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Emission of gaseous nitrogen oxides from an extensively managed grassland in NE Bavaria, Germany
II. Stable isotope natural abundance of N_2O

JENS TILSNER[1,2], NICOLE WRAGE[1,3], JUTTA LAUF[1,4] and GERHARD GEBAUER[1,*]

*[1]Universität Bayreuth, Lehrstuhl für Pflanzenökologie, 95440 Bayreuth, Germany; [2]Current address: Albrecht-von-Haller–Institut für Pflanzenwissenschaften, Abteilung Biochemie der Pflanze, 37073 Göttingen, Germany; [3]Department of Environmental Sciences, Wageningen University and Research Center, 6700 EC Wageningen, The Netherlands; [4]Agrolab, 85416 Langenbach, Germany; *Author for correspondence (e-mail: gerhard.gebauer@uni-bayreuth.de; phone: ++49–921-552060; fax: ++49–921-552564)*

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Abstract. We analysed the stable isotope composition of emitted N_2O in a one-year field experiment (June 1998 to April 1999) in unfertilized controls, and after adding nitrogen by applying slurry or mineral N (calcium ammonium nitrate). Emitted N_2O was analysed every 2–4 weeks, with additional daily sampling for 10 days after each fertilizer application. In supplementary soil incubations, the isotopic composition of N_2O was measured under defined conditions, favouring either denitrification or nitrification. Soil incubated for 48 h under conditions favouring nitrification emitted very little N_2O (0.024 μmol g_{dw}^{-1}) and still produced N_2O from denitrification. Under denitrifying incubation conditions, much more N_2O was formed (0.91 μmol g_{dw}^{-1} after 48 h). The isotope ratios of N_2O emitted from denitrification stabilized at $\delta^{15}N = -40.8 \pm 5.7‰$ and $\delta^{18}O = 2.7 \pm 6.3‰$. In the field experiment, the N_2O isotope data showed no clear seasonal trends or treatment effects. Annual means weighted by time and emission rate were $\delta^{15}N = -8.6‰$ and $\delta^{18}O = 34.7‰$ after slurry application, $\delta^{15}N = -4.6‰$ and $\delta^{18}O = 24.0‰$ after mineral fertilizer application and $\delta^{15}N = -6.4‰$ and $\delta^{18}O = 35.6‰$ in the control plots, respectively. So, in all treatments the emitted N_2O was ^{15}N-depleted compared to ambient air N_2O ($\delta^{15}N = 11.4 \pm 11.6‰$, $\delta^{18}O = 36.9 \pm 10.7‰$). Isotope analyses of the emitted N_2O under field conditions *per se* allowed no unequivocal identification of the main N_2O producing process. However, additional data on soil conditions and from laboratory experiments point to denitrification as the predominant N_2O source. We concluded (1) that the isotope ratios of N_2O emitted from the field soil were not only influenced by the source processes, but also by microbial reduction of N_2O to N_2 and (2) that N_2O emission rates had to exceed 3.4 μmol N_2O m^{-2} h^{-1} to obtain reliable N_2O isotope data.

Introduction

Nitrous oxide (N_2O) is an atmospheric trace gas that at present originates predominantly from microbial transformation of anthropogenic nitrogen (N) inputs into soils (IPCC 1995, 1996; Mosier et al. 1998). Due to its effects on the radiation balance

(Houghton et al. 1995) and on stratospheric ozone (Crutzen 1981), there is considerable interest to reduce N_2O emissions. To enable the evaluation of land use practices with respect to their potential effects on N_2O emissions, N_2O emissions from the two most important N_2O forming soil processes, nitrification and denitrification (Davidson 1991) need to be distinguished.

During nitrification, N_2O can be formed by spontaneous disintegration of nitrogen hydroxide (NOH), an unstable, enzyme-bound intermediate during the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) (Hynes and Knowles 1984; Schmidt and Voerkelius 1989). It can also be produced by nitrifier denitrification, the reduction of NO_2^- by nitrifying bacteria, probably under low-oxygen conditions (Ritchie and Nicholas 1972; Poth and Focht 1985; Voerkelius 1990; Wrage et al. 2001). During denitrification, N_2O is an obligatory intermediate of the complete NO_3^- reduction to N_2 (Payne 1981; Zumft and Kroneck 1990). Not all denitrifying microorganisms, however, carry out all reduction steps. Complete anaerobiosis favours N_2 formation. N_2O can become the main reaction product under low O_2 pressure (Knowles 1982). Generally, intermediate O_2 concentrations at the interface between anoxic and oxic soil favour formation of N_2O by both, nitrification and denitrification (Goreau et al. 1980; Khdyer and Cho 1983; Parkin and Tiedje 1984).

Attributing emitted N_2O to particular source processes is difficult, because different processes can occur simultaneously in close proximity (Robertson and Tiedje 1987; Davidson 1992). The inhibition of nitrification in the presence of 10 Pa acetylene (C_2H_2) or both, nitrification and N_2O reduction in denitrification at 10 kPa C_2H_2 (Klemedtsson et al. 1990; Knowles 1990) (acetylene inhibition method, AIM), disturbs the soil system and can lead to an underestimation of denitrification by blocking the supply of NO_3^-. Results based on the AIM are also uncertain when diffusion into the soil is hindered, e.g. under wet conditions (Arah et al. 1993; Malone et al. 1997). There is currently no inhibition method to study N_2O emission from nitrification by selectively inhibiting denitrification. E.g. adding O_2 suppresses denitrification (Knowles 1982), however, also affects N_2O production from nitrification. ^{15}N tracer techniques are potentially useful to distinguish between nitrification and denitrification, but may have undesired fertilization effects and uncertainties about its homogenous distribution in the soil (Granli and Bøckman (1994) and references therein). Moreover, when adding $^{15}NO_3^-$ tracer, N_2O may result from nitrification or from denitrification of newly formed (unlabelled) NO_3^-. Thus, denitrification may be underestimated. If $^{15}NH_4^+$ is used, any labelled N_2O derived from denitrification of $^{15}NO_3^-$ formed in nitrification would cause an overestimation of N_2O production from nitrification.

