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ORIGINAL ARTICLE

New field technique to determine in-situ gross nitrification rates on an intact 4 m^2 scale on arable land

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Abstract Nitrification is one major part of the terrestrial nitrogen cycle and is responsible for the N supply to microbes and plants. Furthermore, it opens N-loss pathways. Quantifying actual gross rates of nitrification is of growing interest due to the risk of nitrate-N leaching into groundwater. Gross nitrification measurements are often conducted either in disturbed soils or in small intact soil cores. Both approaches can have methodological issues. Our study presents a newly developed technique at an intact 2 × 2 m^2 field scale that was tested extensively on agricultural (sandy) soils. The irrigation technique allowed for a uniform distribution of $^{15}NO_3^-$ using a tracer solution. It further enabled a calculation of gross nitrification rates directly in the field. The gross nitrification rates within the 4 m^2 plots were highly variable. Individual plots showed gross nitrification rates between 3.9 and 17.9 µmol kg^{-1} soil d^{-1}. At the chosen meter scale, the dependency of the nitrification rate on environmental and soil parameters could be observed. Nitrification was influenced by the mean soil temperature during field incubation. Nitrification rates normalized for temperature (20 °C) showed a negative linear correlation with the C/N ratio of the plots ($r^2 = 0.78$).

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Introduction

Nitrification is the microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) and a key process in the soil nitrogen cycle. The significance of nitrification in agricultural ecosystems is well documented (e.g. Cookson et al. 2006). Nitrification is an important process governing N availability for plant uptake and potential off-site N losses. While NH_4^+ will be strongly adsorbed to clay particles and organic matter, NO_3^- is significantly more mobile than NH_4^+ and vulnerable to losses by leaching (Abbasi and Adams 1998). This is especially important if high amounts of N are leached into groundwater. A better understanding of the nitrification process could improve nitrate input and adaptation assessments and with that groundwater protection measures (van Groenigen et al. 2015). In addition, nitrification promotes NO and N_2O formation, either directly as a by-product of NO_3^- formation or indirectly as a producer of substrate for denitrification (Arth et al. 1998). A thorough understanding of the nitrification processes in fields is important for developing effective climate protection strategies (Rütting et al. 2011, van Groenigen et al. 2015, Elrys et al. 2021).

When determining rates of nitrate production, it is necessary to record the gross turnover rate in addition to the net rate. Stark and Hart (1997) observed that net rates poorly predicted the gross nitrate production, because soil microorganisms had the capacity to assimilate the most of the nitrate produced. Loss by denitrification must also be considered. So high gross rates can be masked by high N consumption rates. The standard method for determining gross nitrification rates in soils is the ^{15}N-pool dilution technique (Kirkham and Bartholomew 1954). This method is well established and has been proven to be applicable in the laboratory to a wide range of soils (Murphy et al. 2003). Several studies (e.g. Rütting et al. 2011) point to factors that may impact ^{15}N dilution-determined gross nitrification rates as those control N-transformation processes. This strengthens the necessity of undisturbed experimental designs. Gütlein et al. (2016) found that sieving increases N mineralization whereas storage stimulates nitrification. Furthermore, taking soil cores may lead to an increase in root exudation (Rütting et al. 2011; Frank and Groffman 2009) and hence to higher nitrification rates. Booth et al. (2006) found that soils that are physically disturbed, are altered in N rates compared to intact soil samples. Arnold et al. (2008) criticized laboratory determinations for the following reason: when testing intact cores in the lab, incubations are often performed after cold storage and pre-incubation. This may cause N-cycling rates to differ from field conditions. Staelens et al. (2012) suggest that losses of ^{15}N may occurr via N leaching, gas emission, uptake and transport by roots and mycorrhizae, and diffusion to non-sampled soil that may alter N-transformation rates. Elrys et al. (2021) conducted a meta-analysis of more than 900 observations worldwide, summarizing many of the listed aspects. They found that the C/N ratio is the main controlling factor for the nitrification rate. Apart from the C/N-ratio, temperature is known to govern nitrification rates in soils. Recous et al. (1999) found a clear temperature dependency of gross nitrification rates. They used a Q10-value of 3.17 to calculate nitrification rates normalized for temperature.

Up to now, several studies have used ^{15}N pool dilution for quantifying gross nitrification rates in the field. Most of those studies used soil cores of different sizes (e.g. Davidson et al. 1991; Habteselassie et al. 2006; Dong et al. 2012). Only few worked without cores in intact soils at a plot-scale (e.g. Staelens et al. 2012; Zhu et al. 2013, Munera Echeverri et al. 2022). One reason for the limited number of studies in intact soils is that a uniform and homogeneous distribution of the tracer solution is difficult to realize in the field, in particular with respect to undisturbed, i.e. structured soils. However, according to Murphy et al. (2003) this is a prerequisite for determining gross nitrification rates. Several approaches of ^{15}N tracer applications have been published: e.g. (i) needle injection (e.g. Davidson et al. 1991), (ii) exposure to nitric oxide (NO) or ammonia (NH_3) gas (Stark and Firestone 1995; Murphy et al. 1997), (iii) dry addition (Willison et al. 1998) or (iv) flushing (application on the soil surface followed by irrigation, e.g. Geens et al. 1991). However, all of these approaches have their drawbacks. Needle injection is only applicable on a small spatial scale and can cause artificial macropores. Additionally, the added solution or gas has to propagate from the point of injection into the soil. Applying a multi-point soil injector using a cluster of needles (Hatch et al. 2000) is suggested to improve this method. Besides, an injection of ^{15}N labeled gas is a promising tool despite being seldomly used (Murphy et al. 1997, 2003) because of its specific preparation. Flushing is by far the most common method although it may (i) significantly change the soil water content and solute concentration, (ii) result in anaerobic conditions during flushing, and (iii) causes a preferential flow pattern and with that a spatially inhomogeneous ^{15}N labeling.

As outlined above, several studies have quantified gross nitrification using a ^{15}N pool dilution at the centimeter or decimeter scale (e.g. Davidson et al. 1991; Habteselassie et al. 2006; Rütting et al. 2011; Laine et al. 2018), but not at the meter scale. Because nitrification may already vary over small areas in the field (e.g. Mathieu et al. 2006), the aim of our study was to increase the scale from centimeter and decimeter to meter. Furthermore, we wanted to evaluate its representativeness. For this reason, we addressed two major questions: (i) what is a realistic in-situ nitrification rate in arable fields at a given time and scale? (ii) Which sampling procedure can determine nitrification rates adequately at the meter scale? Our study addressed these aspects by developing a technique to determine gross nitrification rates in the field. We applied this method to determine the spatial variability. In addition, we determined soil parameters

such as the pH, temperature, water content and C/N ratios to test whether they explained the variability of nitrification rates in the field. To our knowledge, our study presents the first results from a larger-scale field experiment on in-situ gross nitrification rates in intact soils.

Materials and methods

Site description

Three experiments were conducted on a total of 12 plots at two different study sites. Site A (Markhausen) is located in the northwest of Lower Saxony, Germany and site B (Fuhrberger Feld) near Hanover, Germany. The experiments were conducted on arable land. Plants were removed from trial plots prior to the experiments to improve the distribution of the tracer solution on the soil surface.

