Biogeosciences Discuss., 12, 12653–12689, 2015
www.biogeosciences-discuss.net/12/12653/2015/
doi:10.5194/bgd-12-12653-2015

Biogeosciences Discussions
Open Access

Application of the ^{15}N-Gas Flux method for measuring in situ N_2 and N_2O fluxes due to denitrification in natural and semi-natural terrestrial ecosystems and comparison with the acetylene inhibition technique

F. Sgouridis[1], **S. Ullah**[1], **and A. Stott**[2]

[1]School of Physical and Geographical Sciences, Keele University, Staffordshire, UK
[2]NERC Life Sciences Mass Spectrometry Facility, Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster, UK

Received: 9 July 2015 – Accepted: 23 July 2015 – Published: 11 August 2015

Correspondence to: F. Sgouridis (f.sgouridis@keele.ac.uk)

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

Abstract

Soil denitrification is considered the most un-constrained process in the global N cycle due to uncertain in situ N_2 flux measurements, particularly in natural and semi-natural terrestrial ecosystems. ^{15}N tracer approaches can provide in situ measurements of both N_2 and N_2O simultaneously, but their use has been limited to fertilised agro-ecosystems due to the need for large ^{15}N additions in order to detect $^{15}N_2$ production against the high atmospheric N_2. For ^{15}N-N_2 analyses, we have used an "in house" laboratory designed and manufactured N_2 preparation instrument which can be interfaced to any commercial continuous flow isotope ratio mass spectrometer (CF-IRMS). The N_2 prep unit has gas purification steps, a copper based reduction furnace, and allows the analysis of small gas injection volumes (4 µL) for ^{15}N-N_2 analysis. For the analysis of N_2O, an automated Tracegas Pre-concentrator (Isoprime Ltd) coupled to an IRMS was used to measure the ^{15}N-N_2O (4 mL gas injection volume). Consequently, the co-efficient of variation for the determination of isotope ratios for N_2 in air and in standard N_2O (0.5 ppm) was better than 0.5 %. The ^{15}N Gas-Flux method was adapted for application in natural and semi-natural land use types (peatlands, forests and grasslands) by lowering the ^{15}N tracer application rate to 0.04–0.5 kg ^{15}N ha^{-1}. For our chamber design (volume / surface = 8 : 1) and a 20 h incubation period, the minimum detectable flux rates were 4 µg N m^{-2} h^{-1} and 0.2 ng N m^{-2} h^{-1} for the N_2 and N_2O fluxes respectively. The N_2 flux ranged between 2.4 and 416.6 µg N m^{-2} h^{-1}, and the grassland soils showed on average 3 and 14 times higher denitrification rates than the woodland and organic soils respectively. The N_2O flux was on average 20 to 200 times lower than the N_2 flux, while the denitrification product ratio ($N_2O/N_2 + N_2O$) was low, ranging between 0.03 and 13 %. Total denitrification rates measured by the acetylene inhibition technique under the same field conditions correlated (r = 0.58) with the denitrification rates measured under the ^{15}N Gas-Flux method but were underestimated by a factor of 4 and this was attributed to the incomplete inhibition of N_2O reduction to N_2 under relatively high soil moisture content. The results show that the ^{15}N Gas-Flux method can

BGD
12, 12653–12689, 2015

be used for quantifying N_2 and N_2O production rates in natural terrestrial ecosystems, thus significantly improving our ability to constrain ecosystem N budgets.

1 Introduction

There has been a renewed interest recently in developing new or enhancing existing measurement approaches for improving our ability to constrain dinitrogen (N_2) fluxes due to denitrification in terrestrial ecosystems (Kulkarni et al., 2014; Lewicka-Szczebak et al., 2013; Wang et al., 2011; Yang et al., 2014). Denitrification, the reduction within soils of nitrogen oxides (NO_3^- and NO_2^-) to NO, N_2O and ultimately N_2 gas, constitutes the most important mechanism for the removal of excess reactive nitrogen (Nr) in terrestrial ecosystems (Galloway et al., 2008; Groffman, 2012). Despite its importance, denitrification is considered the most un-constrained process in the global N cycle (Groffman, 2012; Kulkarni et al., 2008) due to uncertainties in N_2 flux estimations that are likely leading to underestimations of denitrification rates at multiple scales (Butterbach-Bahl et al., 2013). Considering contemporary atmospheric N deposition rates globally including UK (Dore et al., 2012; Galloway et al., 2008; Payne, 2014), the available Nr pool in soils may be greater than the capacity of denitrification for its removal with important consequences of chronic N enrichment of natural terrestrial ecosystems (Galloway et al., 2008; Limpens et al., 2003). Moreover, nitrous oxide (N_2O), an obligate intermediate of denitrification, is a potent greenhouse gas involved in the breakdown of stratospheric ozone (Ravishankara et al., 2009). Therefore, a reliable estimation of the relative magnitude of the major denitrification end products ($N_2 + N_2O$) in soils is crucial in evaluating the role of denitrification as an Nr sink (Kulkarni et al., 2008).

N_2 comprises ~ 78 % of the atmosphere and thus it is extremely difficult to measure small N_2 fluxes from soil against this high background, particularly in natural terrestrial ecosystems (Groffman et al., 2006). Available methods for measuring both N_2 and N_2O are limited and can be categorised into the direct flux and ^{15}N isotope tracer

BGD
12, 12653–12689, 2015

methods (Kulkarni et al., 2014), whilst micrometeorological approaches (Eddy covariance) are impossible in the N_2 rich atmosphere (Felber et al., 2012). The gas-flow soil core method (Burgin and Groffman, 2012; Butterbach-Bahl et al., 2002; Scholefield et al., 1997; Wang et al., 2011) allows the direct measurement of N_2 flux (without the addition of any substrate such as nitrate) from intact soil cores where the soil atmosphere is replaced by a mixture of He/O_2. However, despite the high precision of the technique, cores still need to be extracted from the field and conditioned over lengthy periods of time for the complete removal of N_2 from the soil atmosphere. This method is therefore time and resource intensive which limits its application to intensive temporal and large spatial scales (Kulkarni et al., 2014). Moreover, the gas-flow soil core method cannot discriminate between sources of N_2O thus overestimating the denitrification product ratio (N_2O/N_2+N_2O) (Butterbach-Bahl et al., 2013; Morse et al., 2015). The acetylene inhibition technique (AIT) is also a direct flux method that exploits the ability of acetylene (C_2H_2) at high concentrations (10 % v/v) to inhibit the reduction of N_2O to N_2 (Tiedje et al., 1989), thus total denitrification (N_2+N_2O) is measured in C_2H_2 amended soil cores in situ, whilst N_2 flux is estimated indirectly by difference from un-amended soil cores. Despite its simplicity and cost-effectiveness, the AIT is becoming increasingly unpopular due its several limitations, reviewed in Groffman et al. (2006), that preclude its use for reliable estimates of in situ denitrification rates (Felber et al., 2012).

The ^{15}N Gas-Flux method (Mosier and Klemedtsson, 1994) has the advantage of providing in situ measurements of both N_2 and N_2O simultaneously, thus allowing its application over large temporal and spatial scales. It requires the addition of a ^{15}N-labelled tracer in a soil enclosure in the field which is subsequently covered by a chamber while the chamber headspace is progressively enriched with ^{15}N-N_2 and ^{15}N-N_2O produced by denitrification (Stevens and Laughlin, 1998). Assuming that both N_2 and N_2O originate from the same uniformly labelled soil NO_3^- pool (Stevens and Laughlin, 2001), the true denitrification product ratio can be more accurately estimated as opposed to the direct flux approaches (Bergsma et al., 2001). The ^{15}N Gas-Flux method

BGD
12, 12653–12689, 2015

is suitable for both well and poorly-drained soil applications and allows for broader areal coverage compared to ^{15}N tracer "push-pull" techniques constrained to "point" measurements in fully saturated soils and sediments (Harms and Jones, 2012; Sanders and Trimmer, 2006; Whitmire and Hamilton, 2005). Field applications of the ^{15}N Gas-Flux method so far have been limited to fertilised agro-ecosystems (Baily et al., 2012; Cuhel et al., 2010; Graham et al., 2013) with high ^{15}N tracer application rates (between 10–200 kg N ha^{-1}), with the exception of Kulkarni et al. (2014) who have measured denitrification rates in northern hardwood forests of the US by adding tracer amounts of ^{15}N-labelled nitrate and Morse and Bernhardt (2013) who applied the same technique in intact soil cores collected from mature and restored forested wetlands in North Carolina, USA. These recent studies hold much promise that the ^{15}N Gas-Flux technique can be applied to a range of natural and semi-natural terrestrial ecosystems allowing the quantification of the relative magnitude of N_2 and N_2O fluxes due to denitrification from these under-represented ecosystems.

Natural and semi-natural terrestrial ecosystems in the UK (i.e. peatlands, heathlands, acid grasslands, deciduous and coniferous forests), where there is no fertiliser use and the impact from grazing and commercial forestry is minimal (Mills et al., 2013), along with improved and unimproved grasslands (grazed and/or fertilised) constitute approximately 49 and 85 % of rural land use cover in England and Wales, respectively (Morton et al., 2011). Unlike arable agriculture, these land use types have been poorly investigated for their role in Nr loss through denitrification.

