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# The acetylene inhibition technique to determine total denitrification ( $N_2 + N_2O$ ) losses from soil samples: potentials and limitations

R. Felber<sup>1</sup>, F. Conen<sup>2</sup>, C. R. Flechard<sup>3</sup>, and A. Neftel<sup>1</sup>

<sup>1</sup>Agroscope Reckenholz-Tänikon Research Station ART, Air Pollution/Climate, Reckenholzstrasse 191, 8046 Zürich, Switzerland

<sup>2</sup>Institute of Environmental Geosciences, University of Basel, Bernoullistrasse 30, 4056 Basel, Switzerland

<sup>3</sup>INRA, Agrocampus Ouest, UMR1069 SAS, Rennes, France

Received: 2 February 2012 – Accepted: 6 February 2012 – Published: 13 March 2012

Correspondence to: R. Felber (raphael.felber@art.admin.ch)

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

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## Abstract

The loss of  $N_2$  from intensively managed agro-ecosystems is an important part of the N budget. The monitoring of  $N_2$  emissions at the field scale is impossible due to the high atmospheric background of 78 %, which precludes the measurement of fluxes.

5 The acetylene ( $C_2H_2$ ) inhibition technique is a rather simple, albeit imperfect, method to determine  $N_2$  losses from entire soil cores. Despite serious limitations it is one among very few methodological options to estimate total denitrification at high temporal resolution and on small spatial scale, with limited workload and costs involved. A laboratory system with two different detection systems (photoacoustic IR spectroscopy and gas chromatography) is presented, which allowed parallel measurements of up to 10 7 intact soil cores in air-tight glass tubes in a temperature controlled cabinet (adjusted to field conditions) with an automated  $C_2H_2$  injection.

A survey of total denitrification losses ( $N_2 + N_2O$ ) over 1.5 yr in soil from an intensively managed, cut grassland system in central Switzerland showed a lower bound loss in the range of 6 to 25 kg N ha<sup>-1</sup> yr<sup>-1</sup> (3–13 % of added N), roughly 3.4 times 15 higher than the  $N_2O$  loss.

However, several drawbacks of the  $C_2H_2$  inhibition technique preclude a more precise determination of the total denitrification loss.

## 1 Introduction

20 Nitrogen (N) is an essential nutrient for ecosystem functioning and food production in the world. N availability is one of the main limiting factors controlling the dynamics, biodiversity, functions, and services of many ecosystems (Vitousek et al., 1997). The increased demand for food and energy production on a global scale has altered the nitrogen cycle by introducing ever greater quantities of reactive nitrogen ( $N_r$ ) in the environment. The plant usable N is either in form of nitrate ( $NO_3^-$ ) or ammonium 25 ( $NH_4^+$ ). Only a relatively minor fraction of the N added by fertilisation and atmospheric

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deposition is actually taken up by plants, since various fractions are leached as  $\text{NO}_3^-$  and dissolved organic nitrogen (DON), assimilated by soil microbial biomass, emitted to the atmosphere as  $\text{NH}_3$  and  $\text{NO}$ , or denitrified to  $\text{N}_2\text{O}$  and  $\text{N}_2$ . Thus the increased introduction of  $\text{N}_r$  into ecosystems has also increased the losses of  $\text{N}_r$ , with environmental effects in other terrestrial and aquatic systems (e.g. eutrophication of rivers and lakes) and in the atmosphere (greenhouse gases, aerosol formation) through the so-called N cascade (Galloway et al., 2003).

Galloway et al. (2008) estimated today's anthropogenic nitrogen fixation to  $187 \text{ Tg N yr}^{-1}$ . On the global scale, they estimated that 10–40 % of introduced  $\text{N}_r$  is denitrified and returned to the atmosphere as  $\text{N}_2$ . In Switzerland, using a simple input-output-model Braun et al. (1994) calculated the total loss of N in agricultural systems on a national scale to 29 % (equivalent to  $44 \text{ kg N ha}^{-1}$  agricultural area  $\text{yr}^{-1}$ ) of introduced N.

For a quantitative description of the nitrogen cycle on the field scale, all relevant N fluxes have to be determined. Ammann et al. (2009) emphasised the necessity to measure total N losses to close the N budget and to decrease uncertainties. Even though total denitrification, the reduction within soils and aquifers of nitrogen oxides ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ) to  $\text{NO}$ ,  $\text{N}_2\text{O}$  and ultimately to  $\text{N}_2$ , represents an important part of the N budget, the measurement of  $\text{N}_2$  emissions is difficult due to the high background concentration (78 %) in the atmosphere (Groffman et al., 2006). A reliable and operational experimental approach is at distant prospect and all known approaches suffer from a large degree of uncertainty. Nonetheless, the development of a system where the total  $\text{N}_2$  losses of agro-systems can be estimated in situ is needed.

Various experimental approaches exist to measure  $\text{N}_2$  losses from soils. Groffman et al. (2006) published an overview of approaches to determine N losses in aqueous and terrestrial systems, including:

1. acetylene-based methods (e.g. Tiedje et al., 1989),
2.  $^{15}\text{N}$  tracers (e.g. Hauck and Melsted, 1956),

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3. direct N<sub>2</sub> quantification (e.g. Scholefield et al., 1997; Butterbach-Bahl et al., 2002; Cardenas et al., 2003),
4. mass balance approaches (e.g. Allison, 1955), and
5. molecular approaches.

5 van der Salm et al. (2007) using the C<sub>2</sub>H<sub>2</sub> inhibition technique found that up to 22 % of the applied N was lost due to denitrification. Other studies reported 10–40 % denitrification losses from fertilized maize fields in France (C<sub>2</sub>H<sub>2</sub> inhibition, Jambert et al., 1997), 0–25 % losses from permanent pasture in Switzerland (C<sub>2</sub>H<sub>2</sub> inhibition, Rudaz et al., 1999), and 10–70 % losses from cotton sites in Uzbekistan (direct N<sub>2</sub> quantifica-  
10 tion, Scheer et al., 2009).

The C<sub>2</sub>H<sub>2</sub> inhibition technique is based on the findings by Fedorova et al. (1973) that C<sub>2</sub>H<sub>2</sub> at high concentrations (>0.5 %) in the headspace of pure microbe samples inhibits the microbial reduction of N<sub>2</sub>O to N<sub>2</sub>. Consequently N<sub>2</sub>O emissions measured after addition of C<sub>2</sub>H<sub>2</sub> at high concentration should correspond to the production of N<sub>2</sub>  
15 and N<sub>2</sub>O in the soil sample under consideration.

The C<sub>2</sub>H<sub>2</sub> inhibition method is in principle an easy approach to measure total denitrification. However, limitations of the C<sub>2</sub>H<sub>2</sub> inhibition method are the scavenging (C<sub>2</sub>H<sub>2</sub>-catalysed oxidation) of the intermediate NO, the precursor of N<sub>2</sub>O that itself is the precursor of N<sub>2</sub> (Bollmann and Conrad, 1997) and the suppressed microbial respiration  
20 through C<sub>2</sub>H<sub>2</sub> (Zhang et al., 2009). In addition, inhibition by C<sub>2</sub>H<sub>2</sub> might be incomplete when added C<sub>2</sub>H<sub>2</sub> does not reach the denitrifiers due to limited diffusion in the entire soil samples. This can be minimised by using soil cores with a small diameter to ensure a sufficient diffusion into the core and also into water filled pores.

Clearly, there is at present no scientific consensus as to the reliability and adequacy  
25 of the C<sub>2</sub>H<sub>2</sub> inhibition technique for total denitrification measurements. The present study investigates the plausibility of total N losses from a managed grassland soil, obtained by the C<sub>2</sub>H<sub>2</sub> inhibition method in the laboratory using intact soil cores, and compared to actual N<sub>2</sub>O emissions measured by static chambers in the field.

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The total denitrification (the sum of  $N_2$  +  $N_2O$  fluxes) is estimated here from  $C_2H_2$  incubations of soil samples from the measurement of  $N_2O$  fluxes.

## 2 Material and methods

### 2.1 Field site description and soil samples

5 The experimental grassland site was located at Oensingen in Central Switzerland ( $7^{\circ}44'$  E,  $47^{\circ}17'$  N) at 450 m a.s.l. and was formerly part of the CarboEurope and NitroEurope integrated projects (Ammann et al., 2007, 2009). The region is characterized by a relatively small scale pattern of agricultural fields (grasslands and arable crops). The climate is temperate with an average annual rainfall of about 1100 mm and a mean  
10 annual air temperature of  $9.5^{\circ}C$ . Until November 2001, the field had been under a ley-arable rotation management (common for the region) with a typical rotation period of 8 yr including summer- and winter-wheat, rape, maize and bi- or tri-annual grass-clover mixture. The nitrogen input depended on the crop type and followed the Swiss standard fertilisation practice ( $110 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  on average). In November 2000 the field was  
15 ploughed and in May 2001 sown with grass-clover mixtures typical of permanent grassland under intensive management in Switzerland. The field was cut typically four times per year and was fertilised twice or thrice with solid ammonium nitrate or liquid cattle manure (overall  $230 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). In December 2007 the field was again ploughed up and in May 2008 sown with grass-clover mixture. This was necessary to re-establish a homogenous flora and to restore the productivity of the grassland system.  
20

The soil is classified as Eutri-Stagnic Cambisol (FAO, ISRIC and ISSS, 1998) developed on clayey alluvial deposits. Clay contents between 42 % and 44 % induce a total pore volume of 55 % and a fine pore volume of 32 % (permanent wilting point). Average soil organic carbon contents in the upper 20 cm are  $25\text{--}35 \text{ g kg}^{-1}$  dry soil and the C/N  
25 ratio is around 9.5. The soil pH is around 7.3 (Ammann et al., 2009).