Site A: Markhausen

The study site, Markhausen, is part of the northern Geest, a flat landscape, where soils predominately formed from sandy parent material of glacial fluvial and aeolian origin. The predominant soil texture is moderately fine sand. The study area is under intensive agricultural use. It is further characterized by conventional arable farming and intensive livestock farming. The soils are classified as moderately acidic Gleyic Podzols, which have a fine sandy texture (sand 82%, silt 15%, clay 3%, Fishkis et al. 2020). The groundwater level is at a depth of 2.4 m. The mean annual precipitation is 808 mm and the annual temperature 9 °C.

Site B: Fuhrberger Feld

The Fuhrberger Feld, located approximately 30 km northeast of Hanover is a catchment area for Hanover's drinking water (from groundwater). These soils were formed by Quaternary sands, gravel and intercalated glacial till and loam, respectively. Typical groundwater levels are at 1–2 m depth. The dominant soil types are Podzols and Gleysols (Böttcher et al. 2011). The soil texture of the study area is sandy sand (more than 95% sand). Tilled arable agriculture has been the dominant land use for decades. The Fuhrberger Feld has been studied extensively with respect to water and mass fluxes (e.g. Böttcher et al. 2011; Deurer et al. 2008). The mean annual precipitation is 661 mm and the annual mean air temperature is 9.6 °C.

Experimental set-up

The three field experiments at sites A (1 plot in 2018) and B (3 plots in 2019 and 8 plots in 2021) presented in this study are identical in their basic methodology. However, they differ in some respects due to further methodological development and increasingly advanced objectives. Experiments on site A were performed to test the irrigation and tracer distribution by including the conservative tracer Br. The sampling pattern was supposed to be tested, as well. In 2019, experiments on site B were targeted to have an improved sampling design and better recording of the spatio–temporal extension of gross nitrification rates. In 2021, experiments on site B focussed on spatial heterogeneities of nitrification rates.

All experiments used the same irrigation system and similar low drip irrigation rates between 1.65 and 2.1 mm h^{-1}, simulating continuous rain. This was done in order to achieve a homogeneous distribution of the tracer solution in the soil. The main aim was to replace as much of the unbound soil water as possible by the labeled tracer solution in the topsoil. Again, this was done to ensure a homogeneous distribution throughout the soil. An irrigation system was developed by the Federal Institute for Geosciences and Natural Resources (BGR) which allows for a precise adjustment of watering intensities on an area of approximately 2.6×2.7 m^2. The tracer solution was pumped through 48 hoses with openings positioned in equidistance on a mobile panel by using two peristaltic laboratory pumps (IPC 24, Ismatec, Germany). This panel was repeatedly driven back and forth over the irrigated area by an electric motor (at a pace of ~ 2 cm s^{-1}). Crosswise, the panel was moved repeatedly back and forth (at a pace of ~ 3 cm s^{-1}) by an additional electric motor (see also Fishkis et al. 2020). This ensured a uniform distribution of the tracer solution on the irrigated area, confirmed by tests of the irrigation system that revealed a coefficient of variation of < 10% between the sum of the irrigated water volume on arbitrary chosen areas (75 cm^2) on the irrigated plot in less than one hour.

To prevent boundary effects on the experiment, the trial plot was placed into the inner 2 × 2 m^2 center of the irrigated area. In addition, the experimental site was covered the whole time of the field experiment in order to avoid disturbances by precipitation. In our study, the following time designations were used: t_0 to present the end of irrigation, t_1 as the first soil sampling time (= initial conditions for incubation) and t_2 as the second soil sampling time.

Experiment 1 (Markhausen): set-up and sampling procedure

The irrigation took place on March 12–15, 2018 with an irrigation rate of 1.65 mm h^{-1} and 57 h duration (approx. 90 mm, or 630 l). The irrigation solution was prepared by adding 2445 g $LiBr^-$ (Merck, Darmstadt, Germany), 25 ml of 99.9% ^2H_2O (Cortecnet, Voisins-Le-Bretonneux, France) and 152.9 g ^{15}N-KNO_3 (1.51 mol N) with 15.0 at. % ^{15}N (Cortecnet, Voisins-Le-Bretonneux, France) to 750 l (2.0 mmol l^{-1}) of tap water. The conservative tracer Br was applied to this irrigation solution to validate the method. On March 20–22, 2018, soil was sampled with an Edelman auger at 18 locations in the irrigation plot. The sampling was done to a depth of 50 cm, in increments of 10 cm, and in a regular arrangement to record the initial conditions for incubation (t_1-samples). Boreholes were refilled with quartz-sand after sampling. On April 3–5, 2018 (after an incubation time of 13 days), the soil was sampled again at 18 locations (t_2-samples) and close to the sampling locations of the first (t_1) sampling campaign (7–13 cm distance). The second sampling was conducted to a depth of 50 cm with increments of 10 cm. At each sampling point, approximately 300 g of soil was collected in five depths (0–50 cm in 10 cm increments). TDR-sensors (EasyTest, Lubiln, Poland) were installed at 20 and 50 cm depth to monitor soil moisture and soil temperature during the experiment in 30-minute intervals.

Experiment 2 (Fuhrberg I): set-up and sampling procedure

The field experiment was conducted from October 7–30, 2019. In one field, three plots were investigated and irrigated on October 7, 9 and 11, 2019. The KNO_3 irrigation solution had a concentration of 0.5 mmol N as nitrate with an abundance of 10.0 at. %. 630 l of the solution were irrigated within 43 h at plots 1 and 3 (2.1 mm h^{-1}). In contrast, the irrigation period had a duration of 49 h (1.8 mm h^{-1}) at plot 2. Based on experiences of the first experiment, five modifications were implemented in experiment 2: The sampling points of t_1 and t_2 were placed directly next to each other. A gouge auger (Ø 30 mm) was used. Instead of filling the holes with sand, PVC pipes with appropriate outside diameters were placed into the holes. A sample was combined from 2 subsamples. Only the depth interval from 5 to 25 cm was sampled. The top soil layer (0–5 cm) was discarded during sampling in order to avoid unnatural accumulation of the tracer solution due to water loss by evaporation. Due to the cold weather during the experiments, this concern was unfounded. This was not known at the planning stage. A randomized sampling design (stratified systematic unaligned sampling after Webster and Oliver 2007) was chosen for 16 points.

On October 21, 22 and 23, 2019, soil was sampled to record t_1-conditions at the 16 points of each plot. Soil samples were taken with an N_{min} drill from 5 to 25 cm soil depth. At each time step, two diagonally opposite points were sampled (Fig. 1) based on the "four-quarter" sample division method. On October 28, 29 and 30, 2019 (after an incubation time of 7 days), the soil was sampled again at the 16 locations (t_2-samples), but this time on the other two diagonally opposite points (Fig. 1).

TDR-sensors (EasyTest, Lubiln, Poland) were installed at depths of 5, 15 and 25 cm to monitor soil moisture and soil temperature during the experiment. The soil temperature was monitored with a PT100 Sensor at the soil surface.

