within a period of 302 days, especially in treatments with glucose amendment. Jacinthe et al. (1998) designed a similar experiment with two types of aquifer material over 132 days. The authors reported that heterogeneously distributed “patches” of organic matter induced denitrification in a 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 NO₃⁻. Blicher-Mathiesen and Hoffmann (1999) conducted an experiment with continuously permeated columns as well as static incubations. In both cases, they observed considerable NO₃⁻ removal and net N₂O production, but they also questioned the transferability of these results to parallel investigated field conditions which did not exhibit N₂O accumulation due to an efficient reduction of N₂O to N₂. Differences in net N₂O production between field and laboratory studies were also observed and discussed by Well et al. (2003). By comparing the N₂O 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 N₂O (Obenhuber and Lowrance, 1991; Hénault et al., 2001). As becomes clear at this point, it is uncertain whether laboratory investigations of the kinetics of N₂O production and reduction are applicable to field conditions.

Recently, the magnitude and controls of indirect N₂O fluxes from the groundwater have been investigated by combining field monitoring, process studies and modelling. Research activities were conducted within the unconsolidated sandy Fuhrberger Feld aquifer (FFA) in northern Germany. 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 drinking water supply on the one hand and agricultural activities causing considerable inputs of pollutants via seepage, especially of nitrate, on the other hand (Kölle et al., 1985; Frind et al., 1990). In the FFA, substantial microbially mediated processes and reactions like denitrification and desulfurification (heterotrophic reduction of sulfate, Korom, 1991, 1992; Böttcher et al., 1991) occur, strongly influencing groundwater geochemistry. Autotrophic denitrification with pyrite as an electron donor was identified as the dominant microbial reaction for NO₃⁻ elimination (Kölle et al., 1985) in depths beyond 2–3 m below the groundwater table (Böttcher et al., 1992). This part of the aquifer is denoted as “autotrophic denitrification zone” later on in this paper. The process of autotrophic denitrification was stoichiometrically described by Kölle et al. (1985) and Böttcher et al. (1990) as a reaction mediated by the bacteria *Thiobacillus denitrificans*:



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 the potential of autotrophic denitrification on the basis of pyrite oxidation.

In the case of the near-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 pyrite (Kölle et al., 1983; Böttcher et al., 1991):



Autotrophic denitrification is much more efficient for NO₃⁻ reduction than heterotrophic denitrification in the near-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 NO₃⁻ concentrations are limited to the top few metres of the aquifer, but the rest of the aquifer is almost NO₃⁻-free (Frind et al., 1990; von der Heide et al., 2009).

The recent research activities in the FFA focused on the dynamics of N₂O in the system groundwater – unsaturated zone. Deurer et al. (2008) investigated the accumulation and dynamics of N₂O 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 the groundwater may also act as a sink for N₂O. An extremely high spatial variability of N₂O concentrations in the near-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 N₂O concentrations in the near-surface groundwater. Weymann et al. (2008) determined groundwater N₂O emission factors with respect to initial NO₃⁻ concentrations and assessed these factors related to N₂O accumulation during different stages of the denitrification progress.

In the present study, we investigated the kinetics of N₂O production and reduction in aquifer material from the FFA. The specific objectives of this study are (i) to determine the time courses of NO₃⁻, N₂O and N₂ during long-term laboratory incubation of aquifer material samples, (ii) to evaluate kinetic rate constants of N₂O 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). 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 at the multilevel sampling wells B1 and I1 (see von der Heide et al., 2009). Since the aquifer consists of vertically separated denitrification zones according to the availability of electron donors, i.e. organic carbon and pyrite (von der Heide et al., 2008), we were able to investigate not only the kinetics of N₂O production and reduction during heterotrophic denitrification, but also during the autotrophic pathway. 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 N₂O, NO₃⁻, SO₄²⁻, O₂, DOC, and pH. 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 campaign was conducted in March 2006. Here, we collected the groundwater 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 near-surface groundwater (0.1 m–2.1 m below the groundwater table) and 1.0 m in the subjacent 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 N₂O 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

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

Sample location	Depth interval [m]	Denitrification zone	Organic C	Total N	C-to-N ratio	DOC ¹	C _{hws} ²	Total S	Clay [%]
			[mg kg ⁻¹]			[mg kg ⁻¹]			
B1	2.0–2.6	“heterotrophic”	539.62	17.22	31.34	28.13	n.d. ³	47.35	0.00
B1	2.6–3.0		587.66	40.94	14.35	16.37	n.d. ³	45.79	0.00
B1	3.4–4.0		658.61	39.46	16.69	13.27	n.d. ³	39.65	0.00
II–S1	1.5–2.0		816.12	53.43	15.27	19.27	167.25	44.61	0.00
II–S1	2.0–2.5		609.26	40.22	15.15	16.28	111.80	75.78	0.00
II–S1	2.5–3.0		485.18	67.82	7.15	12.69	109.66	91.55	0.00
II–S2	1.5–2.0		536.64	23.78	22.57	16.40	91.59	24.76	0.00
II–S2	2.0–2.5		506.05	32.39	15.62	17.82	101.66	13.57	0.00
II–S3	1.5–2.0		729.46	42.06	17.34	21.09	113.56	33.72	0.00
II–S3	2.0–2.5		584.57	36.82	15.88	17.73	103.55	41.67	0.00
II–S3	2.5–3.0		527.99	41.40	12.75	13.45	94.90	64.33	0.00
II–1	6.5–7.0	“autotrophic”	556.00	30.00	18.53	8.77	330.20	302.45	0.70
II–2	6.5–7.0		437.95	129.84	3.37	7.65	338.39	265.47	0.95
II–3	6.5–7.0		469.38	52.62	8.92	6.85	351.00	457.96	1.99
II–4	6.5–7.0		714.68	65.07	10.98	9.88	390.00	430.86	2.22
II–5	6.5–7.0		1293.73	94.97	13.62	8.46	258.70	379.89	3.44
II–6	6.5–7.0		1488.87	123.58	12.05	11.87	267.15	396.13	5.09
II–7	6.5–7.0		685.32	39.72	17.25	10.37	284.05	253.24	1.95
II–8	6.5–7.0		461.45	45.33	10.18	8.27	247.00	361.88	1.50
II–9	6.5–7.0		894.72	70.58	12.68	12.27	253.50	376.33	3.55
II–10	6.5–7.0		545.91	41.64	13.11	7.25	318.50	436.03	2.26
II–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; ³ n.d.: not determined

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 and reduced groundwater from the same depth, i.e. sampling included replacing of the original sediment with sediment in the vicinity of the borehole. Thus, sediment with different properties from slightly different areas was sampled. 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 ¹⁵N tracer technique that reaches back to the seminal study of Nömmik (1956) who quantified the gaseous denitrification products from soils receiving K¹⁵NO₃ by mass spectrometry. The approach of anaerobic incubation of NO₃⁻ amended slurries has been extensively used for measuring denitrification and N₂O production (Tiedje, 1994; Hénault et al., 2001; Well et al., 2003, 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 K¹⁵NO₃ test solution (10 mg N L⁻¹; 60 atom% ¹⁵N). 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 N₂, respectively. The duration of each cycle was about 10 minutes. This method was examined carefully prior to the incubation experiment. We also checked the headspace O₂ concentration

