Biogeosciences, 9, 4125–4138, 2012
www.biogeosciences.net/9/4125/2012/
doi:10.5194/bg-9-4125-2012
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Biogeosciences

Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses

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Received: 2 February 2012 – Published in Biogeosciences Discuss.: 13 March 2012
Revised: 27 September 2012 – Accepted: 28 September 2012 – Published: 26 October 2012

Abstract. The loss of N_2 from intensively managed agroecosystems is an important part of the N budget. Flux monitoring of N_2 emissions at the field scale, e.g., by eddy correlation or aerodynamic gradient method, is impossible due to the large atmospheric N_2 background (78 %). The acetylene (C_2H_2) inhibition technique (AIT) is a rather simple and frequently used, albeit imperfect, method to determine N_2 losses from intact soil cores. In principle, AIT allows an estimation of total denitrification at high temporal resolution and on small spatial scales, with limited workload and costs involved. To investigate its potential and limitations, a laboratory system with two different detection systems (photoacoustic IR spectroscopy and gas chromatography) is presented, which allowed simultaneous measurements of up to 7 intact soil cores in air-tight glass tubes in a temperature controlled cabinet (adjusted to field conditions) with automated C_2H_2 injection.

A survey of total denitrification losses ($N_2 + N_2O$) over 1.5 yr in soil cores from an intensively managed, cut grassland system in central Switzerland supports previous reports on severe limitations of the AIT, which precluded reliable estimates of total denitrification losses. Further, the unavoidable sampling and transfer of soil samples to the laboratory causes unpredictable deviations from the denitrification activity in the field.

1 Introduction

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 in form of nitrate (NO_3^-) , ammonium (NH_4^+) and possibly monomeric organic N (Schimel and Bennett, 2004; Näsholm et al., 2009). The relative rate depends on the type of ecosystem. In an intensively fertilized mown grassland system, the N uptake by the plant is largely dominated by the uptake of NO_3^- and to a lesser extent of NH_4^+. Uptake of organic N is likely minor in this ecosystem. On a global scale Galloway and Cowling (2002) indicated that only about 31 % of produced N fertiliser shows up in harvested biomass.

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 be 187 Tg N kg^{-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 a total

loss of N from agricultural systems on a national scale of 29 % (44 kg N ha^{-1} agricultural area yr^{-1}) of introduced N. An empirical classification scheme from Germany indicates for the total annual denitrification a range of 30–50 kg N_2 for the soil type of our experimental field in Oensingen (Gäth et al., 1997).

The objective for this study was to contribute to the determination of all major N fluxes at our grassland site as part of the NitroEurope project (www.nitroeurope.eu). For a quantitative description of the nitrogen cycle on the field scale, we aimed at measuring all significant N fluxes. 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 in a magnitude similar to biological N fixation. For the Oensingen site, Ammann et al. (2009) estimated this fraction (N_2 loss (estimated) to N input) to around 15 %.

Various experimental approaches exist to measure N_2 losses from soils (for comprehensive reviews see Groffman et al. (2006) and Schlesinger (2009). These approaches including: (1) acetylene-inhibition technique (AIT) (e.g. Tiedje et al., 1989), (2) ^{15}N tracers (e.g. Hauck and Melsted, 1956; Rolston et al., 1978, 1982; Mosier et al., 1986), (3) direct N_2 quantification by completely replacing N_2 in the soil samples with, for example, He (e.g. Scholefield et al., 1997; Butterbach-Bahl et al., 2002; Cardenas et al., 2003), and (4) mass balance approaches (e.g. Allison, 1955). Direct flux measurements of N_2 emissions by micrometeorological approaches are impossible due to the high background concentration of N_2 (78 %) in the atmosphere.

There are only few studies that compared AIT and ^{15}N labelling in parallel in the field. Rolston et al. (1982) used a combination of AIT and strong isotope labelling on a well-drained loamy soil on 1 m^2 plots planted with perennial ryegrass. After the application of large amounts of mineral N fertilisers (> 200 kg N ha^{-1}), noticeable N_2O and N_2 fluxes were measured after irrigation with a good correspondence between AIT and isotopic labelling method. Mosier et al. (1986) successfully applied the ^{15}N labelling technique to a moderately well-drained soil planted with corn. In their study 200 kg N ha^{-1} in form of $(NH_4)_2SO_4$ was applied to the surface. Soil cores were extracted and the top 10 cm of these were replaced with ^{15}N-labelled NH_4^+. Again noticeable $^{15}N{-}N_2$ fluxes were measured after irrigation.

More frequently total denitrification losses were measured in the laboratory on soil samples. The AIT is based on the findings by Fedorova et al. (1973) that C_2H_2 at high concentrations (> 0.5 %) in the headspace of pure microbial cultures inhibits the microbial reduction of N_2O to N_2. Consequently N_2O emissions measured after addition of C_2H_2 at high concentration should correspond to the combined production of N_2 and N_2O in the C_2H_2-free soil sample under consideration.

However, there are a number of limitations of the AIT, which most of them are allready listed by Groffman et al. (2006):

1. The scavenging under aerobic conditions (C_2H_2-catalysed oxidation) of NO, the precursor of N_2O, which itself is the precursor of N_2 (Bollmann and Conrad, 1997);

2. the suppressed microbial respiration in the presence of C_2H_2 (Zhang et al., 2009);

3. the inhibition of N_2O reduction to N_2 by C_2H_2 might be incomplete when added C_2H_2 does not reach the denitrifiers due to diffusion barriers within the soil samples (Jury et al., 1982);

4. inhibition of nitrification and nitrifier-denitrification by already low concentrations of C_2H_2. This suppresses the supply of NO_3^- and the production of N_2O by nitrification and nitrifier-denitrification (Seitzinger et al., 1993; Wrage, 2003; Wrage et al., 2004). For short measurement times a suppressed supply of NO_3^- may only have a marginal effect on estimated total denitrification rates;

5. decomposition of C_2H_2 by C_2H_2-degrading microbes (Yeomans and Beauchamp, 1982; Terry and Duxbury, 1985; Topp and Germon, 1986; Flather and Beauchamp, 1992);

6. incomplete inhibition of N_2O reductase by C_2H_2 (Yu et al., 2010; Qin et al., 2012).

This long list of drawbacks suggests that a stable isotope approach might have been preferable to use in our context. However, due to the lack of isotopic measurement facilities in our laboratory, to budget constraints, to the difficulty of obtaining highly labelled slurry for the experiment, but also because we had reservations concerning detection limits and how well the labelled fertiliser would be distributed through the soil column, we opted for the AIT. Despite the aforementioned limitations, the AIT is in principle an easy and relatively inexpensive approach to measure total denitrification. At the beginning of our measurements there was no scientific consensus as to the reliability and adequacy of the C_2H_2 inhibition technique for total denitrification measurements. Groffman et al. (2006) rhetorically asked in their review "Is there still a role for C_2H_2-based methods in denitrification studies? In systems with high NO_3^- concentrations, certainly yes." Our intensively managed grassland system receives over 200 kg N ha^{-1} yr^{-1} and can be regarded as well supplied with inorganic N.

2 Material and methods

2.1 Field site description and soil samples

The experimental grassland site is located at Oensingen in Central Switzerland (7°44′ E, 47°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 characterised 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 annual air temperature of 9.5 °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, oilseed rape, maize and bi- or tri-annual grass-clover mixture. N input depended on the crop type and followed the Swiss standard fertilisation practice (110 kg N ha^{-1} yr^{-1} on average). In November 2000 the field was ploughed and in May 2001 sown with grass-clover mixtures typical of permanent grassland under intensive management in Switzerland. The field was typically cut four times per year and was fertilised twice or thrice with solid ammonium nitrate (NH_4NO_3) or liquid cattle manure (overall 230 kg N ha^{-1} yr^{-1}). In December 2007, the field was again ploughed and in May 2008 sown with a grass-clover mixture. This was necessary to re-establish 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) 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 to 35 g kg^{-1} dry soil and the C / N ratio is around 9.5. The soil pH is around 7.3 (Ammann et al., 2009).