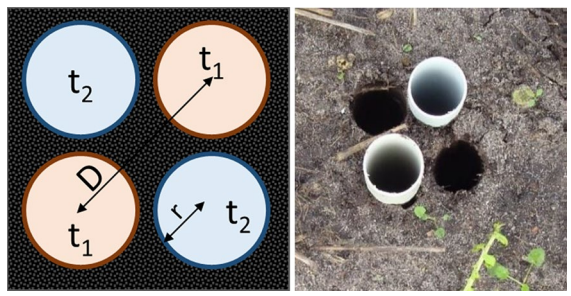

Fig. 1 Sampling scheme, with r (radius) = 1.5 cm and D (distance between t_1 and t_1) = approximately 5 cm

Experiment 3 (Fuhrberg II): set-up and sampling procedure

The 2021 field experiments were conducted from February 22 to March 29, 2021 and were very similar to the 2019 experiments. They were carried out on a total of 8 plots that were distributed across three fields. A ninth plot (plot 2c in field 2) had to be abandoned due to wind damage. The irrigation solution was prepared by adding 25 g ^{15}N-KNO_3 (0.25 mol N) with 10.0 at. % (Sigma Aldrich, Germany) and 25 g KNO_3 (0.25 mol N) with natural abundance to only 600 l water. This resulted in a concentration of 0.82 mmol l^{-1} with 5.2 at. %. The drip irrigation rate was 1.8 mm h^{-1}.

In field 1, plots a, b and c were irrigated on February 23, 25 and March 1, 2021. In field 2, plots a and b were irrigated on March 3 and 5, 2021 whereas in field 3, plots a, b and c were irrigated on March 17, 19 and 22, 2021. The first sampling (t_1) was conducted 4–6 days after the irrigation. The time span between the two sampling times was—depending on weather (and temperature)—between 3 and 5 days. Plots 2a and 2b were the exception, where unfavorable weather conditions caused an extension of 7 and 8 days, respectively.

Soil-moisture sensors (Teros 12; Meter, Munich, Germany) were installed at depths of 10 and 20 cm to monitor soil moisture and soil temperature during the experiment.

Laboratory soil and leachate analysis (experiments 1–3)

On the sampling day, the field fresh material was sieved at 4 mm in the laboratory to homogenize the sample and to avoid losses of ^{15}N. Plant residues were removed by hand during the sieving process. To prepare the soil for nitrate and ammonium measurements, samples were extracted using a 1 M potassium chloride (KCl) solution. 20 g of the field fresh soil material was extracted with 100 ml (experiment 1) or 40 ml (experiments 2 and 3), respectively, of the KCl solution. The mixture was shaken for one hour in an overhead shaker (20 rpm) and then centrifuged for 10 min at 3000 g. To determine NO_3^- concentrations and ^{15}N abundances at BGR, Hanover, Germany, 5 ml of supernatant was analyzed in duplicates using the SPINMAS technique (Stange et al. 2007). The measurements were carried out in an automated sample preparator (SPIN unit) and a GAM 400 quadropole mass spectrometer (InProcess, Bremen, Germany). NH_4^+ measurements were carried out on a subset of samples, but measured NH_4^+ concentrations were below the detection limit of the SPINMAS technique of 0.1 mmol l^{-1} (Stange et al. 2007). The water content in the field fresh soil was determined gravimetrically in 20 g soil.

Total organic carbon (C_{tot}) and nitrogen (N_{tot}) were analyzed in air-dried samples using an Elementar VarioMAX Cube Analyzer (Hanau, Germany). Soil pH and the electrical conductivity was measured in a 1:5 soil to water ratio using a SenTix 41 electrode (Weilheim, Germany) and a TetraCon 325 electrode (Weilheim, Germany), respectively.

The term recovery in this study is defined as the ratio of the calculated amount of the chemical (bromide or ^{15}N-NO_3) in the soil layer to the amount applied by irrigation (e.g. ^{15}N-$NO_{3\text{-layer}}$/^{15}N-$NO_{3\text{-irrigation}}$). To calculate the ^{15}N-amount in a soil layer, the calculated ^{15}N excess (measured ^{15}N abundance—natural abundance), the nitrate concentration, the measured water content and the soil density were used. In experiment 1, calculations for three soil layers of 10 cm thickness each were performed. In experiments 2 and 3, the recovery refers to the entire topsoil (soil layer 0–30 cm). Because open system conditions prevailed when determining the nitrification rates (both leaching below the topsoil and diffusion into the non-irrigated soil area are possible), recovery rates observed here are not comparable with recovery rates in ^{15}N experiments in closed or semi-open systems. Since the volume of irrigation was designed in such a way that the soil solution of the topsoil was completely replaced in any case, a transport of the tracer solution into the subsoil could not be avoided, in fact it was even desired.

Calculation of gross nitrification and consumption rates

To determine gross nitrification rates, the isotope dilution technique was implemented according to Kirkham and Bartholomew (1954) and calculations were conducted as follows:

$$n = \frac{N_0 - N}{t} \times \frac{log\left(H_0N/HN_0\right)}{log\left(N_0/N\right)} \quad (1)$$

where n is the nitrification rate in µmol kg^{-1} d^{-1}, N_0 [µmol kg^{-1}] is the NO_3^--N content at time t_1, N [µmol kg^{-1}] is the NO_3^--N content at t_2, H_0 [µmol kg^{-1}] is the content of the labeled $^{15}NO_3^-$ at time t_1 and H [µmol kg^{-1}] at time t_2, respectively; t [d] is the time of incubation. Consumption rates c were calculated by subtracting net rates from gross rates:

$$c = n - \left(\frac{N - N_0}{t}\right) \quad (2)$$

Nitrification and consumption rates were determined separately for every sampled point.

Calculation of temperature-normalized nitrification rates

The effect of soil temperature on the nitrification rate is commonly described by a temperature factor. In order to compare the individual plots with each other, a temperature-normalized turnover rate was calculated by dividing the determined nitrification rate by the temperature factor (Eq. 3). We used a temperature response function and the parameters of Stange and Neue (2009) for mineral fertilized soil

$$n_{temp\_norm} = \frac{n}{f(T)} = \frac{n}{\left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)^a * e^{a * \frac{T - T_{opt}}{T_{max} - T_{opt}}}} \quad (3)$$

where n_{temp_norm} is the temperature-normalized nitrification rate in µmol kg^{-1} d^{-1}, n is the nitrification rate in µmol kg^{-1} d^{-1}, f(T) is the temperature factor 0–1 [-], T is the averaged soil temperature during the field experiments [°C], T_{max} is the maximum temperature for nitrification of 40 °C, T_{opt} is the optimum temperature of 30 °C, a is the shape parameter of 1.8.

A reference temperature of 20 °C was chosen because laboratory experiments are often carried out at this temperature.

Uniform distribution of ^{15}N-NO_3^- was assessed by investigating the homogeneity of the tracer distribution, i.e. the irrigation procedure and the spatial distribution of the tracer solution in the soil recorded by the conservative tracer Br, soil hydraulic data and the measured distribution data of ^{15}N after irrigation.