(see Sect. 2.4) to ensure that O₂ was sufficiently removed. 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 N₂O by gas chromatography, the other for the ¹⁵(N₂O+N₂) 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 N₂ after sampling. The resulting dilution of the headspace gas was taken into account in the calculation of the ¹⁵(N₂O+N₂) concentrations. Water samples were collected with a syringe. Routinely, we withdrew a 15 mL aliquot for NO₃⁻ analysis. Subsequently, an equivalent amount of the oxygen-free K¹⁵NO₃ test solution was re-injected. The NO₃⁻ concentration of the test solution was adjusted according to the expected sample NO₃⁻ concentration. The oxygen-free K¹⁵NO₃ test solution was manufactured in a 10 L glass tank sealed with a butyl rubber stopper. To establish anoxic conditions, the solution was flushed with N₂ for 1 h using a stainless steel capillary tube and was stirred simultaneously by magnetic stirring. Subsequently, O₂ in the headspace of the tank was examined by GC measurement as described in Sect. 2.4.

2.4 Analytical techniques

The aquifer materials' 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, Hanau, Germany) equipped with a thermal conductivity detector. The precision of the analysis was 0.5%. Total sulfur of the identical samples was analysed with a vario EL III elemental analyser (ELEMENTAR ANALYSENSYSTEME, Hanau, Germany) equipped with a thermal conductivity detector and an UV-absorption photometer. The precision of the analysis was 0.1%.

NO₃⁻ and NO₂⁻ in the water samples from the laboratory incubations were analysed photometrically using a continuous flow analyser (Skalar, Erkelenz, Germany). The measurement precision was 5%. The pH of these samples was

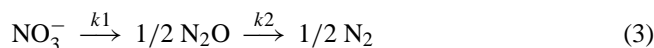
measured with a pH-meter (METROHM 632, Filderstadt, Germany). DOC in the water samples from the laboratory incubations, in cold-water extracts, and hot-water soluble organic carbon (C_{hws}) were analysed as described by Well et al. (2005b). SO₄²⁻ concentrations of the water samples from the laboratory incubations (autotrophic denitrification zone) were determined by potentiometric back-titration using EDTA as a titrant. Prior to back-titration, SO₄²⁻ was precipitated as barium sulfate after addition of barium chloride solution in excess. Please note that it was not possible to measure SO₄²⁻ also in the water samples of the laboratory incubations from the heterotrophic denitrification zone, because the sample volumes after measuring NO₃⁻ and NO₂⁻ were limited and an analysis based on back titration was not possible because of the comparatively low sulfate concentrations.

NO₃⁻, SO₄²⁻, and potassium concentrations 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%. DOC, pH and the O₂ concentration of the groundwater were measured on site as specified by Deurer et al. (2008).

N₂O was measured using a gas chromatograph equipped with an electron capture detector and an auto sampler that was described earlier (Well et al., 2003). The ¹⁵N analysis of (N₂O+N₂) in the headspace gas was conducted following the method specified in Well et al. (1998, 2003). The gas concentrations of the sample solutions (dissolved N₂O and N₂) were calculated according to Henry's laws from the headspace concentrations using the Bunsen absorption coefficients of N₂O and N₂, respectively (Weiss, 1970; Weiss and Price, 1980). The calculation was described in detail by Well and Myrold (1999) and Well et al. (2003). O₂ was monitored throughout the laboratory incubations to ensure anaerobic conditions. We used a gas chromatograph (Fractovap 400, CARLO ERBA, Milano) equipped with a thermal conductivity detector and a packed column (1.8 m length, 4 mm ID, molecular sieve 5 Å). Helium functioned as the carrier gas.

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 the case of our laboratory approach, we consider a two-step reaction chain for N₂O production and N₂O reduction (Holtan-Hartwig et al., 2002) in order to characterise the heterotrophic and autotrophic denitrification process:



It is important to anticipate that concentrations of nitrite (NO₂⁻) were below detection limit during the laboratory incubations, i.e. no reaction limited step between NO₃⁻ and N₂O (*k*₁) occurred. For further discussion see Sect. 4.2.

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 NO₃[−] and N₂O, respectively:

$$\frac{dC_{\text{NO}_3}}{dt} = -k_1 \cdot C_{\text{NO}_3}, \quad (4)$$

$$\frac{dC_{\text{N}_2\text{O}}}{dt} = F \cdot k_1 \cdot C_{\text{NO}_3} - k_2 \cdot C_{\text{N}_2\text{O}} \quad (5)$$

The analytical solutions are:

$$C_{\text{NO}_3}(t) = C_0 \cdot \exp(-k_1 \cdot t), \quad (6)$$

$$(k_1 \neq k_2): C_{\text{N}_2\text{O}}(t) = F \cdot C_0 \cdot \frac{k_1}{(k_2 - k_1)} \cdot [\exp(-k_1 \cdot t) - \exp(-k_2 \cdot t)] \quad (7)$$

$$(k_1 = k_2): C_{\text{N}_2\text{O}}(t) = F \cdot C_0 \cdot k_1 \cdot t \cdot \exp(-k_1 \cdot t), \quad (8)$$

where F is the stoichiometric factor and C_0 is the initial nitrate concentration. We note that the sum of N₂ and N₂O is only a function of k_1 and the analytical solution follows by mass balance considerations:

$$C_{\text{sum}}(t) = C_{\text{N}_2}(t) + C_{\text{N}_2\text{O}}(t) = F \cdot (C_0 - C_{\text{NO}_3}(t)). \quad (9)$$

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. The fitting parameters for the 3-parameter fits were C_0 , k_1 and k_2 , respectively.

To further evaluate the control of NO₃[−] reduction by denitrification we also used a simpler approach which did not include the distinction between N₂O production and reduction and was based on zero-order-kinetics. Reaction rates (D) were derived from the slope of (N₂O+N₂) 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 (N₂O+N₂)-curve. Finally, we used the maximum N₂O concentration during incubation ($c_{\text{N}_2\text{O}_{\text{max}}}$) and the ratio between N₂O and (N₂O+N₂) at maximum N₂O concentration ($c_{\text{N}_2\text{O}_{\text{max}}}$ -to-[N₂O+N₂]) as qualitative indicators for the balance between production and reduction of N₂O.

Since we expected SO₄^{2−} production in the samples from the autotrophic denitrification zone (Kölle et al., 1985), SO₄^{2−} production rates were obtained from the increase of SO₄^{2−} concentrations. The rates were calculated using the difference between the final and the initial SO₄^{2−} concentration depending on time and were converted to N equivalents according to Eq. (1) in order to facilitate a comparison with the denitrification rates.

