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

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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⁻¹ yr⁻¹ (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 NO_3^- and dissolved organic nitrogen (DON), assimilated by soil microbial biomass, emitted to the atmosphere as NH_3 and NO , or denitrified to N_2O and N_2 . Thus the increased introduction of N_r into ecosystems has also increased the losses of 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 Tg N yr^{-1} . On the global scale, they estimated that 10–40 % of introduced N_r is denitrified and returned to the atmosphere as 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 kg N ha^{-1} agricultural area 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 (NO_3^- and NO_2^-) to NO , N_2O and ultimately to N_2 , represents an important part of the N budget, the measurement of 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 N_2 losses of agro-systems can be estimated in situ is needed.

Various experimental approaches exist to measure 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}N tracers (e.g. Hauck and Melsted, 1956),

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3. direct N₂ 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 C₂H₂ 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 (C₂H₂ inhibition, Jambert et al., 1997), 0–25 % losses from permanent pasture in Switzerland (C₂H₂ inhibition, Rudaz et al., 1999), and 10–70 % losses from cotton sites in Uzbekistan (direct N₂ quantifica-
10 tion, Scheer et al., 2009).

The C₂H₂ inhibition technique is based on the findings by Fedorova et al. (1973) that C₂H₂ at high concentrations (>0.5 %) in the headspace of pure microbe samples inhibits the microbial reduction of N₂O to N₂. Consequently N₂O emissions measured after addition of C₂H₂ at high concentration should correspond to the production of N₂
15 and N₂O in the soil sample under consideration.

The C₂H₂ inhibition method is in principle an easy approach to measure total denitrification. However, limitations of the C₂H₂ inhibition method are the scavenging (C₂H₂-catalysed oxidation) of the intermediate NO, the precursor of N₂O that itself is the precursor of N₂ (Bollmann and Conrad, 1997) and the suppressed microbial respiration
20 through C₂H₂ (Zhang et al., 2009). In addition, inhibition by C₂H₂ might be incomplete when added C₂H₂ 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 C₂H₂ inhibition technique for total denitrification measurements. The present study investigates the plausibility of total N losses from a managed grassland soil, obtained by the C₂H₂ inhibition method in the laboratory using intact soil cores, and compared to actual N₂O 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 Ni-
troEurope 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 grass-
land 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
20 a homogenous flora and to restore the productivity of the grassland system.

The soil is classified as Eutri-Stagnic Cambisol (FAO, ISRIC and ISSS, 1998) devel-
oped 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 $\text{N}_2\text{O} + \text{N}_2$ 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 H₂O, CO₂, N₂O, and C₂H₂: SB0527 (1985 ± 40 cm⁻¹) with strong H₂O absorption lines, UA0983 (2270 ± 15 cm⁻¹) with strong CO₂ absorption lines, UA0985 (2215 ± 22 cm⁻¹) with strong N₂O absorption lines, and UA0969 (1254 ± 34.5 cm⁻¹) with C₂H₂ absorption lines. These are broadband filters which result in cross interferences between the different gases (including H₂O) 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 CO₂. However, Flechard et al. (2005) found that the N₂O concentrations indicated by the Innova 1312 output were still heavily dependent on CO₂, H₂O 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_{*i*} is the analog signal measured by filter *i* in mV, *C* stands for the concentration of the gas, and *k*_{gas→*i*} 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 N_2O , 300 ppm to 10 000 ppm CO_2 , and 1 % to 7 % C_2H_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 ± 20 ppb for ambient N_2O 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 μl sample loop. The gas was analysed by an Electron Capture Detector (ECD: N_2O , CO_2) and a Flame Ionisation Detector (FID: C_2H_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) and using 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 N_2O , and 300 ppm to 10 000 ppm CO_2 .