Results

Experiment 1

Soil properties

The soil water content at sampling time t_1 varied from 15.1 to 16.2%, 15.0 to 16.7% and 10.7 to 16.3% for soil depths of 0–10 cm, 10–20 cm and 20–30 cm, respectively. The water content decreased at sampling time t_2 to 13.0–14.4%, 13.7–14.9% and 13.5–15.2%. During incubation, the average soil temperature was 5.9 °C.

The pH values of the topsoil showed moderate acidic conditions (Table 1, minimum pH 4.74, maximum pH 5.04) typical for sandy soils under intensive agricultural management. C_{tot} and N_{tot} showed values of 2.63% C and 0.15% (Table 1).

Distribution of the tracer solution in experiment 1

The average Br^- content in the Ap horizon varied between 4.03 and 4.89 mmol kg^{-1} as well as 3.87 mmol kg^{-1} and 4.70 mmol kg^{-1} in soils sampled at t_1 and t_2, respectively (Fig. 2). In 30–50 cm depth, Br^- contents ranged between 1.88 and 2.75 mmol kg^{-1} as well as between 2.20 and 2.91 mmol kg^{-1} for the two sample times. Results showed that less of the tracer solution reached

Table 1 ^{15}N recovery, pH, C, N and nitrification rates over depths in the plot of experiment 1

Depth	^{15}N Recovery [%], t_1	^{15}N Recovery [%], t_2	pH (H_2O)	C [%]	N [%]	C/N	Gross Nitrification [µmol kg^{-1} d^{-1}]	Net Nitrification [µmol kg^{-1} d^{-1}]
0–10 cm	19.0	18.7	–	–	–	–	9.2 ± 4.7	8.6 ± 12.1
10–20 cm	18.0	15.6	4.9 ± 0.1	2.63 ± 0.08	0.15 ± 0.01	17.8 ± 0.2	7.8 ± 6.4	3.4 ± 8.1
20–30 cm	16.0	15.7	–	–	–	–	5.9 ± 5.6	6.5 ± 9.9

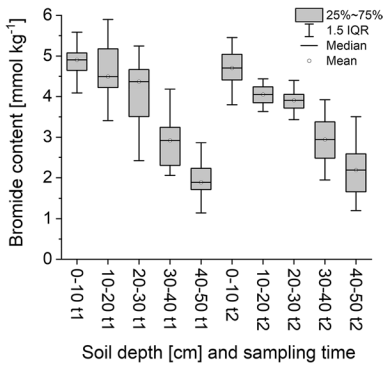

Fig. 2 Box plots of bromide content over the sampled depths at sampling times t_1 and t_2

the subsoil (30–50 cm) compared to the topsoil (0–30 cm).

Gross NO_3^--N transformation rates

Changes in NO_3^--N contents and ^{15}N excesses were sufficient to calculate nitrification rates in the topsoil (0–30 cm). The average increase of NO_3^--N content was 80 µmol N kg^{-1}, corresponding to net nitrification rates of 8.6, 3.4 and 6.5 µmol kg^{-1} d^{-1} for the depths of 0–10, 10–20 and 20–30 cm (Fig. 3). Standard deviations exceeded average values in all depths, which indicates a high variation in the net rates. ^{15}N abundance decreased by 1.39 at. % on average (1.26 in the depth 0–10 cm; 1.34 in 10–20 cm and 1.58 in 20–30 cm) during 13 days of incubation. Summarized ^{15}N recovery in the topsoil decreased only slightly from 53% at time point t_1 to 50.0% at time point t_2. Changes in ^{15}N recovery in the soil depths 0–10 cm and 20–30 cm were almost negligible, reflecting the small consumption rates. The change in ^{15}N recovery in the soil depth 10–20 cm from 18.0 to 15.6% could also be seen in the difference between gross and net rates, i.e. the consumption (Table 1). With 7.6 µmol N kg^{-1} d^{-1} (standard deviation: 5.8), estimated mean gross nitrification rates were very low in the topsoil.

Experiment 2

Soil properties

For the soil samples from plots a, b and c, the soil water content varied at sampling time t_1 from 12.5 to 16.7%, 9.5 to 13.4% and 12.6 to 14.5%, respectively. The water content decreased by 0.6%, 0.5% and 1%, respectively, until sampling time t_2. During incubation, soil temperatures ranged between − 1.8 and 26.7 °C at the soil surface and between 5.8–21.7 °C, 8.5–16.9 °C and 9.2–16.9 °C in 5 cm, 15 cm and 25 cm soil depth, respectively.

The pH values differed significantly between the individual plots and were between 5.4–5.9, 5.0–5.5 and 6.1–6.9 for plots a, b and c, respectively. The electrical conductivity was similar between the plots (50 mS for plots a and b, and 63 mS for plot c). The mean C/N ratio was very similar in the three plots, but C_{tot} and N_{tot} showed differences between the plots (Table 2).

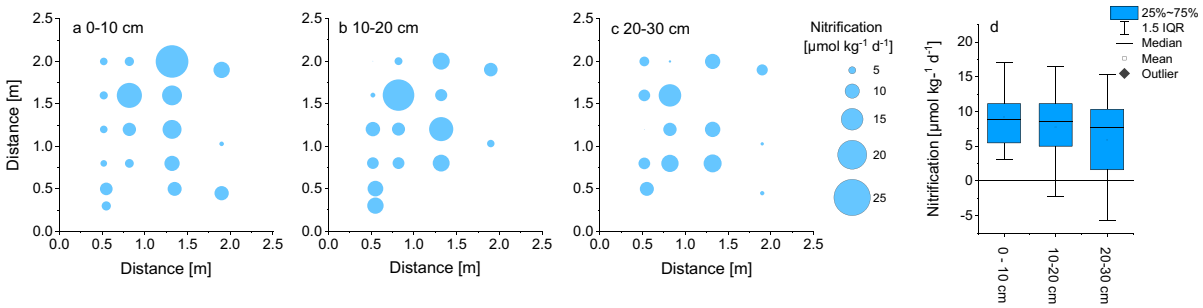

Fig. 3 Spatial distribution of nitrification rates in three depths (**a**) 0–10 cm, (**b**) 10–20 cm and (**c**) 20–30 cm, and (**d**) box plots of nitrification rates at the experimental plots at field A (Markhausen) in spring 2018

Gross NO_3^--N transformation rates

The recovery of ^{15}N in the soil of two of three plots decreased from time t_1 to time t_2 by 7% (plot 2a) and 3% (plot 2b), reflecting the detectable consumption rates. At plot 2c, the changes of the recovery of ^{15}N between t_1 and t_2 were almost negligible, which was also reflected in almost equal gross and net rates. Mean gross nitrification rates were between 9.5 and 13.7 µmol kg^{-1} d^{-1} for the plots (Table 2).