We report total denitrification measurements in four observation periods between July 2008 and December 2009 (Tab. 1). On each sampling occasion seven soil samples of 2 cm diameter and 30 cm length were spatially randomly collected from the field with a gauge auger (model P, Eijkelkamp, NL, www.eijkelkamp.com) and transported within one hour to the laboratory. The small diameter of the soil samples was chosen to minimise the distance between C_2H_2 applied to the headspace and potentially denitrifying hotspots in the soil sample. After inserting the auger into the soil, it was turned once before extraction and we assumed that compaction of the sample can be neglected. For transport we cut the soil cores into 10 cm sections with a knife and then carefully transferred them into plastic bags. At low water content the soil sections could not always be transported as a single piece, but the samples were broken along cracks and agregates were assumed to be still intact. The lower end of the core (20–30 cm) was always intact, hence, the rest of the broken core was filled in the incubator above the intact part for the measurement. They were not tightly fitted into the incubators to ensure a better flow through the measurement system. The samples were taken from spots were no vegetation was growing and were measured at the same temperature (−5 cm) as the soil had when the samples had been taken.

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, remains in the form of N_2O, which is detected in our laboratory set-up by photoacoustic IR spectroscopy (pIRS) or by gas chromatography (GC). The laboratory system allows the quasi-simultaneous measurements of trace gas fluxes from up to seven 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 polyurethane 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 minutes. 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 are unlikely to measurably affect the applied concentration. The sampling frequency of one minute allows a quasi-continuous observation of the gas evolution.

At first, the Innova 1312 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_2O, CO_2, N_2O and C_2H_2: SB0527 ($1985 \pm 40\,cm^{-1}$) with strong H_2O absorption lines, UA0983 ($2270 \pm 15\,cm^{-1}$) with strong CO_2 absorption lines, UA0985 ($2215 \pm 22\,cm^{-1}$) with strong N_2O absorption lines, and UA0969 ($1254 \pm 34.5\,cm^{-1}$) with C_2H_2 absorption lines. These are broadband filters which result in cross interferences between the different gases (including H_2O) 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_2. However, Flechard et al. (2005) found that the N_2O concentrations indicated by the Innova 1312 output were still heavily dependent on CO_2, H_2O 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 labour-intensive calibration procedure and adapted the algorithm used by Flechard et al. (2005).

To further minimise 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 analogue 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):

$$\mathrm{Sig}_i = C_{\mathrm{H_2Ov}} \times k_{\mathrm{H_2Ov}\rightarrow i} + C_{\mathrm{CO_2}} \times k_{\mathrm{CO_2}\rightarrow i} + C_{\mathrm{N_2O}} \times k_{\mathrm{N_2O}\rightarrow i} + C_{\mathrm{C_2H_2}} \times k_{\mathrm{C_2H_2}\rightarrow i} + k_i \quad (1)$$

Sig_i is the analogue signal measured by filter i in mV, C stands for the concentration of the gas and $k_{\mathrm{gas}\rightarrow i}$ 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, indicated by $k_{\mathrm{gas}\rightarrow i}$. 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 $k_{\mathrm{gas}\rightarrow i}$ 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 ng N_2O m^{-2} 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 analyser.

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 and 5000 ppb N_2O, and 300 ppm to 10 000 ppm CO_2.

The sample rate of the Innova 1312 was one minute, the sample rate of the GC around five minutes. However, we did not change the time of the measurement per sample (30 min), yielding in 30 datapoints for the pIRS measurements and 5 for the GC measurements.

2.2.2 Acetylene inhibition measurements

Soil samples were measured without C_2H_2 for at least one or two cycles (referred to as C_2H_2-free fluxes). These fluxes should be comparable to field conditions, albeit with differences resulting from sampling, transportation, change of water content, etc. All samples were then incubated with 5 % C_2H_2 (referred to as C_2H_2-treated fluxes). After the addition of C_2H_2 the samples were measured for 24 h. However, the largest fluxes were measured within the first four to six hours after the addition of C_2H_2. Then the fluxes steadily decreased.

2.2.3 Flux evaluation

The temporal concentration changes (∂C) of N_2O and CO_2 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 C_2H_2 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.

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:

(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

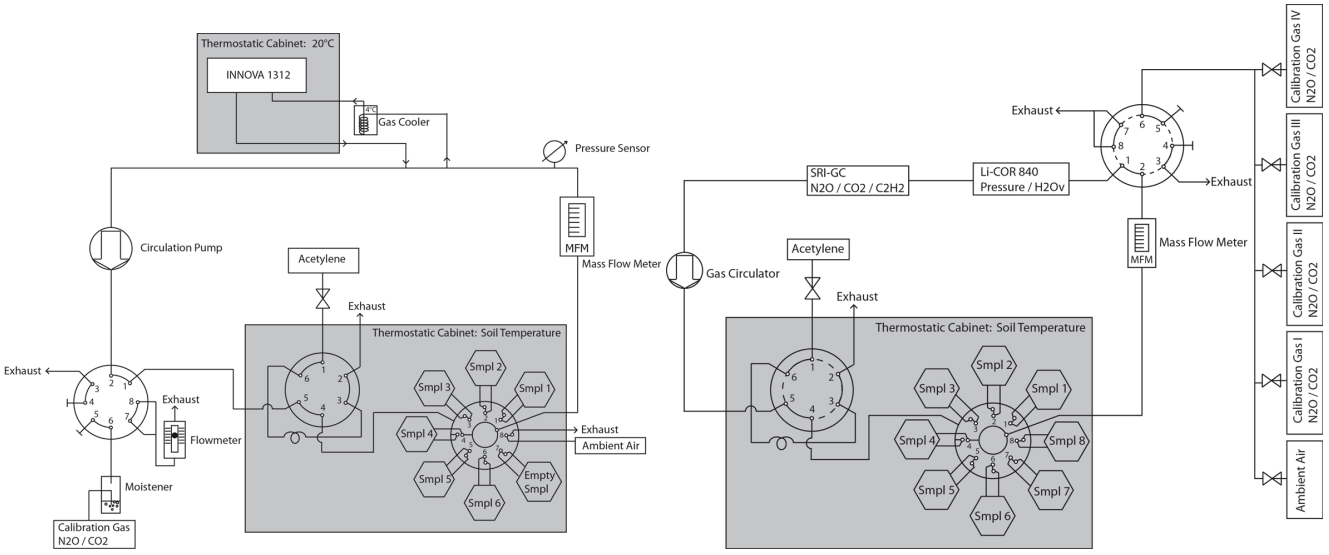

Fig. 1. Instrumental set up: photoacoustic IR gas analyser (Innova 1312, left) or alternatively a gas chromatograph (SRI 8610C GC, right) measures concentration increases of up to seven soil samples in rotation.

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 existent 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.

The mean flux ($\overline{F_s}$) of N_2O from the C_2H_2-treated samples was calculated as the mean of the fluxes measured for each batch of the first 12 hours. The daily mean flux of N_2O from all C_2H_2-treated samples was then calculated as 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 (sd = sd ($\overline{F_s}$) with s = number of samples) was computed. Concerning the uncertainty associated with the daily mean flux, given that N_2O 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:

$$F_{\text{day}} = \bar{x}^*/s^* \ldots \bar{x}^* \times s^* \tag{3}$$

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

2.3 Quasi-continuous N_2O measurements with static chambers

N_2O and CO_2 fluxes were 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 operating 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). N_2O and CO_2 fluxes were determined using a nonlinear approach with the HMR R-package (Pedersen et al., 2010) that is based on a specific application of the nonlinear model (HM) proposed by Hutchinson and Mosier (1981).