2.2.2 Experiments

In the base runs, the samples were measured without C_2H_2 for at least one or two cycles (referred to as C_2H_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 C_2H_2 (referred to as C_2H_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 C₂H₂-free fluxes versus C₂H₂-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 C₂H₂ on the microbial activity and to provide a direct comparison of C₂H₂-free fluxes versus C₂H₂-treated fluxes three special experiments were conducted (referred to as C₂H₂-effect experiments). During these measurements three samples were measured without C₂H₂ for the entire measurement and four were simultaneously incubated with C₂H₂. Before switching from a C₂H₂-treated sample to a C₂H₂-free sample the analytical part of the tubes was flushed by ambient air to avoid the contamination by C₂H₂ of the C₂H₂-free samples.

2.2.3 Flux evaluation

The temporal concentration changes (∂C) of N₂O and CO₂ over time (∂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 C₂H₂ 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 (∂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 N₂O fluxes measured from C₂H₂-treated samples ended up being smaller than in C₂H₂-free soil. Clearly such occurrences should be identified and filtered out from the dataset. The flux results were therefore processed as follows:

1. N₂O fluxes measured from C₂H₂-free samples being higher than from C₂H₂-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 C₂H₂-free samples was regarded as a lower bound of N₂ losses.
2. Fluxes of N₂O in C₂H₂-treated samples being larger than in C₂H₂-free samples indicated that the limitations were at best low or moderate, but it cannot be excluded that the limitations were actually large. The N₂O flux of C₂H₂-treated samples thus represented a lower boundary of total N losses, since not necessary all denitrifying microorganisms were C₂H₂-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 C₂H₂-treated samples. The daily mean flux of all C₂H₂-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 N₂O 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_{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 N₂O measurements with static chambers

N₂O and CO₂ 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 N₂O concentration in the chambers headspace for “zero” flux conditions, i.e. in cases when no increase of N₂O could be measured. The temporal change in the isotopic composition (¹⁵N, ¹⁸O) of the constant N₂O concentration in the headspace allows the discrimination of gross N₂O production and consumption processes (van Groenigen et al., 2005; Vieten et al., 2007).

3 Results

3.1 N₂ and N₂O 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. N₂O fluxes measured with static chambers in the field showed similar patterns as in the years before. Peak N₂O emissions occurred mainly after fertilisation events when soil moisture content was large.

Table 1 shows the total N (N₂ + N₂O) losses, measured as N₂O fluxes, by C₂H₂ inhibition of incubated soil samples, and the corresponding actual N₂O 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 N₂O chamber measurements and in the laboratory data by the C₂H₂ inhibition technique were similar; larger emissions in the field coincided in larger C₂H₂ fluxes in the laboratory. The total N losses varied between 0.3 and 9.5 mg N₂O-N m⁻² d⁻¹ for the period using the pIRS and 0.9 and 86.7 mg N₂O-N m⁻² d⁻¹ for the GC measurements.

In general the loss of N either as N₂O or as N₂ 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 C₂H₂ inhibition of incubated soil samples, was actually smaller than the loss measured by the static chambers in the field (Table 1).

3.2 CO₂ fluxes

CO₂ fluxes measured in the C₂H₂ 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 CO₂ 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 C₂H₂-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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CO₂ fluxes measured from the samples before the C₂H₂ injection did not significantly differ from CO₂ fluxes measured after the injection of C₂H₂ (Fig. 4b). The three data points from the C₂H₂-effect experiment (Fig. 4b: open circles, cf. Sect. 2.2.2) also lie close to the 1/1-line, indicating that the incubation by C₂H₂ did not crucially alter the soil respiratory activity. The upper cluster of measurement points corresponds to the large emission peak observed in the N₂O chamber measurements in August 2009. The large spatial variability of CO₂ 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 N₂O measurements and N₂O/N₂ ratio

The three C₂H₂-effect experiments (Sect. 2.2.2) showed that C₂H₂-treated samples emitted, on average, twice as much N₂O than the C₂H₂-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 N₂O fluxes measured by the chambers in the field and the total N (N₂ + N₂O) loss estimated from C₂H₂-treated samples in the laboratory incubation system. The error bars in both directions demonstrate the typical small scale spatial variability associated with N₂O flux measurements (e.g. Parkin, 1987).