3 Results

3.1 Multilevel well measurements

At the investigated wells, each of the vertical concentration gradients of NO₃[−] and N₂O showed a similar pattern. In the near-surface groundwater, NO₃[−] concentrations initially increased downwards in both profiles to a mean value of 34 mg NL^{−1} in a depth of 3.8 m below the soil surface at B1 and to 30 mg NL^{−1} in a depth of 3.2 m below the soil surface at I1, respectively (Fig. 1a). Below 4 m, where the autotrophic denitrification mainly governs NO₃[−] reduction, NO₃[−] concentrations decreased rapidly and reached zero in a depth of 7 m at both wells. In the case of N₂O (Fig. 1b), we identified two layers where the concentrations were highest: first, there is a zone of N₂O accumulation in the uppermost groundwater coinciding with an “exchange zone” that was recently reported by Deurer et al. (2008). We observed N₂O concentrations up to 1.84 mg NL^{−1} in a depth of 2.0 m below the soil surface at B1 and 1.63 mg NL^{−1} in a depth of 1.6 m below the soil surface (0.54 m below the groundwater table) at I1. Second, Fig. 1b shows a sharp concentration peak in both profiles, consisting of an outstanding value in 5 m and 6 m depth, respectively. Between these layers, N₂O concentrations in the groundwater were substantially lower at both wells, but still up to three orders of magnitude higher than the N₂O concentration in water equilibrated air. In the autotrophic denitrification zone, N₂O concentrations declined rapidly after the sharp peak and were below detection limit in 6 m at B1 and 7 m at I1, respectively. In contrast to the vertical concentration gradients of NO₃[−] and N₂O, the SO₄^{2−} concentration pattern was different at the investigated wells (Fig. 1c). 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 N₂O. Furthermore, the SO₄^{2−} concentrations remained elevated in the deeper groundwater compared to the near-surface groundwater. At B1, these phenomena did not occur during all sampling campaigns (further details will be given in the discussion section).

Ranges of groundwater pH in the profiles indicated acidic conditions and were similar at the investigated wells. The pH ranged between 4.1 and 6.0 at B1 and between 4.9 and 5.7 at I1, respectively (Fig. 1f). O₂ concentrations were highest in the near-surface groundwater. Values up to 3.0 mg L^{−1} (B1) and 4.2 mg L^{−1} (I1) suggested oxic conditions (Fig. 1e). In contrast, practically anoxic conditions dominated in the groundwater of the autotrophic denitrification zone.

3.2 Laboratory incubation: reaction rates, time courses of the N-species, pH, and correlation analysis

The concentration courses of N₂O, of the total denitrification products (N₂O+N₂), and of NO₃[−] are represented in Figs. 2 and 3, respectively. Whereas Fig. 2 refers to heterotrophic

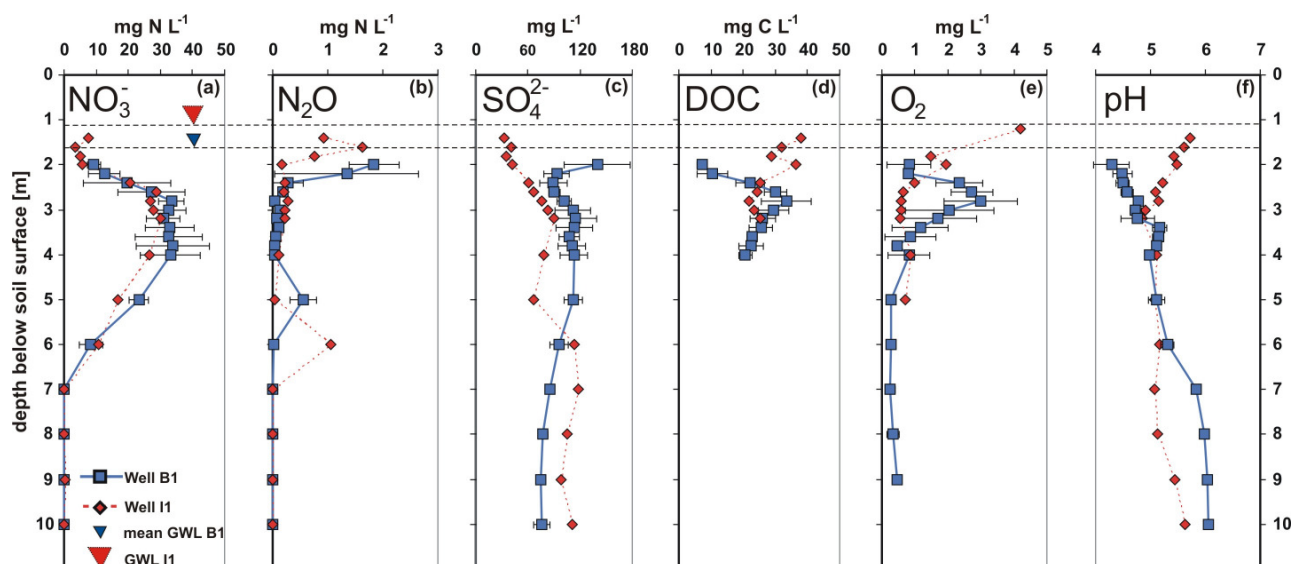


Fig. 1. Vertical gradients of NO_3^- , N_2O , SO_4^{2-} , DOC-, and O_2 concentrations as well as pH at the wells B1 and I1. The data of well B1 are mean values of three sampling campaigns, the error bars denote the standard deviation (only shown when larger than the symbols). The dotted lines indicate the groundwater level at I1 (GWL I1) and the mean groundwater level of the three sampling campaigns conducted at B1 (GWL B1), respectively.

