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.

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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 coefficient 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 cm^3:cm^2) and up to 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 in the same land use types 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 partially attributed to the

46 incomplete inhibition of N_2O reduction to N_2 under relatively high soil moisture content. The
47 results show that the ^{15}N Gas-Flux method can be used for quantifying N_2 and N_2O
48 production rates in natural terrestrial ecosystems, thus significantly improving our ability to
49 constrain ecosystem N budgets.

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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 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

89 can be categorised into the direct flux and ^{15}N isotope tracer methods (Kulkarni et al. 2014),
90 whilst micrometeorological approaches (Eddy covariance) are impossible in the N_2 rich
91 atmosphere (Felber et al. 2012). The gas-flow soil core method (Burgin and Groffman 2012,
92 Butterbach-Bahl et al. 2002, Scholefield et al. 1997, Wang et al. 2011) allows the direct
93 measurement of N_2 flux (without the addition of any substrate such as nitrate) from intact soil
94 cores where the soil atmosphere is replaced by a mixture of He/O_2. However, despite the high
95 precision of the technique, cores still need to be extracted from the field and conditioned over
96 lengthy periods of time for the complete removal of N_2 from the soil atmosphere. This
97 method is therefore time and resource intensive which limits its application to intensive
98 temporal and large spatial scales (Kulkarni et al. 2014). Moreover, the gas-flow soil core
99 method cannot discriminate between sources of N_2O thus overestimating the denitrification
100 product ratio ($N_2O/ N_2 + N_2O$) (Butterbach-Bahl et al. 2013, Morse et al. 2015). The
101 acetylene inhibition technique (AIT) is also a direct flux method that exploits the ability of
102 acetylene (C_2H_2) at high concentrations (10 % v/v) to inhibit the reduction of N_2O to N_2
103 (Tiedje et al. 1989), thus total denitrification ($N_2 + N_2O$) is measured in C_2H_2 amended soil
104 cores *in situ*, whilst N_2 flux is estimated indirectly by difference from un-amended soil cores.
105 Despite its simplicity and cost-effectiveness, the AIT is becoming increasingly unpopular due
106 its several limitations (Groffman et al. 2006), of which the catalytic decomposition of NO in
107 the presence of C_2H_2 under oxic or suboxic conditions in the field (Nadeem et al. 2013) in
108 particular, precludes its use for reliable estimates of *in situ* denitrification rates (Felber et al.
109 2012).

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111 The ^{15}N Gas-Flux method (Mosier and Klemedtsson 1994) has the advantage of providing *in*
112 *situ* measurements of both N_2 and N_2O simultaneously, thus allowing its application over
113 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). 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) and more recently restored peatland soils (Tauchnitz et al. 2015) 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 $^{15}N\text{-}N_2$ 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 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 μL. These injection volumes are much smaller than have previously been reported in the literature.

Studies that have 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, Qin et al. 2014). A 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 compare the validity and applicability 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 (Figure 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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

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

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225 2.2. Field application of the ^{15}N Gas-Flux and AIT techniques

226 *In situ* measurements of N_2 and N_2O were made using static chambers according to the
227 ^{15}N Gas-Flux method (Mosier and Klemedtsson 1994). Five plots were randomly
228 established in June 2013 in each of four study sites in the Ribble - Wyre River catchments
229 (area 1145 km^2; NW England, 53°59'99" N, 2°41'79" W). The study sites were a
230 heathland (R-HL), a deciduous woodland (R-DW), an unimproved grassland (R-UG) and
231 an improved grassland (R-IG). In August 2013, four more study sites were tested in the
232 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 & Ullah (2014).

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 collars were open at the bottom to permit natural water table levels during the measurements. The natural vegetation cover at the soil surface of each installed collar remained unchanged. 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 hours was negligible with the relative standard deviation (RSD) being < 5 %. We did not use a vent tube for pressure equilibration, as suggested by Hutchinson and Mosier (1981), in our chamber design, which could have diluted the chamber headspace with atmospheric N_2, as part of our effort to increase the probability of a detectable ^{15}N-N_2 signal in the chamber headspace. 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 ten injections of equal volume through an equally-spaced grid (4 x 6 cm) 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 - 200 mL) was adjusted between 3 and 5 % of the ambient volumetric water content (see Supplementary Table 1 for detailed data from each sampling plot). Since the volume of the added solution corresponded to a precipitation amount of ≤ 2 mm, the increase of the volumetric water content was considered minor (Tauchnitz et al. 2015).

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 septum of the chamber cover at T = 1h, T = 2h and T ≈ 20h after the tracer injection, while a T = 0h 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).

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

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295 2.3. Flux calculations

296 The ^{15}N content of the N_2 in each 12 mL vial was determined using the IRMS system
297 described above and the ratios R29 ($^{29}N_2/^{28}N_2$) and R30 ($^{30}N_2/^{28}N_2$) were measured in
298 both enriched (T=1, 2 and 20 hours) and reference samples (T=0 hours). The inclusion of
299 air reference standards between every 10 samples indicated an upward drift for R30 over
300 time, potentially due to the formation of NO^+ in the ion source despite the inclusion of the
301 Cu reduction step (Lewicka-Szczebak et al. 2013). Subsequently, every sample batch was
302 drift corrected by fitting a linear regression through the air reference standards and
303 calculating an offset correction for both R29 and R30 (Yang et al. 2014). The minimum
304 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):

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

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 x 10^{-7} and for R30 was 6.1 x 10^{-7} and these values were used to determine if each time step sample was significantly different from ambient reference samples (T=0 hours), 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 hours) and reference samples (T=0 hours). 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:

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

The N_2 flux was then calculated using linear regression between the maximum evolved N_2 and the respective incubation time per plot surface area and was expressed in μg 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 hours) and reference samples (T=0 hours). 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:

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

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

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 x 10^{-5} and 2.9 x 10^{-5} respectively. The second set of gas samples collected at the same time in the field were analysed for total 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

349 N_2O flux due to denitrification of the mixture of the ^{15}N-labelled tracer and the soil N and
350 expressed in μg $N\text{-}N_2O$ m^{-2} h^{-1}. Assuming that the N_2O originates from the same
351 uniformly labelled pool as N_2, the $^{15}X_N$ from N_2O was used to estimate *d* for N_2 using
352 either R30 (Eq. 3) or R29 (Eq. 4), thus lowering the limit of detection for N_2 (Stevens and
353 Laughlin 2001) and allowing measurement of N_2 gas flux from natural terrestrial
354 ecosystems at low ^{15}N-tracer application rates.

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356 Gas samples collected from the intact soil cores with or without acetylene amendment
357 were analysed for N_2O on a GC-μECD (7890A GC Agilent Technologies Ltd., Cheshire,
358 UK) and for CO_2 on a GC-FID (7890A GC Agilent Technologies Ltd., Cheshire, UK)
359 and flux rates were determined by linear regression between 0 and 2 hours. The
360 instrument precision was determined from repeated analyses of 6 ppm N_2O and 200 ppm
361 CO_2 standards respectively (n = 8) and the RSD was <1%.

362

363 2.4. Statistical analysis

364 Using factor analysis on selected soil physico-chemical properties, the samples from the 8
365 field sites were ordinated in three broad land use types: organic soils (C-PB, C-UG, R-
366 HL); forest soils (C-MW, R-DW) and grassland soils (C-IG, R-UG, R-IG) according to
367 Sgouridis and Ullah (2014). All subsequent statistical analyses were performed on the
368 broad land use types rather than individual field sites. The data were analysed for
369 normality and homogeneity of variance with the Kolmogorov-Smirnov test and the
370 Levene statistic respectively and logarithmic transformations were applied as necessary.
371 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).

