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Gross nitrification

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Seasonal variation of gross nitrification rates at three differently treated long-term fertilisations sites

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Abstract

The formation of nitrate (nitrification) in soils is an important process that influences the form of N available for plant uptake and the potential off-site N losses. Gross nitrification is one of the main sources of nitrous oxide (N₂O) and nitric oxide (NO) from soils.

5 A field experiment was designed to verify the idea that gross nitrification rates in soils can be readily predicted by a model approach where seasonal variability is described only by soil moisture and soil temperature and the magnitude of gross nitrification is controlled by the soil organic matter (SOM).

10 Gross nitrification rates were measured by a Barometric Process Separation (BaPS), first described by Ingwersen et al. (1999). The BaPS measurements were validated with the commonly used ¹⁵N pool dilution technique measurements at six times. In general, the rates determined from both measurement approaches were in the same order of magnitude and showed a good correlation.

15 The effects of three different fertilisations (mineral fertiliser, manure and the control) over more than 100 years on gross nitrification rates were investigated. During 2004 soil probes from the long-term “static fertilisation experiment” at Bad Lauchstädt were sampled weekly and were measured in the laboratory under field conditions and subsequently under standardised conditions (16°C soil temperature and –30 kPa matrix potential) with the Barometric Process Separation system (BaPS). Gross nitrification rates determined by the BaPS-method under field conditions showed a high temporal variability and ranged from 5 to 77 μg N h⁻¹ kg⁻¹ dry mass, 2 to 74 μg N h⁻¹ kg⁻¹ dry mass and 0 to 49 μg N h⁻¹ kg⁻¹ dry mass with respect to manure, mineral fertiliser and control. The annual average was 0.32, 0.26 and 0.18 g N a⁻¹ kg⁻¹ dry mass for the manure site, mineral fertiliser site and control site, respectively. On all sites gross nitrification revealed a strong seasonal dynamic. Three different methods (a temperature and soil moisture dependency model from Recous et al., 1998, a multiple linear regression and the method proposed in this paper) were applied for reproducing the measured results. On the manure site 78% to 80%, on the mineral fertiliser site 66%

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to 72% and on the control site 39% to 56% of the observed variations could be explained by the tested models. Gross nitrification rates determined under standardised conditions did not show any seasonal trends but did also however reveal high temporal variability.

1 Introduction

It is commonly accepted that a firm understanding of the basal biogeochemical processes related to the soil N cycle represents the prerequisite to meet the challenge of developing mechanistic models which will prospectively allow a more reliable prediction of N gas emissions from agricultural soils (Murphy et al., 2007). Furthermore, it was already well documented by numerous studies that (based on the following two aspects) the biological process of nitrification represents a key process of N turnover and N removal from agricultural ecosystems (e.g. Vitousek et al., 1979; Currie, 1996; Müller et al., 2004b; Cookson et al., 2006) and should therefore be implemented more in detail in the next generation of N cycle models. First of all nitrification is known to promote nitrogen eluviation from soils, because the less mobile cation ammonium (NH_4^+) is oxidised by nitrifier species in a two step reaction via nitrite (NO_2^-) to the well mobile and leachable anion nitrate (NO_3^-) (Abbasi and Adams, 1998). Secondly, nitrification is also known to be one of the main sources of nitrous oxide (N_2O) and nitric oxide (NO) released from agricultural soils (Russow et al., 2008a) – either directly as a by-product of nitrification (Firestone and Davidson, 1989) or indirectly through denitrification of the nitrate and nitrite supply by nitrification (Robertson and Tiedje, 1987; Khalil and Baggs, 2005; Baggs, 2008).

Numerous environmental parameters such as soil temperature, soil moisture, soil oxygen concentration, SOM content, NH_4^+ availability, pH-value etc. were shown to act as important physiological constraints and therefore to take control of nitrification rates in terrestrial ecosystems (Robertson and Tiedje, 1987; Booth et al., 2005; Cookson et al., 2006; Silva et al., 2005). In spite of the wide range of well known parameters that

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are potentially associated with nitrification rates only some of them appear expedient as model parameters. Booth et al. (2005) for example, identified soil C and soil N content as well as $\text{NH}_4\text{-N}$ availability and the N mineralization rate as the best predictors for modelling the nitrification rate, Cooksen et al. (2006) were able to demonstrate that from a total of 15 investigated soil parameters, soil moisture, soil temperature, NH_4^+ content, NO_3^- content, microbial N mass, microbial respiration rate, and dissolved organic N content were significantly correlated with the gross nitrification rate, whereby the best correlation was observed between gross nitrification rates and gross nitrogen mineralization rates. A significant positive relationship between the soil temperature and nitrification rates was found by Hoyle et al. (2006) and also by Breuer et al. (2002), whereas the latter additionally revealed a negative relation between the soil moisture and nitrification rate with regards to tropical forest soils. Zaman and Chang (2004) also confirmed the temperature dependency of the gross nitrification rate, but pointed out that in spite of moisture-induced variations in the nitrification rate, no consistent trend could be evidenced within a field capacity range of 50 to 100%. Considering the importance of temperature for N turnover in soils it is surprising that only a few studies have quantified the temperature dependency of the gross nitrification in soils (Murphy et al., 2003).

Zaman et al. (1999) demonstrated that the optimum soil water potential for gross nitrification rates was -10 kPa compared with -80 kPa and 0 kPa. Differences in nitrification rates at different water contents was more pronounced in the NH_4^+ fertilized treatment than in the unfertilized control (Zaman et al., 1999). Recous et al. (1999) investigated the influences of climate factors on gross N transformation in arable soils. Their main objective was to quantify the rates of gross mineralisation and immobilisation associated with the decomposition of crop residues. Nevertheless they also calculated the gross nitrification. Recous et al. (1999) argued that the differences in gross nitrification at eight times can be explained by temperature und soil moisture. Jamieson et al. (1998) highlighted the problems of separating the direct impact of temperature and moisture from the indirect impact of changes of N-availability through

mineralization and consuming processes like microbial immobilisation and plant uptake.

Based on these results we hypothesised, that soil temperature and soil moisture would explain most of the seasonal variation of gross nitrification in the field whereas soil organic matter (SOM) (C and N content in the soil and NH_4^+ -N availability) will be determined by the difference between the different sites. The main goals of this research were

1. to validate the gross nitrification measurements from the BaPS system with ^{15}N pool dilution measurements for arable mineral soils extensively,
2. to determine the influence of soil climate condition on the actual gross nitrification at three differently fertilised sites and to attest the causal relation,
3. to parameterize a model approach to explain the temperature and soil moisture relationship with gross nitrification in soils.