denitrification in the near-surface groundwater, Fig. 3 shows the results for the autotrophic denitrification zone. N_2O and ($\text{N}_2\text{O}+\text{N}_2$) were detectable in all samples indicating the general occurrence of denitrification. In contrast, NO_2^- concentrations were found to be below detection limit in all samples throughout the entire incubations of the aquifer slurries. 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), symbolized by the slopes of the ($\text{N}_2\text{O}+\text{N}_2$) curves (Fig. 3), were typically one order of magnitude higher than the rates of heterotrophic denitrification. The coincidence of NO_3^- reduction and ($\text{N}_2\text{O}+\text{N}_2$) production indicate that the mass balance was satisfactory (Figs. 2 and 3). Consequently, NO_3^- concentrations decreased continuously 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 ($c\text{N}_2\text{O}_{\text{max}}$) followed by a decrease that resulted in complete N_2O reduction for the case of autotrophic denitrification. However, the N_2O concentration courses and $c\text{N}_2\text{O}_{\text{max}}$ 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 $c\text{N}_2\text{O}_{\text{max}}$ with depth (Fig. 2, Table 2). Consequently, $c\text{N}_2\text{O}_{\text{max}}$ 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 79, 79 and 59 $\mu\text{g N}_2\text{O-N kg}^{-1}$, respectively. In contrast, $c\text{N}_2\text{O}_{\text{max}}$ was lowest in the topmost depth intervals where it ranged between 2.4 and 23 $\mu\text{g N}_2\text{O-N kg}^{-1}$. The results showed that N_2O concentrations were close to the $c\text{N}_2\text{O}_{\text{max}}$ 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 $c\text{N}_2\text{O}_{\text{max-to-}}(\text{N}_2\text{O}+\text{N}_2)$ ratio (Fig. 3, Table 2). Furthermore, this sample showed by far the highest D_i . $c\text{N}_2\text{O}_{\text{max}}$ of the sample I1–10 was 77 $\mu\text{g N}_2\text{O-N kg}^{-1}$ and thus comparable with the highest $c\text{N}_2\text{O}_{\text{max}}$ values we observed in the samples of the heterotrophic denitrification zone. Apart from these two samples, all the other ones were characterized by considerably higher $c\text{N}_2\text{O}_{\text{max}}$ values (Fig. 3, Table 2) between 239 $\mu\text{g N}_2\text{O-N kg}^{-1}$ (sample I1–5) and 1698 $\mu\text{g N}_2\text{O-N kg}^{-1}$ (sample I1–4).

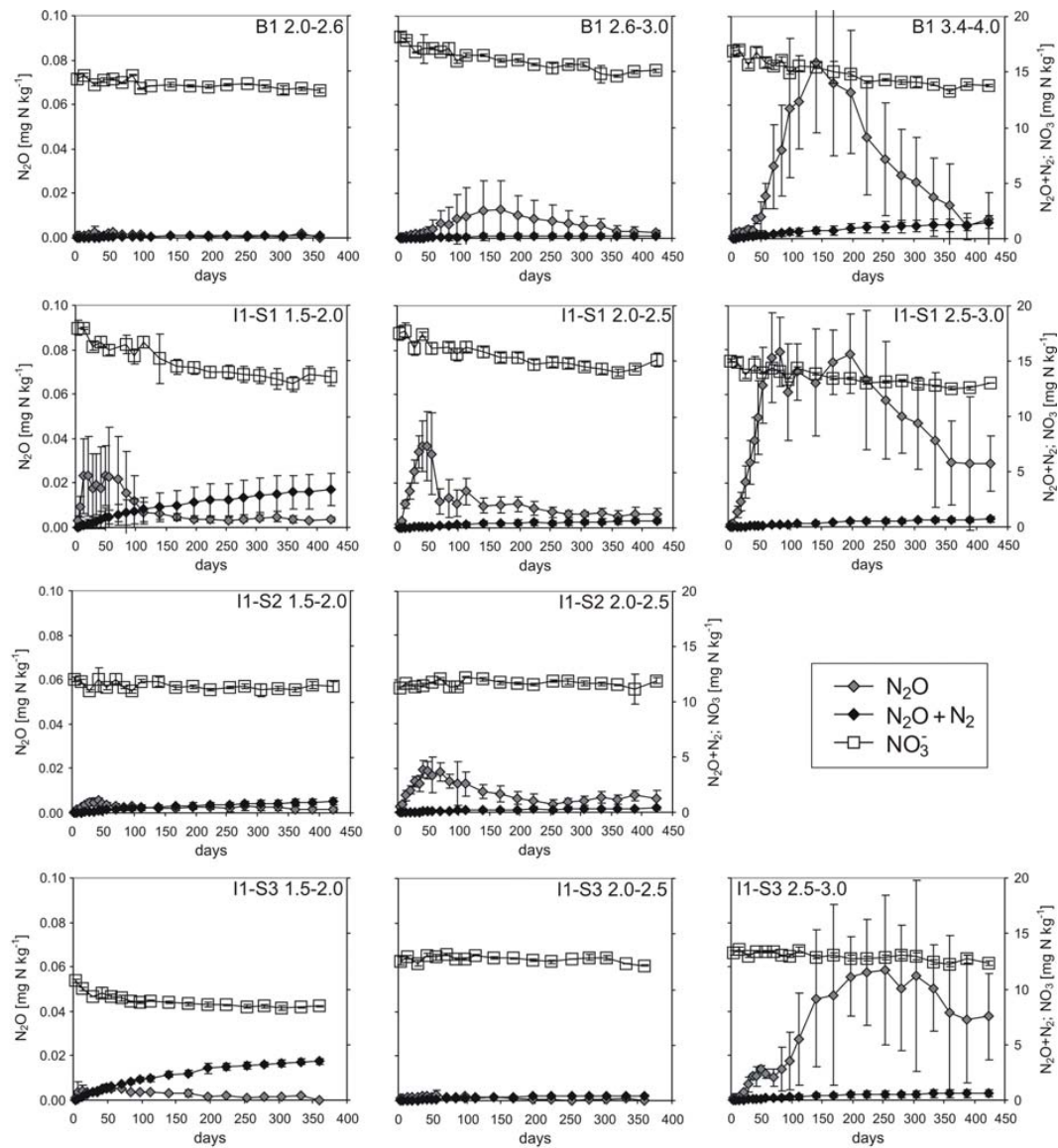


Fig. 2. Concentration courses of N₂O, N₂O+N₂, and NO₃⁻ during long-term anaerobic incubation of aquifer material from the heterotrophic denitrification zone. The symbols denote the means of 4 replications and the error bars represent the standard deviation.

We observed considerable formation of SO₄²⁻ during incubation of the aquifer slurries from the autotrophic denitrification zone. SO₄²⁻ production rates converted to N equivalents ranged between 36 and 185 μg N⁻¹ kg⁻¹ d⁻¹ and were in good agreement with the denitrification rates (Table 2), i.e. were found to be between D_i and D_{max} for the majority of the samples. However, sample I1-4 exhibited a lower and sample I1-6 a slightly higher SO₄²⁻ production rate than predicted by the denitrification rates.

The pH was monitored throughout the entire incubation experiments. Typical ranges were between 4.2 and 5.9. These values indicate similar acidic conditions which we also found in the groundwater at the wells B1 and I1, respectively (Fig. 1f).

