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Towards enhanced sensitivity of the ^{15}N gas flux method for quantifying denitrification in soil

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ABSTRACT

Denitrification is the least studied process of the global N cycle mainly due to the sensitivity required to discriminate small fluxes of soil emitted N_2 against the high atmospheric N_2 background. We aimed to enhance the sensitivity of the ^{15}N Gas Flux method to measure *in situ* denitrification rates by optimising the quantity of ^{15}N–NO_3 tracer applied and by using an artificial atmosphere (containing 5 % N_2, 20 % O_2, 75 % He and 0.11 ppm of N_2O) during field incubation. We first conducted a dose-response laboratory study to assess the stimulation effect of nitrate tracer addition. Subsequently, we developed two novel approaches to measure *in situ* denitrification rates, using either modified static chambers or intact soil cores inside plastic liners; where in both cases the entire headspace was replaced by the artificial atmosphere prior to incubation. Furthermore, we compared the two models of calculations of the ^{15}N Gas Flux method (the "Mulvaney & Boast" and "Arah" models) as well as the calculated ^{15}N enrichment of the soil denitrifying pool based on either N_2 or N_2O isotopologue distribution data. The results showed that doubling the amount of ambient nitrate did not lead to a significant stimulation of denitrification activity in our case. However, excessive amendment of nitrate (e.g. 20 times the ambient levels) increased the denitrification product ratio by stimulating nitrous oxide emission. Our two novel field techniques were successful in measuring *in situ* denitrification rates, however, the liner method was preferred due to a higher success rate of N_2 flux detection (up to 90 %), a higher throughput (up to 24 cores at a time) and improved spatial resolution. Under high-resolution instruments, our N_2 limit of detection was 160 ppb, which is 5-fold better than the original method. The Mulvaney & Boast model performed better than the Arah one and consistently yielded higher fluxes (17 % at maximum), especially for low ^{15}N enrichments of the soil denitrifying pool and short times of incubation. The ^{15}N enrichments calculated with either N_2 or N_2O data differed statistically, but the magnitude of difference was small (4.6 % at maximum). Measuring *in situ* denitrification is imperative to quantify realistic fluxes and the liner method presented here is an inexpensive, reproducible and high-resolution candidate. For increased sensitivity, we recommend using the method of Mulvaney & Boast for N_2O emissions and the resulting ^{15}N enrichment in combination with $^{29}N_2$ data (only) to determine N_2 emissions.

1. Introduction

Denitrification in soil is the sequential reduction of nitrate (NO_3^-) to gaseous dinitrogen (N_2) through microbial respiration under suboxic conditions. This sequential process includes nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) as obligatory intermediates of reaction. Denitrification is considered the major pathway of reactive nitrogen (N) removal in terrestrial systems, which is of particular relevance for

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agricultural land uses (arable and grasslands) receiving large inputs of synthetic and organic N fertilizer (Lassaletta et al., 2014). The last intermediate of this sequential process is nitrous oxide (N_2O), a greenhouse gas with 298 times greater radiative forcing than CO_2 (IPCC, 2013) which is also involved in the depletion of the ozone layer (Ravishankara et al., 2009). Denitrification has the ambiguous role of being both the only natural sink for respiratory reduction of N_2O into N_2 but also a source of N_2O through incomplete denitrification. To guide future N_2O emission reduction strategies and close the nitrogen budget of ecosystems, it is of primary importance to fully characterize this process and particularly the reduction of N_2O to N_2.

Measuring small amounts of soil evolved N_2 against the high atmospheric N_2 background (78 % v/v) is very challenging (Groffman et al., 2006). Historically, denitrification has mainly been measured using three different techniques (Well et al., 2019a): the He/O_2 Gas Flow Soil Core method (He/O_2 GFSC), the Acetylene Inhibition Technique (AIT) and the ^{15}N Gas Flux method (^{15}NGF). Briefly, the He/O_2 GFSC is a laboratory-based incubation method where ambient atmosphere is replaced by a N_2-gas free mix of Helium (He) and dioxygen (O_2) in a tightly sealed vessel (Butterbach-Bahl et al., 2002; Cárdenas et al., 2003; Wang et al., 2013, 2020; Kulkarni et al., 2014; Loick et al., 2016). The AIT uses acetylene to block N_2O reduction to N_2 so that denitrification can be measured as total N_2O production. This technique has been very popular since its original application in the mid 1970's due to its simplicity and low cost but is today considered unsuitable due to several limitations. Amongst others, it catalyses the oxidation of NO and only partially inhibits the reduction of N_2O to N_2 (Seitzinger et al., 1993; Simarmata et al., 1993; Yu et al., 2010; Saggar et al., 2013; Sgouridis et al., 2016). Finally, the ^{15}NGF uses ^{15}N-labelled nitrate tracer applied to soil incubated in gas tight vessels under laboratory conditions or within static chambers in field applications to measure the abundance of ^{15}N atoms in both denitrified N_2 and N_2O molecules (Stevens and Laughlin 2002; Ruser et al., 2006; Baily et al., 2012; Sgouridis and Ullah, 2015; Deppe et al., 2017; Rummel et al., 2021; Liu et al., 2022a). The ^{15}NGF can be used with two main models of calculation, the "Mulvaney & Boast" model (based on Mulvaney and Boast, 1986) or the "Arah" model (based on Arah, 1992), as recently reviewed in Micucci et al. (2023). They both use the distribution of the ^{15}N atoms in the N_2 and N_2O isotopologues (molecules with same atomic identity but different isotopic composition) to derive the fluxes of denitrification, but they differ in their approach. The Mulvaney & Boast model is based on the differences in isotopic ratios before and after incubation while the Arah approach is a mixing model of different ^{15}N pools. They both rely on the hypothesis that the soil denitrifying pool (soil nitrate pool undergoing denitrification) forms a sole pool at isotopic equilibrium after label injection. Since this assumption is most likely violated due to the non-homogenous distribution of the added tracer (but still valid to some extent, Mulvaney and Vanden Heuvel, 1988; Stevens et al., 1997), it is difficult to predict which model is the most adapted.

The main advantage of the ^{15}NGF method is its applicability under *in situ* conditions, which provides more robust estimations of the real dynamics of denitrification compared to laboratory experiments (as in the He/O_2 GFSC). Additionally, coupled with Gas Chromatography (GC) measurements or laser spectroscopy methods for the quantification of total N_2O emission, the ^{15}NGF enables the partitioning of the sources of N_2O between denitrification and other potential sources (Stevens et al., 1997). Since both denitrified N_2 and N_2O emissions are measured with the same technique, the denitrification product ratio R_{N2O}=$N_2O/(N_2 + N_2O)$ is also more accurately determined (Bergsma et al., 2001). Nevertheless, the ^{15}NGF also has limitations and in particular, its sensitivity through Isotope Ratio Mass Spectrometry (IRMS) might not be high enough to discriminate small fluxes of N_2 emissions from soil because of the presence of naturally abundant ^{15}N isotopes in the atmosphere (~0.37 %).

One way to increase the sensitivity of the ^{15}NGF is to combine it with the use of an artificial atmosphere depleted in N_2. As shown in Micucci et al. (2023; Fig. 7), reducing the atmospheric N_2 concentration below 10 % highly increases the sensitivity of the ^{15}NGF. Maintaining this low level under field conditions might be challenging however due to the diffusion of atmospheric N_2 inside the incubation vessel. This was achieved by Well et al. (2019a) using a sophisticated gas chamber system that kept a low N_2 background by continuously flushing both the chamber's headspace and the incubated soil with the artificial atmosphere gas mixture. This very same method has recently been used by Buchen-Tschiskale et al. (2023) to follow N transformations following cattle slurry application. It is however an expensive and complex technique to use, which may preclude its use at wider spatial scales.

Another way to enhance the sensitivity of the ^{15}NGF is to optimize the quantity of ^{15}N tracer applied to reach a 50 % ^{15}N enrichment of the soil denitrifying pool, which leads to a higher abundance of the $^{15}N^{14}N$ isotopologue and thus a higher detection probability via IRMS (Stevens and Laughlin, 2001). We further demonstrate in **Annex I (SI)** why this particular enrichment is ideal for ^{15}NGF; however, it requires to double the quantity of available nitrate (Fig. S2, **SI**). It has been reported that denitrification emissions as well as the product ratio increase with external nitrate inputs (Jarvis et al., 1991; Clayton et al., 1997; Blackmer and Bremner, 1978; Firestone and Tiedje, 1979; Loick et al., 2017; Warner et al., 2019). We thus need to assess if a fertilization effect leading to higher denitrification rates occurs at a 50 % ^{15}N enrichment before using this enrichment for enhanced sensitivity of the ^{15}NGF.