2 Materials and methods

2.1 Soil sampling

Soil sampling (5–10 cm soil depth) was carried out weekly (Mondays between 9 and 12 a.m.) over a period of one year (2004) at three differently treated agricultural sites (manure – D, mineral fertiliser – M, and control – C) that form part of the long-term field study “Static Fertilisation Experiment” at the experimental research station of the Helmholtz Centre for Environmental Research – UFZ in Bad Lauchstädt. The soil was classified as Haplic Chernozem (Altermann et al., 2005). Manure fertiliser and mineral fertiliser is applied at a rate of 30 t farmyard manure ha^{-1} every second year (according to 96 kg N $\text{ha}^{-1} \text{a}^{-1}$) and 111 kg N $\text{ha}^{-1} \text{a}^{-1}$, respectively. The control site remained unfertilised since 1902. Undestroyed soil cores (100 ml) were taken randomly from seven defined locations (sampling area 1 m^2) from each of the three agricultural

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sites. The actual soil temperature in the field was read from three permanently installed soil mercury thermometers at 5, 10, and 20 cm soil depth and this was then used as the incubation temperature in the laboratory. Within 1 to 3 h sampled soil cores were transferred to the laboratory.

5 In order to determine ammonium and nitrate 25 g of soil fresh from the field was extracted with 100 ml 1M KCl stirred on a rotary shaker for 1 h. Suspension was filtered through a fluted filter (0.2 μm , Schleicher & Schuell, Dassel, Germany) and filtrate was analysed on NH_4^+ and NO_3^- with the auto-analyser (Bran & Lübbe, Germany).

10 At frozen soil conditions (2nd, 5th, 9th and 10th week) no soil sample was taken except for in the 5th week. The frozen soil samples were very carefully transported to the laboratory to avoid thawing and to enable a measurement in the BaPS system in a frozen condition.

2.2 Gross nitrification rates determined by the BaPS

15 In general gross nitrification rates were measured by the Barometric Process Separation (BaPS) system, which simultaneously determines denitrification and soil respiration rates.

The theory of the determination of gross nitrification by BaPS technique is based on the determination of the total pressure change, as well as the changes of O_2 - and CO_2 -partial pressure in an isothermal gas tight system. Nitrification lead to a pressure decrease by net oxygen consumption, denitrification lead to a pressure increase, and soil respiration is neutral for pressure (for a respiration coefficient=1). The central equation of the BaPS method is

$$\Delta N_x O_y = \Delta n - \Delta O_2 - \Delta CO_2 \quad (1)$$

25 where $\Delta N_x O_y$ (mol h^{-1}) stands for the rate of N gases produced by denitrification. The symbol Δn (mol h^{-1}) denotes the net rate of the total gas production ($\Delta n > 0$) or consumption ($\Delta n < 0$), and ΔCO_2 (mol h^{-1}) and ΔO_2 (mol h^{-1}) are the net rate of CO_2

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formation and O_2 depletion, respectively, in the closed chamber's atmosphere. Since the total gas production (Δn), the net changes of O_2 (ΔO_2) and CO_2 (ΔCO_2) are measured, the production of N-trace gases ($\Delta N_x O_y$) via denitrification can be calculated. Afterwards gross nitrification rates can be deduced by solving the equation system inversely. Further details including discussion of uncertainties are given by Ingwersen et al., 1999, 2008; Breuer et al., 2002 and Müller et al., 2004a.

Fresh soil cores from the field were put into the BaPS system for 2 to 38 h to acclimatise under field conditions (soil moisture and soil temperature) and subsequently gross nitrification rates were determined during an 8 to 16 h BaPS run. Afterwards the BaPS measurement was repeated under standard conditions (16°C soil temperature, -30 kPa soil matrix potential). For this purpose soil cores were removed from the BaPS system and drained for 5 days by putting them on a ceramic plate at -30 kPa under pressure. If necessary, soil samples were also moisturised with N-free standard rain prior to drainage. Drained soil cores were then put back into the BaPS system and acclimatised for 2 to 38 h at 16°C before measuring.

At each step the water content was controlled gravimetrically and calculated after drying for 24 h at 105°C at the end of all measurements.

The calculation of gross nitrification, denitrification and soil respiration rates was carried out by using the original BaPS software (UMS, München, Germany), but taking into consideration a shortcoming in the calculation of the carbonate equilibrium as published recently by Ingwersen et al. (2008). The ratio of autotrophic nitrification to total nitrification was set to 1, as nitrification can be assumed to be predominantly carried out by autotrophic nitrifiers (Stange and Döhling, 2005). The ratio between N_2O - and N_2 -production by denitrification was set to 1:3 for this soil according to Wolf and Russow (2000).

2.3 Gross nitrification rates determined by the ^{15}N pool dilution technique

For comparison purposes a ^{15}N pool dilution technique (Kirkham and Bartholomew, 1954) was conducted simultaneously with the BaPS approach at 6 randomly selected

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points in time over the 2004 study period. In this context seven fresh soil cores from the field were at first disturbed carefully in small aggregates (<6 mm) and 20 ml ^{15}N solution (5 mM K^{15}NO_3 95.8 at %) was added to the aggregates by spraying it on. During the labelling procedure the soil was simultaneously mixed several times to ensure a homogenous labelling with ^{15}N . Afterwards soil aggregates were refilled in the seven steel cores and placed in the BaPS system for acclimatisation. In order to determine the initial ^{15}N abundance of labelled soil NO_3^- , one soil core was removed from the BaPS system 6 h after ^{15}N labelling and extracted as describe below. The remaining soil cores were measured three times with the BaPS system for a period of 12 h at 16°C . Soil extraction was then repeated with one soil core as follows. From the core 3, PE flasks were each filled with 25 g of fresh soil and 100 ml 1 M KCl solution was added. The samples were stirred on a rotary shaker for 1 h and the suspension was filtered through a fluted filter ($0.2\ \mu\text{m}$, Schleicher & Schuell, Dassel, Germany). $^{15}\text{N}\text{-NO}_3^-$ abundance and NO_3^- concentration in the extracted soil solution was determined by SpinMas (Stange et al., 2007). Sub sample of the remaining 5 soil cores were used to determine the soil water content.

2.4 Determination of N_2O emission

Since the 19th week of 2004 N_2O emissions from the soil cores were additionally measured through running the BaPS system. For this purpose N_2O concentration in the BaPS headspace was analyzed by gas chromatography (GC 14B FID/ECD; Shimadzu) at the beginning and the end of soil core incubation, where the soil N_2O release was calculated as the difference. The current air pressure, air temperature and headspace volume in the BaPS system were taken into consideration, whereas changes in N_2O concentration in soil water were not.