Experiment 3

Soil properties

In field 1, the soil water content varied from 16.2 to 21.0%, in field 2 from 12.9 to 15.9% and in field 3, from 9.2 to 13.7%, respectively. Mean water contents for the two times at the 8 plots are given in Table 3. The water content decreased by 0.5%, 1.0% and 1.3% in field 1, 0.3% and 1.1% in field 2, 0.5%, 0.7% and 0.5% in field 3, respectively, until sampling time t_2. In spring 2021, soil temperatures ranged between 2.5 and 17.7 °C at 10 cm soil depth for all measurements during incubation. The pH values were more acidic than in the experiments before and ranged between 4.1 and 5.4 (Table 4). The mean C/N ratios differed

Table 2 ^{15}N recovery, pH, C, N and nitrification rates the 3 plots of the field 2, experiment 2

	^{15}N Recovery [%], t_1	^{15}N Recovery [%], t_2	pH (H_2O)	C [%]	N [%]	C/N	Gross Nitrification [µmol kg^{-1} d^{-1}]	Net Nitrification [µmol kg^{-1} d^{-1}]
Plot 2a	30.7	23.1	5.6 ± 0.1	2.86 ± 0.13	0.14 ± 0.01	20.5 ± 0.3	13.7 ± 4.9	1.7 ± 9.9
Plot2b	21.3	18.0	5.2 ± 0.1	2.24 ± 0.08	0.11 ± 0.01	20.7 ± 0.9	9.5 ± 6.0	4.7 ± 6.8
Plot 2c	25.9	26.0	6.5 ± 0.2	2.92 ± 0.11	0.14 ± 0.01	20.5 ± 0.5	9.6 ± 5.7	8.2 ± 2.9

Table 3 Mean water contents and mean temperatures in the 8 plots of the three fields, experiment 3

	Field 1			Field 2		Field 3		
	Plot 1a	Plot 1b	Plot 1c	Plot 2a	Plot 2b	Plot 3a	Plot 3b	Plot 3c
Mean water content t_1 [%]	19.5	18.3	18.1	14.5	15.2	12.5	12.6	11.0
Mean water content t_2 [%]	19.0	17.3	16.8	14.2	14.3	12.0	11.9	10.5
Mean temperature [°C]	6.2	5.9	5.1	6.5	6.6	8.1	8.3	9.9
Temperature range [°C]	2.6–12.5	3.3–10.8	2.5–10.9	2.6–13.1	3.6–11.3	4.7–17.7	5.3–14.5	6.1–16.1

Table 4 ^{15}N recovery, pH, C, N and nitrification rates in the 8 plots of the three fields, experiment 3

	^{15}N Recovery [%], t_1	^{15}N Recovery [%], t_2	pH (H_2O)	C [%]	N [%]	C/N	Gross Nitrification [µmol kg^{-1} d^{-1}]	Net Nitrification [µmol kg^{-1} d^{-1}]
Plot 1a	28.0	25.6	4.6 ± 0.1	3.66 ± 0.28	0.19 ± 0.02	19.2 ± 0.3	3.9 ± 2.0	− 0.5 ± 6.2
Plot 1b	42.3	24.4	5.4 ± 0.1	3.17 ± 0.3	0.17 ± 0.02	18.8 ± 0.5	9.5 ± 2.3	− 34.2 ± 15.3
Plot 1c	27.2	22.9	5.2 ± 0.1	3.04 ± 0.28	0.15 ± 0.01	20.1 ± 0.5	5.7 ± 2.1	1.6 ± 4.1
Plot 2a	21.3	18.2	4.9 ± 0.1	2.78 ± 0.27	0.14 ± 0.01	19.7 ± 0.3	6.4 ± 1.2	8.1 ± 4.2
Plot 2b	18.6	14.0	4.4 ± 0.1	2.52 ± 0.21	0.12 ± 0.01	21.3 ± 0.4	3.9 ± 2.0	0.3 ± 5.0
Plot 3a	33.8	15.1	4.3 ± 0.2	1.65 ± 0.16	0.10 ± 0.01	16.3 ± 0.4	17.9 ± 3.5	− 31.8 ± 15.0
Plot 3b	27.9	21.6	4.9 ± 0.1	2.08 ± 0.23	0.12 ± 0.01	16.8 ± 0.3	12.8 ± 2.0	7.1 ± 14.9
Plot 3c	20.7	16.9	4.1 ± 0.1	1.62 ± 0.18	0.09 ± 0.01	18.4 ± 0.3	10.6 ± 7.7	11. 3 ± 18.8

significantly between the individual plots and were between 16.3 and 21.3 (Table 4).

Gross NO_3^--N transformation rates

At six of the eight plots, the recovery of ^{15}N in the topsoil decreased only slightly from time t_1 to time t_2 (Table 4). At the plots in field 1b and 3a, the decrease of recovery was high and corresponded with high negative net rates. We may therefore assume that a high consumption of nitrate, e.g. by denitrification, occurs at these two plots (Fig. 4).

With mean nitrification rates between 3.9 and 17.9 µmol N kg^{-1} d^{-1} at the eight plots (Figs. 5, 6

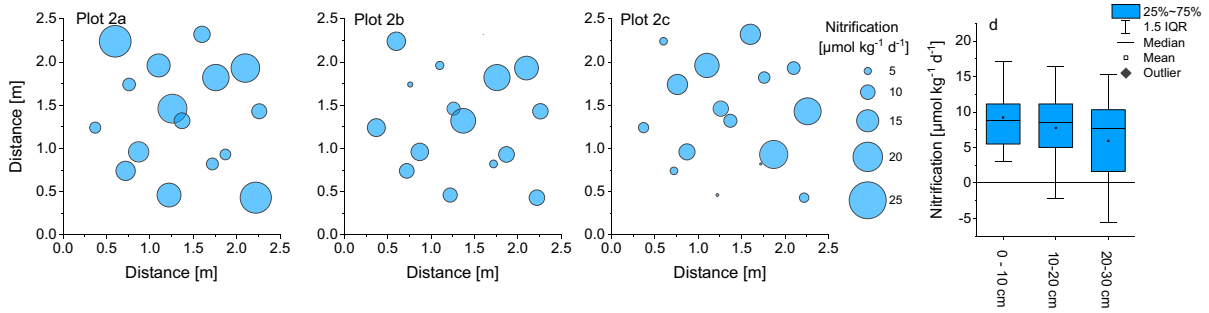

Fig. 4 Spatial distribution of nitrification rates measured in autumn 2019 at three experimental plots (**2a**, **2b**, **2c**) at Fuhrberger Feld, and (**d**) box plots of nitrification rates

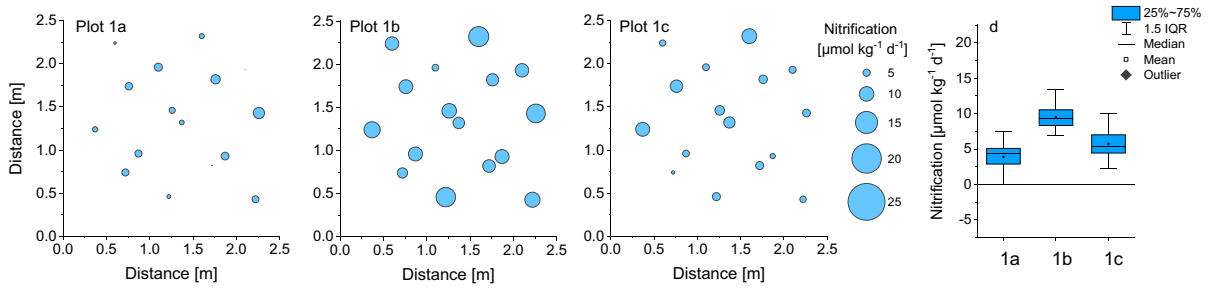

Fig. 5 Spatial distribution of nitrification rates measured in 3 plots (**a**, **b** and **c**) of field 1 at Fuhrberger Feld (in spring 2021), and **d** box plots of nitrification rates