Finally, it is also possible to increase the limit of detection of the ^{15}NGF by combining the N_2 and N_2O isotopologue distribution data. More detail can be found in Micucci et al. (2023) and in this study but briefly, by discarding the measurement of the $^{30}N_2$ molecules (for which the IRMS sensitivity is very limited) and by focusing on the $^{29}N_2$ molecules only; while the ^{15}N enrichment of the soil denitrifying pool is determined with the N_2O data, a better sensitivity can be achieved (Stevens and Laughlin, 2001; Spott et al., 2006; Sánchez-García et al., 2014; Friedl et al., 2020). This approach assumes that both gases originate from the same and sole denitrifying pool (Bergsma et al., 2001) and that no hybrid molecules are emitted (Phillips et al., 2016; Micucci et al., 2023). Using this method enabled Baily et al. (2012) to report a sensitivity 16 times greater than when using $^{29}N_2$ and $^{30}N_2$ data.

In order to address the important methodological gap of accurate quantification of denitrification, we aimed to create a robust, easy to apply at wider spatial scales and inexpensive method for *in situ* measurement of denitrification. Our approach was to optimize the quantity of applied tracer to reach a 50 % ^{15}N enrichment of the soil nitrate pool and to use an artificial atmosphere depleted in N_2 (containing 5 % N_2, 20 % O_2, 75 % He and 0.11 ppm of N_2O) under field conditions. To that end, we developed modified static chambers that could be connected to a gas cylinder containing the artificial atmosphere gas mixture for an initial flush and replacement of the ambient atmospheric headspace prior to incubation. The obvious diffusion issues due to the open-bottom nature of the developed static chambers were not incompatible with this approach. Indeed, keeping a constant N_2 background during the length of the incubation is not necessary if this background can be quantified at each sampling time. Thus, if the N_2 concentration background rises slowly enough, detection could occur for short incubations. As an alternative to the challenges of open-bottom static chambers, we also developed an incubation approach using intact soil cores sampled directly into plastic liners where the bottom and top lids were modified to enable incubation of small soil cores with a similar initial flush of the ambient atmosphere. Our research objectives were thus to:

(i) Assess whether the addition of nitrate tracer stimulates the denitrification process through a dose-response laboratory study (**Experiments 1 and 2**).
(ii) Characterize how fast the N_2 background rises in the developed static chamber and core liner systems, and validate if these two systems enable reliable measurement of denitrification under field conditions (**Experiments 3 and 4**).

(iii) Compare the "Mulvaney & Boast" and "Arah" models for the calculation of the ^{15}NGF to determine if one of them is more adapted than the other (**Experiment 1**).
(iv) Compare the ^{15}N enrichments of the soil denitrifying pool determined with either the N_2 or the N_2O isotopic gas data, confirming that these two gases originate from the same mineral pool (**Experiment 3**).

2. Material and methods

2.1. Laboratory study, impact of the nitrate tracer addition on denitrification fluxes

For the laboratory study, soil was sampled at the Allerton Project in Loddington, Leicestershire UK (52.617659-0.833060), which displays three different agricultural land uses as shown in **Annex II (SI)**.

These agricultural land uses were a conventional arable field (**A1**), an intensively managed grass-clover pasture (**GC1**) and a herbal rich pasture (**H1**) where a mix of 20 grasses, forbs and herbs were planted as an alternative to grass-clover pastures. The exact species mix can be found in **Annex II (SI)**.

2.1.1. Experiment 1: Comparison of the two calculation methods

Under laboratory conditions, we incubated 6 replicates of 50 g of sieved soil from the Herbal pasture (**H1**, sampled April 30, 2021) in 450 mL sealed gas-tight jars with modified lids containing septa. The gas-tightness of the jars was tested by introducing 800 ppm of CO_2 in 6 closed jars and by measuring the CO_2 concentration after 6 h. On average, the CO_2 concentration only dropped by 0.4 %, indicating that the jars were properly sealed. This was verified by observing consistent N_2 levels for every jar (data not shown). ^{15}N-labelled nitrate (as KNO_3, 98 at % ^{15}N, Merck) was added to 5 different treatments targeting a 0, 20, 30, 40 or 50 % ^{15}N enrichment of the total nitrate pool (after tracer application). The quantity of tracer to add in order to reach these enrichments was determined as:

$$n_{add} = \frac{[NO_3^-] \times m_{soil} \times a_p}{0.98 - a_p} \quad (1)$$

where $\boldsymbol{n_{add}}$ is the quantity of $^{15}NO_3$ tracer to add (mol), $\boldsymbol{[NO_3^-]}$ the soil nitrate concentration (mol g^{-1}), $\boldsymbol{m_{soil}}$ the mass of incubated soil (g) and $\boldsymbol{a_p}$ the targeted ^{15}N enrichment of the soil denitrifying pool.

The control treatment (0 %) consisted of addition of deionised water only (at the same volume as the tracer) and thus can only be used to compare total emissions of N_2O. The 20 % enrichment is representative of a standard target in the context of the ^{15}NGF (Mulvaney, 1984) and was used as our minimum enrichment treatment. Gravimetric moisture was also increased up to 45 % in order to increase the probability of detection of N_2O and N_2. Indeed, a preliminary incubation showed no detectable amounts of denitrification products when using a 5 % increase in soil moisture as per the use of the ^{15}NGF. However we did not increase soil moisture further and reach the optimal conditions for denitrification (60–70 %, Wang et al., 2023) in order to stay as close as possible to field conditions. The ^{15}N–NO_3 tracer was diluted in deionised water, bubbled with gaseous N_2 to remove dissolved oxygen; and 9.5 mL of this solution were applied to each jar using 30 syringe injections while gently rotating the jars to ensure homogenous application. Sampling took place at times 0, 3, 6 and 24 h, where 15 mL of gas were sampled from the jars headspace into 12 mL pre-evacuated Exetainer vials (Labco Limited, UK) and 15 mL of He were added to equilibrate for the loss of pressure. Replacing the sampled volume with pure He creates slightly anoxic conditions (19 % O_2 after 6 h) but avoids the introduction of laboratory N_2O inside the jars.

Under this experimental setup, we directly compared the ^{15}N enrichments and N_2O fluxes obtained using either the Mulvaney & Boast or Arah models.

2.1.2. Experiment 2: Dose-response study of the three land uses

The protocol of **Experiment 1** was adapted to enable replacement of the jars headspace with a custom-made artificial atmosphere containing 5 % of N_2, 20 % of O_2, 75 % of He and 0.11 ppm N_2O (CK Isotopes Limited, UK). Using a 6-port gas manifold connected to the artificial atmosphere gas cylinder, the headspaces of six jars were replaced simultaneously 10 times with a gas flow of 400 mL min^{-1} for 10 min before incubation. The same artificial atmosphere was used for headspace pressure equilibration after sampling.

Soil sampling in the **H1**, **GC1** and **A1** land uses occurred on July 9, 2021. For each of them, we added tracer to target a 0, 20 or 50 % ^{15}N enrichment of the soil denitrifying pool.

2.2. Field study: development of a new method for in situ denitrification measurement

To measure denitrification *in situ*, we developed modified static chambers and plastic liners (for incubation of 10 cm long soil cores) that would be compatible with an initial flush of artificial atmosphere prior to incubation. The designs of these modified chambers and liners can be found in **Annex III (SI)**.

2.2.1. Experiment 3: Chamber and liner tests, comparison of the ^{15}N enrichment calculated through N_2 or N_2O isotopic data

2.2.1.1. Static chamber test. The chamber test took place on September 27, 2021 in a 5 year old grass-clover pasture at the Fenswood Farm near Bristol UK (51.421083-2.662733). One week prior to incubation, five chamber collars were inserted 10 cm deep into the soil and soil samples were collected and brought back to the laboratory for determination of the nitrate content. Isotopic solutions targeting a ^{15}N enrichment of 50 % of the soil denitrifying pool and inducing a 5 % moisture increase were prepared.