On three occasions field N₂O emissions were higher than the corresponding laboratory based total denitrification fluxes. This was especially likely for high fluxes after fertilisation where the N₂O fluxes are controlled by available N and accessible energy (carbon assimilates) for the denitrifying microbial communities.

In 2009 the total loss of N₂O + N₂ was dominated by the slurry event around 6 August. Over the measurement period, total N₂O + N₂ losses estimated by C₂H₂ inhibition were in the range of 6 to 26 kg N ha⁻¹ yr⁻¹ (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⁻¹ yr⁻¹ which is 2.4 times higher than the actual field N₂O emission.

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3.4 Isotopic composition of the N₂O concentration for zero flux conditions

Isotopic analysis during flux measurements with small but detectable concentration changes (“zero” flux condition = flux < 1.5 ng N₂O m⁻² s⁻¹) showed a significant change in the ¹⁵N and ¹⁸O isotopic fractions (Fig. 7). Although enrichment factors are associated with large uncertainties, these changes indicated that a “zero” flux did not necessarily mean no N₂O production. In such situations the gross production of N₂O was equal to the gross consumption (i.e. reduction of N₂O to N₂).

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 C₂H₂ should penetrate the sample and reach the active denitrification micro-sites by diffusion, and (3) the produced N₂O 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 CO₂ 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 N₂O fluxes being higher than C₂H₂-treated N₂O 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 N₂O molecule travels until it is reduced to N₂ (Neffel et al., 2000). The chamber N₂O fluxes were controlled from top 5 cm whereas the C₂H₂ inhibition system measured the integral loss from samples from 0–30 cm depth. Furthermore, the likelihood of N₂O escaping to the atmosphere decreased with increasing depth of denitrification as the scale length of N₂O 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 C₂H₂ and CO₂ on the N₂O concentration and therefore severely hampered the accuracy of the flux determination. The detection limit with the pIRS was around 40 ng N₂O m⁻² s⁻¹, i.e. a factor 10 higher than using the GC (3.1 ng N₂O m⁻² s⁻¹) independent of the C₂H₂ 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 C₂H₂ method

The scavenging of intermediate NO by C₂H₂ (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 C₂H₂ led to lower N₂O emission than in C₂H₂-free samples. The comparison of CO₂ fluxes (i.e. soil respiration) before and after the incubation with C₂H₂ (Fig. 4b) indicated that in some cases the fluxes were lower after the application of C₂H₂. This suggested a suppressed microbial activity. However, Payne (1984) reported that the growth and vitality of soil bacteria are not affected by C₂H₂ 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 N₂O 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 C₂H₂ 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 N₂ + N₂O emission by the C₂H₂ inhibition technique is dependent on the distribution of the active aggregates in the soil and can hardly be predicted.

C₂H₂ also inhibits nitrification (Berg et al., 1982) which is the natural supplier of NO₃⁻ (cf. Sect. 4.1). However, the high level of NO₃⁻ 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 NO₃⁻. 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.

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Table 1. Summary of measured N₂O fluxes in laboratory incubations (total denitrification experiments) and in the field at Oensingen (actual N₂O emissions in ambient conditions).

Date	Detector ^a	Unit	C ₂ H ₂ inhibition N ₂ O			Chamber N ₂ O	
			\bar{x}^*/s^*	\bar{x}^*	$\bar{x}^* \times s^{*b}$	\bar{x}	sd ^c
21 Jul 2008	pIRS	mg N ₂ O-N m ⁻² d ⁻¹	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 ₂ O-N m ⁻² d ⁻¹	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 ₂ O-N ha ⁻¹ yr ⁻¹	5.9	9.7	25.5	2.9	

^a pIRS stands for the measurements with the photoacoustic IR instrument and GC for the gas chromatograph measurements.