A non-invasive alternative is the measurement of $^{15}N/^{14}N$ and $^{18}O/^{16}O$ isotope ratios of the emitted N_2O at natural abundance level (Shearer and Kohl 1993). Due to different substrates of N_2O formation and different isotope discrimination processes during key reaction steps, N_2O derived from nitrification and from denitrification may differ isotopically (Wahlen and Yoshinari 1985; Kim and Craig 1990). Sources of N in N_2O from nitrification are NH_4^+ from mineralized soil organic matter, from mineral or organic fertilizer, or from atmospheric deposition. O sources are soil water ($\delta^{18}O \approx -10‰$ in the area of this investigation) and soil air ($\delta^{18}O$

= 23.5‰) (Schmidt and Voerkelius 1989). NH_4^+ from organic manure or soil organic matter in agricultural soils is usually enriched in ^{15}N (see Tilsner et al. (2002)). NH_4^+ from mineral fertilizers has a $\delta^{15}N$ close to zero (see Tilsner et al. (2002)), while NH_4^+ from atmospheric deposition is depleted in ^{15}N (see Bauer et al. (2000)). In N_2O from denitrification, $\delta^{15}N$ and $\delta^{18}O$ depend on the origin of the nitrate. NO_3^- from soil or organic fertilizer nitrification is depleted in ^{15}N when compared to its NH_4^+ precursor (see Tilsner et al. (2002)) and has a $\delta^{18}O$ between 0.8 and 5.8‰ (Durka et al. 1994). In NO_3^- from mineral fertilizers $\delta^{15}N$ is close to 0‰ and $\delta^{18}O$ is close to 23.5‰ (Tilsner et al. 2002). Nitrate from atmospheric deposition is characterized by its high enrichment in ^{18}O ($\delta^{18}O \approx 60$‰; see Durka et al. (1994)). Due to the complex stable isotope composition of its sources and the complex reaction mechanisms, the interpretation of the stable isotope composition of N_2O with regard to its possible sources is more difficult than for other nitrogen compounds (Högberg 1997). The N and O atoms in N_2O not only derive from different sources, but also depend on isotope effects during transformation processes. For example, in denitrification N_2O is not the only possible end product and its isotope composition is affected by the rate of N_2O reduction to N_2. Also, depending on the turnover rate, different intermediates of denitrification may accumulate so that the isotope fractionation effects of normally not rate-limiting reaction steps imprint themselves on the N_2O produced.

Our aim was to determine the source of N_2O emitted over a one-year period from an extensively managed grassland treated with two types of N fertilizer application and an unfertilized control (Tilsner et al. 2002) by analysing the relative N and O isotope abundances of N_2O. N_2O isotope data from the field study were compared to those obtained from soil incubation experiments under controlled laboratory conditions.

Materials and methods

Laboratory experiments

We determined the isotope signature of N_2O emitted from the field soil under conditions favouring either denitrification or nitrification (Table 1). 100 g_{fw} (fw: fresh weight) of homogenized moist soil without stones and roots, corresponding to approximately 70 g_{dw} (dw: dry weight), were incubated for 48 hours in closed 1 l glass vessels. To establish conditions favouring denitrification, the soil samples were incubated in He atmosphere and the water content was adjusted to 80% w/w of soil dw. The N_2O accumulation was measured at 1, 6, 12, 24, 30, 34 and 48 hours after N application by connecting the headspace to a photoacoustic trace gas analyzer (TGA) (Multigas Monitor 1302, Bruel & Kjaer, Ballerup, Denmark) *via* a closed circular tube system. Simultaneously, gas samples were taken from the headspace to determine the $\delta^{15}N$ and $\delta^{18}O$ values of the accumulated N_2O. Due to the highly pressure sensitive measurement chamber of the TGA, the circular tube sys-

Table 1. Experimental conditions as used for laboratory incubations of soil samples from the extensively managed grassland site in NE Bavaria, Germany. 100 g_{fw} of soil were incubated in 1 l glas vessels for 48 h in both experiments. N was added as KNO_3 in experiment 1 and as NH_4Cl in experiment 2. Each treatment is based on three replicates. Incubation was carried out at 22 °C in the dark.

Treatment	N addition	incubation atmosphere
Experiment 1: soil conditions favouring denitrification (80% (w/w) water content)		
1. control	—	He
2. favoured denitrification	100 mg NO_3^--N	He
3. favoured denitrification and inhibition of nitrification	100 mg NO_3^--N	He + 0.01% (v/v) C_2H_2
4. favoured denitrification and inhibition of N_2O reduction	100 mg NO_3^--N	He + 10% (v/v) C_2H_2
Experiment 2: soil conditions favouring nitrification (60% (w/w) water content)		
1. control	—	ambient air
2. favoured nitrification	100 mg NH_4^+-N	ambient air
3. favoured nitrification and test for denitrification (^{15}N labelled NO_3^- pool)	100 mg NH_4^+-N	ambient air
4. favoured nitrification and suppression of denitrification	100 mg NH_4^+-N	O_2

tem was not flushed with He before sampling. Thus, ambient air was introduced into the glass vessels during each TGA measurement, resulting in an increase of the O_2 concentration up to $\sim$1.6% v/v in the headspace. This is still sufficiently anaerobic to effectively favour denitrification (Parkin and Tiedje 1984; Arah et al. 1991). Both, N_2O concentration and isotope ratios, were corrected mathematically for N_2O introduced into the headspace with ambient air by taking into account the volume of the TGA tube system and the concentration and δ values of N_2O in ambient air. Four different treatments with conditions favouring denitrification were applied with three replicates each: (1) unfertilized control; (2) fertilization with 100 mg N as KNO_3 ($\delta^{15}N = -3.3 \pm 1.1‰$, $\delta^{18}O = 20.1‰$; see Table 2); (3) fertilization with 100 mg N as KNO_3 and inhibition of nitrification by addition of 0.01% (v/v) C_2H_2; (4) fertilization with 100 mg N as KNO_3 and inhibition of N_2O reduction by addition of 10% (v/v) C_2H_2 (Table 2). Acetylene was flushed through demineralized water before use to exclude the possibility of a fertilizing effect of acetone traces (Gross and Bremner 1992)

To favour nitrification, 100 g_{fw} (corresponding to $\sim$70 g_{dw}) soil was incubated at a water content of 60% w/w of soil dw, with treatments (1) control: unfertilized, incubated with ambient air; (2) nitrification: fertilized with 100 mg N as NH_4Cl ($\delta^{15}N = -0.3 \pm 0.2‰$,Table 2) and incubated in ambient air; (3) nitrification and test for denitrification: fertilized with 100 mg N as NH_4Cl and incubated in ambient air. Additionally, the soil nitrate pool (15 μmol in 100 g_{fw} of soil) was ^{15}N-