On the day of the incubation, isotopic solutions (~200 mL, 0.23 g L^{-1} of KNO_3, 98 at % ^{15}N, Merck) were applied to soil within the chamber collars using syringes (40 injections; at 0, 2.5, 5 and 10 cm depth). The top chambers were mounted and connected to the artificial atmosphere gas cylinder for a flushing of 15 min at a rate of 2.8 L min^{-1} (ambient atmosphere replaced 5 times). Gas sampling (15 mL) occurred at times 0, 1, 2, 3, 4, 5, 6 and 24 h and we did not replace the sampled volume by an equal volume of artificial atmosphere. The samplings after 4 h of incubation were only used for N_2 background characterization and not flux measurements.

2.2.1.2. Core liner test. The liner test took place on October 15, 2021 in a grass-clover pasture nearby the Allerton Project site. Soil sampling, nitrate content determination and isotopic solution preparation occurred in the same way as for the chamber test. The day before incubation, we sampled five intact cores (10 cm height) inside plastic liners that were left to pre-incubate overnight under environmental conditions in freshly dug holes.

On the day of the incubation, we injected the tracer (~15 mL, 1.16 g L^{-1} of KNO_3, 98 at % ^{15}N, Merck) inside the soil cores using a syringe (24 injections; at 0, 2.5, 5 and 10 cm depth). The liners were then capped and flushed with the artificial atmosphere at a rate of 200 mL min^{-1} for 5 min (headspace replaced 10 times). We directed the gas flow from the top to the bottom of the liners (using a vent needle inserted in the bottom lid septum) to flush the soil pores and avoid the diffusion of soil pore N_2 towards the headspace of the liners. Sampling occurred at times 0, 2, 4, 7, 9 and 24 h, at which times 15 mL of the headspace were sampled and 15 mL of artificial atmosphere were added for pressure equilibration. The samplings after 4 h of incubation were only used for N_2 background characterization and not flux measurements. This setup enabled us to compare the ^{15}N enrichment of the soil denitrifying pool based on the N_2 or N_2O data.

2.2.2. Experiment 4: Confirmation of the liner method for the measurement of denitrification

Experiment 4 took place at the FarmED site, near Shipton-under Wychwood UK (51.869981-1.581136). The experimental layout at the FarmED site consisted of strips of the same soil under herbal pastures of different ages compared to an arable control. We studied the 4-year-old herbal pasture (**H2**) and arable (**A2**) strips (more information can be found in **Annex II**, **SI**). The **A2** land use had been fertilized just before our experiment there, 50 kgN ha^{-1} had been applied a month prior and 70 kgN ha^{-1} ten days prior, using a mix of nitrate (23%), ammonium (30%) and urea (47%) both times.

Experiment 4 took place on the May 19, 2022 and replicated the liner test of **Experiment 3** (see 2.2.1.2) in the **H2** and **A2** land uses with 8 replicates for each.

2.3. Equivalent fertilizer rate of applied ^{15}N tracer

For an easier assessment of a hypothetical fertilizer effect, we calculated an equivalency between tracer application and fertilizer rate for each experiment (Table 1). For the laboratory experiments (where sieved soil was used) we worked out how the amendment of nitrate tracer could be translated as a fertilizer application by assuming that fertilizer was applied to the top 15 cm of soil and that 26 % of a soil volume sampled in the field would be sievable soil (<2 mm) that can be used for laboratory incubation (after subtraction of rocks, roots, etc.; based on results from the present study).

2.4. Gas flux calculations

For each sample, total N_2O concentration was determined by gas chromatography and the isotopic ratios R29 ($^{29}N_2/^{28}N_2$), R30 ($^{30}N_2/^{28}N_2$), R45 ($^{45}N_2O/^{44}N_2O$) and R46 ($^{46}N_2O/^{44}N_2O$) were measured via IRMS. The protocols for these analyses can be found in Annexe IV (SI).

2.4.1. N_2O isotopic calculations

Denitrified N_2O emissions were determined by first correcting the R46 and R45 ratios to account for oxygen isotopes in accordance with Bergsma et al. (2001):

$$R29 = R45 - R17 \tag{2}$$

$$R30 = R46 - (R29)(R17) - R18 \tag{3}$$

where R17 is the $^{17}O/^{16}O$ ratio and R18 is the $^{18}O/^{16}O$ ratio. We used the values of 0.000373 and 0.0020052 respectively, based on Bergsma et al. (2001).

Then, we calculated $\boldsymbol{a_p}$ the ^{15}N enrichment of the soil nitrate pool undergoing denitrification and the coefficient $\boldsymbol{d'}$ (where a prime is used for N_2O emissions and distinguish them from N_2 emissions, see Micucci et al., 2023) which represents the proportion of total N_2O that derives from denitrification, with the method of Mulvaney and Boast (1986). These quantities can also be determined with the method of Arah (1992) and both methods have been used and compared during **Experiment 1**. It should be noted that since the tracer is applied before flushing the headspace with the artificial atmosphere, by the time the first measurement is made (t = 0), soil has been labelled for 15 min and denitrification of the tracer has already started. Although most of the labelled denitrification products are probably flushed with the gas flow, the isotopic ratios of N_2O at time 0 were usually already high. Indeed, after flushing, the average ambient ^{15}N enrichment of the liners headspace was 2.78 %, instead of the atmospheric value of 0.37 %. Therefore, we recommend using a laboratory or field air reference for the R46 and R45 ratios at time 0.

Table 1
Equivalency between added tracer amounts and fertilizer rate for each experiment of this study.

Experiment	Treatment – Enrichment	Added N–NO_3 (kgN ha^{-1})
Exp1	H1 - 20%	0.75
Exp1	H1- 30%	1.28
Exp1	H1- 40%	1.99
Exp1	H1 - 50%	2.99
Exp2	H1 - 20%	0.60
Exp2	H1 - 50%	2.42
Exp2	GC1 - 20%	1.40
Exp2	GC1 - 50%	5.62
Exp2	A1 - 20%	1.03
Exp2	A1 - 50%	4.11
Exp3	Chambers	2.04
Exp3	Liners	12.46
Exp4	H2	1.38
Exp4	A2	23.96

The concentration of denitrification-derived N_2O is obtained as:

$$[N_2O]_{denitrified} = d' \times [N_2O]_{total} \tag{4}$$

where $\boldsymbol{[N_2O]_{total}}$ is the total N_2O concentration measured through GC (in ppm).

We then calculated the N_2O source partitioning coefficient (SPC) as:

$$SPC = \frac{f_{N2O\ denitrified}}{f_{N2O\ total}} \tag{5}$$

where $\boldsymbol{f}$ is the flux of N–N_2O (in µgN kg^{-1} h^{-1} for the jar measurements and in µgN m^{-2} h^{-1} for the chamber and liner measurements).

2.4.2. N_2 isotopic calculations

Since the ratios R30 were under the limit of detection of the IRMS most of the time, denitrified N_2 emissions were determined by using the ^{15}N enrichment of the soil nitrate pool undergoing denitrification ($\boldsymbol{a_p}$) calculated with the N_2O data (with the assumption that N_2 and N_2O derive from the same nitrate pool), and by calculating the coefficient $\boldsymbol{d}$ as in Spott et al. (2006):

$$d = \frac{1}{1 - \frac{R29(1-ap)^2 - 2ap(1-ap)}{R29(1-aa)^2 - 2aa(1-aa)}} \tag{6}$$

where $\boldsymbol{a_a}$ is the ^{15}N enrichment of the atmosphere (~0.37 %) that we recalculated as:

$$a_a = \frac{R_{29}(t=0)}{2 + R_{29}(t=0)} \tag{7}$$

Similarly to N_2O emissions, the concentration of denitrified N_2 is given by:

$$[N_2]_{denitrified} = d \times [N_2]_{background} \tag{8}$$

where $\boldsymbol{[N_2]_{background}}$ is the total N_2 concentration measured through IRMS (in ppm).

Usually, the atmospheric $[N_2]$ background (~780 000 ppm) is used for equation [8], but under the artificial atmosphere conditions, this was not possible. It thus had to be quantified at each time step as shown in the next section.

Finally, we calculated the denitrification product ratio as:

$$R_{N2O} = \frac{f_{N2O\ denitrified}}{f_{N2O\ denitrified} + f_{N2\ denitrified}} \tag{9}$$

where $\boldsymbol{f}$ is a flux of nitrogen (either as N–N_2O or N–N_2; in µgN kg^{-1} h^{-1} for the jar measurements and µgN m^{-2} h^{-1} for the chamber and liner measurements).