2.4 NH_4^+, NO_3^- concentrations measurements and other soil parameter measurements

NO_3^- and NH_4^+ concentrations were measured to estimate the availability of N in the samples in August 2009. Concentrations were measured after dissolving 20 g moist soil in 80 ml 0.01 M $CaCl_2$-solution. Samples were shaken for 30 min and afterwards filtered (Filter Paper Cod: PL 1290150; Filtros Anoia, S. A. Barcelona). The filtrates were analysed at the laboratory of lbu – Labor für Boden- und Umweltanalytik (Thun).

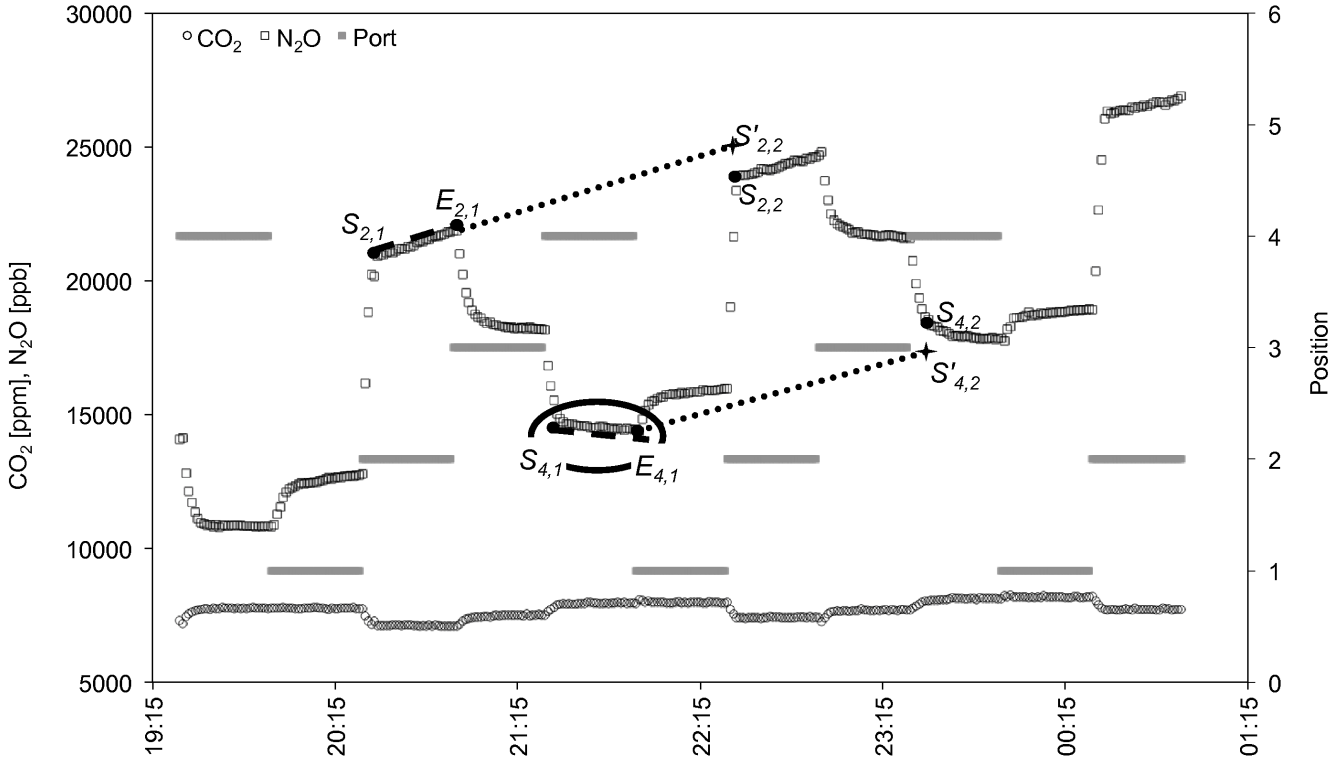

Fig. 2. Temporal concentration evolution of an extended measurement with four soil samples after C_2H_2 was injected (not shown). The dashed and dotted lines indicate the two ways calculating the slope for flux calculations. The concentration at point *S* was corrected for mixing effects. The ellipse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

Soil water content and soil temperature were measured at the Oensingen site at depths −5, −10, −30 and −50 cm since 2004. The used soil moisture sensors (ML2x – ThetaProbe, Delta-T Devices Ltd., UK, www.delta-t.co.uk) integrate over a volume of 18 cm^3. The volumetric water content was then multiplied by the total pore volume (55 %) to obtain the water filled pore space (WFPS). For temperature measurements thermocouples sondes (105T, Campbell Scientific Ltd., UK, www.campbellsci.co.uk) were used.

3 Results

3.1 Estimation and accuracy of N_2O fluxes with the AIT

The Innova 1312 photoacoustic IR instrument showed large interference by C_2H_2 and CO_2 on the N_2O concentration and, therefore, severely hampered the accuracy of the flux determination. The detection limit with the pIRS was around 40 ng $N_2O\,m^{-2}\,s^{-1}$, i.e., a factor 10 higher than with the GC (3.1 ng $N_2O\,m^{-2}\,s^{-1}$), independent of the C_2H_2 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.

3.2 N_2O flux chamber measurements in the field and environmental conditions over the entire observation period

Figure 3 gives an overview of the N_2O flux measured in the field, together with air temperature, soil temperature at 5 cm depth, calculated WFPS at 5 cm depth and rainfall over the entire observation period. The latter two are the most important environmental variables influencing denitrification. The top panel of Fig. 3 shows the management events (i.e., cuts and fertilising events). The amount of rainfall (Fig. 3b) during the measurement period was comparable to other years. The cumulative amount of rain was 664 mm for July to December in 2008 and 1005 mm in 2009. WFPS (Fig. 3b) was similar to other years and no exceptionally dry period occurred. N_2O fluxes measured with static chambers in the field showed similar patterns as in the years before (e.g. Flechard et al., 2005). Peak N_2O emissions occurred mainly after fertilisation events when the soil was moist.

3.3 AIT results

The observation phase was divided into four different periods representative of the different management practices and in