We conducted Spearman rank tests for the partial datasets 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-(N₂O+N₂) 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

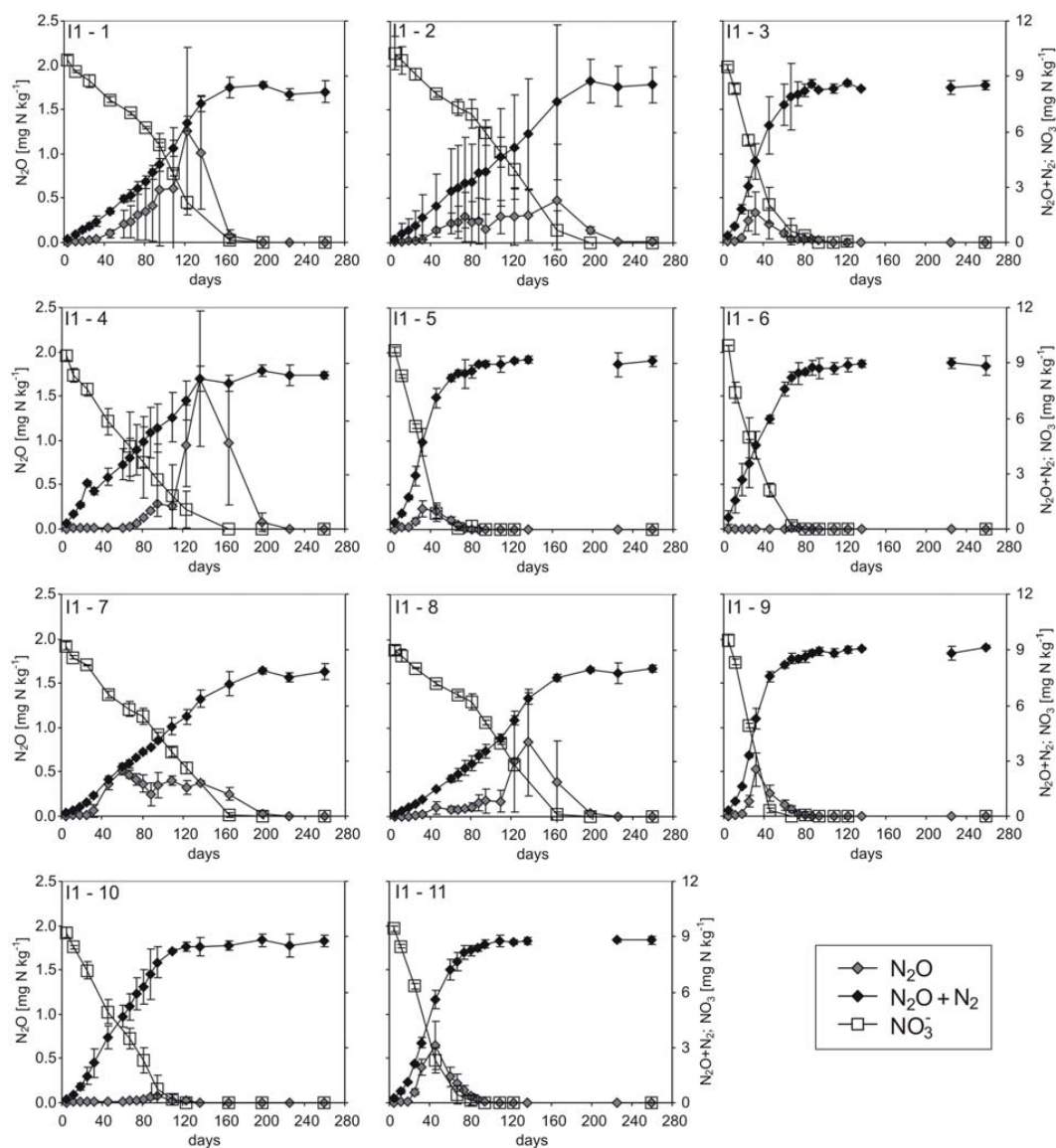


Fig. 3. Concentration courses of N₂O, N₂O+N₂, and NO₃⁻ during long-term anaerobic incubation of aquifer material from the autotrophic denitrification zone. The symbols denote the means of 4 replications and the error bars represent the standard deviation.

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 total sulfur content of the aquifer material, but interestingly also with organic carbon. Organic carbon was highly correlated with the clay content, but not with total 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.

3.3 Kinetic rate constants of N₂O production and reduction

All data sets with the calculated rate constants and the corresponding fitting parameters are listed in Table 4 (sequential 3-parameter fit) and Table 5 (1-step 3-parameter fit). As expected from the time courses of the NO₃⁻ and the (N₂O+N₂) 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

Table 2. Maximum N₂O concentrations (cN₂O_{max}), cN₂O_{max}-to-(N₂O+N₂) ratio, and reaction rates (D_i , D_{max} , SO₄²⁻ PR) 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 (N₂O+N₂) curve. SO₄²⁻ PR represents the sulfate production rate calculated for the samples from the autotrophic denitrification zone in N equivalents.

Sample location	Depth interval [m]	Denitrification zone	cN ₂ O _{max} [μg N kg ⁻¹]	cN ₂ O _{max} -to-(N ₂ O+N ₂) ratio	D_i	D_{max}	SO ₄ ²⁻ PR
					[μg N kg ⁻¹ d ⁻¹]		
B1	2.0–2.6	“heterotrophic”	2.37	0.027	0.46	2.70	n.d. ¹
B1	2.6–3.0		12.75	0.069	0.59	2.46	n.d. ¹
B1	3.4–4.0		79.32	0.110	3.35	13.33	n.d. ¹
I1–S1	1.5–2.0		23.35	0.026	8.61	35.16	n.d. ¹
I1–S1	2.0–2.5		36.79	0.379	1.12	3.97	n.d. ¹
I1–S1	2.5–3.0		79.28	0.415	0.74	4.57	n.d. ¹
I1–S2	1.5–2.0		5.50	0.026	1.61	11.93	n.d. ¹
I1–S2	2.0–2.5		19.44	0.258	0.85	4.67	n.d. ¹
I1–S3	1.5–2.0		5.32	0.004	13.33	30.60	n.d. ¹
I1–S3	2.0–2.5		2.46	0.018	0.43	3.51	n.d. ¹
I1–S3	2.5–3.0		58.53	0.118	0.22	6.52	n.d. ¹
I1–1	6.5–7.0	“autotrophic”	1257.95	0.195	43.19	77.04	60.66
I1–2	6.5–7.0		482.74	0.063	50.93	61.24	58.44
I1–3	6.5–7.0		330.47	0.075	84.65	177.63	125.90
I1–4	6.5–7.0		1698.03	0.208	78.43	166.55	41.64
I1–5	6.5–7.0		239.15	0.051	86.52	257.73	125.50
I1–6	6.5–7.0		11.07	0.001	148.05	156.57	184.60
I1–7	6.5–7.0		520.17	0.192	34.40	61.35	59.08
I1–8	6.5–7.0		836.24	0.131	30.30	39.71	36.01
I1–9	6.5–7.0		525.60	0.100	77.71	283.56	152.15
I1–10	6.5–7.0		77.32	0.010	41.52	100.42	90.41
I1–11	6.5–7.0		647.00	0.115	57.21	168.48	130.41

¹ n.d.: not determined

I1–6, the sample with the highest D_i and practically no N₂O accumulation, yielded an outstanding high value for k_2 , indicating intensive N₂O reduction.