The major challenge in measuring ^{15}N-N_2 at near natural abundance levels is the possibility of interference at m/z 30 ($^{30}N_2$) due to the reaction of oxygen in the ion source with N and the formation of NO^+ ions that also have m/z 30 (Stevens et al., 1993). Commonly, this issue is addressed in continuous flow isotope ratio mass spectrometers (CF-IRMS) with the inclusion of a copper (Cu) oven for reducing O_2 in the gas sample (Russow et al., 1996). Recently, it has been suggested that the interference at m/z 30 can be further reduced by including a molecular sieve column in gas chromatograph IRMS (GC-IRMS) systems to not only separate N_2 and O_2 in the

BGD
12, 12653–12689, 2015

gas sample, but also to quantitatively remove O_2 and other trace gases such as carbon monoxide (Lewicka-Szczebak et al., 2013; Yang et al., 2014). We hypothesise that the precision for m/z 30 determination can be greatly improved by using a custom-built preparative unit for the removal of H_2O, CO_2, N_2O, NO^+ and CO; a device which also permits the micro scale injection of volumes of $< 5\,\mu L$. These injection volumes are much smaller than have previously been reported in the literature.

Studies that have directly compared the ^{15}N Gas-Flux method with the AIT in the field are rare and have exclusively focused on highly fertilised agro-ecosystems with moderate to low soil moisture contents (Aulakh et al., 1991; Mosier et al., 1986; Rolston et al., 1982). These studies have measured comparable denitrification rates by both field techniques, although the relatively low soil moisture contents have probably allowed greater diffusion of C_2H_2 to the anaerobic microsites where denitrification occurs (Malone et al., 1998), whilst the high nitrate application rates have probably favoured nitrate reduction over N_2O reduction (Dendooven and Anderson, 1995) resulting in high denitrification rates from the AIT. Conversely, laboratory studies have shown that the AIT significantly underestimates total denitrification compared to the ^{15}N tracer approach (Yu et al., 2010) and the direct N_2 flux approach (Qin et al., 2012) due to the incomplete inhibition of N_2O reduction to N_2 by C_2H_2 in wet soils (Yu et al., 2010) or in soils with low nitrate content, where N_2O reduction is more energetically favourable (Qin et al., 2013, 2014). A direct comparison of the ^{15}N Gas-Flux method with the AIT under in situ conditions across a range of natural and semi-natural terrestrial ecosystems has not been attempted before. It can provide valuable insights in terms of the validity and applicability of the two field techniques for measuring denitrification rates across broad spatial and temporal scales.

The objectives of the present study were: (1) to determine the precision and suitability of our preparative-IRMS instrumentation for measuring $^{15}N\text{-}N_2$ and $^{15}N\text{-}N_2O$ at low enrichment levels, (2) to adapt the ^{15}N Gas-Flux method for application across natural and semi-natural terrestrial ecosystems and (3) to directly compare the validity and ap-

BGD
12, 12653–12689, 2015

plicability of the ^{15}N Gas-Flux method with the AIT for measuring in situ denitrification rates.

2 Materials and methods

2.1 IRMS system

For N_2 gas isotopic analysis we used an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, UK, Wythenshawe) coupled to an in house built N_2 preparative interface (Fig. 1). Headspace gas (4 µL) was manually injected with a gas tight syringe (SGE Analytical science) into the preparative interface via an open split. Prior to its introduction into the IRMS, the sample was treated as follows: (a) dried by passing through $Mg(ClO_4)_2$ (Elemental Microanalysis Ltd, Devon, UK), (b) CO_2 removed with 0.7–1.2 mm Carbosorb (Elemental Microanalysis Ltd, Devon, UK), (c) N_2O cryogenically trapped under liquid nitrogen, and (d) O_2 removed over a copper-packed reduction furnace heated at 600 °C. The N_2 was then directed towards the triple collectors of the isotope ratio mass spectrometer where m/z 28, m/z 29 and m/z 30 mass ions were measured. Mass/charge ratios for the m/z 28, m/z 29 and m/z 30 nitrogen ($^{28}N_2$, $^{29}N_2$ and $^{30}N_2$) were recorded for each sample at a trap current of 300 µAmps. Instrument stability checks were performed prior to each analysis by running a series of 10 reference pulses of N_2 (BOC special gases) until a standard deviation of fit better than 0.05 ‰ was achieved. Additionally, 10 consecutive injections (4 µL) of atmospheric air were analysed prior to the analysis of actual samples. Precision of the instrument was better than 0.08 ‰ in all quality control tests.

Nitrous oxide was analysed using modified headspace methods described for the analysis of nitrogen gas above. Headspace gas (ca. 4 mL) was injected into a TraceGas™ Preconcentrator coupled to an Isoprime™ IRMS (GV instruments Ltd, UK) whereupon the sample was directed through a series of chemical traps designed to remove H_2O and CO_2. The N_2O was cryogenically trapped under liquid nitrogen.

BGD
12, 12653–12689, 2015

The waste was flushed out of the instrument. The N_2O was further cryofocused in a second liquid nitrogen trap prior to being introduced onto a 25 m × 0.32 mm Poraplot Q gas chromatography column (Chrompack column, Varian, Surrey, U.K). The column separated N_2O from any residual CO_2, and both entered the IRMS via an open split. The retention time between the first eluting CO_2 ($< 2 \times 10^{-10}$ amplitude) and second eluting N_2O peak typically fell in the range between 60–70 s to avoid isobaric interference of the CO_2 with the calculated ^{15}N. The N_2O was directed towards the triple collectors of the isotope ratio mass spectrometer where m/z 44, m/z 45 and m/z 46 mass ions were measured and recorded. Instrument stability checks were performed prior to each analysis by running a series of 10 reference pulses of N_2O (BOC special gases) until a standard deviation of fit better than 0.05 ‰ was achieved. Prior to each sample batch analysis, trace gas N_2O measurements were made on 3 mL × 100 mL flasks containing atmospheric air collected from outside the stable isotope laboratory. $\delta^{15}N$ precisions using the Trace gas Preconcentrator and Isoprime IRMS were better than 0.3 ‰ respectively at 600 µAmp trap current.

2.2 Field application of the ^{15}N Gas-Flux and AIT techniques

In situ measurements of N_2 and N_2O were made using static chambers according to the ^{15}N Gas-Flux method (Mosier and Klemedtsson, 1994). Five plots were randomly established in June 2013 in each of four study sites in the Ribble–Wyre River catchments (area 1145 km^2; NW England, 53°59′99″ N, 2°41′79″ W). The study sites were a heathland (R-HL), a deciduous woodland (R-DW), an unimproved grassland (R-UG) and an improved grassland (R-IG). In August 2013, four more study sites were tested in the Conwy River catchment (area 345 km^2; N. Wales, 52°59′82″ N, 3°46′06″ W) following a similar sampling design. These sites were an acid grassland (C-UG), an ombrotrophic peat bog (C-PB), a mixed deciduous and coniferous woodland (C-MW) and an improved grassland (C-IG). Further details on the location, land management status and major soil properties for all study sites can be found in Sgouridis and Ullah (2014).

BGD
12, 12653–12689, 2015

In each plot a round PVC collar (basal area 0.05 m^2; chamber volume 4 L) was inserted into the soil at c. 10 cm depth 2–4 weeks before the measurement date. The PVC collars were fitted with a circular groove of 25 mm depth to fit in an acrylic cylindrical cover (chamber) providing a gas-tight seal when filled with water (Ullah and Moore, 2011). The gas leak rate from the chamber was determined in the laboratory by placing the sealed collar and chamber over a tray of water, injecting CH_4 (10 ppm), and determining the change in CH_4 concentration within the chamber headspace over time (Yang et al., 2011). The CH_4 concentration change within 24 h was negligible with the relative standard deviation (RSD) being < 5 %. Due to the relatively small volume of the chamber's headspace there was no need for air circulation within the chamber or a vent for pressure equilibration (Mulvaney and Kurtz, 1984). Instead chambers were covered with reflective foil for minimising temperature increase within the chamber headspace during the incubation period (Ullah and Moore, 2011). Labelled $K^{15}NO_3^-$ (98 at. % ^{15}N, Sigma-Aldrich) was applied in each plot via multiple injections of equal volume through an equally-spaced grid using custom-made 10 cm long lumber needles attached to a plastic syringe (Ruetting et al., 2011). The ^{15}N tracer was delivered as the needle was pushed into the soil from the surface up to 10 cm depth aiming to achieve as uniform as possible labelling of the soil volume enclosed by the collar, as required by the ^{15}N gas flux method (Mosier and Klemedtsson, 1994). The volume and concentration of the labelled $K^{15}NO_3^-$ tracer solution was determined from measurements of soil nitrate and moisture content, as well as bulk density adjacent to each plot made during the installation of the collars (Morse and Bernhardt, 2013). Lower application rates (< 0.1 kg N ha^{-1}) were administered to natural study sites (e.g. peat bog, heathland) and higher rates (< 1 kg N ha^{-1}) administered to semi-natural (e.g. unimproved and improved grasslands). The tracer solution (50–250 mL) was adjusted within 5 % of the ambient volumetric water content.

Following the ^{15}N tracer application the collars were covered with the acrylic chamber fitted with a rubber septum for gas sampling. Two sets of gas samples (20 mL each) were collected with a gas tight syringe (SGE Analytical science) through the

BGD
12, 12653–12689, 2015

septum of the chamber cover at $T = 1$ h, $T = 2$ h and $T \approx 20$ h after the tracer injection, while a $T = 0$ h sample was collected immediately after tracer injection above the plot surface before fitting the chamber cover. The gas samples were transferred into pre-evacuated (< 100 Pa) 12 mL borosilicate glass vials with butyl rubber septa (Exetainer vial; Labco Ltd., High Wycombe, United Kingdom) for storage under positive pressure and were analysed within 8 weeks from collection without any significant change of the gas concentration (Laughlin and Stevens, 2003).