2.4.3. Background of N_2

The N_2 concentration background was also determined through IRMS, using the peak height of the $^{28}N_2$ isotopologue. Calibration was

made by diluting pure N_2 in He for the high end of the calibration and our custom artificial atmosphere (5 % N_2) for the low end ($R^2 > 0.95$). The robustness of our N_2 background quantification using IRMS was evaluated by direct comparison of identical manual N_2 dilution samples between our IRMS and an Agilent 7890A (Agilent, Böblingen, Germany) equipped with a thermal conductivity detector (Fig. 1).

2.4.4. Liner and jar flux calculations

When using small incubation vessels, sampling can be rather disruptive. On average, a 15 mL sampling represented about 5 % of the jars headspace and 18 % of the liners headspace. Therefore, 15 mL of artificial atmosphere (or He in the case of **Experiment 1**) had to be replaced to compensate for the change in pressure. We corrected for the sampling error as:

$$C_n = C_n^{'} + \frac{V_{sample}}{V_{headspace}} \left[\left(\sum_{i=o}^{i=n-1} C_i^{'} \right) - nC_{gas\ mix} \right] \quad (10)$$

With $C_0 = C'_0$

Where, $\boldsymbol{C_n}$ is the corrected concentration at the nth measurement (ppm), $\boldsymbol{C'_n}$ is the uncorrected concentration at the n^{th} measurement (ppm) and $\boldsymbol{V}$ is the volume (mL).

A model was derived to account for the leaking of the liners and used to correct the concentrations of the studied gases (**Annex V**, **SI**). We found that on average, neglecting these leaks only resulted in an 8.7 % underestimation of the denitrified N_2O fluxes. In closed systems (jars and liners), the issues of gas diffusion from soil to headspace are much smaller than when using static chambers. Therefore, linear regressions were applied to the data corrected for sampling and leaks, with a criterion $R^2 > 0.90$.

It can also be challenging to consistently sample 10 cm high soil cores when using the liners. A liner has a surface of 16.62 cm^2, but we calculated a corrected surface as:

$$S^* = \frac{m}{h \times BD} \quad (11)$$

where $\boldsymbol{S^*}$ is the corrected surface (cm^2) to account for the differences in heights of soil cores, $\boldsymbol{m}$ is the mass of the soil core (g), $\boldsymbol{h}$ its height (cm) and $\boldsymbol{BD}$ the Bulk Density of the studied soil (g cm^{-3}).

Finally, fluxes were calculated on a per mass basis for the jars and on a per surface basis for the liners.

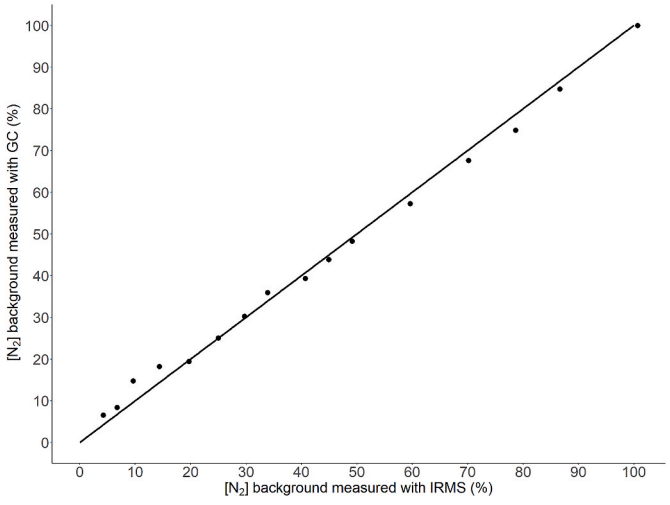

Fig. 1. Direct comparison of the N_2 concentration background measured in identical handmade dilution samples via either IRMS or GC. The 1:1 line is represented in black ($R^2 = 0.9933$).

2.4.5. Static chamber flux calculations

The chambers did not need equilibrium of the pressure after sampling. However, prolonged incubation times can lead to diffusive issues. To mitigate these diffusive errors, we applied the HMR model (Pedersen et al., 2010), using the associated R script (R Core Team, 2023) from CRAN (Comprehensive R Archive Network). This model is based on a revised version of the model of Hutchinson and Mosier (1981). It should be noted that emissions of ^{15}N-labelled molecules induce further diffusive issues that cannot be totally corrected through this model (Well et al., 2019b; Micucci et al., 2023). Fluxes from the chambers were measured on a per surface basis.

2.5. Statistics and modelling

Before statistical analysis, data were tested for normality and homoscedasticity. Normality was tested by visual inspection of a QQ plot combined with a Shapiro-Wilk test (p-value >0.05). If the data were found to be non-normally distributed, they were log-transformed and normality as well as homoscedasticity tests were run again. If the data still did not meet the requirements, non-parametric statistical tests were used (Wilcoxon or Kruskal-Wallis tests). Homoscedasticity was checked with Levene test (p value > 0.05). If data met all the requirements, one-way ANOVA or student-t tests were used to compare means. If an ANOVA showed significant effect of the studied parameter, TuckeyHSD post hoc test was applied to determine which treatments were significantly different. All statistical analyses were performed using statistical packages of R.

All results in this study are given ± standard error.

The non-linear regressions for the modelling of the N_2 background concentration inside the static chambers and liners (**section 3.3.1**) were done using SPSS (IBM Corp. Released, 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp).

3. Results

3.1. Soil properties

For each experiment, soil was analysed with the protocols described in Annexe IV (SI). The different land uses at the Allerton Project site (**H1**, **GC1**, **A1**) were all silt loams, with the two pastures having very similar texture and the arable having a higher proportion of clay (Table 2). On the other hand, the two land uses of the FarmED site (**H2**, **A2**) were clay soils. In July 2021 (**Experiment 2**), the arable **A1** at the Allerton Project site was significantly drier than the pastures (**H1** and **GC1**). Prior to **Experiment 4**, two successive applications of fertilizer in the **A2** field resulted in high levels of nitrate but not of ammonium.

3.2. Laboratory study, impact of the nitrate tracer addition on denitrification fluxes

3.2.1. Experiment 1: Comparison of the two calculation methods

No amount of evolved N_2 could be detected in this experiment and thus, a potential denitrification stimulation could not be fully assessed. The comparison of N_2O emissions is however described in Annexe VI (SI). Another consequence is that the comparison of the two models of calculation had to be done via the denitrified N_2O emissions. The calculation of the ^{15}N enrichment of the soil denitrifying pool differed significantly when calculated with either the "Mulvaney & Boast" or "Arah" model (paired student t-tests for each enrichment and each sampling time, $p < 0.05$). For each replicate, the ^{15}N enrichment calculated with the model of Arah was strictly greater to the one calculated with the model of Mulvaney & Boast (Fig. 2). The difference between the two models decreased with time, the Arah model being on average 14.35, 8.79 and 2.22 % higher after 3, 6 and 24 h respectively (Figs. S8 and SI).

The resulting fluxes also differed significantly between the two

Table 2
Properties of the studied soils. NT = no tested.

	NO_3^- (mgN/kg)	NH_4^+ (mgN/kg)	Gravimetric moisture (%)	WFPS (%)	Bulk density (g/cm^3)	pH	Clay (%)	Silt (%)	Sand (%)
Experiment 1 H1	6.17 ± 0.03	1.93 ± 0.14	21.53	47.21	1.20	6.45	12	75	13
Experiment 2 H1	5.32 ± 0.15	1.93 ± 0.17	29.60	64.9	1.20	6.89	12	75	13
Experiment 2 GC1	12.28 ± 0.23	2.74 ± 0.46	28.74	64.0	1.21	6.45	12	72	16
Experiment 2 A1	8.16 ± 0.10	2.24 ± 0.27	16.78	49.1	1.39	6.72	22	64	14
Experiment 3 Chamber	5.39 ± 0.80	3.73 ± 0.55	26.36	66.3	1.29	NT	NT	NT	NT
Experiment 3 Liner test	25.61 ± 5.41	12.62 ± 3.26	33.16	80.9	1.27	NT	NT	NT	NT
Experiment 4 H2	3.00 ± 0.29	1.99 ± 0.13	19.23	45.5	1.25	7.94	81	10	9
Experiment 4 A2	53.44 ± 10.22	1.67 ± 0.10	15.82	48.4	1.42	7.97	82	13	5