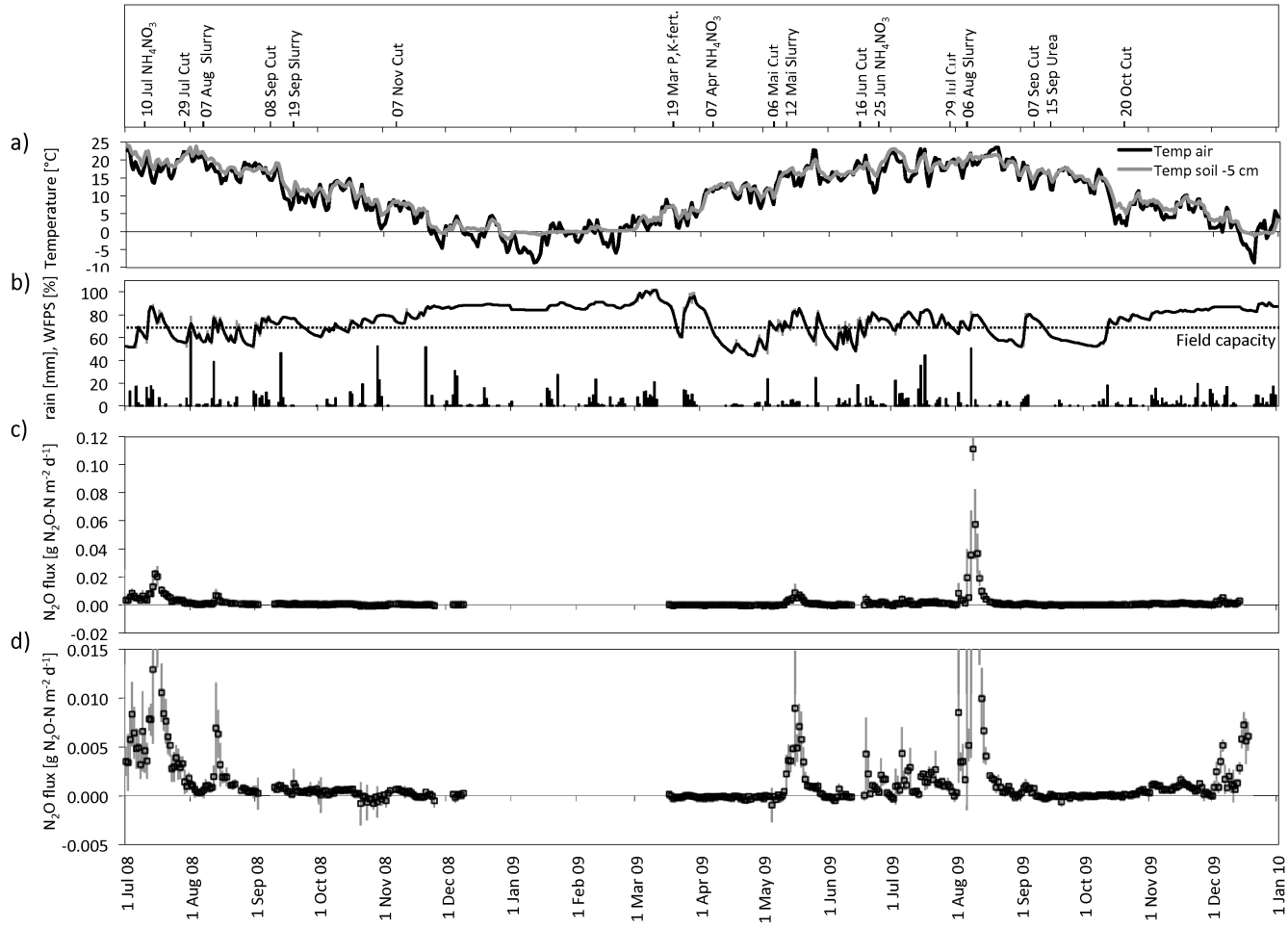

Fig. 3. Results of a 1.5-yr N_2O flux measurement period over intensively managed grassland in Oensingen (CH). Management events are shown in the top panel. **(a)** Air temperature and soil temperature at −5 cm. **(b)** Daily rainfall amount [mm] (bars) and measured WFPS [%] (line) at −5 cm, field capacity is indicated as dashed line. **(c)** Mean N_2O fluxes [g N_2O-N m^{-2} d^{-1}] measured by static chambers in the field. Error bars are standard deviations. **(d)** Same data as **(c)** on a different scale.

different seasons. Figures 4a-d show a detailed view of the WFPS and daily mean N_2O fluxes as measured in the field of the four observation periods when samples for the laboratory incubation experiments were taken. Note the different scales of the y-axis.

The first period from 1 July to 18 August 2008 (Fig. 4a) shows a period where two applications of 30 kg N ha^{-1} in form of NH_4NO_3 took place (10 July and 7 August). N_2O emissions were triggered by rain and were noticeable only when measured WFPS at 5 cm depth was close to, or above field capacity (70 %). N_2O fluxes from C_2H_2-treated samples were larger than from C_2H_2-free samples in all cases.

In the second period (Fig. 4b), only background N_2O emissions were measured. The AIT values show a clear bias towards larger fluxes than the field measurement, but no enhancement of N_2O fluxes was observed after addition of C_2H_2.

During the third period, on 6 August 2009 (Fig. 4c), slurry was applied, adding 32 kg NH_4^+-N ha^{-1} and 13 kg organic-N ha^{-1} to the field. Immediately after application, as well as 4 and 6 days later, soil samples were taken for the AIT measurements. The N_2O chamber flux data showed a first N_2O emission peak lasting less than one day, then two days later the start of a second, larger and longer-lasting peak after a rain event (Fig. 4c and detailed in Fig. 5a). It is the period with the highest N_2O fluxes and a reasonable correspondence of field-measured fluxes and fluxes derived from samples analysed in the laboratory without addition of C_2H_2. Addition of C_2H_2 slightly decreased these fluxes. Table 1 shows the N_2O fluxes measured from C_2H_2-free samples, the total $N(N_2 + N_2O)$ losses, measured as N_2O fluxes from C_2H_2-treated samples, and the corresponding actual N_2O fluxes measured at the same date by the automated flux chambers in the field.

During period 3, four hourly means of N_2O fluxes measured in the field showed two peaks (Fig. 5a). C_2H_2-free and C_2H_2-treated N_2O fluxes from subdivided (10 cm sections) samples showed in sum a similar pattern (Fig. 5b); a first peak right after the slurry application and a second one three days later after rain. However, the samples taken immediately after the slurry application showed higher N_2O fluxes from C_2H_2-free samples than from C_2H_2-treated samples (Tab. 1).

The fourth period showed again small N_2O emissions in the field. C_2H_2-free laboratory samples were twice as high. Addition of C_2H_2 gave a further enhancement of about 30 %.

3.4 CO_2 fluxes

CO_2 fluxes measured in the laboratory incubation system must be attributed to soil heterotrophic respiration, while fluxes measured in the field with flux chambers provided total ecosystem respiration (thus, including an autotrophic component in shoot and root 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 (mostly heterotrophic) respiration fluxes (unpublished data). Therefore, to assess the effect of soil sampling on microbial activity, a comparison of the incubated CO_2 fluxes to 50 % of the ecosystem respiration measured by static chambers in the field is shown in Fig. 6. The 50 % ecosystem respiration was in most cases smaller than the C_2H_2-free CO_2 flux, with the slope of the linear regression around 0.6, indicating a laboratory-based respiration roughly twice as large as the assumed soil respiration in the field.

CO_2 fluxes measured from the samples before the C_2H_2 injection did not significantly differ from CO_2 fluxes measured after the injection of C_2H_2. A similar large spatial variability of CO_2 fluxes found in the field was also seen in the samples measured in the laboratory.

4 Discussion

The results clearly show several problems when attempting to determine N_2 losses from a field by taking soil samples to the laboratory and subjecting them to the AIT. There are issues associated with changing soil properties in the samples taken to the laboratory compared with field conditions. Then there is the usual problem of spatial representativeness (Parkin, 1987). Other issues are directly associated with collateral effects of C_2H_2 on soil processes other than the desired inhibition of the reduction of N_2O to N_2.

4.1 Influence of soil sampling

The measurements were performed in a laboratory setup on intact soil cores with a small diameter (2 cm) and a length of 30 cm. The issue here is to what degree the soil cores can actually be considered to be "intact" in the strictest sense of the word. 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_2H_2 should penetrate the sample and reliably reach the active denitrification micro-sites by diffusion, and (3) the produced N_2O should escape into the headspace as soon as it is produced in the sample. Our admittedly rather optimistic and implicit hypothesis regarding N_2 flux was to assume that the laboratory measurements on intact soil samples without C_2H_2 should give comparable N_2O flux values to those derived from the automated chamber system.

4.2 Spatial considerations

The soil samples were not taken from exactly the same locations as where fluxes were measured with closed chambers (i.e., within the chamber), but randomly in their close vicinity (within 30 m). Spatial variability of N_2O fluxes measured in the field is notoriously high. Our chambers are integrating over a surface area of $0.09\,m^2$. Since our soil samples analysed in the laboratory have a two orders of magnitude smaller surface area ($3.14 \times 10^{-4}\,m^2$), variability will even be larger. N_2O fluxes measured by chambers in the field are controlled by soil conditions in the top 5 cm of the soil due to the short scale length of N_2O in a heavy clay soil (Neftel et al., 2000). The vertical scale length as introduced by Neftel et al. (2000) is a macroscopic property of a uniform soil indicating how far an N_2O molecules in the gas phase can travel before it is consumed. In other words, N_2O molecules produced at greater depth are unlikely to diffuse to the soil surface and be captured by the flux chamber. More likely, they are reduced to N_2 before reaching the soil surface. By contrast, an N_2O molecule produced in a 2 cm diameter soil core has a greater likelihood to escape before reduction because the largest lateral distance to the surface of the core is only 1 cm. Consequently, the laboratory measurements provide N_2O flux values somewhere between the net N_2O emission as measured in the field and the gross N_2O production in the top 30 cm of the soil.