Adjacent to each PVC collar in each plot, two intact soil cores (50 mm I.D., 15 cm long) were extracted from 10 cm depth leaving the top 5 cm void as a headspace volume. The cores were capped on both ends with the top cap fitted with a rubber septum for gas sampling. One set of cores was amended with pure C_2H_2 with 5 mL injected through the septum directly in the middle of the soil core before 10 % of the headspace being also replaced with pure C_2H_2. The second set of cores was not amended with C_2H_2 and both cores were placed back in the ground where they came from. Gas samples (5 mL) were collected with a gas tight syringe (SGE Analytical science) through the septa of the cores at $T = 1$ h and $T = 2$ h after amendment with acetylene. The gas samples were transferred into pre-evacuated (< 100 Pa) 3 mL borosilicate glass vials with butyl rubber septa (Exetainer vial; Labco Ltd., High Wycombe, UK) for storage under positive pressure.

2.3 Flux calculations

The ^{15}N content of the N_2 in each 12 mL vial was determined using the IRMS system described above and the ratios R29 ($^{29}N_2/^{28}N_2$) and R30 ($^{30}N_2/^{28}N_2$) were measured in both enriched ($T = 1$, 2 and 20 h) and reference samples ($T = 0$ h). The inclusion of air reference standards between every 10 samples indicated an upward drift for R30 over time, potentially due to the formation of NO^+ in the ion source despite the inclusion of the Cu reduction step (Lewicka-Szczebak et al., 2013). Subsequently, every sample batch was drift corrected by fitting a linear regression through the air reference standards and calculating an offset correction for both R29 and R30 (Yang et al., 2014). The

BGD
12, 12653–12689, 2015

minimum detectable change (MDC) in R29 and R30 was defined with repeated manual analyses of air reference standards ($n = 10$) and was calculated using the following equation (Matson et al., 2009):

$$\mathrm{MDC} = \mu_{\text{pair diff}} + (2\sigma_{\text{pair diff}}) \quad (1)$$

where μ is the mean difference of all possible unique pairs of air reference standards ($n = 45$) and σ is the standard deviation between sample pairs. The MDC for R29 was 7.7×10^{-7} and for R30 was 6.1×10^{-7} and these values were used to determine if each time step sample was significantly different from ambient reference samples ($T = 0$ h), and if not they were excluded from the flux calculations.

For calculating the total N_2 flux from a uniformly labelled soil nitrate pool when both R29 and R30 are measured, the "non-equilibrium" equations were applied as described by Mulvaney (1984) for estimating first the ^{15}N fraction in the soil NO_3^- denitrifying pool ($^{15}X_N$) as:

$$^{15}X_N = 2(\Delta R30/\Delta R29)/(1 + 2(\Delta R30/\Delta R29)) \quad (2)$$

where ΔR29 and ΔR30 is the difference between R29 and R30 respectively between enriched ($T = 1$, 2 and 20 h) and reference samples ($T = 0$ h). Subsequently, the $^{15}X_N$ allows the quantification of the fraction of the N_2 evolved from the ^{15}N-labelled pool (d) using either the ΔR30 or the ΔR29:

$$d = \frac{\Delta R30}{\left(^{15}X_N\right)^2} \quad (3)$$

$$d = \frac{\Delta R29}{2\left(^{15}X_N\right)\left(1 - ^{15}X_N\right)^2} \quad (4)$$

Using d and the concentration of [N_2] (μg N) in the chamber headspace, the evolved N_2 from the soil pool was calculated:

$$\text{Evolved } N_2 = d[N_2]/(1 - d) \quad (5)$$

BGD
12, 12653–12689, 2015

The N_2 flux was then calculated using linear regression between the evolved N_2 and the incubation time per plot surface area and was expressed in $\mu g\,N\,m^{-2}\,h^{-1}$ representing the total N_2 flux from the mixture of the ^{15}N-labelled tracer and the soil N at natural abundance (Stevens and Laughlin, 1998).

The ^{15}N content of the N_2O in the same 12 mL vials as well as the ratios R45 ($^{45}N_2O/^{44}N_2O$) and R46 ($^{46}N_2O/^{44}N_2O$) were measured in both enriched (T = 1, 2 and 20 h) and reference samples (T = 0 h). The application of the non-equilibrium equations to N_2O is analogous to N_2 after correcting for the naturally occurring oxygen isotopes (Bergsma et al., 2001). Therefore, the ratios R45 and R46 were converted to ratios of R29 and R30 respectively by applying the following equations:

$$R29 = R45 - R17 \quad (6)$$

$$R30 = (R46 - (R29R17)) - R18 \quad (7)$$

where for R17 ($^{17}O/^{16}O$) the value 0.000373 was used and for R18 ($^{18}O/^{16}O$) the value 0.0020052 was used (Bergsma et al., 2001). There was no significant instrumental drift for the ratios R45 and R46 over time. The MDC was defined, for the converted R29 and R30, with repeated automatic analyses of 0.5 ppm N_2O standards (n = 15) as 3.4×10^{-5} and 2.9×10^{-5} respectively. The second set of gas samples collected at the same time in the field were analysed for bulk N_2O on a GC-μECD (7890A GC Agilent Technologies Ltd., Cheshire, UK) and the concentration of [N_2O] (μg N) was used in Eq. (5) to calculate the N_2O flux due to denitrification of the mixture of the ^{15}N-labelled tracer and the soil N and expressed in $\mu g\,N\text{-}N_2O\,m^{-2}\,h^{-1}$. Assuming that the N_2O originates from the same uniformly labelled pool as N_2, the $^{15}X_N$ from N_2O was used to estimate d for N_2 using either R30 (Eq. 3) or R29 (Eq. 4), thus lowering the limit of detection for N_2 (Stevens and Laughlin, 2001) and allowing measurement of N_2 gas flux from natural terrestrial ecosystems at low ^{15}N-tracer application rates.

Gas samples collected from the intact soil cores with or without acetylene amendment were analysed for N_2O on a GC-μECD (7890A GC Agilent Technologies Ltd.,

BGD
12, 12653–12689, 2015

Cheshire, UK) and for CO_2 on a GC-FID (7890A GC Agilent Technologies Ltd., Cheshire, UK) and flux rates were determined by linear regression between 0 and 2 h. The instrument precision was determined from repeated analyses of 6 ppm N_2O and 200 ppm CO_2 standards respectively (n = 8) and the RSD was < 1 %.

2.4 Statistical analysis

Using factor analysis on selected soil physico-chemical properties, the samples from the 8 field sites were ordinated in three broad land use types: organic soils (C-PB, C-UG, R-HL); forest soils (C-MW, R-DW) and grassland soils (C-IG, R-UG, R-IG) according to Sgouridis and Ullah (2014). All subsequent statistical analyses were performed on the broad land use types rather than individual field sites. The data were analysed for normality and homogeneity of variance with the Kolmogorov–Smirnov test and the Levene statistic respectively and logarithmic transformations were applied as necessary. One-Way ANOVA combined with the Hochberg's GT2 post hoc test for unequal sample sizes or the Games–Howell post hoc test for unequal variances was performed for comparing the variance of the means between land use types for all gas fluxes. Pearson correlation was used between log-transformed flux rates. Comparisons between the ^{15}N Gas-Flux and AIT techniques were made with independent samples t test. All statistical analyses were performed using SPSS® 21.0 for Windows (IBM Corp., 2012; Armonk, NY).

3 Results

3.1 IRMS system evaluation

The precision of the IRMS systems was evaluated using repeated analyses of ambient air samples for N_2 (n = 10) injected manually in one batch and repeated analyses of N_2O gas standard at natural abundance and 0.5 ppm concentration (n = 15) using

BGD
12, 12653–12689, 2015

automated injections. The mean measured ratios of R29 and R30 for N_2 and of R45 and R46 for N_2O are shown in Table 1. Measurement precision was defined as the coefficient of variation (%) and it was lower for R29 compared to R30 and lower for R45 compared to R46, but still less than 0.5 % for all four measured ratios. We estimated the ^{15}N atom% abundance for both gases as per Yang et al. (2014) and the precision was less than 0.01 % for N_2 in air and 0.26 % for standard N_2O at natural abundance. The mean measured R30 (5.16×10^{-5}) was higher than the theoretical value of 1.35×10^{-5} for N_2 in ambient air suggesting some interference at m/z 30 potentially due to the formation of NO^+ ions in the ion source of the mass spectrometer despite the inclusion of the Cu reduction oven. The contribution of NO^+ ions (R30 measured – R30 theoretical) was 3.81×10^{-5}, whilst the ratio of R30 theoretical/R30 measured was 0.26. Correcting the R30 ratio for the contribution of NO^+ ions results in a lower "true" precision for the R30 (CV = 1.67 %).