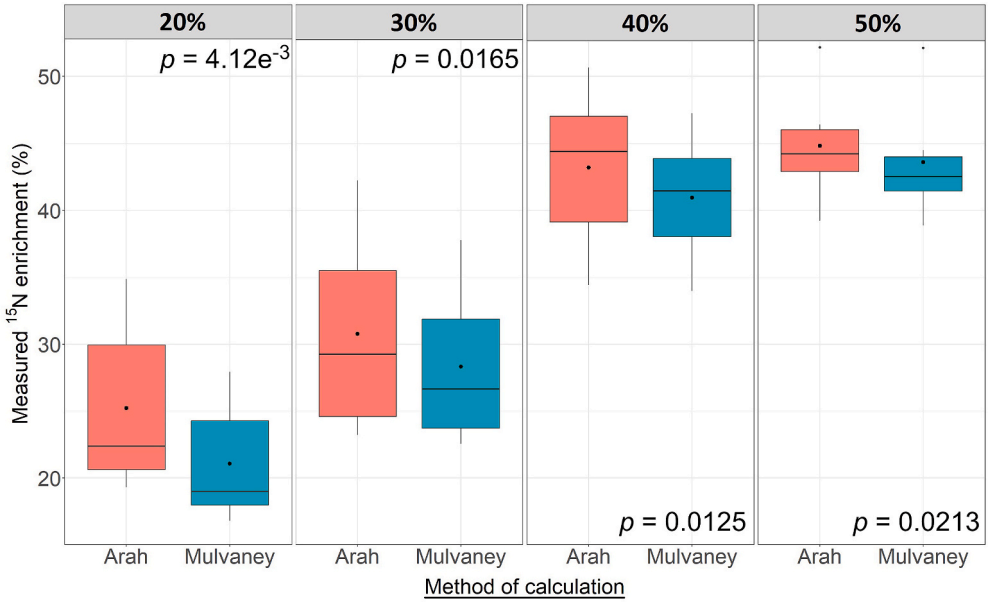

Fig. 2. Comparison of the "Arah" and "Mulvaney & Boast" models for the calculation of the ^{15}N enrichment of the soil denitrifying pool after 6 h of incubation. The plots for the times 3 and 24 h can be found in Fig. S8 (SI). Fig. 2 has been used in Micucci et al. (2023) to illustrate the differences between the two models of calculations but data originate from the present study.

models (paired Wilcoxon tests for each ^{15}N enrichment treatment, $p < 0.05$), with the magnitude of difference decreasing when the ^{15}N enrichment increased. In each case, the model of Mulvaney & Boast yielded a higher flux than the model of Arah. On average, the flux calculated with the Arah model was 17.18, 7.82, 5.22 and 3.24 % lower for the 20, 30, 40 and 50 % ^{15}N enrichment treatments respectively.

3.2.2. *Experiment 2: Dose-response study of the three land uses*

The addition of ^{15}N-labelled nitrate for each treatment resulted in measured ^{15}N enrichments close to the targeted ones (Fig. S9, **SI**).

In **Experiment 2**, the N_2O emissions of the **A1** land use increased slowly and similarly to the other land uses during the first 6 h of incubation; however, every replicate of the three enrichment levels showed very high N_2O concentrations after 24 h (Fig. 3). The SPC after 24 h were (105.5 ± 2.3) % and (103.1 ± 1.1) % for the 20 and 50 % treatments respectively and showed that these large N_2O emissions originated strictly from denitrification.

The fluxes of denitrified N_2O increased for all land uses when targetting a 50% ^{15}N enrichment of the soil denitrifying pool; especially for the **GC1** land use where the average of emissions more than doubled (Fig. 4). These increases were however non-statistically significant (ANOVA, $F_H = 2.19$, $F_{GC} = 2.50$, $F_A = 2.89$, $p > 0.05$) due to the high

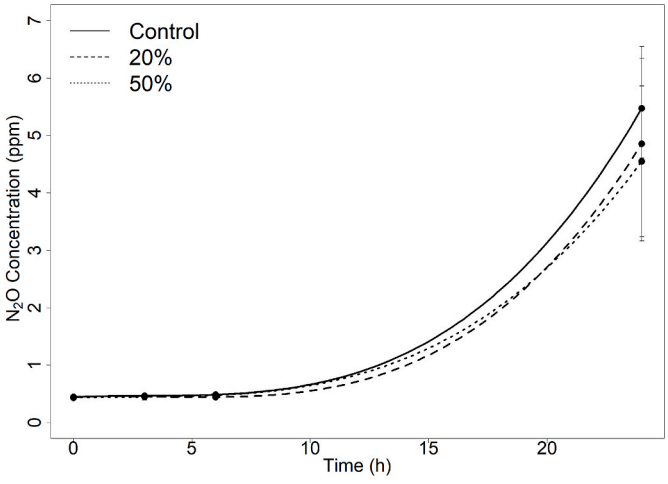

Fig. 3. Mean measured N_2O concentrations of the **A1** land use for the three ^{15}N enrichment treatments (6 replicates) during **Experiment 2**. An exponential model was fitted for better visual assessment. The error bars represent the standard errors of the means.

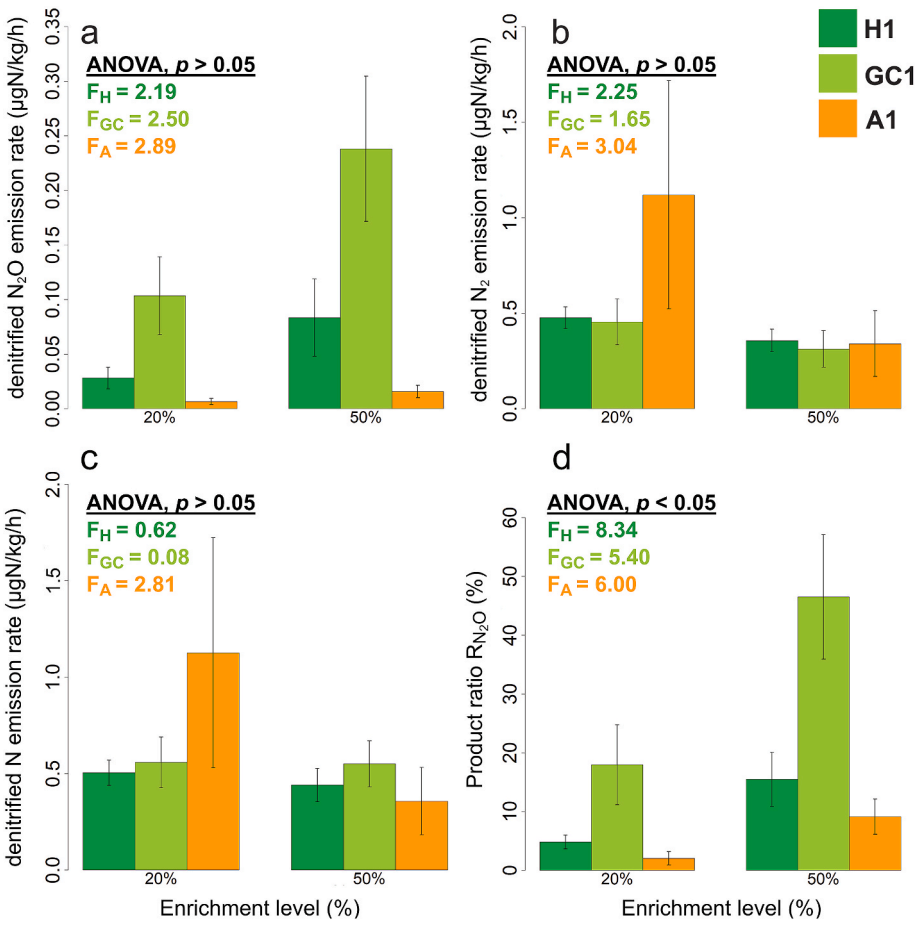

Fig. 4. a) Denitrified N_2O emission rate, b) denitrified N_2 emission rate, c) total denitrification ($N_2O + N_2$) emission rate and d) denitrification product ratios for all treatments during **Experiment 2**. All fluxes are expressed in µgN kg^{-1} h^{-1} while the denitrification product ratios are expressed in %. The error bars represent standard errors of the means.

variability between replicates. The use of the artifical atmosphere gas mix enabled the successful detection of the emissions of N_2 for all replicates. These emissions were relatively steady and did not increase significantly when increasing the amounts of available nitrate (ANOVA, $F_H = 2.25$, $F_{GC} = 1.65$, $F_A = 3.04$, $p > 0.05$). The **A1** land use exhibited higher N_2 emissions under the 20 % treatment, however two replicates at this enrichment level showed abnormally high N_2 emissions. If ignoring these two replicates, this treatment emitted only (0.32 ± 0.06) µgN kg^{-1} h^{-1}, which is comparable to the 50 % treatment emitting (0.34 ± 0.17) µgN kg^{-1} h^{-1}. The N_2 fluxes dominated the denitrification emissions and thus, the fluxes of total denitrification ($N_2O + N_2$) were very comparable to the N_2 fluxes and were also non-statistically different between treatments (ANOVA, $F_H = 0.62$, $F_{GC} = 0.08$, $F_A = 2.81$, $p > 0.05$). The product ratios were inferior to 20 % except for the **GC1** land use at a 50 % ^{15}N enrichment, which reached almost 50 %. These ratios were statistically different between the 20 and 50 % ^{15}N enrichment treatments for all three land uses (ANOVA, $F_H = 8.34$, $F_{GC} = 5.40$, $F_A = 6.00$, $p < 0.05$).