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The measurement campaign lasted 18 months from July 2008 until the end of 2009. On each sampling occasion seven soil samples of 2 cm diameter and 30 cm length were spatially randomly collected from the field with a hand auger and transported within one hour to the laboratory. The sampling frequency was irregular, but intensified during special events when high denitrification rates were expected (large amounts of rainfall, management events like harvest and fertilisation).

## 2.2 Total denitrification measurements

### 2.2.1 Laboratory instrumental set up

The measurement principle of total denitrification (the sum of  $N_2O + N_2$  fluxes), in  $C_2H_2$ -treated soil samples, is based on the assumption that every  $N_2$  molecule which would normally escape from the soil system, actually ends up as  $N_2O$ , which is detected in our laboratory set up by photoacoustic IR spectroscopy (pIRS) or gas chromatography (GC). The laboratory system allows the quasi-simultaneous measurements of trace gas fluxes from up to 7 soil cores in air-tight glass tube incubators in a temperature-controlled cabinet adjusted to field soil temperatures (Fig. 1). The  $C_2H_2$  injection is automated. A circulation pump mixes the gas concentrations in the head space of the glass tube and transports the gas into a gas analyser. The tubing system, attached to the switching valve delivering the samples via polyurethan tubing, can be flushed with ambient air to preclude contamination from samples of the previous batch. In particular this cleaning is used if a mixing of  $C_2H_2$  into a sample without  $C_2H_2$  has to be avoided.

To evaluate emission fluxes, the gas concentrations of  $CO_2$ ,  $N_2O$ , and  $C_2H_2$  are monitored over a period of typically 20–30 min. The increase in  $CO_2$  over time shows the respiratory activity of the sample. A decrease in the  $C_2H_2$  concentration indicates a potential leak in the system as consumption or production processes of  $C_2H_2$  affecting the applied concentration are unlikely. The sampling frequency of one minute allows a quasi-continuous observation of the gas evolution. At first, the Innova 1312

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photoacoustic IR gas analyser (INNOVA Air Tech Instruments, Ballerup, Denmark; www.innova.dk) was used for the concentration measurements. Four wavelength-selective infra-red filters were chosen for the detection of H<sub>2</sub>O, CO<sub>2</sub>, N<sub>2</sub>O, and C<sub>2</sub>H<sub>2</sub>: SB0527 (1985 ± 40 cm<sup>-1</sup>) with strong H<sub>2</sub>O absorption lines, UA0983 (2270 ± 15 cm<sup>-1</sup>) with strong CO<sub>2</sub> absorption lines, UA0985 (2215 ± 22 cm<sup>-1</sup>) with strong N<sub>2</sub>O absorption lines, and UA0969 (1254 ± 34.5 cm<sup>-1</sup>) with C<sub>2</sub>H<sub>2</sub> absorption lines. These are broadband filters which result in cross interferences between the different gases (including H<sub>2</sub>O) in the different filters (Yamulki and Jarvis, 1998).

The Innova 1312 offers an internal compensation for the interference from water vapour and other gases such as CO<sub>2</sub>. However, Flechard et al. (2005) found that the N<sub>2</sub>O concentrations indicated by the Innova 1312 output were still heavily dependent on CO<sub>2</sub>, H<sub>2</sub>O and the temperature of the photoacoustic cell. Clearly the manufacturer's corrections for water vapour interference and cross interferences were not adequate and resulted in very large biases, typically exceeding the biases reported by Yamulki and Jarvis (1998). We therefore developed our own correction algorithm based on a labor-intensive calibration procedure and adapted the algorithm used by Flechard et al. (2005).

To further minimize effects arising from instrument temperature changes and the interference by water vapour, the Innova 1312 was set up in a temperature-controlled cabinet and the sample's water vapour content was lowered by condensation to a constant dew point of 4 °C (cf. gas cooler in Fig.1, left). We recorded and processed the analog photoacoustic signal of the Innova 1312, instead of using the instrument's internally processed/corrected concentration data. The raw signal is composed for each gas species of contributions by several gases (Eq. 1):

$$\text{Sig}_i = C_{\text{H}_2\text{O}} \times k_{\text{H}_2\text{O} \rightarrow i} + C_{\text{CO}_2} \times k_{\text{CO}_2 \rightarrow i} + C_{\text{N}_2\text{O}} \times k_{\text{N}_2\text{O} \rightarrow i} + C_{\text{C}_2\text{H}_2} \times k_{\text{C}_2\text{H}_2 \rightarrow i} + k_i \quad (1)$$

Sig<sub>*i*</sub> is the analog signal measured by filter *i* in mV, *C* stands for the concentration of the gas, and *k*<sub>gas→*i*</sub> defines the absorption strength of the gas in the transmission range of filter *i*. Thus, each gas concentration has a different influence on the raw signal,

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indicated by  $k_{\text{gas}\rightarrow j}$ . We assumed that there were no additional significant contributions to the signal (and thus interferences) by gases other than the ones listed in Eq. (1).

Under stable temperature and water vapour conditions, accurate calibrations using different gas mixtures (300 ppb to 5000 ppb  $\text{N}_2\text{O}$ , 300 ppm to 10 000 ppm  $\text{CO}_2$ , and 1 % to 7 %  $\text{C}_2\text{H}_2$ ) were carried out in order to determine the values of the different  $k_{\text{gas}\rightarrow j}$  parameters of Eq. (1).

The large cross interferences resulted in a precision of  $\pm 20$  ppb for ambient  $\text{N}_2\text{O}$  concentration, and thus a rather high flux detection limit of  $40 \text{ ng N}_2\text{O m}^{-2} \text{ s}^{-1}$  for our laboratory incubation set up. The high detection limit prohibited an accurate measurement of small fluxes, which were detected – and not resolved – for most of the time. In addition the problem of leakage led to very small concentration changes, which were below the detection limit of the pIRS analyzer.

To avoid these difficulties, in July 2009, we changed to a SRI 8610C gas chromatograph (GC) using a 500  $\mu\text{l}$  sample loop. The gas was analysed by an Electron Capture Detector (ECD:  $\text{N}_2\text{O}$ ,  $\text{CO}_2$ ) and a Flame Ionisation Detector (FID:  $\text{C}_2\text{H}_2$ ). The separation of the gases was achieved by a micropacked SilcoSmooth column (2 m, 1 mm ID) containing ShinCarbon stainless steel (mesh 100/120) (Restek Chromatography Products, Bellfonte, US; [www.restek.com](http://www.restek.com)) and using  $\text{N}_2$  as carrier gas.

Bi-polynomial integrals of the ECD and FID peaks were fitted. To overcome instabilities of the ECD signal, an automated calibration with four standard gases before each measurement cycle was implemented. The GC was calibrated with gas mixtures containing between 300 ppb and 5000 ppb  $\text{N}_2\text{O}$ , and 300 ppm to 10 000 ppm  $\text{CO}_2$ .

## 2.2.2 Experiments

In the base runs, the samples were measured without  $\text{C}_2\text{H}_2$  for at least one or two cycles (referred to as  $\text{C}_2\text{H}_2$ -free fluxes) resulting in fluxes which should be comparable to field conditions, albeit with differences resulting from sampling, transportation, change of water content, etc. All samples were then incubated with  $\text{C}_2\text{H}_2$  (referred to as  $\text{C}_2\text{H}_2$ -treated fluxes) for several hours and measured at least four times.

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These experiments do not allow a direct (i.e. simultaneous) comparison of C<sub>2</sub>H<sub>2</sub>-free fluxes versus C<sub>2</sub>H<sub>2</sub>-treated fluxes, due to the short term (a few minutes to a few hours) dynamics of C and N turnover. Therefore, to assess the effect of C<sub>2</sub>H<sub>2</sub> on the microbial activity and to provide a direct comparison of C<sub>2</sub>H<sub>2</sub>-free fluxes versus C<sub>2</sub>H<sub>2</sub>-treated fluxes three special experiments were conducted (referred to as C<sub>2</sub>H<sub>2</sub>-effect experiments). During these measurements three samples were measured without C<sub>2</sub>H<sub>2</sub> for the entire measurement and four were simultaneously incubated with C<sub>2</sub>H<sub>2</sub>. Before switching from a C<sub>2</sub>H<sub>2</sub>-treated sample to a C<sub>2</sub>H<sub>2</sub>-free sample the analytical part of the tubes was flushed by ambient air to avoid the contamination by C<sub>2</sub>H<sub>2</sub> of the C<sub>2</sub>H<sub>2</sub>-free samples.