3.2 Field application of the ^{15}N Gas-Flux method

The ^{15}N tracer application rate ranged between 0.03 and 1 kg ^{15}N ha^{-1} or between 0.1 and 2.2 mg ^{15}N kg^{-1} dry soil and it was lower in the case of the organic soils and higher for the woodland and grassland soils (Table 2). The ^{15}N fraction in the denitrifying pool ($^{15}X_N$), as calculated from the measured isotopic ratios of the N_2O after 1 h of incubation using Eq. (2), ranged between 65 and 93 ^{15}N at. %. The average change of the $^{15}X_N$ with incubation time, indicated by the slope shown in Table 2, was not different from 0 in case of the organic (t test; $t = 0.520$, df = 18, $p > 0.05$) and grassland soils (t test; $t = 0.047$, df = 28, $p > 0.05$), whilst it was significantly below 0 for the woodland soils (t test; $t = 2.917$, df = 18, $p < 0.05$). Separating the woodland soils to C-WL and R-WL sites, only the former displayed a significant negative slope of $^{15}X_N$ with incubation time (t test; $t = 3.306$, df = 8, $p < 0.05$), suggesting that N_2O may be deriving from a second nitrate pool, possibly nitrate produced from the oxidation of NH_4^+ via nitrification, in the C-WL.

BGD
12, 12653–12689, 2015

The evolved N_2 in the chamber headspace increased linearly from 1 to 20 h of incubation in all three land use types (Fig. 2a). The increase was statistically significant after 20 h incubation in GL (ANOVA; $F = 19.8$, $p < 0.01$), whilst due to the high variability among plots, shown by the large error bars at 20 h incubation in Fig. 2a, it was not significant for the OS and WL soils. Similarly, the evolved N_2O also increased linearly between incubation time points (Fig. 2b), and the amount of N_2O accumulated after 20 h was significantly higher than in the previous time points for all land use types (ANOVA; $F_{OS} = 4.6$, $F_{WL} = 5.1$, $F_{GL} = 14.7$, $p < 0.05$). Therefore, N_2 and N_2O flux rates were estimated using linear regression (when $r^2 > 0.95$) between 1 and 20 h incubation using only those time points that were above the MDC values estimated for each gas.

The N_2 flux ranged between 2.4 and 416.6 $\mu g\,N\,m^{-2}\,h^{-1}$ and was significantly different among land use types (Table 3) with the grassland soils showing on average 3 and 14 times higher denitrification rates than the woodland and organic soils respectively (Fig. 3a). A similar pattern was observed for the N_2O flux due to denitrification (range: 0.003–20.8 $\mu g\,N\,m^{-2}\,h^{-1}$) with the grassland soils emitting on average 14 and 120 times more N_2O than the woodland and organic soils respectively (Fig. 3b), whilst the N_2O flux was on average 20 to 200 times lower than the N_2 flux among land use types. Consequently, the denitrification product ratio ($N_2O/N_2 + N_2O$) was low, ranging between 0.03 and 13 % and was highest in the GL and similar between the WL and OS (Fig. 3c).

3.3 Comparison with the AIT

The total denitrification rate measured from the C_2H_2 amended intact soil cores in the same land use types ranged between 0.5 and 325.2 $\mu g\,N\,m^{-2}\,h^{-1}$ and correlated positively with the total denitrification rate (N_2 and N_2O fluxes combined) measured with the ^{15}N Gas-Flux method (Pearson; $r = 0.581$, $n = 25$, $p < 0.01$) following a similar trend among land use types, albeit only the OS being significantly lower than the grassland

BGD
12, 12653–12689, 2015

and woodland soils (Table 3). The AIT denitrification rates were between 3 and 5 times lower than the total denitrification from the ^{15}N Gas-Flux (Fig. 4a) with the difference being significant in woodland (t test; $t = 3.914$, df = 18, $p < 0.01$) and grassland soils (t test; $t = 3.521$, df = 25, $p < 0.01$).

The bulk N_2O flux measured from the un-amended intact soil cores ranged between 0.15 and 86.6 $\mu g\,N\,m^{-2}\,h^{-1}$ and was between 1 and 3 times lower than the total denitrification rate from the C_2H_2 amended cores. There were no significant differences between bulk N_2O fluxes measured with the static chambers and the un-amended intact soil cores (Fig. 4b), which indicated that total N_2O emissions were comparable between the two field techniques. Consequently, estimating the denitrification product ratio from the un-amended and C_2H_2 amended intact soil cores resulted in significantly higher ratios compared to the ^{15}N Gas-Flux approach (Fig. 4c), which were on average between 50 and 60 % and not significantly different among land use types (Table 3).

The mean CO_2 production rate was similar irrespective of whether it was measured in static chambers, in C_2H_2 amended or un-amended intact soil cores (Fig. 5), indicating that soil respiration (including both microbial and plant respiration) was not affected by the measurement technique.

4 Discussion

4.1 IRMS system evaluation

The precision of our trace gas isotope ratio mass spectrometer (TG-IRMS) for manual analysis of ^{15}N-N_2 in gas samples was comparable for both R29 and R30 ratios to the recently developed gas chromatograph-IRMS (GC-IRMS) systems that included a combination of a copper reduction oven and a molecular sieve (Lewicka-Szczebak et al., 2013) or only a molecular sieve (Yang et al., 2014) for the removal of O_2 from the samples. In fact, injecting only a trace amount of headspace gas sample (4 µL), which is less than half of what is used by Lewicka-Szczebak et al. (2013) and ten times less

BGD
12, 12653–12689, 2015

than the required sample volume by Yang et al. (2014), we have reduced the interference at m/z 30 by NO^+ ions by an order of magnitude (3.81×10^{-5}) compared to the value (1.6×10^{-4}) reported by Lewicka-Szczebak et al. (2013). Consequently, correcting the R30 ratio for the NO^+ ions interference led to a CV value of < 2 %, which was significantly lower than the precision reported for natural abundance samples in previous studies (Lewicka-Szczebak et al., 2013; Russow et al., 1996; Stevens et al., 1993), thus constituting a significant improvement in m/z 30 determination in N_2 gas samples with low ^{15}N enrichment. However, the correction of the R30 ratio is only useful for estimating the "true" instrument precision for m/z 30 and is not necessary for calculating N_2 fluxes as shown by Lewicka-Szczebak et al. (2013).

The TraceGas™ Preconcentrator IRMS system used for ^{15}N-N_2O analysis displayed similar precision for the determination of R45 and R46 in standard N_2O gas at circa ambient concentration to a similar system used by Bergsma et al. (2001), while injecting only 4 mL of gas sample as opposed to 0.5 L used by Bergsma et al. (2001). When expressed in delta values ($\delta^{15}N$), the precision of our system was better than 0.05 ‰, which is significantly better than the respective precisions reported in Lewicka-Szczebak et al. (2013) and Yang et al. (2014). Therefore, the improved analytical precision achieved for both ^{15}N-N_2 and ^{15}N-N_2O analyses using smaller sample volumes than previously reported, allowed us to quantify in situ N_2 and N_2O fluxes with low ^{15}N enrichment under field conditions, which was previously not possible.

4.2 Field application of the ^{15}N Gas-Flux method

The minimum detectable N_2 and N_2O fluxes depend on the precision of the IRMS systems, the soil NO_3^- pool enrichment and the incubation parameters, such as the dimensions of the static chamber and the incubation time (Bergsma et al., 2001; Stevens and Laughlin, 2001). For our chamber design, an incubation time of 20 h, and using the estimated MDC values (for both N_2 and N_2O) for calculating a $^{15}X_N$ value of 0.6, the minimum detectable flux rates were 4 $\mu g\,N\,m^{-2}\,h^{-1}$ and 0.2 $ng\,N\,m^{-2}\,h^{-1}$

BGD
12, 12653–12689, 2015

for the N_2 and N_2O fluxes respectively. These were significantly better than the minimum rates (175–900 $\mu g\,N_2\text{-}N\,m^{-2}\,h^{-1}$ and 0.04–0.21 $\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$) reported by Bergsma et al. (2001) and Kulkarni et al. (2014), using similar field ^{15}N tracer approaches, and comparable to the minimum rates measured by a high precision ^{15}N gas flux approach in a laboratory soil incubation (Yang et al., 2014) and the gas-flow soil core method (8 $\mu g\,N_2\text{-}N\,m^{-2}\,h^{-1}$ and < 1 $\mu g\,N_2O\text{-}N\,m^{-2}\,h^{-1}$) by Wang et al. (2011). We have managed to further lower the limit of detection for N_2 and N_2O fluxes due to the high precision of our preparative devices coupled to the IRMS systems, but also by lowering the volume to surface area ratio of our chambers from 16 : 1 to 8 : 1 and by extending the incubation time to approximately 20 h. Most studies using ^{15}N tracers and static chambers in highly fertilised systems typically deploy their chambers between 1 and 2 h (Baily et al., 2012; Cuhel et al., 2010), but it has been shown that longer incubation periods (up to 24 or 48 h) may be needed in case of low ^{15}N enrichment applications in intact soil cores (Morse and Bernhardt, 2013) and laboratory incubations (Yang et al., 2014) for a more precise and accurate detectable $^{15}N\text{-}N_2$ signal. Moreover, an extended incubation period with closed chambers aggregates any diurnal fluctuations of denitrification activity (Aulakh et al., 1991) providing a more accurate daily flux rate. We have demonstrated that the N_2 flux and more importantly the N_2O flux increased linearly with time through the 20 h incubation period, probably as a result of a slow N_2O diffusion rate due to the high water filled pore space (WFPS) (Jury et al., 1982) in our land use types (range: 60–70 %, apart from the C-WL site with an average WFPS of 42 %), and were therefore confident in estimating N_2 and N_2O flux rates by linear regression across the incubation period.