To analyse the fitting results, we chose the data set “I1–S2 2.0–2.5” as a representative example that is shown in Fig. 4. Since the time courses of N₂O concentration result from the balance between production (k_1) and reduction of N₂O (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 (N₂O+N₂) (Eq. 7). 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 N₂O-curve. We emphasize that fitting for best agreement of measured and modeled N₂O curves (compare 1-step 3-parameter fit in Fig. 4 with experimental data) did not yield a satisfactory agreement for the NO₃⁻ and (N₂O+N₂) curves, respectively.

In order to ensure that the cumulative curve of (N₂O+N₂) 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 N₂O+N₂ and in a second step we determined k_2 by the N₂O-curve. As can be seen in Fig. 4b, we then obtained an excellent fit to the time course of (N₂O+N₂). However, the goodness of fit of the N₂O-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 sample I1–S2 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 N₂O- and to the (N₂O+N₂) curves was insufficient, indicating that the constant C_0 (Eqs. 7–9) is not given

Table 3. Spearman rank correlation coefficients between the parameters obtained from the laboratory incubation experiments for the heterotrophic and the autotrophic data set. Clay was not detectable in the aquifer material of the heterotrophic denitrification zone and was thus excluded from the correlation analysis.

	<i>C_{org}</i>	Total N	C-to-N ratio	DOC	<i>C_{hws}</i>	Total S	Clay	<i>cN₂O_{max}</i>	<i>cN₂O_{max}-to-(N₂O+N₂) ratio</i>	<i>D_i</i>
correlation coefficients between parameters of heterotrophic denitrification										
Total N	0.22 ns									
C-to-N ratio	0.27 ns	-0.69 ²								
DOC	0.32 ns	-0.34 ns	0.65 ¹							
<i>C_{hws}</i>	0.67 ¹	0.69 ¹	-0.17 ns	0.45 ns						
Total S	-0.15 ns	0.44 ns	-0.62 ¹	0.44 ns	0.33 ns					
<i>cN₂O_{max}</i>	-0.08 ns	0.51 ns	-0.64 ¹	-0.82 ²	0.02 ns	0.34 ns				
<i>cN₂O_{max}-to-(N₂O+N₂) ratio</i>	-0.56 ¹	0.12 ns	-0.62 ¹	-0.681	-0.21 ns	0.50 ns		0.69 ²		
<i>D_i</i>	0.62 ²	0.24 ns	0.32 ns	0.19 ns	0.55 ns	-0.45 ns		0.15 ns	-0.23 ns	
<i>D_{max}</i>	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 ²
correlation coefficients between parameters of autotrophic denitrification										
Total N	0.33 ns									
C-to-N ratio	0.43 ns	0.54 ¹								
DOC	0.57 ¹	0.15 ns	0.29 ns							
<i>C_{hws}</i>	-0.38 ns	-0.11 ns	-0.20 ns	-0.33 ns						
Total S	0.15 ns	0.13 ns	-0.28 ns	-0.22 ns	0.19 ns					
Clay	0.82 ²	0.49 ns	0.07 ns	0.31 ns	-0.43 ns	0.47 ns				
<i>cN₂O_{max}</i>	-0.20 ns	-0.35 ns	0.04 ns	0.11 ns	0.15 ns	-0.35 ns	-0.48 ns			
<i>cN₂O_{max}-to-(N₂O+N₂) ratio</i>	-0.19 ns	-0.52 ns	0.17 ns	0.16 ns	0.25 ns	-0.38 ns	-0.54 ¹	0.94 ³		
<i>D_i</i>	0.64 ¹	0.66 ¹	0.66 ¹	0.15 ns	0.10 ns	0.53 ¹	0.65 ¹	-0.38 ns	-0.42 ns	
<i>D_{max}</i>	0.65 ¹	0.32 ns	0.32 ns	0.08 ns	-0.08 ns	0.52 ¹	0.72 ²	-0.17 ns	-0.21 ns	0.73 ²

¹ Correlation significant at the 0.05 probability level; ² correlation significant at the 0.01 probability level; ³ correlation significant at the 0.001 probability level; ns: not significant

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 N₂O 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 N₂ depending on depth. Whereas excess N₂ from denitrification was found to be low in the shallow groundwater, i.e. in the heterotrophic zone, the authors reported highest values for excess N₂ (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 N₂O 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 N₂O concentrations in the investigated in situ profiles at the wells B1 and I1 (Fig. 1b). More precisely, we identified a zone of considerable N₂O accumulation close to the groundwater surface which has been already reported by Deurer et al. (2008). Elevated N₂O 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 incubations indicate and Weymann et al. (2008) confirmed, this zone is characterised by low nitrate removal efficiency. The occurrence of N₂O accumulation or N₂O emission combined with low nitrate removal efficiency has also been described in other studies. Hefting et al. (2006) found significant N₂O 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 N₂O 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 NO₃⁻ levels favour N₂O accumulation due to inhibited N₂O reduction to N₂ (Blackmer and Bremner, 1978; van Cleemput, 1998; Šimek and

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 (N₂O+N₂)-data and the N₂O-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	k_2	$R^2(k_1)$	$R^2(k_2)$	$C_{0\text{fit}}$ [mg N kg ⁻¹]	$C_{0\text{fit}}/C_{0\text{exp}}$
		[days ⁻¹]					
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

Cooper, 2002). In the case of the FFA, it has been previously assumed that the pH of <5.5 (Deurer et al., 2008), intermediate O₂ concentrations below 3.15 mg L⁻¹ (Deurer et al., 2008), and the high NO₃⁻ concentrations (von der Heide et al., 2008) are further factors supporting the accumulation of N₂O in the near-surface groundwater of the FFA in addition to the low availability of 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 (Fig. 1f), (iii) intermediate O₂ concentrations (Fig. 1e) and (iv) elevated NO₃⁻ concentrations (Fig. 1a) explains the low nitrate removal efficiency in the near-surface groundwater as well as the considerable N₂O accumulation. Since N₂O concentrations in the unsaturated zone above the groundwater table are low, i.e. similar to ambient N₂O concentrations (von der Heide et al., 2009; Weymann et al., 2009), we want to emphasize that the described N₂O accumulation is a result of the in situ production in the groundwater. In contrast, the

unsaturated zone was identified as an N₂O source for British limestone aquifers (Mühlherr and Hiscock, 1998).