^b \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.

^c \bar{x} and sd are the daily means and standard deviations.

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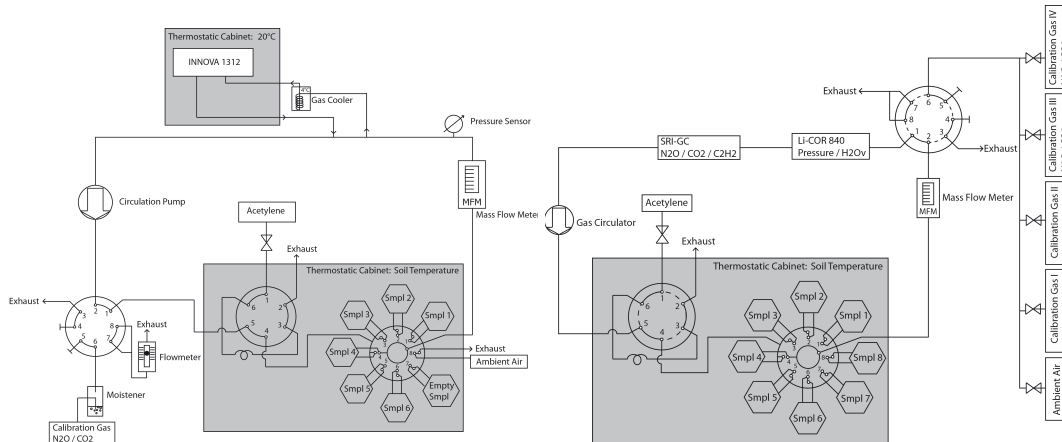


Fig. 1. Instrumental set up: photoacoustic IR gas analyser (Innova 1312, left) or alternatively a gas chromatograph (SRI 8610C GC, right) measures concentration increases of up to seven soil samples in rotation.

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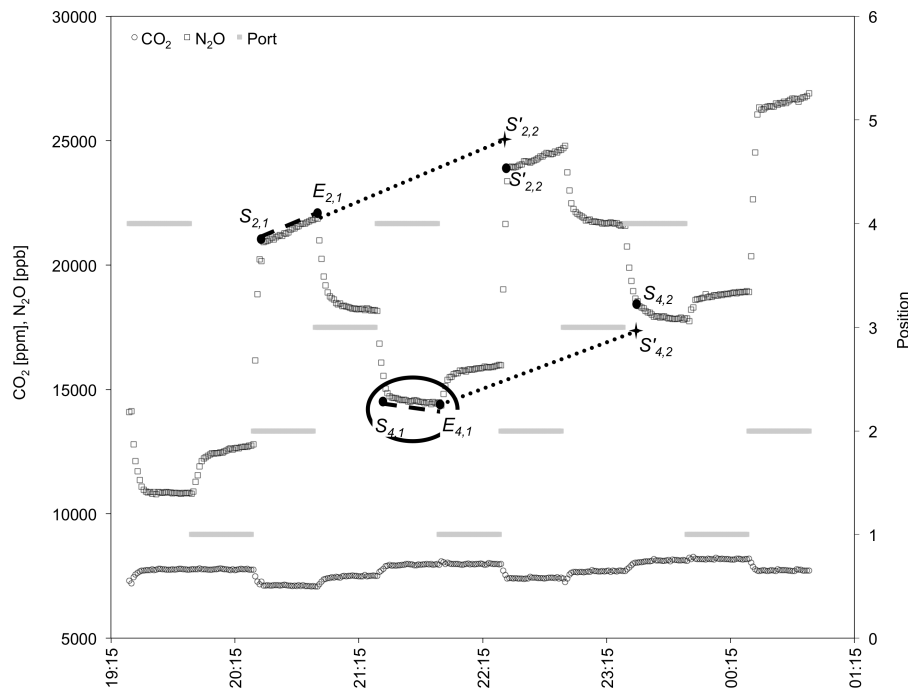