The average ^{15}N tracer application rate (0.04–0.5 $kg\,^{15}N\,ha^{-1}$ or 0.4–1.2 $mg\,^{15}N\,kg^{-1}$ dry soil) across land use types was one to two orders of magnitude lower than previous applications of the ^{15}N Gas-Flux method in highly fertilised agricultural systems (Baily et al., 2012; Bergsma et al., 2001; Cuhel et al., 2010; Graham et al., 2013). For the organic soils, the average tracer application rate reflected the current estimates of daily atmospheric N deposition (0.05 $kg\,N\,ha^{-1}\,d^{-1}$) in the UK (~ 15–20 $kg\,N\,ha^{-1}\,y^{-1}$) (Dore

BGD
12, 12653–12689, 2015

et al., 2012; Payne, 2014), whilst for the grassland soils the tracer application mimicked a daily fertiliser application rate of 0.5 kg N ha^{-1} d^{-1}. Due to the inclusion of the N-rich C-WL site in the woodland soils, tracer application rates were higher than the daily atmospheric N deposition rates to reflect internal N cycling processes (e.g. nitrification) as an additional source of nitrate in these well-drained forest soils.

The major assumptions of the ^{15}N Gas-Flux method and the associated "non-equilibrium equations" are that the denitrifying soil NO_3^- pool is uniformly labelled with ^{15}N and that the N_2 and N_2O originate from the same denitrifying pool (Stevens and Laughlin, 1998). The ^{15}N fraction in the denitrifying pool ($^{15}X_N$), calculated non-destructively from the measured isotope ratios, ranged between 65 and 93 % and was well above the 10 % threshold for the correct application of the "non-equilibrium equations" (Lewicka-Szczebak et al., 2013). However, the calculated $^{15}X_N$ was higher than the estimated soil NO_3^- pool enrichment (range: 13–40 %) suggesting only partial mixing of the added tracer (98 ^{15}N at. %) with the ambient soil nitrate at natural abundance despite the elaborate effort for uniform tracer application with multiple injections across 10 cm soil depth (Ruetting et al., 2011). We did not sample the soil within the chamber collars for directly estimating the $^{15}NO_3^-$ content of the soil pool due to time and budget constraints. However, in cases where destructive soil sampling was used to measure the soil nitrate pool enrichment (Kulkarni et al., 2014), the results were significantly different from the estimated enrichment due to sampling bias of the volume of soil affected by the tracer application. Non-uniform mixing of the ^{15}N label may lead to overestimation of the $^{15}X_N$ and underestimation of the denitrification flux rates (Boast et al., 1988). However, it is unlikely under field conditions to achieve complete mixing of the added tracer with the ambient nitrate; and experimental studies (Mulvaney, 1988; Mulvaney and Van den Heuvel, 1988) have shown that the error is well-constrained and that accurate measurements can be made even with a less-uniformly labelled denitrifying pool. The non-significant change of $^{15}X_N$ with incubation time suggested only one denitrifying pool for both N_2 and N_2O, assuming negligible N_2 production from anammox and co-denitrification (Spott and Stange, 2007), thus permitting the use of the $^{15}X_N$,

BGD
12, 12653–12689, 2015

calculated from the N_2O isotope ratios, for calculating N_2 flux rates using the more reliable R30 measurements (Stevens and Laughlin, 2001). Only in the case of the C-WL well-drained forest site, shown to exhibit the highest nitrification potential (Sgouridis and Ullah, 2014), the slope of $^{15}X_N$ with time was negative suggesting a second co-occurring nitrifying pool for N_2O production. It is therefore possible that N_2 flux rates may be overestimated in C-WL, due to the underestimation of the $^{15}X_N$ from the N_2O data, but Bergsma et al. (1999) showed that temporal changes of the soil NO_3^- pool enrichment are negligible at ^{15}N enrichment levels similar to ours.

We were able to measure appreciable in situ fluxes of both N_2 and N_2O due to denitrification in all three land use types. Our N_2 fluxes from woodland soils compare well with the rates reported in the literature for restored forested wetlands in North America (Morse and Bernhardt, 2013) and with the rates from northern hardwood forests in US (Kulkarni et al., 2014), using ^{15}N tracers at similar application rates to ours. Our results are also comparable to the rates reported from central European forests, under similar atmospheric N deposition rates, using the gas-flow soil core method (Butterbach-Bahl et al., 2002). For the grassland soils, the N_2 fluxes measured in the present study were significantly lower than previous applications of the ^{15}N Gas-Flux method at high fertiliser application rates (Baily et al., 2012; Cuhel et al., 2010; Graham et al., 2013), whilst for the organic soils a comparison with the literature was not possible due to the lack of experimental data on N_2 fluxes. The N_2O fluxes were up to 200 times lower than the N_2 fluxes leading to low denitrification product ratios in all land use types, a result which is in line with the N_2O yields reported from ^{15}N tracer studies in forest (Kulkarni et al., 2014; Morse and Bernhardt, 2013) and grassland soils (Baily et al., 2012; Bergsma et al., 2001).

The significantly higher denitrification rates in grassland soils, compared to organic and forest soils, observed under this study could be supported through the additional supply of reactive nitrogen via fertilisation and the additional inputs of organic C and N through grazing (Cowan et al., 2015; Rafique et al., 2012, van Beek et al., 2010). A relatively high denitrification activity in poorly drained forest soils is usually sustained

BGD
12, 12653–12689, 2015

by the high soil water filled pore space (WFPS) (Liu et al., 2013), while it may be limited by low nitrification rates necessary for supplying the electron acceptors (nitrate) for denitrification to occur (Sgouridis and Ullah, 2014). Conversely, well-drained forest soils with organic-N rich leaf litter may display high nitrification rates, but denitrification activity is often limited by the low WFPS (Sgouridis and Ullah, 2014). Organic soils are naturally nutrient limited and their denitrification potential has been shown to be primarily limited by the availability of nitrate (Francez et al., 2011; Hayden and Ross, 2005; Sgouridis and Ullah, 2014). It should be noted that N_2 fluxes below the detection limit of $4\,\mu g\,N\,m^{-2}\,h^{-1}$ were not used in denitrification rate calculations and this might have led to slight overestimation of rates, particularly for the OS. It is beyond the scope of this paper to discuss in detail the differences in denitrification rates between land use types. The variation of denitrification across natural and semi-natural land use types has been investigated in these sites over two years using the adapted ^{15}N Gas-Flux method and the results are presented in a separate publication (Sgouridis & Ullah, submitted).

4.3 Comparison with the AIT

The total denitrification rates measured with the C_2H_2 amended intact soil cores followed the same trend as the total denitrification (N_2 and N_2O fluxes combined) from the ^{15}N Gas-Flux measurements, while they were on average 168 times lower than the denitrification potential measured in the same land use types in anaerobic soil slurries amended with acetylene and nitrate in a previous study (Sgouridis and Ullah, 2014), thus reflecting lower in situ rates. However, the AIT denitrification rates were between 3 and 5 times lower than the ^{15}N Gas-Flux rates, which was an indication that discrepancies due to overestimation of the soil NO_3^- pool ^{15}N enrichment most likely have not led to serious underestimation of the denitrification rates using the "non-equilibrium equations". Previous comparisons between the AIT and the ^{15}N tracer method in field studies showed no significant difference between the two methods in measuring in situ total

BGD
12, 12653–12689, 2015

denitrification rates when tracer is applied at high fertilisation rates (50–200 kg N ha^{-1}) and relatively low soil moisture contents (WFPS: 40–60 %) (Aulakh et al., 1991; Mosier et al., 1986). Conversely, in laboratory incubations it was shown that the AIT significantly underestimated total denitrification compared to the ^{15}N tracer approach (Yu et al., 2010) and the direct N_2 flux approach (Qin et al., 2012) due to the incomplete inhibition of N_2O reduction to N_2 by C_2H_2 in wet soils (Yu et al., 2010) or in soils with low nitrate content (Qin et al., 2013, 2014). In our study, the soil WFPS ranged between 60 and 70 % in all land use types, with the exception of the C-WL site, whilst ^{15}N-NO_3^- tracer was not added in the intact soil cores with or without acetylene treatment. Adding nitrate to the C_2H_2 amended cores would have been desirable for evaluating directly the priming effect of the added substrate on denitrification rates. However, the lack of tracer addition in the AIT treatments is unlikely to be the cause of underestimation of denitrification as the bulk N_2O flux (including all possible N_2O sources) was not different between the ^{15}N Gas-Flux chamber and the no-C_2H_2 amended intact soil core measurements. If the ^{15}N tracer addition in the static chambers, even at such low rate (< 1 kg ^{15}N ha^{-1}), were to stimulate the denitrification activity, this might have been reflected through high bulk N_2O flux from the chamber compared to the intact cores. Moreover, the disturbance of the soil structure during the extraction of the soil cores and the effect of the acetylene addition to microbial activity were not significant as it was suggested by the similar CO_2 production rates (Aulakh et al., 1991), representing soil respiration (Felber et al., 2012), in the static chambers and the C_2H_2 amended and un-amended intact soil cores. Therefore, we conclude that the AIT underestimated total denitrification rates compared to the ^{15}N Gas-Flux method due to the likely incomplete inhibition of N_2O reduction to N_2 under relatively high soil moisture contents, although the shorter incubation time (2 h for the intact cores) may have limited the ability of C_2H_2 to fully equilibrate within soil pore spaces. Consequently, the estimation of the denitrification product ratio from the un-amended cores (N_2O only) and the C_2H_2 amended cores ($N_2 + N_2O$) is grossly overestimated (Butterbach-Bahl et al., 2013) since the source of N_2O cannot be discriminated with the AIT, whilst the

BGD
12, 12653–12689, 2015

N_2 flux is underestimated due to the incomplete inhibition of N_2O reduction. Therefore, the much lower denitrification product ratio estimated from the ^{15}N Gas-Flux measurements is significantly more reliable and the wider application of this field technique across a range of land use types can have important implications for evaluating the role of denitrification as a reactive nitrogen sink and as a source of N_2O emissions (Butterbach-Bahl et al., 2013; Kulkarni et al., 2008).