392 ## 3. Results

393 ### 3.1. IRMS system evaluation

394 The precision of the IRMS systems was evaluated using repeated analyses of ambient air
395 samples for N_2 (n=10) injected manually in one batch and repeated analyses of N_2O gas
396 standard at natural abundance and 0.5 ppm concentration (n=15) using automated
397 injections. The mean measured ratios of R29 and R30 for N_2 and of R45 and R46 for N_2O
398 are shown in Table 1. Measurement precision was defined as the coefficient of variation
399 (%) and it was lower for R29 compared to R30 and lower for R45 compared to R46, but
400 still less than 0.5 % for all four measured ratios. We estimated the ^{15}N atom% abundance
401 for both gases as per Yang et al. (2014) and the precision was less than 0.01% for N_2 in
402 air and 0.26 % for standard N_2O at natural abundance. The mean measured R30 (5.16×10^{-5})
403 was higher than the theoretical value of 1.35×10^{-5} for N_2 in ambient air suggesting
404 some interference at m/z 30 potentially due to the formation of NO^+ ions in the ion source
405 of the mass spectrometer despite the inclusion of the Cu reduction oven. The contribution
406 of NO^+ ions (R30 measured - R30 theoretical) was 3.81×10^{-5}, whilst the ratio of R30
407 theoretical/ R30 measured was 0.26. Correcting the R30 ratio for the contribution of NO^+
408 ions results in a lower 'true' precision for the R30 (CV = 1.67 %).

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410 ### 3.2. Field application of the ^{15}N Gas-Flux method

411 The ^{15}N tracer application rate was variable between land use types and ranged between
412 0.03 and 1 kg ^{15}N ha^{-1} while it was lower in the case of the organic soils and higher for
413 the woodland and grassland soils (Table 2). Based on the soil nitrate content on the day of
414 the tracer amendments (Table 2), the estimated enrichment of the total soil nitrate pool

was on average between 13 and 25 % (detailed data on the ^{15}N tracer application per field site are shown in Supplementary 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 hour 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-MW and R-DW 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 N_2O production from a second nitrate pool, possibly nitrate produced from the oxidation of NH_4^+ via nitrification, in the C-MW. In cases where the $^{15}X_N$ could be calculated from the N_2 isotope ratio data (woodland and grassland soils; data shown in Supplementary Table 3), this was not significantly different from their respective $^{15}X_N$ calculated from the N_2O isotope ratio data (t-test; $t\text{-}_{WL} = 0.929$, $df = 12$, $p > 0.05$; $t\text{-}_{GL} = 1.511$, $df = 20$, $p > 0.05$).

The linearity of the evolved N_2 and N_2O fluxes in the chamber headspace between 1 and 20 hours of incubation time was evaluated in each sampling plot when all three time steps were above the MDC values (data presented in Supplementary Tables 4 & 5). With respect to the N_2 flux, significant deviation from linearity was observed only in C-MW (mean $r^2 = 0.59$, n = 5), whilst in C-PB, C-UG, R-HL and R-IG the per site analysis was not possible due to missing flux data between time steps. When the data were pooled per

land use type (Figure 2a), the linear increase in the evolved N_2 was statistically significant after 20 hours 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 hours incubation in Figure 2a, it was not significant for the OS and WL soils. Regarding the N_2O flux, this was found to increase linearly with time in all the field sites (Supplementary Table 5), with the exception of the R-IG (mean r^2 = 0.49, n = 4). When data were pooled per land use type (Figure 2b), the amount of N_2O accumulated after 20 hours 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 hours 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 μg 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 (Figure 3a). A similar pattern was observed for the N_2O flux due to denitrification (range: 0.003 - 20.8 μg 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 (Figure 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 (Figure 3c). The change of the denitrification product ratio with incubation time was evaluated in each sampling plot where both N_2 and N_2O fluxes were available (data shown in Supplementary Table 6). Generally, the product ratio increased with increasing incubation time with the exception of the grassland soils, where the maximum product

464 ratio was observed after 2 hours of incubation (ANOVA; $F = 6.11$, $p < 0.05$). This was an
465 indication of some reduction of the denitrification derived N_2O to N_2 during the extended
466 closure period (up to 20 hours).

467

468 3.3. Comparison with the AIT

469 The total denitrification rate measured from the C_2H_2 amended intact soil cores in the
470 same land use types ranged between 0.5 and 325.2 μg N m^{-2} h^{-1} and correlated positively
471 with the total denitrification rate (N_2 and N_2O fluxes combined) measured with the ^{15}N
472 Gas-Flux method (Pearson; $r = 0.581$, $n = 25$, $p < 0.01$) following a similar trend among
473 land use types, albeit only the OS being significantly lower than the grassland and
474 woodland soils (Table 3). The AIT denitrification rates were between 3 and 5 times lower
475 than the total denitrification from the ^{15}N Gas-Flux (Figure 4a) with the difference being
476 significant in woodland (t-test; $t = 3.914$, $df = 18$, $p < 0.01$) and grassland soils (t-test; $t =$
477 3.521, $df = 25$, $p < 0.01$).

478

479 The total N_2O flux measured from the un-amended intact soil cores ranged between 0.15
480 and 86.6 μg N m^{-2} h^{-1} and was between 1 and 3 times lower than the total denitrification
481 rate from the C_2H_2 amended cores. There were no significant differences between bulk
482 N_2O fluxes measured with the static chambers and the un-amended intact soil cores
483 (Figure 4b), which indicated that total N_2O emissions were comparable between the two
484 field techniques. Consequently, estimating the denitrification product ratio from the un-
485 amended and C_2H_2 amended intact soil cores resulted in significantly higher ratios
486 compared to the ^{15}N Gas-Flux approach (Figure 4c), which were on average between 50
487 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 (Figure 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 $^{15}N\text{-}N_2$ 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. This was achieved while injecting 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 than the required sample volume by Yang et al. (2014). Furthermore, the interference at *m/z* 30 by NO^+ ions was reduced 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), unless using the mathematical formulations of Spott and Stange (2007).

The TraceGas^{TM} Preconcentrator IRMS system used for $^{15}N\text{-}N_2O$ 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), but comparable to Well et al. (1998) 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 up to 20 hours, 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 μg N m^{-2} h^{-1} and 0.2 ng N m^{-2} h^{-1} for the N_2 and N_2O fluxes respectively. These were significantly better than the minimum rates (175 - 900 μg N_2-N m^{-2} h^{-1} and 0.04 - 0.21 μg N_2O-N m^{-2} h^{-1}) reported by Bergsma et al. (2001), Kulkarni et al (2014) and Tauchnitz et al (2015), 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 μg N_2-N m^{-2} h^{-1} and < 1 μg N_2O-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

555 the volume to surface area ratio of our chambers from 16:1 to 8:1 (cm^3/cm^2) and by
556 extending the incubation time to approximately 20 hours, for the first time in a field
557 study.