### 2.2.3 Flux evaluation

The temporal concentration changes ( $\partial C$ ) of N<sub>2</sub>O and CO<sub>2</sub> over time ( $\partial t$ ) in the volume of the system are proportional to the exchange fluxes as described in Flechard et al. (2005) for the case of static chambers in the field:

$$F_i = \frac{V}{A} \frac{\partial C_i}{\partial t} \quad (2)$$

Here, in the case of the C<sub>2</sub>H<sub>2</sub> inhibition system,  $V$  stands for the headspace volume of the glass tube incubator plus the volume of circulation tubing, and  $A$  for the soil surface area exposed to the headspace.  $V$  is also equivalent to the volume of the total system minus the volume of the soil sample, and  $A$  is the cross sectional area of the sample.

Figure 2 illustrates the temporal course of a typical measurement batch. The letter  $S$  (Start) stands for the concentration at the time after switching when the sample air is well mixed,  $E$  (End) for the last measurement point. The first subscript refers to the sample (port) number, the second subscript to the measurement iteration. There are two potential ways to calculate fluxes from the observed concentration changes ( $\partial C$ ) in the headspace:

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(A) from the slope when the soil sample is directly being monitored (Fig. 2, dashed lines);

(B) from the concentration change in the headspace between the end point ( $E$ ) of one flux measurement run and the start point ( $S$ ) of the next run (i.e. during the period when the other soil samples are being monitored) (Fig. 2, dotted line).

In the case of method (B) the sample in the analytical loop at the beginning of the measurement (see Fig. 2, point  $S$ , i.e. after switching from the previous soil sample to the current sample), is mixed with the headspace air accumulated during the time the sample was closed. This inevitable mixing is a problem because the circulation (tubing) loop volume is large compared with the headspace volume, and it needs to be mathematically corrected for. Thus the corrected (true) concentration point  $S'_{2,2}$  would lie higher than the actually measured concentration  $S_{2,2}$ , since in the analytical loop a lower concentration was extant and was mixed with the higher concentration of the actual sample. Conversely, for sample 4, the true concentration  $S'_{4,2}$  would actually lie below the measured concentration  $S_{4,2}$ , since the higher concentration of the analytical loop was mixed with the lower concentration of the sample's headspace.

On the other hand, the flux calculation from the directly measured concentration change (method A) can be misleading if a sample with low denitrification rates is measured after a sample with higher denitrification rates, the problem arising from the slow mixing of the amount of gas carried over from the previous sample. This is evident from the comparison of concentration points  $E_{4,1}$  and  $S_{4,2}$  in Fig. 2, showing an increase during the time the sample was not measured, and demonstrating a clear emission, while the slope of the concentration change from  $S_{4,1}$  to  $E_{4,1}$  was negative, apparently indicative of a consumption due to carry-over effects. Therefore, for the flux calculation the second method (B) was judged more stable and was used for calculation throughout this paper.

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## 2.3 Flux data evaluation

It occasionally occurred that N<sub>2</sub>O fluxes measured from C<sub>2</sub>H<sub>2</sub>-treated samples ended up being smaller than in C<sub>2</sub>H<sub>2</sub>-free soil. Clearly such occurrences should be identified and filtered out from the dataset. The flux results were therefore processed as follows:

1. N<sub>2</sub>O fluxes measured from C<sub>2</sub>H<sub>2</sub>-free samples being higher than from C<sub>2</sub>H<sub>2</sub>-treated samples indicated that at least one of the above described limitations (NO scavenging or suppressed microbial activity) was a dominant process. In this case the total N loss measured in C<sub>2</sub>H<sub>2</sub>-free samples was regarded as a lower bound of N<sub>2</sub> losses.
2. Fluxes of N<sub>2</sub>O in C<sub>2</sub>H<sub>2</sub>-treated samples being larger than in C<sub>2</sub>H<sub>2</sub>-free samples indicated that the limitations were at best low or moderate, but it cannot be excluded that the limitations were actually large. The N<sub>2</sub>O flux of C<sub>2</sub>H<sub>2</sub>-treated samples thus represented a lower boundary of total N losses, since not necessary all denitrifying microorganisms were C<sub>2</sub>H<sub>2</sub>-inhibited.

Based on the fluxes calculated by Eq. (2) the mean sample flux ( $\overline{F_s}$ ) was calculated by the mean of the three largest fluxes from C<sub>2</sub>H<sub>2</sub>-treated samples. The daily mean flux of all C<sub>2</sub>H<sub>2</sub>-treated samples was then calculated from the arithmetic mean of all samples ( $\overline{F}_{\text{day}} = \text{mean}(\overline{F_s})$  with  $s$  = number of samples) and the corresponding standard deviation ( $\text{sd} = \text{sd}(\overline{F_s})$  with  $s$  = number of samples) was computed. Concerning the uncertainty associated with the daily mean flux, given that N<sub>2</sub>O fluxes are not normally distributed but rather log-distributed, we provide the range of log- and then back-transformed values (Limpert et al., 2001). The range of daily fluxes ( $F_{\text{day}}$ ) was thus calculated as:

$$F_{\text{day}} = \bar{x}^* / s^* \dots \bar{x}^* \times s^* \quad (3)$$

with  $\bar{x}^* = \overline{F}_{\text{day}} / \sqrt{\omega}$  and  $s^* = e^{\sqrt{\ln \omega}}$ , with  $\omega = 1 + (\text{sd} / \overline{F}_{\text{day}})^2$ .

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## 2.4 Quasi-continuous N<sub>2</sub>O measurements with static chambers

N<sub>2</sub>O and CO<sub>2</sub> fluxes have been monitored in the field at the Oensingen experimental site since 2004 using stainless steel automatic static chambers (side length: 30 cm, height: 25 cm). Up to eight chambers were operated in the field, providing quasi-continuous flux measurements at a regular interval (2 h). The chambers were mounted on PVC frames that were inserted permanently 5 cm into the soil (Conen and Smith, 1998; Flechard et al., 2005).

For special investigations the chambers were manually controlled. This mode was used to follow the isotopic composition of the N<sub>2</sub>O concentration in the chambers headspace for “zero” flux conditions, i.e. in cases when no increase of N<sub>2</sub>O could be measured. The temporal change in the isotopic composition (<sup>15</sup>N, <sup>18</sup>O) of the constant N<sub>2</sub>O concentration in the headspace allows the discrimination of gross N<sub>2</sub>O production and consumption processes (van Groenigen et al., 2005; Vieten et al., 2007).

## 3 Results

### 3.1 N<sub>2</sub> and N<sub>2</sub>O measurements

Figure 3 gives an overview of the flux data measured in the field during the experimental campaign. The amount of rainfall (Fig. 3a) during the measurement was comparable to other years. The cumulative amount of rain was 664 mm for July to December in 2008 and 1005 mm in 2009. Figure 3a also shows the management events (i.e. cuts and fertilizing events). Soil moisture content (Fig. 3b) corresponded to other years and no exceptional dry period occurred during the measurement period. N<sub>2</sub>O fluxes measured with static chambers in the field showed similar patterns as in the years before. Peak N<sub>2</sub>O emissions occurred mainly after fertilisation events when soil moisture content was large.

Table 1 shows the total N (N<sub>2</sub> + N<sub>2</sub>O) losses, measured as N<sub>2</sub>O fluxes, by C<sub>2</sub>H<sub>2</sub> inhibition of incubated soil samples, and the corresponding actual N<sub>2</sub>O fluxes measured at

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the same date by the automatic chambers in the field. The irregular sampling frequency for the inhibition measurements was related to trigger events (heavy rain, fertilizer application, mowing, etc.), when high losses of N were expected.

The overall patterns of the temporal variability in the field N<sub>2</sub>O chamber measurements and in the laboratory data by the C<sub>2</sub>H<sub>2</sub> inhibition technique were similar; larger emissions in the field coincided in larger C<sub>2</sub>H<sub>2</sub> fluxes in the laboratory. The total N losses varied between 0.3 and 9.5 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> for the period using the pIRS and 0.9 and 86.7 mg N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup> for the GC measurements.

In general the loss of N either as N<sub>2</sub>O or as N<sub>2</sub> increased with soil moisture. After fertilisation the losses were very high for a short time, even in summer. In periods with slurry application over dry soil, the loss of N was small (see Fig. 3 around 19 September 2008). Larger losses were not related to the wetness of the soil only, as the level of available nitrate and soil temperature also controlled the magnitude of denitrification.