The N_2O emissions determined were used to calculate the N_2O leakage rate during nitrification which is the amount of $\text{N}_2\text{O}\text{-N}$ emitted per unit of oxidised $\text{NH}_4^+\text{-N}$. The

N₂O leakage rate was calculated as follows:

$$LR_{N_2O} = \frac{F_{N_2O}}{R_{nit}} * 1000 \quad (2)$$

with LR_{N₂O} – leakage rate for N₂O during nitrification [%]; F_{N₂O} – N₂O-emission [μg N kg⁻¹ soil h⁻¹]; R_{nit} – nitrification rate [μg N kg⁻¹ soil h⁻¹].

The leakage rate for N₂O during nitrification (R_{N₂O}) was only calculated when no denitrification was detected during the BaPS run.

2.5 Statistical analysis

Statistical analyses were performed using the STATISTICA 7 software. For the analyses of variance between the different treatments the T-test was applied. The coefficient of variation (COV) is defined as the standard derivation (SD) divided by the mean value and allows a comparison of the variation of measurements by different means.

2.6 Model approach

To determine the effect of soil temperature and water content on the nitrification rates:

1. The climatic factor proposed by Andrén and Paustian (after Recous et al., 1998) was used. The model is based on the exponential relationship between nitrification rates against temperature and soil water potential. Soil water potential in the field probes was estimated from the water content using the retention curve. The reference temperature used here was 10°C. The parameters were recalculated by multiple regression using STATISTICA.
2. A multiple linear regression with two factors (soil temperature and soil moisture was used). For the temperature the incubation temperature of the BaPS system and for the soil moisture the measured gravimetric water content were used.

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3. A two-factor model as described by Stange (2007) was used. Two different functions to describe the temperature response were tested (i.e. the Arrhenius function and the O'Neill function), and the optimum function by. The best results were obtained from the O'Neill function:

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

with $f(T)$ – effect of soil temperature 0–1; T soil temperature [°C]; T_{\max} – maximum temperature of the O'Neill function, fixed at 50°C; T_{opt} – optimal temperature of the O'Neill function [°C]; a – shape parameter of the O'Neill function; used to calculate the temperature response. The soil moisture response was described with the following function:

$$f(M) = 1 - e^{-\left(\frac{M}{M_{\text{crit}}}\right)^b} \quad (4)$$

with $f(M)$ – effect of soil moisture on gross nitrification 0–1; M – soil water content [% g water g⁻¹ soil]; M_{crit} – water content at the inflection point of the curve [% g water g⁻¹ soil]; b – sharp parameter of the function.

For combining temperature and moisture response function the mathematical approach published by Stange (2007) was used. The approach use the harmonic mean and is driven by the idea of a limiting factor as given by the Liebig's law. This was achieved by adding reciprocal values as follows:

$$g(f(M), f(T)) = \frac{2 * R_{\max}}{\frac{1}{f(T)} + \frac{1}{f(M)}} \quad (5)$$

with g – response function; R_{\max} maximal nitrification rate [$\mu\text{g N kg}^{-1} \text{ h}^{-1}$]; f – response functions for each factor (e.g. temperature or moisture).

Estimates of parameters for gross nitrification were conducted using the non-linear parameter estimate procedure in STATISTICA 7.

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3 Results

3.1 Site description

The soil was classified as Haplic Chernozem (Altermann et al., 2005). Its loess substrate comprises 21% clay, 68% silt, and 11% sand and the collected soil revealed a total N (N_t) of 0.19, 0.16 and 0.13% and a total carbon (C_t) content of 2.3, 1.9 and 1.6% for manure, mineral fertilisation, and the control, respectively. Soil samples were air-dried and sieved (2 mm) and the pH values determined for the sites in the distilled water ranged from 6.6 to 6.9, 6.5 to 6.7, and 7.4 to 7.8 for manure, mineral fertilisation, and the control, respectively. Detailed information on this soil type is provided by Altermann et al. (2005).

3.2 Comparison of BaPS and the ^{15}N pool dilution technique

In general, the gross nitrification rates calculated using the BaPS system showed a good correlation compared to values determined by the ^{15}N pool dilution technique (r^2 0.78, 0.95, and 0.88 for manure, mineral fertilisation, and the control, respectively). Nevertheless, both methods revealed strong variations for the gross nitrification rates determined. The COV for standardised gross nitrification rates measured by the BaPS system was 0.22 whereas the COV for standardised gross nitrification rates determined by the ^{15}N pool dilution technique was 0.30. Beyond these fairly large variations in the determination of the gross nitrification rates using both methods it should also be noted, that considering the manure site, gross nitrification rates calculated by the ^{15}N pool dilution technique on average only reached 70% compared to the rates determined by the BaPS system (Fig. 1).

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3.3 Gross nitrification rates on the three differently treated agricultural sites

Under field conditions

With respect to the annual course, a distinct seasonal trend with maximum gross nitrification rates during summer and minimum rates in the winter was observed at all three sites. In general the lowest gross nitrification rates were determined under frozen conditions at the beginning of February, whereas the highest rates were observed between calendar week 20 and 29.

Gross nitrification rates under field conditions determined by the BaPS system ranged from 5 to $77 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, from 2 to $74 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, from 0 to $49 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ for the manure site, mineral fertiliser site, and the control, respectively. On average the highest gross nitrification rate was found at the site fertilized with manure ($38 \pm 21 \mu\text{g N kg}^{-1} \text{h}^{-1}$), whereas the lowest mean gross nitrification rate was found at the unfertilised control site ($21 \pm 13 \mu\text{g N kg}^{-1} \text{h}^{-1}$). The mineral fertilizer site showed a mean gross nitrification rate of $30 \pm 17 \mu\text{g N kg}^{-1} \text{h}^{-1}$. The observed differences between the sites are statistically significant (t-test, $p < 0.01$). The coefficient of variation (COV) was comparable on the three sites with 0.55, 0.57, and 0.61 for the manure site, mineral fertiliser site, and the control, respectively.