Table 2. $\delta^{15}N\text{-}N_{total}$ of slurry and calcium ammonium nitrate and $\delta^{15}N\text{-}NH_4^+$, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values of calcium ammonium nitrate as used as fertilizers for the field experiment and $\delta^{15}N\text{-}NH_4^+$, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values of the KNO_3 or NH_4Cl as used in the laboratory incubations (n = 3 for $\delta^{15}N$ and n = 1 for $\delta^{18}O$ measurements). Annual means were calculated as averages of the three fertilizations weighted by the respective amounts of applied N.

experiment	fertilizer	$\delta^{15}N$ [‰]	$\delta^{18}O$ [‰]
fertilization on 6 June 1998	slurry N_{total}	8.6	
(day 0)	mineral fertilizer N_{total}	–0.7	
	mineral fertilizer NH_4^+	–2.6	
	mineral fertilizer NO_3^-	1.8	*no data*
fertilization on 19 Sept. 1998	slurry N_{total}	7.1	
(day 84)	mineral fertilizer N_{total}	–0.7	
	mineral fertilizer NH_4^+	–2.6	
	mineral fertilizer NO_3^-	1.8	*no data*
fertilization on 15 March 1999	slurry N_{total}	8.6	
(day 262)	mineral fertilizer N_{total}	–0.7	
	mineral fertilizer NH_4^+	0.7	
	mineral fertilizer NO_3^-	0.5	21.3
annual means	slurry N_{total}	8.3	
	mineral fertilizer N_{total}	–0.7	
	mineral fertilizer NH_4^+	–1.5	
	mineral fertilizer NO_3^-	1.4	
incubation experiments	KNO_3	–3.3 ± 1.1	20.1
	NH_4Cl	–0.3 ± 0.2	

enriched to a $\delta^{15}N$ of $\sim$ 2760‰ by adding 0.16 μmol $K^{15}NO_3$ tracer (99% ^{15}N enriched). (4) nitrification and suppression of denitrification: fertilization with 100 mg N as NH_4Cl, incubation in 100% O_2 atmosphere (Table 1). The four treatments were run in three replicates, each.

Soil NO_3^- and NH_4^+ concentrations and the corresponding $\delta^{15}N$ values were determined for one sample of homogenized and untreated soil before the start of each experiment and for each incubated soil sample after each experiment, as described by Tilsner et al. (2002).

Design of the field experiment and gas sampling

The site of the field experiment is a meadow which is mown 2–3 times per year for silage and hay production and receives three slurry applications of 10–15 m^3 ha^{-1} annually. The soil of this grassland is a clay loam with a pH_{KCl} of 5.7 ± 0.2 and a pH_{H_2O} of 6.3 ± 0.1 in the topmost 10 cm 4 × 4 m plots were fertilized with either slurry (75 kg N ha^{-1}), mineral fertilizer (calcium ammonium nitrate, 74 kg N ha^{-1}), or left unfertilized as control, with 4 replicates per treatment. The fertilizers were applied in three doses between June 1998 and March 1999, in parallel with the slurry applications by the farmer. Details about the field site and the experiment are

in Tilsner et al. (2002). The relative N and O isotope abundances of the applied fertilizers are shown in Table 2.

Gas samples for N_2O isotope analysis were taken from June 1998 to April 1999 in combination with N_2O emission measurements (Tilsner et al. 2002). Each fertilizer application was followed by daily sampling for 10 days with at least one sample per plot and per day. Otherwise, gas samples were taken approximately once every two to four weeks except for 12 weeks between September and December 1998, when the meadow was completely flooded due to heavy rainfall.

Gas samples for isotope analyses were collected by including glas vessels (volume of 100–120 ml) in a closed circular tube system used for N_2O emission measurements (Tilsner et al. 2002). Every 10 minutes, the air in this closed system was circulated between a closed chamber in which the N_2O emitted from the soil was accumulating for 40 min, and a TGA. Thus, the vessels were flushed with sample air from the measurement chamber five times before being closed and taken to the laboratory for further analysis.

Measurement of N_2O isotope ratios

N and O isotope ratios were determined directly from N_2O gas at m/z 44, 45 and 46 by use of a gas chromatograph-isotope ratio mass spectrometer coupling (GC-IRMS) (Hewlett-Packard GC 5890 series II, Wilmington, USA; Combustion Interface II and gas-IRMS delta S, both Finnigan MAT, Bremen, Germany). CO_2 and H_2O were removed in a NaOH trap (Carbon Dioxide Absorbent, Lüdi AG, Flawil, Switzerland) and N_2O was purified and pre-concentrated from 100 to 120 ml air samples by cryo-focussation in a liquid N_2 trap for 8 min (PreCon, Finnigan MAT), as described by Brand (1995). As a laboratory standard, N_2O gas (99.9990%, Linde, Munich, Germany) from a lecture bottle was used. For N isotope ratio calibration purposes this N_2O standard gas was reduced to N_2 on a Ni (99,98%) surface at 1150 °C in the Combustion Interface II and measured against an N_2 laboratory standard gas, which had previously been calibrated against the reference substances N1 and N2 provided by the IAEA (Vienna, Austria). For ^{18}O, the laboratory N_2O standard gas was calibrated against a CO_2 reference gas (Voerkelius 1990) previously calibrated against the reference substances NBS 16 to 18 provided by the IAEA. The internal reproducibility of the measurement system is typically ± 0.15‰ for N and ± 0.30‰ for O. Isotope ratios are presented as δ values, which are defined as:

$$\delta x = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \quad [‰] \tag{1}$$

where δx is the δ value of the heavy isotope x and R is the ratio of heavy isotope (at%, atom percent) to light isotope (at%). The international standards are N_2 in ambient air for $\delta^{15}N$ (Mariotti 1983) and standard mean ocean water (Vienna-SMOW) for $\delta^{18}O$, respectively.

The δ values measured in sample air have to be corrected for N_2O in the ambient air already present in the measurement chamber when it is closed (Gebauer et al., in preparation):

$$\delta x_{emitted} = \frac{\delta x_{measured} \times c(N_2O)_{measured} - \delta x_{ambient\ air} \times c(N_2O)_{ambient\ air}}{c(N_2O)_{measured} - c(N_2O)_{ambient\ air}} \quad [‰] \quad (2)$$

where δx is the δ value of the heavy isotope x (‰), c is the N_2O concentration (ppm) and the indices "emitted", "measured" and "ambient air" indicate newly emitted N_2O, total N_2O in the chamber and N_2O in ambient air, respectively.

Statistical methods

To calculate mean δ values weighted by both, emission rate and time, the time between subsequent samplings (Δt_i) was used as the time-weighting factor:

$$\Delta t_i = 0.5 \quad (t_{i+1} - t_{i-1}) \quad (3)$$

where t_i is the time (days since the first fertilizer application in June 1998) of the i^{th} sampling. Differences between experimental treatments were compared by one-way ANOVA. When effects were significant at the 0.05 level, multiple comparisons of means by the LSD test were executed. In the laboratory experiments, N_2O concentration changes with time were analyzed by linear regression when no obvious increase was visible.