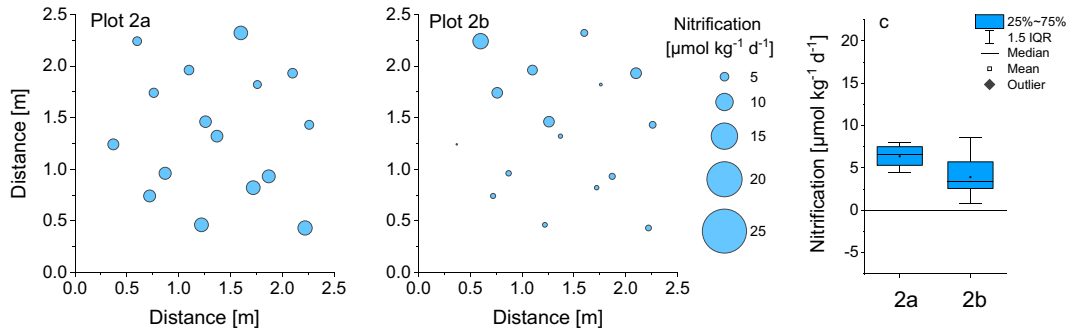

Fig. 6 Spatial distribution of nitrification rates measured at 2 experimental plots (**a** and **b**) of field 2 at Fuhrberger Feld (in spring 2021) (same field was investigated in autumn 2019), and (**c**) box plots of nitrification rates

and 7), the observed rates were very low and comparable with the rates recorded in experiments 1 and 2 (Figs. 3 and 4). However, the standard deviations for the individual plots in experiment 3 were lower than in the previous experiments. Plot 3c is an exception and showed a pronounced bipartite distribution. In plot 3c, the regions of low and high rates were well separated within the plot (Fig. 7 right). Despite being a region of significantly low nitrification, the highest overall nitrification rates were observed in plots 3 a-c. This coincides with the similar C/N ratios of 16.3, 16.8 and 18.4, respectively, observed on the three plots.

Discussion

Requirements for the application of the pool dilution method in the field

In the context of N turnover, variabilities in the distribution of nitrate concentrations observed in our study were in accordance with published variation ranges (e.g. Mathieu et al. 2006). In the subsoil, different observations were made: a strict gradient in the Br content showed that less soil water was replaced by tracer solution and an increase of the Br content over time could be found in these horizons. The observation of homogenous water transport in the topsoil and preferential flow in the subsoil (induced by different bulk densities) was confirmed by Diehl (unpublished). He investigated residence times and flow paths in the unsaturated zone at the same plot by soil hydraulic investigations. For this reason, we only focused on the topsoil in our experiments.

Similar recorded Br contents at times t_1 and t_2 demonstrate that a change in the $^{15}N\text{-}NO_3^-$ and nitrate concentration at times t_1 and t_2 was predominantly caused by turnover rates. In addition, it can be assumed from the similarity that no major changes took place between the two time points, t_0 and t_1, too. This suggests that an almost even distribution of the tracer solution in the Ap horizon was achieved by the irrigation due to the very low irrigation rate. During the experiment, no indications for hydrophobicity were found as no surface water formation was observed. This, however, was observed in irrigation system trials on other plots, where the irrigation system stopped and remained at the same place.

Regardless of the—for field experiments—homogeneous application and infiltration of the ^{15}N tracer solution into soil, sampling at time t_1 already showed a high variability of ^{15}N abundance and nitrate concentrations. This clearly showed that nitrification can be very variable at a small scale.

The staggering of the experiments and the adjustment of the experimental design allowed for an evaluation of the development. The time periods used in the experiments, the equilibrium time (from t_0 to t_1) and finally the incubation time (from t_1 to t_2) will be discussed as follows.

The time required to establish a moisture equilibrium in the soil varied between 6 days (experiment 1), 10–12 days (experiment 2) and 4–6 days (experiment 3). Since the equilibrium adjustment in the soil proceeds asymptotically and becomes slower with time, there is no ideal time. However, the results of experiments 1 and 2 showed that the decrease in moisture changed from an exponential to a quasi-linear course after 3–4 days. At the same time, it must be taken into account that soil processes labeling the NH_4^+ pool

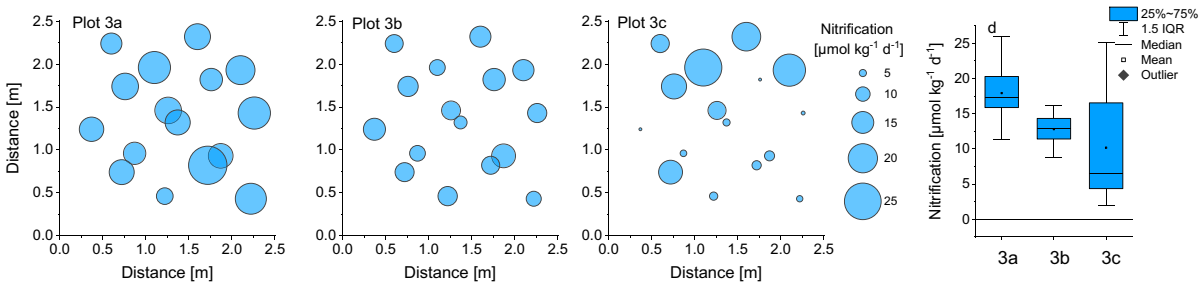

Fig. 7 Spatial distribution of nitrification rates measured at three experimental plots (**a**, **b** and **c**) of field 3 at Fuhrberger Feld (in spring 2021), and (**d**) box plots of nitrification rates

with ^{15}N (e.g. remineralization) was already possible at this point in time. The comparison between the calculated rates in field 2 of experiment 2 (autumn 2019) and experiment 3 (spring 2021) showed higher rates for autumn. In our view, the higher temperatures were a possible reason for this. However, it also shows that a labeling of the ammonium pool could not be ruled out. This applies especially to the equilibrium time, which was twice as long in experiment 2. Although this did not exclude the possibility that the calculation was affected (e.g. by remineralization or DNRA), the nitrification rates determined with longer equilibrium time and longer incubation times in experiments 1 and 2 matched those determined with significantly shorter equilibrium and incubation times (experiment 3). We therefore assume that an equilibrium time of 4 days is a good compromise for the temperatures and soil conditions that prevailed during our field trials. Future studies should, however, focus on the influence of this equilibrium time span on the nitrification rates.