Under standard conditions

Gross nitrification rates under standard conditions (16°C and -30 kPa matrix potential) (Fig. 3) ranged from 30 to $83 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, 19 to $67 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, and 18 to $53 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ with respect to the manure site, the mineral fertiliser site, and the control site, respectively. Furthermore, mean gross nitrification rates were $49 \pm 12 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, $43 \pm 10 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$, and $31 \pm 8 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ for the manure site, the mineral fertiliser site, and the control, respectively. High variations in the nitrification rates were observed at each site but the COV were almost equal between the three sites ranging from 0.24 to 0.25. As expected the observed COV

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under standard conditions are clearly smaller than in the measurements under field conditions. A small seasonal trend was only visible with respect to the control site. For the fertilised plots no clear trends or seasonality could be observed.

3.4 Modelling gross nitrification rates

5 Three different models were tested to explain the seasonal variability in gross nitrification rates in the field. Multiple linear regression analyses could explain the seasonal variability in the gross nitrification rates with 78%, 72%, and 47% for the manure site, the mineral fertiliser site, and the control, respectively. The new parameterised approach developed by André and Paustian (Recous et al., 1999; Ambus, 2005) could be
10 used to explain 78%, 72%, and 39% (M, D, and C, respectively) of the variability due to soil temperature and soil moisture.

The proposed approach (Eqs. 3 to 5) could explain 56 to 80% of the observed variations (Table 1). Measured and simulated mean values were almost equal (D: $37.2 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ vs. $37.6 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$; M: $29.8 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ vs. $29.8 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$; C: $21.4 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ vs. $21.5 \mu\text{g N h}^{-1} \text{kg}^{-1} \text{DM}$ for both
15 simulated and observed mean values, respectively). Nevertheless, the COV of the simulated gross nitrification rates (D: 0.49, M: 0.51, C: 0.47) were smaller than for those of the observed rates on all sites.

3.5 N_2O emissions

20 Determined N_2O emissions showed a very high temporal variability and ranged from 0 to $192 \text{ ng N h}^{-1} \text{kg}^{-1}$ dry matter (DM), 0 to $372 \text{ ng N h}^{-1} \text{kg}^{-1} \text{DM}$, and 0 to $16 \text{ ng N h}^{-1} \text{kg}^{-1} \text{DM}$ with respect to the manure fertiliser site, the mineral fertiliser site, and the control site. N_2O emissions were log-normal distributed and the emission from the control plot (C) differs significantly from the fertilised plots (D and M). The geometric mean emission rates were $8.8 \text{ ng N h}^{-1} \text{kg}^{-1}$, $10.7 \text{ ng N h}^{-1} \text{kg}^{-1}$, and $3.3 \text{ ng N h}^{-1} \text{kg}^{-1}$
25 (D, M, and C respectively). With respect to the N_2O emission rates determined at

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30 days from May to December, denitrification was observed at 6 to 9 times whereas only nitrification was witnessed at the remaining 21 to 24 times by the BaPS system. On days where denitrification was detected, the geometric mean N_2O emission rates were 10.7, 14.5, and $3.0 \text{ ng N h}^{-1} \text{ kg}^{-1} \text{ DM}$ with respect to manure, mineral fertiliser, and the control sites.

Geometric mean N_2O emission rates discarding the days where no denitrification was detected are 8.1, 9.7, and $3.4 \text{ ng N h}^{-1} \text{ kg}^{-1}$ for the manure, mineral fertiliser, and control sites, respectively. The calculated N_2O leakage rates during nitrification therefore ranged from 0.001 to 3.8‰ (Fig. 4), and were significantly site-specific (t-test, $p < 0.05$) with average values from 0.38, 0.96 and 0.20‰ for the manure, mineral fertiliser, and the control sites, respectively. N_2O leakage rates at the mineral fertiliser site were correlated with temperature and soil moisture (multiple regression: r^2 0.588, β_{temp} 0.415, β_{moisture} 0.766) whereas on the manure site N_2O leakage rates depended only on the temperature (r^2 0.435, β_{temp} 0.630). Neither of the two parameters correlated with N_2O leakage rates on the control plot.

4 Discussion

4.1 Comparison of the BaPS method and the ^{15}N pool dilution technique

To the best of our knowledge this was the first extensive validation of both approaches with respect to agriculturally-used soils. A two point validation for agricultural soils was published very recently (Ingwersen et al., 2008). In general a good correlation could be observed between the results of the BaPS measurements and the ^{15}N pool dilution technique (r^2 0.78, 0.95, and 0.88 for manure, mineral fertilisation, and the control, respectively). Notably, the gross nitrification rates calculated using the BaPS system depend greatly on the input parameter soil pH-value. The pH-values were measured in water after conducting the BaPS measurement, but were not determined in situ during the experiments. Ingwersen et al. (2008) pointed out that BaPS nitrification rates using

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the current pH value (measured in water) conformed better with the ^{15}N pool dilution technique measurements than BaPS calculations using potential pH values (measured in CaCl solution). Soil pH values measured using both methods can strongly differ i.e. in the investigated soil by up to 0.5 pH units (Altermann et al., 2005) which leads to differences of up to 50% in the calculated gross nitrification rates (Ingwersen et al., 2008). This can be observed in particular for the control site where the soil pH value ranged between 7.4 and 7.8 (measured in water) and thus might be responsible for the observed differences in gross nitrification rates determined by the BaPS approach and the ^{15}N pool dilution technique, respectively. One central assumption of the BaPS approach is that mineralization, nitrification, and denitrification are the only processes involved in the gas household in the enclosed soil system. To test this assumption, the change in the methane concentration as well as the N_2 uptake during a BaPS run was additionally determined. No significant change could be observed in the methane concentration between the beginning and the end of the BaPS incubation (not illustrated) and the $^{15}\text{N}_2$ uptake was smaller than the detection limit. Therefore it can be concluded that the impact of both gases was negligible considering the observed differences in gross nitrification rates determined by the BaPS approach.

Furthermore, ^{15}N pool dilution is based on a number of assumptions: (1) no isotopic discrimination, (2) no re-mineralization of added labelled N, (3) constant process rates during incubation, and (4) similar behaviour of added and native N pools (Murphy et al., 2003). Mathieu et al. (2007) found that isotopic fractionation can be neglected if enrichment was higher than 0.6%, as was the case in this study. We paid specially attention to distributing the ^{15}N as uniform as possible, firstly by using very homogenous soil and secondly by adding the ^{15}N solution through spraying. However, a completely homogenous mixture of the isotope with the soil inorganic N pool was virtually impossible. Given the heterogeneity of soils it is highly unlikely that the applied $^{15}\text{NO}_3^-$ will generate an immediate equilibrium with the indigenous soil nitrate. Recent investigations challenged the one pool theory (Stevens et al., 1997; Spott et al., 2006), and at least for NO_2 – more than one pool was proven in this soil (Russow et al., 2008b).