Results

Laboratory experiments

Under conditions favouring denitrification, the field soil produced significant amounts of N_2O (0.91 μmol g_{dw}^{-1} after 48 h of incubation) in all treatments (Figure 1) except for the control (0.20 μmol g_{dw}^{-1} after 48 h of incubation), which differed significantly from all other treatments. Treatments that received NO_3^- additions showed a linear increase in N_2O concentration. After 48 h, the amount of applied NO_3^- in these three treatments was reduced by about 15% (data not shown). In the control treatment, the N_2O concentration in the headspace increased during the first 24 h of the incubation (linear regression: positive slope with $r^2 = 0.97$ for sample 1 and $r^2 = 0.99$ for samples 2 and 3). During the remaining 24 h of the incubation, the N_2O concentration in the headspace decreased or remained constant (linear regression: slope negative with $r^2 = 0.95$ for samples 2 and 3 or close to zero with $r^2 = 0.84$ for sample 1). Here, almost all the NO_3^- present in the soil at the start of the incubation was gone at the end of the incubation (data not shown). This indicates substrate limitation and subsequent consumption of previously

formed N_2O for the control. In the other, not substrate-limited treatments, isotope ratios of N_2O quickly stabilized at constant values of $\delta^{15}N = -40.8 \pm 5.7‰$ and $\delta^{18}O = 2.7 \pm 6.3‰$ (Figure 1). Neither 0.01% nor 10% C_2H_2 treatments changed the N_2O formation rate or the isotopic composition in the denitrification experiment (LSD tests, $p = 0.05$).

N_2O production was much lower under conditions favouring nitrification in all treatments (Figure 1). The treatments with NH_4^+ addition produced 0.024 μmol N_2O g_{dw}^{-1} after 48 h of incubation, which equals less than 3% of the N_2O production in the denitrification experiment. The N_2O concentration in the headspace increased for 24 h and then stagnated. However, no more than 5–7% (350–470 μmol) of the applied NH_4^+ was used after 48 h (data not shown). The nitrate content of the soil increased only slightly from 15 μmol (start of the incubation) to 38 ± 3 μmol per vessel (after 48 h) in all treatments (data not shown).

Incubation under O_2 atmosphere further reduced N_2O production (significantly different from other treatments with NH_4^+ addition, but not from controls) (Figure 1). For this treatment, $\delta^{18}O$-N_2O was not measured, because of the high O_2 content of the headspace gas mixture. Labelling of the inherent nitrate pool of the soil with trace amounts (1% of pool size) of $^{15}NO_3^-$ resulted in considerably ^{15}N-enriched N_2O ($\delta^{15}N \approx 900‰$).

Field experiment

δ values of N_2O in ambient air varied between $\delta^{15}N = -1.6 \pm 12.0‰$ and $\delta^{18}O = 39.1 \pm 8.2‰$ in the summer (June – August 1998) and $\delta^{15}N = 15.8 \pm 2.6‰$ and $\delta^{18}O = 33.3 \pm 2.8‰$ in the spring (March – April 1999), with annual means of $\delta^{15}N = 11.4 \pm 11.6‰$ and $\delta^{18}O = 36.9 \pm 10.7‰$ (Table 3). δ values of N_2O emitted from the soil scattered in a range of several thousand ‰ in both, positive and negative directions after correction for ambient air contamination (Equation 2). This scattering is unrealistic, because δ values of natural substances are known to usually vary within narrow ranges of about 20 to −20‰ for $\delta^{15}N$ and about 30 to −30‰ for $\delta^{18}O$ (see Ehleringer and Rundel (1988)). There are only very few reports for δ values of natural compounds that exceed these narrow ranges, e.g. $\delta^{15}N$ of about −60‰ in N_2O from nitrification (Yoshida 1988) or $\delta^{18}O$ of about 60‰ in NO_3^- from atmospheric deposition (Durka et al. 1994). Plotting of δ values against the corresponding N_2O emission rates (Figure 2), showed that the scattering only occurred below a threshold emission rate of approximately 2–5 μmol N_2O m^{-2} h^{-1} and fell into a more reasonable range of about ± 50‰ at higher emission rates. Equation (2) is based on the assumption that isotope and concentration differences between N_2O in ambient air and N_2O accumulated in the closed chamber are due to emission of N_2O from the soil. However, at very low emission rates, these differences decrease while the measurement error increases, until both become indistinguishable. To remove erroneous data, a threshold emission rate of 3.4 μmol N_2O m^{-2} h^{-1} was established (see Appendix). All data measured at lower emission rates were discarded. Sufficiently high emission rates occurred only immediately after fertilizer applications (Figure 3). No data at all remained for the control plots after