3.3. Field study: development of a new method for denitrification measurement

3.3.1. Experiment 3: Chamber and liner tests, comparison of the ^{15}N enrichment calculated through N_2 or N_2O isotopic data

As expected, the diffusion of atmospheric N_2 inside the chambers occurred at a faster rate than inside the liners (Fig. 5). The N_2 concentration profiles inside these two systems followed a Fickian model and data in Fig. 5 were fitted with the following model equation, in accordance with Pedersen et al. (2010):

$$C(t) = \varphi + (C_0 - \varphi)e^{(-\kappa t)} \quad (12)$$

where C is the concentration of N_2 inside the chamber (in %), φ is the N_2 concentration outside the chamber (assumed constant at 78 %) and κ is an experimental constant (which depends on the coefficient of diffusion of N_2 as well as the chamber dimensions). This parameter was determined via a non-linear regression using **equation [12]**.

The flushing did not work for one of the chambers (Fig. 5, grey in the left panel). The average N_2 concentration background for the chambers after flushing was about (8 ± 0.7) % which is close to the 5 % contained in the artificial atmosphere mixture. However, this background evolved rapidly and rose to an average of (29 ± 6) % after an hour and (43 ± 7) % after 2 h. In comparison, the liners started with an average background of (10 ± 0.5) % in N_2 but only rose to (18 ± 0.6) % after 2 h. They reached an average of (34 ± 2) % after 9 h which is still less than the chambers after 2 h.

The chamber and liner incubations took place in different locations and at different times. Although we can compare the evolution of the N_2 backgrounds, we cannot compare denitrification activities. Through chambers, denitrification emitted an average of (20.02 ± 7.96) µgN m^{-2} h^{-1} of N_2O as well as (2768 ± 775) µgN m^{-2} h^{-1} of N_2, with an average source partitioning coefficient of (21.59 ± 4.09) % and a product ratio of (1.23 ± 0.55) %.

Through liners, denitrification emitted an average of (1100 ± 343) µgN m^{-2} h^{-1} of N_2O as well as (257 ± 55) µgN m^{-2} h^{-1} of N_2, with an average source partitioning coefficient of (94.27 ± 1.92) % and a

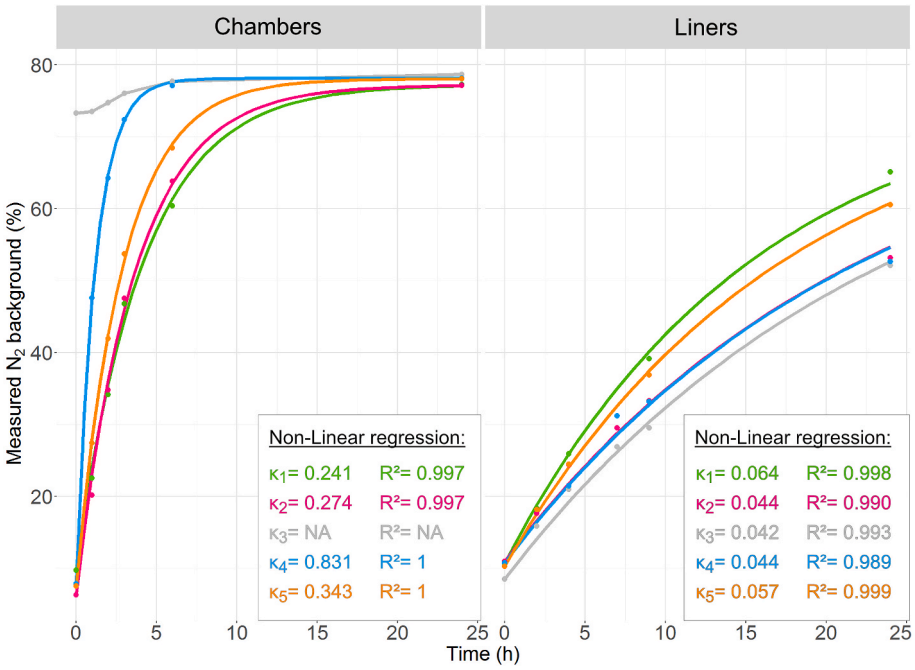

Fig. 5. Evolution of the N_2 background concentration inside the modified static chambers and liners, following flushing of the headspace with the artificial atmosphere gas mix. Each profile represents a replicate (5 for both chambers and liners). The data were fitted with a Fickian model (see equation [12]) for which the parameter κ has been calculated using a non-linear regression. This parameter is given for each replicate along with the coefficient of determination (R^2) in the figure legends.

product ratio of (76.81 ± 4.45) %. The N_2O emissions clearly dominated the denitrification fluxes in this case.

During this first test of the liners, heterogeneity of soil was not taken into account in the calculation of the tracer solution concentrations. Indeed, a subsequent test revealed that only 26 % of a core contained inside a liner was sievable soil (<2 mm, after subtraction of rocks, roots, etc.). This resulted in large excesses of tracer application. Nonetheless, the resulting high enrichments of the soil denitrifying pools coupled with high sensitivity (due to the low N_2 background) allowed us to obtain both reliable R29 and R30 data and to compare the calculated a_p based on either the N_2O or N_2 data (Fig. 6).

The ^{15}N enrichment of the soil denitrifying pool calculated with either the N_2 or N_2O data were significantly different (paired student t-tests for both times of sampling; $p < 0.05$). For each replicate, the ^{15}N enrichment of the soil denitrifying pool derived from the N_2O data was strictly greater to the one derived from the N_2 data. The N_2O data predicted an average a_p of (93.48 ± 1.72) % after 2 h and (93.09 ± 1.91) % after 4 h. In comparison, the N_2 data predicted respectively (89.64 ± 1.90) % and (88.83 ± 2.10) %. As shown by Fig. S2 (SI), in order to reach an a_p of 93 %, one needs to add approximately 20 times the quantity of originally present nitrate, compared to only 1 time for an a_p of 50%.

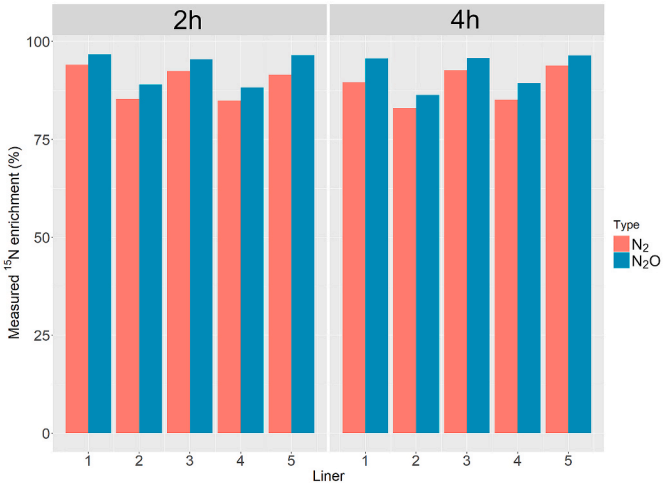

Fig. 6. Comparison of the ^{15}N enrichment of the soil denitrifying pool (a_p) measured with either the N_2 or N_2O data during an *in situ* incubation after 2 h (left) and 4 h (right). The method of Mulvaney & Boast was used for both N_2O and N_2. These enrichments are given individually for each replicate and for the two times of sampling.