5 Conclusions

The analytical precision for both ^{15}N-N_2 and ^{15}N-N_2O analyses was greatly improved by using smaller sample volumes than previously reported, thus allowing us to quantify in situ N_2 and N_2O fluxes with low ^{15}N enrichment under field conditions, which was previously not possible. The ^{15}N Gas-Flux method was applied for the first time across a range of natural and semi-natural land use types at ^{15}N tracer application rates mimicking current estimates of atmospheric N deposition (natural systems) or grassland fertiliser application rates and yielded analytically valid flux rates for both N_2 and N_2O in all the land use types. A possible limitation of the ^{15}N Gas-Flux method when applied at low ^{15}N enrichment levels is the uncertainty associated with the estimation of the soil NO_3^- pool enrichment that may result in the underestimation of denitrification rates. However, the direct field comparison of the ^{15}N Gas-Flux method with the AIT suggested that the AIT further underestimates in situ denitrification rates most likely due to the incomplete inhibition of N_2O reduction to N_2 under relatively high soil moisture contents. Consequently, the ^{15}N Gas-Flux method, as applied in our study, constitutes a more reliable field technique for measuring in situ denitrification rates across a range of terrestrial ecosystems and can significantly improve our understanding of the role of denitrification as a reactive nitrogen sink.

Acknowledgements. The authors are grateful to Edward Ritchie and Richard Rhodes for granting us permission to access their land, as well as the National Trust in Conwy, the Abbeystead

BGD
12, 12653–12689, 2015

Estate in the Trough of Bowland and the Forestry Commission in Gisburn Forest for their guidance and advice. We are also thankful to Ravindi Wanniarachchige at Keele University for her help during field sampling and laboratory analysis. This research was funded by the UK Natural Environment Research Council grant (NE/J011541/1) awarded to Keele University and supported by a "grant in kind" from the NERC Life Sciences Mass Spectrometry Facility Steering Committee.

References

Aulakh, M., Doran, J., and Mosier, A.: Field-evaluation of 4 methods for measuring denitrification, Soil Sci. Soc. Am. J., 55, 1332–1338, 1991.

Baily, A., Watson, C. J., Laughlin, R., Matthews, D., McGeough, K., and Jordan, P.: Use of the ^{15}N gas flux method to measure the source and level of N_2O and N-2 emissions from grazed grassland, Nutr. Cycl. Agroecosys., 94, 287–298, 2012.

Bergsma, T., Bergsma, Q., Ostrom, N., and Robertson, G.: A heuristic model for the calculation of dinitogen and nitrous oxide flux from nitrogen-15-labeled soil, Soil Sci. Soc. Am. J., 63, 1709–1716, 1999.

Bergsma, T., Ostrom, N., Emmons, M., and Robertson, G.: Measuring simultaneous fluxes from soil of N(2)O and N(2) in the field using the (15)N-Gas "nonequilihrium" technique, Environ. Sci. Technol., 35, 4307–4312, 2001.

Boast, C., Mulvaney, R., and Baveye, P.: Evaluation of N-15 tracer techniques for direct measurement of denitrification in soil, 1. Theory, Soil Sci. Soc. Am. J., 52, 1317–1322, 1988.

Burgin, A. J. and Groffman, P. M.: Soil O-2 controls denitrification rates and N_2O yield in a riparian wetland, J. Geophys. Res.-Biogeo., 117, G01010, doi:10.1029/2011JG001799, 2012.

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.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S.: Nitrous oxide emissions from soils: how well do we understand the processes and their controls?, Philos. T. R. Soc. B, 368, doi:10.1098/rstb.2013.0122, 2013.

Cowan, N. J., Norman, P., Famulari, D., Levy, P. E., Reay, D. S., and Skiba, U. M.: Spatial variability and hotspots of soil N_2O fluxes from intensively grazed grassland, Biogeosciences, 12, 1585–1596, doi:10.5194/bg-12-1585-2015, 2015.

BGD
12, 12653–12689, 2015

Cuhel, J., Simek, M., Laughlin, R. J., Bru, D., Cheneby, D., Watson, C. J., and Philippot, L.: Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity, Appl. Environ. Microb., 76, 1870–1878, 2010.
Dendooven, L. and Anderson, J.: Maintenance of denitrification potential in pasture soil following anaerobic events, Soil Biol. Biochem., 27, 1251–1260, 1995.
Dore, A. J., Kryza, M., Hall, J. R., Hallsworth, S., Keller, V. J. D., Vieno, M., and Sutton, M. A.: The influence of model grid resolution on estimation of national scale nitrogen deposition and exceedance of critical loads, Biogeosciences, 9, 1597–1609, doi:10.5194/bg-9-1597-2012, 2012.
Felber, R., Conen, F., Flechard, C. R., and Neftel, A.: Theoretical and practical limitations of the acetylene inhibition technique to determine total denitrification losses, Biogeosciences, 9, 4125–4138, doi:10.5194/bg-9-4125-2012, 2012.
Francez, A., Pinay, G., Josselin, N., and Williams, B. L.: Denitrification triggered by nitrogen addition in Sphagnum magellanicum peat, Biogeochemistry, 106, 435–441, 2011.
Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S., and Sutton, M. A.: Transformation of the nitrogen cycle: recent trends, questions and potential solutions, Science, 320, 889–892, 2008.
Graham, C. J., van Es, H. M., and Melkonian, J. J.: Nitrous oxide emissions are greater in silt loam soils with a legacy of manure application than without, Biol. Fert. Soils, 49, 1123–1129, 2013.
Groffman, P.: Terrestrial denitrification: challenges and opportunities, Ecological Processes, 1, 11, 2012.
Groffman, P. M., Altabet, M. A., Bohlke, 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.
Harms, T. K. and Jones Jr., J. B.: Thaw depth determines reaction and transport of inorganic nitrogen in valley bottom permafrost soils, Glob. Change Biol., 18, 2958–2968, 2012.
Hayden, M. and Ross, D.: Denitrification as a nitrogen removal mechanism in a Vermont peatland, J. Environ. Qual., 34, 2052–2061, 2005.
Jury, W., Letey, J., and Collins, T.: Analysis of chamber methods used for measuring nitrous-oxide production in the field, Soil Sci. Soc. Am. J., 46, 250–256, 1982.
Kulkarni, M. V., Groffman, P. M., and Yavitt, J. B.: Solving the global nitrogen problem: it's a gas!, Front. Ecol. Environ., 6, 199–206, 2008.

BGD
12, 12653–12689, 2015

Kulkarni, M. V., Burgin, A. J., Groffman, P. M., and Yavitt, J. B.: Direct flux and N-15 tracer methods for measuring denitrification in forest soils, Biogeochemistry, 117, 359–373, 2014.
Laughlin, R. J. and Stevens, R. J.: Changes in composition of nitrogen-15-labeled gases during storage in septum-capped vials, Soil Sci. Soc. Am. J., 67, 540–543, 2003.
Lewicka-Szczebak, D., Well, R., Giesemann, A., Rohe, L., and Wolf, U.: An enhanced technique for automated determination of N-15 signatures of N_2, ($N_2 + N_2O$) and N_2O in gas samples, Rapid Commun. Mass Sp., 27, 1548–1558, 2013.
Limpens, J., Berendse, F., and Klees, H.: N deposition affects N availability in interstitial water, growth of Sphagnum and invasion of vascular plants in bog vegetation, New Phytol., 157, 339–347, 2003.
Liu, X., Chen, C. R., Wang, W. J., Hughes, J. M., Lewis, T., Hou, E. Q., and Shen, J.: Soil environmental factors rather than denitrification gene abundance control N_2O fluxes in a wet sclerophyll forest with different burning frequency, Soil Biol. Biochem., 57, 292–300, 2013.
Malone, J., Stevens, R., and Laughlin, R.: Combining the N-15 and acetylene inhibition techniques to examine the effect of acetylene on denitrification, Soil Biol. Biochem., 30, 31–37, 1998.
Matson, A., Pennock, D., and Bedard-Haughn, A.: Methane and nitrous oxide emissions from mature forest stands in the boreal forest, Saskatchewan, Canada, Forest Ecol. Manag., 258, 1073–1083, 2009.
Mills, R. T. E., Tipping, E., Bryant, C. L., and Emmett, B. A.: Long-term organic carbon turnover rates in natural and semi-natural topsoils, Biogeochemistry, 118, 257–272, 2013.
Morse, J. L. and Bernhardt, E. S.: Using N-15 tracers to estimate N_2O and N_2 emissions from nitrification and denitrification in coastal plain wetlands under contrasting land-uses, Soil Biol. Biochem., 57, 635–643, 2013.
Morse, J. L., Duran, J., Beall, F., Enanga, E. M., Creed, I. F., Fernandez, I., and Groffman, P. M.: Soil denitrification fluxes from three northeastern North American forests across a range of nitrogen deposition, Oecologia, 177, 17–27, 2015.
Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Wadsworth, R., and Simpson, I. C.: Final Report for LCM2007 – the New UK Land Cover Map, Centre for Ecology & Hydrology, CS Technical Report 11/07, 112 pp., UK, 2011.
Mosier, A. R. and Klemedtsson, L.: Measuring denitrification in the field, in: Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties, edited by: Weaver, R. W., An-