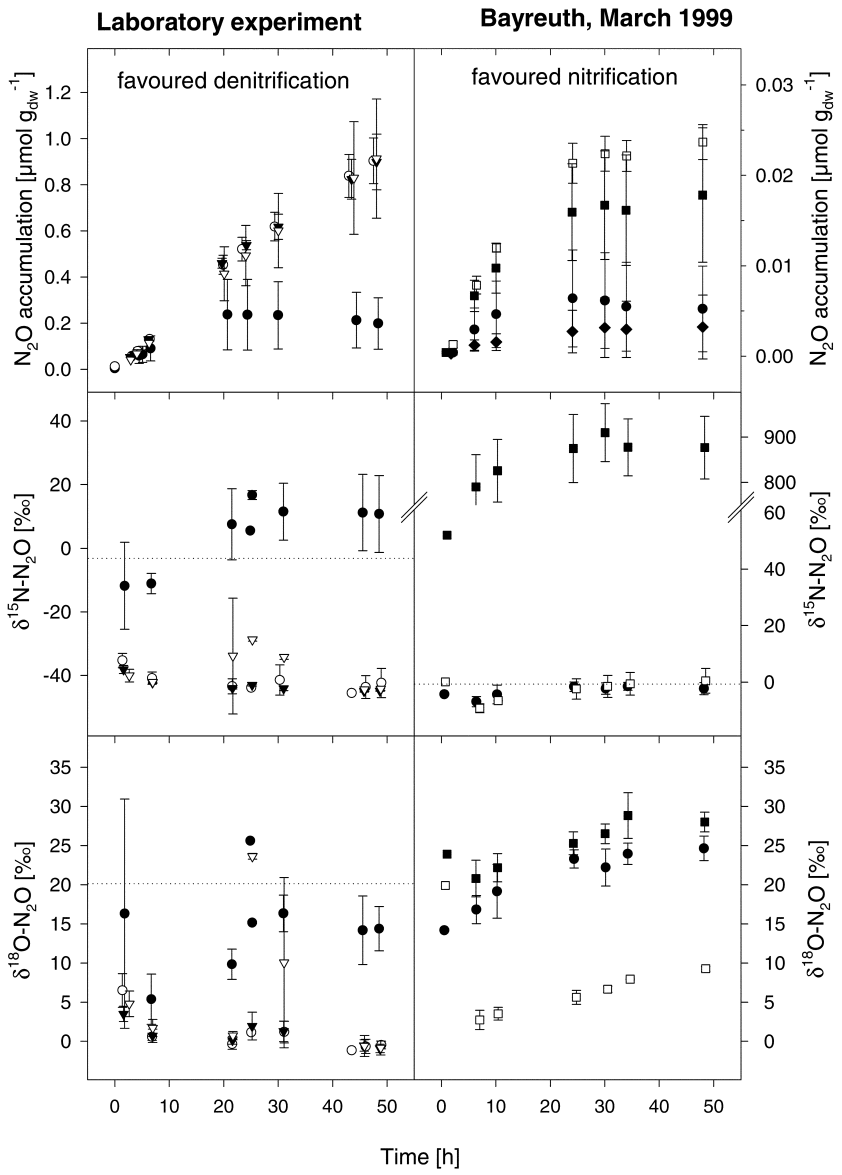

Figure 1. : Accumulation of N_2O in soil incubation experiments under conditions favouring either denitrification or nitrification and $\delta^{15}N$ and $\delta^{18}O$ values of the accumulated N_2O. Left column: favoured denitrification (80% soil water content, NO_3^- application, except for the control): ● control; ○ 100 mg NO_3^--N; ▼ 100 mg NO_3^--N, 0.01% C_2H_2; ▽ 100 mg NO_3^--N, 10% C_2H_2. Right column: favoured nitrification (60% soil water content, NH_4^+ application, except for the control): ● control; □ 100 mg NH_4^+-N; ■ 100 mg NH_4^+-N, $^{15}NO_3^-$-tracer; ◆ 100 mg NH_4^+-N, 100% O_2. Note the different scale for denitrification and nitrification experiments in the N_2O accumulation and $\delta^{15}N$ graphs. Error bars represent standard deviations (n = 3). Where no error bars are visible, they are smaller than the symbols. Dotted lines indicate the $\delta^{15}N$ and $\delta^{18}O$ values of the added KNO_3 and NH_4Cl, respectively.

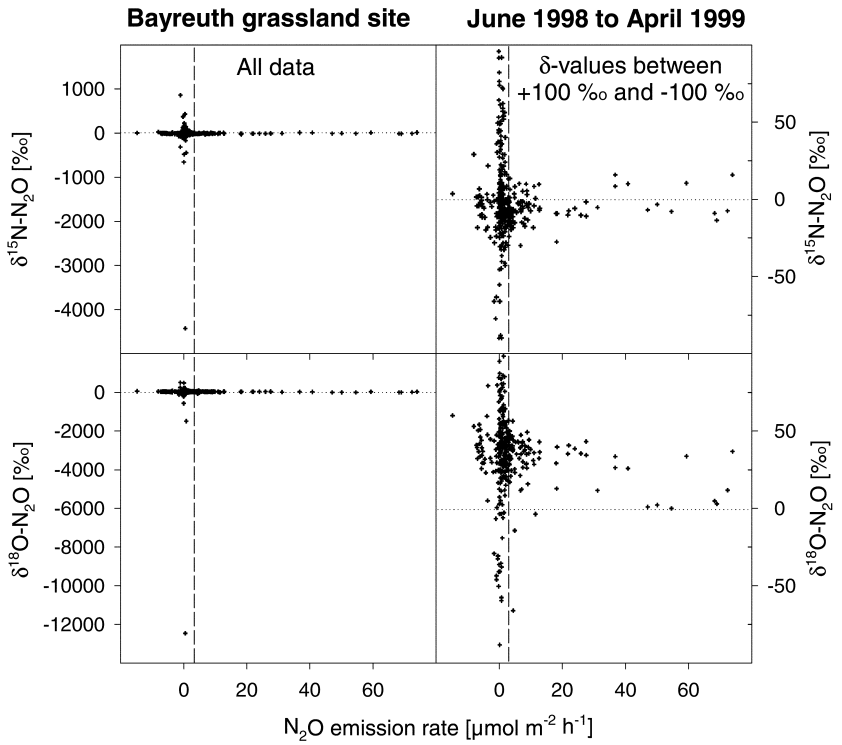

Figure 2. : $\delta^{15}N$ and $\delta^{18}O$ values of N_2O plotted against their coresponding N_2O emission rates obtained during the one-year field experiment. The left graphs show all IRMS data, the right graphs show only those in a range of −100 to +100‰. The vertical dashed lines represent the threshold N_2O emission rate of 3.4 μmol m^{-2} h^{-1} below which data are erroneous due to calculation artefacts (see section *Results* and Appendix for details).

the third fertilization (for results of the emission measurements see Tilsner et al. (2002)).

After exclusion of erroneous data, the remaining δ values fall into a range of $\delta^{15}N$ = −40 to 20‰ and $\delta^{18}O$ = 0 to 50‰. No temporal trends, significant differences between treatments or correlations between δ values and emission rates were detectable. Time and emission rate weighted annual mean δ values of the emitted N_2O were $\delta^{15}N$ = −4.6‰ and $\delta^{18}O$ = 24.0‰ for plots receiving mineral N applications, $\delta^{15}N$ = −8.6‰ and $\delta^{18}O$ = 34.7‰ for slurry fertilized plots and $\delta^{15}N$ = −6.4‰ and $\delta^{18}O$ = 35.6‰ for control plots, respectively (Table 3). For all treatments the emitted N_2O was ^{15}N-depleted compared to N_2O in ambient air.