558

559 Most studies using ^{15}N tracers and static chambers in highly fertilised systems typically
560 deploy their chambers between 1 and 2 hours (Baily et al. 2012, Cuhel et al. 2010,
561 Tauchnitz et al. 2015), but it has been shown that longer incubation periods (up to 24 or
562 48 hours) may be needed in case of low ^{15}N enrichment applications in intact soil cores
563 (Morse and Bernhardt 2013) and laboratory incubations (Yang et al. 2014) for a more
564 precise and accurate detectable ^{15}N-N_2 signal. However, it should be noted that in these
565 cases where an extended incubation period was employed, the soil cores or slurries did
566 not allow the subsoil diffusion of the evolved N_2 and N_2O back into the soil pore spaces
567 (Clough et al. 2005). The open-bottom, un-vented static chamber design used in this study
568 may have allowed some loss of the evolved N_2 and N_2O through downward subsoil
569 diffusion and/or reduction of gas exchanges at the soil-atmosphere interface due to
570 positive pressure build up in the chamber headspace. However, we have demonstrated
571 that the N_2 flux and more importantly the N_2O flux increased linearly with time through
572 the 20 hour incubation period, probably as a result of a slow N_2O diffusion rate due to the
573 high water filled pore space (WFPS) (Jury et al. 1982) in our field sites (Mean WFPS: C-
574 PB = 70 ± SE 3.21 %; C-UG = 66 ± SE 1.58 %; R-HL = 69 ± SE 2.00 %; C-MW = 42 ±
575 SE 0.76 %; R-DW = 65 ± SE 1.79 %; R-UG = 64 ± SE 1.41 %; C-IG = 60 ± SE 1.45 %;
576 R-IG = 61± SE 2.46 %). In the case of the C-MW, the N_2 flux may have been
577 underestimated due to a faster decrease in the gas concentration gradient between the soil
578 surface and the chamber headspace as a result of higher air-filled porosity (Healy et al.
579 1996) and the subsequent diffusion of N_2 back into subsoil. In the case of the R-IG, where

580 N_2O flux was not found linear up to 20 hours incubation, some of the N_2O may have been
581 diffused into the subsoil and further reduced to N_2 (Clough et al. 2005), thus leading to an
582 underestimated N_2O flux rate. In this study, we have chosen to report flux rates based on
583 linear regression up to 20 hours incubation period (where available), for comparison
584 purposes between land use types exhibiting marked differences in potential denitrifer
585 activity (Sgouridis and Ullah 2014). It has been shown that a linear flux model is less
586 sensitive to noisy datasets hovering close to the limit of detection (particularly the OS
587 land use type in our case), in spite of the possibility of underestimation of true fluxes
588 (Levy et al. 2011). However, when our objective was to estimate annual in situ flux rates
589 of N_2 and N_2O due to denitrification from natural and semi-natural land use types
590 between April 2013 and October 2014 (Sgouridis and Ullah 2015), the flux rate
591 estimation was based on the maximum evolved N_2 and N_2O rate at any valid (above the
592 MDC) time step, thus reporting maximum flux rates per land use type to possibly avoid
593 the risk of underestimation. Therefore, we suggest using varying incubation times under
594 field conditions to capture a more reliable ^{15}N signal, particularly for N_2 gas, from sites
595 exhibiting significant seasonal variability of flux rates.

596

597 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
598 soil) across land use types was one to two orders of magnitude lower than previous
599 applications of the ^{15}N Gas-Flux method in highly fertilised agricultural systems (Baily et
600 al. 2012, Bergsma et al. 2001, Cuhel et al. 2010, Graham et al. 2013) and in restored
601 peatland soils (Tauchnitz et al. 2015). The estimated enrichment of the total soil NO_3^-
602 pool was variable (2 – 40 %, Supplementary Table 2) and this wide range was due to the
603 fact that the tracer concentration was calculated based on the previous campaign's soil
604 nitrate data, which in some cases did not reflect the soil nitrate content on the day of the

605 tracer application a month later. It should be noted that the soil nitrate enrichment levels
606 reported in this study correspond to the high end of the average soil NO_3^- pool enrichment
607 (10 – 15 %, Supplementary Table 2) for the period April 2013 to October 2014, which is
608 presented in a separate publication (Sgouridis and Ullah 2015). To our knowledge, only
609 Kulkarni et al. (2014) have applied the ^{15}N Gas-Flux method in the field with soil nitrate
610 enrichment levels (5 %) lower than in our study, but this had as a consequence poorly
611 detected ^{15}N-N_2 fluxes. Nevertheless, for the organic soils the average tracer application
612 rate corresponded to current estimates of daily atmospheric N deposition (0.05 kg N ha^{-1}
613 d^{-1}) in the UK (~ 15 - 20 kg N ha^{-1} y^{-1}) (Dore et al. 2012, Payne 2014), whilst for the
614 grassland soils the tracer application mimicked a daily fertiliser application rate of 0.5 kg
615 N ha^{-1} d^{-1}. Due to the inclusion of the N-rich C-MW site in the woodland soils, tracer
616 application rates were higher than the daily atmospheric N deposition rates, thus also
617 reflecting internal N cycling processes (e.g. nitrification) as an additional source of nitrate
618 in these well-drained forest soils. Therefore, the application of the ^{15}N tracer at these low
619 rates should not be expected to enrich the soil nitrate pool, and potentially enhance the
620 denitrification activity, in excess of the amount of nitrogen normally deposited via natural
621 processes and common management practices.

622

623 The major assumptions of the ^{15}N Gas-Flux method and the associated 'non-equilibrium
624 equations' are that the denitrifying soil NO_3^- pool is uniformly labelled with ^{15}N and that
625 the N_2 and N_2O originate from the same denitrifying pool (Stevens and Laughlin 1998).
626 The ^{15}N fraction in the denitrifying pool ($^{15}X_N$), calculated non-destructively from the
627 measured isotope ratios, ranged between 65 and 93 % and was well above the 10 %
628 threshold for the correct application of the 'non-equilibrium equations' (Lewicka-
629 Szczebak et al. 2013). However, the calculated $^{15}X_N$ was higher than the estimated total

630 soil NO_3^- pool enrichment (range: 2 - 40 %) suggesting only partial mixing of the added
631 tracer (98 ^{15}N at %) with the ambient soil nitrate at natural abundance despite the
632 elaborate effort for uniform tracer application with multiple injections across 10 cm soil
633 depth (Ruetting et al. 2011). Wu et al. (2011) have optimised the number of injections and
634 the volume of tracer needed to achieve homogeneous labelling of a soil core (diameter 15
635 cm; height 20 cm) and reported that 38 injections of 4 mL volume each were necessary.
636 We have used only 10 injections of 5- 20 mL volume (depending on the soil water
637 content of each land use type) to minimise the disturbance of the soil matrix, particularly
638 in highly porous media such as peatland soils, and this may have affected the
639 homogeneous distribution of the tracer. We were not able to sample the soil within the
640 chamber collars for directly estimating the $^{15}NO_3^-$ content of the soil pool due to time and
641 budget constraints. However, in cases where destructive soil sampling was used to
642 measure the soil nitrate pool enrichment (Kulkarni et al. 2014), the results were
643 significantly different from the estimated enrichment due to sampling bias of the volume
644 of soil affected by the tracer application. Non-uniform mixing of the ^{15}N label may lead to
645 overestimation of the $^{15}X_N$ and underestimation of the denitrification flux rates (Boast et
646 al. 1988). However, it is unlikely under field conditions to achieve complete mixing of
647 the added tracer with the ambient nitrate; and experimental studies (Mulvaney 1988,
648 Mulvaney and Van den Heuvel 1988) have shown that the error is well-constrained and
649 that accurate measurements can be made even with a less-uniformly labelled denitrifying
650 pool. The non-significant change of $^{15}X_N$ with incubation time suggested only one
651 denitrifying pool for both N_2 and N_2O, assuming negligible N_2 production from anammox
652 and co-denitrification (Spott and Stange 2007). Moreover, the similar $^{15}X_N$ values
653 obtained from both the N_2 and the N_2O isotope ratio data for the woodland and grassland
654 soils (Supplementary Table 3), was an additional indication that the effect of hybrid N_2