The high variation in the nitrification rates determined compared to other studies using BaPS could be explained by the low nitrification rate in these soils. Nitrifications rates in this study were in the range of BaPS measurements from two other agricultural soils (Ingwersen et al., 2008) but were one order lower than BaPS measurements determined in forest soils (Ingwersen et al., 1999; Breuer et al., 2002; Stange, 2007) or in an old grassland soil (Müller et al., 2004a). The BaPS system was developed for forest soils, especially for the litter layer where a high turnover rate was observed (Ingwersen et al., 1999). Therefore this validation is important and it should be noted that the applicability to measure nitrification rates in mineral soil is limited on surface soils.

4.2 Gross nitrification rates under field conditions

Mean nitrification rates are highly linearly correlated with C_t and N_t values from the three field sites. Of course the three values are too low to generalize these findings. Determined gross nitrification rates of the three differently treated sites agree with the rates from other studies (Table 2). Nevertheless, average rates (36, 29, and $21 \mu\text{g N kg}^{-1} \text{h}^{-1}$) were lower compared to former studies and this might be caused by lower mean temperatures as published in previous studies. Furthermore, most of the former studies focused directly on the influence of nitrogen addition on nitrification rates and thus higher rates can be expected (Murphy et al., 2007).

It should be considered that processes in the N-cycle of soils (e.g. N-mineralisation, nitrification NH_4^+ - and NO_3^- immobilisation) are closely linked together (Corre et al., 2002) and therefore it is difficult to separate the direct and indirect impact of climatic factors on the process of the N cycle (Jamieson et al., 1998). Nitrification depends upon the substrate availability of ammonium and oxygen. The short mean residence time (MRT) for NH_4^+ in our soil demonstrates that NH_4^+ availability is determined more by N-mineralisation than by the size of the NH_4^+ pool in the soil. Therefore the response of measured actual nitrification rates on climate factors may also reflect the response of N-mineralisation. A clear response to soil temperature was observed in the mea-

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surements under field conditions, but the highest nitrification rates were not observed at the highest temperatures. It is possible that the time interval of the highest temperatures (calendar week 30 to 34) the nitrification rate was limited by the substrate deficit, although no index is given in the measurements under standard condition at this time.

5 Therefore we assumed that the nitrification rate was reduced by the high temperature itself. Other studies also found a decrease in the Q_{10} value with an increasing temperature or even a decrease in gross nitrification rates at higher temperatures (e.g. Zaman and Chang, 2004; Stange, 2007) when the chosen temperature interval was large.

The optimum temperature found on the control site was unexpectedly low (17°C) in this study. These findings contradict the observation made by Recous et al. (1998) who observed the highest nitrification rate at the highest temperature (20.4°C) and a strong temperature response in the interval from 4°C to 21°C (a Q_{10} value 3.17). Normally, the optimal temperatures for microbial processes in the field are adapted to the maximal temperatures in the field (Malhi and McGill, 1982; Stark and Firestone, 1996).
15 Following the hypothesis that microorganisms are adapted to their optimal temperature range at the climate conditions of their habitat (Nozhevnikova et al., 2001; Fierer et al., 2003) we must expect similar temperature responses for the three sites. Nevertheless the model can only explain the observed temporal variation at the control site to 56% and of course the highest uncertainties must be expected at this site because the lowest nitrification rates are determined here. It is possible that the optimum temperature
20 found at the control site is an artefact due to the high variance in the determination of gross nitrification rates. Substrate availability can also be a reason, due to the stronger competition at higher temperatures on this unfertilised plot.

25 During the two summer periods decreasing soil water content was observed. In particular in the first period, when soil moisture fell below 16 % (w/w) a decrease in the gross nitrification rates was measured. Nevertheless, as also observed previously by Recous et al. (1998) the influence of soil moisture is much smaller than the influence of temperature. Our results illustrated that microbial activity is only affected in extreme dry or wet conditions, and has a wide interval of soil moisture where moisture is not limiting.

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The humidity factor for the observed soil water content in this study between 19.7 and 28.5% (w/w) only varied from 0.8 to 1.0 whereas the temperature factor changed by a factor of 5. However, 2004 was not a dry year and it should be considered that no strong moisture changes could be observed in 2004. The small soil moisture interval in the field observations may explain some of the distinct uncertainties with respect to soil moisture response. Laboratory studies with manipulated soil moisture have found increasing nitrification with increasing soil moisture (e.g. Khalil and Baggs, 2005), as is expected in microbial processes, if O₂ availability is not limited.

4.3 Gross nitrification rates under standard conditions

Observed nitrification rates under standard conditions averaged 49, 43, and 31 $\mu\text{g N kg}^{-1} \text{ h}^{-1}$ and are in the range observed by other studies at similar soil temperature and soil moisture conditions (see Table 2). The measurements showed great variation and consequently COV's between 0.24 and 0.25 were calculated. The COV of measurements under standard conditions was only marginally higher than the COV in the BaPS measurements due to the BaPS validation against the ¹⁵N pool dilution technique (COV: 0.22). It is assumed that this COV is due to the uncertainties in the BaPS measurement system itself. Therefore most of the observed variability over the time in the measurements under standardised condition (0.24 and 0.25) is caused by the determination uncertainty of the BaPS system rather than by seasonal variation or trends. The poor seasonal trend with respect to the control site is caused by lower nitrification rates in the first ten weeks. The comparability of gross nitrification measurements under field and standard conditions was illustrated by the fact, that the measured rates under standard condition are similar to the calculated rates with the model approach from 50, 39 and 33 $\mu\text{g N kg}^{-1} \text{ h}^{-1}$ using the parameterisation of the measurement under field conditions and mean soil moisture (20%, 18.8%, and 17.6% for manure, mineral fertilisation, and the control, respectively) and 16°C as an input parameter. This comparability of the measurement results under field conditions (measured 4 to 40 h after sampling) and standard conditions (including a one-week storage

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in the laboratory to adjust the matrix potential to -30 kPa) confirmed our assumption, that the nitrification rate is not altered too much by storage and is consequently more or less constant for 8 days under a constant condition.