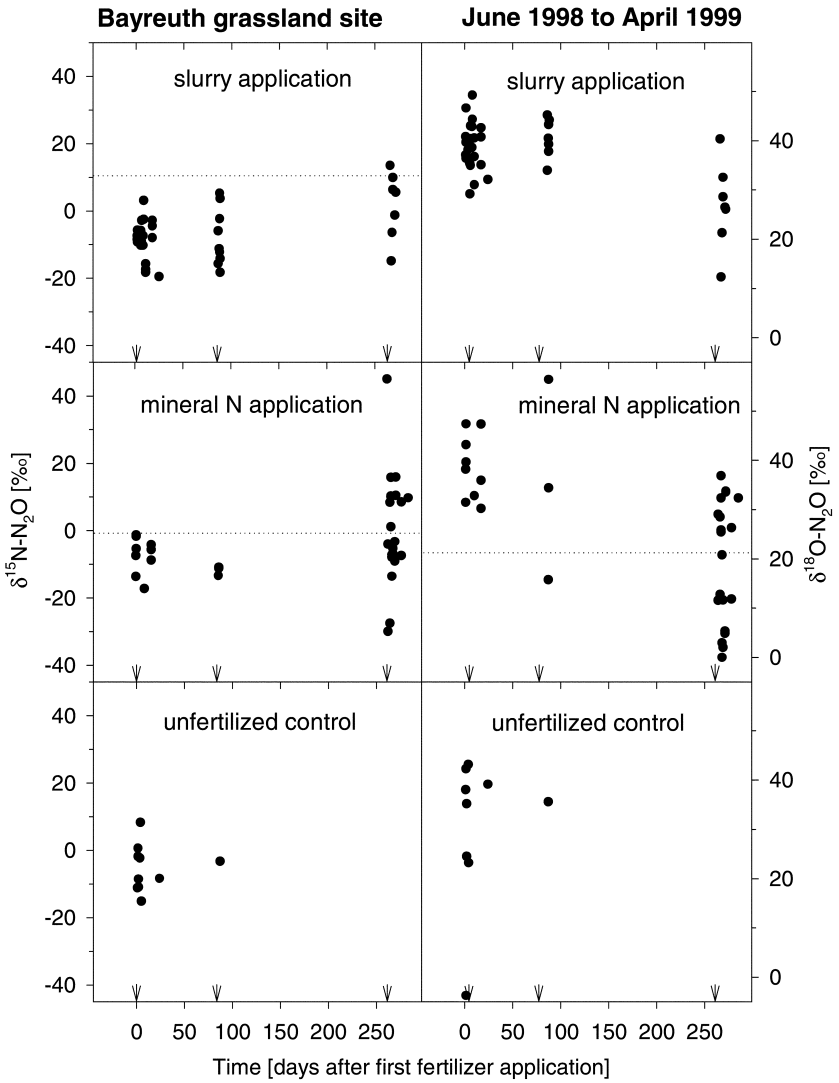

Figure 3. : $\delta^{15}N$ and $\delta^{18}O$ values of N_2O measured at emission rates $\geqslant$ 3.4 μmol N_2O m^{-2} h^{-1} in dependence on the time after fertilizer application. Symbols represent individual measurements. Note the different scale for $\delta^{15}N$ and $\delta^{18}O$. Fertilizer applications on 6 June 1998, 19 September 1998 and 15 March 1999 are indicated by arrows. Dotted lines indicate annual mean $\delta^{15}N\text{-}N_{total}$ values of the respective fertilizers. For $\delta^{18}O$, the dotted line indicates the $\delta^{18}O\text{-}NO_3^-$ of the mineral fertilizer used for the third fertilizer application.

Table 3. Means[1] of $\delta^{15}N$ and $\delta^{18}O$ values of N_2O in ambient air and of N_2O emitted from an extensively managed grassland in NE Bavaria, Germany, with three different types of treatments: slurry application, mineral N application and unfertilized control. Only IRMS data measured at N_2O emission rates at or above 3.4 μmol m^{-2} h^{-1} are included (see section *Results* and Appendix for explanation).

period	treatment	$\delta^{15}N$ [‰]	$\delta^{18}O$ [‰]
1 year	slurry	–8.6	34.7
	mineral N	–4.6	24.0
	control	–6.4	35.6
	ambient air[2]	11.4 ± 11.6	36.9 ± 10.7
after fertilization in June 1998	slurry	–8.3	39.5
	mineral N	–8.2	36.6
	control	–6.4	35.6
	ambient air[2]	–1.6 ± 12.0	39.1 ± 8.2
after fertilization in September 1998	slurry	–9.5	30.3
	mineral N	–16.6	17.9
	control	*no data*	*no data*
	ambient air[2]	15.5 ± 4.9	48.0 ± 14.7
after fertilization in March 1999	slurry	3.0	26.3
	mineral N	–0.6	18.6
	control	*no data*	*no data*
	ambient air[2]	15.8 ± 2.6	33.3 ± 2.8

[1]means are weighted by time and emission rate

[2]arithmetic mean of daily averages ± SD (n = 23 for the entire year, 10 for the June 1998, 4 for the September 1998 and 9 for the March 1999 fertilizations, respectively).

Discussion

Denitrification laboratory experiment

The linear increase of the N_2O concentration and the slight decrease of the soil nitrate content after NO_3^- addition indicate that denitrification took place and was not substrate-limited. With increasing NO_3^- availability, the ratio of N_2O/N_2 from denitrification usually increases (Blackmer and Bremner 1978; Mosier et al. 1983). Therefore, N_2O probably was the main end product of denitrification under the conditions of this experiment. This allows to interpret the N_2O isotope ratios of $\delta^{15}N = -40.8 \pm 5.7$‰ and $\delta^{18}O = 2.7 \pm 6.3$‰ as representative of the isotope signature of N_2O derived from denitrification without further reduction of N_2O to N_2. The $\delta^{15}N$ value corresponds to a depletion of −37.5‰ against the applied NO_3^- ($\delta^{15}N = -3.3 \pm 1.1$‰) and both, this depletion and the $\delta^{15}N$ and $\delta^{18}O$ values, agree with previous studies (Voerkelius 1990; Webster and Hopkins 1996). Due to discrimination against ^{15}N and ^{18}O, reduction of N_2O to N_2 would again lead to an increase in both, $\delta^{15}N$-N_2O and $\delta^{18}O$-N_2O.