4.3 Soil aeration

Assuming that soil respiration in the field constitutes as a rule of thumb about 50 % of the ecosystem respiration, a systematic increase of soil respiration must have occurred in the samples measured in the laboratory (see Fig. 6). It is unclear whether this is actually due to an increased O_2 supply, or to an enhanced supply of easily degradable soil organic matter (e.g., freshly cut roots).

The studied soil is characterised by a bimodal pores size distribution (Flechard et al., 2007) with mainly large pores and then many fine pores and relatively few pores in the intermediate range. The "natural" complexity of this soil dictates that the active respiration sites is exposed to a very wide range of oxygen concentration ranging from purely aerobic

Table 1. Summary of flux measurement results. Range and log-transformed means of N_2O fluxes from C_2H_2-free and C_2H_2-treated measurements and corresponding daily chamber N_2O fluxes.

		C_2H_2-free N_2O mg N_2O-N m^{-2} d^{-1}			C_2H_2-treated N_2O mg N_2O-N m^{-2} d^{-1}			$N_2O/(N_2O+N_2)$	WFPS %	Chamber N_2O mg N_2O-N m^{-2} d^{-1}	
Date	Detector[a]	lower	$\bar{x}$*	upper[b]	lower	$\bar{x}$*	upper[b]			$\bar{x}$	sd[c]
21 Jul 2008	pIRS	−0.37	−0.67	−1.23	0.95	1.06[§]	1.18	−0.63	68.7	6.0	1.8
29 Jul 2008	pIRS	−0.01	−0.06	−0.32	0.49	0.72[§]	1.04	−0.08	55.3	1.4	1.3
5 Aug 2008	pIRS	1.61	2.41	3.62	4.41	6.48[§]	9.51	0.37	62.8	0.3	0.2
11 Aug 2008	pIRS	0.01	0.03	0.17	0.49	0.81[§]	1.33	0.04	56.2	0.8	0.4
14 Aug 2008	pIRS	0.33	0.62	1.17	1.54	2.08[§]	2.81	0.30	65.5	6.3	2.5
period 1	mean	0.31	0.47	0.68	1.58	2.23[§]	3.17	0.21	61.7	**3.0**	**3.3**
	median	0.01	0.03	0.17	0.95	1.06[§]	1.30	0.03	62.8	1.4	
19 Mar 2009	pIRS	0.60	1.40	3.30	0.36	0.99	2.75	1.41	81.9	−0.3	0.2
7 Apr 2009	pIRS	0.09	0.20	0.41	0.35	0.58[§]	0.97	0.34	65.2	−0.1	0.2
17 Apr 2009	pIRS	0.25	0.65	1.68	0.27	0.51	0.95	1.28	49.6	0.0	0.1
22 Apr 2009	pIRS	0.15	0.53	1.83	0.11	0.19	0.35	2.76	48.7	−0.5	0.3
period 2	mean	0.27	0.69	1.81	0.27	0.57	1.26	1.22	61.6	−0.2	0.4
	median	0.20	0.59	1.76	0.31	0.54	0.96	1.09	62.8	−0.2	
31 Jul 2009	GC	3.33	7.28	15.92	5.11	11.03[§]	23.82	0.66	67.2	−0.009	0.6
3 Aug 2009	GC	13.99	25.05	44.85	6.81	15.17	33.78	1.65	73.7	3.5	1.0
6 Aug 2009	GC	41.05	67.51	111.03	14.26	21.69	32.99	3.11	68.2	19.4	20.8
10 Aug 2009	GC	9.31	13.41	19.33	11.28	18.20[§]	29.36	0.74	79.5	57.6	25.0
12 Aug 2009	GC	6.57	10.06	15.41	10.25	15.46[§]	23.32	065	78.0	19.0	5.6
period 3	mean	14.85	24.66	41.31	9.54	16.31	28.65	1.51	73.3	19.9	33.1
	median	9.31	13.41	19.33	10.25	15.46[§]	29.36	0.87	78.0	19.0	
26 Oct 2009	GC	0.65	2.00	6.13	0.87	2.66[§]	8.12	0.75	79.2	0.2	0.1
2 Nov 2009	GC	1.76	4.35	10.76	0.12	0.28	0.66	15.69	80.2	1.1	0.4
6 Nov 2009	GC	0.36	0.85	2.04	2.36	4.91[§]	10.22	0.17	81.9	1.0	0.4
period 4	mean	0.92	2.40	6.31	1.12	2.62[§]	6.33	0.92	77.7	0.75	0.5
	median	0.65	2.00	6.13	0.87	2.66[§]	8.12	0.75	79.2	1.0	

[a] pIRS stands for the measurements with the photoacoustic IR instrument and GC for the gas chromatograph measurements.
[b] $\bar{x}$* is the log-transformed daily means. Lower and upper are the lower and upper end of the daily flux calculated as suggested by Limpert et al. (2001).
[c] $\bar{x}$ and sd are the daily means and standard deviations.
[§] Measurement days when C_2H_2-treated fluxes were larger than C_2H_2-free fluxes.

to fully anaerobic conditions. In the coarser pores CO_2 concentrations rarely exceed a few percent. Accordingly, O_2 concentrations will not be less than a few percent below atmospheric concentrations. The half-saturation constant (k_m) for soil respiration is < 1 %. Hence, it makes almost no difference to respiration when a soil sample was exposed to, say, 18 % O_2 in the field and is then measured at 20.5 % in the laboratory.

Denitrification occurs in aerated soils in microsites where potential O_2 demand locally exceeds O_2 supply. O_2 concentrations can drop from atmospheric concentrations at an aggregate surface to zero in a distance of a few millimetres (Hojberg et al., 1994). Such gradients most likely persists when the aggregate is taken from the field (e.g., surrounded by 18 % O_2) to the laboratory (20.5 % O_2).

Roots severed during sampling may bleed easily degradable carbon sources into the soil that fuel O_2 demand, as suggested by the larger than expected soil respiration (Sect. 3.4). This counter-balance enhanced O_2 concentrations in the macropore space due to the soil sampling. Hence, we do not expect that changes in soil sample aeration between field and laboratory significantly altered denitrification rates in poorly aerated centres of soil aggregates.

4.4 N_2O fluxes

Nitrous oxide fluxes measured by static chambers in situ in the field were generally not correlated to fluxes measured from C_2H_2-free samples in the laboratory. The differences between the flux estimates by the two methods are examined for the four measurement periods identified in Fig. 4.

Period 1: in this period, the N_2O fluxes measured in the field systematically exceeded the laboratory based values from C_2H_2-free samples. It is a summer period with soil water contents in the field around field capacity. The soil in Oensingen had apparently enough active denitrification sites that were anaerobic and produced N_2O that reached the