BGD
12, 12653–12689, 2015

gle, J. S., and Bottomley, P. S., Soil Science Society of America, Inc., Wisconsin, USA, 1047–1065, 1994.
Mosier, A., Guenzi, W., and Schweizer, E.: Field denitrification estimation by N-15 and acetylene inhibition techniques, Soil Sci. Soc. Am. J., 50, 831–833, 1986.
Mulvaney, R. L.: Determination of 15N-labeled dinitrogen and nitrous oxide with triple-collector mass spectrometers, Soil Sci. Soc. Am. J., 48, 690–692, 1984.
Mulvaney, R.: Evaluation of N-15 tracer techniques for direct measurement of denitrification in soil, 3. Laboratory studies, Soil Sci. Soc. Am. J., 52, 1327–1332, 1988.
Mulvaney, R. and Kurtz, L.: Evolution of dinitrogen and nitrous-oxide from N-15 fertilized soil cores subjected to wetting and drying cycles, Soil Sci. Soc. Am. J., 48, 596–602, 1984.
Mulvaney, R. and Van den Heuvel, R.: Evaluation of N-15 tracer techniques for direct measurement of denitrification in soil, 4. Field studies, Soil Sci. Soc. Am. J., 52, 1332–1337, 1988.
Payne, R. J.: The exposure of British peatlands to nitrogen deposition, 1900–2030, Mires and Peat, 14, 1–9, 2014.
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.
Qin, S., Yuan, H., Dong, W., Hu, C., Oenema, O., and Zhang, Y.: Relationship between soil properties and the bias of N_2O reduction by acetylene inhibition technique for analyzing soil denitrification potential, Soil Biol. Biochem., 66, 182–187, 2013.
Qin, S., Yuan, H., Hu, C., Oenema, O., Zhang, Y., and Li, X.: Determination of potential N_2O-reductase activity in soil, Soil Biol. Biochem., 70, 205–210, 2014.
Rafique, R., Anex, R., Hennessy, D., and Kiely, G.: What are the impacts of grazing and cutting events on the N_2O dynamics in humid temperate grassland?, Geoderma, 181–182, 36–44, 2012.
Ravishankara, A. R., Daniel, J. S., and Portmann, R. W.: Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century, Science, 326, 123–125, 2009.
Rolston, D., Sharpley, A., Toy, D., and Broadbent, F.: Field measurement of denitrification, 3. Rates during irrigation cycles, Soil Sci. Soc. Am. J., 46, 289–296, 1982.
Ruetting, T., Huygens, D., Staelens, J., Mueller, C., and Boeckx, P.: Advances in N-15-tracing experiments: new labelling and data analysis approaches, Biochem. Soc. T., 39, 279–283, 2011.

BGD
12, 12653–12689, 2015

Russow, R., Stevens, R., and Laughlin, R.: Accuracy and precision for measurements of the mass ratio 30/28 in dinitrogen from air samples and its application to the investigation of N losses from soil by denitrification, Isotopes Environ. Health Stud., 32, 289–297, 1996.

Sanders, I. A. and Trimmer, M.: In situ application of the $^{15}NO_3^-$ isotope pairing technique to measure denitrification in sediments at the surface water–groundwater interface, Limnol. Oceanogr., 4, 142–152, 2006.

Scholefield, D., Hawkins, J., and Jackson, S.: 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.

Sgouridis, F. and Ullah, S.: Denitrification potential of organic, forest and grassland soils in the Ribble-Wyre and Conwy River catchments, UK, Environ. Sci. -Process Impacts, 16, 1551–1562, 2014.

Spott, O. and Stange, C. F.: A new mathematical approach for calculating the contribution of anammox, denitrification and atmosphere to an N_2 mixture based on a ^{15}N tracer technique, Rapid Commun. Mass Sp., 21, 2398–2406, 2007.

Stevens, R. and Laughlin, R.: Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils, Nutr. Cycl. Agroecosys., 52, 131–139, 1998.

Stevens, R. J. and Laughlin, R. J.: Lowering the detection limit for dinitrogen using the enrichment of nitrous oxide, Soil Biol. Biochem., 33, 1287–1289, 2001.

Stevens, R. J., Laughlin, R. J., Atkins, G. J., and Prosser, S. J.: Automated determination of ^{15}N-labeled dinitrogen and nitrous oxide by mass spectrometry, Soil Sci. Soc. Am. J., 57, 981–988, 1993.

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.

Ullah, S. and Moore, T. R.: Biogeochemical controls on methane, nitrous oxide, and carbon dioxide fluxes from deciduous forest soils in eastern Canada, J. Geophys. Res.-Biogeo., 116, G03010, doi:10.1029/2010JG001525, 2011.

van Beek, C. L., Pleijter, M., Jacobs, C. M. J., Velthof, G. L., van Groenigen, J. W., and Kuikman, P. J.: Emissions of N_2O from fertilized and grazed grassland on organic soil in relation to groundwater level, Nutr. Cycl. Agroecosys., 86, 331–340, 2010.

BGD
12, 12653–12689, 2015

Wang, R., Willibald, G., Feng, Q., Zheng, X., Liao, T., Brueggemann, N., and Butterbach-Bahl, K.: Measurement of N_2, N_2O, NO, and CO_2 emissions from soil with the gas-row-soil-core technique, Environ. Sci. Technol., 45, 6066–6072, 2011.

Whitmire, S. L. and Hamilton, S. K.: Rapid removal of nitrate and sulfate in freshwater wetland sediments, J. Environ. Qual., 34, 2062–2071, 2005.

Yang, W. H., Teh, Y. A., and Silver, W. L.: A test of a field-based N-15-nitrous oxide pool dilution technique to measure gross N_2O production in soil, Glob. Change Biol., 17, 3577–3588, 2011.

Yang, W. H., McDowell, A. C., Brooks, P. D., and Silver, W. L.: New high precision approach for measuring N-15-N_2 gas fluxes from terrestrial ecosystems, Soil Biol. Biochem., 69, 234–241, 2014.

Yu, K., Seo, D., and DeLaune, R. D.: Incomplete acetylene inhibition of nitrous oxide reduction in potential denitrification assay as revealed by using 15N-nitrate tracer, Commun. Soil Sci. Plan., 41, 2201–2210, 2010.

BGD
12, 12653–12689, 2015

Table 1. Measured ratios of R29 and R30 for N_2 in ambient air (n = 10), ratios of R45 and R46 in standard N_2O gas (0.5 ppm concentration, n = 15) and ^{15}N at. % abundance calculated from the respective ratios for both gases. SD; standard deviation, CV; coefficient of variation.

	R29 (N_2)	R30 (N_2)	R45 (N_2O)	R46 (N_2O)	^{15}N at. % (N_2)	^{15}N at. % (N_2O)
Mean	$7.38\ 10^{-3}$	$5.16\ 10^{-5}$	$8.00\ 10^{-3}$	$2.21\ 10^{-3}$	$3.71\ 10^{-1}$	$3.88\ 10^{-1}$
SD	$2.77\ 10^{-7}$	$2.26\ 10^{-7}$	$1.25\ 10^{-5}$	$1.04\ 10^{-5}$	$2.09\ 10^{-5}$	$1.01\ 10^{-3}$
CV (%)	0.00	0.44	0.16	0.47	0.01	0.26

BGD
12, 12653–12689, 2015

Table 2. The areal application rate of the ^{15}N tracer, the calculated $^{15}X_N$ value from N_2O and the slope of the $^{15}X_N$ change with incubation time in the three land use types. Data are means with standard errors in parentheses.

Land Use Type	Application rate ($kg\,^{15}N\,ha^{-1}$)	$^{15}X_N$ (%)	$^{15}X_N$ slope
Organic Soil (n = 3)	0.04 (0.015)	90 (1.5)	0.003 (0.0054)
Woodland (n = 2)	0.62 (0.406)	79 (8.3)	−0.007 (0.0025)
Grassland (n = 3)	0.51 (0.190)	81 (8.4)	0.000 (0.0037)

BGD
12, 12653–12689, 2015

Table 3. Comparison of mean flux rates and ratios between land use types for the two field methods using One-Way ANOVA. All variables are log-transformed. F; F statistic, P; probability level.