In denitrification both, N and O in N_2O, originate from NO_3^-. However, at least 8% of the O atoms are exchanged with soil water during N_2O formation, depend-

ing on the turnover rate (Weeg-Aerssens et al. 1987). The actual fraction of O atoms from the applied KNO_3 ($\delta^{18}O$ = 20.1‰) that were exchanged with soil water ($\delta^{18}O$ = −10‰, Schmidt and Voerkelius (1989)) is unknown. Assuming the minimal exchange rate of 8%, the mixed substrate value would be $\delta^{18}O$ = 17.7‰ and the measured $\delta^{18}O$-N_2O value would represent an ^{18}O depletion of ca. 15‰. However, exchange of a greater fraction of the O atoms would result in a more negative substrate $\delta^{18}O$ value and the observed $\delta^{18}O$-N_2O could also be interpreted as a smaller ^{18}O depletion or even an ^{18}O enrichment against the substrate. Both, depletion and enrichment are possible, because the reaction pathway of NO_3^- reduction to N_2O includes inter-molecular isotope effects, leading to ^{18}O-depletion of the product, as well as intra-molecular isotope effects (preferential release of ^{16}O during NO_2^- reduction), leading to an ^{18}O-enrichment. In a sequential reaction pathway, an inter-molecular isotope effect can only result in an isotope discrimination in the pathway's final product, if not all of the available substrate is consumed, i.e. if intermediates accumulate. At a high turnover rate, only the rate limiting step contributes to the overall isotope effect. An intra-molecular effect, on the other hand, is independent of the turnover rate, because all reacting molecules are subjected to the discrimination. Slow and steady denitrification under substrate-limited conditions will, therefore, make an ^{18}O enrichment more likely. Rapid turnover after sudden addition of substrate, as in this experiment, will probably result in accumulation of intermediates and ^{18}O depleted N_2O (Shearer and Kohl 1988; Voerkelius 1990). Here, with the exchanged fraction of O atoms unknown, a conclusive interpretation of the $\delta^{18}O$ data is not possible.

The ^{15}N discrimination also depends on the process turnover rate. Considering the reduction of NO_3^- to N_2O, only inter-molecular effects are involved, leading to a ^{15}N depletion against the substrate. However, this depletion is more pronounced when intermediates accumulate and isotope effects of other reaction steps than the normally rate-limiting nitrate reduction become relevant. Therefore, a denitrification process rapidly starting after sudden substrate addition will lead to more negative $\delta^{15}N$-N_2O values than one steadily progressing with continual substrate availability (Mariotti et al. 1982; Voerkelius 1990). Considering also the reduction of N_2O to N_2, an inter-molecular isotope effect can once again cause an increase in $\delta^{15}N$-N_2O. Voerkelius (1990) found a ^{15}N fractionation of −4.2‰ for N_2O reduction. Vogel et al. (1981) reported that N_2 formed in incubation experiments with denitrifiying groundwater samples was depleted by −30‰ against the NO_3^- substrate, which is only 7.5‰ more positive than the ^{15}N-depletion of N_2O. The isotope effect of N_2O reduction thus appears to be small compared to that of N_2O production from NO_3^-. Thus, N_2O from denitrification can usually be expected to have more negative $\delta^{15}N$ values than its substrate. However, increasing N_2O concentrations lead to a greater isotope fractionation in N_2O reduction (Voerkelius 1990) and thus, more positive $\delta^{15}N$-N_2O values, as observed in the controls of this experiment. Summing up, a variety of different factors affect N_2O isotope composition, making it necessary that field experiments should always be accompanied by laboratory incubations of soil samples under a range of different conditions to determine soil specific characteristics of N_2O isotope composition.

Nitrification did not contribute significantly to N_2O production, as demonstrated by the absence of any effect of 10 Pa or 10 kPa C_2H_2 on N_2O formation rate or isotopic composition. Diffusion of C_2H_2 into the soil may have been ineffective, because of the high soil water content. But even so, at least in the 10 kPa acetylene incubation, diffusion of only 0.1% of the applied acetylene into the soil were sufficient to block nitrification. However, the C_2H_2 concentration in the soil air may not have been high enough to block N_2O reduction. Inhibition of this step by 10 kPa C_2H_2 has previously been found to be ineffective in wet soils (Malone et al. 1997).

Nitrification laboratory experiment

NH_4^+ consumption and an increase in soil NO_3^- prove that nitrification took place in all treatments of this experiment. However, under conditions favouring nitrification, the small N_2O emissions alone indicate that N_2O production in nitrification was much lower than in denitrification. Higher NH_4^+ consumption (350–470 μmol) than net NO_3^- formation ($\sim$ 15 μmol) indicates that denitrification might have occurred as well. The ^{15}N labelling of the soil NO_3^- pool resulted in considerably ^{15}N enriched N_2O, proving that denitrification, indeed, contributed significantly to the N_2O production. Furthermore, N_2O production levelled off in all NH_4^+ fertilized treatments, despite of more than 90% of the applied NH_4^+ still being present. If N_2O mainly derived from denitrification even under nitrification-favouring conditions, the rate of NO_3^- supply through nitrification could have been limiting for the N_2O production. Finally, lower N_2O production under 100% O_2 than in ambient air suggests inhibition of denitrification, although nitrifier denitrification could also have been affected. Thus, even the little N_2O emitted under conditions favouring nitrification cannot be completely ascribed to nitrification.

The experiment demonstrated clearly that even when nitrification took place, denitrification was a significant source of N_2O. Since the N_2O emitted in this incubation experiment could not be attributed to nitrification and since N_2O consumption probably occurred simultaneously, it was not possible to determine the isotope signature of N_2O from nitrification. In nitrification, N_2O-N comes from NH_4^+, with a δ value depending on the NH_4^+ source. NH_4^+ is oxidized to hydroxylamine (NH_2OH) with O_2 from soil air ($\delta^{18}O$ = 23.5‰, Schmidt and Voerkelius (1989)), so that O in N_2O formed via NOH disintegration should derive solely from that source. The second O atom for the oxidation of NH_2OH to NO_2^- originates from soil water ($\delta^{18}O \approx -10$‰, Schmidt and Voerkelius (1989)). N_2O from nitrifier denitrification should, therefore, have an intermediate $\delta^{18}O$ value of approximately 6.5‰.

Little data has been published on the isotope signature of N_2O derived from nitrification. Yoshida (1988) and Webster and Hopkins (1996) used pure cultures of *Nitrosomonas europaea* and *N. multiformis* and found a very strong ^{15}N depletion of more than 60‰ against the substrate. However, these data cannot be compared directly to a field situation, because NO_2^- accumulated during the experiment (Voerkelius 1990). This is not the case under field conditions, because NH_4^+ oxidation is

the rate limiting step. When NO_2^- accumulates, the inter-molecular isotope effect of the NO_2^- reduction step during nitrifier denitrification can contribute to the overall isotope discrimination, leading to a much greater ^{15}N depletion than under field conditions. Both, Voerkelius (1990) and Webster and Hopkins (1996), investigated the isotope signature of N_2O from nitrification in soil incubation experiments, but neither of these studies included additional tests for denitrification (100% O_2 or $^{15}NO_3^-$ labelling). In conclusion, the isotope composition of N_2O from nitrification has yet to be measured accurately. A possible approach could be to focus on the δ values of N_2O formed within minutes after NH_4^+ addition, before NO_3^- formation stimulates denitrification.