4.4 Modelling gross nitrification rates

5 With the exception of modelling the measured nitrification rate at the control plot, all of the tested approaches could explain the variation with a very similar accuracy. This is probably due to the fact that proposed approach differ more under extreme conditions compared to the conventional approaches as close to the optimum. On the control plot the differences in the approaches could be explained by the observation that the mea-
10 sured nitrification rate do not increased at temperatures over 20°C . We can therefore conclude that the combined approach from Stange (2007) led to no better results than the classical multiplying approaches using the climatic factor and the multiple regres-
15 sion. Changing the response function for temperature (Arrhenius vs. O'Neill function) definitely has more of an effect than changing the combining approach. The tempera-
20 ture response could be better described by the O'Neill function especially on the control plot where the nitrification rate decreased at temperatures over 20°C . Compared to the more common Arrhenius function, the O'Neill function is an optimum function and does not work at temperatures over the parameter T_{max} . This function is more suitable in characterising microbiological processes at high temperatures than the monotonically
25 increasing Arrhenius function which was developed for chemical reactions, but not for microbial processes. In this study the O'Neill function was used because many investigations dealing with a temperature response observed a decrease in the Q_{10} value with an increasing temperature or even a decrease in gross nitrification rates at higher temperatures (e.g. Zaman and Chang, 2004; Stange, 2007) when the temperature interval selected was large. Nevertheless, at low temperatures both the O'Neill and the Arrhenius function act very similar and a proxy Q_{10} value can be deviated from the values of the O'Neill function. Therefore these results are also in agreement with the results from Recous et al. (1998) who only investigated the nitrification rate for a small

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interval from 4°C to 21°C and found a strong temperature response with a Q_{10} value of 3.17.

Even more differences than between the model approaches were observed between the three sites. The observed differences between the optimised parameters for temperature and soil moisture response for the three sites cannot be explained at the moment. It was assumed that climatic conditions were the same on all plots over the 100 years of different fertiliser management, because the plots are a maximum of 150 m from each other. Parameters of the soil moisture function are uncertain however, caused by the small response of the measurements in the soil moisture interval observed in the field in 2004. Therefore an interpretation of the parameter is more or less speculative. Optimised values for the maximal nitrification rate (R_{\max} Eq. 5 and Table 1) were in a similar ratio together (1:0.8:0.5 for manure fertiliser (D), mineral fertiliser (M) and the control (C), respectively) as the mean measured values (1:0.8:0.6) indicated that the climatic response averaged over the whole year was similar on the three sites. Temperature responses for both of the fertilised plots were similar, although the unfertilised control plot had a smaller optimum temperature (17°C). This unexpectedly low optimum temperature was discussed earlier on.

To compare the coefficient of variation of measurements under field conditions with the measurements at standardised conditions the climatic influence was eliminated. For this model results were used to normalise the measurements under field conditions, e.g. if the climate factor explained all of the variations in the measurements the normalised nitrification was 1 for each day and consequently the COV was 0. The COV of the normalised measurements were 0.33, 0.32 and 0.37 for D, M and C respectively, and consequently higher than the COV of measurements under standardised conditions (0.25 and 0.24). This contradicts the observation that standardised nitrification rates of measurements under field conditions and under standardised conditions are the same (see earlier on). It was therefore assumed that the climatic conditions in the field prior to sampling influence the actual rate determined for the nitrification rate but also the temperature sequence before. Consequently the relatively constant tempera-

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ture and moisture conditions in the laboratory over 1 week in order to standardise the probes to a matrix potential of -30 kPa may cause the smaller variation in the nitrification rates observed in measurements under standardised conditions. Of course to verify this hypothesis, specific manipulation experiments are necessary, Cookson et al. (2002) have in fact already demonstrated that the preincubation temperature can influence the nitrification rate. They proposed that the observed increase in gross rates is caused by a depletion of the intermediate substrate, which restricts the pool of potential mineralisable N. A shift in the function and composition of microbial communities with changing temperature has also been discussed by Zogg et al. (1997).

4.5 N_2O leakage rate during nitrification

N_2O production in this soil is highly variable with time and depended on soil temperature and soil moisture. The high variation is caused by different processes involved in N_2O production but mainly nitrification and denitrification (Mathieu et al., 2006). A separation of processes is therefore essential to interpret dependencies correctly (Baggs, 2008). A primitive approach was used in this study to distinguish between N_2O that was produced by nitrification or denitrification. The results derived from the BaPS system for the denitrification rate were recorded, in spite of the fact that up to date no validation has been carried out for the denitrification measurement by BaPS. Müller et al. (2004a) found that denitrification also occurred if BaPS calculated negative denitrification rates (set to zero). Nevertheless, results might be in a realistic order. Differences in N_2O fluxes between the sites were determined more by denitrification than by nitrification, because the N_2O flux differed on days where nitrification only was occurring by a factor of 10, compared to days with denitrification by a factor of 30.

N_2O production in these soils is not correlated with the gross nitrification rate. The leakage rates (N_2O emission/gross nitrification) where no denitrification was observed (see Sect. 2) ranged from 0.001 to 3.8%. This high variability cannot be explained by climatic conditions such as soil moisture and/or soil temperature. Furthermore, Ambus (2005) observed no correlation between gross nitrification and N_2O emission and

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found highly variable leakage rates between 0.04 and 2.9‰. Measured ratios based on gross nitrification rates are scarce, because most N₂O production related to net nitrification (Mathieu et al., 2006). Due to the fact that gross nitrification is greater than net nitrification, leakage rates related to the net rates tend to represent an upper limit for the values related to gross nitrification. Nevertheless the leakage rates for net nitrification collected by Mathieu et al., 2006 (values between 0.2 and 10‰) are smaller than the leakage rates in the studies where gross nitrification was determined (0.5 to 23.2‰) (Mathieu et al., 2006). This demonstrates that our knowledge on leakage rates is still not profound. High leakage rates (>10‰) were found by Morkved et al. (2007) for peat and by Watson and Mills (1998), but it should be noted that in the latter study the detected N₂O might have been produced by denitrification and high amounts of NH₄⁺ and NO₃⁻ was added to the soil. In the peat soil Morkved et al. (2007) found an increasing leakage rate with a decreasing pH-value. In two mineral soils the leakage rates (0.3 to 0.9‰) were in the middle of our range and the leakage rate increased with an increasing pH-value (Morkved et al., 2007).