On five measurement days, the total loss of N, as estimated by C<sub>2</sub>H<sub>2</sub> inhibition of incubated soil samples, was actually smaller than the loss measured by the static chambers in the field (Table 1).

### 3.2 CO<sub>2</sub> fluxes

CO<sub>2</sub> fluxes measured in the C<sub>2</sub>H<sub>2</sub> inhibition system can be assimilated to soil heterotrophic respiration, while field chamber fluxes provided total ecosystem respiration. Earlier investigations of soil- vs. total ecosystem-respiration at the Oensingen grassland site had shown a difference in the order of 50 % between ecosystem respiration and soil respiration fluxes (Ammann et al., 2006). Therefore, to assess the effect of soil sampling on microbial activity, a comparison of the incubated CO<sub>2</sub> fluxes to 50 % of the ecosystem respiration measured by static chambers in the field is shown in Fig. 4a. The 50 % ecosystem respiration is in most cases smaller than the C<sub>2</sub>H<sub>2</sub>-free flux, with the slope of the linear regression around 0.5, indicating a laboratory-based respiration roughly twice as large as the assumed field soil respiration.

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CO<sub>2</sub> fluxes measured from the samples before the C<sub>2</sub>H<sub>2</sub> injection did not significantly differ from CO<sub>2</sub> fluxes measured after the injection of C<sub>2</sub>H<sub>2</sub> (Fig. 4b). The three data points from the C<sub>2</sub>H<sub>2</sub>-effect experiment (Fig. 4b: open circles, cf. Sect. 2.2.2) also lie close to the 1/1-line, indicating that the incubation by C<sub>2</sub>H<sub>2</sub> did not crucially alter the soil respiratory activity. The upper cluster of measurement points corresponds to the large emission peak observed in the N<sub>2</sub>O chamber measurements in August 2009. The large spatial variability of CO<sub>2</sub> fluxes found in the field was also seen in the samples measured in the laboratory (large error bars in Fig. 4a and b).

### 3.3 N<sub>2</sub>O measurements and N<sub>2</sub>O/N<sub>2</sub> ratio

The three C<sub>2</sub>H<sub>2</sub>-effect experiments (Sect. 2.2.2) showed that C<sub>2</sub>H<sub>2</sub>-treated samples emitted, on average, twice as much N<sub>2</sub>O than the C<sub>2</sub>H<sub>2</sub>-free samples (Fig. 5), hence the inhibition of the last step in the denitrification was active, even if the efficiency of the inhibition remains uncertain.

Figure 6 shows the relationship between the N<sub>2</sub>O fluxes measured by the chambers in the field and the total N (N<sub>2</sub> + N<sub>2</sub>O) loss estimated from C<sub>2</sub>H<sub>2</sub>-treated samples in the laboratory incubation system. The error bars in both directions demonstrate the typical small scale spatial variability associated with N<sub>2</sub>O flux measurements (e.g. Parkin, 1987).

On three occasions field N<sub>2</sub>O emissions were higher than the corresponding laboratory based total denitrification fluxes. This was especially likely for high fluxes after fertilisation where the N<sub>2</sub>O fluxes are controlled by available N and accessible energy (carbon assimilates) for the denitrifying microbial communities.

In 2009 the total loss of N<sub>2</sub>O + N<sub>2</sub> was dominated by the slurry event around 6 August. Over the measurement period, total N<sub>2</sub>O + N<sub>2</sub> losses estimated by C<sub>2</sub>H<sub>2</sub> inhibition were in the range of 6 to 26 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Table 1), i.e. 3–13 % of added N per year (Table 1). The median over the entire experiment was 9.7 kg N ha<sup>-1</sup> yr<sup>-1</sup> which is 2.4 times higher than the actual field N<sub>2</sub>O emission.

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### 3.4 Isotopic composition of the N<sub>2</sub>O concentration for zero flux conditions

Isotopic analysis during flux measurements with small but detectable concentration changes (“zero” flux condition = flux < 1.5 ng N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) showed a significant change in the <sup>15</sup>N and <sup>18</sup>O isotopic fractions (Fig. 7). Although enrichment factors are associated with large uncertainties, these changes indicated that a “zero” flux did not necessarily mean no N<sub>2</sub>O production. In such situations the gross production of N<sub>2</sub>O was equal to the gross consumption (i.e. reduction of N<sub>2</sub>O to N<sub>2</sub>).

## 4 Discussion

### 4.1 The dilemma of measuring total denitrification while reducing the denitrification potential

The acetylene inhibition technique was performed in a laboratory set up on intact soil cores with a small diameter (2 cm) over the first 30 cm of the soil. The size of the soil cores was a compromise between the following aspects: (1) the samples, although measured in the laboratory, should be as close as possible to field conditions, (2) the C<sub>2</sub>H<sub>2</sub> should penetrate the sample and reach the active denitrification micro-sites by diffusion, and (3) the produced N<sub>2</sub>O should escape into the headspace as soon as it is produced in the sample.

Inevitably soil sampling altered the condition of the sample in several ways:

1. Aeration was enhanced as the bulk part of the measured soil was closer to the surface and might have enhanced the microbial activity by an increase of oxygen resulting in a higher loss of N and a boosted CO<sub>2</sub> production. The magnitude of this effect was checked by comparing the ecosystem respiration measured in the field with the respiration of the laboratory samples. Assuming that soil respiration in the field constitutes as a rule of thumb about 50 % of the ecosystem respiration,

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a systematic increase of soil respiration must have occurred in the samples measured in the laboratory, but it is unclear whether this is actually due an increased  $O_2$  supply, or to an enhanced supply of easily degradable soil organic matter (e.g. freshly cut roots).

2. Plant activity was changed due to sampling (e.g. roots were cut). Active plants are a source of energy through root exudates for micro-organisms as well as mycorrhiza. This effect could not be quantified as the alteration might go in both directions, decrease or augmentation of  $N_2O$  production (Gillam et al., 2008).
3. The incubation with  $C_2H_2$  affected the soil microbial community in different ways.  $C_2H_2$  also potentially inhibits nitrification processes (Berg et al., 1982) and thus the supply of nitrate for denitrification may have been reduced or interrupted. As nitrate was in a steady state before the addition of  $C_2H_2$ , suppression of the inflow (i.e. the oxidation of  $NH_4^+$  to  $NO_3^-$ ) reduced the  $NO_3^-$  level at a timescale comparable to the turnover time. To limit the magnitude of this effect, the measurements were conducted as quickly as possible without a long lasting incubation period. High level of  $C_2H_2$  (5%) might suppress microbial activity in general (Zhang et al., 2009), but as stated above we did not observe a systematic change in soil respiration activity by the addition of  $C_2H_2$ .

Overall there were many effects that potentially reduced  $N_2O$  production in the  $C_2H_2$ -treated samples compared to the true  $N_2O$  and  $N_2$  production. For an estimation of the mean total N loss from one sample, the three highest fluxes were taken, assuming that for these runs the  $C_2H_2$  had sufficient time to enter the denitrification spots, and  $N_2O$  could be released from the sample before consumption.

## 4.2 Spatial considerations

The results comparing  $C_2H_2$ -treated fluxes to chamber fluxes should be treated with care since the samples used in the  $C_2H_2$  inhibition measurements were not taken

from the same locations as the chambers (i.e. within the chamber), but randomly in their close vicinity (within 30 m). There are different explanations for the occasional observations of field chamber N<sub>2</sub>O fluxes being higher than C<sub>2</sub>H<sub>2</sub>-treated N<sub>2</sub>O fluxes (see Fig. 6 and Table 1). Differences may occur due to different scale lengths and spatial heterogeneities of the field. The scale length represents the mean distance a N<sub>2</sub>O molecule travels until it is reduced to N<sub>2</sub> (Neffel et al., 2000). The chamber N<sub>2</sub>O fluxes were controlled from top 5 cm whereas the C<sub>2</sub>H<sub>2</sub> inhibition system measured the integral loss from samples from 0–30 cm depth. Furthermore, the likelihood of N<sub>2</sub>O escaping to the atmosphere decreased with increasing depth of denitrification as the scale length of N<sub>2</sub>O molecules in the soil is at most a few centimetres for the Oensingen soil type (Neffel et al., 2000).

### 4.3 Estimation and accuracy of the total N loss

The Innova 1312 photoacoustic IR instrument showed large interference by C<sub>2</sub>H<sub>2</sub> and CO<sub>2</sub> on the N<sub>2</sub>O concentration and therefore severely hampered the accuracy of the flux determination. The detection limit with the pIRS was around 40 ng N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, i.e. a factor 10 higher than using the GC (3.1 ng N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) independent of the C<sub>2</sub>H<sub>2</sub> level. Therefore using a GC allowed the determination of small background fluxes, which occur most of the time, although the problem of leakage could not be solved by changing the trace gas detection device. The two ways to calculate fluxes (cf. Sect. 2.4) offer a quality check of the flux determination. For samples with a strong denitrification activity both approaches yielded comparable results (see Fig. 2: sample 2). By contrast, for weak denitrification activities the evolution of the direct increase of the headspace concentration was reduced due to an imperfect mixing of the carrying over gas (Fig. 2: sample 4).