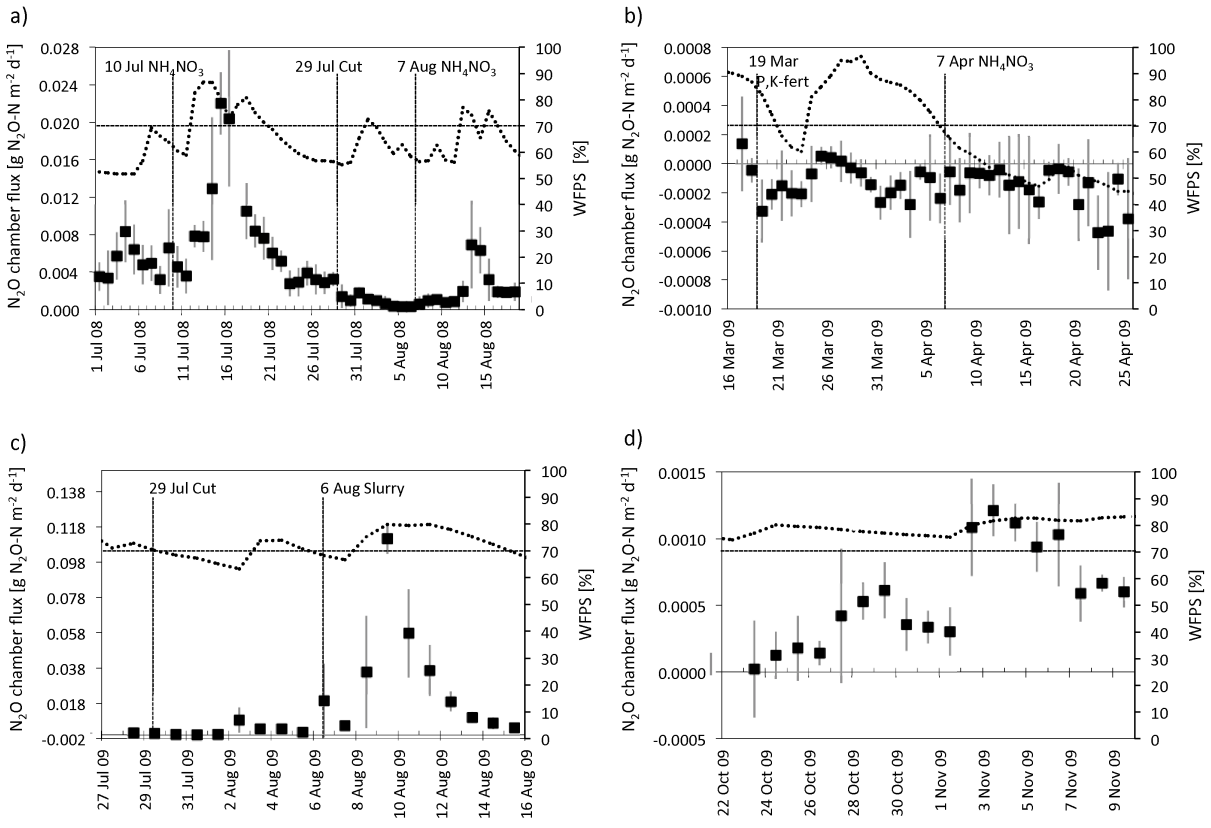

Fig. 4. Four typical measurement periods. **(a)** Moderate N_2O peak triggered by rain following application of mineral fertiliser. **(b)** Background N_2O exchange from wet to dry conditions, with indication of small uptake. **(c)** Double N_2O emission peak after slurry application (first peak nitrification, second peak denitrification). **(d)** Background N_2O fluxes above field capacity. The horizontal dashed line indicates field capacity ($\sim$ 70 %). Note the different scales of the axis.

surface. Taking soil samples has inevitably cut roots causing a bleeding of exudates and has increased the anaerobic zones in the laboratory samples, thus promoting complete denitrification.

Period 2: this is an early spring period with soil water contents above field capacity at the beginning and below field capacity from the mid to the end of the period. During the beginning the NO_3^- level was probably low since neither NO_3^- nor NH_4^+ was applied for a longer period. After the application of NH_4NO_3 on 7 April the soil water content decreased below field capacity. N_2O fluxes measured in the field indicate small uptake values and overall very small fluxes in either direction. Despite the application of mineral fertiliser this points to a strong N-substrate limitation, so that the still active denitrifiers use N_2O as electron acceptor. The C_2H_2-free laboratory samples showed enhanced fluxes, a clear bias to larger emission fluxes than the field measurement. One explanation is that the mean path length to the atmosphere in which the concentrations are measured is smaller in the laboratory samples and chances for further reductions are smaller. Interpretation necessarily will remain speculative as we only can compare bulk values (i.e., net N_2O fluxes) of two different systems with no further means to distinguish different processes. As the samples were taken after the dormant season aeration and the cutting of plant roots may have made available labile carbon sources that could have triggered mineralisation that in turn might trigger nitrification and denitrification activity.

Period 3: this is a period dominated by an N_2O emission triggered by the application of slurry. Applied ammonia was rapidly nitrified and a high NO_3^- level was maintained throughout the period. High NO_3^- levels favour a large N_2O to N_2 ratio of the emission. In this period laboratory based and field based N_2O fluxes are statistically not different. As such events dominate the fluxes they might erroneously suggest that laboratory measurements that are focused on trigger events are generally representative for field conditions.

Period 4: this autumn period is characterised by wet conditions with WFPS above field capacity. The high water content in the field implies diffusion limitation on produced N_2O to reach the atmosphere. The chances that N_2O produced is further reduced to N_2 is larger in the soil under the chamber, than in the laboratory soil samples where the mean distance to the headspace is small. Consequently, N_2O fluxes

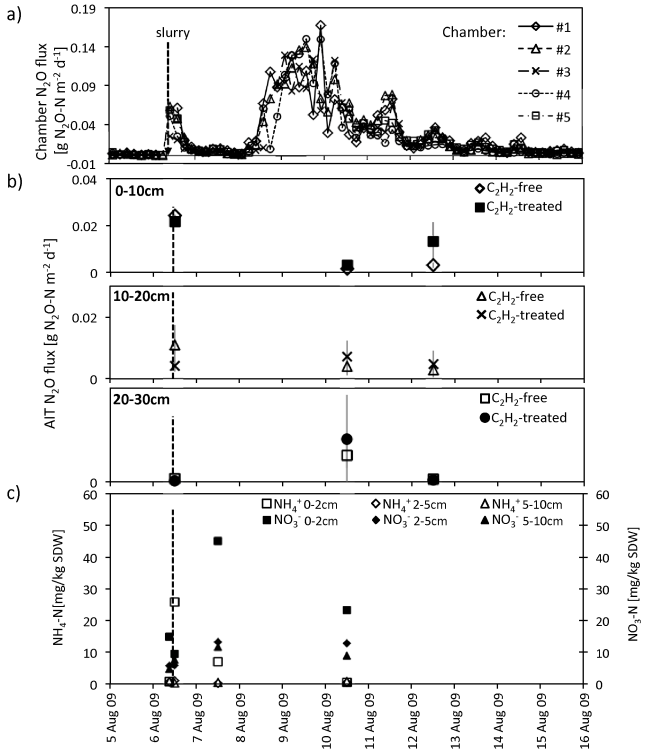

Fig. 5. (a) Time series of N_2O chamber fluxes measured in the field, **(b)** from samples measured by AIT from different soil depths, **(c)** and ammonium (NH_4^+) and nitrate (NO_3^-) concentrations.

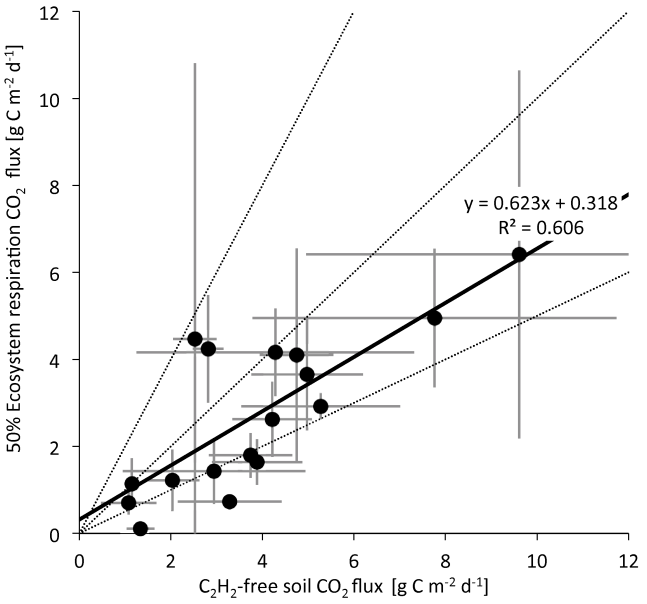

Fig. 6. Comparison of CO_2 fluxes from C_2H_2-free soil samples incubated in the laboratory to 50 % ecosystem respiration fluxes (assumed to be commensurate with soil respiration) measured in the field. Data and error bars are means and standard deviations ($\bar{x} \pm$sd). The outer lines show slopes of 0.5 and 1.5. The black solid line is the linear regression.

measured in the field were smaller than those determined in the laboratory.