The question of the incubation time (from t_1 to t_2) should also be considered critically. Since the ideal time period depends on the magnitude of nitrification, it remains difficult to predict. If it is too short, the uncertainties in calculating the rate increase because the change in ^{15}N abundance remains small. If it is too long, the NH_4^+ pool could be labelled by remineralization or other processes such as dissimilatory nitrate reduction to ammonium (DNRA). Wang et al. (2016) showed that immobilization of nitrate and DNRA were negligible in most studied temperate grassland, except the soils with high soil organic carbon (SOC > 4%) or high mean annual precipitation. In our experiments, the incubation time varied from 13 days (experiment 1) to 3 days in the last plot of experiment 3. Since the incubation time was adjusted to the temperatures prevailing during the time, higher rates were to be expected on the plots with shorter incubation times. The incubation times were in the range of other field experiments (Laine et al. 2018; Murphy et al. 2003). Laine et al. (2018) used an incubation time of 7 days, whereas Murphy et al. (2003) suggested 2–6 days for the two subsequent soil extractions (assuming laboratory temperatures of at least 20 °C). Due to low ammonium concentrations in the investigated soils, it was not possible to determine the ^{15}N abundance in ammonium with the SPINMAS technique. However, there were no indications that substantial amounts of ^{15}N had entered the ammonium pool from the nitrate pool. Bengtson and Bengtsson (2005) showed that remineralization of microbial N occurred mainly at high NH_4^+ concentrations and further enhanced microbial growth due to N-fertilization. We therefore assumed that at low temperatures (below 10 °C) and in sandy soils, an incubation period of between 4 and 6 days would be favorable, and up to 10 days would be possible. If in the future the workload can be significantly minimized by pooled sampling. Several sampling campaigns should be conducted over a period of up to 20 days in order to investigate the dependency of rate calculation on the incubation time in the field. The large labeled area of 2×2 m^2 provides very good prerequisites for this.

Replacing the soil solution by irrigation water led to changes in the chemical composition and consequently to changes in nitrification. A possible disturbance due to an input of substances seems to be low as the irrigation water had drinking water quality and hence a low ion content. It resembled rainwater. On the other hand, a possible mobilization of substances by irrigation must be considered. This particularly affected easily soluble ions, which were washed out. However, ammonium, the substrate of nitrification, was adsorbed by soil and was therefore only slightly washed out. In addition, a large part of the ammonium that was nitrified in our experiments was delivered by N-mineralization during the incubation period. Hence, we consider the change in conditions for nitrification to be small. In addition, the method offers the possibility to control the ionic strength or to add substrate such as ammonium or amino acids.

NO_3^--N transformation rates

Throughout all of our experiments, we recorded low nitrification rates for the given boundary conditions (agricultural soil, mean soil temperature between 5.1 and 13.9 °C, no plant uptake). With 3.9–17.9 µmol kg^{-1} d^{-1}, the rates strongly deviated from values of 253 µmol kg^{-1} d^{-1} given as mean rates in a review by Stange and Neue (2009) and 400 µmol kg^{-1} d^{-1} for agricultural soils by Elrys et al. (2021). Gross nitrification rates determined by barometric process separation (BaPS) under field conditions ranged from 3.4 to 126.8 µmol kg^{-1} d^{-1} for the mineral fertilizer site and 0–84.0 µmol kg^{-1} d^{-1} and over unfertilized (> 100 years) control site

(mean ± standard deviation; 53 ± 31, 38 ± 22, respectively) (Stange and Neue 2009). As the BaPS method is a very different method for determining gross nitrification, Stange and Neue (2009) compared BaPS results on 18 samples using the ^{15}N pool dilution technique. They found high levels of agreement. In many studies (e.g. Silva et al. 2005; Cookson et al. 2006), minimal rates in individual experiments lay far above the rate determined here. Laine et al. (2018) compared the gross nitrification between a ploughed and no-tilled boreal clay soil in a 9-day field experiment using a 'virtual soil core approach'. The nitrification was 12-fold higher in a ploughed field compared to one with no-till. With 20 µmol kg^{-1} d^{-1} (ploughed) and 1.6 µmol kg^{-1} d^{-1} (no-till), this compared well to our rates. Cookson et al. (2002) observed that nitrification rates in untreated soils fluctuated between 17.9 and 81.4 µmol kg^{-1} d^{-1} during an observation time of 34 days (mean 55.9 µmol kg^{-1} d^{-1}). Studies that found greater mean nitrification rates conducted experiments either in finer textured soils with higher NH_4^+ contents (Davidson et al. 1991; Ruppel et al. 2006) or at higher temperatures (Hatch et al. 2000). Ruppel et al. (2006) additionally added ^{15}N-fertilizer as $(^{15}NH_4)_2SO_4$ to enrich the inorganic N-pool. This provided an additional substrate for microbes (Davidson et al. 1991). Very high gross rates of up to 10.4 mg kg^{-1} d^{-1} (corresponding 742 µmol kg^{-1} d^{-1}) were measured by Dong et al. (2012). These high rates might have resulted from different agricultural management techniques.

Spatial heterogeneity of nitrification rates

Bengtson et al. (2006) demonstrated that in a mixed beech-oak forest, gross N turnover rates correlated within a distance of a few metres. However, our attempts failed to fit a model to a semi-variogramme of nitrate concentrations or ^{15}N abundances in order to calculate the spatial distribution by kriging. No range with spatial dependency could be observed at the 2 × 2 m^2 scale. Hence, we had to assume that the individual points are stochastically independent. The microscale heterogeneity is the reason why it was virtually impossible to consider the two destructive samples t_1 and t_2 as one pair. In fact, studies addressing the variability of nitrification rates at this scale are only possible if non-destructive sampling methods for nitrate concentration and ^{15}N abundance are used. Being aware of this problem, we still formed pairs to illustrate this heterogeneity. The observed gross nitrification rates in all 12 plots varied substantially across the 2 × 2 m^2 plots. In principle, the method has proven to be suitable to map the variability of nitrification in the field. Whether the investigation scale of 2 × 2 m^2 is representative for assessing field-scale gross nitrification rates is adequate or not, could not be conclusively evaluated by our experimental set-up. The fact that the variability within a 2 × 2 m^2 plot was significantly higher than between plots, and the differences between two plots correlated with differences in soil properties, suggests that the meter scale seemed appropriate. In addition, most soil sampling scales are even smaller than our set-up. This suggests that our experimental set-up represented natural soil conditions and rates better than earlier studies. In the light of the strong site-specific differences of previously published gross nitrification rates, which are several orders of magnitude higher (Elrys et al. 2021), it is difficult to explain the similarity of nitrification rates across our investigated fields. Similar conditions during the incubation periods as well as the restriction to sandy soils of one region (Northern Germany) could be the reason for the small differences between the fields. Manipulation experiments with NH_4^+-fertilizer applications would be useful to test and improve the measuring system and to analyze whether the system may determine rates in the ranges of other previous studies.

For future studies, we suggest that rates at individual sampling points should not be determined. Instead, a 2 × 2 m^2 plot could considered as a representative section for the soil conditions present there. Pooled samples could be taken and mixed, e.g. pooling 25 subsamples per plot. This way high measuring efforts may be reduced. This also provides the possibility to investigate further questions, such as the effect of the incubation length or ammonium fertilization on nitrification rates.