655 fluxes was negligible and thus it was appropriate to use the $^{15}X_N$, calculated from the N_2O
656 isotope ratios, for calculating N_2 flux rates using the more reliable R30 measurements
657 (Stevens and Laughlin 2001). Only in the case of the C-MW well-drained forest site,
658 shown to exhibit the highest nitrification potential (Sgouridis and Ullah 2014), the slope
659 of $^{15}X_N$ with time was negative suggesting dilution of the ^{15}N-labelled soil NO_3^- pool by
660 the oxidation of the ambient ammonium (nitrification). It is therefore possible that N_2 flux
661 rates may be overestimated in C-MW, due to the underestimation of the $^{15}X_N$, but
662 Bergsma et al. (1999) showed that temporal changes of the soil NO_3^- pool enrichment are
663 negligible at ^{15}N enrichment levels similar to ours.

664

665 We were able to measure appreciable *in situ* fluxes of both N_2 and N_2O due to
666 denitrification in all three land use types. Our N_2 fluxes from woodland soils compare
667 well with the rates reported in the literature for restored forested wetlands in North
668 America (Morse and Bernhardt 2013) and with the rates from northern hardwood forests
669 in US (Kulkarni et al. 2014), using ^{15}N tracers at similar or lower application rates to
670 ours. Our results are also comparable to the rates reported from central European forests,
671 under similar atmospheric N deposition rates, using the gas-flow soil core method
672 (Butterbach-Bahl et al. 2002). For the grassland soils, the N_2 fluxes measured in the
673 present study were significantly lower than previous applications of the ^{15}N Gas-Flux
674 method at high fertiliser application rates (Baily et al. 2012, Cuhel et al. 2010, Graham et
675 al. 2013), whilst for the organic soils our rates were significantly lower than the ones
676 reported by Tauchnitz et al. (2015) since their ^{15}N tracer application rate (30 kg N ha^{-1})
677 was 300 times higher than ours. The N_2O fluxes were up to 200 times lower than the N_2
678 fluxes leading to low denitrification product ratios in all land use types, a result which is
679 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). It is likely that the denitrification product ratio in the grassland soils has been underestimated due to the extended incubation period (up to 20 hours), during which some of the denitrification derived N_2O may have diffused back into the soil and was further reduced to N_2. Therefore, we would recommend that in soils displaying high denitrification activity (e.g. improved grasslands) the incubation period should not exceed 2 hours for a more accurate estimation of the $N_2O/ N_2 + N_2O$ ratio. In the present study we have compared the in situ denitrification rates between three major land use types using an extended field incubation period to increase the probability of detecting a reliable $^{15}N\text{-}N_2$ signal, particularly under conditions of low denitrifier activity due to seasonality of denitrification and/or inherent capacity of soils (for example organic and deciduous forest soils). However, these rates should be considered conservative since confounding issues such as subsoil diffusion and non-homogeneous labelling of the soil nitrate pool may in some cases have led to underestimations of the in situ denitrification rates.

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. The AIT denitrification rates were between 3 and 5 times lower than the ^{15}N Gas-Flux rates despite the fact that the AIT intact soil cores were

capped at the bottom, thus not allowing any subsoil diffusion of the evolved gases due to denitrification. Therefore, the AIT rates should have been higher than the ^{15}N Gas-Flux rates if serious underestimation was occurring due to subsoil diffusion in the open-bottom static chambers, which was not the case. Adding nitrate to the C_2H_2 amended cores would have been desirable for directly evaluating the priming effect of the added substrate on denitrification rates. The ^{15}N tracer addition to the static chambers corresponded to the amounts of N naturally deposited in these land use types either via management practices and/or atmospheric deposition, thus avoiding excessive N fertilisation of the sampling plots. However, it cannot be conclusively argued that the same amount of applied nitrate would not have led to similar denitrification rates between the AIT and the ^{15}N Gas-Flux methods. 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 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, Qin et al. 2014). In our study, the soil WFPS ranged between 60 and 70 % in all land use types, with the exception of the C-MW site (mean WFPS 42 %), whilst the $^{15}N\text{-}NO_3^-$ tracer application rate was low (< 1 kg N ha^{-1}). 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 could argue that it is possible 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 (2h for the intact cores) may have limited the ability of C_2H_2 to fully equilibrate within soil pore spaces. Other confounding factors such as the catalytic decomposition of NO in the presence of C_2H_2 (Nadeem et al. 2013) may have also contributed to the lower denitrification rates measured by the AIT.. This study has confirmed some of the drawbacks of the AIT as a quantification method of in situ denitrification rates compared to the^{15}N Gas-Flux .

The estimation of the denitrification product ratio using the AIT method, from the un-amended cores (N_2O only) and the C_2H_2 amended cores (N_2 + N_2O), is usually overestimated since the source of N_2O cannot be discriminated with the AIT, whilst the N_2 flux is underestimated due to the incomplete inhibition of N_2O reduction (Butterbach-Bahl et al. 2013). This was confirmed in the present study for all the land use types and even the maximum denitrification product ratio after 2 hours incubation in the case of the grassland soils (23 %), was still significantly lower than the respective ratio from the AIT (50 %). 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. Conclusion

753 The analytical precision for both ^{15}N-N_2 and ^{15}N-N_2O analyses was greatly improved by
754 using smaller sample volumes than previously reported, thus allowing us to quantify *in*
755 *situ* N_2 and N_2O fluxes with low ^{15}N enrichment under field conditions, which was
756 previously not possible. The ^{15}N Gas-Flux method was applied for the first time across a
757 range of natural and semi-natural land use types at ^{15}N tracer application rates mimicking
758 current estimates of atmospheric N deposition (natural systems) or grassland fertiliser
759 application rates and yielded analytically valid flux rates for both N_2 and N_2O in all the
760 land use types. A possible limitation of the adapted ^{15}N Gas-Flux method when applied at
761 low ^{15}N enrichment levels is the uncertainty associated with the estimation of the soil NO_3^-
762 pool enrichment and the possibility for subsoil diffusion of the evolved gases in cases of
763 extended incubation (> 2 hr) that may result in the underestimation of denitrification rates.
764 Comparing the ^{15}N Gas-Flux method with the AIT confirmed the drawbacks of the AIT as
765 a reliable quantification method of in situ denitrification rates. Moreover, the AIT
766 methodoverestimates the denitrification product ratio compared to the ^{15}N Gas-Flux
767 method. The ^{15}N Gas-Flux methodholds much promise as a more reliable field technique
768 for measuring *in situ* denitrification rates and its wider application across a range of
769 terrestrial ecosystems can lead to its refinement and improvement and in the long termcan
770 significantly contribute to our understanding of the role of denitrification as a reactive
771 nitrogen sink.
772
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7. 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 N2O and N-2 emissions from grazed grassland, Nutr. Cycling Agroecosyst., 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 N2O yield in a riparian wetland, Journal of Geophysical Research-Biogeosciences, 117, G01010, 2012.