The leakage rates in our study are within the range reported by the BaPS measurements in forest soils (Ingwersen et al., 1999; Breuer et al., 2002). Breuer et al. (2002) have published information on leakage rates from 0.73 to 9.13‰ with a mean value of 1.9‰. Firestone and Davidson (1989) expected the leakage rate to vary under changing environmental conditions, although the control of this ratio was opaque up to now. Khalil et al. (2004) showed a positive, linear correlation between the gross nitrification and the N₂O emitted by nitrification, whereas the leakage rate (slope of the regression) was highly dependent on O₂ concentration. Leakage rates in the laboratory study from Khalil et al. (2004) were one magnitude higher (1.6 to 14.8‰) than our study. Since Khalil et al. (2004) demonstrated that O₂ concentration has a high influence on the N₂O leakage rate it could be that the observed variation in our leakage rates are caused by a high variability of O₂ concentration in our soil. Although O₂ concentration in the headspace only varies very slightly during and between measurements it is still possible that the soil O₂ concentration differs greatly because of differences in the soil

structure and soil pore space of the undisturbed soil cores used. Most of the studies (e.g. Khalil et al., 2004; Morkved et al., 2007; Mathieu et al., 2006) work with sieved soil and consequently the soil structure was destroyed. Measurements in undestroyed soil samples (e.g. Ingwersen et al., 1999; Breuer et al., 2002; Ambus, 2005) found leakage rates in the same order as this study and also high variability.

5 Conclusions

Based on our current state of knowledge Barometric Process Separation (BaPS) is a suitable method for determining high nitrification rates, also in mineral soils. It is limited to measure small rates as normally observed in the winter time or in subsoil layers. Furthermore BaPS enables a combination of laboratory measurements under near-to-field conditions without adding nitrogen or destroying the soil structure, which is essential for filling in the gap in existing knowledge between laboratory studies and virtual field conditions (Robertson and Groffmann, 2007). The determination of nitrification rates using the BaPS system will be improved considerably if in situ pH measurements were conducted during incubation. This is particularly important for agricultural soils, where the pH value is mostly neutral and in some cases above 7 the pH value is a critical factor in the BaPS calculation.

We can conclude that soil temperature is the most important driver in the seasonal variation of gross nitrification in the field. The comparison between field probes and the same probes under standard conditions demonstrate the causal relationship between climate conditions and seasonal variation in gross nitrification and confirm our hypothesis. Using the model approach presented we were able to simulate gross nitrification rates for the cores of black earth soil investigated under field conditions.

No significant correlation of N_2O emission to gross nitrification, or N_2O leakage rates (N_2O emission/gross nitrification) to soil temperature and soil moisture could be observed. This implies that we are still a long way from understanding the process of N_2O -emissions under field conditions. In contrast to our results, a clear positive rela-

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relationship between nitrification rates and N₂O-emissions due to nitrification was found in laboratory studies (e.g. Khalil et al., 2004). We assume that such differences are mainly due to laboratory sample preparation, since N₂O-emission measurements under laboratory conditions were commonly conducted on disturbed and sieved soil samples with artificially added nutrients (e.g. ammonium). Laboratory studies alone are therefore insufficient for describing N₂O-emissions in the field. Hence, a focus on the virtual field conditions (e.g. the soil structure, nutrient and O₂ availability) is urgently required, and the BaPS system will be a useful tool for this purpose.

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Table 1. Model parameters used for the simulation of gross nitrification and the resulting coefficient of determination.

	R_{\max} [$\mu\text{g N kg}^{-1} \text{ soil h}^{-1}$]	T_{opt} [$^{\circ}\text{C}$]	a []	M_{crit} [% g water g^{-1} soil]	b []	r^2
Manure fertiliser	89	31	3.8	20	1.9	0.7977
Mineral fertiliser	64	30	4.0	17	1.4	0.6583
Control	44	17	29.8	35	1.6	0.5573

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Table 2. Summary of published gross nitrification rates in agricultural soils. * Standardised condition 16°C and field capacity (FC) (–6 to –30 kPa matrix potential).

Author	Region	Location	Culture	Treatment	Additional variations	Rates [µg N kg ⁻¹ h ⁻¹]			comment
						Min	Max	(16°C, FC)* mean rate	
Cookson et al. (2006)	Australia	field	wheat	organic	variation in soil moisture and temp.	19	119	62	four times in the vegetation period
						23	123	65	
						15	123	69	
Hoyle et al. (2006)	Western Australia	lab	wheat/legume	integrated	variation in soil moisture and temp.	21	167	89	four times in the vegetation period
						33	246	113	
						29	183	46	
Ambus (2005)	Denmark	field	grass-clover pasture	conventional with stubble burnt	temperature range 5°–40°C	142	229	172	four times in the vegetation period
						125	233	192	
						96	358	203	
Silva et al. (2005)	Oklahoma	field	old grassland	1-year-old	moisture 59–69% WFPS	8	58	28	four times in the vegetation period
						8	58	28	
						8	58	28	
Khalil et al. (2004)	France	lab	maize	0 kPa O ₂	moisture 59–69% WFPS	0	0	0	five measurements over the time
						50	71	64	
						113	175	139	
Zaman Chang (2004)	Australia	lab	agroforestry	0.35 kPa O ₂	moisture 56–72% WFPS	150	350	254	five measurements over the time
						179	642	367	
						29	633	394	
Zaman Chang (2004)	Australia	lab	agroforestry	4.3 kPa O ₂	moisture 20–29°C	9	55	31	five measurements over the time
						15	123	62	
						24	138	76	
Cookson Murphy (2004)	Western Australia	lab	pasture	20.4 kPa O ₂	bare ground	142	271	206	incubated at 20°C, 75 FC
						79	142	110	
						4	119	65	
Cooksen et al. (2002)	New Zealand	lab	cereal	control	sandy loam/sandy clay loam	21	717	305	15 observations in 160 days
						35	150	55	
						35	150	55	
Recous et al. (1999)	France	field	control	control	temperature range 2°–15°C	13	40	22	eight times in the vegetation period
						13	40	22	
						13	40	22	
Zaman et al. (1999)				straw amended	moisture 19.7–28.5% temperature 4.4°–20.5°C, moisture 19.7–28.5%	13	40	22	six observations in 90 days
						13	40	22	
						13	40	22	
Watson Mill (1998)	Northern Ireland	lab	grassland	control	control, dairy shed effluent and NH ₄ ⁺ added	25	52	42	six observations in 90 days
						27	46	37	
						27	46	37	
MEAN						100 kg N ha ⁻¹ a ⁻¹			15°C, 38.2% H ₂ O/DM
						200 kg N ha ⁻¹ a ⁻¹			
						300 kg N ha ⁻¹ a ⁻¹			
						400 kg N ha ⁻¹ a ⁻¹			
						500 kg N ha ⁻¹ a ⁻¹			
53	206	88	148						