Comparison of gross and net rates

While Dong et al. (2012) found a high correlation between gross nitrification and consumption, but hardly any net nitrification, Elrys et al. (2021) confirmed a high correlation between net and gross nitrification. Stark and Hart (1997) did not find the latter in their study. Their correlation of net and gross rate

was very low ($R^2 = 0.09$). As pointed out earlier, we found different scenarios, i.e. similar net and gross rates (= no consumption), net rates near zero (= high consumption) as well as values in-between in our study. Stark and Hart (1997) assumed that an addition of NO_3^- to the tracer may increase consumption rates due to microbial assimilation. Although different nitrate consumption processes cannot be separated in this study, it should be emphasized that microbial nitrate immobilization is certainly one of the most important. Due to the unsaturated conditions, even during irrigation and because we investigated sandy soils, denitrification was not expected to occur at high rates. However, hotspots as described in Parkin (1987) may contribute to the variability in nitrate consumption. Parkin (1987) showed that less than 1 per mill of 100 g soil is responsible for over 85% of the observed denitrification rate. However, with a sampling size of about 300 g soil, we assume that we do not capture this very small-scale variability. It is worth noting that even on the size-scale of the plots (2×2 m^2), we still observed significant variabilities in net rates (and thus in the process of consumption).

Reasons for the observation of low rates and dependency of nitrification rates on environmental and soil parameters

Especially in agricultural soils with high nitrifier abundance, substrate availability is a determining factor for the magnitude of the nitrification rate. In our study, exchangeable NH_4^+ was not detectable (by the SPINMAS technique) in any soil samples, not even in the Ap horizon. We therefore assume that nitrification in the investigated soils is substrate-limited. A good correlation between nitrification and N mineralization (ammonification) was found by Booth et al. (2005). Other studies showed that nitrification processes were stronger related to N mineralization than to amounts of NH_4^+ in soil (e.g. Stange and Neue 2009).

Apart from substrate availability, temperature is known to be a factor affecting nitrification rates in soil. Stange and Neue (2009) observed the lowest nitrification rates during winter, when temperatures were at a minimum. Cookson et al. (2002) also demonstrated that gross nitrification rates decreased with decreasing temperature in the range of 2–15 °C. This is consistent with temperatures measured during incubation in our study. Nevertheless, authors of both studies point out that the temperature sensitivity of gross nitrification rates should always be interpreted in the context of available substrate. Therefore the effect of temperature may be caused indirectly due to the temperature depended N-mineralization (ammonification) and resulting substrate limitation.

The mean nitrification rates of the 12 plots investigated in our study showed a trend with the mean temperature during the study period t_1-t_2 (Fig. 8). However, the general trend cannot be statistically validated by regression analysis. At a significance level of 0.05, the slope was not significantly different from zero. For study field 2, which was investigated for the first time in autumn 2019 and for the second time in spring 2021, factor 2 between mean soil temperatures may also explain factor 2.1 in the calculated nitrification rates. This shows how important comparable temperatures are when comparing nitrification rates of individual studies. Thus, in order to compare the individual plots with each other, a temperature-normalized turnover rate at 20 °C was calculated for each plot (Fig. 9). The normalized rates showed a strong correlation with the C/N ratios observed in the plots. Furthermore, the determined slope was significantly different from zero at a significance level of 0.05. Thus, we were able to confirm the above mentioned observations of Elrys et al. (2021). In a meta-study, they collected nitrification rates from all over the world and also showed that nitrification rates depended strongly on the C/N ratio.

The soil water content controled nitrification processes because of its significance for the diffusional supply of NH_4^+ and oxygen for microbes (Robertson et al. 1999; Norton and Stark 2011). In our study, its influence on nitrification rates was expected to be

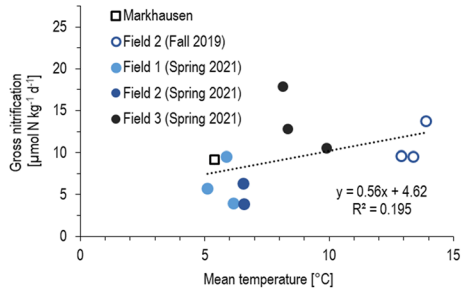

Fig. 8 Observed nitrification rates at the 12 plots measured in this study as a function of the average soil temperature between sampling times t_1 and t_2

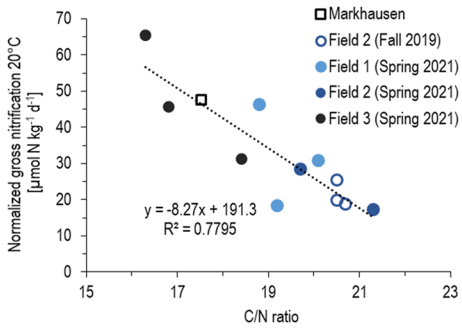

Fig. 9 Observed temperature-normalized nitrification rates at the 12 plots as a function of the average C/N ratios of the plots

minimal due to an adequate water supply after irrigation. Water contents were in the range of the field capacity and consequently in an optimal range for nitrification. Cookson et al. (2006) could not find a relation between the soil moisture and microbial community structures in sandy soils.

The determined pH values of the investigated plots range between 4.1 and 6.5. This acidic to nearly neutral conditions are typical for sandy soils under agricultural use. Since a rather wide range is covered, it is somewhat surprising that no correlation between the pH values of the plots and the nitrification rates could be observed. Many studies show that the pH can be an important factor influencing nitrification (Zhang et al. 2015; Elrys et al. 2021). Yet Booth et al. (2005) found no clear effect of pH on nitrification.

Conclusions and outlook

The developed irrigation technique with a low irrigation rate and a prevention of preferential flow allowed for a uniform distribution of $^{15}NO_3^-$ using a tracer solution in the topsoil. The technique allowed for a calculation of reliable gross nitrification rates based on the pool dilution approach directly in the field. Our calculated gross nitrification rates seemed realistic as the newly applied technique fulfilled the criteria for ^{15}N pool dilution and only had a minimum impact on the investigated system. Our study provides a basis for further in-field investigations of gross nitrification as well as for source identification of N_2O emission and/or quantification of N_2 emission.

A disadvantage was that the soil water content was defined by irrigation and could not be varied in the field. In addition, the time required for equilibration and, if necessary, longer incubation times might underestimate gross nitrification and should be investigated additionally in the future.

The chosen irrigation scale of 2×2 m^2 appeared to be more appropriate for field investigations of gross nitrification rates than for cores only. We provided a profound dataset for the observed scale of 2×2 m^2 and suggest for future studies that pooled samples could be taken to calculate the gross nitrification rate for 2×2 m^2 plots.

The shift from decimeter to meter scale appeared to lead to a better robustness of the calculated rates. However, subsequent studies should also address a scale of at least an order of magnitude higher (e.g. 100 m^2 or ha) in order to cover realistic field scales.

Novel methodological developments are necessary to improve the estimation of the spatial distribution of gross nitrification rates. Non-destructive sampling methods should be used to measure in exactly the same places as it became evident in our ^{15}N pool dilution study that samples t_1 and t_2 were too independent of each other to represent a common point. Future developments and new field methods will show whether field rates can be further confirmed and are comparable to laboratory measurements.

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