N_2O fluxes measured without C_2H_2 in the laboratory strongly deviated from N_2O fluxes measured in the field. Hence, a reliable comparison of a strongly log-normally distributed parameter such as denitrification rate representatively in the field with 2 cm diameter cores is difficult. In addition, severing roots alters the soil condition in the sampled cores.

4.5 Effect of C_2H_2 addition

The incubation with C_2H_2 affected the soil microbial community in different ways. Below we discuss the most critical points.

4.5.1 Suppression of nitrification and nitrifier-denitrification

C_2H_2 inhibits nitrification and nitrifier-denitrification processes (Berg et al., 1982; Wrage, 2003). The further supply of NO_3^- for denitrification is interrupted. Consequently N_2O production by these two processes will not take place. We believe the major part of N_2O emissions at our field site are from denitrification for two reasons. i) Large N_2O emission events mainly occur when WFPS exceeds field capacity (∼70 % WFPS), indicative of denitrification being the source process (Fig. 7). ii) A high NO_3^- to NH_4^+ ratio. The Oensingen grassland soil generally shows a ratio of NO_3^- to NH_4^+ of 10 to 1 or larger that remains fairly constant over time (Fig. 5c). In cases where strong nitrification occur, a sytematic increase of the NO_3^- to NH_4^+ ratio would be expected, which is not the case. Suppression of NO_3^- formation by nitrification due to the addition of C_2H_2 has only a minor influence on the bulk NO_3^- level in the soil over the duration of the AIT measurements.

Only for a short period after application of reduced N fertiliser, the NO_3^- to NH_4^+ ratio is temporarily reversed. In our measurements we traced very rapid nitrification in the field (indicated by a rapid decrease in soil NH_4^+ concentrations, though root uptake and microbial immobilisation could also have contributed part of the NH_4^+ sink), but we only observed small to moderate N_2O emissions. Figure 5 illustrate this behaviour.

On 6 August 2009, slurry was applied, adding 32 kg N ha^{-1} in the form of NH_4^+ and 13 kg organic-N ha^{-1} on the field. Immediately after application, soil samples were taken for the AIT measurements, as well as 4 and 6 days later. A first N_2O emission peak with a short duration of less than one day was observed in the field. Then two days later a second, larger and longer-lasting peak after a rain event was observed. As the laboratory measurements of the samples taken immediately after the slurry application

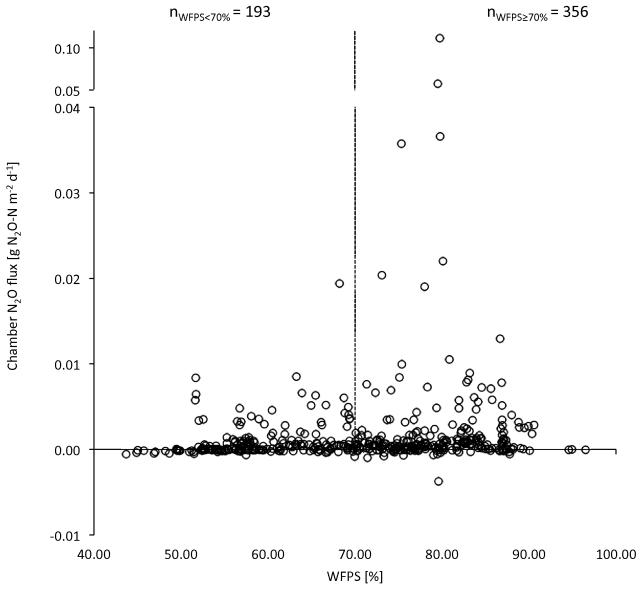

Fig. 7. N_2O fluxes measured in the field in relation to measured water-filled pore space (WFPS) at −5 cm.

showed much higher N_2O fluxes without C_2H_2 than with C_2H_2 and the NH_4^+ concentration in the top soil layer exceeded the NO_3^- concentration, we interpret that the first peak observed in the field has potentially an important contribution from nitrification and/or nitrifier-denitrification and that the second peak is due to denitrification. Note that the first peak contributed a very minor fraction to the cumulated N_2O emission over the 10 days after slurry application. On August 6th, the laboratory N_2O fluxes without C_2H_2 were much higher than the values with C_2H_2, but were in the same range as measurements in the field. This indicates that nitrification and/or nitrifier-denitrification was probably inhibited by the addition of C_2H_2. For the next two sampling dates on 10 August and 12 August nitrification should not have played a major role, as NH_4^+ in the soil was already nitrified.

4.6 Influence on microbial activity

High level of C_2H_2 (5 %) might suppress microbial activity in general (Zhang et al., 2009), but we did not observe a systematic change in soil respiration activity by the addition of C_2H_2. The comparison of CO_2 fluxes (i.e., soil respiration) before and after the incubation with C_2H_2 indicated that only in some cases the fluxes were smaller after the application of C_2H_2, but we did not observe a systematic change in soil respiration activity. Payne (1984) reported that the growth and vitality of soil bacteria are not affected by C_2H_2 application if sufficient NH_4^+ is available in the substrate.

4.7 N_2O / N_2 ratio

As the C_2H_2 inhibition techniques commonly used (e.g. Rudaz et al., 1999) determines the N_2 loss as the difference between N_2O emission without and with C_2H_2 treatment, it is convenient to characterise the N_2 loss with a scaling factor of the measured N_2O emissions. In situations where enhanced N_2O fluxes are observed, such an approach is meaningful.

Most of the time, N_2O fluxes in the field are very small and close or below the detection limit of the measuring system (Neftel et al., 2007) and frequently N_2O uptake is reported. N_2O uptake is diffusion limited and, therefore, can only be small (Neftel et al., 2010). Nevertheless, they demonstrate, as N_2O is believed to be an obligatory precursor of N_2 in the denitrification process, that small N_2 fluxes might persist over longer time periods when N_2O fluxes cannot be detected. In such situations, N_2 fluxes cannot be estimated by a scaling factor of N_2O emissions.

4.8 Can we infer any meaningful results from the AIT?

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, N_2 fixation, harvested N, wet and dry deposition) allow only an indirect determination of the sum of the denitrification N losses and the storage change in the soil. The latter could be constrained by the C-budget, so that an estimate of $40 \pm 30\,kg\,N\,ha^{-1}\,yr^{-1}$ for the total denitrification resulted.

In the literature several estimations of N_2 losses have been presented. van der Salm et al. (2007) using the C_2H_2 inhibition technique found that up to 22 % of the applied N was lost due to denitrification. Other studies reported 10–40 % denitrification losses from fertilised maize fields in France (C_2H_2 inhibition, Jambert et al., 1997), 0–25 % losses from permanent pasture in Switzerland (C_2H_2 inhibition, Rudaz et al., 1999).

Our initial, and admittedly rather optimistic and implicit, hypothesis regarding N_2 flux was that the laboratory measurements on intact soil samples, without C_2H_2 treatment, should give comparable N_2O flux values to those derived from the automated chamber system in the field, since in our experiment N_2O fluxes were measured in the laboratory at the temperature and soil water content of the field conditions.

Had this been confirmed, the AIT could have been applied to samples with similar N_2O production and consumption characteristics and would potentially have provided reliable estimates for the total denitrification losses. Clearly, for our grassland site at Oensingen, this hypothesis was not verified. Although our earlier estimation of the range (6 to $26\,kg\,N\,ha^{-1}\,yr^{-1}$) of a lower bound given in the discussion paper of N_2 losses were commensurate as the N_2 fluxes necessary to close the N budget of the site ($40 \pm 30\,kg\,N\,ha^{-1}\,yr^{-1}$), they cannot reduce the large

uncertainty in this budget because of the various limitations we discussed and we cannot be certain whether we can indicate a lower bound.