3.3.2. *Experiment 4: Confirmation of the liner method for the measurement of denitrification*

During this experiment, we took specific care to reach the targeted ^{15}N enrichment (50 % for both **H2** and **A2** treatments). We reached an average enrichment of (58.86 ± 2.36) % for **H2** and (37.54 ± 5.30) % for **A2** (Fig. S10, **SI**). The higher variability in the **A2** treatment can be correlated with the variability in nitrate content (Figs. S10 and SI) resulting from recent application of fertilizer.

Denitrification was successfully measured *in situ* using the liner method (5 replicates out of 8 showed detectable N_2 for both the **H2** and **A2** treatments) and was dominated by N_2 emissions (Fig. 7) as shown by the average product ratios of (11.05 ± 1.80) % in **H2** and (32.30 ± 9.74) % in **A2**. The N_2O emissions were dominated by denitrification in the case of **H2** but not in the case of **A2**, as shown by the SPC of (56.35 ± 7.77) % and (41.88 ± 6.05) % respectively (Fig. 7).

4. Discussion

4.1. *Fertilization effect*

We did not observe higher total denitrifying activity (N_2O + N_2) when increasing the amount of available nitrate under laboratory conditions (**Experiment 2**), but rather a non-significant increase in N_2O emissions. This could be due to the fact that, as shown in Table 1, the amendments of nitrate in our study were too low to stimulate

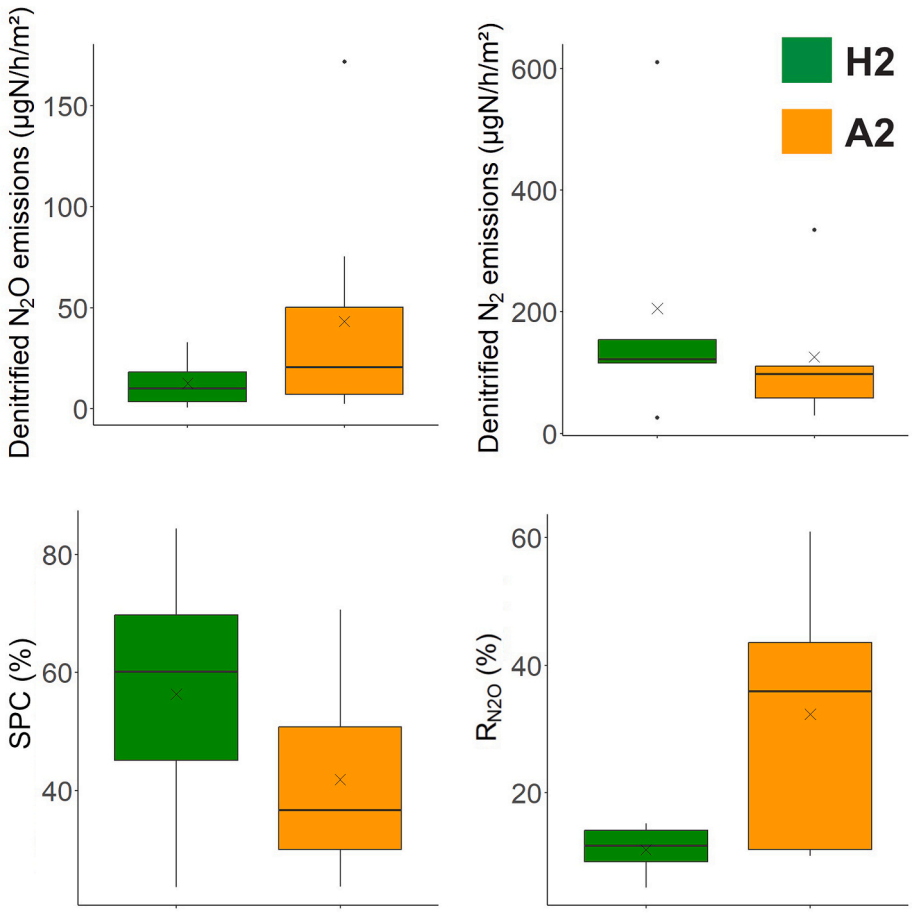

Fig. 7. a) Fluxes of denitrified N_2O, b) fluxes of denitrified N_2, c) N_2O source partitioning coefficient and d) denitrification product ratios for **H2** and **A2** during **Experiment 4**. All fluxes are expressed as µgN kg^{-1} h^{-1} while the source partitioning coefficients and denitrification product ratios are expressed in %. The crosses in the middle of the boxes represent the averages.

denitrification significantly. Indeed, some studies reported higher denitrifying activity under amendment of nitrate, but after several applications of at least 75 kgN ha^{-1} (e.g. Jarvis et al., 1991; Loick et al., 2017), which do not really compare with the present rates. Or it could be that the peak of emissions after amendment had not been reached yet. For example, Loick et al. (2017) observed a peak of denitrification emissions 3–5 days after application of nitrate. If our experiments followed the same pattern, our approach would not measure an artefact due to tracer application, but still background fluxes labelled with isotopes. Our laboratory trials for denitrification stimulation only focused on the first 24 h after label application, a more thorough assessment in time (i.e. during the first week) would enable to verify this hypothesis. The very high N_2O concentrations observed after 24 h for the **A** soil in **Experiment 2** were most likely due to a wetting effect and not to nitrate amendment. This particular emission pattern did not affect the results (see Annexe VI, SI).

We did however observe significantly different denitrification product ratios in laboratory (**Experiment 2**). This result seems to be confirmed in **Experiment 3** where incorrect application of large amounts of $^{15}N–NO_3$ tracer (20 times the quantity of ambient nitrates) resulted in large emissions of denitrified N_2O as well as high denitrification product ratios (77 % on average). The denitrification product ratio depends on many parameters but mainly on the nitrate concentration (Clayton et al., 1997; Blackmer and Bremner, 1978; Firestone and Tiedje, 1979; Warner et al., 2019). It seems that under higher nitrate conditions, microbes tend to favour N_2O over N_2 and incomplete denitrification is promoted.

In the context of enhancing the current sensitivity of the ^{15}NGF, increasing the quantity of $^{15}N–NO_3^-$ tracer applied only makes sense if it does not create significant artefacts. In a recent meta-analysis, Scheer et al. (2020) reported a mean denitrification product ratio of (12.40 ± 3.1) % for soils under natural vegetation and (10.90 ± 2.0) % for agricultural soils. The denitrification product ratio measured in **Experiment 4** for the herbal pasture (**H2**) was 11 % and thus fell in the expected range; while the average a_p reached was 40 %. During the chamber test (**Experiment 3**), the denitrification product ratio was only 1.2 % with an average a_p of 63 % and thus, does not seem to have been stimulated. The **A2** soil in **Experiment 4** showed a high product ratio (32 %) for an agricultural soil but this could rather be explained by recent amendment of fertilizer than tracer application. Indeed, this field received 2 applications of nitrate (11.57 kgN ha^{-1} a month prior as well as 17.36 kgN ha^{-1} ten days prior, along with similar quantities of ammonium and urea, see **section 2.2.2**). This accumulation of nitrate may have resulted in a saturation that drove this high product ratio. The addition of nitrate tracer (23.96 kgN ha^{-1} equivalent fertilizer rate) resulted in less than doubling the amount of present nitrate. We hypothesize here that the product ratio of 32 % observed in the **A2** soil during **Experiment 4** is representative of a product ratio for an arable soil after fertilization events and that the addition of nitrate tracer did not impact significantly this ratio, as it did when adding 20 times the quantity of ambient nitrates in **Experiment 3** (product ratio of 77 %).

Overall, frequent fertilization of agricultural soils along with rotations in arable systems (involving nitrogen-fixing legumes) maintains elevated nitrate levels. Consequently, it appears that denitrification is not constrained by nitrate availability in these types of systems and that using ^{15}N enrichments up to 50 % is a good way to derive product ratios

representative of the selected fields.