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#### 4.4 Reliability of the C<sub>2</sub>H<sub>2</sub> method

The scavenging of intermediate NO by C<sub>2</sub>H<sub>2</sub> (Bollmann and Conrad, 1997) as well as an incomplete inhibition led to systematic underestimations of total denitrification, but the magnitude of the underestimation remains unknown. The first effect, i.e. the scavenging of NO was most probably the reason that the addition of C<sub>2</sub>H<sub>2</sub> led to lower N<sub>2</sub>O emission than in C<sub>2</sub>H<sub>2</sub>-free samples. The comparison of CO<sub>2</sub> fluxes (i.e. soil respiration) before and after the incubation with C<sub>2</sub>H<sub>2</sub> (Fig. 4b) indicated that in some cases the fluxes were lower after the application of C<sub>2</sub>H<sub>2</sub>. This suggested a suppressed microbial activity. However, Payne (1984) reported that the growth and vitality of soil bacteria are not affected by C<sub>2</sub>H<sub>2</sub> application if sufficient ammonium is available in the substrate. The investigation by Russow et al. (2000) could not confirm the findings of Bollmann and Conrad (1997) claiming that NO is released from the denitrifying bacteria. Russow et al. (2000) concludes that NO is not a free precursor of N<sub>2</sub>O formation via denitrification. Hence, if the NO does not escape the denitrifying bacteria, it is not missing for denitrification and a scavenging effect cannot take place. Furthermore Murray and Knowles (2003) argued that the scavenging of NO by the C<sub>2</sub>H<sub>2</sub> catalyzed reaction does not cause a serious underestimation of long-term measurements of active denitrification in anaerobic soils containing adequate carbon and nitrate sources. Also Groffman et al. (2006) considered the acetylene inhibition technique as a robust technique for soils with a high inorganic nitrogen supply. These contradicting results suggest that the degree of underestimation of N<sub>2</sub> + N<sub>2</sub>O emission by the C<sub>2</sub>H<sub>2</sub> inhibition technique is dependent on the distribution of the active aggregates in the soil and can hardly be predicted.

C<sub>2</sub>H<sub>2</sub> also inhibits nitrification (Berg et al., 1982) which is the natural supplier of NO<sub>3</sub><sup>-</sup> (cf. Sect. 4.1). However, the high level of NO<sub>3</sub><sup>-</sup> in our samples from intensively managed grassland (data not shown) was likely not limiting, suggesting that denitrifiers were not crucially affected by a possibly reduced contribution of nitrification to any further supply of NO<sub>3</sub><sup>-</sup>. The transportation to denitrifying micro-sites and more crucial the inhibition

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by  $C_2H_2$  may therefore have led to a rather small reduction of the  $NO_3^-$  level in the sample and the denitrification activity during the time interval following sampling and incubation.

Jarvis et al. (2001) concluded that the  $C_2H_2$  inhibition technique in jars underestimates denitrification and overestimates net mineralisation. Other studies comparing the  $C_2H_2$  inhibition technique to the  $^{15}N$  labelling technique showed a difference of 10 % (Malone et al., 1998) to 30 % (Myrold, 1990). Our results and uncertainties are in the same order of magnitude as the results presented by Michel et al. (2011) based on the atmosphere replacement technique.

Reasons for this difference are not only found in the various measurement techniques but also in different environmental factors (Rudaz et al., 1999), in different soil cultivation practices (Rudaz et al., 1999), in soil moisture content (Maag and Vinther, 1996; Weier et al., 1993), nitrate content (Weier et al., 1993), soil temperature (Maag and Vinther, 1996), in varying availability of organic carbon (Weier et al., 1993) or different soil types (Maag and Vinther, 1996).

In our experiment  $N_2O$  fluxes are measured at ambient soil temperature. By contrast van der Salm et al. (2007) measured the total denitrification always at  $20^\circ C$  with 5 %  $C_2H_2$  incubated for 48 h, followed by a temperature correction to the actual soil temperature (cf. van der Salm et al., 2007, therein Eq. 3). Reported denitrification rates are about three times higher than our values. The difference in the results might therefore be attributed to the different measurement temperatures and different soil parameters (heavier soil with about the double of organic C than the Oensingen soil).

#### 4.5 $N_2O/N_2$ ratio

As the  $C_2H_2$  inhibition techniques commonly used (e.g. Rudaz et al., 1999) determine the  $N_2$  loss as the difference between  $N_2O$  emission without and with  $C_2H_2$  treatment, it is convenient to characterize the  $N_2$  loss with the ratio of  $N_2O/N_2$  emission. Our measurements showed that total denitrification  $N_2$  losses occurred also during period when chamber  $N_2O$  fluxes in the field indicated fluxes at or below the detection limit

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(Fig. 7). For such conditions the  $N_2O/N_2$  ratio is not applicable.  $N_2O$  flux is believed to be an obligatory precursor of  $N_2$ , thus any emission of  $N_2$  must be accompanied by a commensurate  $N_2O$  production (although not necessarily by a net emission to the atmosphere). The fraction of  $N_2O$  production that does not reach the atmosphere is reduced to  $N_2$ .

#### 4.6 $N_2$ losses derived from N budget

Ammann et al. (2009) proposed a complete N budget of the Oensingen grassland site. The N fluxes that were actually measured ( $NH_3$ ,  $NO_x$ ,  $NO_3^-$ ,  $NH_4^+$ ,  $N_2O$ , harvested N, wet and dry deposition) allow only an indirect determination of the sum of the N losses via denitrification and the storage change in the soil. The latter could be constrained by the C-budget, so that an estimate of  $40 \pm 30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for the total denitrification resulted. This is in the same range as the estimated N loss in the range of 6 to  $26 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  based on the  $C_2H_2$  inhibition method. Nonetheless, uncertainties are close to 100 %.

### 5 Conclusions

An attempt to experimentally estimate the loss of  $N_2$  using the  $C_2H_2$  inhibition technique of an intensively managed, fertilised, mown grassland is presented. The lower bound total denitrification loss of  $N_2$  is estimated to be in the range of 6 to  $26 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , albeit with uncertainties close to 100 %.

There does not seem to exist at present any reliable method for the field determination of  $N_2$  losses and it needs to be stressed that only a lower estimate of the  $N_2$  loss can at best be provided, as most of the known artefacts tend toward an underestimation of the total denitrification fluxes.

Losses of N from agricultural grasslands are highly variable. The temporal and spatial variability in this study was dependent on various factors and hinted at large

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inter-annual differences. No fixed  $N_2O/N_2$  ratio could be determined, as the measurements shows that the ratio is highly variable depending on meteorological conditions, soil properties, and management effects, and likewise subject to a very large uncertainty.

- 5 *Acknowledgements.* We would like to thank Markus Jocher for the assistance in the laboratory (setting up the acetylene inhibition system) and Michael Schibig for the assistance during the soil sampling and incubations. For funding we gratefully thank the EU project NitroEurope (Contract 017841).

## References

- 10 Allison, F. E.: The enigma of soil nitrogen balance sheets, *Adv. Agron.*, 7, 213–250, 1955. 2854  
Ammann, C., Flechard, C. R., Neftel, A., and Fuhrer, J.: Partitioning of NEE and ecosystem respiration of a managed temperate grassland based on eddy covariance and chamber measurements, Open Science Conference on the GHG Cycle in the Northern Hemisphere, Sissi-Lassithi, Crete, 14–18 November 2006, available at: [http://www.bgc-jena.mpg.de/bgc-processes/ceip/conference/abstracts/PA\\_poster/ammann.htm](http://www.bgc-jena.mpg.de/bgc-processes/ceip/conference/abstracts/PA_poster/ammann.htm), 2006. 2863  
15 Ammann, C., Flechard, C. R., Leifeld, J., Neftel, A., and Fuhrer, J.: The carbon budget of newly established temperate grassland depends on management intensity, *Agr. Ecosyst. Environ.*, 121, 5–20, 2007. 2855  
Ammann, C., Spirig, C., Leifeld, J., and Neftel, A.: Assessment of the nitrogen and carbon budget of two managed temperate grassland fields, *Agr. Ecosyst. Environ.*, 133, 150–162, 2009. 2853, 2855, 2870  
20 Berg, P., Klemetsson, L., and Rosswall, T.: Inhibitory effect of low partial pressures of acetylene on nitrification, *Soil Biol. Biochem.*, 14, 301–303, 1982. 2866, 2868  
Bollmann, A. and Conrad, R.: Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide, *Soil Biol. Biochem.*, 29, 1067–1077, 1997. 2854, 2868  
25 Braun, M., Hurni, P., and Spiess, E.: Phosphor- und Stickstoffüberschüsse in der Landwirtschaft und Para-Landwirtschaft, Nummer 18 in Schriftenreihe der FAC Liebefeld, Eid-