As mentioned in Sect. 3.1, we observed a sharp N₂O concentration peak in both profiles in the autotrophic denitrification zone in depths of 5 m and 6 m, respectively (Fig. 1). 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 NO₃⁻ load of the groundwater was still high (concentrations between 11 and 23 mg N L⁻¹) when it came in contact with the pyrite present in the autotrophic denitrification zone. Accordingly, N₂O was produced within an intensive nitrate removal caused by autotrophic denitrification. But, in contrast to the N₂O accumulation in the near-surface groundwater, we assume that the sharp N₂O concentration peak in both profiles is an indicator for rapid N₂O reduction which hampered an accumulation

of N₂O 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 NO₃⁻ and N₂O in depths below 5 m and 6 m, respectively. Thus, the autotrophic denitrification zone clearly functioned as a sink for N₂O. This is comparable with the findings of Blicher-Mathiesen and Hoffmann (1999) who reported an effective nitrate removal in a riparian fen without N₂O accumulation. It is important to note that the high nitrate removal efficiency in the autotrophic denitrification zone is most likely caused by practically anoxic conditions (Fig. 1e) and the high reactivity of the microcrystalline pyrite components (Kölle et al., 1985). Rivett et al. (2008) also identified O₂ and electron donor concentration and availability as the primary factors governing denitrification in groundwater. Furthermore, these authors pointed out that reduced iron (Fe²⁺) may serve as an electron donor for autotrophic denitrification in groundwater. Kölle et al. (1983) showed that there is some evidence for this reaction in the FFA. However, the mediating bacterium *Gallionella ferruginea* does require a small amount of O₂ for growth (Rivett et al., 2008) which is not a condition that is implicitly true for the predominantly anoxic autotrophic denitrification zone of the FFA. Since sulfate production and denitrification of nitrate obtained from the laboratory incubations are mostly in satisfactory agreement, we assume that reduced iron is probably of minor importance as an electron donor for denitrification compared to pyrite.

In summary, the vertical courses of NO₃⁻ and N₂O concentrations at the investigated wells plausibly reflect the occurrence of the separated denitrification zones in the aquifer. Taking the SO₄²⁻ concentrations into account (Fig. 1c), this conception is only confirmed by the gradient of I1. At this well, the increase of the SO₄²⁻ concentrations reflect the considerable sulfate formation capacity of the autotrophic zone (Table 2; Kölle et al., 1985). This was not observed at well B1. Low potassium concentrations between 2.0 and 2.5 mg L⁻¹ (well I1: typically 13.5-to-17.0 mg L⁻¹) indicate that the aquifer at this well is recharged by groundwater that infiltrated at forest or pasture areas. This groundwater is characterised by significantly lower concentrations of SO₄²⁻, N₂O, and NO₃⁻ 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 autotrophic denitrification zone at well B1. Furthermore, we found comparatively high SO₄²⁻ concentrations in the near-surface groundwater at the well B1 (Fig. 1c). This can be attributed to the intensive use of fertilisers containing sulfate ((NH₄)₂SO₄; (NH₄)₂SO₄·NH₄NO₃; Hansen, 2005). Thus, we do not interpret those high SO₄²⁻ concentrations as a result of autotrophic denitrification, because of the exhaustion of pyrite in the near-surface groundwater (von der Heide et al., 2008).

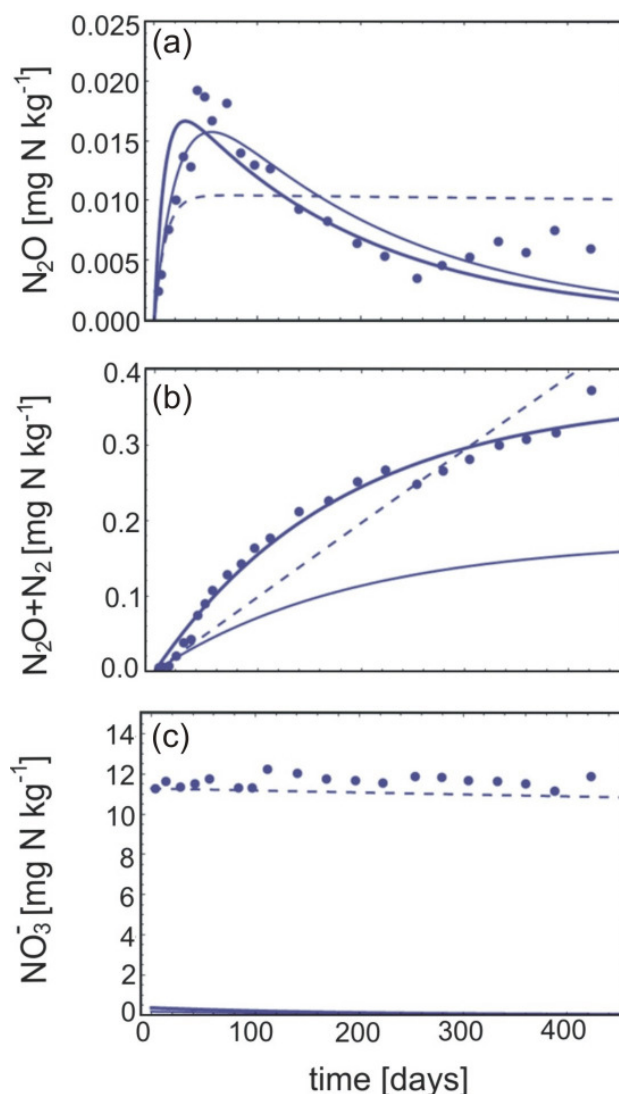


Fig. 4. Comparison between experimental N₂O- (a), (N₂O+N₂)- (b), and NO₃⁻ (c) concentrations (solid circles) and fitting curves (thick solid line: sequential 3-parameter fit; thin solid line: 1-step 3-parameter fit; dashed line: sequential 2-parameter fit) for the data set I1-S2 2.0–2.5 (heterotrophic 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.

4.2 Kinetics of N₂O production and reduction during long-term laboratory incubation

The results showed convincingly the substantial difference between the N₂O kinetics of heterotrophic and autotrophic denitrification. Among the factors governing denitrification, the initial NO₃⁻ concentration, anaerobic conditions, and pH were similar for the heterotrophic and the autotrophic aquifer material during anaerobic incubation. Variation in process dynamics between the heterotrophic and the autotrophic process was thus mainly caused by the differences in the electron

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	k_2	R^2	$C_{0\text{fit}}$ [mg N kg ⁻¹]	$C_{0\text{fit}}/C_{0\text{exp}}$
		[days ⁻¹]				
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

donors, i.e. organic carbon and pyrite 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 NO₃⁻ 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 NO₃⁻ 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 CL⁻¹ and were predominantly higher than the critical lower threshold of about

2–7 mg CL⁻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). We assume that labile, bioavailable DOC fractions were consumed in the unsaturated soil before entering the groundwater as it was shown for subsoils (McCarty and Bremner, 1992). However, von der Heide et al. (2010) reported significant negative correlations between DOC and N₂O concentrations in the near-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 N₂O accumulation by

decreasing bioavailability of DOC. This would require that DOC functions as an electron donor for the NO₃⁻-to-N₂O step of denitrification, but to lesser extent for the N₂O-to-N₂ step. As our data supply no evidence to confirm or contradict this, further research into the effect of DOC on N₂O accumulation in groundwater is needed.

In contrast to heterotrophic denitrification, the autotrophic process was not limited by its electron donor pyrite, but by its electron acceptor NO₃⁻. The availability of pyrite was sufficient to eliminate NO₃⁻ and N₂O completely in all samples. Hence, we stress that the laboratory incubations also confirm the role of autotrophic denitrification to function as a sink for NO₃⁻ and N₂O.