Fig. 2. Temporal concentration evolution of an extended measurement with four soil samples after C_2H_2 was injected (not shown). The dashed and dotted lines indicate the two ways calculating the slope for flux calculations. The concentration at point S was corrected for mixing effects. The ellipse illustrates 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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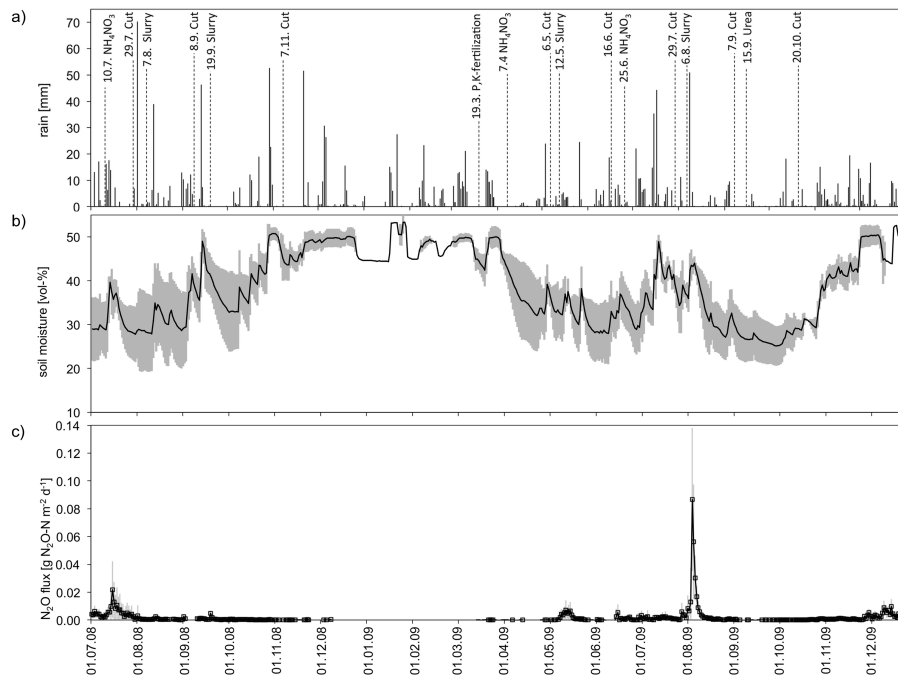


Fig. 3. Results of a 1.5-yr N₂O flux measurement period over intensively managed grassland in Oensingen (CH). **(a)** Daily rainfall amount [mm] and management events (dashed lines). **(b)** Mean soil moisture [vol-%] from 0–30 cm. **(c)** Mean N₂O fluxes [g N₂O-N m⁻² d⁻¹] measured by static chambers in the field. Error bars are standard deviations.

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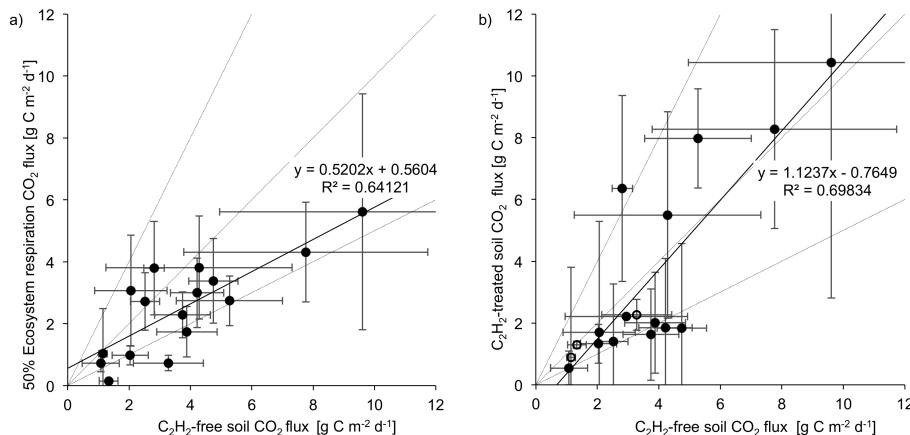