5 Conclusions

An attempt to experimentally estimate the loss of N_2 using the AIT of an intensively managed, fertilised, mown grassland was made. The different artifacts associated with the AIT preclude a reliable estimate of N_2 losses. This failure might also partially be due to the characteristics heavy clay soil at our grassland site.

Not only was the AIT itself flawed, but also the unavoidable sampling and transfer of soil samples to the laboratory yielded unpredictable deviations from the denitrification activity in the field. Unfortunately, at present there does not exist any easy, cost-effective and reliable method for an operational and continuous field determination of N_2 losses, even though this loss constitutes an important part of the N budget at this site and in many agricultural soils.

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. Further we would like to thank the reviewers Reinhard Wells, Michael Dannenmann, Nicole Wrage and the anonymous reviewer for many helpful inputs and comments.

For funding we gratefully thank the EU project NitroEurope (Contract 017841).

Edited by: H. van Grinsven

References

Allison, F. E.: The enigma of soil nitrogen balance sheets, Adv. Agron., 7, 213–250, 1955.

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.

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.

Berg, P., Klemedtsson, L., and Rosswall, T.: Inhibitory effect of low partial pressures of acetylene on nitrification, Soil Biol. Biochem., 14, 301–303, 1982.

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.

Braun, M., Hurni, P., and Spiess, E.: Phosphor- und Stickstoffüberschüsse in der Landwirtschaft und Para-Landwirtschaft, Nummer 18 in Schriftenreihe der FAC Liebefeld, Eidgenössische Forschungsanstalt für Agrikultur und Umwelthygiene, Liebefeld-Bern, Switzerland, 1994.

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.

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.

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.

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.

Flather, D. H. and Beauchamp, E. G.: Inhibition of the fermentation process in soil by acetylene. Soil Biol. Biochem., 24, 905–911, 1992.

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.

Flechard, C. R., Neftel, A., Jocher, M., Ammann, C., Leifeld, J., and Fuhrer, J.: Temporal changes in soil pore space CO_2 concentration and storage under permanent grassland, Agr. Forest Meteorol., 142, 66–84, 2007.

Galloway, J. N. and Cowling, E. B.: Reactive nitrogen and the world: 200 years of change, Ambio, 31, 64–71, 2002.

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.

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

Gäth, S., Antony, F., Becker, K. W., Geries, H., Höper, H., Kersebaum, C., and Nieder, R.: Bewertung der standörtlichen Denitrifikationsleistung und des Mineralisations-/Immobilisationspotenzials von Böden und Bodennutzungssystemen, Mitt. Dt. Bodenkdl. Ges., 85, 1373–1376, 1997.

Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., 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.

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.

Hojberg, O., Revsbach, N. P., and Tiedje, J. M.: Denitrification in Soil Aggregates Analyzed with Microsensors for Nitrous Oxide and Oxygen, Soil Sci. Soc. Am. J., 58, 1691–1698, 1994.

Hutchinson, G. L. and Mosier, A. R.: Improved soil cover method for field measurement of nitrous oxide fluxes, Soil Sci. Soc. Am. J., 45, 311–316, 1981.

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, 1997.

Jury, W. A., Letey, J., and Collins, T.: Analysis of chamber methods used for measuring nitrous oxide production in the field, Soil Sci. Soc. Am. J., 46, 250–255, 1982.

Limpert, E., Stahel, W. A., and Abbt, M.: Log-normal distributions across the sciences, Keys and clues, Bioscience, 51, 341–352, 2001.

Mosier, A. R., Guenzi, W. D., and Schweizer, E. E.: Soil losses of dinitrogen and nitrous oxide from irrigated crops in northeastern Colorado, Soil Sci. Soc. Am. J., 50, 344–348, 1986.

Näsholm, T., Kielland, K., and Ganeteg, U.: Uptake of organic nitrogen by plant. New Phytol., 182, 31–48, 2009.

Neftel, A., Blatter, A., Schmid, M., Lehmann, B., and Tarakanov, S. V.: An experimental determination of the scale length of N_2O in the soil of a grassland, J. Geophys. Res-Atmos., 105, 12095–12103, 2000.

Neftel, A., Flechard, C., Ammann, C., Conen, F., Emmenegger, L., and Zeyer, K.: Experimental assessment of N_2O background fluxes in grassland systems, Tellus, 59B, 470–482, 2007.

Neftel, A., Ammann, C., Fischer, C., Spirig, C., Conen, C., Emmenegger, L., Tuzson, B., and Wahlen, S.: N_2O exchange over managed grassland: Application of a quantum cascade laser spectrometer for micrometeorological flux measurements, Agr. Forest Meteorol., 150, 775–785, 2010.

Parkin, T. B.: Soil microsites as a source of denitrification variability, Soil Sci. Soc. Am. J., 51, 1194–1199, 1987.

Payne, W. J.: Influence of acetylene on microbial and enzymatic assays, J. Microbiol. Meth., 2, 117–133, 1984.

Pedersen, A. R., Petersen, S. O., and Schelde, K.: A comprehensive approach to soil-atmosphere trace-gas flux estimation with static chambers, Eur J. Soil Sci., 61, 888–902, 2010.

Qin, S., Hu, C., and Oenema, O.: Quantifying the underestimation of soil denitrification potential as determined by the acetylene inhibition method. Soil Biol. Biochem., 47, 14–17, 2012.

Rolston, D. E., Hoffman, D. L., and Toy, D. W.: Field measurement of denitrification: I. Flux of N2 and N2O, Soil Sci. Soc. Am. J., 42, 863–869, 1978.

Rolston, D. E., Sharpley, A. N., Toy, D. W., and Broadment, F. E.: Field measurement of dentitrification. III. Rates during irrigation cycles, Soil Sci. Soc. Am. J., 46:289–296, 1982.

Rudaz, A. O., Walti, E., Kyburz, G., Lehmann, P., and Fuhrer, J.: Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation with management, soil water content and soil temperature, Agr. Ecosyst. Environ., 73, 83–91, 1999.

Schimel, J. P. and Bennett, J.: Nitrogen mineralization: Challanges of a changing paradigm, Ecology, 85, 591–602, 2004.

Schlesinger, W. H.: On the fate of anthropogenic nitrogen, P. Natl. Acad. Sci. USA, 106, 203–208, 2009.

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.

Seitzinger, S. P., Nielsen, L. P., Caffrey, J., and Christensen, P. B.: Denitrification measurements in aquatic sediments: A comparison of three methods, Biogeochemistry, 23, 147–167, 1993.

Terry, R. E. and Duxbury, J. M.: Acetylene decomposition in soils, Soil Sci. Soc. Am. J., 49, 90–94, 1985.

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.

Topp, E. and Germon, J. C.: Acetylene metabolism and stimulation of denitrification in an agricultural soil, Appl. Environ. Microb., 52, 802–806, 1986.

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.

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 alternation of the global nitrogen cycle, Causes and consequences, Ecol. Appl., 7, 737–750, 1997.

Wrage, N.: Pitfalls in measuring nitrous oxide production in nitrifiers, PhD Thesis Wageningen University, Wageningen, 2003.

Wrage, N., Velthof, G. L., Oenema, O., and Laanbroek, H. J.: Acetylene and oxygen as inhibitors of nitrous oxide production in Nitrosomonas europaea and Nitrosospira briensis: a cautionary tale, Fems Microbiol. Ecol., 47, 13–18, 2004.

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

Yeomans, J. C. and Beauchamp, E. G.: Acetylene as a possible substrate in the denitrification process, Can. J. Soil Sci., 62, 137–144, 1982.

Yu, K., Seo, D. C., and DeLaune, R. D.: Incomplete acetylene inhibition of Nitrous Oxide reduction in potential denitrification assay as revealed by using 15N-Nitrate tracer, Comm. Soil Sci. Plant Anal., 41, 2201–2210, 2010.

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.