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 autotrophic denitrification zone. On the one hand, lignitic pebbles which are nonuniformly distributed throughout the 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 N₂O 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 pyrite is 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. The similar rates of SO₄²⁻ production and denitrification (Table 2) seem to support this assumption and confirm ongoing autotrophic denitrification for the samples from the autotrophic denitrification zone. However, sample I1–4 indicated that there is also potential for heterotrophic denitrification because the denitrification rates D_i and D_{\max} can not be completely explained by the comparatively lower SO₄²⁻ production rate. Hence, the reactivity of the lignitic pebbles and the question, to what extent a possible heterotrophic process in the autotrophic denitrification zone contributes to total denitrification, remain subjects of uncertainty.

NO₂⁻ did not accumulate during our incubation experiments, i.e. it was not detectable in any of the samples which were simultaneously analysed for NO₃⁻. We attribute this phenomenon to the pH, which was typically between 4.2 and 5.9 throughout the entire incubation experiments (data not shown). Van Cleemput (1998) stated that NO₂⁻ accumulation is favoured by high pH and high ammonium concentrations and – vice versa – is not stable in the presence of converse conditions (pH < 6.0). Furthermore, Laverman et al. (2010) did not observe NO₂⁻ production in batch experiments during incubation of river sediment slurries. NO as the precursor of N₂O is a further possible intermediate in the denitrification reaction chain. However, Firestone and Davidson (1989) reported that very little, if any NO, was detected as a product of denitrifying cultures. For soils, several studies revealed that NO from soils is mainly produced by nitrification of ammonium while N₂O mainly originates from the denitrification of nitrate (Davidson et al., 1993; Skiba et al., 1997; Russow et al., 2008). Furthermore, laboratory incubation experiments based on a kinetic ¹⁵N isotope method did not reveal NO as a free intermediate in the denitrification pathway (Russow et al., 2000). This is attributed to the limitation of diffusion in soil environments with high water content, i.e. to conditions favouring denitrification. NO produced is therefore rapidly reduced before it can escape from the cells (Skiba et al., 1997). We conclude that such “diffusion limitation” is even more valid for our laboratory approach with saturated aquifer slurries and that accumulation of NO was very unlikely. Since there is no evidence for the occurrence of NO₂⁻ and NO during our incubation experiments, we argue that the simplified two-step model of the denitrification pathway (Eq. 3) is valid for our laboratory approach.

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 N₂O production and reduction was clearly at the reduction side, yielding negligible N₂O 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 NO₃⁻ 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 NO₃⁻ which did not occur during our experiments due to the poor nitrate removing efficiency of heterotrophic denitrification. Furthermore, the

predominantly linear time courses of NO₃⁻ and (N₂O+N₂) during autotrophic denitrification (Fig. 3) indicate that the reaction kinetics is rather described by a zero-order than by a first-order model. Pättsch (2006) and Konrad (2007) reported in agreement that both kinetics can occur in one aquifer. Therefore, using only one modeling approach may include uncertainties (Pättsch, 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 N₂O more precisely. Moreover, an improved approach should also be able to incorporate the kinetics of NO₂⁻ reductase and NO reductase and to take into account pH and O₂ as governing parameters. This would enlarge the flexibility of the model and thus its applicability to a wider range of environmental conditions.

4.3 Transferability of laboratory incubations to field conditions

Did the laboratory experiments and the respective kinetic constants reflect denitrification and N₂O accumulation which are present in the groundwater? This question is generally 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 N₂O (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. 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 in situ concentration gradients (Fig. 1a) 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 N₂O, the subject of transferability has to be considered more differentially. For the autotrophic zone that was investigated at well I1, the field measurements yielded a maximum N₂O 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 $c_{\text{N}_2\text{O}_{\max}}$ 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 N₂O if the NO₃⁻ pool is exhausted, since N₂O was completely consumed during the last stage of denitrification progress (Figs. 1 and 3). On the other hand, $c_{\text{N}_2\text{O}_{\max}}$ measured during laboratory incubation of the heterotrophic aquifer material were considerably lower than the maximum N₂O concentrations we evaluated in the field. Whereas the median of the $c_{\text{N}_2\text{O}_{\max}}$ values measured during laboratory incubation (Table 2) was 0.02 mg N kg⁻¹, the averaged maximum N₂O 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 N₂O-fractions as a result of laboratory incubation in most of the investigated soils. Blicher-Mathiesen and Hoffmann (1999) also observed higher N₂O concentrations during their laboratory experiments due to a differing reduction pattern that supported N₂O accumulation. Another disagreement between laboratory and field data is evident from the increasing $c_{\text{N}_2\text{O}_{\max}}$ values of N₂O with depth during laboratory incubation (Fig. 2), whereas the field data indicate the highest N₂O accumulation in the uppermost groundwater (Fig. 1b; 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 N₂O production and reduction? 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, storage, and aerobic conditions during collection. This may alter the conditions for the microbial communities. For example, Laverman et al. (2010) reported that homogenized sediment slurries support higher denitrification rates compared to intact sediment “flow-through reactors”, due to an increase in the contact between microorganisms and the substrate. Another aspect is the difference in O₂ concentrations. Whereas O₂ concentrations were elevated in the near-surface groundwater (Fig. 1e), the laboratory incubations were carried out under anoxic conditions. We assume that the “intermediate” O₂ concentrations in the groundwater (Deurer et al., 2008) contribute to the considerable in situ N₂O accumulation, whereas anoxic

conditions present during the laboratory incubation experiment possibly supported a further reduction of N₂O to N₂. Thus, the transferability of the results obtained from the laboratory incubations to the in situ conditions should be interpreted with care for the heterotrophic denitrification zone. In fact, the influence of the mentioned 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 pyrite 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 N₂O 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 N₂O 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 N₂O 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 electron donor as well as anoxic conditions induce 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

N₂O is produced in the near-surface groundwater of the FFA as an intermediate of heterotrophic denitrification as well as in depths beyond 2–3 m below the groundwater table due to autotrophic denitrification. The heterotrophic process is limited by the availability of the electron donor organic carbon, low pH, and oxic conditions, yielding a low denitrification capacity. Field measurements indicated considerable N₂O accumulation especially in the uppermost groundwater. In contrast, laboratory incubations of aquifer material showed substantially lower N₂O concentrations than measured in the field. Thus, the transferability of the laboratory results to

the field scale is limited. We conclude that the discrepancy between field and laboratory conditions is due to the susceptibility of the sensitive heterotrophic process to sampling activities, storage of aquifer material, different O₂ levels and differences in spatial scales. The autotrophic process is characterised by a high denitrification capacity and not limited by its electron donor pyrite and the presence of oxygen, respectively. Laboratory and field data were found to be in good agreement showing that the autotrophic zone functions as a sink for N₂O. 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 useful to describe the kinetics of N₂O production and reduction more properly.

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