Butterbach-Bahl, K., Willibald, G. and Papen, H.: Soil core method for direct simultaneous determination of N-2 and N2O 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?, Philosophical Transactions of the Royal Society B-Biological Sciences, 368, 2013.

Clough, T., Sherlock, R., Rolston, D.: A review of the movement and fate of N_2O in the subsoil. Nutr.Cycling Agroecosyst. 72, 3-11, 2005.

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 N2O and N-2 Emissions and Denitrifier Community Size and Activity, Appl. Environ. Microbiol., 76, 1870-1878, 2010.

Dendooven, L. and Anderson, J.: Maintenance of Denitrification Potential in Pasture Soil Following Anaerobic Events, Soil Biology & Biochemistry, 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, 2012.

831 Felber, R., Conen, F., Flechard, C. R. and Neftel, A.: Theoretical and practical limitations of
832 the acetylene inhibition technique to determine total denitrification losses, Biogeosciences, 9,
833 4125-4138, 2012.

834 Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R.,
835 Martinelli, L. A., Seitzinger, S. and Sutton, M. A.: Transformation of the Nitrogen Cycle:
836 Recent trends, questions and potential solutions, Science, 320, 889-892, 2008.

837 Graham, C. J., van Es, H. M. and Melkonian, J. J.: Nitrous oxide emissions are greater in silt
838 loam soils with a legacy of manure application than without, Biol. Fertility Soils, 49, 1123-
839 1129, 2013.

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

843 Groffman, P.: Terrestrial denitrification: challenges and opportunities, Ecological Processes,
844 1, 11, 2012.

845 Healy, R. W., Striegel, R. G., Russel, T. F., Hutchinson, G.L. and Livingston, G. P.:
846 Numerical evaluation of static-chamber measurements of soil-atmosphere gas exchange:
847 Identification of physical processes, Soil Sci. Soc. Am. J., 60, 740-747, 1996.

848 Hutchinson, G. L. and Mosier, A. R.: Improved Soil Cover Method for Field Measurement of
849 Nitrous Oxide Fluxes, Soil Sci. Soc. Am. J., 45, 311-316, 1981.

850 Jury, W., Letey, J. and Collins, T.: Analysis of Chamber Methods used for Measuring
851 Nitrous-Oxide Production in the Field, Soil Sci. Soc. Am. J., 46, 250-256, 1982.

852 Kulkarni, M. V., Burgin, A. J., Groffman, P. M. and Yavitt, J. B.: Direct flux and N-15 tracer
853 methods for measuring denitrification in forest soils, Biogeochemistry, 117, 359-373, 2014.

854 Kulkarni, M. V., Groffman, P. M. and Yavitt, J. B.: Solving the global nitrogen problem: it's
855 a gas!, Frontiers in Ecology and the Environment, 6, 199-206, 2008.

856 Laughlin, R. J. and Stevens, R. J.: Changes in composition of nitrogen-15-labeled gases
857 during storage in septum-capped vials, Soil Sci. Soc. Am. J., 67, 540-543, 2003.

858 Levy, P. E., Gray, A., Leeson, S. R., Gaiawyn, J., Kelly, M.P.C., Cooper, M.D.A., Dinsmore,
859 K. J., Jones, S. K. and Sheppard, L. J.: Quantification of uncertainty in trace gas fluxes
860 measured by the static chamber method, Eur. J. Soil Sci., 62, 811-821, 2011.

861 Lewicka-Szczebak, D., Well, R., Giesemann, A., Rohe, L. and Wolf, U.: An enhanced
862 technique for automated determination of N-15 signatures of N-2, (N-2+N2O) and N2O in
863 gas samples, Rapid Communications in Mass Spectrometry, 27, 1548-1558, 2013.

864 Limpens, J., Berendse, F. and Klees, H.: N deposition affects N availability in interstitial
865 water, growth of Sphagnum and invasion of vascular plants in bog vegetation, New Phytol.,
866 157, 339-347, 2003.

867 Malone, J., Stevens, R. and Laughlin, R.: Combining the N-15 and acetylene inhibition
868 techniques to examine the effect of acetylene on denitrification, Soil Biology &
869 Biochemistry, 30, 31-37, 1998.

870 Matson, A., Pennock, D. and Bedard-Haughn, A.: Methane and nitrous oxide emissions from
871 mature forest stands in the boreal forest, Saskatchewan, Canada, For. Ecol. Manage., 258,
872 1073-1083, 2009.

873 Mills, R. T. E., Tipping, E., Bryant, C. L. and Emmett, B. A.: Long-term organic carbon
874 turnover rates in natural and semi-natural topsoils, Biogeochemistry, 1-16, 2013.

875 Morse, J. L. and Bernhardt, E. S.: Using N-15 tracers to estimate N2O and N-2 emissions
876 from nitrification and denitrification in coastal plain wetlands under contrasting land-uses,
877 Soil Biology & Biochemistry, 57, 635-643, 2013.

878 Morse, J. L., Duran, J., Beall, F., Enanga, E. M., Creed, I. F., Fernandez, I. and Groffman, P.
879 M.: Soil denitrification fluxes from three northeastern North American forests across a range
880 of nitrogen deposition, Oecologia, 177, 17-27, 2015.

881 Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Wadsworth, R. and
882 Simpson, I. C.: Final Report for LCM2007 - the new UK Land Cover Map, Centre for
883 Ecology & Hydrology, 2011.

884 Mosier, A. R. and Klemedtsson, L.: Measuring denitrification in the field, in: Methods of Soil
885 Analysis, Part 2, Microbiological and Biochemical Properties, Weaver, R. W., Angle, J. S.
886 and Bottomley, P. S. (Eds.), Soil Science Society of America, Inc., Wisconsin, USA, 1047,
887 1994.

888 Mosier, A., Guenzi, W. and Schweizer, E.: Field Denitrification Estimation by N-15 and
889 Acetylene Inhibition Techniques, Soil Sci. Soc. Am. J., 50, 831-833, 1986.

890 Mulvaney, R. L.: Determination of 15N-Labeled Dinitrogen and Nitrous Oxide With Triple-
891 collector Mass Spectrometers., Soil Sci. Soc. Am. J., 48, 690-692, 1984.

892 Mulvaney, R.: Evaluation of N-15 Tracer Techniques for Direct Measurement of
893 Denitrification in Soil .3. Laboratory Studies, Soil Sci. Soc. Am. J., 52, 1327-1332, 1988.

894 Mulvaney, R. and Van den Heuvel, R.: Evaluation of N-15 Tracer Techniques for Direct
895 Measurement of Denitrification in Soil .4. Field Studies, Soil Sci. Soc. Am. J., 52, 1332-
896 1337, 1988.

897 Nadeem, S., Dorsch, P. and Bakken, L. R.: Autoxidation and acetylene-accelerated oxidation
898 of NO in a 2-phase system: Implications for the expression of denitrification in ex situ
899 experiments, Soil Biol. Biochem., 57, 606-614, 2013.

900 Payne, R. J.: The exposure of British peatlands to nitrogen deposition, 1900-2030, Mires and
901 Peat, 14, 04, 2014.

902 Qin, S., Hu, C. and Oenema, O.: Quantifying the underestimation of soil denitrification
903 potential as determined by the acetylene inhibition method, Soil Biology and Biochemistry,
904 47, 14-17, 2012.