Fig. 4. Comparison of CO₂ fluxes from C₂H₂-free soil samples incubated in the laboratory to (a) 50 % ecosystem respiration fluxes (assumed to be commensurate with soil respiration) measured in the field and to (b) CO₂ fluxes from C₂H₂-treated (y-axis) samples. Open circles indicate fluxes measured from the C₂H₂-effect experiment (Sect. 2.2.2). Data and error bars are means and standard deviations ($\bar{x} \pm sd$). The outer lines show slopes of 0.5 and 1.5. The black solid line is the linear regression.

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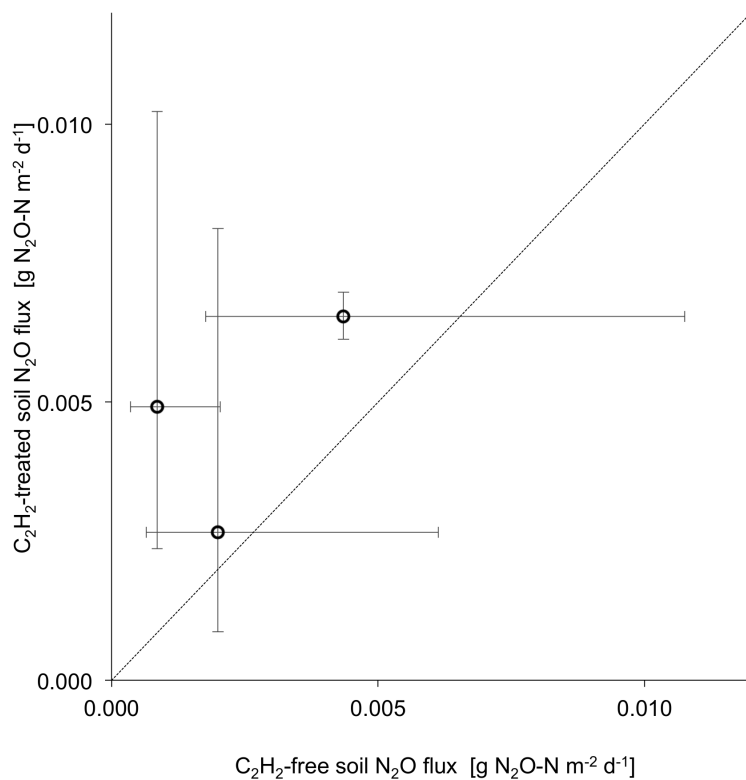


Fig. 5. Comparison of N₂O fluxes measured with C₂H₂ inhibition to fluxes measured without C₂H₂ inhibition during special experiments with parallel measurements of 3 C₂H₂-treated and 4 untreated samples. Error bars for the C₂H₂ incubation system indicate the range of the fluxes.