Field experiment

The δ values of N_2O from ambient air agree with the range reported by other authors (Yoshida and Matsuo 1983; Yoshinari 1990; Kim and Craig 1993). Even though individual soil emissions of isotopically enriched N_2O occurred, the mean $\delta^{15}N$-N_2O values weighted by time and emission rate were depleted against the respective samples of N_2O from ambient air for the entire year as well as for each fertilizer application (Table 3). Annual and June 1998 average $\delta^{18}O$ values of the emitted N_2O agreed with those of the respective samples from ambient air, but September 1998 and March 1999 values were also depleted (Table 3). The reasons why N_2O in air is usually enriched in heavy isotopes, while all known sources produce isotopically depleted N_2O, are still unknown (Kim and Craig 1993). Seasonal variation of ambient air N_2O isotope composition as an effect of climatic conditions has also been reported (Yoshida and Matsuo 1983), but there is not yet enough information available to allow the interpretation of our data with respect to such aspects.

The laboratory experiments show that N_2O was most likely produced by denitrification throughout the field experiment, because the field soil emitted very little N_2O under conditions favouring nitrification and even that derived at least partially from denitrification. Moreover, the generally wet soil conditions during fall, winter and spring (after the September and March fertilizer applications) that favour denitrification support this assumption. Also, after fertilizer applications the mineral N fertilized plots, where NO_3^- was immediately available, reached their maximal N_2O emission rate faster than the slurry fertilized plots, where nitrification had to proceed first in order to supply substrate for denitrification (for emission data see Tilsner et al. (2002)). Nevertheless, nitrification could have been the soil process with the highest N turnover despite not being the most important source of N_2O. Since N_2O is an obligatory intermediate of denitrification, the "leakage" in this process is expected to be higher than in nitrification (Firestone and Davidson 1989).

The isotope data obtained in the field experiment give no conclusive indication towards one particular process as a dominant N_2O source, especially since no definite information is available on δ values of N_2O from nitrification. Rather, the data show considerable variation in all treatments after each fertilizer application, demonstrating a very high spatial inhomogeneity of N_2O forming processes in the soil.

This has to be taken into account for further field experiments using stable isotopes for the distinction of N_2O from nitrification or denitrification. The lowest δ values found here ($\delta^{15}N = -30.0‰$ and $\delta^{18}O = -3.7‰$) agree with those from the denitrification laboratory experiment and correspond to depletions by approximately −30‰ against soil nitrate for both isotopes (data not shown, see Tilsner et al. (2002)). It has been pointed out above that less isotopically depleted, or in the case of ^{18}O even enriched, N_2O can be formed during denitrification as well. Furthermore, fractionation against ^{15}N during the reduction of N_2O to N_2 in a wet soil with hindered diffusion would result in a ^{15}N-enrichment of the remaining N_2O in the soil.

In conclusion, the analysis of stable isotopes at natural abundance levels can become a valuable tool for the identification of N_2O sources in the field. Difficulties encountered here were mostly due to the low N_2O emission rates. Therefore, further experiments on soils with higher N_2O fluxes should be carried out to improve the yield of data which are unbiased by calculation thresholds. The method may eventually remain restricted to sites with sufficiently high emission rates or requires further methodological improvement. Additionally, more research is needed on the isotope signature of N_2O derived from nitrification. The recently established position specific isotope analysis of the two N atoms of N_2O (Brenninkmeijer and Röckmann 1999) will likely improve identification of source processes based on δ values of the emitted gas. Isotope analyses of N_2O should always be combined with data on the isotope signature of the respective sources of N_2O production to calculate isotope fractionations.

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Appendix

Calculation of a threshold emission rate for reliable isotope data

The emission rate below which the intrinsic errors of the measurement system and the actual ‰ difference of $\delta x_{emitted}$ and $\delta x_{ambient\ air}$ become indistinguishable de-

pends on both, the source process and the isotope in consideration. To obtain one minimum emission rate that could be used for all isotope data, the isotopic differences between $\delta x_{emitted}$ and $\delta x_{ambient\ air}$ were "standardized": Means of both, $\delta^{15}N_{emitted}$ and $\delta^{18}O_{emitted}$, corrected for ambient air and weighted by time and emission rate were calculated for each of the three fertilization periods. Then, differences between weighted means of $\delta x_{emitted}$ and the corresponding mean $\delta x_{ambient\ air}$ values were calculated. The mean of these differences was −8.4‰. Thus, for the calculation of the threshold emission rate, δvalues were standardized as $\delta x_{ambient\ air} \equiv 0‰$ and $\delta x_{emitted} \equiv -8.4‰$. Equation (2) then yields:

$$\delta x_{measured} = \delta x_{emitted} + \frac{c_{ambient\ air}}{c_{measured}} \times (\delta x_{ambient\ air} - \delta x_{emitted}) \quad (4)$$

Considering the intrinsic errors of the measurement system, $\delta x_{measured}$ can be expressed as a minimum deviation from $\delta x_{ambient\ air}$ and Equation (4) solved to give the minimum value for $c_{measured}$: Although the error of the IRMS alone is smaller, the actual measurement error can be around ± 1–2‰, because the measurement error of the TGA and errors resulting from the sampling and the often low IRMS signal intensity (< 1 V) add to it. This also implies that the measurement error increases for low fluxes. It can be assumed that measurement errors plus natural variability in the δ values can result in a maximum difference of $\delta x_{ambient\ air} - \delta x_{measured} \approx 4‰$ without any actual N_2O emission. The minimum emission rate should therefore be such that $\delta x_{measured} \leq \delta x_{ambient\ air} - 4‰$. With $c_{ambient\ air} = 0.327 \pm 0.059$ ppm (annual mean in this study), Equation (4) yields $c_{measured} \geq 0.624$ ppm. Assuming a linear increase starting at $c_{ambient} = 0.327$ ppm at t_0 over an accumulation time of 40 minutes during which the soil chambers were closed, $c_{measured}$ corresponds to a minimum emission rate of 3.4 μmol $N_2O\ m^{-2}\ h^{-1}$. As can be seen from Figure 2, this value agrees well with the borderline between randomly scattering and interpretable isotope data. Therefore, for all further discussion only δ values measured at emission rates ≥ 3.4 μmol $N_2O\ m^{-2}\ h^{-1}$ (24% of all data) were considered.

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