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genössische Forschungsanstalt für Agrikultur und Umwelthygiene, Liebefeld-Bern, Switzerland, 1994. 2853

Butterbach-Bahl, K., Willibald, G., and Papen, H.: Soil core method for direct simultaneous determination of  $N_2$  and  $N_2O$  emissions from forest soils, *Plant Soil*, 240, 105–116, 2002. 2854

Cardenas, L. M., Hawkins, J. M. B., Chadwick, D., and Scholefield, D.: Biogenic gas emissions from soils measured using a new automated laboratory incubation system, *Soil Biol. Biochem.*, 35, 867–870, 2003. 2854

Conen, F. and Smith, K. A.: A re-examination of closed flux chamber methods for the measurement of trace gas emissions from soils to the atmosphere, *Eur. J. Soil Sci.*, 49, 701–707, 1998. 2862

Fedorova, R. I., Milekhina, E. I., and Il-Yukhina, N. I.: Evaluation of the method of “gas metabolism” for detecting extraterrestrial life. Identification of nitrogen-fixing microorganism, *Izv. Akad. Nauk. SSR*, 6, 797–806, 1973. 2854

Flechard, C. R., Neftel, A., Jocher, M., Ammann, C., and Fuhrer, J.: Bi-directional soil/atmosphere  $N_2O$  exchange over two mown grassland systems with contrasting management practices, *Glob. Change Biol.*, 11, 2114–2127, 2005. 2857, 2859, 2862

Galloway, J. N., Aber, J. D., Willem Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B., and Cosby, B. J.: The nitrogen cascade, *Bioscience*, 53, 341–356, 2003. 2853

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., and Seitzinger, S. P., Sutton, M. A.: Transformation of the Nitrogen Cycle, *Recent Trends, Questions, and Potential Solutions*, *Science*, 320, 889–892, 2008. 2853

Gillam, K. M., Zebarth, B. J., and Burton, D. L.: Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration, *Can. J. Soil Sci.*, 88, 133–143, 2008. 2866

Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., and Kana, T. M., Nielsen, L. P., and Voytek, M. A.: Methods for measuring denitrification, *Diverse approaches to a difficult problem*, *Ecol. Appl.*, 16, 2091–2122, 2006. 2853, 2868

Hauck, R. D. and Melsted, S. W.: Some aspects of the problem of evaluating denitrification in soils, *Soil Sci. Soc. Am. Pro.*, 20, 361–364, 1956. 2853

Jambert, C., Serça, D., and Delmas, R.: Quantification of N-losses as  $NH_3$ ,  $NO$ , and  $N_2O$  and  $N_2$  from fertilized maize fields in southwestern France, *Nutr. Cycl. Agroecosys.*, 48, 91–104,

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- Jarvis, S. C., Hatch, D. J., and Lovell, R. D.: An improved soil core incubation method for the field measurement of denitrification and net mineralization using acetylene inhibition, *Nutr. Cycl. Agroecosys.*, 59, 219–225, 2001. 2869
- 5 Limpert, E., Stahel, W. A., and Abbt, M.: Log-normal distributions across the sciences, *Keys and clues*, *Bioscience*, 51, 341–352, 2001. 2861
- Maag, M. and Vinther, F. P.: Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures, *Appl. Soil Ecol.*, 4, 5–14, 1996. 2869
- 10 Malone, J. P., Stevens, R. J., and Laughlin, R. J.: Combining the N-15 and acetylene inhibition techniques to examine the effect of acetylene on denitrification, *Soil Biol. Biochem.*, 30, 31–37, 1998. 2869
- Michel, K., Augustin, J., Butterbach-Bahl, K., and Zechmeister-Boltenstern, S.: N gas emissions from soil – comparison of three measuring systems for the direct determination of N<sub>2</sub> and N<sub>2</sub>O, *NitroEurope General Assembly, Edinburgh, United Kingdom, 11–15 April 2011, NEU11 C-S1*, 2011. 2869
- 15 Murray, R. E. and Knowles, R.: Production of NO and N<sub>2</sub>O in the presence and absence of C<sub>2</sub>H<sub>2</sub> by soil slurries and batch cultures of denitrifying bacteria, *Soil Biol. Biochem.*, 35, 1115–1122, 2003. 2868
- 20 Myrold, D. D.: 15N techniques, *Denitrification in Soil and Sediment, Book Series, Fems. Symp.*, 56, 181–198, 1990. 2869
- Neftel, A., Blatter, A., Schmid, M., Lehmann, B., and Tarakanov, S. V.: An experimental determination of the scale length of N<sub>2</sub>O in the soil of a grassland, *J. Geophys. Res-Atmos.*, 105, 12095–12103, 2000. 2867
- 25 Parkin, T. B.: Soil microsites as a source of denitrification variability, *Soil Sci. Soc. Am. J.*, 51, 1194–1199, 1987. 2864
- Payne, W. J.: Influence of acetylene on microbial and enzymatic assays, *J. Microbiol. Meth.*, 2, 117–133, 1984. 2868
- Rudaz, A. O., Walti, E., Kyburz, G., Lehmann, P., and Fuhrer, J.: Temporal variation in N<sub>2</sub>O and N<sub>2</sub> fluxes from a permanent pasture in Switzerland in relation with management, soil water content and soil temperature, *Agr. Ecosyst. Environ.*, 73, 83–91, 1999. 2854, 2869
- 30 Russow, R., Sich, I., and Neue, H. U.: The formation of the trace gases NO and N<sub>2</sub>O in soils by the coupled processes of nitrification and denitrification, results of kinetic 15N tracer

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investigations, *Chemosphere Global Change Sci.*, 2, 359–366, 2000. 2868

Scheer, C., Wassmann, R., Butterbach-Bahl, K., Lamers, J., and Martius, C.: The relationship between  $N_2O$ , NO, and N-2 fluxes from fertilized and irrigated dryland soils of the Aral Sea Basin, Uzbekistan, *Plant Soil*, 314, 273–283, 2009. 2854

5 Scholefield, D., Hawkins, J. M. B., and Jackson, S. M.: Development of a helium atmosphere soil incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during denitrification, *Soil Biol. Biochem.*, 29, 1345–1352, 1997. 2854

Tiedje, J. M., Simkins, S., and Groffman, P. M.: Perspectives on measurement of denitrification in the field including recommended protocols for acetylene based methods, *Plant Soil*, 115, 261–284, 1989. 2853

10 van der Salm, C., Dolfing, J., Heinen, M., and Velthof, G. L.: Estimation of nitrogen losses via denitrification from a heavy clay soil under grass, *Agr. Ecosyst. Environ.*, 119, 311–319, 2007. 2854, 2869

van Groenigen, J. W., Zwart, K. B., Harris, D., and van Kessel, C.: Vertical gradients of  $\delta^{15}N$  and  $\delta^{18}O$  in soil atmospheric  $N_2O$  – temporal dynamics in a sandy soil, *Rapid Commun. Mass Sp.*, 19, 1289–1295, 2005. 2862

Vieten, B., Blunier, T., Neftel, A., Alewell, C., and Conen, F.: Fractionation factors for stable isotopes of N and O during  $N_2O$  reduction in soil depend on reaction rate constant, *Rapid Commun. Mass Spectrom.*, 21, 846–850, 2007. 2862

20 Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., and Tilman, D. G.: Human alteration of the global nitrogen cycle, Causes and consequences, *Ecol. Appl.*, 7, 737–750, 1997. 2852

Weier, K. L., Doran, J. W., Power, J. F., and Walters, D. T.: Denitrification and the dinitrogen nitrous-oxide ratio as affected by soil-water, available carbon, and nitrate, *Soil Sci. Soc. Am. J.*, 57, 66–72, 1993. 2869

25 Yamulki, S. and Jarvis, S. C.: Automated chamber technique for gaseous flux measurements, Evaluation of a photoacoustic infrared spectrometer-trace gas analyzer, *J. Geophys. Res.*, 104, 5463–5469, 1998. 2857

30 Zhang, T. Y., Xu, X. K., Luo, X. B., Han, L., Wang, Y. H., and Pan, G. X.: Effects of acetylene at low concentrations on nitrification, mineralization and microbial biomass nitrogen concentrations in forest soils, *Chinese Sci. Bull.*, 54, 296–303, 2009. 2854, 2866

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**Table 1.** Summary of measured N<sub>2</sub>O fluxes in laboratory incubations (total denitrification experiments) and in the field at Oensingen (actual N<sub>2</sub>O emissions in ambient conditions).