905 Qin, S., Yuan, H., Dong, W., Hu, C., Oenema, O. and Zhang, Y.: Relationship between soil
906 properties and the bias of N2O reduction by acetylene inhibition technique for analyzing soil
907 denitrification potential, Soil Biol. Biochem., 66, 182-187, 2013.

908 Qin, S., Yuan, H., Hu, C., Oenema, O., Zhang, Y. and Li, X.: Determination of potential
909 N2O-reductase activity in soil, Soil Biology & Biochemistry, 70, 205-210, 2014.

910 Ravishankara, A. R., Daniel, J. S. and Portmann, R. W.: Nitrous Oxide (N2O): The Dominant
911 Ozone-Depleting Substance Emitted in the 21st Century, Science, 326, 123-125, 2009.

912 Rolston, D., Sharpley, A., Toy, D. and Broadbent, F.: Field Measurement of Denitrification
913 .3. Rates during Irrigation Cycles, Soil Sci. Soc. Am. J., 46, 289-296, 1982.

914 Ruetting, T., Huygens, D., Staelens, J., Mueller, C. and Boeckx, P.: Advances in N-15-tracing
915 experiments: new labelling and data analysis approaches, Biochem. Soc. Trans., 39, 279-283,
916 2011.

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

920 Scholefield, D., Hawkins, J. and Jackson, S.: Development of a helium atmosphere soil
921 incubation technique for direct measurement of nitrous oxide and dinitrogen fluxes during
922 denitrification, Soil Biology & Biochemistry, 29, 1345-1352, 1997.

923 Sgouridis, F. and Ullah, S.: Denitrification potential of organic, forest and grassland soils in
924 the Ribble-Wyre and Conwy River catchments, UK, Environ. Sci. -Process Impacts, 16,
925 1551-1562, 2014.

926 Sgouridis, F. and Ullah, S.: The relative magnitude and controls of *in situ* N_2 and N_2O fluxes
927 due to denitrification in natural and semi-natural terrestrial ecosystems using ^{15}N tracers,
928 Environ. Sci. Technol., doi**:** 10.1021/acs.est.5b03513, 2015.

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

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

934 Stevens, R. J., Laughlin, R. J., Atkins, G. J. and Prosser, S. J.: Automated determination of
935 ^{15}N-labeled dinitrogen and nitrous oxide by mass spectrometry, Soil Sci. Soc. Am. J., 57,
936 981-988, 1993.

937 Stevens, R. and Laughlin, R.: Measurement of nitrous oxide and di-nitrogen emissions from
938 agricultural soils, Nutr. Cycling Agroecosyst., 52, 131-139, 1998.

939 Tauchnitz, N., Spott, O., Russow, R., Bernsdorf, S., Glaser, B. and Meissner, R.: Release of
940 nitrous oxide and dinitrogen from a transition bog under drained and rewetted conditions due
941 to denitrification: results from a 15N nitrate-bromide double-tracer study, Isotopes in
942 Environmental and Health Studies, 51, 300-321, 2015.

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

946 Ullah, S. and Moore, T. R.: Biogeochemical controls on methane, nitrous oxide, and carbon
947 dioxide fluxes from deciduous forest soils in eastern Canada, J. Geophys. Res. -Biogeosci.,
948 116, G03010, 2011.

949 Wang, R., Willibald, G., Feng, Q., Zheng, X., Liao, T., Brueggemann, N. and Butterbach-
950 Bahl, K.: Measurement of N-2, N2O, NO, and CO2 Emissions from Soil with the Gas-Row-
951 Soil-Core Technique, Environ. Sci. Technol., 45, 6066-6072, 2011.

952 Well, R., Becker, K-W., Langel, R., Meyer, B. and Reineking, A.: Continuous flow
953 equilibration for mass spectrometric analysis of dinitrogen emissions, Soil Sci. Soc. Am. J.,
954 62, 906-910, 1998.

955 Wu, H., Dannenmann, M., Fanselow, N., Wolf, B., Yao, Z., Wu, X., Bruggemann, N., Zheng,
956 X., Han, X., Dittert, K. and Butterbach-Bahl, K.: Feedback of grazing on gross rates of N
957 mineralisation and inorganic N partitioning in steppe soils of Inner Mongolia, Plant Soil, 340,
958 127-139, 2011.

959 Yang, W. H., McDowell, A. C., Brooks, P. D. and Silver, W. L.: New high precision
960 approach for measuring N-15-N-2 gas fluxes from terrestrial ecosystems, Soil Biology &
961 Biochemistry, 69, 234-241, 2014.

962 Yang, W. H., Teh, Y. A. and Silver, W. L.: A test of a field-based N-15-nitrous oxide pool
963 dilution technique to measure gross N2O production in soil, Global Change Biol., 17, 3577-
964 3588, 2011.

965 Yu, K., Seo, D. and DeLaune, R. D.: Incomplete Acetylene Inhibition of Nitrous Oxide
966 Reduction in Potential Denitrification Assay as Revealed by using 15N-Nitrate Tracer,
967 Commun. Soil Sci. Plant Anal., 41, 2201-2210, 2010.

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Tables

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

Table 2: The ambient soil nitrate pool, the ^{15}N tracer application rate, the estimated enrichment of the total soil nitrate pool, 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	Ambient NO_3^- (kg N ha^{-1})	Tracer application rate (kg ^{15}N ha^{-1})	Enrichment of total soil NO_3^- pool (%)	$^{15}X_N$ (%)	$^{15}X_N$ slope
Organic Soil (n=3)	0.53 (0.44)	0.04 (0.02)	25 (11.8)	90 (1.5)	0.003 (0.0054)
Woodland (n=2)	3.86 (2.42)	0.62 (0.41)	13 (0.7)	79 (8.3)	-0.007 (0.0025)

Grassland (n=3)	1.81 (0.96)	0.51 (0.19)	24 (5.1)	81 (8.4)	0.000 (0.0037)

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

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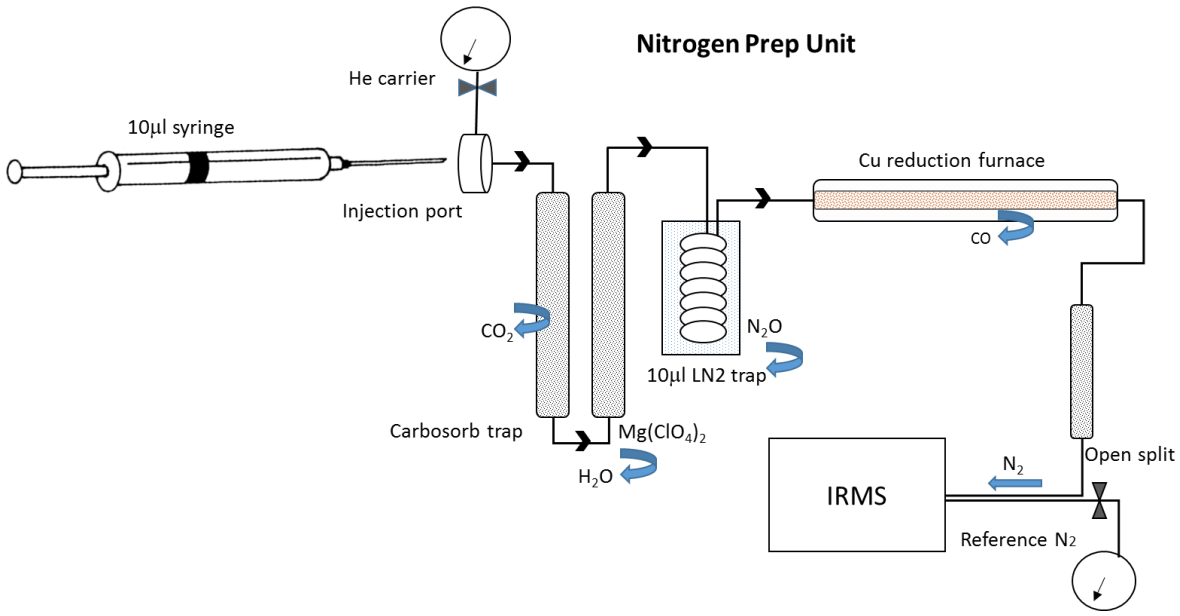
999 **Figures**