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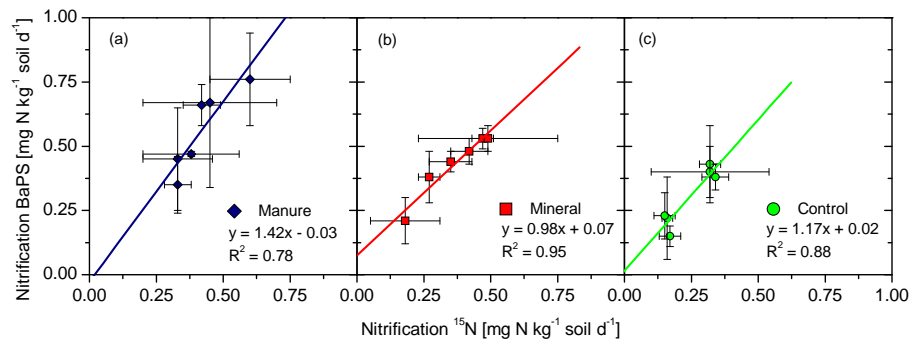
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Fig. 1. Comparison of gross nitrification rates measured with The BaPS and the ^{15}N pool dilution technique for the three field sites (a) manure fertiliser (b) mineral fertiliser and (c) control (without fertiliser). Parameter of the linear regression and the r^2 value are given in the figures.

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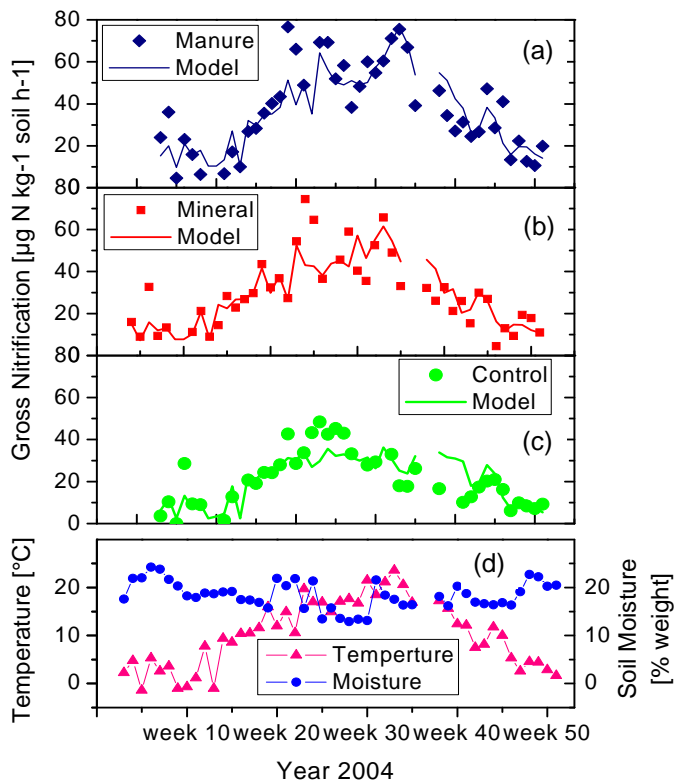


Fig. 2. Comparison of simulated and gross nitrification rates measured with BaPS on the three sites **(a)** manure, **(b)** mineral fertilised, and **(c)** the control (unfertilised for over 100 years). **(d)** Seasonal variation in the soil temperature and soil moisture (averaged value for the three sites) in 2004.

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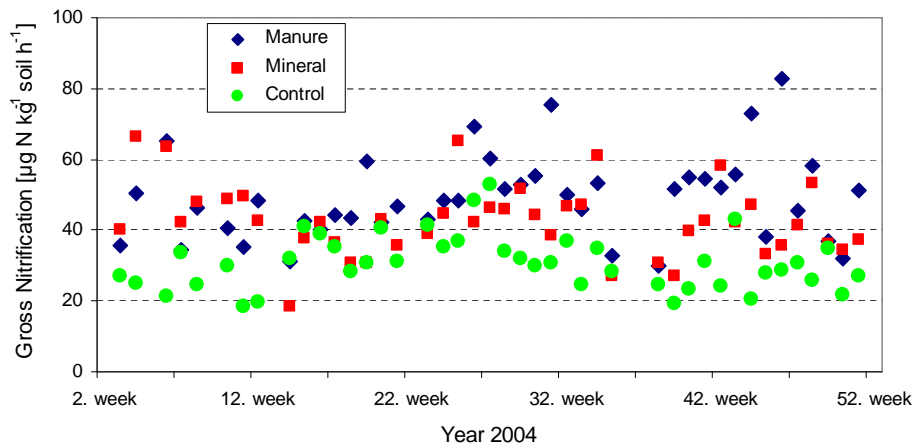


Fig. 3. Measured nitrification rates on the three sites manure, mineral fertilised, and the control (unfertilised for over 100 years) under standardised conditions (16°C, –30 kPa).

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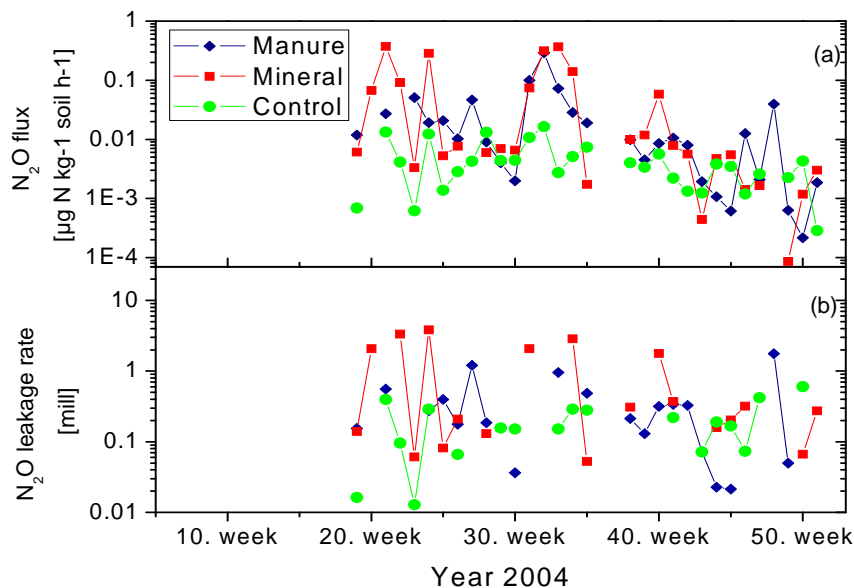
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Fig. 4. (a) N_2O production in the soil cores from the three differently fertilised sites, (b) N_2O leakage rate by nitrification (N_2O production/gross nitrification *1000) in the soil cores from the three sites manure, mineral fertilised, and the control (unfertilised for over 100 years) respectively. Please note the logarithmic scale of the y axis.

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