Date	Detector <sup>a</sup>	Unit	C <sub>2</sub> H <sub>2</sub> inhibition N <sub>2</sub> O			Chamber N <sub>2</sub> O	
			$\bar{x}^*/s^*$	$\bar{x}^*$	$\bar{x}^* \times s^{*b}$	$\bar{x}$	sd <sup>c</sup>
21 Jul 2008	pIRS	mg N <sub>2</sub> O-N m <sup>-2</sup> d <sup>-1</sup>	1.0	1.1	1.2	5.0	7.3
29 Jul 2008	pIRS		0.5	0.7	1.0	2.3	3.5
5 Aug 2008	pIRS		4.4	6.5	9.5	0.5	0.3
11 Aug 2008	pIRS		0.6	1.0	1.5	0.4	0.3
14 Aug 2008	pIRS		1.6	2.2	2.9	1.2	0.7
19 Mar 2009	pIRS		0.5	1.3	3.3	0.0	0.1
7 Apr 2009	pIRS		0.3	0.6	1.0	0.0	0.1
17 Apr 2009	pIRS		0.6	0.9	1.4	0.0	0.1
22 Apr 2009	pIRS		1.0	1.1	1.3	na	na
31 Jul 2009	GC		mg N <sub>2</sub> O-N m <sup>-2</sup> d <sup>-1</sup>	5.1	11.0	23.8	0.1
3 Aug 2009	GC	13.5		24.7	45.3	3.8	1.4
6 Aug 2009	GC	66.1		75.7	86.7	8.4	9.3
10 Aug 2009	GC	11.3		18.2	29.4	56.2	41.2
12 Aug 2009	GC	10.4		15.7	23.6	16.6	8.0
26 Oct 2009	GC	0.9		2.7	8.1	0.1	0.1
2 Nov 2009	GC	6.1		6.5	7.0	0.8	0.8
6 Nov 2009	GC	2.4	4.9	10.2	0.8	0.8	
Yearly emissions median		kg N <sub>2</sub> O-N ha <sup>-1</sup> yr <sup>-1</sup>	5.9	9.7	25.5	2.9	

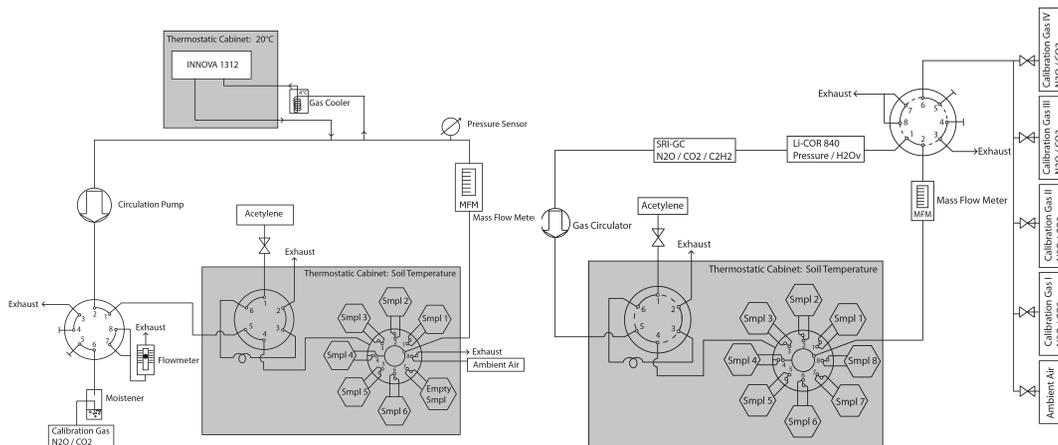
<sup>a</sup> pIRS stands for the measurements with the photoacoustic IR instrument and GC for the gas chromatograph measurements.

<sup>b</sup>  $\bar{x}^*$  and  $s^*$  are the log-transformed daily means and standard deviations.  $\bar{x}^*/s^*$  is the lower and  $\bar{x}^* \times s^*$  the upper end of the calculated flux.

<sup>c</sup>  $\bar{x}$  and sd are the daily means and standard deviations.

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**Fig. 1.** Instrumental set up: photoacoustic IR gas analyser (Innova 1312, left) or alternatively a gas chromatograph (SRI 8610C GC, right) measures concentration increases of up to seven soil samples in rotation.

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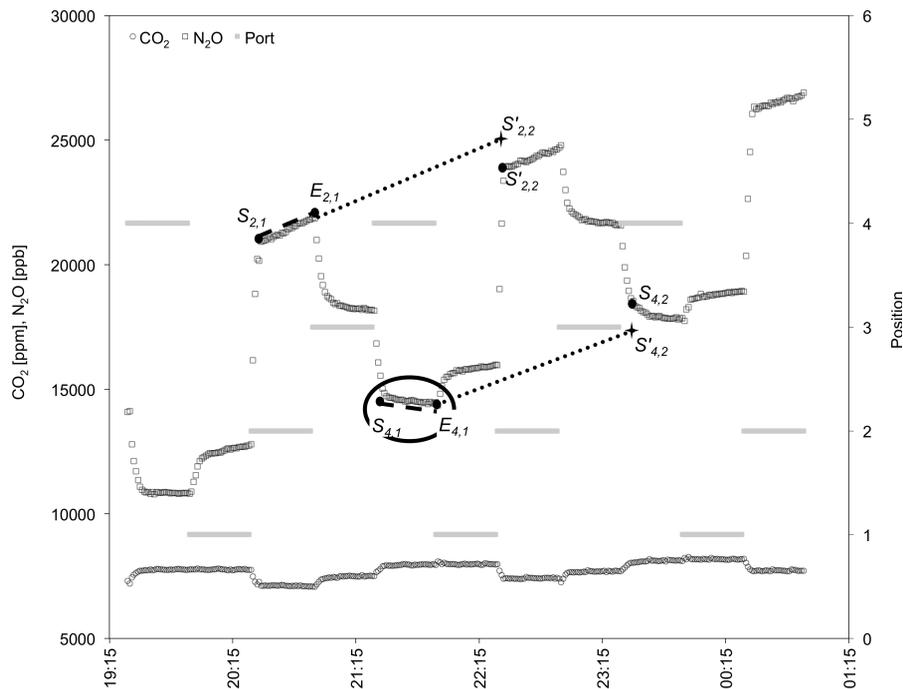
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**Fig. 2.** Temporal concentration evolution of an extended measurement with four soil samples after  $C_2H_2$  was injected (not shown). The dashed and dotted lines indicate the two ways calculating the slope for flux calculations. The concentration at point  $S$  was corrected for mixing effects. The ellipse illustrates the problem of carry-over effects; although  $N_2O$  is produced in sample 4 (evidenced by the increased concentration since the previous flux measurement), no concentration increase could be measured directly during the current run.

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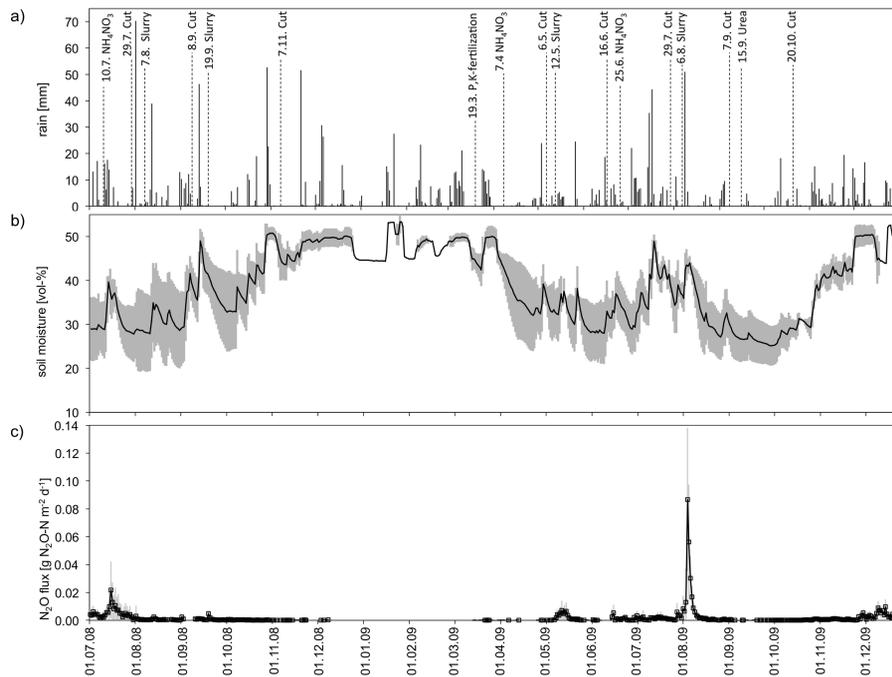
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**Fig. 3.** Results of a 1.5-yr N<sub>2</sub>O flux measurement period over intensively managed grassland in Oensingen (CH). **(a)** Daily rainfall amount [mm] and management events (dashed lines). **(b)** Mean soil moisture [vol-%] from 0–30 cm. **(c)** Mean N<sub>2</sub>O fluxes [g N<sub>2</sub>O-N m<sup>-2</sup> d<sup>-1</sup>] measured by static chambers in the field. Error bars are standard deviations.