^{15}N Gas-Flux	F	P
Denitrification	19.4	< 0.001
N_2O emission	31.1	< 0.001
$N_2O/(N_2 + N_2O)$	7.4	< 0.01
Total bulk N_2O	19.4	< 0.001
CO_2 production	19.8	< 0.001
AIT		
Denitrification	12.7	< 0.001
Total bulk N_2O	9.4	< 0.01
$N_2O/(N_2 + N_2O)$	0.3	> 0.05
CO_2 production (un-amended cores)	11.2	< 0.001
CO_2 production (C_2H_2 amended cores)	11.7	< 0.001

BGD
12, 12653–12689, 2015

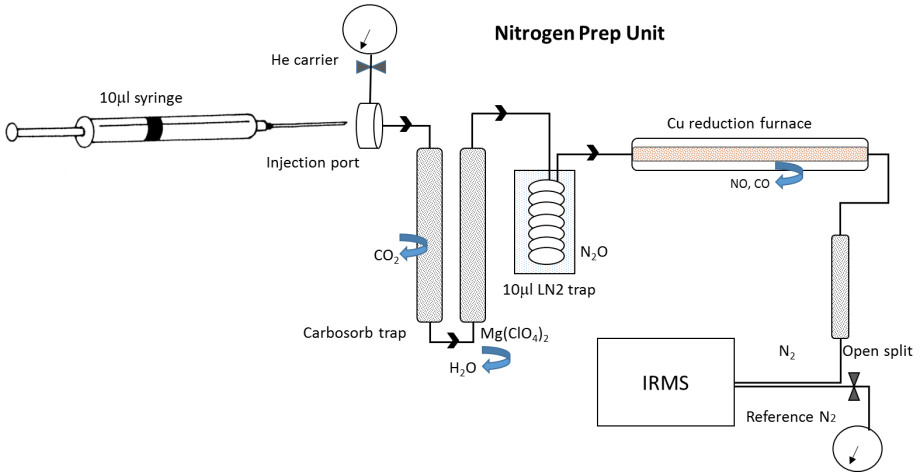

Figure 1. Schematic of the ^{15}N-N_2 analysis system.

BGD
12, 12653–12689, 2015

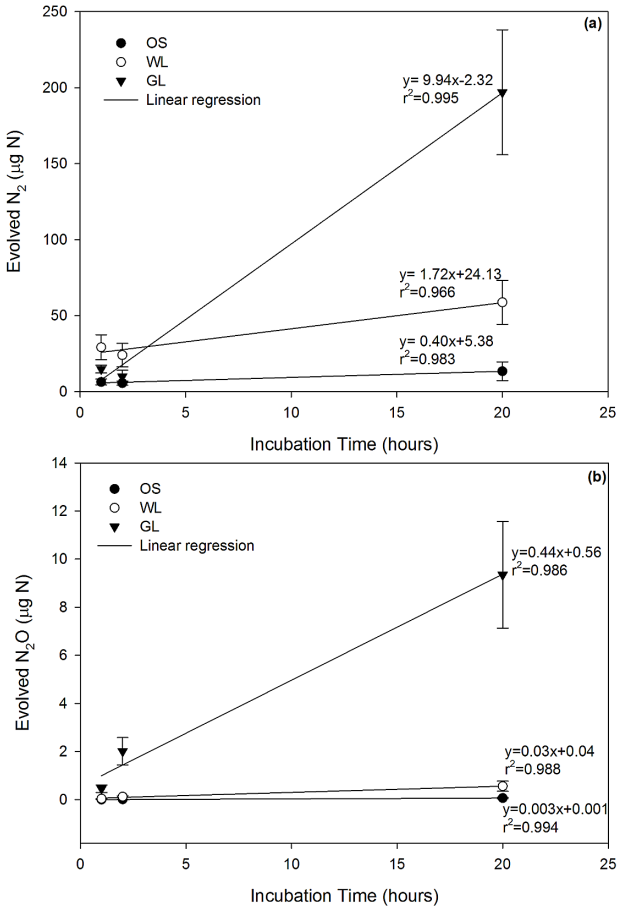

Figure 2. Evolved **(a)** N_2 and **(b)** N_2O gas measured between 1, 2 and 20 h incubation time points using the ^{15}N Gas-Flux method in the organic soil (OS), woodland (WL) and grassland (GL) land use types. Data points are means and the error bars represent standard errors.

BGD
12, 12653–12689, 2015

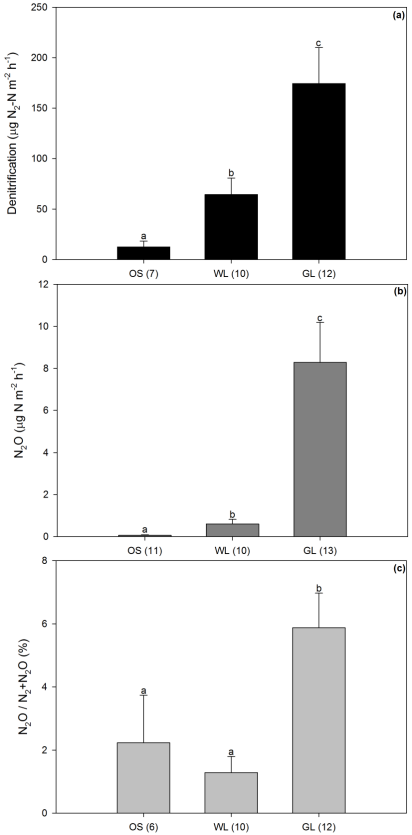

Figure 3. Mean rates of: **(a)** N_2 flux, **(b)** N_2O emission due to denitrification and **(c)** the denitrification product ratio $N_2O/(N_2+N_2O)$ in the three land use types (OS; organic soils, WL; woodland and GL; grassland). Same lower case letters indicate no significant differences ($p > 0.05$) between land use types according to One-way ANOVA and the Games–Howell post hoc test. The sample size (n) is given in parenthesis for each land use type on the x axis. Error bars represent standard errors.

BGD
12, 12653–12689, 2015

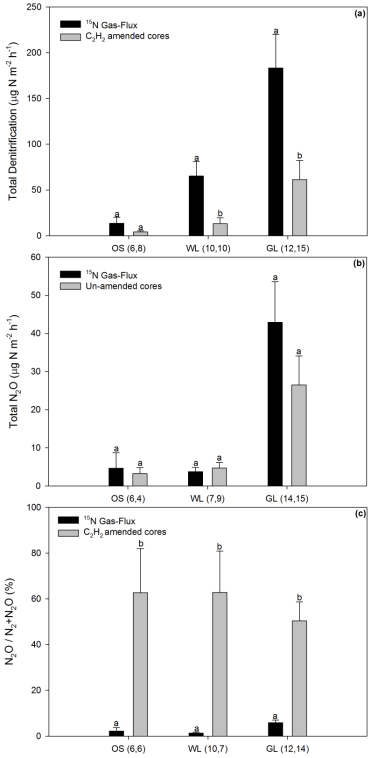

Figure 4. (a) Mean total denitrification measured with the ^{15}N Gas-Flux method and the AIT, **(b)** Mean bulk N_2O emission measured in the static chambers of the ^{15}N Gas-Flux method and in un-amended intact soil cores and **(c)** the denitrification product ratio $N_2O/(N_2 + N_2O)$ with the ^{15}N Gas-Flux method and the AIT in the three land use types (OS; organic soils, WL; woodland and GL; grassland). Same lower case letters indicate no significant differences ($p > 0.05$) between measurement methods according to independent samples t test. The sample size (n) is given in parenthesis for each land use type and each method on the x axis. Error bars represent standard errors.

BGD
12, 12653–12689, 2015

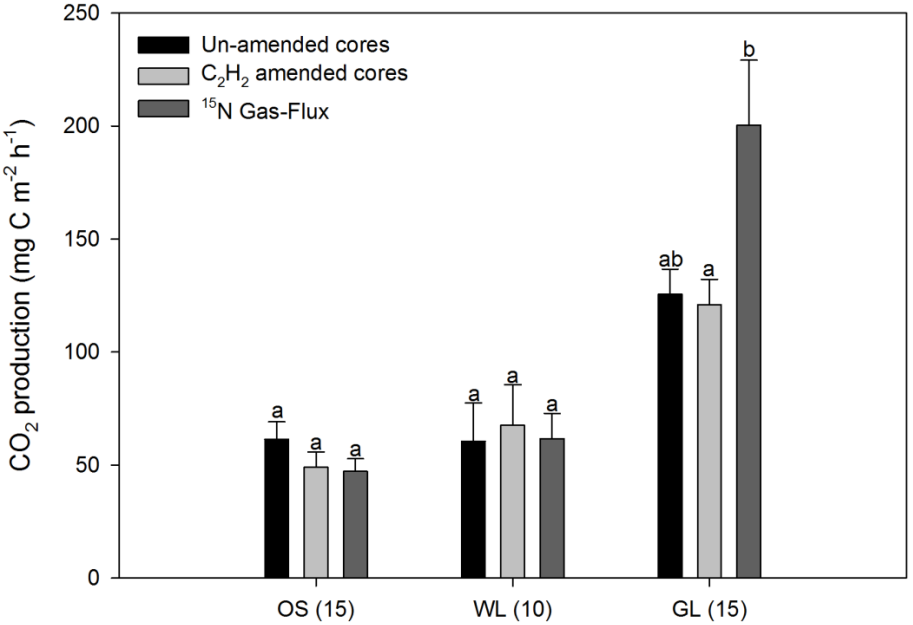

Figure 5. Mean CO_2 production measured in the static chambers of the ^{15}N Gas-Flux method, in un-amended and C_2H_2 amended intact soil cores in the three land use types (OS; organic soils, WL; woodland and GL; grassland). Same lower case letters indicate no significant differences ($p > 0.05$) between measurement methods according to independent samples t test. The sample size (n) is given in parenthesis for each land use type on the x axis. Error bars represent standard errors.

BGD
12, 12653–12689, 2015