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1001 **Figure 1**: Schematic of the ^{15}N-N_2 analysis system

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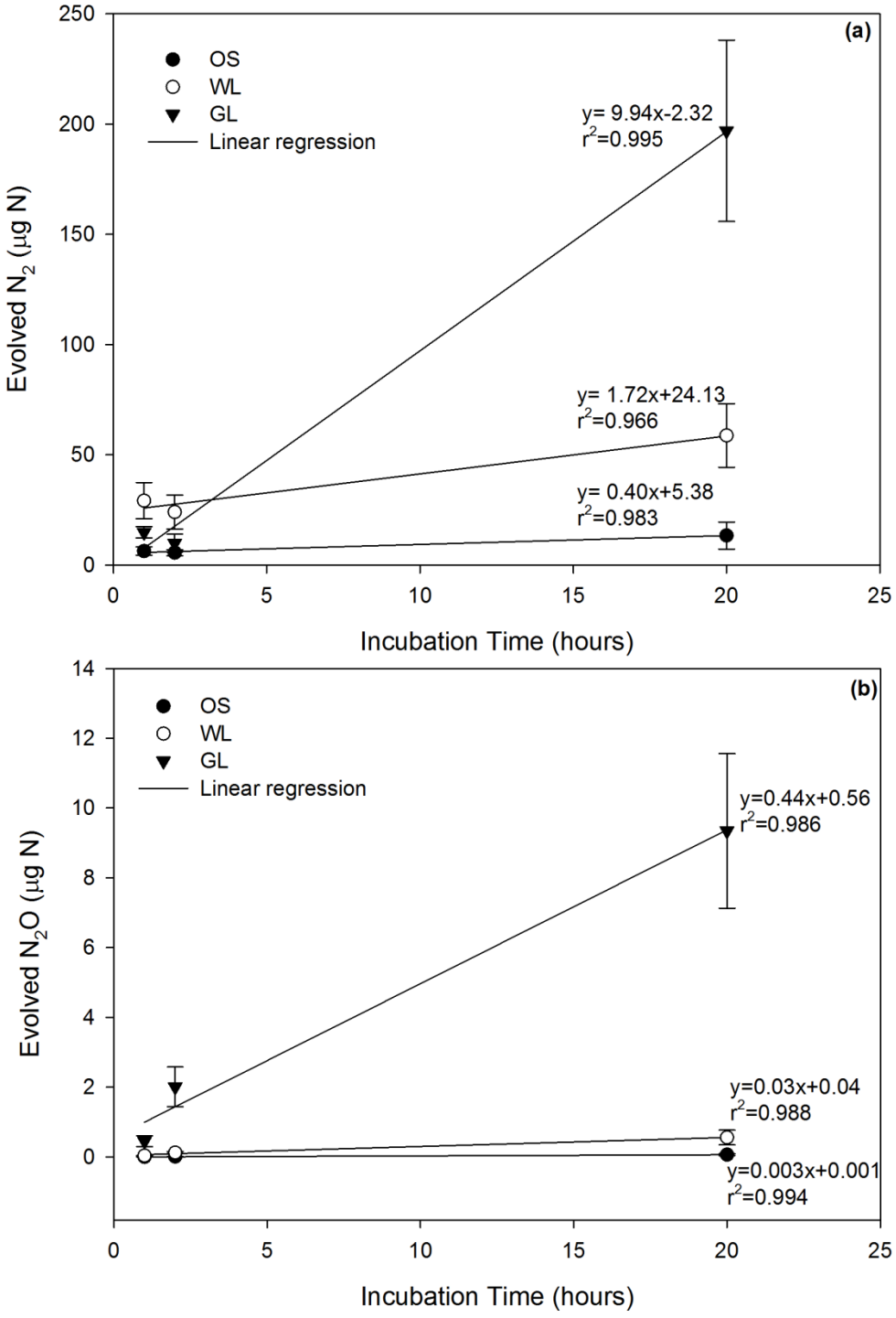


Figure 2: Evolved (a) N_2 and (b) N_2O gas measured between 1, 2 and 20 hours incubation time points using the ^{15}N Gas-Flux method in the organic soil (OS), woodland (WL) and grassland (GL) land use types. Data points are means and the error bars represent standard errors.