4.2. A new in situ method of denitrification measurement

On average, the N_2 concentration background inside the chambers was 29 and 43 % after one and 2 h of incubation respectively (**Experiment 3**). Using data from Micucci et al. (2023) shows that this theoretically results in 2.7 and 1.8 times greater sensitivities respectively compared to regular static chambers. On the other hand, the liners sustained the artificial atmosphere background for longer (Fig. 5), which, combined with a smaller headspace (83 mL compared to 5.3 L for the chambers), offered a higher chance of N_2 flux detection. Well et al. (2019a) reported a sensitivity of 10 ppb for (N_2 + N_2O) fluxes, which was considered 80-fold better than the regular ^{15}NGF method, with an IRMS for which the R29 repeatability was 5×10^{-7} (one standard deviation). We calculated the limit of detection (LOD) of our liner method using **equation [6]** and data from **Experiment 4**, such as the average R29 ratio at time 0 and our IRMS repeatability for R29 (one standard deviation $\sim 1.5 \times 10^{-6}$), the average ^{15}N enrichment of the atmosphere (a_a ~0.37%), a ^{15}N enrichment of the soil denitrifying pool $a_p = 50$ %, and the average N_2 concentration background observed after 2 h (18 %). The resulting LOD was 480 ppb of N_2 (equivalent flux of 4 g N ha^{-1} day^{-1}). If using an IRMS with the same repeatability as Well et al. (2019a), the LOD drops to 160 ppb of N_2 (equivalent flux of 1.3 g N ha^{-1} day^{-1}); which is 16 time greater than Well et al. (2019a) LOD and thus approximately 5 times better than the regular ^{15}NGF. This LOD was however sufficient to enable N_2 flux detection. Indeed, during **Experiment 4**, 62.5 % of our replicates showed detectable N_2 fluxes, but over a one-year field campaign (unpublished results), the detection was successful 90 % of the time. Furthermore, the liner approach easily enabled the simultaneous incubation of 16 cores (even 24 cores during the field campaign), where the method of Well et al. (2019a) has a much smaller throughput, resulting in a poorer spatial resolution. Their approach is however a flow-through method, which is considered as more reliable for flux estimations (Kostyanovsky et al., 2018) and which reduces the diffusion issues caused by the accumulation of gas inside the chamber (Well et al., 2019a). Compared to the He/O_2 GFSC method, our liner approach enables to get *in situ* flux estimations and is less disruptive, since the soil cores are only flushed for 5 min rather >16 h (as in Scholefield et al., 1997; Butterbach-Bahl et al., 2002; Cárdenas et al., 2003; Wang et al., 2020 for example). This limits the drying effect on soil and probably offers more accurate results.

In terms of technical aspects, the liners only isolate the top 10 cm of soil, however, these 10 cm are within the tillage zone where most of the denitrification activity is supposedly occurring due to higher organic matter and nitrate contents, root exudates, plant litter, fertilizer application etc. (Well et al., 2019b; Groffman et al., 2009). It is at this point unclear how to upscale liner fluxes; this approach would probably work best in combination with regular static chambers on top of undisturbed soil. The source partitioning coefficient and denitrification product ratio measured determined through the cores could be applied to the total N_2O emissions measured from the chambers. This is similar to the approach of Wang et al. (2020) or Bizimana et al. (2022) who applied laboratory derived product ratios to field chamber measurements; although in our case, the denitrification metrics would be determined under *in situ* conditions and thus would be more accurate.

Another important aspect of our method is the application of tracer. In our case, we incubated the soil cores immediately after injection to maximise the chances of N_2 flux detection. However, allowing time for the tracer to diffuse within the soil matrix would allow for a more homogenous distribution of the ^{15}N atoms. As shown by Boast et al. (1988), Arah (1992) and recently reviewed by Micucci et al. (2023), heterogeneity of the label distribution leads to an overestimation of the ^{15}N enrichment of the soil denitrifying pool. Data from **Experiment 4** showed that on average, the a_p at times 0 were overestimated by 36 % and 40 % relative to their values at times 2 and 4 h respectively (data not shown). These results highlight the progressive diffusion of the label inside the soil matrix. Given the high success rate of the liner approach, it is likely that allowing time for tracer dispersion will still guarantee a good detection rate of N_2 fluxes and could be a way to improve our method and limit the measurement of eventual artefacts. Similarly, the soil cores inside the liners are much more sensitive to soil heterogeneity than the incubated soil under the chambers. We found that only 26 % of the core contained inside a liner was sievable soil (<2 mm). This needs to be taken into account when preparing the tracer solutions. It can be added that sampling the small headspace of the liners can potentially draw soil pore air. Since the pores and headspace are flushed with the artificial atmosphere, it should not cause much disturbance in theory. One way to mitigate this effect could be to sample times 0 with atmospheric air (outside of the liner) and only sample the final time of incubation inside the liner. Finally, the liners were slightly leaking (as shown by the N_2 background concentration, Fig. 5); in the case of denitrified N_2O fluxes, this only resulted in an 8.7 % underestimation according to our diffusion model (**Annex IV**, **SI**). Given the complexity of this model and its reliance on flux linearity, a case could be made to ignore diffusive losses for liner incubation. Finally, the reduction of the N_2 atmospheric concentration can drive diffusive fluxes from soil pores to the liners headspace, which tend to overestimate N_2 emission rates. Nonetheless, the use of isotopes enables the discrimination of newly produced biogenic N_2 and mitigates this overestimation.

4.3. Comparison of the "Mulvaney & Boast" and "Arah" models

On average, the model of Mulvaney & Boast yielded ^{15}N enrichment values closer to the targets and with less variability (Fig. 2); which tends to demonstrate that this model performs better. The difference between the two models decreased as the ^{15}N enrichment of the soil denitrifying pool and the time of incubation both increased. For the 20% treatment, the Arah model yielded fluxes that were on average 17.18 % smaller to the ones calculated with the model Mulvaney & Boast, which represents a non-negligible error. To our knowledge, this is the first time that these two models are compared. The Arah model is more often found in the literature (Russow et al., 1996; Spott et al., 2006; Tauchnitz et al., 2015; Buchen et al., 2016; Krause et al., 2017; Rummel et al., 2021) due to its simplicity. It does however perform similarly to the Mulvaney & Boast model for higher ^{15}N enrichments of the soil denitrifying pool (3.24 % difference for the 50 % treatment) and should not make a significant difference at these levels.

4.4. Comparison of the ^{15}N enrichment calculated via either N_2 or N_2O isotopic data

The ^{15}N enrichment of the soil denitrifying pool calculated with either the N_2 or N_2O data during the liner test of **Experiment 3** were significantly different. Fig. 6 shows however that the magnitude of difference was almost negligible, the N_2 data yielding an average ^{15}N enrichment only 4.6 % lower at maximum (after 4 h of incubation). The ^{15}N enrichments of the soil denitrifying pool derived from the N_2O data were strictly greater to the ones derived from N_2 data in every case. This is contrary to the observations of Warner et al. (2019, SI) who reported ^{15}N enrichments from N_2 being consistently higher than the ones calculated with N_2O. Buchen et al. (2016) observed mixed trends but most of the time, the ^{15}N enrichment based on N_2 was the highest. Nonetheless, we present further evidence that the ^{15}N enrichment calculated via N_2O is a good proxy for the ^{15}N enrichment of a single denitrifying soil nitrate pool, and should be used for enhanced sensitivity of the N_2 flux measurement via the ^{15}NGF (Baily et al., 2012).

5. Conclusion

Combining the ^{15}NGF method to an N_2-depleted artificial atmosphere is the most promising way to increase its sensitivity, ensuring

robust, reproducible and reliable *in situ* denitrification rate measurements. The two approaches presented in this study are suitable candidates for such measurements, but the liner approach was preferred due to its better sensitivity, throughput and spatial resolution. For both methods, the sensitivity can further be enhanced by targeting a 50 % ^{15}N enrichment of the soil denitrifying pool and by calculating this enrichment through the N_2O isotopic data with the model of Mulvaney and Boast. Furthermore, our *in situ* experiments showed that using a reasonable amount of tracer (i.e. not more than doubling the quantity of ambient soil nitrate) should not impact the product ratio in a significant way. One of the key benefits of the liner method is the ability to reliably quantify true *in situ* denitrification product ratios and source partitioning coefficients. Combined with static chamber N_2O flux measurements, these two metrics could be used to derive field-scale denitrification fluxes and help improve N budget quantification. Finally, a better sensitivity could probably be achieved by using longer cores and allowing more time for tracer to diffuse within the soil matrix before starting the incubation.

CRediT authorship contribution statement

Gianni Micucci: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fotis Sgouridis:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Niall P. McNamara:** Writing – review & editing, Conceptualization. **Stefan Krause:** Writing – review & editing, Supervision. **Iseult Lynch:** Writing – review & editing, Supervision. **Felicity Roos:** Writing – review & editing, Supervision. **M. Glória Pereira:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Sami Ullah:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Towards enhanced sensitivity of the 15N Gas Flux Method for quantifying denitrification in soil (Original data) (Mendeley Data)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109421.

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