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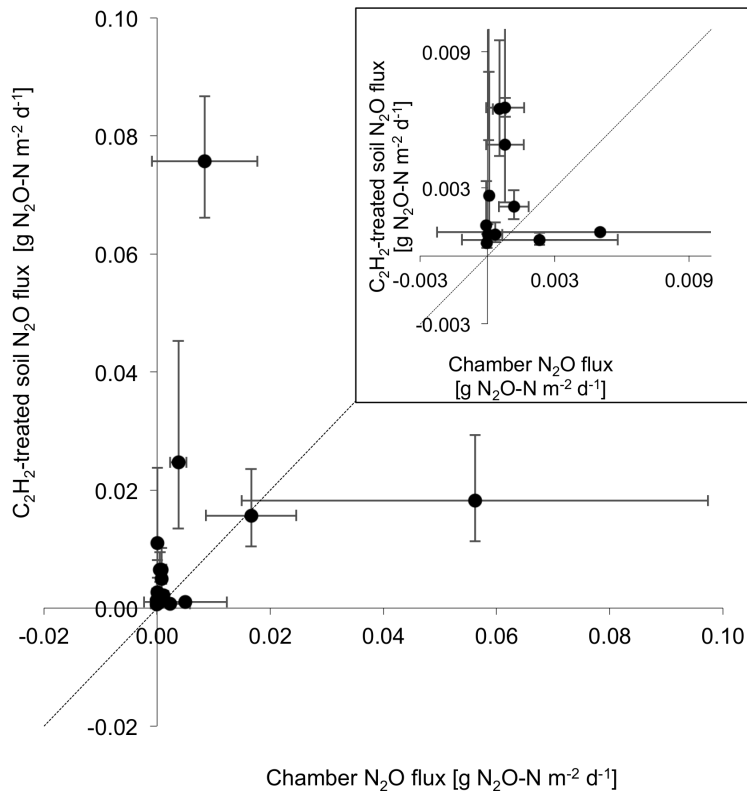


Fig. 6. An anti-correlation between N₂O fluxes measured by the static chambers to the total N loss fluxes measured by the C₂H₂ inhibition technique. Error bars for the C₂H₂ incubation system indicate the range of the fluxes, those for the chamber measurements regarding $\bar{x} \pm s.d.$

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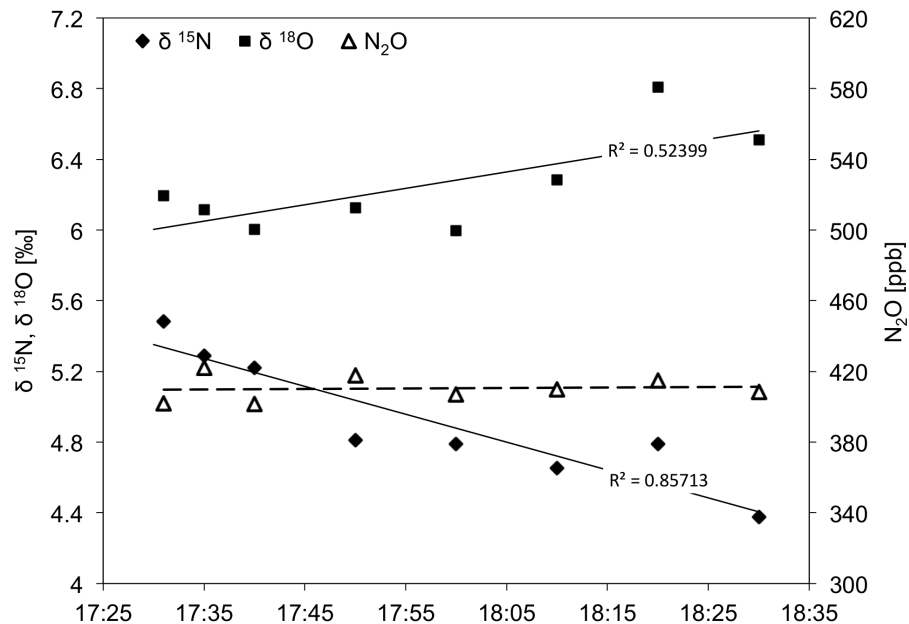


Fig. 7. Significant changes in $\delta^{15}\text{N}$ (-1‰) and $\delta^{18}\text{O}$ (0.4‰) measured in N_2O over an hour in a static chamber, indicating a clear N turnover, although no significant N_2O flux could be measured ($<1.5 \text{ ng N}_2\text{O m}^{-2} \text{ s}^{-1}$).

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