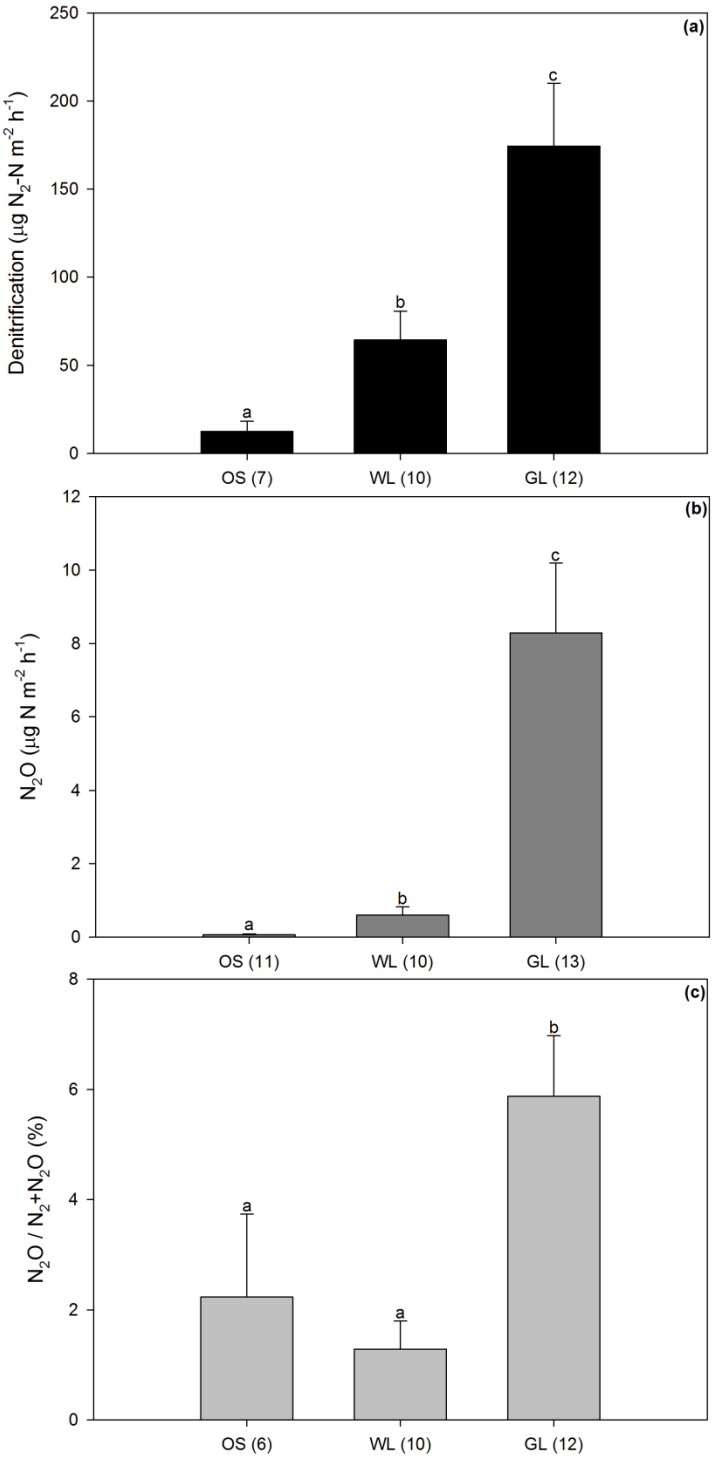


Figure 3: Mean rates of: (a) N_2 flux, (b) N_2O emission due to denitrification and (c) the denitrification product ratio N_2O/ (N_2 + N_2O) in the three land use types (OS; organic soils, WL; woodland and GL; grassland). Same lower case letters indicate no significant differences (p > 0.05) between 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.

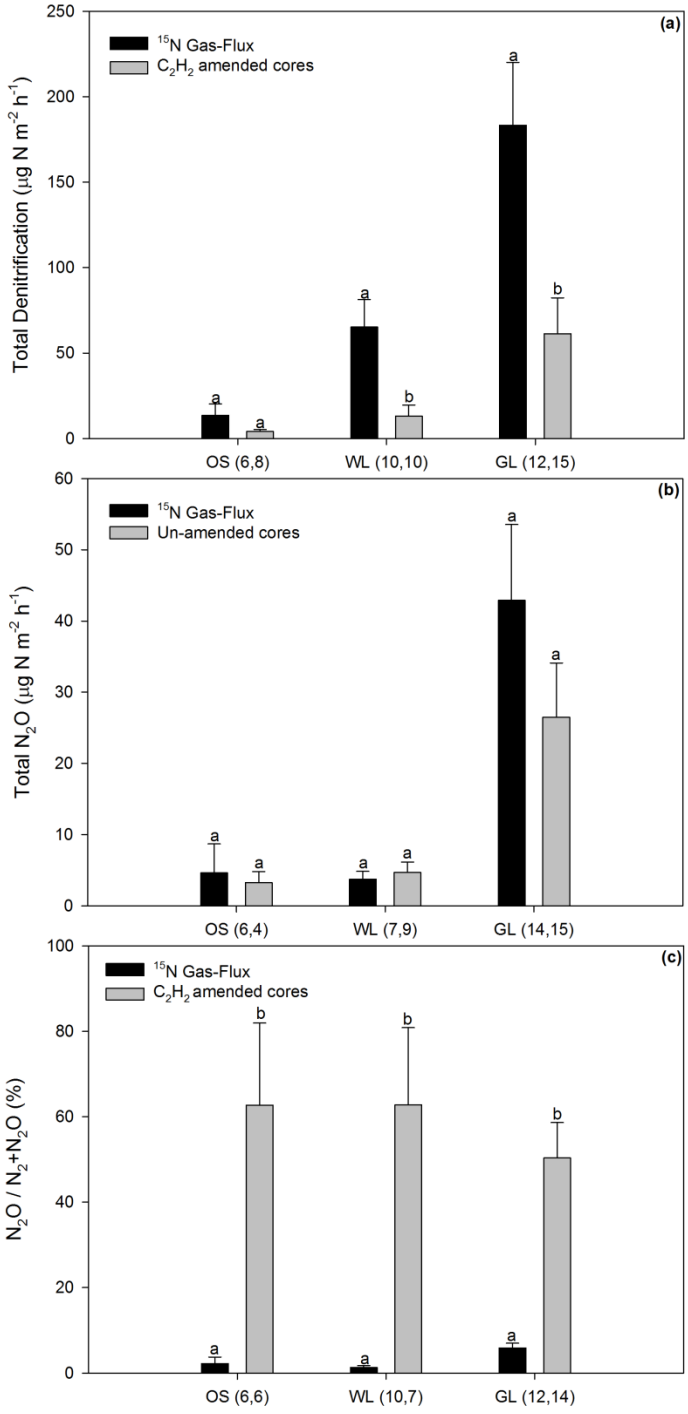
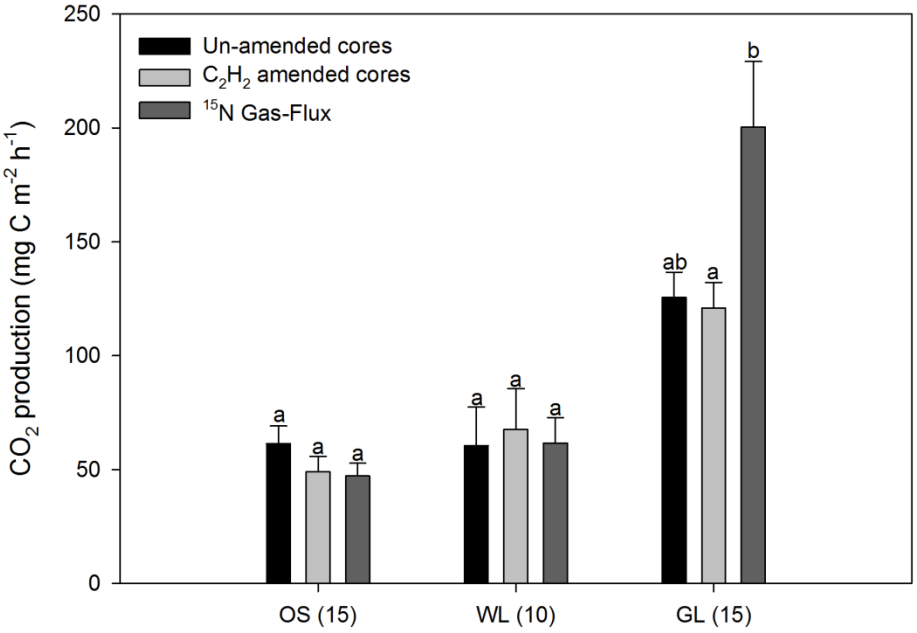


Figure 4: (a) Mean total denitrification measured with the ^{15}N Gas-Flux method and the AIT, (b) Mean bulk N_2O emission measured in the static chambers of the ^{15}N Gas-Flux method and in un-amended intact soil cores and (c) the denitrification product ratio $N_2O/ (N_2 + N_2O)$ with the ^{15}N Gas-Flux 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.



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1037 **Figure 5**: Mean CO_2 production measured in the static chambers of the ^{15}N Gas-Flux
1038 method, in un-amended and C_2H_2 amended intact soil cores in the three land use types (OS;
1039 organic soils, WL; woodland and GL; grassland). Same lower case letters indicate no
1040 significant differences ($p > 0.05$) between measurement methods according to independent
1041 samples t-test. The sample size (n) is given in parenthesis for each land use type on the x-
1042 axis. Error bars represent standard errors.

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