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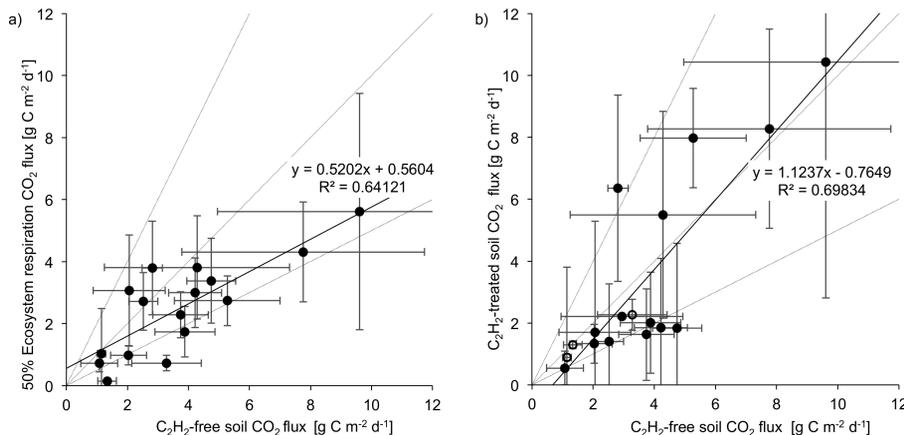
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**Fig. 4.** Comparison of CO<sub>2</sub> fluxes from C<sub>2</sub>H<sub>2</sub>-free soil samples incubated in the laboratory to (a) 50 % ecosystem respiration fluxes (assumed to be commensurate with soil respiration) measured in the field and to (b) CO<sub>2</sub> fluxes from C<sub>2</sub>H<sub>2</sub>-treated (y-axis) samples. Open circles indicate fluxes measured from the C<sub>2</sub>H<sub>2</sub>-effect experiment (Sect. 2.2.2). Data and error bars are means and standard deviations ( $\bar{x} \pm \text{sd}$ ). The outer lines show slopes of 0.5 and 1.5. The black solid line is the linear regression.

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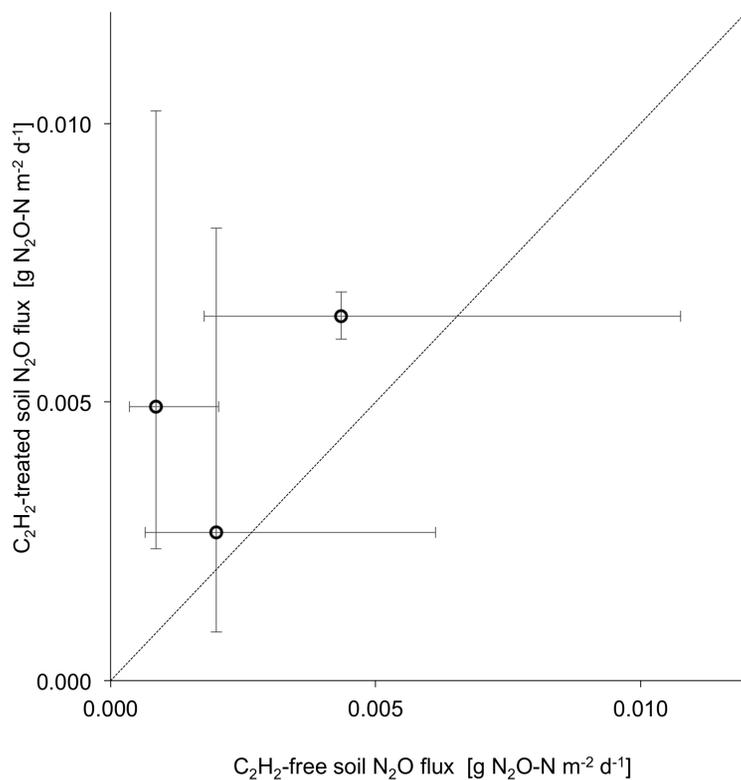
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**Fig. 5.** Comparison of N<sub>2</sub>O fluxes measured with C<sub>2</sub>H<sub>2</sub> inhibition to fluxes measured without C<sub>2</sub>H<sub>2</sub> inhibition during special experiments with parallel measurements of 3 C<sub>2</sub>H<sub>2</sub>-treated and 4 untreated samples. Error bars for the C<sub>2</sub>H<sub>2</sub> incubation system indicate the range of the fluxes.

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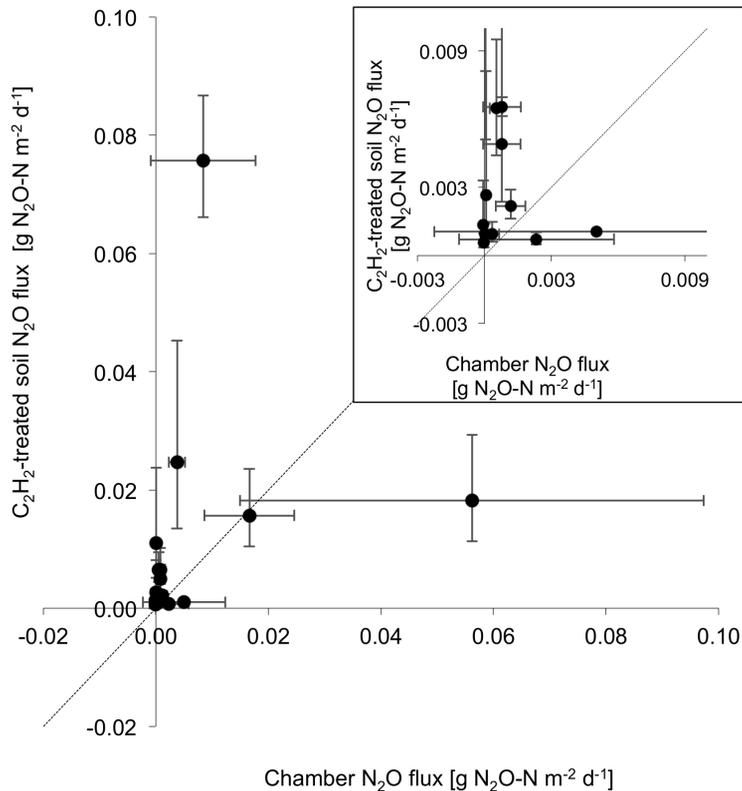
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**Fig. 6.** An anti-correlation between N<sub>2</sub>O fluxes measured by the static chambers to the total N loss fluxes measured by the C<sub>2</sub>H<sub>2</sub> inhibition technique. Error bars for the C<sub>2</sub>H<sub>2</sub> incubation system indicate the range of the fluxes, those for the chamber measurements regarding  $\bar{x} \pm s.d.$

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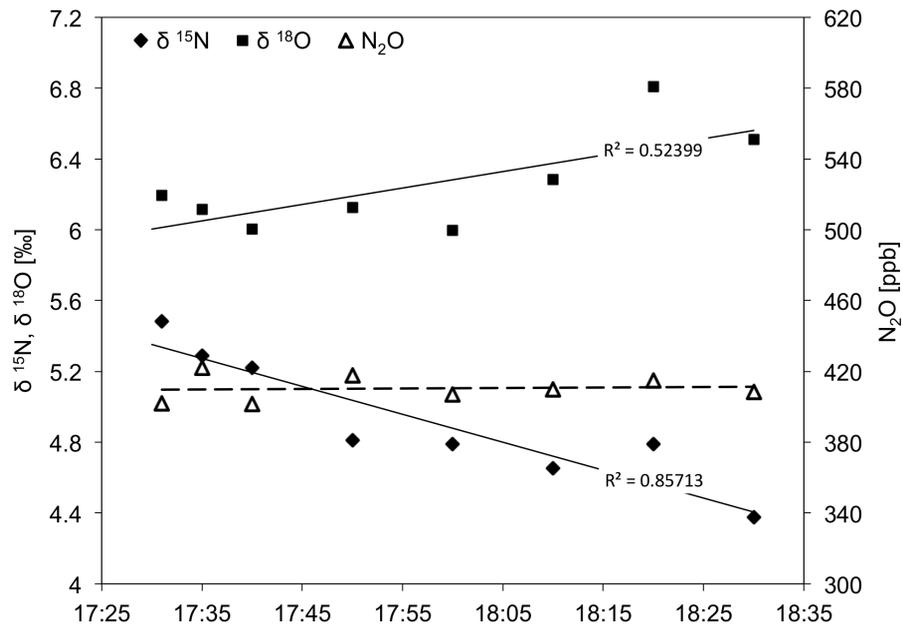
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**Fig. 7.** Significant changes in  $\delta^{15}\text{N}$  ( $-1\text{‰}$ ) and  $\delta^{18}\text{O}$  ( $0.4\text{‰}$ ) measured in  $\text{N}_2\text{O}$  over an hour in a static chamber, indicating a clear N turnover, although no significant  $\text{N}_2\text{O}$  flux could be measured ( $<1.5 \text{ ng N}_2\text{O m}^{-2} \text{ s}^{-1}$ ).

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